BIOCHEMICAL, PHYSIOLOGICAL AND AGRONOMIC RESPONSE OF VARIOUS SWEET POTATO CULTIVARS/VARIETIES TO DROUGHT STRESS IN RAINOUT SHELTERS AND FIELD CONDITIONS

By

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2014

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ABSTRACT

Drought is and will always be an issue in the cultivation of plants. Some plants have the ability to withstand a drought conditions to a certain degree while others, with other useful attributes, fail dismally. The value of testing genotypes for the ability to tolerate drought cannot be underestimated and will enhance the progress in the selection of drought tolerant genotypes. Thus, the objective of this study was to investigate the physiological, biochemical and agronomical reaction of sweet potato plants to drought and the procedures which could be used to test for sweet potato drought tolerance in the field. This was made possible through the creation of an environment at ARC-Roodeplaat in which sweet potato plants could be subjected to drought stress conditions.

Thirty five sweet potato genotypes were planted in three trials in rainout shelters and open fields to analyze their physiological, biochemical and agronomical responses to drought stress. The majority of the genotypes were selected breeding lines with some cultivars from America, Peru and South Africa. These genotypes were chosen due to their range of traits for incorporation in crosses in the sweet potato breeding programme of the Agricultural Research Council (ARC). Drought stress conditions on the plants were induced through selective irrigation practices. In Trial 1 control plants were cultivated at field capacity while drought stressed plants received 60% and 30% of the amount of water of the control, respectively. In Trial 2, genotypes were planted in the field and under rainout shelters respectively. The field plantings acted as the control and received normal rain and irrigation while the rainout shelter planting received irrigation corresponding to 30% of field capacity. The plants in Trial 3 were subjected to control and drought conditions with the drought stressed plants receiving 30% of the water of the control. Leaf harvesting and phenotypical measurements were conducted twice during the trial period i.e. 60 and 120 days after planting. The drought stress impacted the growth of the sweet potato plants significantly. Canopy cover and stem length were severely influenced by the drought stress and resulted in huge declines of the respective values in all trails. Canopy cover values declined by more than twice compared to the control while stem length values were reduced by up to 10 times compared to the control.

Antioxidant systems with particular reference to ascorbate peroxidase (AP), super oxide dismutase (SOD) and glutathione reductase (GR) reacted to the stress imposed and increased significantly. It was observed that values of the respective antioxidant enzyme systems increased sharply in the latter part of the trial and that the increase was also more intense at severe stress. The analysis of the antioxidant system made it possible to distinguish between the genotypes regarding their reaction to the stress.

Results for carbon discrimination experiments in all the trials indicated that a significant decline in values took place as the drought stress increased. The decline appeared to be slightly more pronounced as the stress progressed. Also, as in the case of the antioxidant systems, it was possible to distinguish between genotypes even in the control treatments. The plants responded to the drought stress to the effect that a similar trend, (compared to the antioxidants), was observed with regards to stomatal conductance although genotypical differentiation was not possible in any of the stress conditions. It was demonstrated in the trials that the relative water content (RWC) values in the leaves of plants subjected to drought stress declined significantly between water treatments.

Drought stress in the three trials had a severe impact on the nitrate reductase (NR) activity in the leaves of the plants. The decline in values were substantial but no significant differences could be detected between the genotypes except for the breeding line 2005-1-16 and cultivars Purple Sunset, Beauregard and Zapallo. Slight non-significant differences were observed between the genotypes at mild stress conditions but the severe stress conditions proved too harsh. Significant increases in the proline content of the sweet potato plants subjected to drought stress resulted in differentiation between the genotypes in Trial 1 and Trial 2, especially during the latter stages of the trials and at severe stress.

Large reductions, up to 97%, of root yield were detected in the three trials. It appeared that the severe stress treatment proved too harsh to accomplish significant differences between the genotypes in all the trials. In Trial 1 the genotype Resisto differed significantly from the other genotypes and seemed to tolerate the drought the best in the mild stress conditions. Water use efficiency (WUE) values did allow for discrimination between the genotypes in Trial 1. A large decline in WUE values were observed in Trial 2 in general, although a few breeding lines 2005-7-4, 2006-4-4 and

2006-7-7 were prominent with high WUE values and could be recommended for use in a breeding programme. In Trial 3 the cultivar Bophelo and 199062.1 also exhibited higher WUE values which correlate well with yield data obtained from the same Trial. This could also prove valuable in the selection process.

Due to the fact that multiple traits make a valuable contribution to the decision-making process in the selection for possible screening methods, statistical correlation was undertaken to establish possible relationships between traits. Good correlation was found between yield, stomatal conductance and WUE in Trial 1. This confirmed the assumption that a drop in stomatal conductance will result in lower root yield. Proline correlated also very well with the antioxidant enzyme levels of GR and AP which indicates that while the antioxidant enzymes play a role in combatting oxidants proline aid in possible prevention of moisture loss and stabilization of cell membrane structures.

In Trial 2 good correlation was observed between yield, LAI, NR and CCI and to a lesser extent carbon-13 discrimination. This confirmed the belief that a decrease in LAI and CCI should have a negative effect on the yield due to less canopy cover and less chlorophyll for the capture of sunlight for photosynthesis. Results from Trial 3 also indicated good relationships between proline, GR and AP, as well as good relationships between yield, WUE, carbon discrimination and stomatal conductance (g_s).

It can hereby be concluded that the reaction of sweet potatoes to drought stress revealed results that can be of help for use in the future to successfully establish a protocol whereby successful selection of genotypes can be made in a biochemical, physiological and agronomical way. The study also provided proof that some of the approaches and procedures used in these trials can be successfully implemented in the drought screening of sweet potato.

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LIST OF ABBREVIATIONS

ABA	-	Abscisic acid
AGPase	-	ADP-glucose pyrophosphorylase
ANOVA	-	Analysis of Variance
AOS	-	Active oxygen species
AP	-	Ascorbate peroxidase
ARC-Roodeplaat	-	Agricultural Research Council – Roodeplaat
C	-	Carbon
CO_2	-	Carbon dioxide
C_3 plants	-	Carbon 3 plants
^{12}C	-	Carbon-12 isotope
^{13}C	-	Carbon-13 isotope
CRBD	-	Complete Randomized Block Design
CCI	-	Chlorophyll content index
$^{13}CO_2$	-	Carbon-13 isotope carbon dioxide
FAO	-	Food and Agricultural Organization of United Nations
DAP	-	Days after planting
DW	-	Dry weight
EDTA	-	Ethylene diamine tetraacetic acid
g	-	Gram/s
g_s	-	Stomatal conductance
GR	-	Gluthathion reductase
GSSG	-	Glutathione disulfide
H	-	Hydrogen
HCl	-	Hydrochloric acid
H_2O	-	Water

H_2O_2	-	Hydrogen peroxide
LAI	-	Leaf area index
LR	-	Large rainout shelter
LSD	-	Least Significant Difference
mg	-	Milligram
ml	-	Milliliter
mM	-	Millimole
Mt	-	Megatonne
N	-	Nitrogen
NaCl	-	Sodium chloride
NADP	-	Nicotinamide adenine dinucleotide phosphate
NADPH	-	Nicotinamide adenine dinucleotide phosphate, reduced
NH_3	-	Ammonia
nm	-	Nanometer
NR	-	Nitrate reductase
OA	-	Osmotic adjustment
O_2^-	-	Oxygen radical
PDB	-	Pee Dee Belimnite
P5CS	-	Δ 1-pyrroline-5-carboxylate synthetase
pH	-	Hydrogen ion potential
PXD	-	Peroxidase
RED	-	Reductase
ROI	-	Reactive oxygen intermediates
RWC	-	Relative water content
SOD	-	Super oxide dismutase
SR	-	Small rainout shelter
t/ha	-	Tons per hectare

T1	-	Time 1
T2	-	Time 2
TMT	-	Treatment
Tris	-	Tris(hydroxymethyl)aminomethane
TW	-	Turgescent weight
U	-	Unit/s
W	-	Weight
WHO	-	World Health Organization
WUE	-	Water use efficiency

GENERAL INTRODUCTION

Sweet potato, *Ipomoea batatas* L. (Lam.), is internationally an important crop in the food chain **(MARTIN, 1988)**. It is currently ranked 7th in the world regarding production **(FAO, 2014)**. One of the advantages of sweet potato as a staple crop is that it is relatively easy to grow as well as the fact that it is relatively free of pests and diseases **(MARTIN, 1988)**. One of the reasons for its popularity in Africa and specifically sub-Saharan Africa is that it can be grown in relatively poor soils and needs little attention regarding cultivation during the growing season. It is a nutritious crop with high starch as well as vitamin content, in particular vitamin A, which is abundant in orange fleshed varieties. Apart from being available as a food source for human consumption, sweet potato is an excellent source of animal feed.

It is claimed that due to the lack of knowledge about the water requirements of crops, 70% of the yield is lost **(LOPES, ARAUS, VAN HEERDEN and FOYER, 2011)**. This can be minimized with adequate knowledge of variety choice and correct cultivation practices. Water is an important entity for plant growth. Some plant species have the ability to survive and produce with low quantities of water while other species require an abundance of water to grow optimally.

Although sweet potato is a drought tolerant crop, it is susceptible to drought especially in the early stages of growth. **VAN HEERDEN and LAURIE (2008)** have shown that the growth of the sweet potato plant is severely retarded during drought conditions, which affects yield. This agrees with the findings of **MARTIN and JONES (1986)**.

Various researchers such as **EKANAYAKE and COLLINS (2004)** and **ABIDIN, VAN EEUWIJK, STAM, STRUIK, MALOSETTI, MWANGA, ODONGO, HERMANN and CAREY (2005)** have conducted trials on sweet potato using normal rainfall as the water supply or using an irrigation system to force certain physiological and agronomical outcomes from the varieties. These outcomes can then be used for selection of varieties for future use.

Due to the impact of climate change around the world, the importance of choosing drought tolerant varieties is becoming a priority for producers. Producers, specifically informal producers, are faced with a number of factors to overcome for successful production **(FAO, 2013)**. These factors include choosing the correct varieties to adapt to conditions like poor fertilization, insufficient pest management and most importantly an infrequent supply of water for irrigation. Research has an important part to play here to assist the choice and release of better varieties for the commercial market.

Although little work has been conducted regarding the development of methodology of the assessment of drought tolerance in sweet potato, an appreciable number of scientists have made large contributions in the search for an optimal process of developing successful methodology for screening drought tolerant sweet potato. **EKANAYAKE and COLLINS (2004)** utilized a line source sprinkler system to create drought conditions from which they investigated the effect of drought on the yield and leaf water potential of eight sweet potato genotypes. **HAIMERONG and KUBOTA (2003)** undertook drought experiments in pots, with sweet potato, whereby the photosynthetic system of the plant, leaf area development, leaf water potential, stomatal conductance and soil water potential were investigated. **SUNG (1981)** found that although the movement of sweet potato stomata was not affected by water stress, nitrate reductase activity was reduced as soil water potential decreased. The optimum solution for the discovery of methodology in drought tolerance assessment of sweet potato would be to have plants grown in field conditions whereby irrigation is supplied without the interference of normal rainfall.

In order to establish an efficient screening method to assess drought tolerance in sweet potato, parameters such as stomatal control, antioxidant enzyme activity, leaf area development, free proline content, chlorophyll content, carbon isotope discrimination, leaf water content and water use efficiency have to be identified early in the growth season of the plant. This will save time and costs when the final genotype is to be selected. These parameters can then be used singly or in combination to formulate a successful method to be used in a breeding programme. Several studies have been conducted where these

parameters have been investigated on a number of crops such as potato **(DEBLONDE and LEDENT, 2001)**, sugar beet **(MONTI, AMADUCCI, PRITONI and VENTURI, 2006)**, wheat **(AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)**, and cotton **(LEIDI, LOPEZ, GORHAM and GUTIÉRREZ, 1999; DE BRITO, SOFIATTI, DE ANDRADE LIMA, DE CARVALHO and DA SILVA FILHO, 2011).**

An additional reason why sweet potato is being consumed is the fact that it contains antioxidants, i.e. β-carotene and vitamin C **(RAUTENBACH, FABER, LAURIE and LAURIE, 2010).** These antioxidants also provide a basis on which the plant can defend itself against stress conditions. Various authors have demonstrated the presence and value of these antioxidants in sweet potatoes and their differences in activity between varieties **(TEOW, VAN-DEN TRUONG, MCFEETERS, THOMPSON, PECOTA and YENCHO, 2007; RUMBAOA, CORNAGO and GERONIMO, 2009; KIM, KIM, LEE and KWAK, 2009).**

Objective of the study

There are few investigations regarding the response of sweet potato to drought especially using the above mentioned parameters. The main objective of this study was to try and understand why certain varieties have the ability to withstand drought conditions while others are susceptible. The sweet potato breeding programme at ARC-Roodeplaat consists of parent clones (breeding lines), containing one or more traits, that are used in the process of establishing desired cultivars. The aim was also to identify breeding lines and cultivars that exhibit traits that will result in the production of sweet potato varieties that produce a high root yield with high quality under drought conditions. The approach was to evaluate varieties in drought and normally irrigated conditions, to analyze their performance using the following approaches:

1. Determine the influence that drought has on agronomical aspects such as yield;

2. Determine the influence that drought has on the morpho-physiological variables of the plant i.e. stomatal conductance, canopy coverage, chlorophyll content, stem length and relative water content;

3. Determine the influence that drought has on biochemical enzyme systems such as the antioxidant enzymes glutathione reductase, ascorbate peroxidase and superoxide reductase, as well as the influence on the enzyme nitrate reductase, the important amino acid proline and the total carotenoid content;

4. Determine the applicability of carbon isotope values in the assessment of the varieties for drought and water use efficiency; and

5. Determine possible correlations between different parameters evaluated during the study.

CHAPTER 1
LITERATURE REVIEW

1.1. History and importance of sweet potato

The history of sweet potato (*Ipomoea batatas* (L) Lam) dates back to prehistoric times when it was cultivated in tropical America. It is suggested that its origin is in Peru, where dried roots have been dated to 8000 – 10000 years before present using radiocarbon data **(ENGEL, 1970)**. Christopher Columbus found the sweet potato in the West Indies in 1492 and introduced it, on his return, to Spain and consequently the rest of Europe. Sweet potato was introduced to northern Africa and India via the transfer of European breeding lines from Europe, while Mexican breeding lines found their way to the Philippines via the Spanish trade routes. **O' BRIEN, (1972)** believes that the sweet potato crop was introduced to Mozambique and Angola with the aid of the Portuguese. Information has it that sweet potato was brought to China from India and Burma in 1594 **(HO, 1955)**. Currently sweet potato cultivars/breeding lines, which were improved by local breeders in the respective countries i.e. USA, Peru and Kenya, are being used in other countries as part of national breeding programmes for drought tolerance and quality improvement.

Sweet potato is a very important crop for human consumption due to its nutritional value. It has the advantage over other crops of providing a quick yield during a short growth period and providing stable productivity, little affected by climatic factors **(HAIMEIRONG and KUBOTA, 2003)**. Sweet potatoes are typically rich in carbohydrates and vitamin A, thereby contributing to the nutritional requisite for a healthy diet, while other favorable nutritional characteristics include; a good source of Vitamin C, average amounts of protein, dietary fiber and significant amounts of several additional vitamins and minerals **(KALLOO and BERGH, 1993)**. Considerable quantities of sweet potatoes are used to produce starch and alcohol products adding to its value for humans and animals in addition to its use as a normal food. The crop is normally grown between 40 degrees north and 32 degrees south depending on the climate although it can be grown almost anywhere in the world where temperatures are favorable for sweet potato production

(CARDI, 2010). It is a perennial plant but is cultivated as an annual. Sweet potato is grown in developing countries due to its advantage of a short growing season and few pests and diseases that seem to affect its growth.

Sweet potato is the seventh most produced food crop in the world, surpassed only by wheat, rice, corn, potato, barley, and cassava. The annual world production is a predicted 106 Megatons (Mt) with 88 Mt produced in Asia, 3 Mt produced in the America's and 1.5 Mt produced in Africa respectively. China is the country with the highest production in the world annually harvesting 81 Mt. **(FAO, 2014)**.
In South Africa the production is a fraction of the total world production with a total of about 60 000 tons of which 1700 tons are exported **(DAFF, 2013)**.

1.2. Drought and sweet potato

Drought is one of the most devastating stresses for the production of crops internationally. Drought, amongst other environmental factors, contributes to about 75% of crop diversity loss each year in large parts of the world **(FAO, 2010)**, mainly due to inadequate knowledge of varieties and environmental conditions. Drought tolerance is in general defined as the identity given to a cultivar that shows a relatively small yield reduction upon exposure to drought **(VISSER, 1994)**. There are different mechanisms by which a plant may be rendered drought tolerant. Plants can (1) escape drought by shutting down non-vital processes until the drought period has passed, (2) plants may have the ability to tolerate dry periods through the upholding of an appreciable water balance by means of osmotic adjustment. A deeper root system can aid in this process **(VISSER, 1994)**. Sweet potato, even though a hardy crop by nature, is faced with production constraints that include drought stress. Drought stress is one of the most limiting factors to plant growth and yield, particularly in arid and semi-arid regions. In most parts of southern Africa the rainfall is seasonal and unreliable, and the evapo-transpiration rate very high **(ROUAULT and RICHARD, 2003)**. Drought causes stomatal closure and as a result it reduces carbon dioxide uptake for photosynthesis which in turn affects the yield and growth of the plant **(JONES and CORLETT, 1992)**. Drought tolerance in sweet potato is a complex

physiological trait **(EKANAYAKE and COLLINS, 2004)**, and the effects of drought stress on sweet potatoes are considered to be variety specific.

Although sweet potato can be a relatively drought tolerant crop, the biggest effect of drought susceptibility is encountered at the early growth stages. This is taking into consideration that adventitious roots are formed within a few days after planting. Should drought conditions be introduced very soon after planting the plant would not be able to develop its full complement of roots to ensure optimum production. **SARASWATI, JOHNSTON, COVENTRY and HOLTUM (2004)** executed drought experiments with sweet potato varieties in pots. They found that in stressed sweet potato plants, the varieties showed different reactions to the stress. The leaves exhibited different leaf water potentials related to the stress, but they could not find any significant differences between the varieties. The results from a drought stress experiment executed by **HOLWERDA and EKANAYAKE (1991)** showed that sweet potato plants subjected to drought stress exhibited a significantly higher root:shoot ratio than the well watered plants which might indicate good shoot development. They further noted the importance is the establishment of a good root structure for the establishment of a proper yield.

VAN HEERDEN and LAURIE (2008) reported severe reduction in yield of the cultivar Resisto during drought stress. They found that reduced yield after drought stress was caused by limited expansion and senescence of the leaf canopy, as well as a reduced photosynthetic rate per unit leaf area. Also stomatal conductance revealed large varietal differences in sweet potato **(VAN HEERDEN and LAURIE, 2008)**.

Drought is a common phenomenon around the world and also in sweet potato growing areas even though it is extensively grown in tropical and sub-tropical areas. Little research has been conducted on the effects of drought stress on photosynthesis in sweet potato. This is perhaps not surprising because until recently the starch accumulating capacity of the storage root has been regarded as the main determinant of sweet potato yield **(HAIMEIRONG and KUBOTA, 2003)**. Many studies have also shown that the activity of the storage roots (sink organs) in sweet potato is one of the chief determinants of storage

root production and that the activity of ADP-glucose pyrophosphorylase (AGPase) is key in governing starch synthesis in developing storage roots **(NAKATANI and KOMEICHI, 1992)**. As a consequence, efforts to improve sweet potato yield in the past mainly focused on the enhancement of sink organ function and capacity rather than source organ function and high photosynthetic rates **(YATOMI, KUBOTA, SAITOU and AGATA, 1996)**. Later evidence, however, indicated that sink activity in sweet potato roots is actually restricted by source function and that improvement of leaf photosynthetic activity and sucrose supply from the shoot is a prerequisite for enhancing AGPase activity and thus storage root production **(TSUBONE, KUBOTA, SAITOU and KADOWAKI, 2000)**. It is therefore important, in the case of sweet potato, to also investigate the effects of drought on the performance of physiological and biochemical processes in the leaf that will have an effect on root formation where AGPase activity plays a role.

Recently, attempts to improve drought tolerance in plants have been made through conventional selection and breeding techniques. Scientists have mentioned that selection is convenient and practical if the plant species possesses distinctive indicators of drought resistance that can be seen at whole plant, tissue and cellular levels. However, it has been found that there are no well-defined plant indicators for drought tolerance that could be practically used by plant breeders to improve drought tolerance in a number of important agricultural crops **(ASHRAF and HARRIS, 2004)**. This then opens up the opportunity to investigate the possibility of identifying possible drought stress indicators at an early growth stage of the sweet potato plant to aid conventional breeding.

1.3. Testing for drought tolerance in sweet potato

The screening for drought tolerance in varieties entails using of a combination of various methods. These methods can either be used to confirm previous results or new methodology can be tested to enhance the probability of successful screening. In the case of proline, which is often used as parameter for drought tolerance **(DE RONDE, VAN DER MESCHT and STEYN, 2000; RODRÍGUEZ-DELFÍN, POSADAS, LEÓN-VELARDE, MARES and QUIROZ, 2012)** the question arises will it be as prevalent in sweet potato as in the case of protein-rich species hinting to the reasoning that proline will not act as an important substance in the process of withstanding drought conditions.

Due to the fact that the roots of the sweet potato plant are primarily storage organs with large water holding capacity, this might also play a role in the total water status of the plant, especially during the night when a lot of recovery from drought stress takes place.

1.3.1. Water management and cultivation practices to induce drought stress in sweet potato

In order to execute proper drought stress experiments, proper water management, i.e. the correct irrigation methodology must be applied. Researchers have followed different means of creating moisture stress in the soil for sweet potato. A popular way of cultivating sweet potato for this purpose is by means of planting in pots. Through artificial temperature regulation as well as a protected environment, optimum conditions can be created for growth. The disadvantage is that an artificial environment is created from which the results cannot always be extrapolated to the field.

SARASWATI, JOHNSTON, COVENTRY and HOLTUM (2004) planted sweet potato cuttings in five liter pots of which the soil was allowed to dry out gradually over the growth period. Depending on the soil type used in the experiment, which will determine the tempo of stress induction together with the regulated temperature, this can indicate the difference in reaction of genotypes regarding the development of moisture stress at different stages. The possible disadvantage of conducting these experiments in pots is that some plants cannot withstand the rapid drought stress imposed in the pot system which, in contrast, might simulate a lengthy stress in the field. The authors did not mention the soil water monitoring system, although final soil water determination was conducted. This makes it difficult to relate morphological stages to soil water conditions. **SUNG (1981)** cultivated sweet potato plants in plots that received different amounts of water monitored by soil water potential, while **LEWTHWAITE and TRIGGS (2012)** cultivated sweet potato in an area with sub-optimal precipitation and supplemented the soil moisture, of the drought stressed plants, by means of overhead irrigation when the plant available water, calculated from the available soil moisture, was severely depleted.

In the case of moisture stress trials being conducted in pots, various approaches have been used. **HAIMEIRONG and KUBOTA (2003)** gradually decreased the amount of irrigation to the drought stressed plants and the status of the drying was determined by means of a psychrometer as well as a dewpoint meter. A well-established method for determining soil water content has been used by **CHOWDHURY, ANTONY and KUMAR (2008)** who planted sweet potato in plots. This entailed the collection of soil samples from different depths and determining the soil water thereof. Irrigation was given based on the waterpan/evaporation ratio.

EKANAYAKE and COLLINS (2004), VAN HEERDEN and LAURIE (2008) used a line-source sprinkler system whereby the varieties were assigned randomly to the main plots which were perpendicular to the line-source. This allowed the soil to be irrigated at different levels. In the case of **EKANYAKE and COLLINS (2004)** soil water was estimated using daily evapo-transpiration rates. Water catch pans were allocated to all the plots to monitor the amounts of water at each irrigation time. **VAN HEERDEN and LAURIE (2008)** made use of capacitance probes located in the soil, at a depth of 1.2m, to measure the soil water content. Depleted soil water was then replenished to the required levels by means of irrigation through calibrated nozzles in the line-source system.

1.3.2 Methodology to assess the influence of drought on canopy cover and plant height in sweet potato plants

1.3.2.1. Leaf Canopy cover

Leaf area index (LAI, m^2/m^2) is a representation of the coverage of leaf material in an environment related to the ground surface area of the measurement. When a crop is grown in the field, one of the reliable methods to determine the effect of abiotic stress on the plants is to use the leaf area index (LAI). **JONCKHEERE, FLECK, NACKAERTS,**

MUYS, **COPPIN, WEISS and BARET (2004)** claim that the LAI of vegetation depends on species composition, development stage, and the season it is growing in.
The LAI is also strongly dependent on the usual site conditions and the management practices. On the other hand the LAI will also be dependent on the morphology of the leaf and the plant as a whole. The sum of these factors, combined with the difference in assessment methods, may therefore lead to widely varying LAI-values as is demonstrated in the relevant literature. According to **GOWER, KUCHARIK and NORMAN (1999)** there are two methods for determining LAI; namely the direct and the indirect methods. The direct method involves the destructive (harvesting leaves from the crop for a specific area) or non-destructive techniques (collect leaf litter from a specific area where the crop is growing).

SOMDA and KAYS (1990), BHAGSARI and ASHLEY (1990) and **NEDUNCHEZHIYAN, BYJU, and RAY (2012)** studied the above-ground morphological aspects of sweet potato growth (with regard to canopy cover) in detail from the cutting stage over the growth period by measuring single leaf area, petiole position, node length and overall stem length. This unfortunately resulted in a destructive way of measuring the canopy cover. In the direct method the LAI represents the amount of leaf material in an environment and is geometrically defined as the total one-sided area of photosynthetic tissue per unit ground surface area. **ARKEBAUER, WALTER-SHEA, MESARCH, SUYKER and VERMA (2009)** have speculated that leaf area and canopy structure play important roles in controlling energy, carbon and water vapour exchange between the vegetation and the atmosphere.
One of the indirect methods of determining LAI is by using instruments called plant canopy analyzers calculating the LAI by comparing differential light measurements above and below the canopy. LAI is therefore a valuable index in identifying sweet potato growth and development. It is known that water stress on plants can cause leaf area to decrease. The decrease in leaf area is caused by a lack of water to be used by the vital systems in the plant. Before the use of modern instruments, leaf area index was measured using single leaves in a predetermined area over the surface area measured. Currently various instruments are available to measure LAI. The LI-COR® LAI 2200 meter contains two

optical lenses by which above and below foliage readings are taken. The AccuPAR AP-80 makes use of 80 sensors inside a probe to provide values for above and below foliar measurements.

1.3.2.2. Stem height

If canopy development is influenced by drought stress then it is reasonable to argue that plant growth will be inhibited in general. **YIN, PENG, ZANG, ZHU and LI, (2005)** have observed large decreases in plant height of poplar trees planted in pots where the water application was reduced from field capacity quantities. Large decreases in stem length were observed by **VAN HEERDEN and LAURIE (2008)** when sweet potato varieties were deprived of soil water. Significant decreases in the stem length of potato were observed **(HEUER and NADLER, 1998)** when plants were only given 60% of their normal requirement.

1.3.3. The biochemical effect of drought on the activity of antioxidant enzymes and chlorophyll content in the leaves of sweet potato plants

1.3.3.1 Antioxidant enzymes

A vital response during drought stress is the reported increase of abscisic acid concentrations which favorably stimulates the closure of stomatal cells. This has an effect on the availability of CO_2 resulting in the misdirection of electrons in the photo systems. This process leads to the formation of reactive oxygen species **(BOWLER, MONTAGU and INZE, 1992)**. The formation of these species can lead to oxidative injury but high levels of antioxidants can prevent cell damage.

All plants are subjected to abiotic stresses during their life cycles. Some of these stresses are of a severity that damage plant tissue and have adverse effects on growth and yield. ROI (reactive oxygen intermediates), which contribute to the damage on plant tissues, are partially reduced forms of atmospheric oxygen **(MITTLER, 2002)**. To combat such adverse effects, plants harbor defensive mechanisms which act as antioxidants. Three of the main antioxidant systems that exist in plants are the SOD (superoxide dismutase),

PXD (peroxidase) and RED (reductase, especially glutathione) systems **(KIM, KIM, LEE and KWAK, 2009)**.

The superoxide dismutase system contributes to the antioxidant system by catalyzing the conversion of the highly active $O_2^{\cdot -}$ to H_2O_2 which in turn is converted to water by means of the catalytic enzyme **(DALTON, RUSSELL, HANUS, PASCOE and EVANS, 1986)**. The hydrogen peroxide is then removed by glutathione reductase, dehydroascorbate reductase and ascorbate peroxidase. The removal of superoxide radicals by glutathione reductase mainly takes place in the chloroplasts **(FOYER and HALLIWELL, 1976)**.

The peroxidase system reduces H_2O_2 to water using ascorbate as an electron donor **(LU, DENG and KWAK, 2010)** and the reductase system collaborates with the peroxidase system to remove the hydrogen peroxide through a process known as the Halliwell-Asada pathway. **DALTON, RUSSEL, HANUS, PASCOE and EVANS, (1986); BOWLER, VAN MONTAGU and INZE, (1992); LIN, CHAO, YANG, CHENG, LO and CHANG, (2006)** correctly claimed that very little has been conducted to study the response of the anti-oxidative system in sweet potato in response to drought conditions. Evidence of response of anti-oxidant systems from other crops is then used to predict a response in sweet potato.

1.3.3.2. Chlorophyll content

Chlorophyll forms an integral part of the photosynthetic system of the plant. Chlorophylls of different forms play important roles as part of the photosynthetic apparatus of all phototrophic organisms. Higher plants contain chlorophyll a (the major, yellow-green pigment), chlorophyll b (blue-green), accessory pigments and several additional forms of chlorophyll **(RICHARDSON, DUIGAN and BERLYN, 2002)**. Both chlorophyll a and b pigments are associated with light harvesting processes at the antenna. The estimation of chlorophyll during abiotic stress has implications for drought in the sense that the chlorophyll values might predict the severity of the condition **(SILLA, GONZÁLEZ-GIL, GONZÁLEZ-MOLINA, MEDIAVILLA and ESCUDERO, 2010)**.

As drought stress increases, the concentration of carbon dioxide in the intercellular spaces (C_i) in the leaf decreases due to stoma closure **(MAFAKHERI, SIOSEMARDEH, BAHRAMNEJAD, STRUIK and SOHRABI, 2010)** and hence a decrease in photosynthesis. The build-up of oxygen relative to the decreased levels of C_i causes the potential formation and build-up of free radicals in the chloroplast. The free superoxide radicals can then attack protein structures and membrane lipids causing the breaking up of cells. This break up of cell membranes can cause the leakage of chlorophyll from the chloroplasts causing a decline in concentration. **ITURBE-ORMAETXE, ESCUREDO, ARRESE-IGOR and BECANA (1998)** found that severe drought stress inhibits the photosynthesis of plants through changes in the chlorophyll content, damaging the photosynthetic apparatus by affecting the chlorophyll components. Various researchers have also shown that drought stress has a negative impact on chlorophyll content of the leaves **(MAFAKHERI, SIOSEMARDEH, BAHRAMNEJAD, STRUIK and SOHRABI, 2010; REDDY, CHAITANYA and VIVEKANANDAN, 2004)**.

In recent years the development of instruments that can measure chlorophyll content non-destructively has enabled researchers to handle a much larger quantity of samples compared to the physical extraction chlorophyll method in the laboratory. **RICHARDSON, DUIGAN and BERLYN (2002)** investigated the possible correlation between values of physically extracted chlorophyll and the index values of samples obtained from optical measurements. They used leaves of a North American tree ranging from very green to very yellow in color. These researchers found there were acceptable correlations between the extracted chlorophyll content and the values obtained from the handheld chlorophyll meters.

A very important issue to consider during the measurement of chlorophyll is leaf age, either for extraction or by means of non-destructive measurement. Research work relating to this was undertaken by **SILLA, GONZÁLEZ-GIL, GONZÁLEZ-MOLINA, MEDIAVILLA and ESCUDERO (2010)** on the leaves of four different oak species. In relation to the findings of the mentioned authors, a strong non-linear relationship was found between the extracted chlorophyll concentration and those obtained from optical

measurements. They also found that when comparing these optical values with each other, it should be for leaves of the same age.

1.3.4. Assessment of the effect of drought on the level of relative water content, carbon isotope discrimination, proline concentration and stomatal conductance in leaves of sweet potato plants.

1.3.4.1. Relative water content

Leaf water status in plants is closely related to the physiological processes in the plant **(BLUM, ZHANG, NGUYEN, 1999; RAHBARIAN, AFSHARMANESH and SHIRZADI, 2010)**. Water deficit in plants originates from the loss of water in the growth medium. Relative water content (RWC) is an acceptable measure to determine the water status in plants and therefore to calculate the water deficit. When water loss is taking place through the stomata, the plant has the ability, or not, to adjust to the water loss replacing the lost quantity to a certain extent. This is called osmotic adjustment (OA) and is dependent on the soil water content as well as the transpiration rate. Although water potential does not account for the osmotic adjustment in the plant, it is a useful tool in the estimation of plant water status regarding water transport in the plant. **BLUM (2005)** explained that osmotic adjustment is a powerful means of conserving cellular hydration in drought conditions and that RWC displays the effect of osmotic adjustment in this regard. That is why RWC is thought of as an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA.

The relative water content method has long been in use **(BARRS and WEATHERLEY, 1962; SMART and BINGHAM, 1974)** and has recently gained increasing attention. The method is simple. It estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity, which is a measure of water deficit in the leaf **(SMART and BINGHAM, 1974)**. Normal values of RWC range

between 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves. In most crop species the typical RWC at about wilting is around 60% to 70%.

CHOWDHURY, ANTONY and KUMAR (2008) conducted a sweet potato trial where plants were grown in pots and subjected to various water regimes. Significant differences were observed in the RWC of leaves harvested from plants grown at a higher moisture content compared to those grown at a lower moisture content. **LU, DENG and KWAK (2010)** also noted severe declines in RWC of leaves of sweet potato transgenic and non-transgenic plants grown in pots.

1.3.4.2. Carbon isotope ratio, discrimination and WUE.

In life the three most important elements that influence plant growth, function and distribution are carbon (C), hydrogen (H) and nitrogen (N) of which H is the most abundant. Carbon exists in the air and the soil in two major isotope forms i.e. ^{12}C and ^{13}C of which ^{12}C is the most abundant **(DAWSON, MAMBELLI, PLAMBOECK, TEMPLER and TU, 2002)**.

Numerous studies have shown that discrimination (Δ) between ^{12}C and ^{13}C exists during plant function **(PITA, SORIA, CAÑAS, TOVAL and PARDOS, 2001; DEBLONDE, HAVERKORT and LEDENT, 1999; MONNEVEUX, REYNOLDS, TRETHOWAN, GONZÁLEZ-SANTOYO, PEÑA and ZAPATA, 2005)**. The reason for Δ is that during the normal growth of the plant, CO_2 is taken up from the atmosphere and the lighter ^{12}C is preferred by the plant to the heavier ^{13}C which results in a Δ against the ^{13}C. Discrimination of the ^{13}C is normally linked to the internal versus atmospheric carbon ratio and is calculated as $^{13}C/^{12}C$ **(DAWSON, MAMBELLI, PLAMBOECK, TEMPLER and TU, 2002; BLOCH, HOFFMANN and MÄRLÄNDER, 2006)**.

Variation in Δ of the carbon isotope in relation to water content of the soil has been observed by several researchers **(SHAHEEN and HOOD-NOWOTNY, 2005; PITA, SORIA, CAÑAS, TOVAL and PARDOS, 2001; SAYRE, ACEVEDO and AUSTIN, 1995)**.

Drought is a major limitation in crop production and **MONTI, AMADUCCI, PRETONI and VENTURI (2006)** have shown that carbon isotope discrimination can be a powerful and easy to use tool for selecting genotypes with high water use efficiency. In a study regarding the relation of Δ to yield, **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** showed that leaf Δ decreased with increasing water deficits and found this to relate to decrease of stomatal conductance.

The water use efficiency (WUE) of a plant refers to the ability of the plant in using the available soil water to produce biomass. WUE is dependent on many factors including the genotype, soil water conditions, temperature and humidity. **FARQUHAR, EHLERINGER and HUBICK (1989)** proposed that Δ values might play a role as an indicator for WUE in C_3 plants. This can be reasoned due to the fact that that Δ is correlated with stomatal conductance in general. Stomatal conductance will decrease with a decrease in water availability. **LEIDI, LÓPEZ, GORHAM and GUTIÉRREZ (1999)** in cotton found a decrease in Δ values with increase in water stress. This was followed by a decrease in stomatal conductance and photosynthesis.

1.3.4.3. Stomatal conductance

Stomatal conductance concerns the cooperation between carbon assimilation and water loss by transpiration **(LUDLOW, 1980)**. The stomata regulate the loss of water via transpiration at times of drought stress. Conductance is measured either at the adaxial or abaxial side of the leaf by means of a leaf conductance meter in $mmol/m^2s^{-1}$. All leaves contain stomata (the microscopic pores); openings in the leaf through which gasses diffuse in and out. Stress-inducing environmental changes not only damage the photosynthetic process but also affect stomatal movement, light absorption and the biochemical pathways of CO_2 fixation.

It is well known that the stomata close gradually with increased drought stress which is followed by a reduction in photosynthetic rates **(BAHAR, YILDIRIM and BARUTCULAR, 2009; GONZÁLEZ, MARTÍN and AYERBE, 1999)**. Although the response of stomata to environmental and physiological factors is complex, it is known that stomatal conductance

varies with leaf irradiance, leaf temperature, atmospheric water vapour pressure deficit and CO_2 concentration **(COWAN and FARQUAR, 1977; BUCKLEY and MOTT, 2002)**.

Literature also provides information that stomatal conductance depends on guard cell and epidermal turgor **(WU, SHARPE and SPENCE, 1985; MENCUCCINI, MAMBELLI and COMSTOCK, 2000; FRANKS, BUCKLEY, SHOPE and MOTT, 2001)**, and that regulation of turgor in these cells requires metabolic energy **(FARQUHAR and WONG, 1984)**. Leaf turgor also depends on the balance between loss of water through transpiration and supply of water to the leaf from the soil **(COWAN and FARQUAR, 1977; MOTT and PARKHURST, 1991; MAIER-MAERCKER, 1998; FRANKS, BUCKLEY, SHOPE and MOTT, 2001)**.

BAHAR, YILDIRIM and BARUTCULAR (2009) have reported positive relations between stomatal conductance and yield in bread wheat. These relations are not clear in durum wheat. **GONZÁLEZ, MARTÍN and AYERBE (1999)** also found positive correlations between stomatal conductance and yield when eight barley genotypes were subjected to water stress. Whether these relations are true in the case of sweet potato will be investigated in this study.

1.3.5. The effect of drought on the nitrate reductase activity and proline levels in sweet potato leaves and the carotenoid content in roots.

1.3.5.1 Nitrate reductase

Nitrate reductase is one of the most intensively studied enzymes in the plant metabolic system **(AHMAD and ABDIN, 1999; SUNG, 1981, FRESNEAU, GHASHGHAIE and CORNIC, 2007)**. Nitrogen is normally available to the plant in the nitrate form but cannot be used before reduction to nitrite takes places. The nitrate reductase enzyme catalyzes the rate limiting step in the overall process of nitrate assimilation which involves the reduction of nitrate to nitrite **(KAISER, WEINER and HUBER, 1999).** Nitrite is then reduced to NH_3 which can be incorporated into glutamate.

The protein manufacturing process in the plant is directly dependent on the conditions of how the opening and closing of stomata is influenced and hence photosynthesis is negatively affected (**(HSIAO, 1973)**. This effect on photosynthesis has a negative effect on protein synthesis and therefore also affects all metabolic processes where proteins are involved, and consequently the activity of the nitrate reductase enzyme.

It was found by **FOYER, VALADIER, MIGGE and BECKER (1998)** that the introduction of drought led to a rapid decrease in nitrate reductase activity in maize. **FERRARIO-MÉRY, VALADIER and FOYER (1998)** found that the decrease in nitrate activity, during the first three days of drought in tobacco, was the result of a decrease in nitrate reductase protein concentration. **KRČEK, SLAMKA, OLŠOVSKÁ, BRESTIČ and BENČÍKOVÁ (2008)** reported that levels of nitrate reductase activity in spring barley were considerably higher in the leaves that received optimum water quantities compared to plants in the drought treatment.

Due to the fact that nitrate reductase is more dependent on the decline in rate of photosynthesis **(KAISER, BRENDLE-BEHNISCH, 1991)**, it can be reasoned that the more the plant has the ability to uphold the photosynthetic rate, the better the plant can uphold nitrate reductase activity and hence nitrate metabolism.

1.3.5.2. Proline as possible indicator for drought stress in sweet potato

Proline as a measure of stress has been studied in many crops **(MONREAL, JIMÉNEZ, REMESAL, MORILLO-VELARDE, GARCÍA-MAURIÑO and ECHEVARRÍA, 2007; KNIPP and HONERMEIER, 2006; CLAUSSEN, 2005)** and found to be indicative of plants experiencing stress.

Proline is highly soluble in water and acts as a compatible osmolyte **(KAVI KISHOR, SANGAM, AMRUTHA, SRI LAXMI, NAIDU, RAO, RAO, REDDY, THERIAPPAN AND SREENIVASULU, 2005)**, meaning it has the ability to maintain the osmotic balance in a certain environment. **HAMILTON and HECKATHORN (2001)** found that under NaCl stress the complex II of the photosynthetic system is protected by the presence of proline.

In experiments with cotton **(DE RONDE, VAN DER MESCHT and STEYN, 2000)**, free proline concentrations increased with the decrease in water content in the soil. **KOCSY, LAURIE, SAZALAI, SZILÁGYI, SIMON-SARKADI, GALIBA and DE RONDE (2005)** found the cotton plants which withstood the drought stress had higher free proline contents. In a study of sweet potato plants subjected to salinity stress conducted in a hydroponic system, **RODRÍGUEZ-DELFÍN, POSADAS, LEÓN-VELARDE, MARES and QUIROZ (2012)** detected significant increases in proline content in the leaves and the roots. Based on the above-mentioned findings, and the fact that very little knowledge is available regarding the role of proline during drought stress in sweet potatoes, it is relevant for the present study to include such investigations.

1.3.6. The influence of drought on root yield, uppergrowth mass and carotene content of roots of sweet potato plants subjected to drought

1.3.6.1. Yield

It is well known that yield is influenced by drought. The severity of the drought will determine the extent of yield loss. **GONZÁLEZ, MARTÍN, and AYERBE (1999)** reported that all barley genotypes tested under severe stress conditions, produced lower yield compared to the controls. By means of subjecting sweet potato cultivars to normal irrigated and drought conditions **EKANAYAKE and COLLINS (2004)** were able to classify cultivars with regard to their ability to adapt to drought. Some cultivars were able to produce slightly larger yields than the mean and some were clearly not able to withstand the drought conditions. The effect of drought was also very clear in a study by **LEWTHWAITE and TRIGGS (2012)** who analyzed the performance of cultivars planted in two locations receiving different degrees of irrigation. Significant differences were observed in most of the genotypes, with the higher irrigated ones producing a higher yield; although some genotypes did not show any difference.

1.3.6.2. Carotenoid content of roots

The flesh of most sweet potato varieties are normally white in colour, but a recent trend is to produce more orange fleshed varieties. The reason for that is the higher concentration of β-carotene in the roots of the orange fleshed sweet potatoes **(LOW, ARIMOND, OSMAN, CUNGUARA, ZANO and TSCHIRLEY, 2007)**. β-Carotene (which represents more than 90% of total carotenoids in sweet potato roots) is the precursor of vitamin A **(PURCELL and WALTER, 1968).** Due to the health benefits of Vitamin A, the use of orange fleshed sweet potato as a source of dietary vitamin A is therefore promoted to resource-poor households **(BOVELL-BENJAMIN, 2007)**. Globally 190 million (33.3%) children under the age of 5 years are vitamin A deficient, with a high prevalence of 44% in Africa **(WHO, 2009)**.

Although carotene in the roots as such does not play a role in the tolerance towards drought, it was reported that carotene and more specifically β-carotene values increase during drought **(RAUTENBACH, FABER, LAURIE and LAURIE, 2010; LAURIE, FABER, VAN JAARSVELD, LAURIE, DU PLOOY and MODISANE, 2012)**. Determination of the carotenoid content in the roots can thus possibly aid as an indicator for drought stress in sweet potato.

Correct selection for drought tolerant sweet potato breeding lines by the sweet potato breeder is very important. Successful selection should save time and money and will result in the reaching of the ultimate goal in identifying a drought tolerant sweet potato genotype containing high β-carotene content. It is therefore important to identify efficient and cost saving methodologies that will support the selection process.

CHAPTER 2

INFLUENCE OF DROUGHT STRESS ON GROWTH OF DIFFERENT SWEET POTATO LINES AND CULTIVARS: CANOPY COVERAGE AND STEM LENGTH

2.1. INTRODUCTION

Drought is a global factor that inhibits the growth of plants and leads to yield losses as a consequence **(CATTIVELLI, RIZZA, BADECK, MAZZUCOTELLI, MASTRANGELO, FRANCIA, MARÉ, TONDELLI and STANCA, 2008)**. Research has been ongoing to identify varieties in production crops to minimize losses. One of the factors that has a big influence on the canopy development of a plant is drought. Although some species and more specific cultivars do have the ability to withstand drought for certain periods of time, it is important that these cultivars must have the ability to produce a proper yield notwithstanding poor canopy development in the drought condition.

It is known that leaf area decreases as water stress increases in sweet potato plants **(NEDUNCHEZHIYAN, BYJU and RAY, 2012; LEWTHWAITE and TRIGGS, 2012)**. Leaf area index (LAI) which measures canopy cover is an indication of the leaf material in an ecosystem or trial area. This can provide an indication of the effectiveness of photosynthesis, an estimate of soil water availability, and some other processes that might be a link between the plant and the environment **(BRÉDA, 2003)**.

LEWTHWAITE and TRIGGS (2012) noticed with sweet potato cultivars planted in the field, that clones which showed a large reduction in canopy cover under water deficit also experienced very large reductions in yield. **VAN HEERDEN and LAURIE (2008)** also noticed large reductions in yield for the cultivar Resisto which could be directly linked to low LAI values. **NEDUNCHEZHIYAN, BYJU and RAY (2012)** planted sweet potato in unused rice plots and observed a large significant decrease in LAI readings obtained from plants receiving less irrigation.

The values of LAI , which run parallel to the photosynthetically active radiation received by the plant, decreased significantly in an irrigated treatment compared to the rain fed treatment as noted by **GOMES, CARR and SQUIRE, (2005)** in sweet potato.

If canopy development is influenced by water stress then it is reasonable to argue that plant growth will be inhibited in general. **YIN, PENG, ZANG, ZHU and LI (2005)** have observed large decreases in plant height of poplar trees planted in pots where the water application was reduced from field capacity quantities. Large decreases in stem length were observed by **VAN HEERDEN and LAURIE, 2008** in sweet potato cultivars that were deprived of soil water. Significant decreases in the stem length of potato were observed when plants were only given 60% of their normal water requirement **(HEUER and NADLER, 1998)**.

2.2. AIM

The aim of the trial was to investigate whether drought stress influenced the canopy growth and stem length of sweet potato plants irrigated with different quantities of water.

2.3. MATERIALS AND METHODS

2.3.1 Trial design, maintenance and variety choice

Three drought stress trials (Trial 1, Trial 2 and Trial 3) were executed at the Agricultural Research Council Institute for Vegetable and Ornamental Plant Research (ARC-Roodeplaat) (25.604˚S, 28.345˚E; 1189 m altitude) over a time span of two years (2009/2010 season and 2010/2011 season). Trial 1 (2009/2010 season) and Trial 3 (2010/2011 season) were executed using a large rainout shelter (LR)(Figure 2.1), while Trial 2, was executed in both small rainout shelters and in open fields, (Figure 2.2;2.3).

Figure 2.1: Large rainout shelter used in Trial 1 and 3 for screening sweet potato genotypes for drought tolerance.

Figure 2.2: Small rainout shelter used in Trial 2 for screening sweet potato genotypes for drought tolerance.

Figure 2.3: Sweet potato genotypes grown in open field conditions used as control in Trial 2 for drought screening.

Trial 1 and Trial 3 involved a split plot design with four cultivars (Appendix 2-A) planted in the 2009/2010 season and eight cultivars (Appendix 2-D) planted in the 2010/2011 season respectively. During the 2009/2010 season a second Trial (Trial 2) was executed in two small rainout shelters (SR) (drought stress treatment) and the open field (control treatment). Thirty five sweet potato breeding lines and cultivars were planted in a complete randomized block design (CRBD) with two repeats in small rainout shelters (Appendix 2-B) and two repeats in the adjacent field representing a control Trial (Appendix 2-C). Water management in all the trials was conducted through the monitoring of soil water content by means of a capacitance probe. Readings were taken daily and soil water calculated. Plants were irrigated by means of a line-source overhead irrigation system through spray nozzles (Figure 2.4).

Figure 2.4: Irrigation boom in large rainout shelter for the supply of various irrigation quantities. Blue arrows indicate some nozzle locations.

Nozzles, with different aperture sizes, were used to apply the respective water quantities. In Trial 1 the 100% (control) irrigation treatment received the full complement of plant available water once soil water had depleted to 70%, while the other two treatments received 60% (mild stress) and 30% (severe stress) of the calculated water that the 100% treatment received. This resulted in continuous drying out of the soil in the 60% and 30% treatments. In Trial 1 the border row plants were planted between the irrigation treatments as well as around the Trial area to prevent water contamination from the outside as well as from other water treatment regimes. In Trial 3, where only two water application regimes were used, the plants received either 30% of what the 100% was given as the stress treatment or 100% as the control. Border row plants were planted between the irrigation treatments and around the Trial area to ensure uniform irrigation and to lessen external environmental influences. In Trial 3, three plants per row were planted 30 cm apart with six rows per repeat (18 plants/plot). Each treatment consisted of six rows, 80 cm apart, with border rows at the sides and in the middle of the Trial area. Fertilizer was applied to the soil as per recommendation (Appendix 2-E).

In Trial 2, fertilizer was added to the soil as per recommendation (Appendix 2-F). Each repeat, per treatment, consisted of five plants established 30 cm apart with rows 80 cm apart from each other. Five cultivars were planted per row and each repeat consisted of seven rows. Border rows were planted to minimize external environmental factors. Water management was conducted through the monitoring of soil water content by means of a capacitance probe. Readings were taken daily and soil water calculated. Soil in the SR was irrigated by means of overhead sprinkler irrigation, keeping the soil water availability at 30%. Irrigation in the adjacent field containing the control plants took place, also overhead, when plant available water had depleted by 30%. Normal rainfall that irrigated the adjacent field trial was recorded. Only two water treatment levels were chosen due to space limitation as well as to minimize labor on the physical measurements.

Top cuttings were used for all plantings and specifically two internodes (three eyes) were planted into the soil to allow the growth point and three eyes above the soil surface after planting.

Figure 2.5: Sweet potato cuttings indicating the eyes and internodes with (A) and without (B) leaves. Red arrow indicates growth point. Blue arrow indicates eye without leaf. Yellow arrow indicates eye with leaf. Amber arrow indicates an internode. Cutting (B) will be planted in the soil.

2.3.2. Measurement of canopy coverage (LAI) and stem length in the three trials.

Measurement of LAI took place twice during the Trial period namely at 60 and 120 days after planting (DAP). Readings were conducted with a Li-Cor 2200 plant canopy analyzer (LI-COR, Lincoln, Nebraska, USA) according to the manufacturer's recommendations. A total of five readings were conducted at each repeat between 10 am and 2 pm.

Stem length was measured at the same time as LAI measurements. A piece of string was used for measurement and then the value was read against a calibrated measuring tape. This was used because runners are not straight. Three plants per repeat were identified and the stem length was measured non-destructively from the point of soil contact to the apical tip. Stem length measurements for Trial 2 were not determined due to labor constraints.

2.4. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to analyze the data for each Trial (1, 2 and 3) separately. The data was analyzed as a split-plot with main plots, two or three water treatments and sub plots 4 to 35 cultivars replicated in two or three blocks to test for significant effects. The repeated measurements over time were included in the ANOVA as a sub-subplot factor. Means of significant effects were separated using Fishers' t-LSD (least significant difference) at the 5% level of significance. Statistical analyses were conducted using GenStat *for Windows* 15th Edition (VSN International, Hemel Hampstead, UK).

2.5. RESULTS AND DISCUSSION

2.5.1 Canopy cover

2.5.1.1. Trial 1

Significant effects were detected in LAI with a combination of time and water treatments (Appendix 2-G). The LAI values of four cultivars were compared to each other while being submitted to three different water applications (Figure 2.6). All the cultivars experienced the effect of a deprived soil water system especially between the control and mild stress treatments. The same tendency was observed by **LEWTHWAITE and TRIGGS** (**2012)**

where sweet potato genotypes were planted under both drought and wet conditions. **NEDUNCHEZHIYAN, BYJU and RAY (2012)** also observed a significant increase in vine length, LAI and fodder yield of sweet potato plantings receiving different levels of irrigation. At 60 DAP cultivars Isondlo and W-119 were the only cultivars to experience a significant decrease in canopy cover between the 100% (control) and 60% (mild stress) treatment namely 91 and 85% reduction respectively. Although Purple Sunset and Resisto, according to Figure 2.6, showed the steepest declines, their values did not differ significantly with those of W-119. At T1 no significant decline in LAI values was noticed between the 60% (mild stress) and 30% (severe stress) treatment.

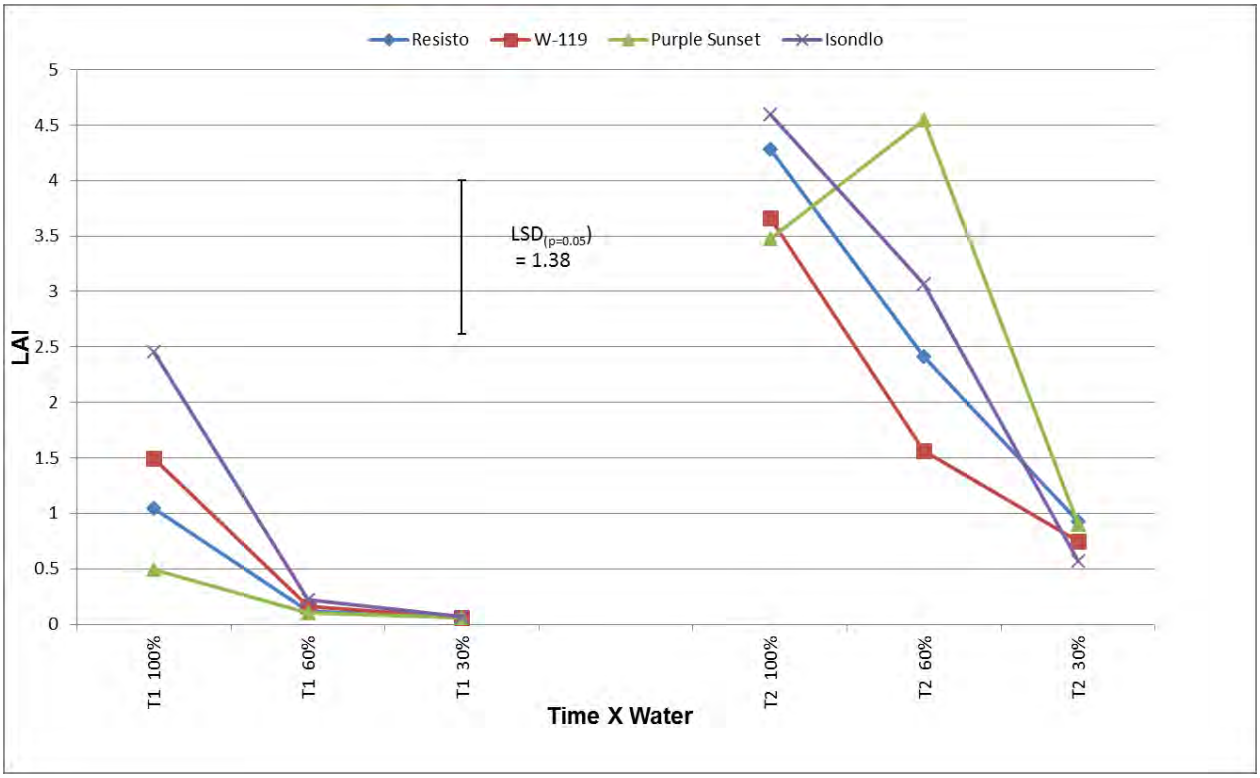

Figure 2.6. Time X Water X Variety interaction means of LAI values for four sweet potato cultivars submitted to drought treatment in Trial 1. $LSD_{(p=0.05)}$ = 1.38; Each measurement is the mean of 5 measurements with 3 repeats per cultivar. T1 = 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

A significant increase in LAI values was also observed from T1 to T2 (Figure 2.6) which can be attributed to the plants still being in the above-ground exponential growth phase. This is in contrast to the observation of **NEDUNCHEZHIYAN, BYJU and RAY (2012)** who

observed a decline in LAI values from 95 days after planting until harvest. No significant differences in the canopy cover could be detected between the cultivars at T2 in the control treatment, but significant difference could be detected in the mild stress treatment. Cultivars Purple Sunset and Isondlo were least affected by the drought stress in the mild stress treatment and probably have a better ability to withstand the drought conditions. All cultivars experienced a large decline in LAI values when comparing the mild to the severe stress treatment, with Purple Sunset and W-119 experiencing the worst and least, respectively.

No significant difference in LAI values between the cultivars could be detected at T2 for the severe stress treatment. This was not unexpected due to the severe stress the plants received as well as the plants were nearing the end of their growth cycle. During a study comparing the performance of three sweet potato cultivars and nine clones regarding yield, **LEWTHWAITE and TRIGGS (2012)** found a significant decline in canopy cover over the season due to drought conditions. Although **VAN HEERDEN and LAURIE (2008)** did not measure canopy cover, large reductions in shoot growth were detected which led to a decrease in canopy cover.

The increase and significant difference in LAI values between T1 and T2 at 100% (Figure 2.6) probably was due to the continued growth of the plants and to the fact that they were relatively small at the first measurement. A sharp decline in values in the mild stress and severe stress treatment had to do with the drought conditions that influenced growth. Purple Sunset did not show a decline at mild stress which might be due to better strategies for coping with the drought condition, although at severe stress the cultivar had the same response as the other three cultivars.

2.5.1.2. Trial 2

Thirty five sweet potato cultivars were grown in two small rainout shelters as well as in an adjacent field. The plants were subjected to drought stress while the plants in the field were treated as control plants which received normal irrigation quantities as rain water.

The LAI values of the 35 cultivars planted in the small rainout shelters (SR's) and the open field were compared to each other and significant differences between the treatments were obtained (Appendix 2-H). This correlates with results obtained by **LEWTHWAITE and TRIGGS (2012)** and **NEDUNCHEZHIYAN, BYJU and RAY (2012)** who observed significant differences in LAI values of sweet potato plantings at different soil water levels. In Table 2.1 significant differences in LAI values indicate the potential of a cultivar or breeding line to withstand drought conditions. At T1 five genotypes did not display significant differences between the control and the stress treatment namely Isondlo, 2006-4-4, 2006-7-3, Impilo and Lethlabula. This is in contrast to T2 where 16 of the 35 cultivars did not show significant differences between the control and the stress. Isondlo, 2006-7-3 and Lethlabula did not differ significantly, between the stress and control, at both T1 and T2 which gives an indication that the cultivars might have the ability to adapt to the drought stress conditions.

Entries that showed significant differences between the control and the stress treatment at T1 and at T2 included Purple Sunset, 2005-1-11, 2005-11-3, 2005-12-2, 2005-2-2, 2005-7-4, 2006-14-4, 2006-2-4, 2006-3-4, Beauregard, Tanzania, W-119 and Zapallo. When comparing the cultivars used in Trial 1 and 3 with the same cultivars used in this trial, some interesting results were seen. The cultivar Resisto displayed a slightly higher value at T2 in Trial 2 which was consistent with the results obtained in Trial 1. The decline in LAI values that Resisto displayed was also consistent with the results obtained by **VAN HEERDEN and LAURIE (2008)** although only vine length was measured.

Table 2.1. LAI values of 35 sweet potato cultivars subjected to drought stress in Trial 2.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	5.39	3.02	5.37	3.12
Purple Sunset	5.07*	1.52	4.09	3.62
2005-1-11	5.08*	2.07	5.54	3.05
2005-1-16	5.16*	2.47	7.00*	2.80
2005-11-3	5.50*	0.83	3.87	1.47
2005-12-2	5.79*	1.24	4.88	2.41
2005-16-1	8.55*	1.95	6.34*	3.16
2005-2-2	5.10*	2.20	6.36	4.03
2005-3-10	7.40*	2.41	8.07*	3.55
2005-3-13	5.81*	1.88	4.97*	2.33
2005-4-1	5.05*	0.46	5.58*	1.90
2005-5-5	7.42*	0.50	7.66*	2.24
2005-7-4	7.15*	3.27	4.67	3.05
2006-14-4	3.99*	1.59	4.55	2.10
2006-15-1	6.59*	1.09	5.49*	1.84
2006-2-4	4.74*	1.79	5.76	3.51
2006-3-4	6.20*	2.99	4.48	3.49
2006-4-4	4.00	2.18	5.77*	1.55
2006-4-5	5.77*	2.67	7.67*	1.57
2006-6-2	5.83*	1.47	5.14*	2.36
2006-7-3	4.61	2.77	3.98	2.11
2006-7-7	6.19*	1.41	5.26*	2.46
2006-7-8	5.98*	1.37	7.12*	2.93
Beauregard	4.43*	1.87	4.56	2.26
Blesbok	4.68*	1.07	5.20*	1.77
Bosbok	4.94*	1.90	5.12*	2.11
Impilo	5.07	3.64	6.25*	2.80
Jewel	6.65*	1.77	6.41*	3.09
Lethlabula	4.84	2.73	4.89	3.04
Ndou	5.45*	0.97	4.69*	0.86
Phala	6.21*	1.51	5.33*	1.25
Resisto	5.98*	1.00	4.37*	1.58
Tanzania	5.16*	1.80	5.28	2.86
W-119	4.79*	1.57	3.11	3.10
Zapallo	4.22*	0.43	5.37	3.42
Mean	5.56	1.81	5.43	2.53

* indicates a significant difference at a 5% level between the control and stress for a specific Time X Water X Variety combination, $LSD_{(p=0.05)}$ = 2.46; Control= control treatment; Stress = severe stress treatment. T1 = 60 days after planting, T2 = 120 days after planting. $MSE_{df=70}$=1.44

Variation amongst the breeding lines with regard to LAI values between the control and stress could be attributed to the fact that the breeding lines were selected for various traits and not only for displaying canopy cover values during drought treatment **(Chapter 1)**. The tendency to increase LAI values in the stress treatment from T1 to T2 might indicate better adaptability towards the stress conditions for specific varieties. The contrary may also be true so that varieties displaying a decline in LAI values exhibit sensitivity towards the drought conditions.

2.5.1.3. Trial 3

The LAI values of eight sweet potato cultivars were compared to each other in two different water treatments during the growth season. This resulted in significant differences detected between the cultivars (Appendix 2-I). As expected the plants subjected to severe stress displayed lower values compared to the control treatment displaying a clear pattern at either of the two periods of measurement. (Figure 2.7) These values also correlate with the findings of **LEWTHWAITE and TRIGGS (2012)** and **NEDUNCHEZHIYAN, BYJU and RAY (2012)** who also observed a decline in canopy cover with the decline in soil water levels.

A significant decline in LAI values, between the control and severe stress treatment, were seen for most of the cultivars (Figure 2.7) where Mvuvhelo displayed the biggest difference at severe stress and Resisto the least. The sudden increase in LAI values for Mvuvhelo showed in Figure 2.7 could be due to either reader error or possible water contamination during irrigation. Water contamination could take place through improper nozzle function. It is also observed, in the control, that the LAI values of all the cultivars except Mvuvhelo were lower at T2 compared to T1 which agree with the findings of **SOKOTO, MAGAJI and SINGH (2007)** who also found a decline in LAI values in sweet potato plants in the latter part of the trial at 84 days after planting.

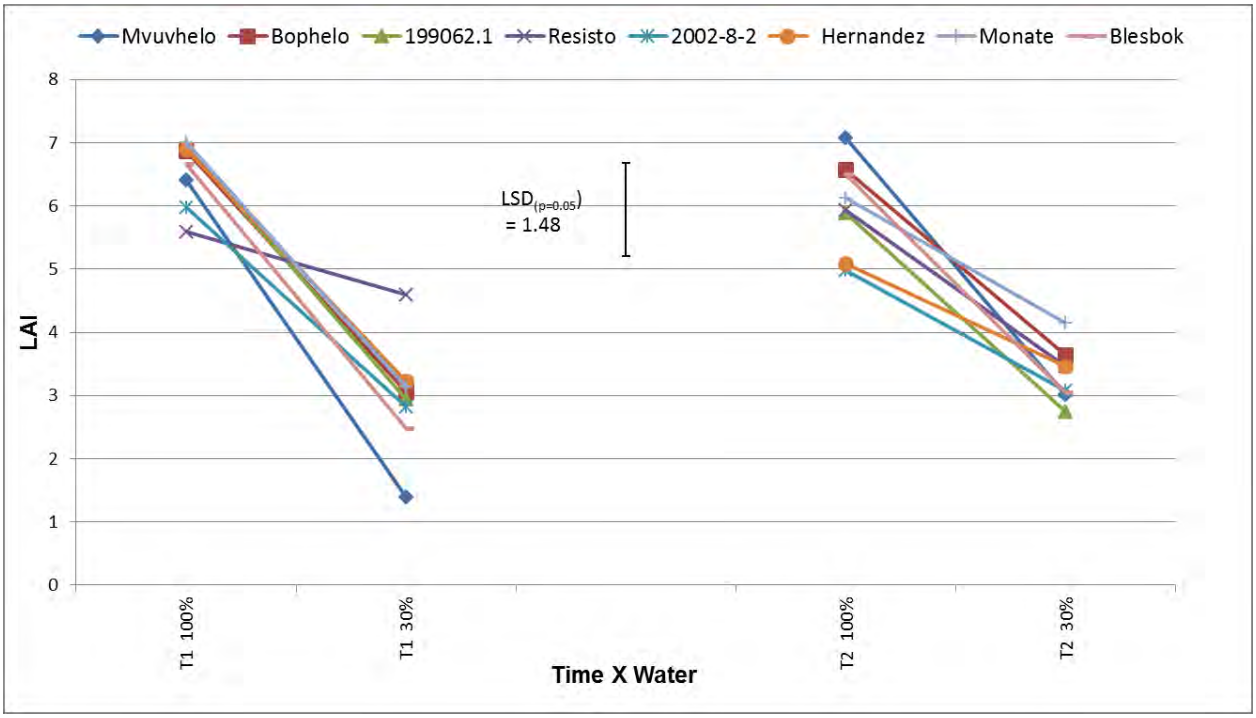

Figure 2.7. Time X Water X Variety interaction means for LAI values for eight sweet potato cultivars subjected to drought stress in Trial 3. Each value is the mean of 5 measurements with 3 repeats per cultivar. $LSD_{(p=0.05)}$ = 1.48, T1 = 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 30% = severe stress treatment

The same tendency was observed in the study that **BHAGSARI and ASHLEY (1990)** did on the relationship of sweet potato LAI to yield. They indicated that there was a decline in LAI measurements from between 60 and 105 days until 143 days after planting. **SOMDA and KAYS (1990)** when investigating the growth of sweet potato also found a decline in canopy cover of sweet potato plants after 10 -12 weeks during normal growth. In this trial the second measurement was conducted at 120 days after planting. It could be argued that the plants at this time were already in an aging state which could contribute to leaf shedding and the production of smaller leaves due to less water being used. At severe stress the majority of the cultivars displayed higher LAI values at 120 DAP, however, only Mvuvhelo showed significant higher differences compared to the 30% treatment at T1.

This could be due to adaption ability that cultivar Mvuvhelo harbors to withstand the drought condition better than the other cultivars by switching off non-essential processes to survive the drought. Bophelo, 199062.1, Resisto, 2002-8-2, Hernandez, Monate and Blesbok did not display significant changes in LAI values comparing the severe stress treatment values from T1 to T2 which could be the result of increased senescence (**SOKOTO, MAGAJI and SINGH, 2007)** as a result of the drought.

2.5.2. Stem length

2.5.2.1. Trial 1

The stem length values (Figure 2.8) give an indication of the severity of the stress imposed on the cultivars planted in Trial 1. Significant differences in stem length were detected between the cultivars in the mild stress treatment and the control treatment (Appendix 2-J). In the control treatment there was a significant continuation in stem growth for all the cultivars, except Resisto, as seen at both T1 and T2. This indicated that the plants were well watered and did not experience any stress which is consistent with findings by **SAPETA, COSTA, LOURENC¸O, MAROCO, VAN DER LINDE and OLIVEIRA (2013)**. A significant decrease in stem length in all the cultivars was observed when comparing the control to the severe stress treatment. This is consistent with results obtained by **NEDUNCHEZHIYAN, BYJU and RAY (2012)** who observed a decline in vine length when sweet potato plantings were subjected to different amounts of irrigation. **DEBLONDE and LEDENT (2001)** also observed a significant decline in stem length values in potato cultivars grown in a drought condition. At the control as well as mild stress conditions, Purple Sunset outperformed all the other cultivars significantly but at severe stress the difference to the other cultivars was nonsignificant. This is an indication that the growth of Purple Sunset is heavily retarded at low soil water conditions, as with the other cultivars, but grows well in mild stress conditions. At T2 this cultivar performed even better having significantly out grown the other cultivars even in the mild stress treatment. It again shows its sensitivity to drought conditions at severe stress conditions.

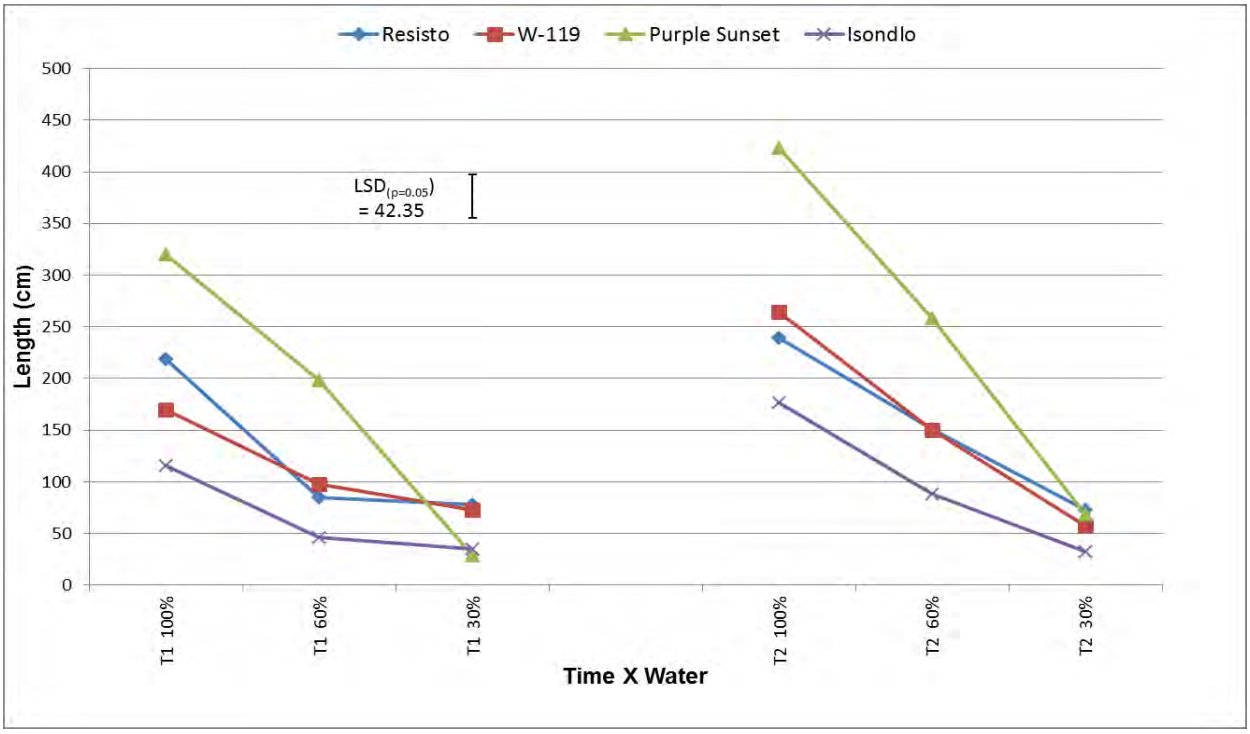

Figure 2.8. Time X Water X Variety interaction means for stem length of four sweet potato cultivars subjected to drought in Trial 1. $LSD_{(p=0.05)}$ = 42.35 Each value is the mean of 3 measurements with 3 repeats per cultivar. T1 = 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment.

In Figure 2.8 it is also evident the cultivar Isondlo is a relatively slow grower as the stem length measurements showed little increase in the control treatment of T1 compared to T2, when compared to the other cultivars. The stem growth of this cultivar was also severely hampered by the drought experienced in the severe stress treatment.

It is clear that cultivar Purple Sunset outperformed the other cultivars in the water application levels except in the severe stress treatment. This is quite interesting as in Figure 2.6, Purple Sunset also displayed good canopy cover at mild stress during the second measurement. This might indicate that the cultivar does have the ability to adapt to drought at acceptable levels of soil water and could be rated as drought tolerant in these specific growth conditions.

2.5.2.2. Trial 3

Stem length measurements between the cultivars and breeding lines for drought and control treatments were compared and found to differ significantly (Appendix 2-K). Stem length values can give an indication of the success that a specific cultivar has to overcome the drought condition regarding canopy growth **(DEBLONDE and LEDENT, 2001)**. In Figure 2.9 the cultivar Blesbok showed the greatest ability to uphold growth throughout the experiment. This is interesting since this cultivar did not show any potential in terms of canopy expansion (Figure 2.7) in the same experiment. This might give an indication that shoot growth is continuing despite the drought and that smaller leaves are formed to minimize the exposure to excess transpiration.

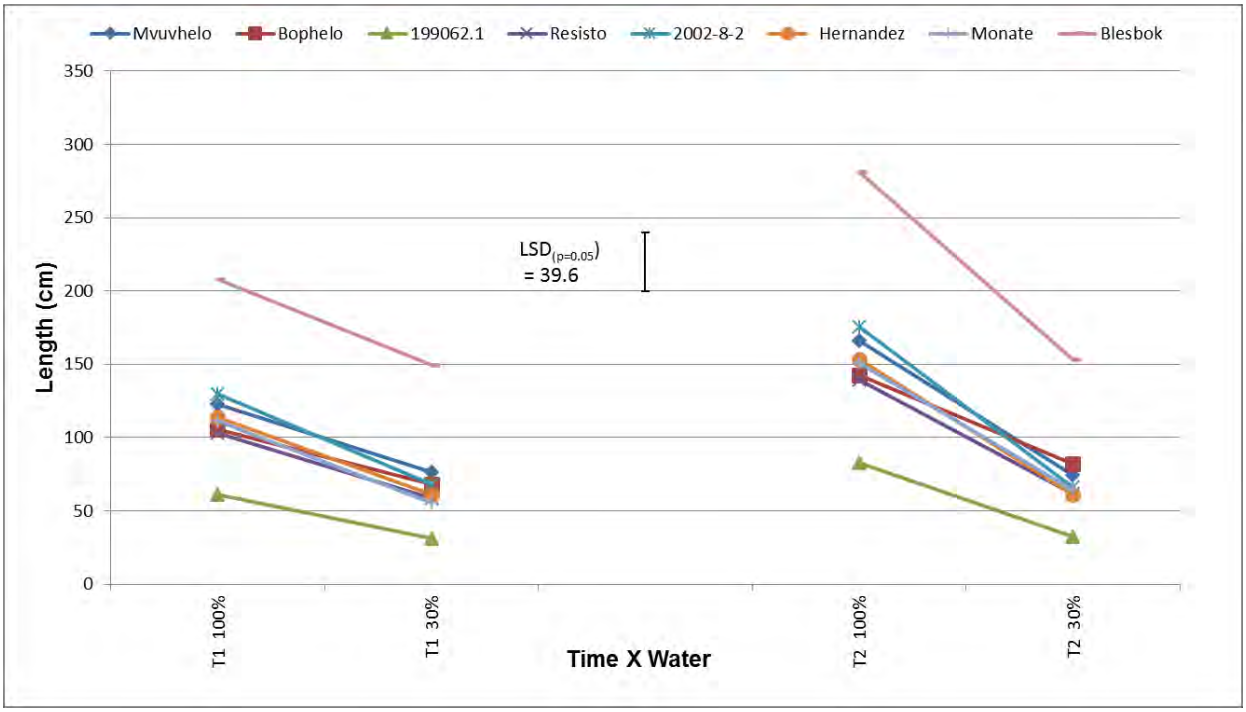

Figure 2.9. Time X Water X Variety interaction means for stem length of eight cultivars subjected to drought in Trial 3. $LSD_{(p=0.05)}$ = 39.6 Each value is the mean of 3 measurements with 3 repeats per cultivar. T1 = 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 30% = severe stress treatment

While the cultivar 199062.1 displayed the shortest stems in all the treatments and Blesbok the longest in the specific treatments, no significant differences were observed between the cultivars Mvuvhelo, Bophelo, Resisto, 2002-8-2, Hernadez and Monate at either of the treatments or time of measurement. When visualizing the results shown in Figure 2.9

it is clear that the plants, as in Trial 1, were able to grow unaffected in the control treatment. These findings differ from the results obtained by **NEDUNCHEZHIYAN, BYJU and RAY (2012)** who indicated a slight decline in shoot length and LAI values throughout the growth season. It is noticeable that there was a sharp increase in growth shown by the cultivar Blesbok, at the control treatment from T1 to T2 that outclassed all the other cultivars. Unfortunately the growth in the severe stress treatment for both T1 and T2 was retarded for Blesbok. This raises the question whether it has good canopy development as well. Despite this promising outlook it was shown in Figure 2.6 that there was a significant difference between the LAI values of this cultivar in the control and severe stress treatment. This then confirms earlier suggestions than the plants produce long stems in the severe stress treatment but the leaves stay small. Significant differences between the control treatment values of the first measurement compared to the severe stress treatment values of either measurements in cultivars Resisto, 2002-8-2, Hernandez, Monate and Blesbok were observed which indicate that these cultivars were affected by the drought causing a retardation in growth which was similar to findings of **PACE, CRALLE, EL- HALAWANY, COTHREN and SENSEMAN (1999)** in their experiments with cotton.

2.6. CONCLUSIONS

The canopy cover of the sweet potato cultivars was affected by the drought conditions imposed in the three trials seen in declining values from control to mild stress and/or severe stress treatments. Although the plants grew reasonably well at mild stress, the severe stress treatment proved too harsh to provide the plant with any chance to adapt to drought.

It was also evident that there was no stress imposed in the control treatment as the plants continue to develop a larger canopy at T2 although in some cases it was not significant. It was shown in Trial 3, that the plants in the severe stress treatment, although experiencing heavily retarded growth, continued to grow despite diminishing soil water quantities. This suggests that a reasonable amount of root growth has taken place

ensuring water uptake or that adaption has forced the plant to shut down certain mechanisms to ensure survival.

Stem growth was severely retarded at severe stress for all the trials with little to nonsignificant differences between the cultivars. This implies that this stress could be too severe and that the mild stress treatment would be more appropriate for distinguishing between the cultivars. From these result is also evident that stem length is a parameter that can be used in conjunction with other screening parameters but not alone. An example is where the cultivar Blesbok showed promising results with regard to shoot growth in drought conditions but had poor canopy cover under the same conditions.

2.7. APPENDICES

Appendix 2-A: Trial layout for Trial 1 during the 2009/2010 season.

R1, R2, R3 indicates the repeat of each cultivar. BR = Border rows. C1 = Resisto*, C2 = W-119*, C3 = Purple Sunset**, C4 = Isondlo**

Plastic canvas is also situated on the side of the control and severe stress treatments to prevent water contamination from outside the Trial area, as well as between treatments.

	100%		**60%**		**30%**	
BR	BR	BR	BR	BR	BR	BR
BR	C1R1	BR	C3R1	BR	C2R1	BR
BR	C3R1	BR	C1R1	BR	C4R1	BR
BR	C4R1	BR	C2R1	BR	C3R1	BR
BR	C2R1	BR	C4R1	BR	C1R1	BR
BR	C1R2	BR	C2R2	BR	C4R2	BR
BR	C4R2	BR	C3R2	BR	C1R2	BR
BR	C2R2	BR	C1R2	BR	C3R2	BR
BR	C3R2	BR	C4R2	BR	C2R2	BR
BR	C1R3	BR	C4R3	BR	C1R3	BR
BR	C2R3	BR	C3R3	BR	C2R3	BR
BR	C3R3	BR	C2R3	BR	C3R3	BR
BR	BR	BR	BR	BR	BR	BR

* Origin: USA
** Origin: Bred at ARC-Roodeplaat

Appendix 2-B: Trial layout Trial 2 (SR) during the 2009/2010 season. BR = Border rows. The numbers were allocated to the following varieties/cultivars:

1:Blesbok, **2**:2006-4-5**, **3**:2006-4-4**, **4**:2005-3-13**, **5**:2006-3-4**, **6**:2005-12-2**, **7**:Tanzania, **8**:2005-5-5**, **9**:W-119*, **10**:2005-1-11**, **11**:2005-11-3**, **12**:2005-4-1**, **13**:Jewel*, **14**:2005-1-16**, **15**:Bosbok**, **16**:Resisto*, **17**:Phala**, **18**:2005-16-1**, **19**:2006-14-4**, **20**:2005-7-4**, **21**:Ndou**, **22**:2006-15-1**, **23**:Lethlabula**, **24**:2006-6-2**, **25**:2006-2-2**, **26**:2006-7-3**, **27**:Isondlo**, **28**:2006-7-8**, **29**:Impilo**, **30**:Zapallo***, **31**:2005-3-10**, **32**:Beauregard*, **33**:Purple Sunset**, **34**:2006-7-7**, **35**:2006-2-4**

Repeat 1

	BR	BR	BR	BR	BR	
BR	34	12	5	11	10	BR
BR	24	4	32	9	33	BR
BR	31	35	17	30	16	BR
BR	26	14	3	21	15	BR
BR	18	25	19	1	7	BR
BR	20	23	8	27	29	BR
BR	6	28	22	2	13	BR
	BR	BR	BR	BR	BR	

Repeat 2

	BR	BR	BR	BR	BR	
BR	27	14	6	5	17	BR
BR	30	21	7	9	22	BR
BR	19	4	13	26	33	BR
BR	11	15	31	29	28	BR
BR	10	34	12	8	32	BR
BR	2	1	16	35	23	BR
BR	24	18	3	20	25	BR
	BR	BR	BR	BR	BR	

* Origin: USA; ** Origin: ARC-Roodeplaat; *** Origin: Peru; **** Origin: East Africa

Appendix 2-C: Trial layout of Trial 2 of the control in the adjacent field to SR. BR = Border rows. The numbers were allocated to the following varieties/cultivars:

1:Blesbok**, **2**:2006-4-5**, **3**:2006-4-4**, **4**:2005-3-13**, **5**:2006-3-4**, **6**:2005-12-2**, **7**:Tanzania****, **8**:2005-5-5**, **9**:W-119**, **10**:2005-1-11**, **11**:2005-11-3**, **12**:2005-4-1**, **13**:Jewel***, **14**:2005-1-16**, **15**:Bosbok**, **16**:Resisto*, **17**:Phala**, **18**:2005-16-1**, **19**:2006-14-4**, **20**:2005-7-4**, **21**:Ndou**, **22**:2006-15-1**, **23**:Lethlabula**, **24**:2006-6-2**, **25**:2006-2-2**, **26**:2006-7-3**, **27**:Isondlo**, **28**:2006-7-8**, **29**:Impilo**, **30**:Zapallo, **31**:2005-3-10**, **32**:Beauregard:, **33**:Purple Sunset**, **34**:2006-7-7**, **35**:2006-2-4**

Repeat 1

	BR	BR	BR	BR	BR	
BR	3	14	7	12	19	BR
BR	32	27	8	16	34	BR
BR	31	24	22	25	1	BR
BR	10	33	9	2	23	BR
BR	13	21	11	17	6	BR
BR	35	18	29	20	5	BR
BR	30	4	26	15	28	BR
	BR	BR	BR	BR	BR	

Repeat 2

	BR	BR	BR	BR	BR	
BR	19	6	32	8	34	BR
BR	28	29	4	7	12	BR
BR	17	24	31	15	5	BR
BR	18	25	21	2	13	BR
BR	1	33	22	11	20	BR
BR	14	23	27	16	30	BR
BR	9	26	3	10	35	BR
	BR	BR	BR	BR	BR	

* Origin: USA; ** Origin: ARC-Roodeplaat; *** Origin: Peru; **** Origin: East Africa

Appendix 2-D: Trial layout for Trial 3 in the 2010/2011 season. R1, R2 and R3 indicate the respective repeat of each cultivar. BR = border row. C1 = Mvuvhelo**, C2 = Bophelo**, C3 = 199062.1**, C4 = Resisto*, C5= 2002-8-2**, C6= Hernandez*, C7= Monate**, C8 = Blesbok**

	30% treatment		100% treatment	
BR	BR	BR	BR	BR
BR	C6R1	BR	C4R1	BR
BR	C1R1	BR	C5R1	BR
BR	C3R1	BR	C8R1	BR
BR	C8R1	BR	C7R1	BR
BR	C2R1	BR	C2R1	BR
BR	C7R1	BR	C6R1	BR
BR	C4R1	BR	C1R1	BR
BR	C5R1	BR	C3R1	BR
BR	C7R2	BR	C7R2	BR
BR	C6R2	BR	C2R2	BR
BR	C5R2	BR	C3R2	BR
BR	C3R2	BR	C1R2	BR
BR	C2R2	BR	C5R2	BR
BR	C4R2	BR	C6R2	BR
BR	C8R2	BR	C4R2	BR
BR	C1R2	BR	C8R2	BR
BR	C4R3	BR	C1R3	BR
BR	C7R3	BR	C5R3	BR
BR	C1R3	BR	C8R3	BR
BR	C5R3	BR	C4R3	BR
BR	C2R3	BR	C2R3	BR
BR	C8R3	BR	C6R3	BR
BR	C3R3	BR	C7R3	BR
BR	C6R3	BR	C3R3	BR

* Origin; USA; ** Origin; ARC-Roodeplaat

Appendix 2-E: Fertilizer application to soil in Trial 1 and 3.

Analysis of soil:

P	K	K	Ca	Ca	Mg	Mg	Na	Na	R	pH
mg/kg	mg/kg	me/100g	mg/kg	me/100g	mg/kg	me/100g	mg/kg	me/100mg	ohm	
56.3	262	0.6701	2431	12.1307	310	2.5514	33.8	.1470	1370	7.78

According to the above analysis the following recommendations regarding fertilizer application was provided by Mr A. van den Bergh, Chief Technician, Crop Science, ARC-Roodeplaat

1. Initial fertilizer:
   1:0:1 (37) 500 kg/ha

2. Top dressing
   14, 30 days after planting: $(NH_4)_2\ SO_4$ – 130 kg/ha
   20, 40 days after planting: K_2SO_4 – 200 kg/ha

Appendix 2-F: Fertilizer application to soil in Trial 2.

Analysis of soil

P	K	K	Ca	Ca	Mg	Mg	Na	Na	R	pH
mg/kg	mg/kg	me/100g	mg/kg	me/100g	mg/kg	me/100g	mg/kg	me/100mg	ohm	
118.5	143	0.3657	1673	8.3483	229	1.8848	16.11	0.0701	1480	8.18

According to the above analysis the following recommendations regarding fertilizer application was provided by Mr A. van den Bergh, Chief Technician, Crop Science, ARC-Roodeplaat

1. Initial fertilizer:
   1:0:1 (37) 500 kg/ha

2. Top dressing
   14, 30 days after planting: $(NH_4)_2\ SO_4$ – 130 kg/ha
   20, 40 days after planting: K_2SO_4 – 200 kg/ha

Appendix 2-G: ANOVA of Trial 1 for LAI.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMT	1	773.744	773.744	51.98	0.019
Residual	2	29.773	14.887	12.15	
VARIETY	34	96.097	2.826	2.31	0.002
VARIETY.TMT	34	73.200	2.153	1.76	0.024
Residual	68	83.337	1.226	0.85	
TIME	1	6.170	6.170	4.27	0.042
VARIETY.TIME	34	35.949	1.057	0.73	0.840
TIME.TMT	1	12.842	12.842	8.89	0.004
VARIETY.TIME.TMT	34	37.507	1.103	0.76	0.805

Appendix 2-H: ANOVA of Trial 2 for LAI.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMT	2	61.6020	30.8010	41.09	0.002
Residual	4	2.9983	0.7496	0.79	
VARIETY	3	2.8550	0.9517	1.00	0.417
TMT.VARIETY	6	11.4991	1.9165	2.01	0.118
Residual	18	17.1750	0.9542	1.89	
TIME	1	74.4403	74.4403	147.36	<.001
TIME.TMT	2	15.4703	7.7352	15.31	<.001
TIME.VARIETY	3	4.2885	1.4295	2.83	0.060
TIME.TMT.VARIETY	6	4.6266	0.7711	1.53	0.212

Appendix 2-I: ANOVA of Trial 3 for LAI.

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
WATER	1	237.3499	237.3499	107.78	0.009
Residual	2	4.4042	2.2021	2.17	
VARIETY	7	7.2183	1.0312	1.02	0.442
WATER.VARIETY	7	15.8911	2.2702	2.24	0.061
Residual	28	28.4383	1.0157	2.36	
TIME	1	0.1520	0.1520	0.35	0.557
TIME.WATER	1	4.8187	4.8187	11.19	0.002
TIME.VARIETY	7	7.9676	1.1382	2.64	0.032
TIME.WATER.VARIETY	7	6.0203	0.8600	2.00	0.093

Appendix 2-J: ANOVA of Trial 1 for stem length.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMT	2	415155.1	207577.5	172.71	<.001
Residual	4	4807.4	1201.9	2.28	
VARIETY	3	163702.8	54567.6	103.62	<.001
TMT.VARIETY	6	85258.8	14209.8	26.98	<.001
Residual	18	9479.3	526.6	0.86	
TIME	1	33153.6	33153.6	53.97	<.001
TIME.TMT	2	14407.6	7203.8	11.73	<.001
TIME.VARIETY	3	4404.6	1468.2	2.39	0.094
TIME.TMT.VARIETY	6	5225.0	870.8	1.42	0.249
Residual	24	14743.3	614.3		

Appendix 2-K: ANOVA of Trial 3 for stem length**.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMT	1	11.002604	11.002604	58.33	0.017
Residual	2	0.377240	0.188620	2.01	
VARIETY	7	14.300383	2.042912	21.80	<.001
TMT.VARIETY	7	0.653113	0.093302	1.00	0.455
Residual	28	2.624042	0.093716	20.56	
TIME	1	1.233067	1.233067	270.51	<.001
TIME.TMT	1	0.893204	0.893204	195.95	<.001
TIME.VARIETY	7	0.118850	0.016979	3.72	0.005
TIME.TMT.VARIETY	7	0.132913	0.018988	4.17	0.002
Residual	32	0.145867	0.004558		

CHAPTER 3

THE BIOCHEMICAL EFFECT OF DROUGHT: ACTIVITY OF ANTIOXIDANT ENZYMES AND CHLOROPHYLL CONTENT IN THE LEAVES OF SWEET POTATO PLANTS

3.1. INTRODUCTION

3.1.1. Antoxidant enzymes

All plants need mechanisms to protect their physiological processes when adverse environmental conditions occur. Due to differences in genotypic composition, plants have different means to survive these conditions. It has been reported by **LEI, YIN and LI, (2006)** that, during drought, levels of abscisic acid (ABA) increased dramatically in poplar trees which favorably stimulated the closure of stomata. The authors also reported an increase in superoxide and hydrogen peroxide in the leaves of drought stressed plants and speculated that in both cases the ABA could have acted as a messenger to activate the antioxidant system in the plant.

The production of active oxygen species (AOS) in plants subjected to adverse conditions such as drought is a reality. These AOS can cause serious damage to the plant cells by means of injuries called oxidative stress injury. To keep the damage to a minimum, plants can prevent the damage, capture the oxidative radical species causing the damage and destroy them or repair the damage already caused **(SIES, 1993)**.

Through the production of the antioxidant enzyme species superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate reductase (AP) plants reduce the concentrations of these AOS and prevent further damage to the cell structure and function **(FOYER, LOPEZ-DELGADO, DAT and SCOTT, 1997)**. When plants are stressed due to drought conditions, the closure of stomata leads to the misdirecting of electrons which lead to the formation of the superoxide radical, H_2O_2 and the anion radical **(SIES, 1993)**. The SOD enzyme catalyzes the dissolving of the radical to produce peroxide and oxygen

(ELSTNER and HEUPEL 1976). Peroxide is eliminated by means of peroxidases of which AP, that is mainly located in the chloroplasts, is a prime example. This process involves the removal of H_2O_2 in collaboration with GR through a process called the Halliwell-Asada pathway (**DALTON, RUSSELL, HANUS, PASCOE and EVANS, 1986)**. AP, GR as well as SOD are located in the chloroplasts of the plant **(MITTLER, 2002)**. This amplifies the importance of the chloroplasts in the physiology of the plant apart from harboring the site for photosynthesis and ultimately energy production for metabolism in the plant. The GR enzyme is also in the mitochondria and the cytoplasm where it assists with the reduction of super radicals. The AP and GR enzyme complexes are integrated in a H_2O_2 scavenging system in the leaves and increases significantly during drought stress to sugar beet **(SAYFZADEH and RASHIDI, 2002)**.

It was found by **KIM, KIM, LEE and KWAK (2009)** and **LIN, CHAO, YANG, CHENG, LO and CHANG (2006)** that the presence of antioxidants in sweet potato is a reality although very little research has been undertaken in the investigation of individual antioxidant enzyme systems in sweet potato with regard to drought stress. Most research has focused on the accumulative antioxidant response by testing scavenging activity, carotenoid concentration and reducing power in sweet potato leaves **(LIN, CHAO, YANG, CHENG, LO and CHANG, 2006; RUMBAOA, CORNAGO and GERONIMO, 2009)**. This means that individual enzymes like SOD, AP and GR were not analyzed individually in drought simulated conditions. This supports the proposed research to investigate what influence drought will have on these enzymes.

3.1.2. Chlorophyll content

Chlorophyll forms an integral part of the photosynthetic system of the plant. Chlorophylls of different form play an important role as part of the photosynthetic apparatus of all phototrophic organisms **(KHALEGHI, ARZANI, MOALLEMI, BARZEGAR, 2012; LI, WU and CHEN, 2012)**. AP, GR as well as SOD are located in the chloroplasts **(MITTLER, 2002)** which gives more reason to have the concentration of chlorophyll in leaf material analyzed to obtain a possible correlation between chlorophyll and antioxidant activity.

Higher plants contain chlorophyll a (the major, yellow-green pigment), chlorophyll b (blue-green), accessory pigments and several additional forms of chlorophyll. Both chlorophyll a and b are associated with light harvesting processes at the antenna. In research often both methods, i.e. chlorophyll extraction by means of solvents and direct chlorophyll measurement in the field, are used **(SILLA, GONZÁLEZ-GIL, GONZÁLEZ-MOLINA, MEDIAVILLA and ESCUDERO, 2010; KHALEGHI, ARZANI, MOALLEMI and BARZEGAR, 2012; VAN DEN BERG and PERKINS, 2004)**. The advantage of direct chlorophyll measurement is that it saves time especially when a large number of samples have to be measured but the question always remains about the reliability of the readings. Various researchers **(RICHARDSON, DUIGAN and BERLYN, 2002; CASTELLI, CONTILLO and MICELI, 1996; SILLA, GONZÁLEZ-GIL, GONZÁLEZ-MOLINA, MEDIAVILLA and ESCUDERO, 2010)** have tested the reliability of the chlorophyll instruments versus the physical concentration values obtained via extraction. The results were very consistent and it was shown that there was a very good correlation between the two methodologies. The only deviation observed was when chlorophyll content index (CCI) values became too high and the correlation started to fade. **RICHARDSON, DUIGAN and BERLYN (2002)** were even able to formulate a calibration equation from the correlation to convert index values to chlorophyll content (mg/cm^2). This was found to be species specific. **BIBER (2007)** evaluated three wetland plant species and found a better correlation between the CCI values and the extracted chlorophyll concentrations in species that had flatter leaves, which might be due to better reflection and transmittance of the light off the instruments. In terms of water stress **KHALEGHI, ARZANI, MOALLEMI and BARZEGAR, (2012)** found good correlation between the chlorophyll a and total chlorophyll contents compared to the CCI values obtained from the leaves of olive plants subjected to stress.

This provides the basis to argue that the values from extracted chlorophyll can be substituted by the use of the chlorophyll content apparatus to predict the physiological plant status. It has been reported that the chlorophyll content in plants subjected to drought fluctuates through the drought period. **MENSAH, OBADONI, ERUOTOR and ONOME-IRIEGUNA (2006)** studied the drought effect on sesame plants and reported

that the chlorophyll content increased and then stabilized as the drought increased. The same tendency was observed by **(NIKOLAEVA, MAEVSKAYA, SHUGAEV and BUKHOV, 2010)** who found a slight but nonsignificant increase in chlorophyll content followed by a significant decrease in wheat plants subjected to drought stress. Since the antioxidant system of the plant is also situated in the chloroplast, there is speculation that the activity of the antioxidant system will diminish as the chlorophyll is destroyed due to drought conditions. It has been shown that AP, SOD and GR in general, increase in activity as the drought condition worsens **(LEI, YIN and LI, 2006; MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI, 2011)**. However, it is not clear to what degree the plants can be stressed to result in the degradation of these enzyme systems.

3.2. AIMS

The aims of this part of the study was to investigate if the activity of three antioxidant enzymes (SOD, GR and AP) is influenced by drought stress in the three trials and to what degree. Due to the fact that these antioxidants are present in the chloroplast it was also the aim to investigate if drought has an influence on chlorophyll concentration and if the enzyme activities and chlorophyll concentration are related to each other.

3.3. MATERIALS AND METHODS

3.3.1. Plant material

Cultivars were planted in the rainout shelters and open field as explained in Section 2.3.1. Leaves (5^{th} leaf from the apical tip) were harvested from the randomly selected plants (control and stress). This was performed twice during the growth season, before sunrise and the material was quickly frozen and freeze-dried. Freeze dried leaves were stored at -80ºC.

3.3.2 Enzyme extraction

The extraction method of **MALAN, GREYLING and GRESSEL (1990)** was used with minor modifications. Freeze-dried plant material (40 mg), instead of 100 mg, was homogenized in liquid nitrogen and suspended in 1 ml of 50 mM phosphate extraction

buffer (pH 7) containing 0.1 mM EDTA and 2% w/w polyvinylpolypirrolidone. It was found that β-mercaptoethanol and bovine serum albumin could be excluded from the mixture. The homogenate was centrifuged and supernatant used for the individual enzyme analysis.

3.3.2.1 Superoxide dismutase (SOD)

The activity determination was executed using the method of **ELSTNER and HEUPEL (1976)**. The results were based on the formation of nitrite from hydroxylammonium chloride in the presence of SOD. A solution was prepared containing 65 mM potassium phosphate buffer, xanthine reagents and hydroxyl ammonium chloride. After the addition of the enzyme extract the mixture was incubated at 25ºC for 20 min. The addition of sulphanilic acid and α-naphtylamine and subsequent 20 min incubation period allowed for colour formation that was determined spectrophotometrically at 530 nm with a Multiscan EX multiplate reader (MTX Lab Systems, VA, USA).

Preparation of buffers and reaction mixtures for enzyme analysis was conducted according to **SPREETH (2001)**.

3.3.2.2. Ascorbate peroxidase (AP)

The activity of AP was determined following the method of **DALTON, RUSSELL, HANUS, PASCOE and EVANS (1986)**. Phosphate buffer (50 mM) and ascorbic acid (0.25 mM) were mixed with the enzyme extract. The reaction was started by adding 1 mM H_2O_2 and following the reduction for 1 min measured at 265 nm with a Beckman Coulter DU 800 UV/Vis spectrophotometer (Beckman Coulter Inc, USA).

Preparation of buffers and reaction mixtures was conducted according to **SPREETH (2001)**. Enzyme activities were expressed as activity/µg protein.

3.3.2.3. Glutathione reductase (GR)

The GR activity (Table 3.2) was determined using the method of **CARLBERG and MANNERVIK (1985)**. Reaction solutions and buffers were prepared as outlined in Table 3.1. The enzyme was extracted according to the procedure described in Section 3.3.2 whereafter an aliquot of the extraction mixture was added to a mixture of oxidized glutathione (GSSG) (0.25 mM), Tris (50 mM) and EDTA (0.5 mM). NADPH (0.125 mM)

was added to the solution (Table 3.2) and the oxidation of NADPH followed spectrophotometrically at 340 nm over a period of 1 min. Enzyme activities were expressed as enzyme units (U)/ g dry weight.

Preparation of buffers and reaction mixtures was conducted according to **SPREETH (2001)**.

Table 3.1 Preparation of reaction solutions for glutathione reductase (GR) determination

Buffer/Reaction Mixture	**Stock**	**Remarks**
Tris (0.5 mM)	2.42 g/200 ml	Adjust to pH 7.8 with HCl
EDTA (0.5 mM)	0.0744 g/150 ml	
Glutathione (oxidized) (GSSG) 0.25 mM	0.0122 g/4 ml	Prepare fresh
NADPH (0.125 mM)	0.00832 g/4 ml	Prepare fresh
Glutathione reductase enzyme	10 µl/90 µl H_2O	Prepare fresh

Table 3.2 Volumes of reaction solutions for determination of GR activity per sample.

Buffer/Reaction mixture	**Volumes**	**Remarks**
Glutathione oxidized (0.25 mM)	100 µl	
Tris (50 mM)	1 ml	
EDTA (0.5 mM)	750 µl	
Plant extract	25 µl	Keep on ice
Mix well and calibrate spectrophotometer at 340 nm, add NADPH		
NADPH (0.125 mM)	50 µl	
Standard: GR enzyme	25 µl	

3.3.3. Chlorophyll measurements

Chlorophyll measurements were achieved by using a CCM 200 chlorophyll reader (Opti-Sciences). The standard protocol as indicated by the manufacturers for calibration and readings were conducted during the two measurement periods namely 60 and 120 days

after planting (DAP). The 5th leaf from the apical tip was read from 3 stems of randomly selected plants per repeat.

3.4. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on all the data to test for treatment differences as well as possible differences between cultivars. Treatment means as well as interaction means were separated using Fishers' t-test least significant difference (LSD) at the 5% level of significance. Statistical analysis was conducted using, GenStat *for Windows* 15th Edition (VSN International, Hemel Hampstead, UK) by the ARC Biometry Unit. The ANOVA tables for the different variables are presented in Appendix A-K.

3.5. RESULTS AND DISCUSSION

3.5.1. Ascorbate peroxidase (AP)

3.5.1.1. Trial 1

The drought stress introduced to the cultivars in the LR during Trial 1 resulted in an increase in AP activity over the treatments at each of the leaf collection periods, T1 and T2, respectively (Figure 3.1). Peroxidase levels, in soybean plants subjected to drought stress, observed by **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)** seem to follow the same trend although peroxidase activity values lessened as the stress continued. Significant differences were observed between the drought and stress treatments with regard to the Time X Water combination (Appendix 3-A). This is in contradiction with findings of **SAYFZADEH and RASHIDI (2002)** who could not find significant differences in the Time X Water combination during the response of sugar beet to drought stress. The increase is expected as AP activity in general increases as the drought condition is increasing (**LEI, YIN and LI, 2006)**. The cultivar W-119 and Resisto experienced a nonsignificant increase in AP activity at the first sampling. The cultivar, Purple Sunset experienced a significant increase in AP activity from the mild stress to the severe stress treatment after a non-significant decrease from the control to mild stress treatment which agrees with the findings of **MASOUMI, DARVISH,**

DANESHIAN, NORMOHAMMADI and HABIBI (2011) who also observed a decrease in peroxidase levels in soybean leaves at a higher drought stress level. The cultivar Isondlo also experienced a significant increase in AP activity after, in severe stress conditions after the first measurement. A significant decline in AP values was observed after the second measurement in cultivar Isondlo at T2. This might indicate that the defensive mechanism of the sweet potato cultivars regarding AP does not react to the drought stress or that protein breakdown commenced. At T2 however Purple Sunset showed an increase in AP activity in mild stress conditions which declined in the severe stress treatment. This could be ascribed to the increase in drought the plants experienced at mild stress but the severe stress treatment proved to be too severe causing a steep decline in activity. This phenomenon was also reported by **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011).** Although **SPREETH (2001)** did not indicate the drought conditions in the soil with experiments on cowpeas, the AP concentration also fluctuated nonsignificantly as the drought condition worsened with cowpeas grown in the glasshouse.

In Figure 3.1 it is shown that a significant increase in AP values was detected in all the cultivars comparing the control treatment at T1 with the control treatment at T2. **KIM, KIM, LEE and KWAK, 2009** found similar increases in antioxidant enzyme activity in aging sweet potato leaves under control conditions, which is probably delaying the senescence process by acting against oxidants. This increase of AP activity, under normal growing conditions, also agrees with the finding of **DALTON, RUSSELL, HANUS, PASCOE and EVANS (1986)** who observed increases in AP in soybean plants grown in non-stressed conditions.

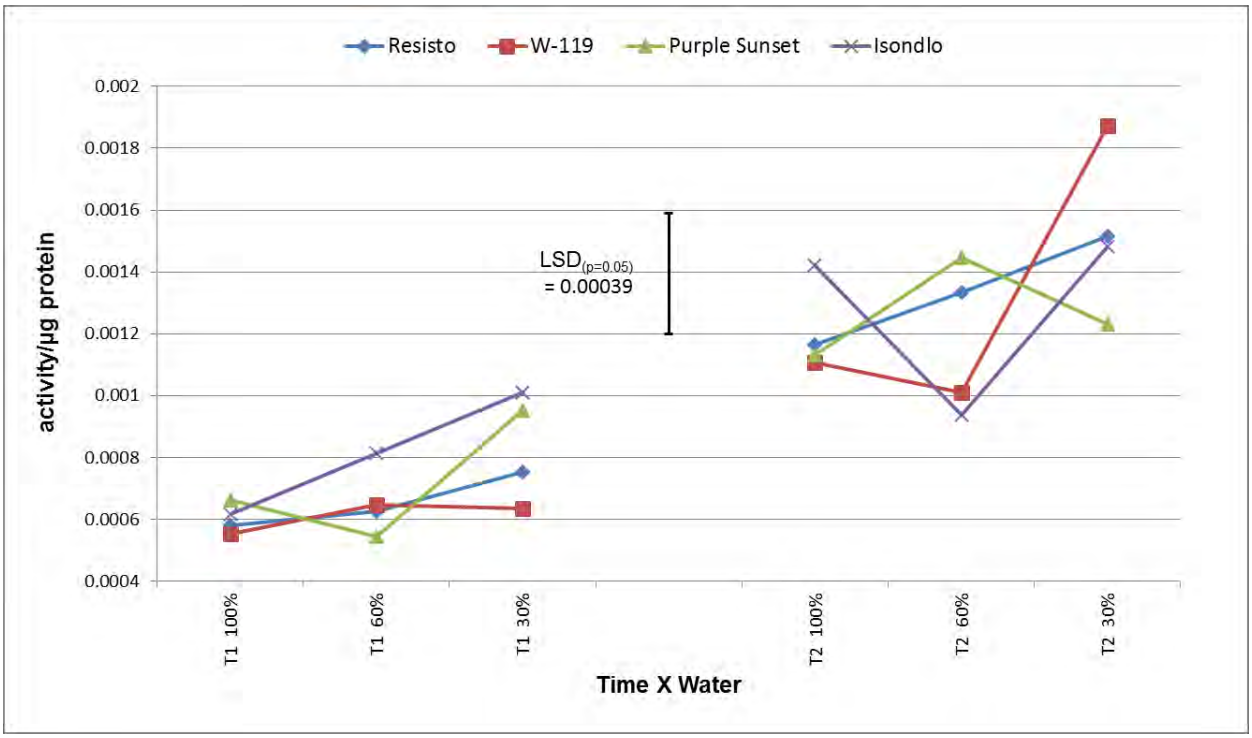

Figure 3.1: Ascorbate peroxidase activities of four sweet potato cultivars subjected to drought stress in Trial 1. $LSD_{(p=0.05)}$ = 0.00039. T1= 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

The plants could be preparing themselves for possible H_2O_2 scavenging although it is still growing in normal conditions. The increase in AP in the present study might also be due to the aging of the plants during the trial as suggested by **KIM, KIM, LEE and KWAK (2009)**. At the second time of measurement the cultivars showed a general increase in AP activity in severe stress conditions. The cultivar Isondlo exhibited a significant decline in AP activity in mild stress conditions which could be either due to possible water contamination, causing non-stressed conditions, or that this specific cultivar did not suffer particularly during this period of the trial. A nonsignificant decline at mild stress was also detected in cultivar W-119, which was followed up by a significant increase in activity in the severe stress treatment. This shows that the plant is experiencing the stress and is reacting to it by increasing the AP to prepare for possible H_2O_2 scavenging. The activity of AP in Resisto gradually increased from control to severe stress conditions with the only

significant difference in the severe stress treatment. This indicates that the cultivar was sensitive to the drought at both times although more pronounced at T2.

3.5.1.2. Trial 2

Table 3.3 demonstrates the impact of drought on the AP system in the plants. Significant differences were observed between the genotypes and the sampling times (Appendix 3-B). The AP values of the 35 sweet potato cultivars and breeding lines did not show any significant differences at T1 between the control and the stress although increasing trends were observed. This observation agrees with the finding of **BAI, SUI, GE, SUN, LU and ZHOU (2006)** and **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** who observed only a significant increase in AP in maize and wheat respectively when subjected to mild and severe drought stress. At 120 DAP (lengthened stress period) significant differences were observed with Purple Sunset, 2005-1-11, 2005-4-1, 2005-5-5, 2006-15-1, 2006-7-7 and the cultivars Blesbok, Lethlabula and Zapallo which could lead to more successful prevention of H_2O_2 formation. The other lines and cultivars in the trial showed increases in AP activity from the control to the severe stress treatment but these were non significant.

All the cultivars and lines except 2005-2-2, 2005-7-4, 2006-14-4, 2006-3-4, 2006-4-4, 2006-4-5, 2006-6-2, 2006-7-8, Beauregard and Jewel exhibited significant differences between the values obtained at T1 and T2 for the stress treatment which could lead to effective tolerance through the stress. The reason for the non significant differences exhibited by the rest of the genotypes could be either that the cultivars and lines did not experience the stress as the others did or that their peroxidase system is not reacting to the stress. **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** also did not observe any significant increase in AP activity in

Table 3.3: Effect of drought on the ascorbate peroxidase activity (activity/µg protein) of 35 sweet potato cultivars and lines subjected to drought stress in Trial 2. Values calculated are the average of two repeats and three samples per repeat.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	0.000327	0.000494	0.000758	0.001463#
Purple Sunset	0.000289	0.000436	0.00112	0.002162*#
2005-1-11	0.000207	0.000312	0.001182	0.002281*#
2005-1-16	0.000288	0.000435	0.000732	0.001413#
2005-11-3	0.000210	0.000316	0.000929	0.001792#
2005-12-2	0.000088	0.000132	0.000823	0.001589#
2005-16-1	0.000338	0.000511	0.000741	0.001431#
2005-2-2	0.000274	0.000413	0.000536	0.001034
2005-3-10	0.000177	0.000268	0.000609	0.001175#
2005-3-13	0.000264	0.000399	0.000496	0.000958#
2005-4-1	0.000271	0.000410	0.000974	0.001880*#
2005-5-5	0.000128	0.000193	0.001030	0.001988*#
2005-7-3	0.000167	0.000252	0.000633	0.001222#
2005-7-4	0.000693	0.001046	0.000456	0.000880
2006-14-4	0.000249	0.000377	0.000626	0.001209
2006-15-1	0.000121	0.000183	0.001185	0.002287*#
2006-2-4	0.000150	0.000227	0.000508	0.000980#
2006-3-4	0.000307	0.000464	0.000539	0.001040
2006-4-4	0.000503	0.000760	0.000808	0.001559
2006-4-5	0.000415	0.000626	0.000777	0.001499
2006-6-2	0.00064	0.000966	0.000797	0.001538
2006-7-7	0.000183	0.000276	0.001036	0.001999*#
2006-7-8	0.000338	0.000511	0.000723	0.001396
Beauregard	0.000300	0.000453	0.000296	0.000571
Blesbok	0.000318	0.000480	0.001270	0.002452*#
Bosbok	0.000268	0.000405	0.001294	0.002498#
Impilo	0.000227	0.000342	0.000704	0.001359#
Jewel	0.000486	0.000734	0.000566	0.001093
Lethlabula	0.000331	0.000500	0.001122	0.002165*#
Ndou	0.000359	0.000543	0.000734	0.001417#
Phala	0.000691	0.001043	0.000945	0.001823*
Resisto	0.000189	0.000285	0.000671	0.001294#
Tanzania	0.000249	0.000376	0.000855	0.001649#
W-119	0.000297	0.000449	0.000833	0.001608#
Zapallo	0.000114	0.000172	0.001231	0.002376*#
Mean	0.000299	0.000451	0.000815	0.001574

* indicates significant difference between the control and the stress at 120 DAP at P≤ 0.05

# indicates significant differences in AP activity between the stress at 60 DAP and 120 DAP of the cultivars and breeding lines at P≤ 0.05, MSE $_{(df=70)}$ = 0.000224

$LSD_{(p=0.05)}$ = 0.0009; control = control treatment, stress = severe stress treatment.

T1 = first leaf harvest at 60 days after planting, T2 = second leaf harvest at 120 days after planting

wheat plants subjected to drought. The AP activity however increased significantly on subsequent re-watering of drought stressed wheat plants.

Differences between the AP values of the genotypes in the control treatment at T1 and T2 were observed and could be ascribed to the aging of leaves as outlined by **KIM, KIM, LEE and KWAK (2009)** who showed that AP activity increased with leaf age. At 120 DAP the plants were considerably "older" than at T1 and this could have led to higher AP activity levels although the selected leaf is the same distance from the growth tip. This could also be true for the stressed plants although the stress would enhance the formation of oxidative species and hence the elevated activity of AP.

3.5.1.3 Trial 3

The exposure of sweet potato plants in Trial 3 to drought, caused significant (Appendix 3-C) increases in AP activity in most of the leaves especially during T2 (Figure 3.2). This correlates with the findings of **BAI, SUI, GE, SUN, LU and ZHOU (2006)** who observed increases in peroxidase activity levels through the growth cycle of maize plants subjected to drought. This suggested that the plants increase their defensive response regarding the stress **BAI, SUI, GE, SUN, LU and ZHOU (2006)**. Sweet potato line 2002-8-2 was the genotype that showed a significant increase in AP activity from the control to severe stress conditions giving rise to the possible increase in H_2O_2 levels during the drought condition which then can be neutralized by the AP action. Cultivars Mvuvhelo, 199062.1, Monate and Blesbok also showed increases in AP activity but proved nonsignificant; indicating that the stress was affecting the antioxidant system, specifically the AP enzyme system, to a very low degree. This was also indicated by **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** who observed non-significant increases in AP activity when wheat plants were subjected to drought stress. The sweet potato plants in the control treatment of T1 did not show any significant difference to the plants in the control treatment at T2 which is contradictory to the results in Trial 1. The reason might be due to the difference in genotypes that were used in this Trial that did not display the expected results. These genotypes, since all genotypes are

not the same, could be at a different growth stage than those in Trial 1 contributing to the nonsignificant difference in AP values between the two times of measurement.

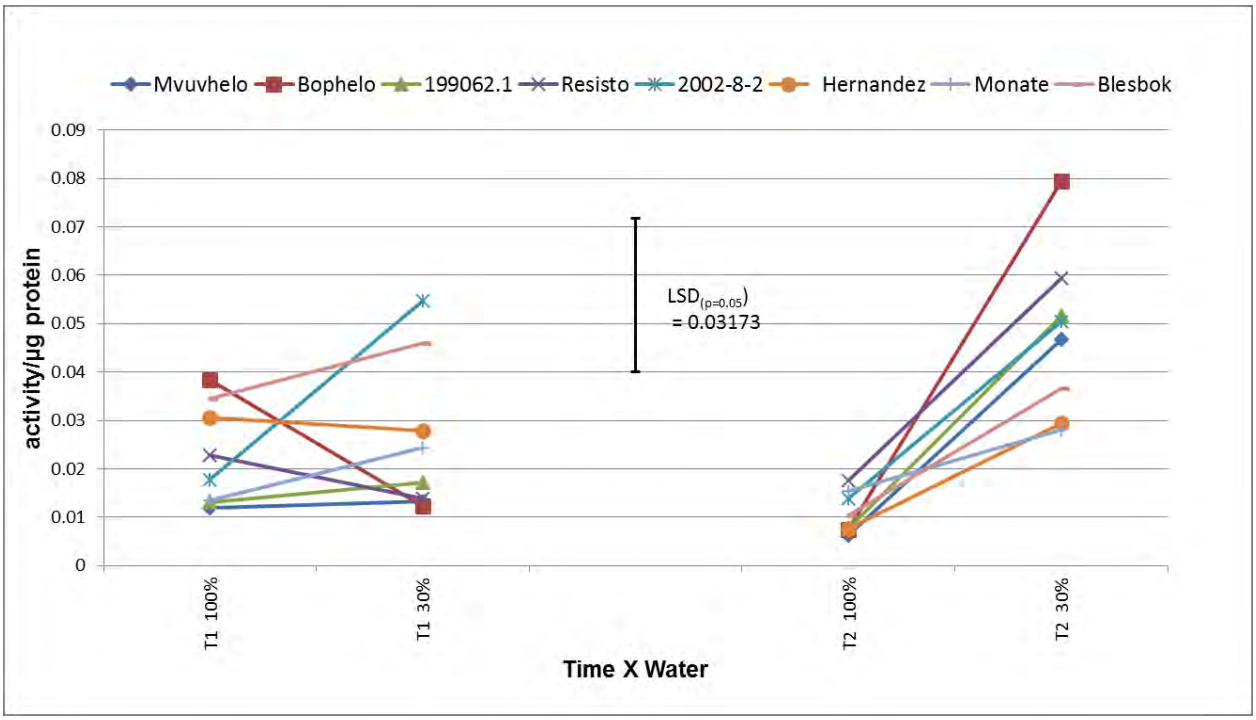

Figure 3.2. Reaction of eight sweet potato cultivars to drought with regard to ascorbate peroxidase activity in Trial 3. $LSD_{(p=0.05)}$ = 0.03173; T1 = 60 days after planting, T2 = 120 days after planting, 100% = control treatment, 30% = severe stress treatment

At T2 the majority of the cultivars experienced an increase in AP activity. This gives rise to the probability that the drought stress resulted in the formation of reactive oxidant species that forces the plants to react by elevating the activity of AP **(ASADA, 1999)**. The cultivar Bophelo showed the most intense reaction to the stress at T2 with regard to AP activity, showing a significant difference (increase) compared to Hernandez, Monate and Blesbok. Bophelo did not show a significant response to the drought stress at T1 but the severity of the stress forced the plants to react by increasing the levels of AP activity at T2. The small nonsignificant increase in AP activity from T1 to T2 in the severe stress treatment for Monate and Hernandez, could be the result of very little response from the peroxidase antioxidant system of the plants to ensure the decomposition of H_2O_2. No explanation can be rendered for the relative low average AP values in the severe stress

treatment compared to the average AP values in the control treatment at 120 DAP other than the fact that the sweet potato plants did not react intensely to the stress to detectable increased levels of AP.

Although the majority of sweet potato cultivars and lines exhibited moderate elevated levels of AP during the drought stress it appears that peroxidase is not one of the antioxidant pathways sweet potato plants use to decrease the AOS levels during drought stress periods.

3.5.2. Superoxide dismutase (SOD)

3.5.2.1. Trial 1

SOD is an antioxidant enzyme which can be produced during abiotic stress conditions to protect cells from the damaging effects of oxygen free radicals **(VAN DER MESCHT, DE RONDE, SLABBERT and OELOFSE, 2007)**. During Trial 1 increases in overall activity of SOD was observed in all the cultivars in both periods, T1 and T2, of measurement (Figure 3.3). This was also found by **SAYFZADEH and RASHIDI (2002)** in sugar beet when subjected to drought although the contrary was observed by **BAI, SUI, GE, SUN, LU and ZHOU (2006)** in maize plants under drought conditions. The cultivar W-119 showed the most continuous significant increase in activity while SOD levels in Purple Sunset and Resisto only started increasing from the mild to the severe stress treatment. This could be due to the fact that the plants only really started experiencing stress during mild stress conditions. No significant differences could be detected between the cultivars at any of the treatments in either T1 or T2 (Appendix 3-D) This could either be that the cultivars react in the same way with regard to SOD towards the stress or it might be that the sweet potato does not react to the stress using the SOD system as such and that the values then could be the result of aging.

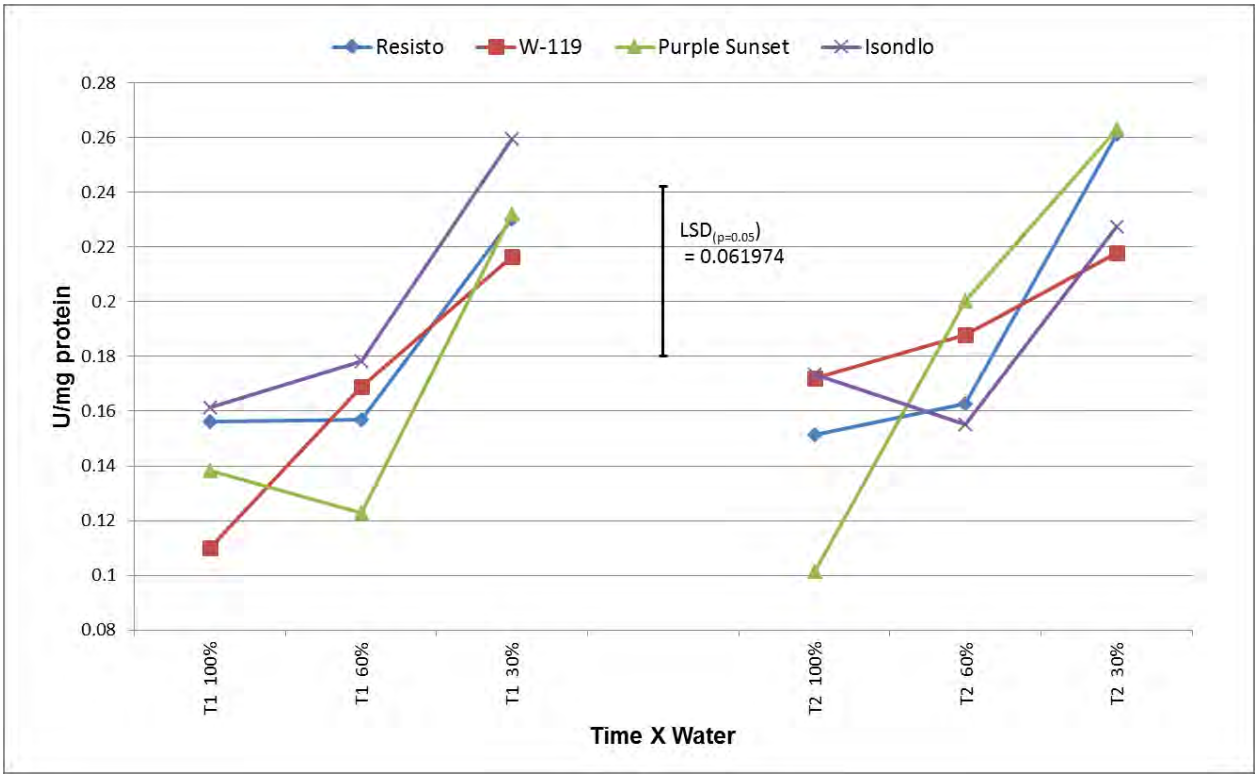

Figure 3.3 Reaction of four sweet potato cultivars to drought stress with regard to super oxide dismutase (SOD) enzyme activity in Trial 1. $LSD_{(p=0.05)}$ = 0.069; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

3.5.2.2. Trial 2

Sweet potato plants were subjected to drought stress and SOD activities determined. At T1 no significant differences between the control and stressed plants could be detected although the breeding line 2005-1-11 showed an increase in activity. The extended stresses at T2 resulted in significant increases in SOD activity in all the cultivars and breeding lines (Table 3.4). This indicated that the plants are reacting to the stress and probably sensing the increased production of oxygen radicals hence the increase in SOD activity. The activity of SOD seemed to increase over time which agrees with the finding of **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)**. They found a continuous increase of SOD activity in soybean cultivars as the drought stress increased.

Table 3.4: SOD values (U/mg protein) in the leaves of 35 sweet potato cultivars and breeding lines subjected to drought in Trial 2.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	0.135	0.193	0.182	0.360*
Purple Sunset	0.123	0.176	0.165	0.325*
2005-1-11	0.152	0.217*	0.147	0.291*
2005-1-16	0.121	0.173	0.132	0.261*
2005-11-3	0.130	0.186	0.148	0.293*
2005-12-2	0.146	0.209	0.151	0.291*
2005-16-1	0.114	0.163	0.143	0.296*
2005-2-2	0.150	0.214	0.157	0.271*
2005-3-10	0.122	0.175	0.143	0.283*
2005-3-13	0.155	0.222	0.166	0.351*
2005-4-1	0.136	0.195	0.157	0.287*
2005-5-5	0.145	0.207	0.169	0.316*
2005-7-4	0.157	0.225	0.113	0.270*
2006-14-4	0.129	0.184	0.161	0.318*
2006-15-1	0.126	0.181	0.135	0.267*
2006-2-4	0.146	0.209	0.136	0.268*
2006-3-4	0.162	0.232	0.122	0.242*
2006-4-4	0.127	0.182	0.167	0.329*
2006-4-5	0.155	0.222	0.142	0.279*
2006-6-2	0.135	0.194	0.151	0.298*
2006-7-3	0.130	0.186	0.158	0.311*
2006-7-7	0.121	0.173	0.138	0.272*
2006-7-8	0.122	0.174	0.180	0.354*
Beauregard	0.106	0.152	0.127	0.251*
Blesbok	0.147	0.210	0.172	0.339*
Bosbok	0.160	0.229	0.149	0.294*
Impilo	0.140	0.201	0.154	0.305*
Jewel	0.146	0.209	0.097	0.192*
Lethlabula	0.152	0.218	0.175	0.346*
Ndou	0.149	0.213	0.154	0.303*
Phala	0.169	0.241	0.148	0.291*
Resisto	0.145	0.208	0.149	0.293*
Tanzania	0.141	0.203	0.150	0.295*
W-119	0.159	0.227	0.163	0.322*
Zapallo	0.128	0.183	0.163	0.322*
mean	0.139	0.200	0.150	0.297

***** indicates a significant difference at 5% level between the control and SR for a specific Time X Water X Variety combination.

$LSD_{(p=0.05)}$ = 0.091; control = control treatment, stress = severe stress treatment in small rainout shelter. T1 = 60 days after planting, T2 = 120 days after planting. $MSE_{(df=72)}$ = 0.001.

Although an increase in SOD was detected at T2 there seems to be very little difference between cultivars and breeding lines. It is speculated that this can either be due to the fact that the plants are stretched to a maximum in their response to the drought stress or that the plants do not differ genetically regarding SOD production.

3.5.2.3. Trial 3

Increase in SOD activity was also observed in sweet potato cultivars subjected to drought stress in Trial 3 (Appendix 3-F). This is similar to findings by **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)** who found increases in SOD activity in soybean cultivars subjected to drought stress. Significant differences between the cultivars were observed at T1 and T2 for the severe stress treatment although much less in the severe stress treatment at T2 (Figure 3.4). The values of SOD activity at severe stress at T2 in general were also higher and could possibly be ascribed to more drought experienced by the cultivars and the resulting increase in O_2 radical levels in the leaves. During T1 the cultivar Hernandez showed a significant increase in SOD activity in severe stress conditions that is possibly the result of increased accumulation of reactive oxygen species to be reflective of an increased stress condition **(SELOTE and KHANNA-CHOPRA, 2006)**. The cultivar Bophelo showed a nonsignificant decline in SOD activity at T1 from the control to the severe stress treatment, which could be the result of either water contamination i.e. water accidently coming from the mild stress treatment area resulting in a termination of stress or an unfortunate mishap in leaf identification, younger leaves might display lower SOD levels than older leaves (**KIM, KIM, LEE and KWAK, 2009)**, for the analysis procedure.

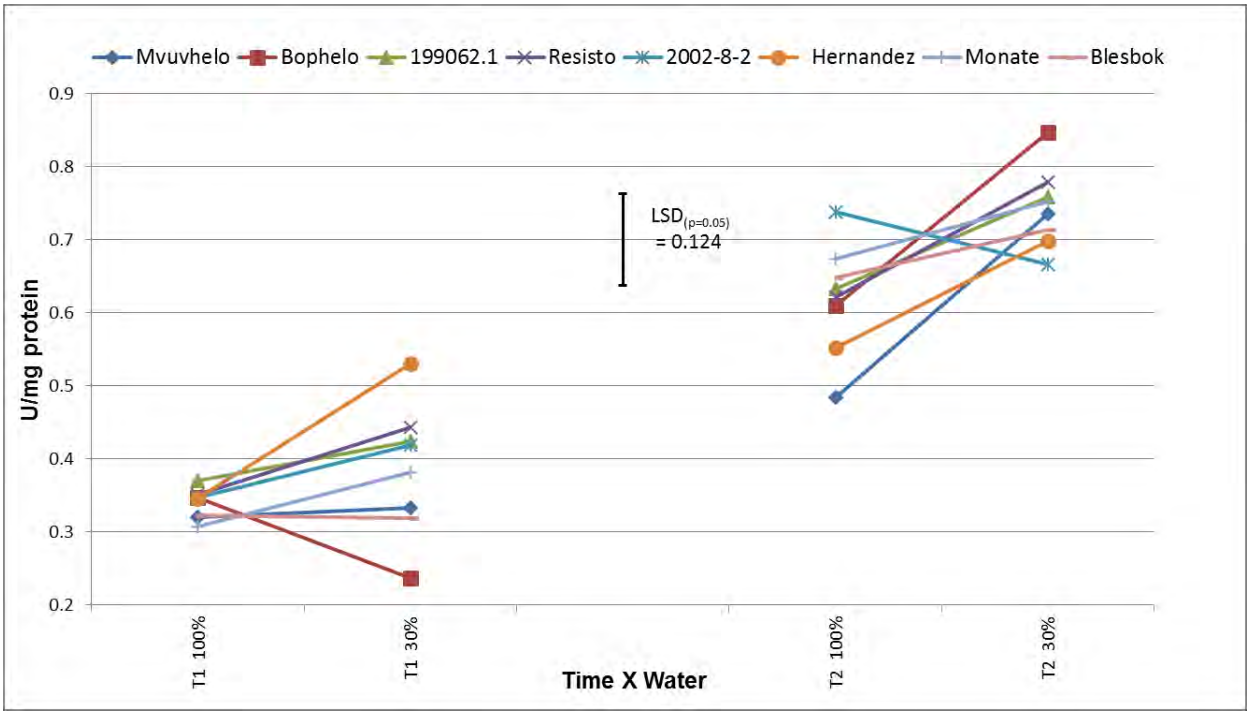

Figure 3.4 Activity levels of the SOD enzyme in the leaves of eight sweet potato cultivars and lines subjected to control and drought stress conditions in Trial 3. $LSD_{(p=0.05)}$ = 0.124; T1 = 60 days after planting; T2 = 120 days after planting; 100% = control treatment; 30% = severe stress treatment

In general the gradient of increase in SOD activity from the control to the severe stress treatment at T2 when compared to T1 was much steeper. This emphasizes the more intense drought stress experienced by the plants during the later stages of the Trial and the tendency to react more against free oxygen radicals.

3.5.3. Glutathione reductase (GR)

3.5.3.1 Trial 1

An increase in GR in the plant due to stress is an indication of the plant's ability to defend itself against AOS and to keep them under control **(LEI, YIN and LI 2006)**. In Trial 1 a significant increase in GR was observed at both times from the control treatment to the severe stress treatment (Figure 3.5). At the severe stress treatment of T2 the cultivars Resisto, Purple Sunset and W-119 showed a higher, although nonsignificant, GR activity compared to Isondlo, which is probably due to the fact that they experience the stress more intensely in the beginning. At T2, however the GR activity of Isondlo increased,

although not significantly, so that a nonsignificant difference between the four cultivars was observed. No significant difference was observed between the cultivars in the control treatment of either T1 or T2 which was also the case in the mild stress treatment. The activity of GR of the cultivar Purple Sunset was lower in the severe stress treatment of T2 than the severe stress treatment of T1, although not significantly so, which could be the result of possible protein denaturation due to the extended stress.

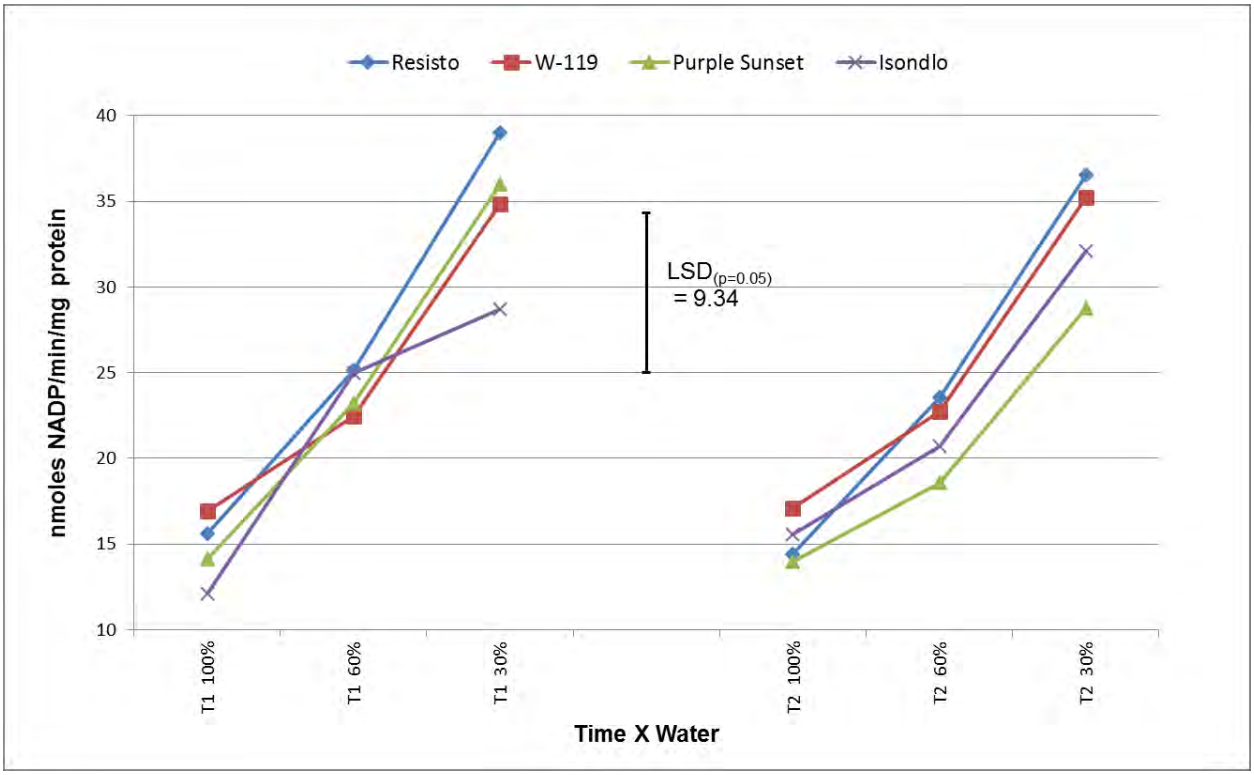

Figure 3.5 Levels of glutathione reductase activity in the leaves of four sweet potato cultivars subjected to drought stress in Trial 1. $LSD_{(p=0.05)}$ = 9.23; T1 = 60 days after planting. T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

The fact that no significant differences could be detected in the severe stress treatment for T2 could mean that the plants of the four cultivars react the same to the drought stress with regard to GR activity. **SPREETH, (2001)** also found limited differences between cowpea lines subjected to drought stress which emphasizes the possibility of nonsignificant differences between genotypes during drought stress.

3.5.3.2 Trial 2

Significant increases in GR activity, between control and stress, were observed for almost all the cultivars and breeding lines in Trial 2 over both T1 and T2 (Table 3.5). It was also observed that the activity of GR increased at T2 indicating that the plants are reacting to increased production of AOS **(MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)**. This provides an indication that these plants might display some tolerance to the stress **(MALAN, GREYLING and GRESSEL, 1990).** This could prove valuable for use in future selections for drought tolerant genotypes.

GR activity levels stayed relatively stable from T1 to T2 in the control treatment with the exception of breeding line 2006-14-4, which displayed significantly elevated levels of GR activity suggesting a stress condition. This could be due to experimental error. The relative low increase in GR activity from control to stress in the breeding lines 2005-2-2, 2006-7-8 and cultivars Impilo and Lethlabula at both time frames could be that the four genotypes did not respond to the stress by means of the GR pathway or could prove to be drought sensitive since as **MALAN, GREYLING and GRESSEL, (1990)** have shown that sensitive maize lines produced very little GR.

Table 3.5. Glutathione reductase activity (nmole NADPH/min/mg protein) in the leaves of 35 sweet potato cultivars and breeding lines subjected to drought in Trial 2.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	33.39	45.78*	25.05	52.03*
Purple Sunset	13.49	64.3*	27.25	57.37*
2005-1-11	19.54	49.01*	23.51	51.74*
2005-1-16	20.04	50.03*	25.97	66.82*
2005-11-3	19.96	50.18*	21.05	45.41*
2005-12-2	23.75	71.28*	17.95	51.71*
2005-16-1	14.36	61.52*	16.98	46.52*
2005-2-2	17.94	19.73	16.28	33.12*
2005-3-10	15.78	43.3*	21.26	52.15*
2005-3-13	16.34	53.47*	21.67	56.3*
2005-4-1	19.74	48.72*	21.49	61.44*
2005-5-5	16.73	35.9*	21.22	38.55*
2005-7-3	17.01	41.28*	18.59	44.66*
2005-7-4	16.66	42.62*	28.72	62.11*
2006-14-4	32.49	18.56	52.65	33.49*
2006-15-1	19.37	38.31*	18.21	58.25*
2006-2-4	18.52	38.36*	24.41	42.15*
2006-3-4	15.58	35.45*	19.87	48.19*
2006-4-4	14.08	50.85*	19.20	49.23*
2006-4-5	17.62	30.75	15.55	54.11*
2006-6-2	17.41	34.59*	11.32	48.81*
2006-7-7	16.37	35.11*	18.71	56.08*
2006-7-8	21.84	32.2	17.36	33.14*
Beauregard	24.19	40.55*	20.10	32.43
Blesbok	14.38	39.48*	16.47	33.33*
Bosbok	15.60	36.54	17.11	42.86*
Impilo	24.13	26.47	18.84	31.59*
Jewel	15.95	67.90*	16.32	37.93*
Lethlabula	14.65	23.59	11.59	30.44*
Ndou	17.32	41.29*	28.64	35.62
Phala	19.33	38.52*	21.77	46.01*
Resisto	13.75	45.23*	9.84	43.80*
Tanzania	19.53	38.09*	15.21	42.23*
W-119	9.13	34.85*	20.10	40.42*
Zapallo	12.69	35.30*	13.01	40.55*
mean	18.24	41.68*	20.37	45.73*

***** indicates a significant difference at a 5% level between the control and stress for a specific Time X Water X Variety combination.

$LSD_{(p=0.05)}$ = 13.71; T1 = 60 days after planting, T2 = 120 days after planting. control = control water treatment, stress = severe stress treatment

$MSE_{(df=46)}$=49.09

3.5.3.3 Trial 3

The GR levels of the cultivars subjected to drought stress in Trial 3 showed an increase from the control to the severe stress treatment at both T1 to T2 (Figure 3.6). Although the GR increase between the control and severe stress treatment was to the same extent for each of the cultivars the simultaneous increase in SOD activity (Figure 3.4) established confirms the possibility of drought tolerance **(MALAN, GREYLING and GRESSEL, 1990)**. Significant differences in GR activity between the cultivars were also detected in the control treatment of T1. This could possible indicate the genotypic diversity between the cultivars regarding GR activity at control conditions. At T1 the cultivar Mvuvhelo showed an increase in GR activity although this was not significantly different from the value in the control treatment.

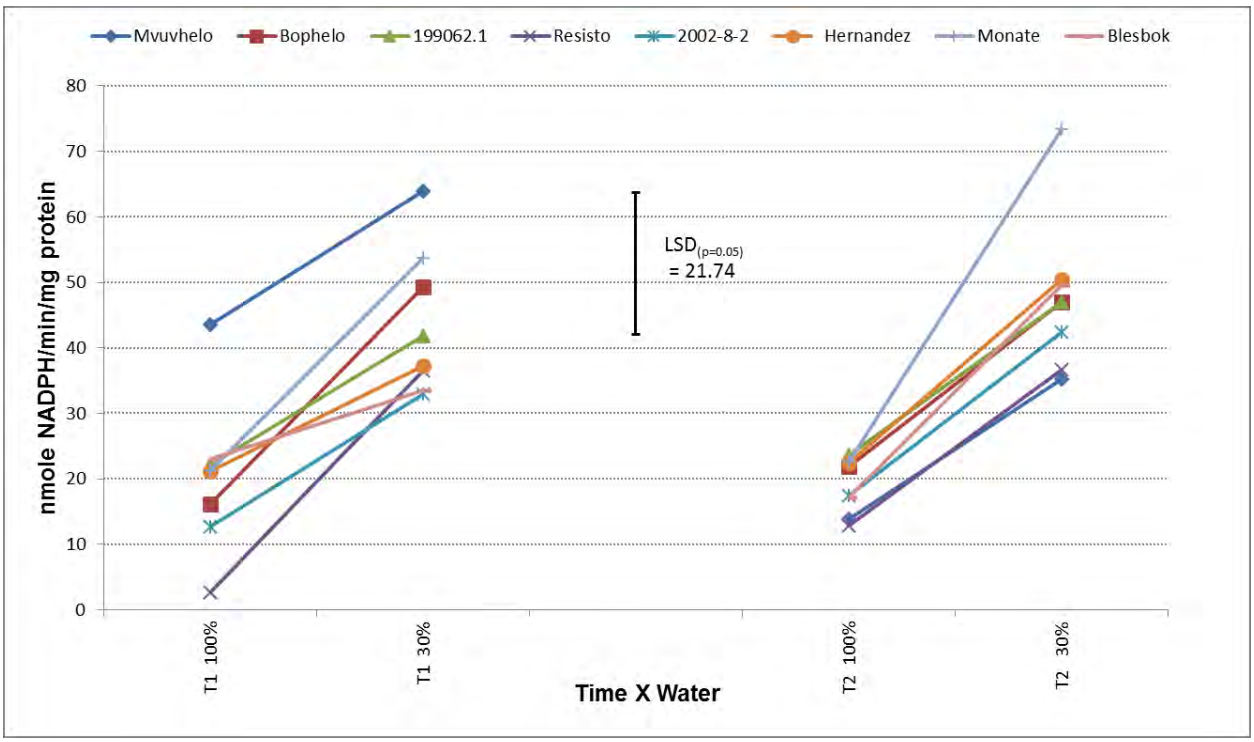

Figure 3.6. Glutathione reductase values in the leaves of eight sweet potato cultivars subjected to drought in Trial 3. $LSD_{(p=0.05)}$ = 21.74; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment

The cultivar Monate did show a significant increase in GR from the control to the severe stress treatment at T2. The nonsignificant difference between values in severe stress conditions at T1 and T2 could indicate that the plants have reached their maximum response to the stress at T2 and that a possible degradation of the GR protein was to follow. **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)** have observed a decline in GR activity in drought experiments which could indicate tissue degradation that leads to a decline in GR values.

3.5.4 Chlorophyll content

3.5.4.1 Trial 1

Chlorophyll content index (CCI) values were measured in the leaves of sweet potato plants in control and drought stress environments. All the genotypes except Purple Sunset showed an increase in chlorophyll content at mild stress where after a significant decline in the chlorophyll content in Resisto and Isondlo was observed at severe stress conditions which agrees with the findings of **NIKOLAEVA, MAEVSKAYA, SHUGAEV and BUKHOV (2010)** who found increases of chlorophyll content in the leaves of wheat plants subjected drought. The cultivar W-119 also showed a decline in in chlorophyll content from the mild stress to the severe stress condition but was nonsignificant. A decrease in CCI was observed for all the cultivars at T2 in all the treatments (Figure 3.7). This could be due to the closure of stomata resulting from the moisture loss the plants experienced leading to the formation of AOS that can assist in the degradation of the chlorophyll **(LEI, YIN and LI 2006; SIES 1993)**. It was also showed by **MIHAILOVIĆ, LAZAREVIĆ, DŽELETOVIĆ, VUČKOVIĆ and DURDEVIĆ (1997)** that during drought the activity of chlorophyllase increased with the simultaneous loss of chlorophyll in wheat plants.

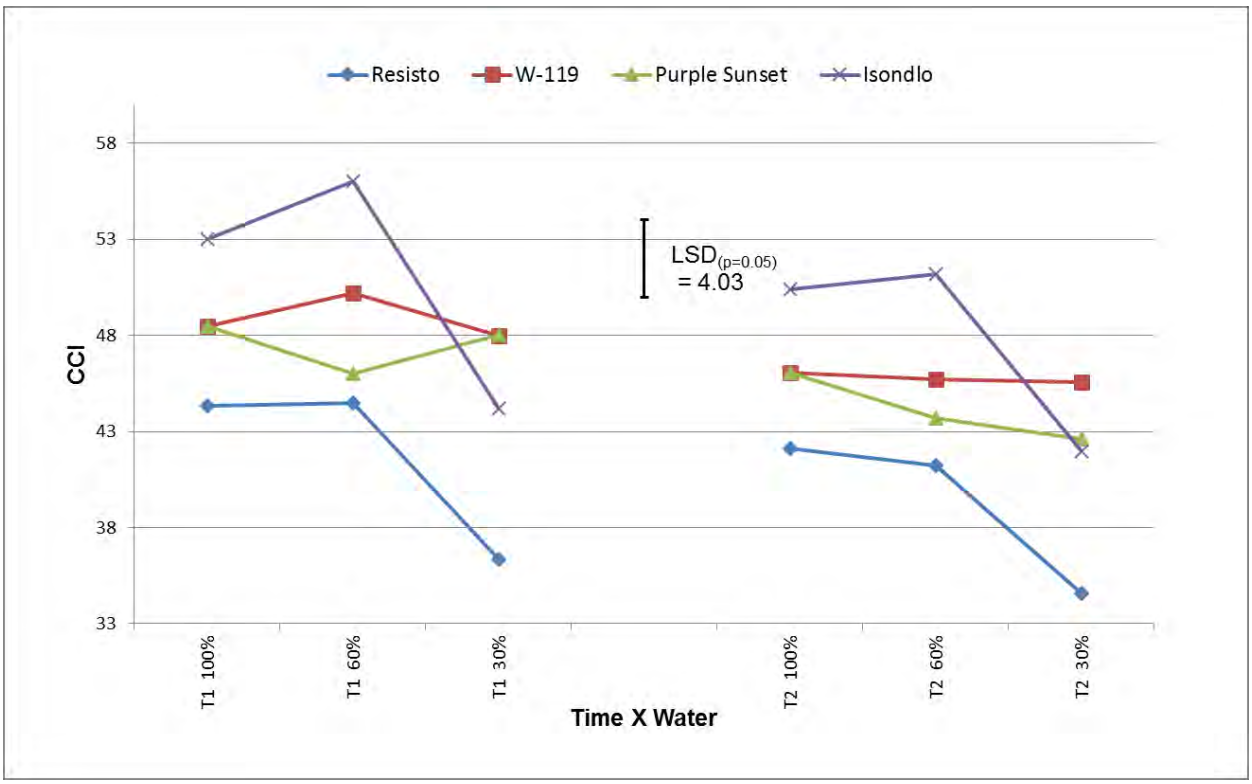

Figure 3.7. Chlorophyll values of four sweet potato cultivars subjected to drought in Trial 1. $LSD_{(p=0.05)}$ = 4.03; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

All the cultivars differed significantly in chlorophyll content in the control conditions at both times of measurement, except for W-119 and Purple Sunset, probably due to the differences in their genetic composition. This significant difference became more pronounced as the Trial continued and the stress increased. The cultivars Resisto and Isondlo experienced the same degree of chlorophyll loss at T2 although visual scanning could not detect any color differences in the leaves.

3.5.4.2 Trial 2

A significant decline in chlorophyll content between the control and stressed plants was found with the cultivar Isondlo and the breeding lines 2005-16-1 and 2006-2-4 during T1 which stabilized at T2. Although the majority of genotypes indicated a decline in chlorophyll content no significant decrease was observed. In contrast it was observed that the genotypes 2005-11-3, 2005-7-4, 2006-4-4, 2006-7-3, Blesbok and Bosbok, showed a significant decrease in chlorophyll content comparing the severe stress treatment of T1 to T2. This could mean that the plants of these genotypes were not affected by the stress or that the antioxidant system were efficient enough to preserve important enzyme systems crucial for the manufacturing of chlorophyll.

This suggested that the stress had little negative effect on the chlorophyll content in this trial. This is confirmed by an observed significant increase in the chlorophyll content values of the cultivars Purple Sunset, Impilo and Ndou and the breeding lines 2006-14-4 and 2006-6-2. The increase is this specific case is not uncommon and has been observed by **NIKOLAEVA, MAEVSKAYA, SHUGAEV and BUKHOV (2010)** in wheat cultivars subjected to drought. The chlorophyll content however, started to decrease as the drought prolonged. The chlorophyll content of Zapallo also declined significantly from the control to severe stress treatment at T1. This is despite an increase in antioxidant activity during the stress which usually should indicate a higher chlorophyll content **(MALAN, GREYLING and GRESSEL, 1990)**.

Table 3.6. Chlorophyll content (CCI) in the leaves of 35 sweet potato cultivars and breeding lines subjected to control and severe drought stress conditions in Trial 2.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	58.30	53.73*	50.43	51.90
Purple Sunset	54.50	52.08	43.05	50.97*
2005-1-11	44.12	43.05	39.37	41.75
2005-1-16	49.97	52.32	41.25	48.90
2005-11-3	52.75	51.57	44.37	44.28
2005-12-2	53.62	50.18	49.92	46.28
2005-16-1	58.22	49.45*	49.32	49.42
2005-2-2	50.37	49.98	39.70	45.12
2005-3-10	44.45	43.63	41.25	40.70
2005-3-13	51.92	47.53	44.63	46.20
2005-4-1	56.92	52.33	44.10	49.18
2005-5-5	51.55	50.98	45.33	50.93
2005-7-4	52.18	53.02	43.72	46.75
2006-14-4	55.50	54.5	47.35	53.62*
2006-15-1	48.40	52.18	47.97	50.65
2006-2-4	59.33	52.22*	52.38	54.25
2006-3-4	55.72	53.98	52.30	54.20
2006-4-4	51.45	51.55	46.37	42.35
2006-4-5	52.82	53.23	45.63	49.72
2006-6-2	56.37	54.68	43.65	55.08*
2006-7-3	49.73	48.20	46.13	40.65
2006-7-7	58.78	52.67	50.03	54.75
2006-7-8	50.42	45.50	50.40	46.55
Beauregard	52.53	49.42	47.42	47.97
Blesbok	58.32	56.18	44.18	49.30
Bosbok	58.45	58.33	47.12	52.93
Impilo	55.62	52.13	45.32	52.38*
Jewel	49.23	46.88	46.12	46.35
Lethlabula	54.85	56.92	48.80	53.18
Ndou	59.55	56.82	46.50	56.68*
Phala	47.32	47.47	43.20	43.95
Resisto	48.83	50.22	42.13	44.93
Tanzania	54.85	52.00	51.48	56.52
W-119	48.52	50.55	43.40	48.45
Zapallo	54.77	49.90	51.02	44.23*
mean	51.14	51.29	46.15	48.88

* indicates a significant difference at 5% level between the control and stress for a specific Time X Water X Variety combination. $LSD_{(p=0.05)}$ = 6.35; control = control treatment, Stress = severe stress treatment. T1 = 60 days after planting, T2 = 120 days after planting

$MSE_{(df=70)}$=5.35

3.5.4.3 Trial 3

The chlorophyll content of the plants subjected to drought stress in Trial 3 at T1 did not decline significantly from the control to the severe stress treatment (Figure 3.8). This was also observed by **MENSAH, OBADONI, ERUOTOR and ONOME-IRIEGUNA (2006)** with sesame plants with various water regimes where the stressed plants only showed significant differences in chlorophyll content from the control at an advance stage of drought stress. The cultivar Hernandez exhibited an increase in chlorophyll content, although nonsignificant, from control to severe stress conditions. **NIKOLAEVA, MAEVSKAYA, SHUGAEV and BUKHOV (2010)** found similar results when subjecting wheat plants to drought stress. The cultivars displayed various levels of chlorophyll content in the control treatment, which extended to the severe stress treatment at T1. This could be ascribed to the genetic difference between the cultivars which will affect the photosynthetic capacity of the different cultivars. A significant and more powerful decline in chlorophyll content values was observed at T2 in all the stressed plants. It is speculated that this could possibly have an effect on the photosynthetic systems of the plant and may have caused the reduction in growth seen in the canopy and stem development. The breakdown of chlorophyll may also have an influence on the intensity of the antioxidant enzyme system hence the relative low values recorded.

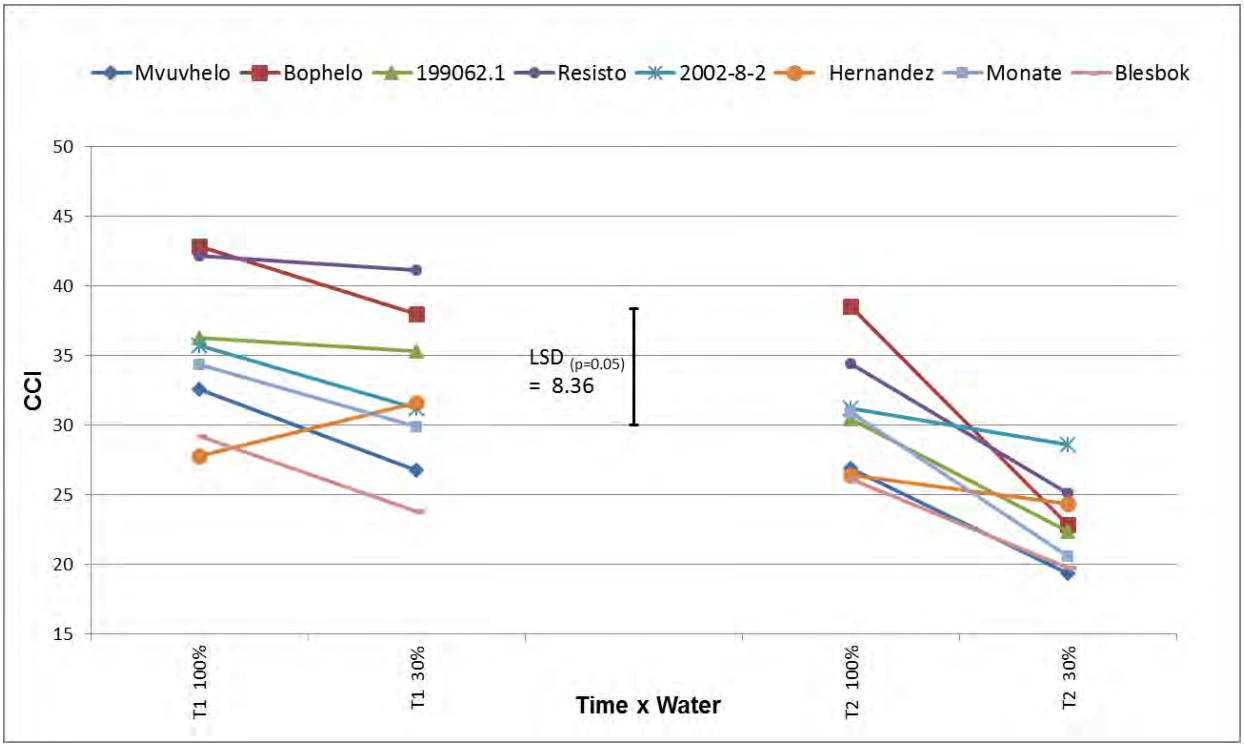

Figure 3.8 Chlorophyll content values of eight sweet potato cultivars subjected to drought stress in Trial 3. $LSD_{(p=0.05)}$ = 8.36; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment

3.6. CONCLUSIONS

Drought stress imposed on sweet potato plants in the three trials affected the activity of the antioxidant enzymes AP, SOD and GR to various degrees.

The role of AP was apparent in the three trials and the three trials have shown that the cultivars and breeding lines only reacted significantly to the stress once the stress became severe. In all the trials a significant increase in AP activity was observed which corresponds with the findings of **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** in wheat and **LEI, YIN and LI (2006)** in poplar. These results show that sweet potato do indeed elevate AP levels in reaction to drought stress to combat tissue damage.

An increase of superoxide production in the sweet potato plants was shown in all three trials which resulted in the strong elevated levels of SOD activity. This finding is similar to

results of **BAI, SUI, GE, SUN, LU and ZHOU (2006)** in maize, **LEI, YIN and LI (2006)** in poplar and **MASOUMI, DARVISH, DANESHIAN, NORMOHAMMADI and HABIBI (2011)** in soybean. However, **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** found the SOD levels declined in wheat plants subjected to drought stress. This may imply that the SOD enzyme does not play such a big role in the antioxidant system in wheat. From the present study it is clear that for sweet potato subjected to drought stress, the SOD enzyme could be an important mechanism in combatting the formation of AOS.

It is clear that from the onset of drought stress the sweet potato plants experienced elevated levels of GR activity in all the trials. The activity of GR is dependent on the NADPH availability meaning that an increase in GR activity will produce more NADP. NADP can then accept electrons from photosystem I and aid in the reduction of superoxide **(BOWLER, VAN MONTAGU and INZE, 1992)**. Although the chlorophyll levels dropped and could lead to fewer photosystem I sites available, the GR activity still increased giving rise to the argument that the plant still had the ability to combat the formation of AOS.

Drought stress had a significant effect on the chlorophyll content of the sweet potato cultivars and breeding lines in two of the three trials. Although all three trials are different from each other with respect to seasonal conditions, it seemed that the drought did not have a negative effect on the chlorophyll content of the plants in Trial 2 in general. This could either be due to the selection of breeding lines and cultivars used in the trial that seemed more robust against the stress resulting in minimum chlorophyll loss, or that the stress did affect the plants but they managed to retain their chlorophyll content because of the influence of antioxidant enzyme systems.

From the above results is became clear that sweet potato plant under drought stress do indeed react to the stress via the antioxidant systems (apart from the fact that chlorophyll degradation also takes place) and that these systems might be a tool to determine drought tolerance amongst cultivars and breeding lines. It is suggested that these studies also be executed with respect to the recovery after rewatering.

3.7. APPENDICES

Appendix 3-A: ANOVA for Trial 1 for AP.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	9.620E-08	4.810E-08	1.27	
REP.Wplot stratum					
WATER	2	1.161E-06	5.805E-07	15.36	0.013
Residual	4	1.512E-07	3.781E-08	0.56	
REP.Wplot.Splot stratum					
VARIETY	3	5.591E-08	1.864E-08	0.28	0.842
WATER.VARIETY	6	2.975E-07	4.958E-08	0.74	0.628
Residual	18	1.214E-06	6.743E-08	1.22	
REP.Wplot.Splot.TIME stratum					
TIME	1	6.578E-06	6.578E-06	119.07	<.001
TIME.WATER	2	7.946E-08	3.973E-08	0.72	0.497
TIME.VARIETY	3	1.853E-07	6.176E-08	1.12	0.361
TIME.WATER.VARIETY	6	1.234E-06	2.057E-07	3.72	0.009
Residual	24	1.326E-06	5.525E-08		

Appendix 3-B: ANOVA for Trial 2 for AP.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	1.451E-05	1.451E-05	14.46	0.063
Residual	2	2.008E-06	1.004E-06	6.01	
REP.Wplot.VARIETY stratum					
VARIETY	34	9.899E-06	2.911E-07	1.74	0.026
VARIETY.TMT	34	9.254E-07	2.722E-08	0.16	1.000
Residual	68	1.136E-05	1.670E-07	0.75	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	4.702E-05	4.702E-05	209.90	<.001
VARIETY.TIME	34	1.393E-05	4.098E-07	1.83	0.017
TIME.TMT	1	6.425E-06	6.425E-06	28.68	<.001
VARIETY.TIME.TMT	34	1.186E-06	3.487E-08	0.16	1.000
Residual	70	1.568E-05	2.240E-07		

Appendix 3-C: ANOVA for Trial 3 for AP.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0009681	0.0004840	0.39	
REP.Wplot stratum					
WATER	1	0.0097486	0.0097486	7.93	0.106
Residual	2	0.0024578	0.0012289	5.01	
REP.Wplot.Splot stratum					
VARIETY	7	0.0031622	0.0004517	1.84	0.118
WATER.VARIETY	7	0.0014881	0.0002126	0.87	0.544
Residual	28	0.0068678	0.0002453	0.69	
REP.Wplot.Splot.TIME stratum					
TIME	1	0.0005453	0.0005453	1.53	0.225
TIME.WATER	1	0.0067637	0.0067637	18.98	<.001
TIME.VARIETY	7	0.0041370	0.0005910	1.66	0.155
TIME.WATER.VARIETY	7	0.0053749	0.0007678	2.15	0.066
Residual	32	0.0114023	0.0003563		

Appendix 3-D: ANOVA for Trial 1 for SOD.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		0.009002	0.004501	0.79	
REP.Wplot stratum						
WATER	2		0.005316	0.002658	0.47	0.658
Residual	4		0.022859	0.005715	4.98	
REP.Wplot.Splot stratum						
VARIETY	3		0.017529	0.005843	5.09	0.010
WATER.VARIETY	6		0.006220	0.001037	0.90	0.514
Residual	18		0.020647	0.001147	1.13	
REP.Wplot.Splot.TIME stratum						
TIME	1		0.051625	0.051625	50.70	<.001
TIME.WATER	2		0.012122	0.006061	5.95	0.010
TIME.VARIETY	3		0.015708	0.005236	5.14	0.009
TIME.WATER.VARIETY	6		0.020241	0.003373	3.31	0.021
Residual	19	(5)	0.019347	0.001018		

Appendix 3-E: ANOVA for Trial 2 for SOD.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	0.746060	0.746060	27.63	0.034
Residual	2	0.053997	0.026998	11.46	
REP.Wplot.VARIETY stratum					
VARIETY	34	0.067698	0.001991	0.85	0.700
VARIETY.TMT	34	0.006556	0.000193	0.08	1.000
Residual	68	0.160193	0.002356	2.29	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	0.204779	0.204779	198.78	<.001
VARIETY.TIME	34	0.071552	0.002104	2.04	0.006
TIME.TMT	1	0.130003	0.130003	126.20	<.001
VARIETY.TIME.TMT	34	0.006563	0.000193	0.19	1.000
Residual	70	0.072112	0.001030		

Appendix 3-F: ANOVA for Trial 3 for SOD.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.002656	0.001328	16.48	
REP.Wplot stratum					
TMT	1	0.174384	0.174384	2164.21	<.001
Residual	2	0.000161	0.000081	0.01	
REP.Wplot.Splot stratum					
VARIETY	7	0.064405	0.009201	1.44	0.228
TMT.VARIETY	7	0.062967	0.008995	1.41	0.240
Residual	28	0.178543	0.006377	1.04	
REP.Wplot.Splot.TIME stratum					
TIME	1	2.454260	2.454260	401.32	<.001
TIME.TMT	1	0.035152	0.035152	5.75	0.023
TIME.VARIETY	7	0.111698	0.015957	2.61	0.030
TIME.TMT.VARIETY	7	0.125828	0.017975	2.94	0.017
Residual	32	0.195693	0.006115		

Appendix 3-G: ANOVA for Trial 1 for GR.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	248.02	124.01	4.15	
REP.Wplot stratum					
TMT	2	4348.32	2174.16	72.80	<.001
Residual	4	119.45	29.86	0.70	
REP.Wplot.Splot stratum					
VARIETY	3	157.90	52.63	1.23	0.328
TMT.VARIETY	6	101.90	16.98	0.40	0.871
Residual	18	770.02	42.78	1.72	
REP.Wplot.Splot.TIME stratum					
TIME	1	23.91	23.91	0.96	0.337
TIME.TMT	2	30.43	15.21	0.61	0.551
TIME.VARIETY	3	66.48	22.16	0.89	0.460
TIME.TMT.VARIETY	6	68.33	11.39	0.46	0.833
Residual	24	597.36	24.89		

Appendix 3-H: ANOVA for Trial 2 for GR.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum						
TMT	1		41670.63	41670.63	264.88	0.004
Residual	2		314.64	157.32	3.63	
REP.Wplot.VARIETY stratum						
VARIETY	34		7592.03	223.30	5.16	<.001
VARIETY.TMT	34		8456.56	248.72	5.74	<.001
Residual	66	(2)	2858.11	43.30	0.88	
REP.Wplot.VARIETY.TIME stratum						
TIME	1		667.48	667.48	13.60	<.001
VARIETY.TIME	34		3208.29	94.36	1.92	0.020
TIME.TMT	1		64.07	64.07	1.31	0.259
VARIETY.TIME.TMT	34		2652.12	78.00	1.59	0.071
Residual	46	(24)	2258.03	49.09		

Appendix 3-I: ANOVA for Trial 3 for GR.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1110.9	555.5	535.35	
REP.Wplot stratum					
WATER	1	16355.4	16355.4	15763.14	<.001
Residual	2	2.1	1.0	0.01	
REP.Wplot.Splot stratum					
VARIETY	7	3628.5	518.4	2.74	0.027
WATER.VARIETY	7	1039.1	148.4	0.78	0.606
Residual	28	5299.2	189.3	1.00	
REP.Wplot.Splot.TIME stratum					
TIME	1	44.5	44.5	0.24	0.630
TIME.WATER	1	176.7	176.7	0.94	0.340
TIME.VARIETY	7	3428.1	489.7	2.60	0.031
TIME.WATER.VARIETY	7	706.8	101.0	0.54	0.801
Residual	32	6032.7	188.5		

Appendix 3-J: ANOVA for Trial 1 for CCI.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.249E+00	1.625E+00	0.13	
REP.Wplot stratum					
TMT	2	3.463E+02	1.732E+02	14.39	0.015
Residual	4	4.815E+01	1.204E+01	1.09	
REP.Wplot.Splot stratum					
VARIETY	3	8.191E+02	2.730E+02	24.68	<.001
TMT.VARIETY	6	3.672E+02	6.120E+01	5.53	0.002
Residual	18	1.991E+02	1.106E+01	1611.91	
REP.Wplot.Splot.TIME stratum					
TIME	1	1.007E+02	1.007E+02	14668.29	<.001
TIME.TMT	2	2.277E-01	1.139E-01	16.59	<.001
TIME.VARIETY	3	5.385E-01	1.795E-01	26.16	<.001
TIME.TMT.VARIETY	6	2.414E-01	4.024E-02	5.86	<.001
Residual	24	1.647E-01	6.863E-03		

Appendix 3-K: ANOVA for Trial 2 for CCI.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum						
TMT	1		13.680	13.680	0.18	0.710
Residual	2		149.333	74.666	7.51	
REP.Wplot.VARIETY stratum						
VARIETY	34		2963.095	87.150	8.76	<.001
VARIETY.TMT	34		521.924	15.351	1.54	0.065
Residual	68		676.485	9.948	1.15	
REP.Wplot.VARIETY.TIME stratum						
TIME	1		1547.979	1547.979	178.41	<.001
VARIETY.TIME	34		419.883	12.349	1.42	0.107
TIME.TMT	1		368.211	368.211	42.44	<.001
VARIETY.TIME.TMT	34		342.591	10.076	1.16	0.295
Residual	69	(1)	598.679	8.677		

Appendix 3-L: ANOVA for Trial 3 for CCI.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	353.48	176.74	2.17	
REP.Wplot stratum					
TMT	1	682.67	682.67	8.36	0.102
Residual	2	163.24	81.62	2.63	
REP.Wplot.Splot stratum					
VARIETY	7	1383.36	197.62	6.36	<.001
TMT.VARIETY	7	219.45	31.35	1.01	0.447
Residual	28	870.61	31.09	2.41	
REP.Wplot.Splot.TIME stratum					
TIME	1	1149.55	1149.55	88.99	<.001
TIME.TMT	1	140.65	140.65	10.89	0.002
TIME.VARIETY	7	205.37	29.34	2.27	0.054
TIME.TMT.VARIETY	7	95.78	13.68	1.06	0.411
Residual	32	413.39	12.92		

CHAPTER 4

THE IMPACT OF DROUGHT STRESS ON THE CARBON DISCRIMINATION, STOMATAL CONDUCTANCE AND RELATIVE WATER CONTENT OF SWEET POTATO CULTIVARS AND BREEDING LINES.

4.1. INTRODUCTION

Drought is a major limitation in crop production and carbon isotope discrimination can be a powerful and easy to use tool for selecting genotypes with high water use efficiency **(MONTI, AMADUCCI, PRETONI and VENTURI, 2006)**. The use of carbon (C) isotopes as an ecological indicator of plant function is derived from the link between environment quality and the biochemical discrimination against $^{13}CO_2$ during gas exchange. In C_3 plants, like sweet potato, discrimination against ^{13}C by the Rubisco enzyme is coupled to photosynthesis by means of the ratio of intercellular to atmospheric CO_2 concentrations **(DAWSON, MAMBELLI, PLAMBOECK, TEMPLER and TU, 2002)**.

FARQUHAR, O'LEARY and BERRY (1982) have shown that during the photosynthetic process the naturally occurring ^{13}C isotope is being discriminated against (there is a preference for ^{12}C) and that the degree of discrimination is comparative to the fixed carbon per unit of water transpired. This means that the plants low in ^{13}C will generally have low water use efficiency**. LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** showed that leaf carbon isotope discrimination decreased (less preference for ^{12}C) with increasing water deficits and found this to relate to a decrease of stomatal conductance. This was also confirmed by **DEBLONDE, HAVERKORT and LEDENT (1999)** who found significant, although small, effects of treatments on carbon discrimination values of potato cultivars grown in drought conditions. It has been observed that carbon discrimination varies in response to soil moisture **(TOKATLIDIS, TSIALIS, XYNIAS, TAMOUTSIDIS and IRAKLI, 2004; DEBLONDE and LEDENT, 2001; AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)**. One of the well-known parameters used to evaluate plants for their ability to adapt to drought conditions is determining water use efficiency (WUE).

WUE can be determined through various methodologies like the ratio of total dry mass to water use **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** or the nett assimilation of carbon related to total yield calculated **(PITA, SORIA, CANÄAS, TOVAL and PARDOS, 2001)**. According to **FARQUHAR and RICHARDS (1984)** carbon discrimination can be used to predict water use efficiency and can therefore be used as a screening tool for genotypes. This has been demonstrated in sugar beet **(MONTI, AMADUCCI, PRITONI and VENTURI, 2006)**; wheat **(SAYRE, ACEVEDO and AUSTIN, 1995)** and cotton **(LEIDI, LOPEZ, GORHAM and GUTIÉRREZ, 1999)**. Since little or no studies have been conducted regarding the WUE of sweet potato with respect to the use of carbon isotope ratio/discrimination it is important to conduct such experiments to facilitate future research.

Water deficit in plants results from the loss of water in the growth medium. Relative water content (RWC) is an applicable measurement to determine the water status in plants and therefore to calculate the water deficit. When water loss is taking place through the stomata the plant has the ability to adjust to the water loss replacing the lost to a certain extent. This is called osmotic adjustment and is dependent on the soil water content as well as the transpiration rate. Although water potential does not account for the osmotic adjustment in the plant, it is a useful tool in the estimation of plant water status regarding water transport in the plant.

BLUM (2005) explained that osmotic adjustment is a powerful means of conserving cellular hydration in drought conditions and that RWC displays the effect of osmotic adjustment in this regard. That is why RWC is thought of as an appropriate estimate of plant water status in terms of cellular hydration under the possible influence of both leaf water potential and osmotic adjustment (OA). The method has long been in use **(BARRS and WEATHERLEY, 1962; SMART and BINGHAM, 1974)** and due to its simplicity is gaining increased popularity. It estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity. It is a measure of water deficit in the leaf.

Normal values of RWC range between 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves. In most crop species the typical RWC at about wilting is around 60 to 70%, with exceptions **(BARRS and WEATHERLEY, 1962)**. **CHOWDHURY, ANTONY and KUMAR (2008)** completed a sweet potato trial where plants were grown in pots and subjected to various water regimes. Significant differences were observed in the RWC of leaves harvested from plants grown at a higher moisture content compared to those grown at a lower moisture content.

Stomatal conductance is about the relationship between carbon assimilation and water loss by transpiration **(LUDLOW, 1980)**. The stomata regulates the loss of water via transpiration at times of drought stress. Conductance is measured either at the adaxial or abaxial side of the leaf by means of a leaf conductance meter in $mmol/m^2s^{-1}$. All leaves contain stomata (the microscopic pores of the leaf) which are openings in the leaf through which gasses are diffusing in and out. Stress-inducing environmental changes not only damage the photosynthetic process but also affect stomatal movement, light absorption and the biochemical pathways for CO_2 fixation. It is well known that the stomata close gradually with increased drought stress which is followed by a reduction in photosynthetic rates.

Although the response of stomata to environmental and physiological factors is complex, it is known that stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapour pressure deficit and CO_2 concentration **(COWAN and FARQUHAR, 1977; BUCKLEY and MOTT, 2002)**. Literature also provides information that stomatal conductance depends on guard cell and epidermal turgor **(WU, SHARPE and SPENCE, 1985; FRANKS, BUCKLEY, SHOPE and MOTT, 2001)**, and that regulation of turgor in these cells requires metabolic energy **(FARQUHAR and WONG, 1984)**. Leaf turgor also depends on the balance between loss of water through transpiration and supply of water to the leaf from the soil **(COWAN AND FARQUHAR, 1977; MOTT and PARKHURST, 1991** and **FRANKS, BUCKLEY, SHOPE and MOTT, 2001)**. **BAHAR, YILDIRIM and BARUTCULAR (2009)** have reported that positive relations between g_s (stomatal conductance) and yield in bread wheat have been found;

but these relations are not clear in durum wheat. Whether these relations are true in the case of sweet potato was investigated in this study.

4.2. AIMS

Due to the widespread use of carbon ratio and discrimination values in the prediction of WUE for plant species it was investigated whether such trends are applicable to sweet potato. It has been reported that some plant species do not react to drought stress and thereby caused a closure of the stomata which had a direct influence on the trend in carbon ratio and discrimination values. This was also investigated with regard to sweet potato. It was also investigated whether the drought stress has any effect in sweet potato with regard to relative water content. This could provide insight if the cultivars have the ability to adjust their osmotic potential if needed.

4.3. MATERIALS AND METHODS

4.3.1. Carbon ratio analysis

Leaf samples were collected from the Trial areas from both the control and drought stressed treatments. Leaf material was freeze-dried and a five milligram sample subjected to analysis. Analysis were conducted at the Department of Archeology, University of Cape Town and were carried out on a Thermo Delta V stable light isotope ratio mass spectrometer interfaced via a Conflo IV with a Thermo Flash 2000 elemental analyser. Working standards were combusted regularly and the results of these analyses were used to normalize the sample results against international standards. The results are reported relative to the standards, VPDB for carbon and Air for nitrogen. The ratio of carbon isotopes were determined as $\delta^{13}C$ calculated relative to the Vienna Chicago PDB (Pee Dee Belemnite) marine lime stone standard. Discrimination values were calculated using the respective carbon isotope ratio values as supplied by the University of Cape Town. Carbon isotope discrimination (Δ) was calculated as:

$$\Delta\ (‰) = (\delta_{air} - \delta_{plant})/\ (1 + \delta_{plant} / 1000)$$

where δ_{air} is the carbon isotope ratio of the air ~ -8‰, and δ_{plant} is the carbon isotope ratio of the leaf sample.

4.3.2. Relative water content

The fifth fully expanded leaf from the apical tip of sweet potatoes, was collected, pre-dawn, from the control and drought stressed plants. The method of **SMART and BINGHAM (1974)** with slight modifications was followed. Five leaf discs were cut from these leaves with a corkborer and the initial weight determined. The leaf discs were placed in small containers containing deionized water and allowed to incubate for four hours at room temperature. The leaf discs were blotted dry and weighed again to determine turgescent weight. The bottles containing the leaf discs were then placed in an oven and incubated for a period of 24 hours at 90ºC. After incubation and a cooling down period of one hour, the leaf discs were weighed again to obtain dry weight.

The RWC was calculated using the following formula:

RWC = [(W-DW)/ (TW-DW)] * 100

Where: W = initial weight

DW = dry weight

TW = turgescent weight

Relative water content was not measured in Trial 2 due to time constraints.

4.3.3. Stomatal conductance

The measurement of stomatal conductance was conducted according to manufacturer's instructions with a SC-1 stomatal conductance meter from Decagon, Pullman, WA, USA. The fifth fully expanded leaf from the tip was used for measurement and three leaves of three random selected plants were measured for both the control and drought-stressed plants of each repeat.

4.4. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on all the data to test for treatment differences as well as possible differences between cultivars. Treatment means as well as interaction means were separated using Fishers' t-test least significant difference (LSD) at the 5% level of significance. Statistical analysis was conducted using, GenStat *for Windows* 15th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk, by the ARC Biometry Section. For the stomatal conductance the data was log transformed, in Trial 1, 2 and 3, to normalize the data and stabilize the variances.

4.5. RESULTS AND DISCUSSION

4.5.1. Carbon isotope discrimination (∆) analysis

4.5.1.1. Trial 1

The measurement of stable carbon isotope ratios as well as discrimination has become a powerful tool for assessing plant performance under field conditions **(DAWSON, MAMBELLI, PLAMBOECK, TEMPLER and TU, 2002)**. Significant differences ($P \leq 0.05$) were detected between the water treatments as well as the genotypes (Appendix 4-A). Drought stress in Trial 1 (Figure 4.1) led to a significant decline in ∆ values for the cultivar Isondlo at T1 correlating with observations made by **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** in showing a decline in ∆ values in sugar beet subjected to drought stress. The cultivars W-119 and Purple Sunset also experienced a decline in ∆ values but these were not significant. The cultivar Resisto experienced a significant increase in ∆ at T1 from the mild stress treatment to the severe stress treatment after a nonsignificant decline from the control treatment to the mild stress treatment. This is in contrast to the findings of **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** who found lower ∆ values in drought-stressed sugar beet plants.

All the cultivars at T2 experienced a significant decline in ∆ values over the different treatments. This is in correlation with the findings of **AKHTER, SABIR, LATEEF, ASHRAF and HAQ, (2008); BLOCH, HOFFMANN and MÄRLÄNDER, (2006); DEBLONDE, HAVERKORT and LEDENT, (1999); LEIDI, LOPEZ, GORHAM and**

GUTIÉRREZ, (1999); MONNEVEUX, REYNOLDS, TRETHOWAN, GONZÁLEZ-SANTOYO, PEÑA and ZAPATA, (2005) and SAYRE, ACEVEDO and AUSTIN, (1995) who also observed a decline in Δ values when wheat, cotton and potato plants respectively were subjected to lengthy drought stress conditions indicating consistent effects of water stress on Δ values.

Significant differences were detected between the cultivars at T2 in severe stress conditions, with the cultivar Purple Sunset experiencing the lowest while Resisto, W-119 and Isondlo exhibited higher values resulting in the former discriminating less against the heavier isotope **(AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)**. The lower Δ value for Purple Sunset could be an indication that this cultivar has the ability to accumulate more ^{13}C during the stress period and thus accumulate more mass and hence used the available water better. Although the intercellular CO_2 concentration was not measured in this experiment it can be assumed that the plants experienced an increased incorporation of ^{13}C during the process of photosynthesis hence the lower discrimination values **(AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)**.

The decline in Δ values for Isondlo and Purple Sunset is almost at the same rate, although different in value. This exhibits the characteristics of less discrimination at higher water stress and therefor possibly displays the ability to use the available water better and thus develop a greater biomass. This is confirmed by **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** who observed that in cotton plants subjected to drought, a negative correlation existed between Δ and specific leaf weight indicating a possible increase in biomass as Δ values decline. The sudden increase in Δ for Resisto at T1 (significant) and T2 (nonsignificant) could possibly be due to water contamination for the specific plant when leaf harvest took place.

An explanation for the difference in Δ values of the control treatment and the mild stress treatment, which were lower at T1 than in T2, could be due to the duration of the Trial and that the plants specifically reacted to the stress that caused a decrease in ^{13}C accumulation at that specific time. At T2 the stress probably resulted in the conservation of energy whereby the plants made better use of the water available hence the lower

levels of discrimination. This finding is in accordance with **SAYRE, ACEVEDO and AUSTIN (1995)** who found a decrease in Δ for wheat plants subjected to more stress.

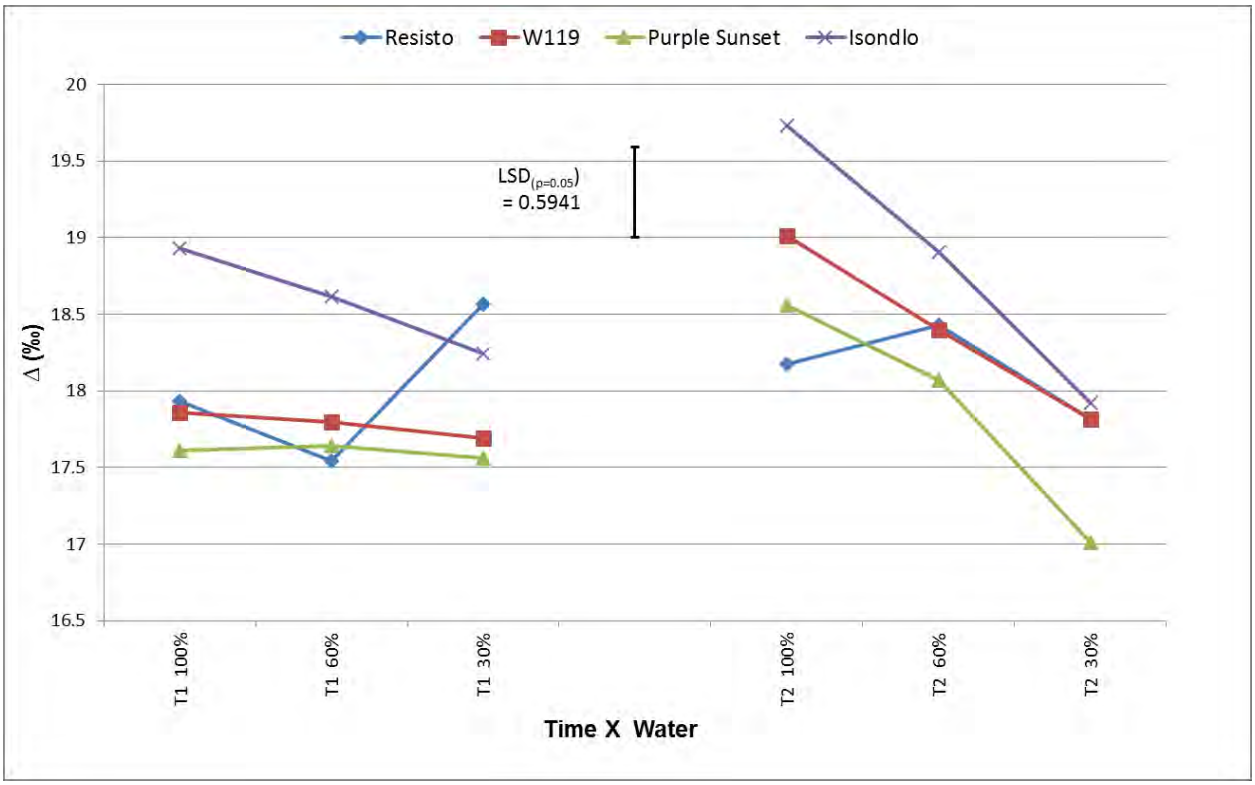

Figure 4.1. Mean carbon isotope discrimination (Δ) values in leaves of four sweet potato cultivars under drought stress conditions in Trial 1. $LSD_{(p=0.05)}$ = 0.5941; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment.

4.5.1.2. Trial 2

Carbon isotope discrimination (Δ) values in small rainout shelters (Table 4.1) were constantly lower, with the exception of 2006-4-4, than the control, at both harvest times, although the difference was not always significant. These findings correlate with the results obtained by **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** who also observed a decline in Δ values in potato plants subjected to drought. In both time frames (T1 and

T2) there were significant differences ($P \leq 0.05$) in Δ between the 35 sweet potato cultivars and breeding lines when comparing the control with stressed plants (Appendix 4-B). The differences were more pronounced at T2 than at T1. This was confirmed by **SAYRE, ACEVEDO and AUSTIN (1995)** who found lower discrimination in drier soil with a positive correlation with yield. This might give rise to the contention that some genotypes have the ability to present lower Δ values even at T1, which can cause increased incorporation of ^{13}C during the process of photosynthesis **(AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)** while others do not have this characteristic. This could be of an advantage since the plant has already adapted to the conditions earlier during the drought stress period and can then accumulate biomass more efficiently. It also seems that some genotypes only develop the ability to exhibit lower Δ values at T2 which then can be a disadvantage since crop losses can be higher. This phenomenon was also observed by **BLOCH, HOFFMANN and MÄRLÄNDER (2006); DEBLONDE, HAVERKORT and LEDENT (1999); LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999); MONNEVEUX, REYNOLDS, TRETHOWAN, GONZÁLEZ-SANTOYO, PEÑA and ZAPATA (2005) and SAYRE, ACEVEDO and AUSTIN (1995)** who observed a decline in Δ values due to lengthened drought stress of potato, wheat and cotton plants respectively. It was also observed that in general, the Δ values of the control and small rainout shelters over time, for the majority of the cultivars and breeding lines, were slightly higher at the second harvest. **TSIALTAS and MASLARIS (2006)** found increases in Δ values over time during the growth season of sugar beet in control conditions which correlate with findings in Trial 2 however the reason for the nonsignificant increase in Δ for the stressed plants is uncertain.

Table 4.1. Mean leaf Δ (‰) for 35 sweet potato cultivars and breeding lines subjected to control and drought conditions.

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	19.087	18.458	18.357	18.307
Purple Sunset	17.916	17.287	18.568	17.903
2005-1-11	19.403	17.495*	20.195	18.41*
2005-1-16	18.584	17.414	19.831	18.645
2005-11-3	19.114	17.737*	20.076	18.145*
2005-12-2	18.952	17.724*	19.231	17.831*
2005-16-1	19.801	17.523*	19.599	18.560
2005-2-2	19.269	18.408	19.825	19.746
2005-3-10	18.202	17.354*	19.008	17.535*
2005-3-13	18.880	17.296*	19.083	17.719*
2005-4-1	18.817	17.347*	19.931	17.118*
2005-5-5	19.259	17.344*	20.183	18.086*
2005-7-4	17.638	17.587	18.366	18.305
2006-14-4	17.949	17.528	19.882	17.548*
2006-15-1	18.467	18.387	20.235	18.371*
2006-2-4	19.366	18.211	18.778	18.732
2006-3-4	18.16	17.849	18.98	18.132
2006-4-4	19.032	18.181	18.915	18.918
2006-4-5	19.111	18.646	19.404	19.077
2006-6-2	19.040	17.807	18.533	19.106
2006-7-3	18.047	17.366	19.47	17.633*
2006-7-7	18.274	17.668	18.846	17.807
2006-7-8	19.084	18.365	19.669	18.865
Beauregard	17.634	17.099	18.55	18.108
Blesbok	17.595	17.263	19.576	17.779*
Bosbok	18.622	18.375	18.989	18.525
Impilo	18.386	17.62	19.191	17.938*
Jewel	19.024	17.375*	18.478	17.926
Lethlabula	18.704	18.199	19.126	18.447
Ndou	18.826	18.321	20.453	17.722*
Phala	17.83	17.082	18.777	17.438*
Resisto	19.201	18.516	19.695	18.384*
Tanzania	18.459	16.423*	18.541	16.54*
W-119	19.223	17.533	19.817	18.684
Zapallo	19.187	17.993	19.767	17.956*
Mean	18.68	17.73	19.31	18.16

* indicates a significant difference at 5% level between the control and stress for a specific Variety X Water X Time combination

$LSD_{(p=0.05)}$ = 1.24; control = control treatment, stress = severe stress treatment. T1 = 60 days after planting, T2 = 120 days after planting. $MSE_{(df=70)}$ = 0.2583

4.5.1.3. Trial 3

Significant differences in carbon discrimination values were observed for the Water X Variety combination (Appendix 4-C). Changes in carbon isotope discrimination (Δ) are displayed in Figure 4.2. A decrease in Δ values was observed for all the cultivars, from the control treatment to the severe stress treatment, at both times of leaf harvest. This tendency was also seen in Trial 1 and confirms the decrease in Δ values in sweet potato subjected to drought in these trials. As mentioned earlier these findings were confirmed by **AKHTER, SABIR, LATEEF, ASHRAF and HAQ (2008); BLOCH, HOFFMANN and MÄRLÄNDER (2006); DEBLONDE, HAVERKORT and LEDENT (1999); LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999); MONNEVEUX, REYNOLDS, TRETHOWAN, GONZÁLEZ-SANTOYO, PEÑA and ZAPATA (2005) and SAYRE, ACEVEDO and AUSTIN (1995)** who also observed declines in Δ values in potato, wheat and cotton plants subjected to drought. Significant differences between the Δ values in the control treatment and the severe stress treatment at T1 were observed with cultivars 199062.1 and Hernandez having the lowest values. The cultivar Hernandez continued to display a low isotope discrimination value at T2 while 199062.1 had an increase in Δ value. The lower Δ value for Hernandez indicated that the cultivar assimilated more ^{13}C **(AKHTER, SABIR, LATEEF, ASHRAF and HAQ, 2008)** at the second harvest as well while more discrimination occurred in 199062.1 as the stress increased. This might lead to a possible decline in biomass (**LEIDI, LOPEZ, GORHAM and GUTIÉRREZ, 1999)** as the stress increases. No significant difference in carbon isotope discrimination could be observed between the cultivars Mvuvhelo, Resisto, Blesbok and Monate in the severe stress treatment for both times which could mean that these cultivars cannot be ranked genotypically with regard to carbon assimilation alone during drought stress.

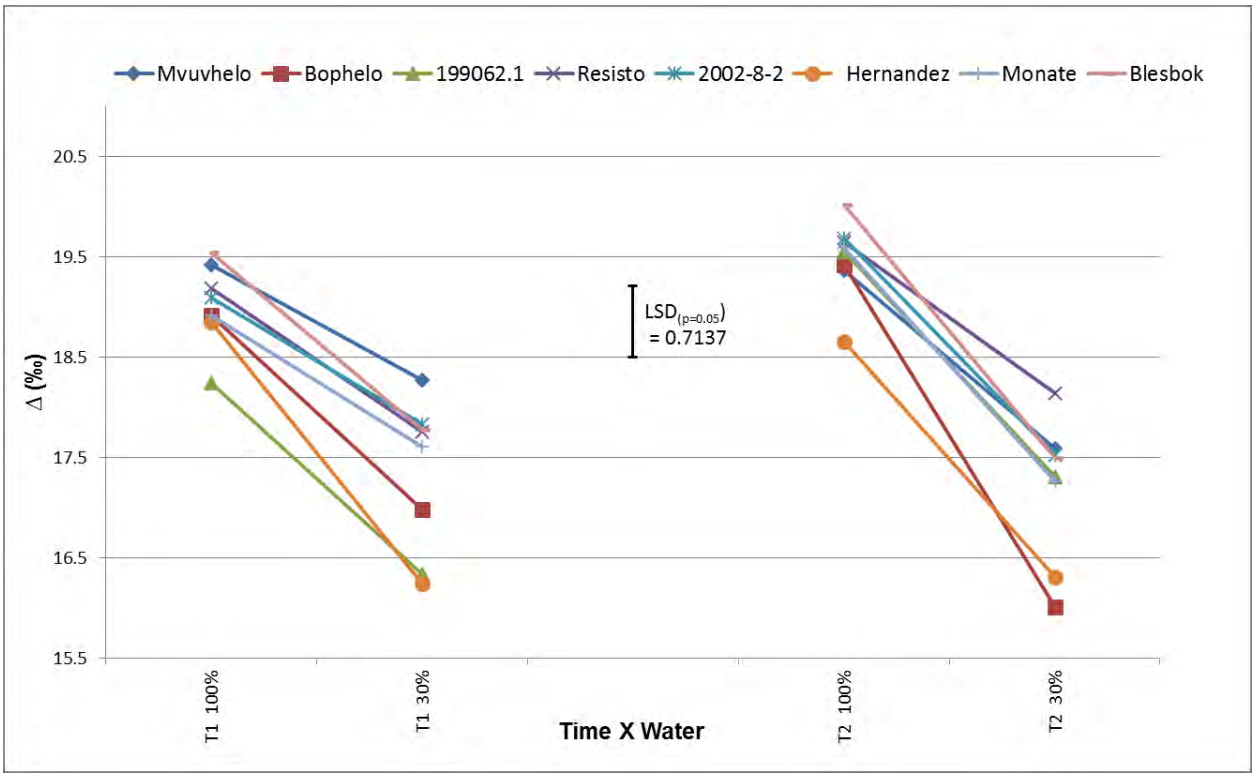

Figure 4.2. ^{13}C isotope discrimination (Δ) values in leaves of eight sweet potato cultivars subjected to drought and control conditions in Trial 3. $LSD_{(p=0.05)}$ = 0.7137; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment. Each value is the mean of 3 measurements with 3 repeats per cultivar.

4.5.2. Stomatal conductance

Stomatal conductance between the control and severe stress treatments declined as the trials continued. This was expected since the soil continues to dry out and the leaf water potential will be having an influence on stomatal conductance **LIANG, ZHANG, SHAO and ZHANG (2002)**.

4.5.2.1. Trial 1

Stomatal conductance significantly decreased at T1 and T2 with a more pronounced decline at T2 (Figure 4.3).

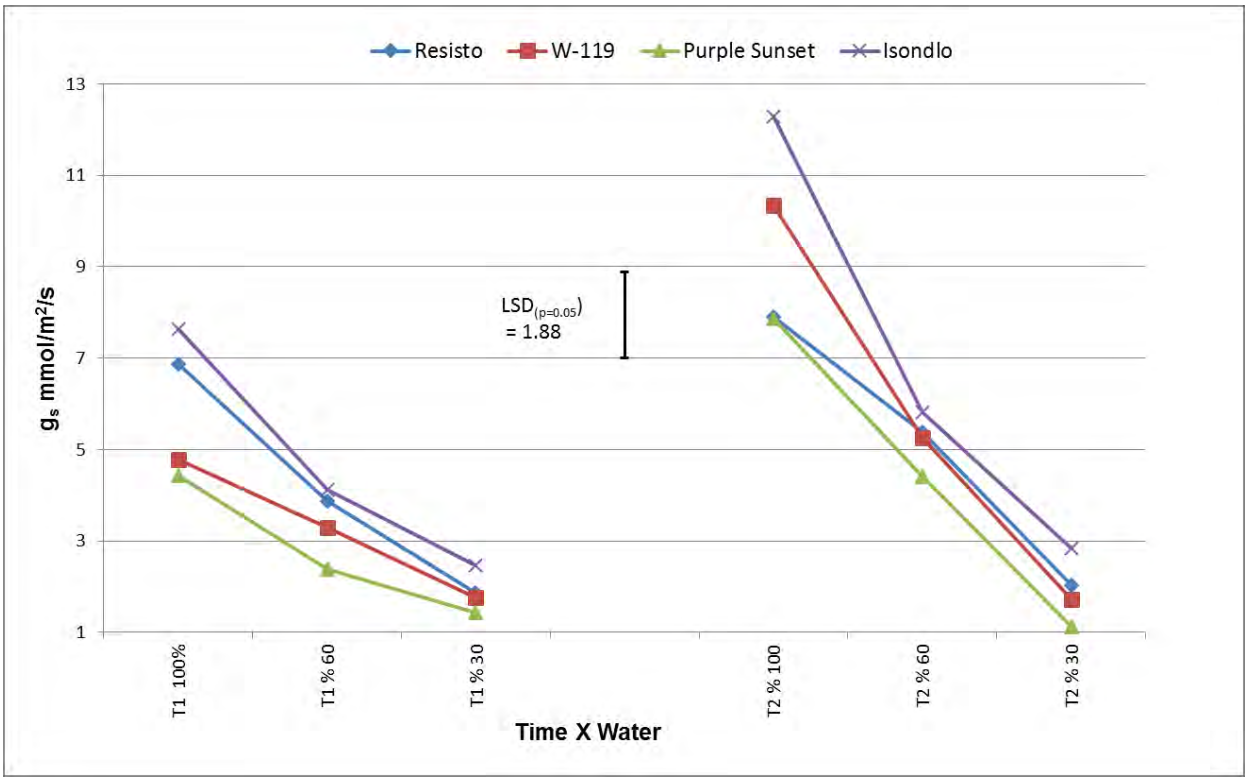

Figure 4.3 Stomatal conductance differences in leaves of four sweet potato cultivars subjected to drought in Trial 1. $LSD_{(p=0.05)}$ = 1.8; Each value is the mean of 3 measurements with 3 repeats per cultivar. T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

The same tendencies were reported by **BLOCH, HOFFMANN and MÄRLÄNDER (2006); LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** and **VAN HEERDEN and LAURIE (2008)** who reported a severe decline in stomatal conductance values for sugar beet, cotton and sweet potato respectively when subjected to drought. Differences in stomatal conductance values between cultivars became less pronounced as the stress increased at T1 which is confirmed through observations by **GONZÂLEZ, MARTÕÂN and AYERBE (1999)** on barley genotypes subjected to drought stress. The less pronounced declines in T1 is probably due to the early stage in the trial and the drought had only been experienced for a relatively short time since the start of the experiment. A significant difference was observed between the cultivars in the control treatment which probably exhibit their genotypic differences in non-stressed conditions. As the stress

progressed fewer differences were observed between the cultivars until the severe stress condition where no significant difference was observed. This was also observed by **GONZÁLEZ, MARTÍN & AYERBE (1999**) and **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** during the evaluation of barley and potato cultivars subjected to drought conditions respectively. The nonsignificant difference in the severe stress treatment (Appendix 4-D) was observed at both T1 and T2 which could be due to the severe stress experienced by the plants at both stages forcing stomatal closure to preserve water loss and indicating that water loss was already severe at T1.

4.5.2.2. Trial 2

A significant decline ($P \leq 0.05$) in conductance values was also observed in Trial 2 (Table 4.2) where 35 sweet potato cultivars and breeding lines were subjected to drought stress conditions (Appendix 4-E). The difference between control and stress became more pronounced as the stressed progressed at 120 DAP. This was demonstrated by **VAN HEERDEN and LAURIE (2008)** where the difference in stomatal conductance values between the control and stress of the cultivar Resisto became increasingly larger as the stress progressed. The breeding lines 2005-1-16, 2005-2-2, 2005-4-1, 2006-2-4, 2006-3-4, 2006-4-4, 2006-4-5, 2006-6-2 and cultivars Impilo, Jewel and Lethlabula did not show significant differences between the control and stress early in the trial at T1 which could be due to successful osmotic adjustment (**BLUM, 2005)** but, probably was due to an increase in stress. Significant differences in stomatal conductance between the control and stress were observed at T2 for all the genotypes except Isondlo and Purple Sunset. This is somewhat in conflict with the findings of **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** who observed less differences between the genotypes as the stress progressed while subjecting sugar beet genotypes to drought stress. It is shown that stomatal conductance values in Trial 2, from the

Table 4.2 Stomatal conductance (g_s) in mmole/m^2/s, of 35 sweet potato cultivars and breeding lines subjected to drought and control conditions

	T1		T2	
Cultivar	**Control**	**Stress**	**Control**	**Stress**
Isondlo	14.87	7.95*	11.75	3.36
Purple Sunset	12.01	5.34*	15.36	5.03
2005-1-11	12.89	4.83*	14.64	1.41*
2005-1-16	11.17	7.21	17.2	4.95*
200511-3	13.44	4.59*	14.75	2.86*
2005-12-2	13.1	5.9*	14.29	4.19*
2005-16-1	12.1	5.48*	15.09	5.23*
2005-2-2	13.11	7.73	15.76	3.38*
2005-3-10	10.86	4.53*	14.38	3.59*
2005-3-13	17.50	6.38*	13.27	4.00*
2005-4-1	11.18	6.87	15.12	3.83*
2005-5-5	14.58	4.10*	14.57	4.56*
2005-7-4	11.67	5.42*	10.41	2.97*
2006-14-4	14.56	7.27*	16.61	4.33*
2006-15-1	10.85	4.34*	17.57	4.61*
2006-2-4	11.36	5.82	10.31	1.76*
2006-3-4	13.37	7.85	15.82	5.64*
2006-4-4	11.15	7.20	15.45	4.49*
2006-4-5	13.39	9.20	16.77	4.43*
2006-6-2	13.03	9.35	15.91	1.66*
2006-7-3	15.18	5.98*	16.7	3.46*
2006-7-7	14.31	5.36*	14.21	1.70*
2006-7-8	12.81	5.39*	12.13	3.78*
Beauregard	13.8	6.26*	13.37	4.82*
Blesbok	12.01	4.37*	14.13	2.57*
Bosbok	15.05	4.93*	14.32	3.03*
Impilo	13.37	7.45	13.28	2.13*
Jewel	11.83	6.02	15.57	5.36*
Lethlabula	13.66	7.66	16.04	4.04*
Ndou	15.15	4.74*	15.53	1.51*
Phala	13.98	6.95*	14.11	3.81*
Resisto	15.00	5.19*	13.21	2.82*
Tanzania	13.54	5.71*	15.54	5.11*
W-119	12.98	5.36*	13.38	4.79*
Zapallo	14.29	7.35*	12.83	2.57*
mean	13.23	6.17*	14.55	3.65*

* indicates a significant difference at P≤0.05 level between the control and stress for a specific Time X Water X Variety combination

$LSD_{(p=0.05)}$ = 6.24; control = control treatment, stress = severe stress treatment.

T1 = 60 days after planting, T2 = 120 days after planting. $MSE_{(df=70)}$ = 5.802

control treatment, is higher than in Trial 1 which could be the result of normal rainfall resulting in more vigorous growth of the plants compared to more controlled conditions in a rainout shelter where all the treatments are experimentally controlled.

4.5.2.3. Trial 3

Severe reduction (significant) in stomatal conductance, from the control to stress treatment, in the sweet potato plants in Trial 3 were observed (Figure 4.4). This correlates with the findings of **VAN HEERDEN and LAURIE (2008)** who observed a clear difference between conductance of the control compared to values of the stressed sweet potato plants. No significant difference could be observed between the genotypes at either control or severe stress treatments at both times although significant reductions between the control and severe stress treatments were observed at T1 and T2 (Appendix 4-F).

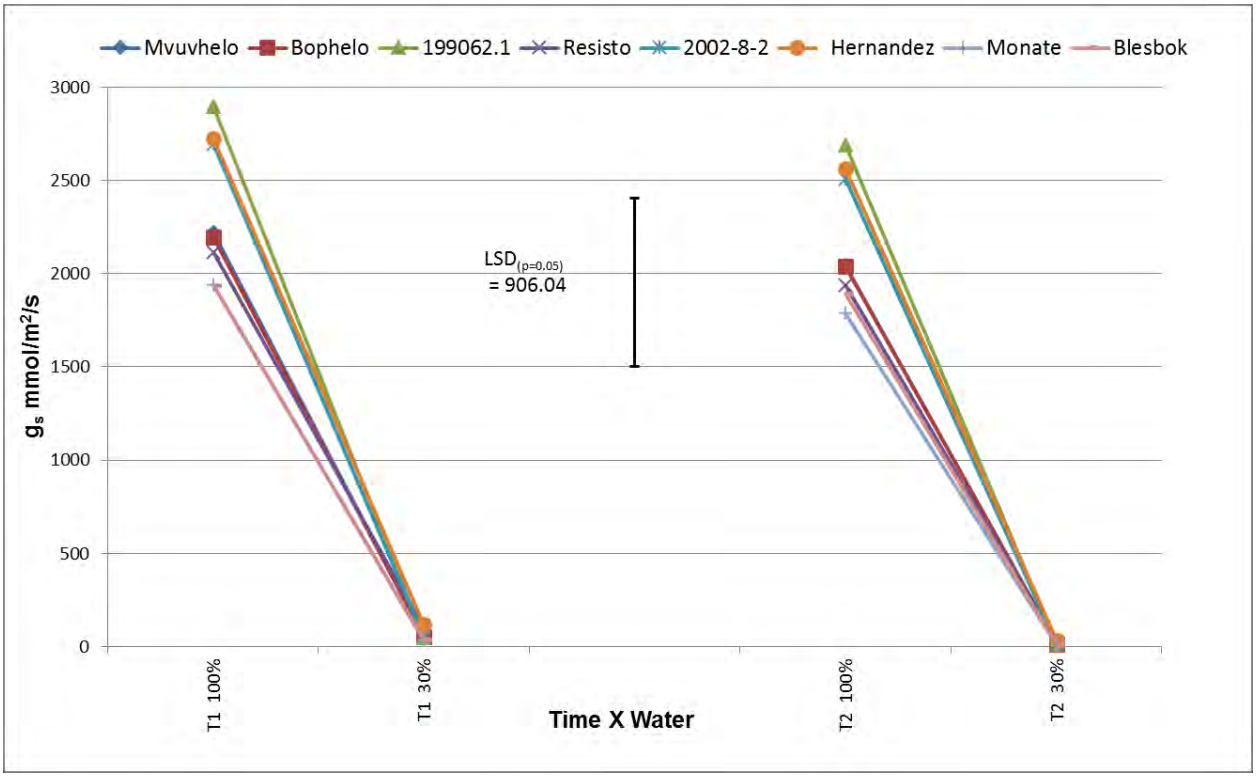

Figure 4.4 Stomatal conductance differences in the leaves of eight sweet potato cultivars and breeding lines subjected to drought in Trial 3. $LSD_{(p=0.05)}$ = 906.04; Each value is the mean of 3 measurements with 3 repeats per cultivar. T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment

These findings correlate with observations made by **LIU, ANDERSEN, JACOBSEN and JENSEN (2005)** early in a drought experiment while testing soybean cultivars for drought tolerance. The non-significant differences between the genotypes at T2 in severe stress conditions could be due to the severe water deficiency in the soil causing the plants to close their stomata to prevent moisture loss to such an extent that all the plants were almost at the same level of survival. Due to the use of untransformed data, the non-significant differences between the cultivars are not clearly discernable as in the case of Trials 1 and 2 where the data had been transformed due to high variability.

4.5.3. Relative water content (RWC)

4.5.3.1. Trial 1

Significant differences ($P \leq 0.05$) in relative water content (RWC) were observed between some of the genotypes in the control treatment of T1 (Figure 4.5). This in contrast to the observations made by **RAHIMIA, MADAH HOSSEINIB, POORYOOSEFC and FATEH (2010)** who could not find significant differences between the two plantago genotypes in control conditions. The differences became non-significant at mild stress and severe stress conditions at the same time of leaf harvest. This was also observed by **RAHIMIA, MADAH HOSSEINIB, POORYOOSEFC and FATEH (2010)** who found that although the differences between the control and stressed plantago plants became more evident no differences could be detected between the genotypes**.** Significant reductions in RWC were observed between the control and the severe stress treatments at T1, as expected. This indicated that the plants were losing water due to the depletion of soil water. Although **BLOCH, HOFFMANN and MÄRLÄNDER (2006)** found genotypic differences in relative water content values at a certain water regime, no significant differences in relative water content were found between the different regimes when sugar beet genotypes were subjected to drought conditions.

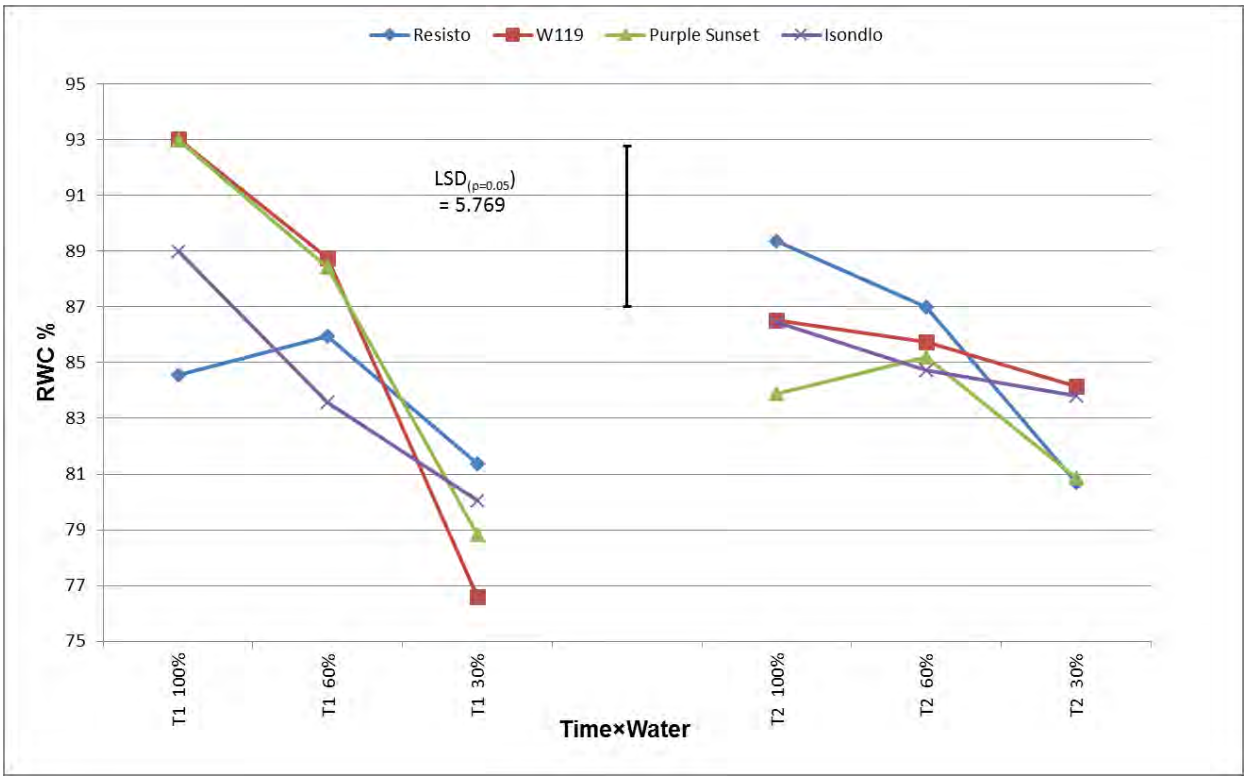

Figure 4.5 Relative water content of leaves of four sweet potato cultivars subjected to drought stress in Trial 1. $LSD_{(p=0.05)}$ = 5.76; Each value is the mean of five leaf discs per leaf of three leaves per plant and three repeats per treatment. T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

At T2 the decline from the control treatment towards the severe stress treatment was less intense and probably due to increased osmotic adjustment (OA) in the plants helping to maintain the water content **(BLUM, 2005)**. The cultivar W-119 displayed the highest RWC (~ 93%) together with Purple Sunset but seemed to lose a lot of leaf water by displaying the lowest RWC (~ 76%) in the severe stress treatment at T1. The cultivar seems to make some osmotic adjustment which indicated that the RWC increased significantly in severe stress conditions at T2. It must be mentioned that leaf samples, for RWC analysis, are harvested in the morning before sunrise. This might contribute to the elevated water content in the leaves despite the severe water stress the plants are experiencing. The roots might act as a reservoir for water under such conditions and aid in the partial restoration of water content in the plant.

4.5.3.2. Trial 3

The effects of drought on the RWC on eight sweet potato cultivars are displayed in Figure 4.5. No significant differences between the genotypes could be detected at T1 while significant differences became apparent at T2 (Appendix 4-H).

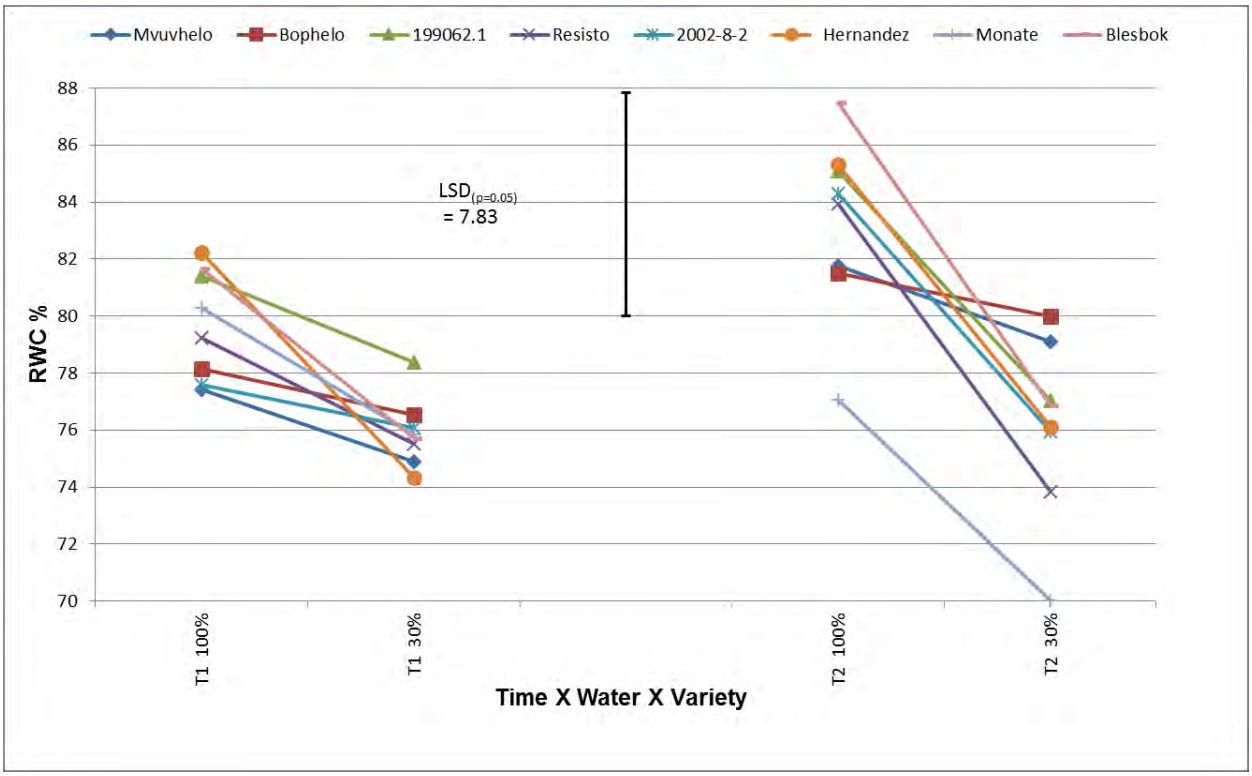

Figure 4.6. Relative water content values of leaves of eight sweet potato cultivars subjected to drought stress in Trial 3. $LSD_{(p=0.05)}$ = 7.83; Each value is the mean of five leaf discs from three leaves per plant and three repeats per treatment. T1=60 days after planting, T2=120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress 30% treatment

The effect of the drought was less pronounced early in the experiment, exhibiting smaller differences in RWC between the control and severe stress treatments. This was also seen in drought experiments **RAHIMIA, MADAH HOSSEINIB, POORYOOSEFC and FATEH (2010)** conducted with plantago species. The differences became more pronounced during the latter stages of the Trial probably due to the stress having a larger impact. The cultivar Bophelo displayed the highest RWC value while the cultivar Monate showed the lowest RWC value of 70% in severe stress conditions. Although it was found by **BARRS**

and WEATHERLEY (1962) that wilting will take place at the level of 70% RWC it was not observed during the harvest before sunrise. Despite the severe stress imposed on the plants RWC values remained reasonably high in the severe stress treatment of T2 giving rise to the argument that reasonable recovery is taking place overnight when the plants are in a less active phase.

4.6. CONCLUSIONS

The data obtained in the present study supported the following conclusions:

1. Genotypic variation was found in sweet potatoes subjected to drought conditions using carbon isotope discrimination data. The range of Δ values obtained from these trials correlates well with the accepted borders for C_3 plants **(GRIFFITHS, 1992)**. The relationship with other physiological and agronomic (yield, water use efficiency) parameters will aid in the selection process of parameters to be considered as possible indicators for screening sweet potato genotypes under water stress.
2. It was difficult to establish genotypic differences using stomatal conductance as a screening parameter. This was probably due to the fact that either the genotypes are very closely related or that the stress conditions were not selected more discreetly. A treatment between severe and mild stress might provide the possibility for better screening.
3. RWC did not appear to be a successful indicator for the screening of sweet potato genotypes in drought conditions. Recovery during night conditions possibly alleviated water deficit in the leaves due to water availability in the roots. Day time harvesting of the leaf material could have an adverse effect on the RWC values when heat stress could also become a factor in the screening factor.

4.7. APPENDICES

Appendix 4-A: ANOVA for Trial 1 for ^{13}C discrimination.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.5148	0.2574	1.25	
REP.Wplot stratum					
WATER	2	5.0589	2.5294	12.33	0.019
Residual	4	0.8208	0.2052	1.78	
REP.Wplot.Splot stratum					
VARIETY	3	9.0955	3.0318	26.37	<.001
WATER.VARIETY	6	3.2027	0.5338	4.64	0.005
Residual	18	2.0698	0.1150	0.95	
REP.Wplot.Splot.TIME stratum					
TIME	1	1.8454	1.8454	15.17	<.001
TIME.WATER	2	4.5223	2.2611	18.59	<.001
TIME.VARIETY	3	0.6176	0.2059	1.69	0.195
TIME.WATER.VARIETY	6	1.0056	0.1676	1.38	0.263
Residual	24	2.9186	0.1216		

Appendix 4-B: ANOVA for Trial 2 for ^{13}C discrimination.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	76.8373	76.8373	22.60	0.042
Residual	2	6.8011	3.4006	7.77	
REP.Wplot.VARIETY stratum					
VARIETY	34	48.1940	1.4175	3.24	<.001
VARIETY.TMT	34	20.8170	0.6123	1.40	0.120
Residual	68	29.7518	0.4375	1.69	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	19.4981	19.4981	75.49	<.001
VARIETY.TIME	34	9.0631	0.2666	1.03	0.444
TIME.TMT	1	0.6261	0.6261	2.42	0.124
VARIETY.TIME.TMT	34	13.9098	0.4091	1.58	0.053
Residual	70	18.0810	0.2583		

Appendix 4-C: ANOVA for Trial 3 for ^{13}C discrimination.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
WATER	1	93.5755	93.5755	222.31	0.004
Residual	2	0.8419	0.4209	3.15	
REP.Wplot.Splot stratum					
VARIETY	7	18.2509	2.6073	19.53	<.001
WATER.VARIETY	7	4.1975	0.5996	4.49	0.002

Appendix 4-D: ANOVA for Trial 1 for stomatal conductance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	2	416.071	208.035	91.69	<.001
Residual	6	13.614	2.269	2.42	
REP.Wplot.Splot stratum					
VARIETY	3	46.379	15.460	16.51	<.001
TMT.VARIETY	6	13.674	2.279	2.43	0.067
Residual	18	16.851	0.936	0.70	
REP.Wplot.Splot.TIME stratum					
TIME	1	60.893	60.893	45.44	<.001
TIME.TMT	2	39.500	19.750	14.74	<.001
TIME.VARIETY	3	6.709	2.236	1.67	0.200
TIME.TMT.VARIETY	6	11.321	1.887	1.41	0.252
Residual	24	32.165	1.340		

Appendix 4-E: ANOVA for Trial 2 for stomatal conductance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	2339.707	2339.707	9.81	0.089
Residual	2	476.967	238.483	69.25	
REP.Wplot.VARIETY stratum					
VARIETY	34	124.999	3.676	1.07	0.400
VARIETY.TMT	34	161.750	4.757	1.38	0.129
Residual	68	234.173	3.444	0.59	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	1011.219	1011.219	174.29	<.001
VARIETY.TIME	34	145.243	4.272	0.74	0.836
TIME.TMT	1	114.410	114.410	19.72	<.001
VARIETY.TIME.TMT	34	96.651	2.843	0.49	0.988
Residual	70	406.146	5.802		

Appendix 4-F: ANOVA for Trial 3 for stomatal conductance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2830714.	1415357.	1.16	
REP.Wplot stratum					
WATER	1	118566635.	118566635.	97.07	0.010
Residual	2	2443030.	1221515.	2.65	
REP.Wplot.Splot stratum					
VARIETY	7	2829411.	404202.	0.88	0.536
WATER.VARIETY	7	2652159.	378880.	0.82	0.576
Residual	28	12884653.	460166.	283.80	
REP.Wplot.Splot.TIME stratum					
TIME	1	251557.	251557.	155.14	<.001
TIME.WATER	1	75325.	75325.	46.46	<.001
TIME.VARIETY	7	17657.	2522.	1.56	0.185
TIME.WATER.VARIETY	7	12527.	1790.	1.10	0.385
Residual	32	51886.	1621.		

Appendix 4-G: ANOVA for Trial 1 for relative water content.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.87	0.93	0.19	
REP.Wplot stratum					
TMT	2	495.24	247.62	50.48	0.001
Residual	4	19.62	4.90	0.52	
REP.Wplot.Splot stratum					
VARIETY	3	32.00	10.67	1.12	0.366
TMT.VARIETY	6	146.58	24.43	2.57	0.056
Residual	18	170.96	9.50	0.59	
REP.Wplot.Splot.TIME stratum					
TIME	1	2.23	2.23	0.14	0.714
TIME.TMT	2	245.67	122.84	7.59	0.003
TIME.VARIETY	3	156.77	52.26	3.23	0.040
TIME.TMT.VARIETY	6	77.29	12.88	0.80	0.583
Residual	24	388.58	16.19		

Appendix 4-H: ANOVA for Trial 3 for relative water content.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	97.06	48.53	12.76	
REP.Wplot stratum					
TMT	1	144.34	144.34	37.94	0.025
Residual	2	7.61	3.80	0.15	
REP.Wplot.Splot stratum					
VARIETY	7	194.82	27.83	1.07	0.409
TMT.VARIETY	7	57.77	8.25	0.32	0.940
Residual	28	729.26	26.05	1.14	
REP.Wplot.Splot.TIME stratum					
TIME	1	73.60	73.60	3.23	0.082
TIME.TMT	1	540.19	540.19	23.74	<.001
TIME.VARIETY	7	157.24	22.46	0.99	0.458
TIME.TMT.VARIETY	7	221.92	31.70	1.39	0.242
Residual	32	728.07	22.75		

CHAPTER 5

THE EFFECT OF DROUGHT STRESS ON NITRATE REDUCTASE ACITIVITY AND PROLINE CONCENTRATIONS IN THE LEAVES OF SWEET POTATO CULTIVARS AND BREEDING LINES.

5.1. INTRODUCTION

Nitrate reductase is one of the most intensively studied enzymes in the plant metabolic system. The enzyme catalyzes the rate limiting step in the overall process of nitrate assimilation involving the reduction of nitrate to nitrite **KAISER, WEINER and HUBER (1999)**. Nitrogen is normally available to the plant in the nitrate form but cannot be used before reduction to nitrite takes places.

The protein manufacturing process in the plant is directly dependent on the conditions that controls the opening and closing of stomata. The aperture of the stomata will influence the photosynthesis processes. This influence on photosynthesis has a negative effect on protein synthesis and therefore will also affect all processes where proteins are involved in the growth process. It was reported by **FOYER, VALADIER, MIGGE and BECKER (1998)** that in maize the introduction of drought leads to a rapid decrease in nitrate reductase activity. **FERRARIO-MÉRY, VALADIER and FOYER (1998)** found that the decrease in nitrate reductase activity, during the first 3 days of drought in tobacco, was the result of a decrease in the nitrate reductase protein. **KRČEK, SLAMKA, OLŠOVSKÁ and BENČÍKOVÁ (2008)** also found that levels of nitrate reductase activity were considerably higher in the leaves that received optimum water quantities compared with drought treated plants. Due to the fact that nitrate reductase is more dependent on the decline in rate of photosynthesis according to **KAISER and BRENDLE- BEHNISCH (1991)**, it can be reasoned that the more the plant has the ability to uphold the photosynthetic rate the better the plant can uphold nitrate reductase activity and hence nitrate metabolism.

Proline as a measure of stress has been studied in many crops **(MONREAL, JIMÉNEZ, REMESAL, MORILLO-VELARDE, GARCÍA-MAURINO and ECHEVARRÍA (2007); KNIPP and HONERMEIER (2006)** and **CLAUSSEN (2005)** and reported to be indicative of the stress experienced by plants. Proline is soluble in water and acts as a compatible osmolyte **(KAVI KISHOR, SANGAM, AMRUTHA, SRI LAXMI, NAIDU, RAO, RAO, REDDY, THERIAPPA and SREENIVASULU, 2005)**, meaning it has the ability to maintain the osmotic balance in a certain environment. **HAMILTON and HECKATHORN (2001)** found that under NaCl stress complex II of the photosynthetic system is protected by the presence of proline. In experiments with cotton **DE RONDE, VAN DER MESCHT and STEYN (2000)** also found that free proline concentrations increased with the decrease in water content in the soil. **KOCSY, LAURIE, SAZALAI, SZILÁGYI, SIMON-SARKADI, GALIBA and DE RONDE (2005)** found that plants which withstood the drought stress had a higher free proline content. Due to these findings and the fact that very little knowledge is available regarding the role of proline during drought stress in sweet potatoes, it is of importance to conduct such investigations.

5.2. AIM

The aim thus was to investigate the effect of drought stress on the activity of nitrate reductase as well as the levels of free proline in the leaves of sweet potato cultivars.

5.3. MATERIALS AND METHODS

Sampling of sweet potato leaves took place twice during the Trial before sunrise. Thirty leaves, fifth from the apical tip, were harvested from each cultivar/repeat for each of the treatments and immediately stored at -80ºC and subsequently freeze-dried. Samples from the freeze-dried leaves were taken for analysis.

5.3.1. Nitrate reductase analysis

Each sample (0.04 g) was extracted according to the method of **PROSSER, PURVES, SAKER and CLARKSON (2001)**. Each leaf tissue sample, in triplicate, was ground in liquid nitrogen. Extraction was conducted in buffer containing 50 mM MOPS-NaOH pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, and 0.1% (v/v) Triton X-100. The extracts were centrifuged at 20000g for 2 min and the supernatants used immediately for the assays in a reaction mixture containing 1 ml of 0.1 M potassium phosphate buffer, 0.2 ml 0.1 M KNO_3, 0.5 ml 1.36 mM NADPH, 0.2 ml enzyme extract. Reaction mixtures were incubated at 27ºC for 15 min and the reaction stopped by the addition of 1 ml 1% w/v sulfanilamide in 1.5 M HCl according to the method of **HAGEMAN and FLESHER (1960)**. N-(1 napthyl) ethylene diamine hydrochloride reagent was added (I ml of 0.02 % w/v) and the contents mixed by inverting the tubes. The absorbancy was determined by reading each sample against its own blank (complete except for NADPH) in a Beckman DU 800 spectrophotometer at 540 nm and the activity calculated as µmole NO_2/g/h.

5.3.2. Proline analysis

The free proline content was determined from the supernatant following the procedure of **BATES, WALDREN and TEARE (1973)**, as described by **DE RONDE, SPREETH and CRESS (2000)**.

5.4. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was performed on all the data to test for treatment differences as well as possible differences between cultivars. Treatment means as well as interaction means were separated using Fishers' t-test least significant difference (LSD) at the 5% level of significance. Statistical analysis was conducted using, GenStat *for Windows* 15th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk, by the ARC Biometry Section.

5.5. RESULTS AND DISCUSSION

5.5.1 Nitrate reductase (NR) activity

Nitrate reductase is a key enzyme in the nitrogen assimilation pathway that is actively controlled during drought **(FRESNEAU, GHASHGHAIE and CORNIC**, **2007; CARAVACA, ALGUACIL, HERNÁNDEZ and ROLDÁN, 2005; FOYER, VALADIER, MIGGE and BECKER, 1998)**. It has been demonstrated by **PANDEY, BAIG and BHATT (2012)** that nitrate reductase is negatively influenced by the reduced rate of photosynthesis in drought stressed oats.

5.5.1.1. Trial 1

The introduction of drought on four sweet potato cultivars in Trial 1 caused significant ($P \leq 0.05$) (Appendix 5-A) reduction in NR activity in the leaves of all the cultivars over the 2 drought conditions at both times (Figure 5.1). This is in agreement with the findings of **ASHRAF and IRAM (2005)** who reported significant and severe reduction in NR activity in the leaves of two legume genotypes subjected to drought stress. This implied that the fixation of nitrogen was severely impaired and should have a negative influence on the synthesis of the enzyme **(MORILLA, BOYER and HAGEMAN, 1973)** as well as possible inhibition of enzyme activity **SUNG (1981)**. **SUNG (1981)** found a linear relationship between nitrate reductase activities and leaf water potential in sweet potato leaves during drought stress indicating that a severe stress accompanied by plant water loss resulted in a rapid decline in nitrate reductase activity. No significant differences were observed between the cultivars at T1 for all the treatments. This was also observed in the leaves of legumes subjected to drought stress by **ASHRAF and IRAM (2005)** although significant differences between the species were indicated in root material. Significant differences were observed in nitrate reductase activity between all the treatments at T1 as well as T2. The decrease in activity at T1 between the control and mild stress treatments had to do with the lower soil water content present in mild stress. The activity of NR was also significantly lower in the mild stress treatment at T2 compared to mild stress treatment at T1 in some cases. This was expected and lower NR activity was also observed by **FOYER, VALADIER, MIGGE and BECKER (1998)**; **FRESNEAU, GHASHGHAIE and**

CORNIC (2007); **MORILLA, BOYER and HAGEMAN (1973)**; **SUNG 1981** and **WIDMANN, GEBAUER, RENDER and ZIEGLER (1993)** in maize, wheat and sweet potato genotypes respectively when subjected to lengthy periods of drought stress. **SHARMA and DUBEY (2005)** also confirmed this subjecting rice seedlings to drought conditions by using an osmoticum. This is expected since the soil is continuously drying out from the bottom in the mild stress treatment creating a more intense drought condition at T2 than T1. Significant difference between some of the genotypes in the mild stress treatment at T2 could be observed with the cultivar Sunset Purple displaying the highest and Isondlo the lowest activity.

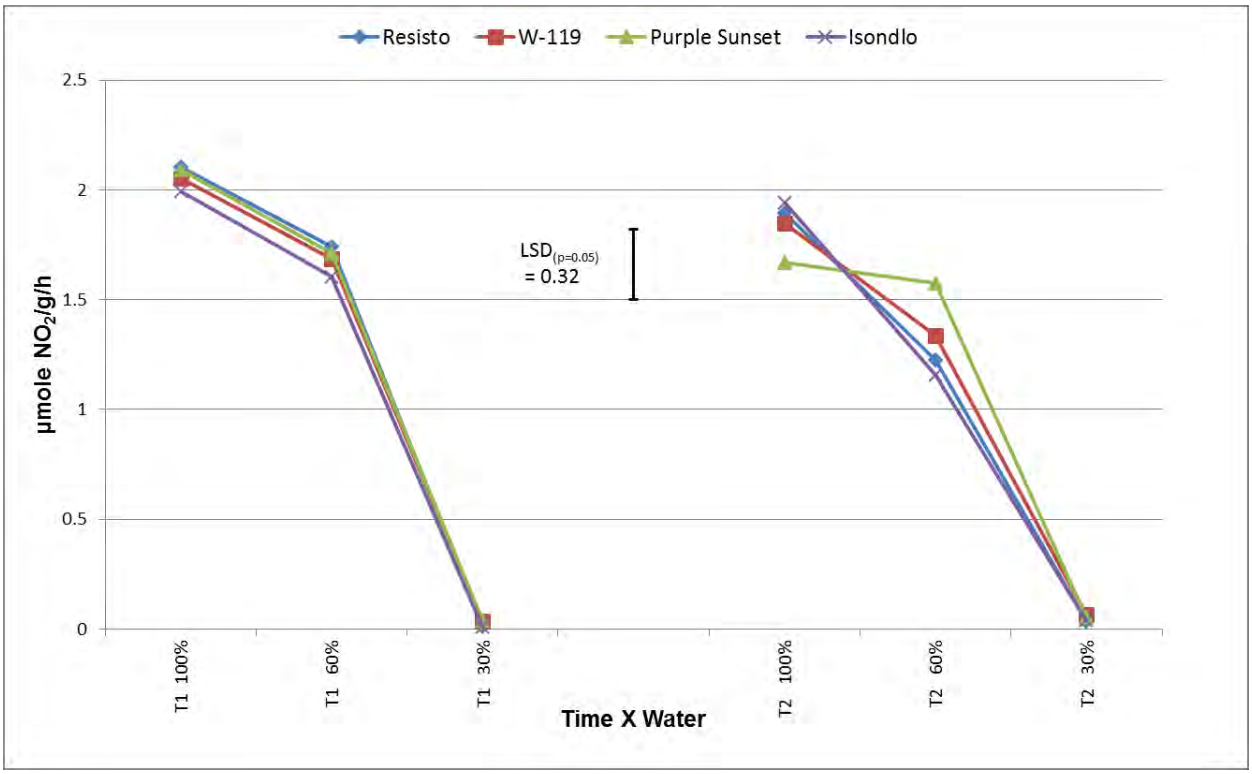

Figure 5.1. Activity of nitrate reductase displayed in amount of NO_2 consumed of four sweet potato genotypes subjected to drought stress in Trial 1. $LSD_{(p=0.05)}$ = 0.32; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

The decrease in activity of NR of the cultivar Sunset Purple in the mild stress treatment at T2 was less pronounced than the mild stress treatment at T1 which could possibly be the result of a slight adjustment of the plants to the stress. **WIDMANN, GEBAUER, RENDER and ZIEGLER (1993)** reported similar results in succulent plants where

alleviated levels of NR were observed at certain times during drought stress. The severe stress treatment appeared to be very harsh since the activity declined to almost zero and no significant difference could be detected between the cultivars at any time interval. Similar results were reported by **WIDMANN, GEBAUER, RENDER and ZIEGLER (1993)** and **ASHRAF and IRAM (2005)** with legumes and succulents respectively confirming that severe water stress will eventually result in non-significant differences between genotypes.

5.5.1.2. Trial 2

Under water deficit (Table 5.1) nitrate reductase (NR) activity was reduced significantly ($P \leq 0.05$) (Appendix 5-B) between the control and stress treatments in all the cultivars and breeding lines at both T1 and T2 between control and stress. The NR activity was slightly lower at T2 than at T1 although not significantly so. No significant difference between the genotypes wase detected in the stress treatment at either T1 nor T2 which possibly also had to do with the low levels of stomatal conductance the leaves experienced. **FRESNEAU, GHASHGHAIE and CORNIC (2007)** noted that low levels of internal CO_2 concentrations aided in the decrease of NR activity. **SIVASANKAR, ROTHSTEIN and OAKS (1997)** on the other hand have observed that a decrease in NR activity was evident due to a reduced flow of nitrate to the leaves due to a reduced water flow in which the nitrate is dissolved. **FERRARIO-MÉRY, VALADIER and FOYER (1998)** also found that the rapid loss of observed NR activity could be due to NR denaturation or degradation.

Table 5.1 Nitrate reductase activities (µmole NO_2/g/h) in 35 sweet potato cultivars and breeding lines subjected to control and drought conditions in the field and small rain out shelters.

	T1		T2	
Variety	**Control**	**Stress**	**Control**	**Stress**
Isondlo	1.4120	0.0085*	1.7968	0.0034*
Purple Sunset	1.6792	0.0091*	1.5401	0.0062*
2005-1-11	1.4794	0.0078*	1.7489	0.0024*
2005-11-3	1.4797	0.0092*	1.7650	0.0035*
2005-1-16	1.8833	0.0069*	1.5372	0.0061*
2005-12-2	1.6000	0.0096*	1.4526	0.0018*
2005-16-1	1.7721	0.0094*	1.5673	0.0042*
2005-2-2	1.7655	0.0092*	1.6433	0.0025*
2005-3-10	1.6047	0.0072*	1.6371	0.0032*
2005-3-13	1.5772	0.0082*	1.8718	0.0034*
2005-4-1	1.6030	0.0084*	1.4758	0.0018*
2005-5-5	1.5394	0.0052*	1.8121	0.0027*
2005-7-4	1.7182	0.0095*	1.6787	0.0023*
2006-14-4	1.5291	0.0080*	1.5035	0.0025*
2006-15-1	1.8794	0.0093*	1.6727	0.0036*
2006-2-4	1.5774	0.0064*	1.8007	0.0036*
2006-3-4	1.5995	0.0062*	1.5983	0.0029*
2006-4-4	1.8845	0.0075*	1.5278	0.0035*
2006-4-5	1.9383	0.0058*	1.8894	0.0046*
2006-6-2	1.3446	0.0053*	1.4738	0.0032*
2006-7-3	1.7696	0.0045*	1.8403	0.0037*
2006-7-7	1.6538	0.0053*	1.2848	0.0042*
2006-7-8	1.6278	0.0080*	1.4620	0.0027*
Beauregard	1.7573	0.0068*	1.4855	0.0069*
Blesbok	1.7739	0.0069*	1.7634	0.0046*
Bosbok	1.5961	0.0080*	1.5114	0.0038*
Impilo	1.4670	0.0047*	1.5116	0.0052*
Jewel	1.6818	0.0065*	1.4021	0.0046*
Lethlabula	1.4560	0.0067*	1.7401	0.0024*
Ndou	1.4876	0.0028*	1.2956	0.0047*
Phala	1.7994	0.0073*	1.4524	0.0037*
Resisto	1.7551	0.0014*	1.4890	0.0024*
Tanzania	1.4707	0.0060*	1.7128	0.0033*
W-119	1.4169	0.0026*	1.2797	0.0016*
Zapallo	1.6630	0.0023*	1.4175	0.0061*
mean	1.6355	0.0067	1.5897	0.0036

* means significant difference between the control and stress for a specific Time X Treatment X Water combination. $LSD_{(p=0.05)}$ = 0.38; Control = control treatment, Stress= severe stress treatment. T1 = 60 days after planting, T2 = 120 days after planting $MSE_{(df=70)}$ = 0.023

5.5.1.3. Trial 3

Drought had a negative effect on nitrate reductase (NR) activities in sweet potato leaves shown in Figure 5.2. A severe significant (Appendix 5-C) decline in NR activity was observed at severe stress at T1 which is consistent with findings of **FERRARIO-MÉRY, VALADIER and FOYER (1998)** who could not detect any activity in seven-week-old tobacco plants deprived of water for five days. It must be mentioned that the tobacco trial was conducted in pots that creates the platform to obtain drought stress conditions at a much earlier time than field trials. In Trial 3 the time frame to create drought conditions took somewhat longer due to simulated field conditions. Significant genotypic differences were displayed in the control treatment at both T1 and T2. The activity of nitrate reductase at T2 in the control treatment was significantly lower than the control treatment at T1 for some of the cultivars. This could be due to the aging of the leaves **AHMAD, FAZILI, HAQUE, KHAN and ABDIN (2010)**.

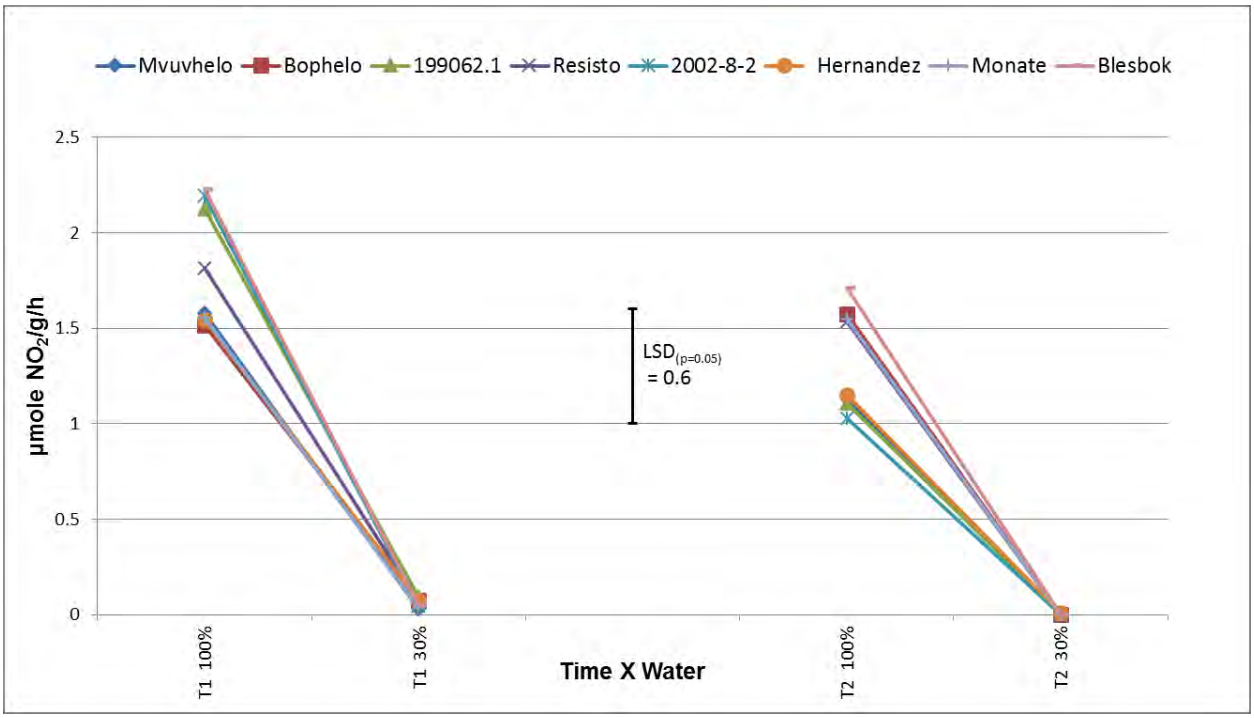

Figure 5.2. Activity of nitrate reductase enzyme displayed through the amount of NO_2 consumed in eight sweet potato cultivars as a result of drought stress in Trial 3. $LSD_{(p=0.05)}$ = 0.6; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment

No significant differences could be detected between the genotypes in the severe stress conditions at either T1 or T2 at which time the activities were already very low, possibly due to protein degradation **(FERRARIO-MÉRY, VALADIER and FOYER, 1998; FOYER, VALADIER, MIGGE and BECKER, 1998)**. This reduction in NR will have an effect on the growth of the plant as nitrogen is an important component in the formation of numerous substances in the plant. The effect that drought has in this regard is shown in Chapter 2. To possibly determine the genotypic difference between the cultivars during a drought condition with regard to nitrate reductase, it is recommended that the drought imposed be of such a level to exhibit a successful partial inhibition of NR activity to expose possible genotypic differences.

5.5.2. Proline analysis

An increase in proline levels has been reported in many crops due to drought (**MONREAL, JIMÉNEZ, REMESAL, MORILLO-VELARDE, GARCÍA-MAURINO and ECHEVARRÍA, 2007**(sugar beet); **VENDRUSCOLO, SCHUSTER, PILEGGI, SCAPIM, MOLINARI, MARUR and VIEIRA, 2007**(wheat); **DE RONDE, VAN DER MESCHT and STEYN, 2000**(cotton) and **DE RONDE, CRESS, KRÜGER, STRASSER and VAN STADEN 2004)**(soybean).

5.5.2.1. Trial 1

An increase in free proline content, in all the trials, was observed in the leaves of four sweet potato cultivars subjected to drought conditions (Figure 5.3). Proline levels were lower at T2 than T1 in control conditions but the differences were non-significant. Significant differences (Appendix 5-D) between genotypes in the mild stress treatment at T2 were observed which was not evident in T1. This could be ascribed to the plants reacting to the desiccated environments and starting to produce proline in excess amounts to aid in preventing the dehydration of the leaf cells. Genotypic differences were detected in the severe stress treatment of T1, mild stress treatment of T2 and severe stress treatment of T2. The large amount of free proline produced in the severe stress treatment at T2 was probably the result of protein breakdown as the stress was quite severe at that specific time **(DE RONDE, VAN DER MESCHT and STEYN, 2000)**. The

cultivar Purple Sunset reacted to the stress in such a way that a five-fold increase in free proline was observed at T2 whereas the increase towards in severe stress conditions at T2 was probably also due to protein degradation since the other three cultivars displayed more practical values which correlate well with values obtained by **RODRÍGUEZ-DELFÍNA, POSADAS, LEÓN-VELARDE, MARES and QUIROZ, (2012)**. Free proline levels peaked in mild stress conditions at T2 for the cultivar Isondlo and then declined. This could be the result of either water contamination (from neighboring irrigation nozzles), allowing proline levels to decrease, or possible enzyme denaturation causing the termination of proline production. The cultivar Resisto also experienced an increase in proline concentration which slowed down in the severe stress treatment at T2. Differences between the genotypes in the severe stress treatment at T1 correlated well with the differences observed in the mild stress treatment at T2. This might be that the stresses the plants experienced, at severe stress at T1, were more or less at the same intensity levels as the plants in in the mild stress condition at T2. The proline values in the severe stress treatment at T2 shows that water deficit plays a big role in the increase in free proline levels in sweet potato leaves in these trials. The increase was probably due to the physiological adjustment i.e. osmotic adjustment, the plant is making to counter cell and substance damage in the leaves. **GUBIŠ, VAŇKOVÁ, ČERVENÁ, DRAGÚŇOVÁ, HUDCOVICOVÁ, LICHTNEROVÁ, DOKUPIL, JUREKOVÁ (2007)** reported that the osmotic potential of leaf sap from transgenic tobacco plants, transformed with proline P5CS gene, declined to a lesser degree under water stress conditions compared to those of control plants thereby reducing the risk of cell damage.

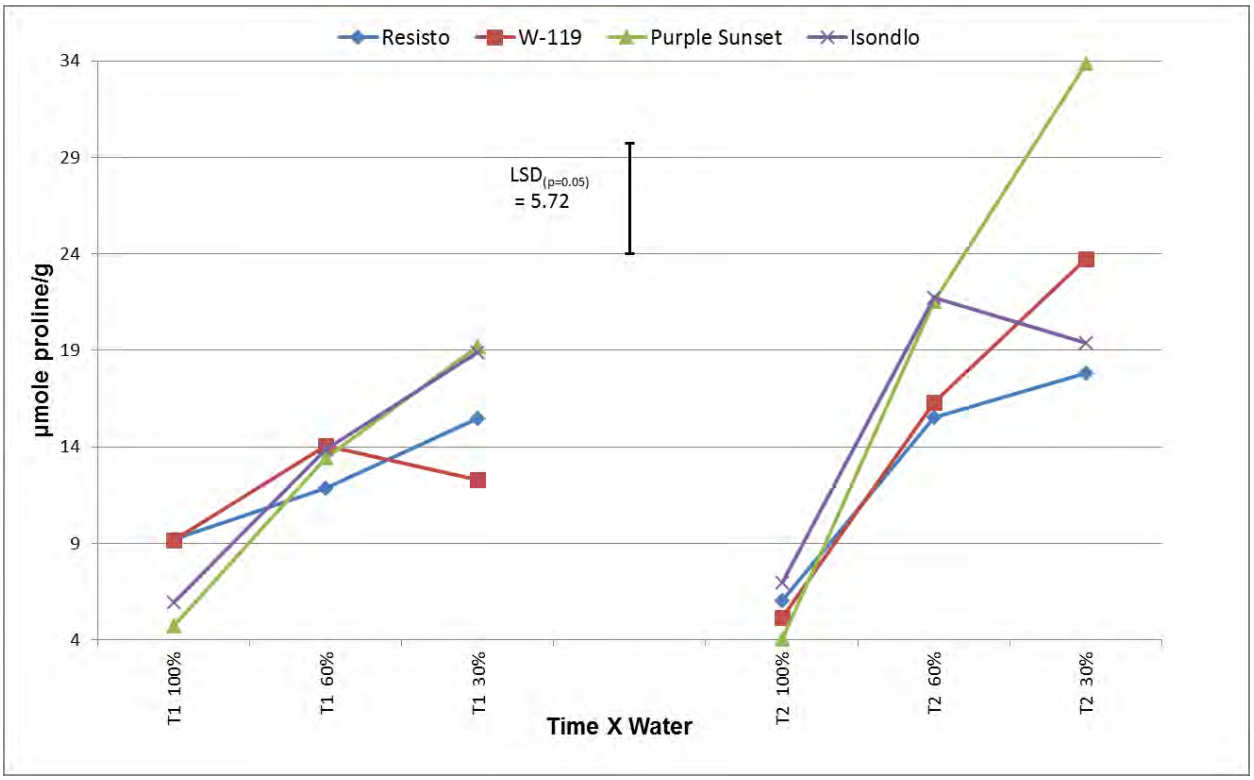

Figure 5.3. Proline content in leaves of four sweet potato cultivars subjected to drought stress in Trial 1. $LSD_{(p=0.05)}$ = 5.72; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

5.5.2.2. Trial 2

The drought imposed on the 35 cultivars and breeding lines resulted in a drastic increase in free proline levels at both periods of leaf harvest (Table 5.2). Significant differences were observed between the genotypes in the 30% treatment at T2 with the breeding line 2006-14-4 having reacted the most to the drought condition showing the highest proline concentration and Purple Sunset exhibiting the lowest proline concentration. This might aid in the search for cultivars and breeding lines during the breeding programme to select genotypes that can successfully tolerate drought. Proline levels in 25 of the 35 cultivars at T2 in the severe stress treatment were slightly higher than those at T1 although non-significant.

Table 5.2 Proline content (µmole proline/g dry weight) in sweet potato leaves analyzed from drought stressed plants in Trial 2.

	T1		T2		Increase
Variety	**Control**	**Stress**	**Control**	**Stress**	**fold**
Isondlo	2.35	11.39*	4.27	15.32*	3.6
Purple Sunset	1.74	14.45*	2.31	11.85*	5
2005-1-11	1.78	14.61*	4.08	16.22*	4
2005-11-3	3.15	17.21*	4.85	15.87*	3
2005-1-16	2.73	13.24*	2.07	15.55*	7.5
2005-12-2	1.49	14.86*	3.73	13.66*	3.6
2005-16-1	2.40	13.29*	1.95	13.85*	7
2005-2-2	2.22	12.41*	1.21	15.98*	13
2005-3-10	4.73	16.10*	4.60	12.48*	2.71
2005-3-13	4.52	14.32*	1.85	15.46*	8
2005-4-1	3.39	15.06*	2.77	13.79*	4.8
2005-5-5	1.85	15.74*	3.56	12.45*	3.5
2005-7-4	3.26	13.87*	3.12	14.36*	4.6
2006-14-4	3.63	14.07*	3.52	20.05*	5.6
2006-15-1	4.40	12.72*	1.03	17.23*	16
2006-2-4	2.55	17.54*	5.73	18.86*	3
2006-3-4	5.36	14.17*	1.46	17.98*	12
2006-4-4	3.26	15.67*	5.16	16.27*	3
2006-4-5	2.25	16.09*	2.81	18.49*	6.5
2006-6-2	3.26	14.03*	3.79	19.01*	5
2006-7-3	2.12	11.85*	1.91	17.73*	9
2006-7-7	4.61	14.71*	6.94	16.22*	2
2006-7-8	2.85	14.57*	3.61	14.85*	4
Beauregard	2.96	12.42*	3.48	15.36*	4
Blesbok	3.54	13.93*	2.79	14.22*	5
Bosbok	1.99	16.13*	1.47	13.44*	9
Impilo	3.09	14.53*	3.24	16.74*	5
Jewel	4.33	12.43*	1.39	13.54*	9.7
Lethlabula	2.37	11.56*	3.41	14.28*	4
Ndou	3.51	16.49*	2.99	15.58*	5
Phala	3.07	12.30*	3.54	14.30*	4
Resisto	2.44	14.96*	1.06	17.09*	16
Tanzania	2.00	19.06*	1.17	16.23*	14
W-119	2.11	16.15*	1.29	14.12*	10
Zapallo	4.17	12.84*	1.59	14.85*	9
mean	3.01	14.42	2.96	15.52*	5

* means significant difference between the control and stress for a specific Time X Water X Variety combination $LSD_{(p=0.05)}$ = 6.56; Control = control treatment, Stress = severe stress treatment.
T1 = 60 days after planting, T2 = 120 days after planting. Increase fold indicates the increase of free proline from control to stress at 120 DAP.
$MSE_{(df=70)}$ = 14.28

Although the average increase in free proline concentration was 5 fold of that of the control, spikes of up to 16 fold in increase was observed during the trial which probably was the result of protein degradation. The drastic increase in proline levels was probably also due to protein breakdown due to the severe drought stress the plants experienced. **BOKHARI and TRENT (1985)** observed large increases (15 fold) in free proline content in grasses grown in pots and subjected to water stress.

The results are a good indication of the stress experienced by the genotypes and might be an aid in combination with other parameters for drought tolerance selection.

5.5.2.3. Trial 3

Significant (Appendix 5-F) increases ($P \leq 0.05$) in free proline concentrations from the control to the severe stress treatments were observed in Trial 3 where eight sweet potato cultivars and breeding lines were subjected to drought (Figure 5.4). Significant genotypic differences in free proline were observed in the control and severe stress treatments at T1 while genotypic differences were only observed in the severe stress treatment at T2. Significant increases in general, up to five fold between the control and the severe stress treatments were observed in T2 compared to the 2.5 fold increase in T1. This indicates that the plants have experienced a drought condition that lead to the increase of proline production either via the transport of free proline from the root system **(ARMENGAUDA, THIERY, BUHOT, MARCH and ARNOULD SAVOURÉ, 2004)**; increased enzymatic production **(HARE and CRESS, 1997)** or the breakdown of protein **(BOKHARI and TRENT, 1985)**. In the severe stress treatment at T1 the cultivar Bophelo produced the highests level of free proline whith Mvuvhelo the lowest. The increase in proline for Bophelo was almost 3 fold which indicates that the plant is reacting to the stress by producing more free proline, probably to assist the plant in combatting dehydration.

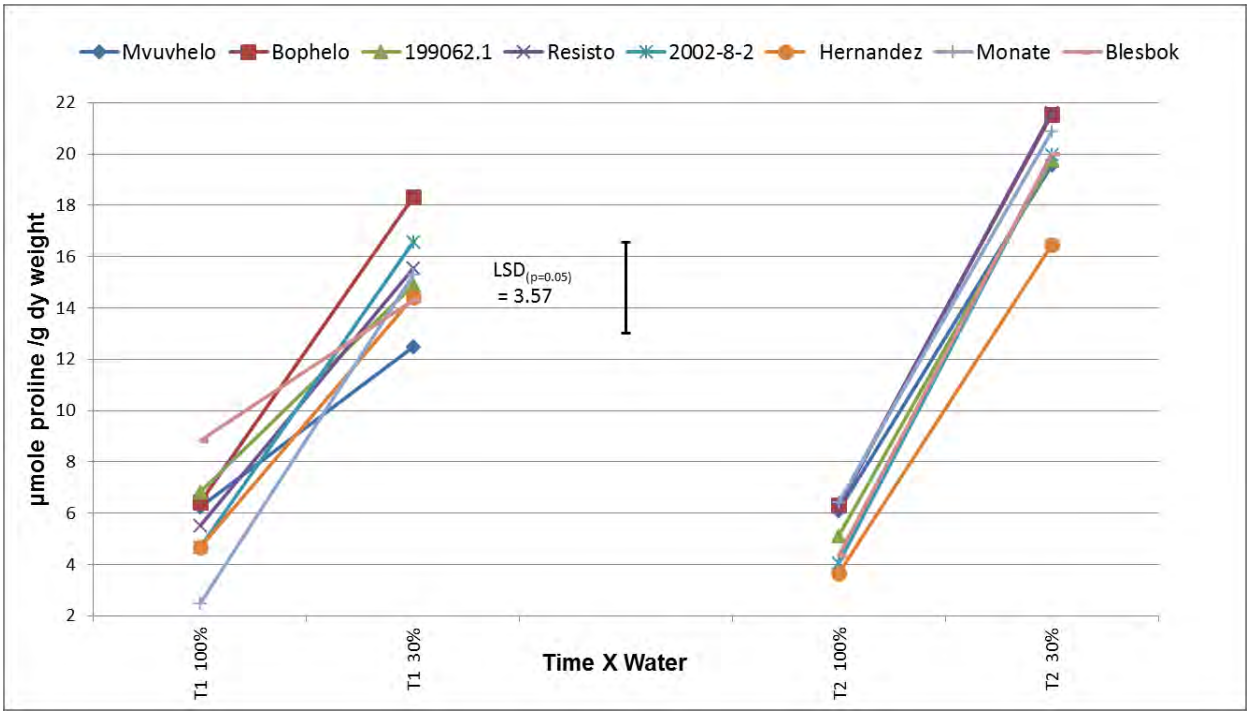

Figure 5.4. Proline concentrations in the leaves of eight sweet potato cultivars and breeding lines subjected to drought in Trial 3. $LSD_{(p=0.05)}$ = 3.57; T1 = 60 days after planting, T2 = 120 days after planting. 100% = control treatment, 30% = severe stress treatment

5.6. CONCLUSIONS

Overall, drought stress proved to have a negative effect on the activity of nitrate reductase in sweet potato genotypes subjected to drought. Although significant differences were observed between the genotypes in control and mild stress conditions, severe stress conditions resulted in non-significant differences which could indicate that the genotypes have difficulty in adapting to the stress. Proline concentrations in the majority of the genotypes increased as the drought progressed thus signifying that adjustment is made by the plants to survive the conditions. Proline proved to be a good indicator of ability to adapt to drought stress for the majority of the genotypes tested, whereby they could also be significantly differentiated from each other in the drought stress conditions by means of analysis.

5.7. APPENDICES

Appendix 5-A: ANOVA of Trail 1 for nitrate reductase activity.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.00598	0.00299	0.12	
REP.Wplot stratum					
TMT	2	45.74695	22.87348	895.27	<.001
Residual	4	0.10220	0.02555	0.46	
REP.Wplot.Splot stratum					
VARIETY	3	0.04046	0.01349	0.24	0.864
TMT.VARIETY	6	0.21888	0.03648	0.66	0.682
Residual	18	0.99336	0.05519	1.71	
REP.Wplot.Splot.TIME stratum					
TIME	1	0.62938	0.62938	19.47	<.001
TIME.TMT	2	2.75813	1.37906	42.66	<.001
TIME.VARIETY	3	0.01417	0.00472	0.15	0.931
TIME.TMT.VARIETY	6	0.20870	0.03478	1.08	0.404
Residual	24	0.77587	0.03233		

Appendix 5-B: ANOVA of Trial 2 for nitrate reductase activity.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	180.86943	180.86943	268.02	0.004
Residual	2	1.34968	0.67484	24.71	
REP.Wplot.VARIETY stratum					
VARIETY	34	0.95955	0.02822	1.03	0.443
VARIETY.TMT	34	0.94522	0.02780	1.02	0.463
Residual	68	1.85677	0.02731	1.18	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	0.04186	0.04186	1.81	0.183
VARIETY.TIME	34	0.81579	0.02399	1.04	0.437
TIME.TMT	1	0.03182	0.03182	1.38	0.245
VARIETY.TIME.TMT	34	0.82652	0.02431	1.05	0.420
Residual	70	1.61865	0.02312		

Appendix 5-C: ANOVA of Trial 3 for nitrate reductase activity.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.3753	0.1877	1.03	
REP.Wplot stratum					
WATER	1	57.9001	57.9001	318.89	0.003
Residual	2	0.3631	0.1816	1.39	
REP.Wplot.Splot stratum					
VARIETY	7	0.8133	0.1162	0.89	0.527
WATER.VARIETY	7	0.8046	0.1149	0.88	0.533
Residual	28	3.6507	0.1304	0.85	
REP.Wplot.Splot.TIME stratum					
TIME	1	1.6615	1.6615	10.79	0.002
TIME.WATER	1	1.0290	1.0290	6.69	0.014
TIME.VARIETY	7	1.0080	0.1440	0.94	0.493
TIME.WATER.VARIETY	7	0.9808	0.1401	0.91	0.511
Residual	32	4.9254	0.1539		

Appendix 5-D: ANOVA of Trial 1 for free proline concentrations.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	8.05	4.02	0.65	
REP.Wplot stratum					
TMT	2	2181.99	1090.99	176.46	<.001
Residual	4	24.73	6.18	0.38	
REP.Wplot.Splot stratum					
VARIETY	3	120.76	40.25	2.50	0.092
TMT.VARIETY	6	348.40	58.07	3.61	0.016
Residual	18	289.48	16.08	1.52	
REP.Wplot.Splot.TIME stratum					
TIME	1	241.73	241.73	22.83	<.001
TIME.TMT	2	456.11	228.05	21.54	<.001
TIME.VARIETY	3	96.97	32.32	3.05	0.048
TIME.TMT.VARIETY	6	166.02	27.67	2.61	0.043
Residual	24	254.14	10.59		

Appendix 5-E: ANOVA of Trial 2 for free proline concentrations.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	10051.11	10051.11	894.30	0.001
Residual	2	22.48	11.24	1.47	
REP.Wplot.VARIETY stratum					
VARIETY	34	211.23	6.21	0.81	0.745
VARIETY.TMT	34	152.83	4.50	0.59	0.955
Residual	68	520.90	7.66	0.54	
REP.Wplot.VARIETY.TIME stratum					
TIME	1	19.30	19.30	1.35	0.249
VARIETY.TIME	34	136.79	4.02	0.28	1.000
TIME.TMT	1	23.12	23.12	1.62	0.207
VARIETY.TIME.TMT	34	187.05	5.50	0.39	0.998
Residual	70	999.68	14.28		

Appendix 5-F: ANOVA of Trial 3 for free proline concentrations.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.940	0.970	1.53	
REP.Wplot stratum					
TMT	1	869.908	869.908	1374.38	<.001
Residual	2	1.266	0.633	0.13	
REP.Wplot.Splot stratum					
VARIETY	7	561.475	80.211	15.98	<.001
TMT.VARIETY	7	152.201	21.743	4.33	0.002
Residual	28	140.510	5.018	0.67	
REP.Wplot.Splot.TIME stratum					
TIME	1	703.443	703.443	93.89	<.001
TIME.TMT	1	1118.678	1118.678	149.31	<.001
TIME.VARIETY	7	262.044	37.435	5.00	<.001
TIME.TMT.VARIETY	7	94.884	13.555	1.81	0.120
Residual	32	239.747	7.492		

CHAPTER 6

THE EFFECT OF DROUGHT STRESS ON YIELD, WATER USE EFFICIENCY AND TOTAL CAROTENOID CONTENT OF SWEET POTATO CULTIVARS AND BREEDING LINES.

6.1. INTRODUCTION

Drought is one of the major environmental factors limiting yield of crops. **(DE BRITO, SOFIATTI, DE ANDRADE, DE CARVALHO and DA SILVA FILHO, 2011)**.

The question one needs to ask is to what degree a specific drought affects yield. **GONZÁLEZ, MARTÍN, and AYERBE (1999)** reported that all barley genotypes tested under severe stress conditions produced lower yield compared to the controls. By means of subjecting sweet potato cultivars to normally irrigated and drought conditions, **EKANAYAKE and COLLINS (2004)** were able to classify the cultivars with regard to their ability to adapt to drought. Some cultivars were able to produce slightly larger yields than the mean and some were clearly not able to withstand the drought conditions. The effect of drought was also very clear in a study by **LEWTHWAITE and TRIGGS (2012)** who analyzed the performance of cultivars planted in two locations receiving different degrees of irrigation. Significant differences were observed in most of the genotypes, with the higher irrigated ones producing a higher yield; although some genotypes did not show any difference. **VAN HEERDEN and LAURIE (2008)** reported a severe reduction in sweet potato root yield with severe water reduction. There was a parallel decline in biomass. In pot experiments, to screen sweet potato cultivars, **SARASWATI, JOHNSTON, COVENTRY AND HOLTUM (2004)** also found severe reductions in biomass which was associated with a reduction in the wet/dry root mass.

When subjected to drought stress, plants use water sparingly due to natural adaptation **(BLUM, 2005)**. In order for the plant to survive drought stress mechanisms have to be switched on to conserve water and energy. The extent to which these mechanisms will be switched on depends on the ability of the plant to adapt to the drought conditions and therefor use water efficiently.

MUNOZ, VOLTAS, ARAUS, IGARTUA and RAMAGOSA (1998) showed that plants with a high yield potential usually have low water use efficiency (WUE) due to the high quantities of water used to produce the crop. Water use efficiency of a crop is generally defined as the ratio of total biomass to the total amount of water consumed **(SINCLAIR, TANNER and BENNETT, 1984)**. **GOMES and CARR (2003)** found with sweet potato grown in the field that the water use efficiencies were less during the rainy season than during the dry season for both vine and storage root yields.

The physiology of the plant responding to drought stress is rather complex and various modifications are displayed by the plant, during these responses. According to **DAMATTA (2004)** an example of an approach to identify and improve genotypes is to identify the traits that can contribute to drought tolerance of plant species and use these to identify promising genotypes for breeding programmes. Depending on the ultimate aim of the breeder, the choice of trait(s) selection will be influenced by the decision on the final genotype product. Relationships and correlations of these might be of great help in shortening the time period during the identification process of the preferred genotype (**SAYRE, ACEVEDO and AUSTIN, 1995)**.

The flesh of most sweet potato varieties is normally white in color, but the recent trend is to produce more orange fleshed varieties. This requires a higher concentration of β-carotene in the roots **(LOW, ARIMOND, OSMAN, CUNGUARA, ZANO and TSCHIRLEY, 2007)**. β-Carotene is the precursor of vitamin A **(PURCELL and WALTER, 1968).** This therefore favors the use of orange fleshed sweet potato as a less expensive source of dietary vitamin A for resource-poor households **(BOVELL-BENJAMIN, 2007)**. Globally 190 million (33.3%) children under the age of 5 years are vitamin A deficient, with a high prevalence of 44% in Africa **(WHO, 2009)**.

Although carotene in the roots as such does not play a role in the tolerance towards drought, it was reported that carotene and more specifically β-carotene values increase during drought **(RAUTENBACH, FABER, LAURIE and LAURIE, 2010; LAURIE, FABER, VAN JAARSVELD, LAURIE, DU PLOOY and MODISANE, 2012)**.

Determination of the carotenoid content can thus possibly aid as an indicator for drought stress in sweet potato over and above the other criteria the genotype should adhere to for possible selection as a candidate for breeding. Total carotenoid analysis were conducted in the sweet potato roots of the three trials due to the fact that β-carotene (pre-Vitamin A) contributes up to 90% of the total carotenoid content in the root **(VAN JAARSVELD, MARAIS, HARMSE, NESTEL and RODRIGUEZ-AMAYA, 2006)**.

6.2. AIM

The aim of this part of the study was to observe the response of sweet potato cultivars to drought stress with regard to yield and the consequent WUE with regard to storage root production. The analysis of the results may give an indication to assist in identifying cultivars harboring the ability to adapt to drought conditions, especially regarding yield. The aim was also to analyze the carotene content of the roots of the respective cultivars and breeding lines to determine a possible link with drought tolerance.

6.3. MATERIALS AND METHODS

6.3.1. Yield

Storage roots of trial plants (excluding border plants) were harvested manually, at the end of each trial, for each cultivar and for each repeat per treatment. The root mass was determined and the yield calculated in tons per hectare (t/ha).

6.3.2. Water use efficiency (WUE)

WUE was calculated by using the ratio of total root mass to the total amount of water consumed for each of the treatments **(SINCLAIR, TANNER and BENNETT, 1984)**. The total root yield of each cultivar and breeding line was determined and the WUE calculated according to the following formula: kg/ha/mm, where, ha, is the total area planted per repeat per cultivar and breeding line and, mm, was the amount of irrigation and/or rainfall the specific treatment received during the Trial period.

6.3.3. Carotenoid content

Sample preparation, extraction of carotenoids and determination were conducted according the method of **KIMURA, CINTIA, KOBORI, RODRIGUEZ-AMAYA and NESTEL, (2007).** Carotenoid samples were analyzed using a Beckman® DU800 spectrophotometer. Total carotenoid content was calculated according to the following formula:

Total carotenoid content (µg/g) =

$$\frac{\text{A x volume (ml) x } 10^4}{A_{1\,cm}^{1\%} \text{ x sample weight (g)}}$$

where A= absorbance; volume = total volume of extract (50 or 25 mL); $A_{1\,cm}^{1\%}$ = absorption coefficient of β−carotene in petroleum ether (2592).

6.4. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to analyze the data for each Trial (1, 2 and 3) separately. The data were analyzed as a split-plot with main plots, 2 or 3 water treatments and sub plots 4 to 35 cultivars replicated in 2 or 3 blocks to test for significant effects. The repeated measurements over time were included in the ANOVA as a sub-subplot factor. Means of significant effects were separated using Fishers' t-LSD (least significant difference) at the 5% level of significance. Statistical analyses were conducted using GenStat *for Windows* 15th Edition (VSN International, Hemel Hampstead, UK).

6.5. RESULTS AND DISCUSSION

6.5.1 Yield

6.5.1.1. Trial 1

Roots of the sweet potatoes were harvested in a large rainout shelter (LR) 6 months after the start of the Trial. Figure 6.1 displays the results of the yield obtained.

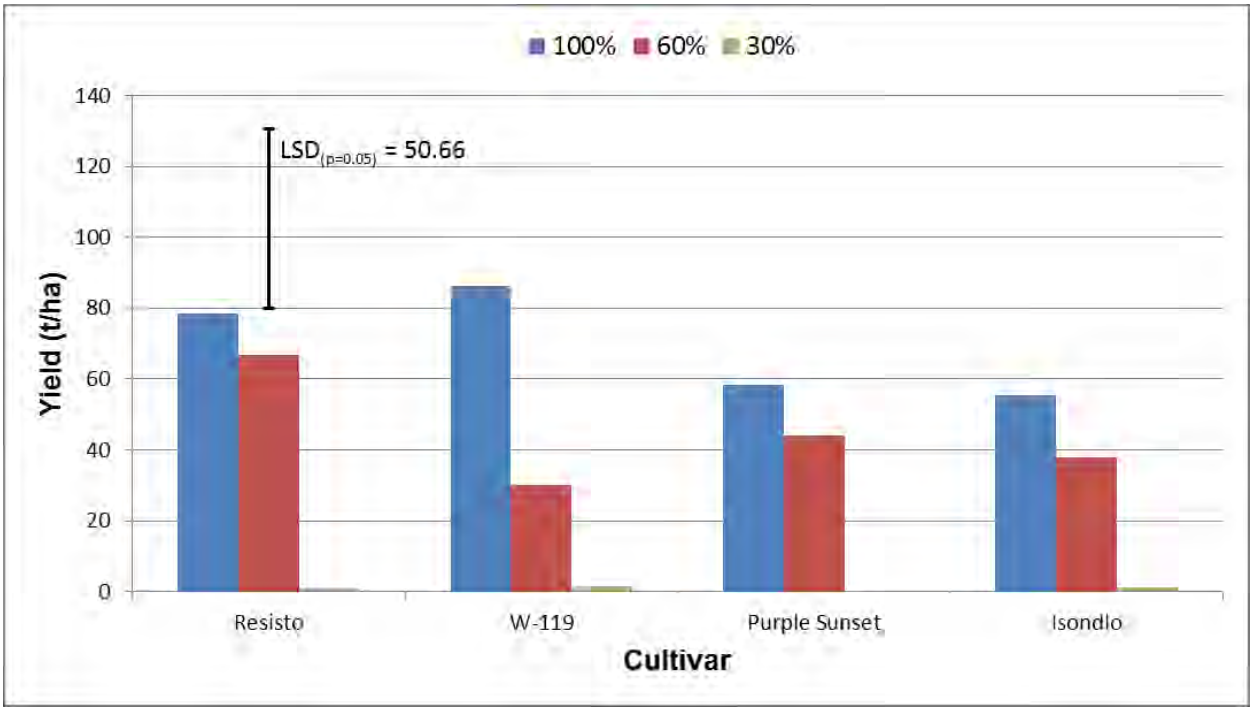

Figure 6.1 Root yield (t/ha) of four sweet potato cultivars subjected to drought stress conditions in Trial 1. $LSD_{(p=0.05)}$ = 50.66; Each value is the combined mass of the roots of 18 plants per cultivar for 3 repeats. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment

Figure 6.1 shows that the drought stress had a severe impact on the yield of all sweet potato cultivars displaying significant differences (Appendix 6-A) between the different treatments. This is confirmed by the findings of **EKANAYAKE and COLLINS (2004), LEWTHWAITE and TRIGGS (2012)** and **GOMES, CARR and SQUIRE (2005)** who found significant decreases in yield in sweet potatoes exposed to drought. In comparison with the findings of **EKANAYAKE and COLLINS (2004),** who managed to classify genotypes according to their reaction to drought, genotypic differences was not observed here. Non-Significant reduction in yield was observed between all the cultivars in the severe stress treatment while a significant reduction in yield was observed with the cultivar W-119 in the mild stress treatment. **LEWTHWAITE and TRIGGS (2012)** also observed severe significant declines in root yield of sweet potato cultivars in the field during water deficit conditions. Although W-119 produced the highest yield in control conditions it displayed high sensitivity towards drought in yielding a decline of 99% in severe stress conditions. Although no significant difference could be detected between the cultivars, Resisto,

Purple Sunset and Isondlo showed less sensitivity in that their yield ratios were better when comparing the control to the mild stress treatments. Less water could be used for the production of Resisto since a reduction of 40% in irrigation resulted in only a 15% loss in yield. This was confirmed by results shown by **VAN HEERDEN and LAURIE (2008)** who subjected two sweet potato cultivars to drought stress conditions. This indicated that these cultivars could adapt better to the lower quantities of water applied. From the results it appeared that the severe stress treatment was too harsh with regard to the provision of sufficient yield data which is not contributing to the criteria for use in the selection criteria for screening.

6.5.1.2. Trial 2

Sweet potato root yield declined severely in Trial 2 from control to drought stress conditions (Table 6.1). These results correlate well with results obtained in Trial 1 and confirmed the results obtained by **EKANAYAKE and COLLINS (2004), LEWTHWAITE and TRIGGS (2012)** and **GOMES, CARR and SQUIRE (2005)** who found large reductions in sweet potato yield in drought conditions**.** Root yield in general from Trial 2 showed a lower average total yield in the control (100%) trial area compared to Trial 3 from the same season. The reason for this could possibly be the over irrigation (irrigation plus rain fed) in control of Trial 2 resulting in a lower total yield. With regard to the drought stress treatment Resisto experienced a reduction in yield which amounting to 96% which is comparable to results found in Trial 1. This also correlates well with the findings of **VAN HEERDEN and LAURIE (2008)** who observed a reduction of 80% in yield when the cultivar Resisto was subjected to drought stress. A significant reduction (Appendix 6-B) in yield from the control to the stressed plants were observed for all the cultivars. Due to relatively low losses in yield in drought stress conditions the genotypes 2005-2-2, 2005-7-4, 2006-4-4 and 2006-7-7 indicated significant promise for adapting to drought conditions. The cultivar 2005-1-11 produced the highest and Tanzania the lowest yield under control conditions while the breeding line 2005-7-4 produced the highest and Tanzania the lowest yield in severe stress conditions. The breeding lines 2005-11-3, 2005-4-1 and cultivars Blesbok, Ndou, Resisto and Tanzania experienced

Table 6.1. Total yield (t/ha) of 35 sweet potato cultivars subjected to control and drought stress conditions

	Treatment		
Cultivar	**Control**	**Stress**	**% reduction**
Isondlo	45.29	9.06	79.9
Purple Sunset	47.62	7.73	83.7
2005-1-11	78.49	12.25	84.3
2005-11-3	41.06	1	97.5
2005-1-16	42.16	6.59	84.3
2005-12-2	35.2	2.63	92.5
2005-3-13	50.12	9.99	79.9
2005-16-1	66.7	5.66	91.5
2005-2-2	55.31	14.29	74.4
2005-3-10	53.31	4.26	91.9
2005-4-1	50.78	2.06	95.9
2005-5-5	45.52	7.03	84.5
2005-7-4	62.47	17.02	72.7
2006-14-4	37.5	6.73	82.0
2006-15-1	44.46	4.43	90.0
2006-2-4	37.6	6.69	82.2
2006-3-4	56.91	12.52	78.0
2006-4-4	52.85	13.59	74.4
2006-4-5	50.75	9.79	80.7
2006-6-2	29.87	7.33	75.4
2005-7-3	38.69	7.99	79.3
2006-7-7	43.19	10.99	74.5
2006-7-8	35.06	8.13	76.8
Beauregard	60.47	7.63	87.3
Blesbok	34.13	0.87	97.4
Bosbok	62.74	8.59	86.3
Impilo	63.04	11.19	82.2
Jewel	49.75	6.36	87.2
Lethlabula	70.76	16.38	76.8
Ndou	62.3	3.26	94.7
Phala	51.91	5.69	89.0
Resisto	40.36	1.66	95.8
Tanzania	29.3	0.7	97.6
W-119	34.13	4.6	86.5
Zapallo	72.53	4.4	93.9
Mean	49.49	7.40	85.0

$LSD_{(p=0.05)}$ = 10.922 indicated differences between the control and stress. Control = control treatment; Stress = severe stress treatment. Each value is the combined mass of five plants, per cultivar and breeding line, for 2 repeats calculated for the planting area for both the control and stress. $MSE_{(d.f.= 68)}$= 19.29

the biggest decline in yield due to the stress conditions while the breeding line 2005-7-4 displayed the least which would be an advantage to breeders in selecting a cultivar/breeding line suiting the criteria for an approved cultivar.

6.5.1.3. Trial 3

Significant (Appendix 6-C) reduction in yield between the control and severe stress treatments in all the cultivars and breeding lines were observed (Figure 6.2). The reduction in yield observed with the cultivar Resisto correlates well with results obtained by **VAN HEERDEN and LAURIE (2008)** who found a large reduction in yield in Resisto in drought conditions that was parallel to a decrease in shoot lengths. This indicated that the results were repeatable. **PRABAWARDANI (2007)** and **LEWTHWAITE and TRIGGS (2012)** also reported significantly large reductions in sweet potato yield during drought stress. **ABIDIN, VAN EEUWIJK, STAM, STRUIK, MALOSETTI, MWANGA, ODONGO, HERMANN and CAREY (2005)** also indicated significant reductions in sweet potato yield where growth took place in drought prone areas despite a high rainfall. They found that 2 drought spells played a significant role in reducing the yield.

This is in comparison of conditions in the current trial where drought was enforced throughout the growth period. In Trial 3 the cultivar 199062.1 produced a significantly higher (112 t/ha) yield in control conditions while the cultivar Hernandez displayed the lowest (42.8 t/ha). Due to their superiority regarding yield, in control conditions, it would be an advantage to select 199062.1, Mvuvhelo and Bophelo to be incorporated into a breeding programme.

No significant differences were visible between the genotypes regarding yield in severe stress conditions. The non-significant differences between the genotypes in the severe stress treatment where Resisto displayed the lowest and Bophelo and 199062.1 the highest yield respectively raises the question whether the severe stress might be too harsh to aid in the in the choice of a selection method for drought tolerant genotypes.

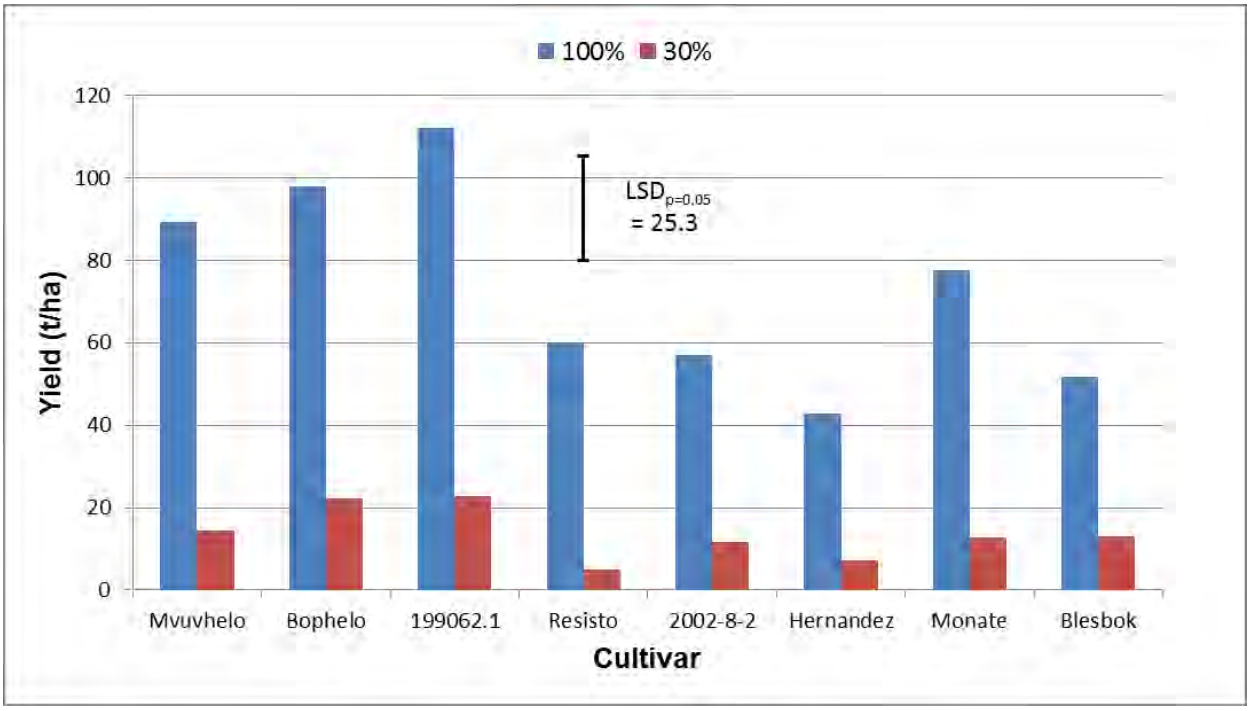

Figure 6.2. Root yield (t/ha) of eight sweet potato cultivars and breeding lines subjected to drought stress conditions in Trial 3. $LSD_{(p=0.05)}$ = 25.3; 100% = control treatment, 30% = severe stress treatment. Each value is the combined mass of the roots of 18 plants per cultivar for 3 repeats calculated from the area planted.

6.5.2. WUE

6.5.2.1 Trial 1

No significant differences were observed between the cultivars for each of the different treatment levels. Water use efficiency (WUE) declined as the stress progressed, from the control to the severe stress treatment, in all the genotypes (Figure 6.3). Yield of the same genotypes has also been shown in Figure 6.1 to decline with stress. This correlates with the reasoning of **BLUM (2005)** who argued that water use efficiency will decline with a reduction in yield. Significant declines (Appendix 6-D) were observed in WUE values, from the control to the severe stress treatment of the cultivars Resisto and Purple Sunset. The high yielding genotype W-119 displayed a smaller decrease in water use efficiency in mild stress conditions, compared to the control, in comparison to the other cultivars. This indicates that the cultivar might use water more efficiently in the mild stress conditions. **BLUM (2005)** has proved by using a high yielding wheat cultivar that an

increase in water use efficiency under drought stress conditions generally constitutes to high yield.

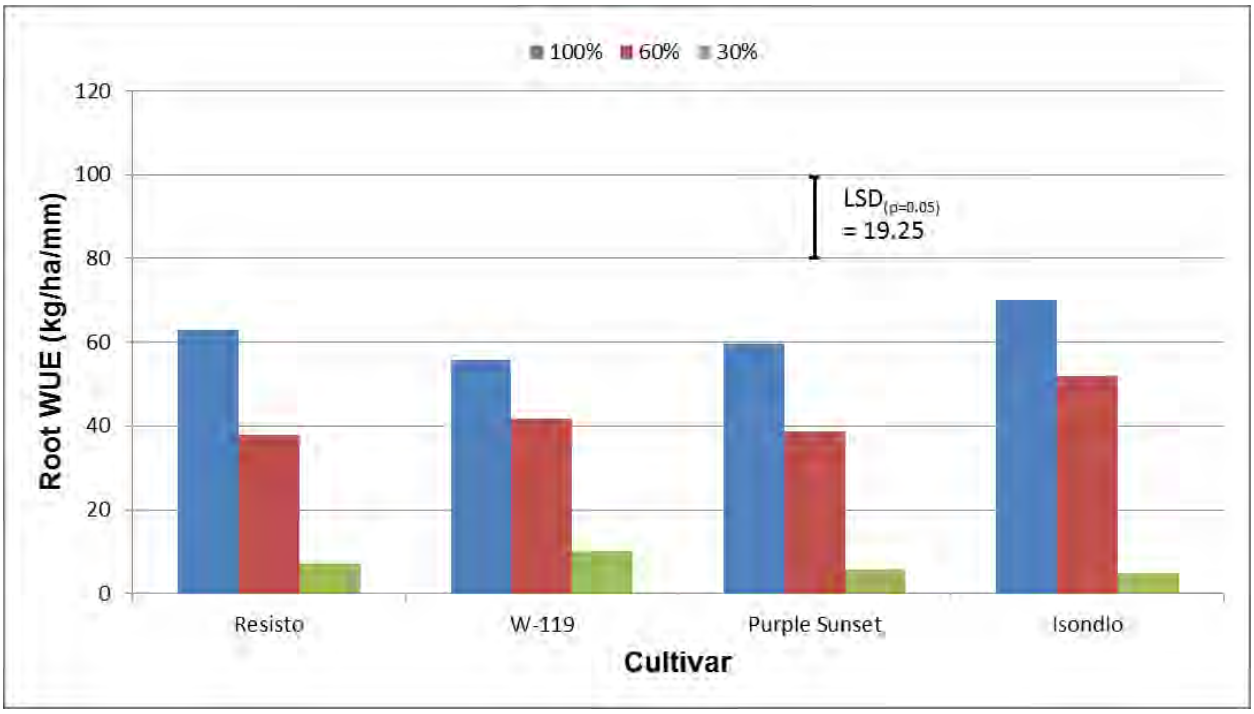

Figure 6.3 Water use efficiency (WUE) of four sweet potato cultivars subjected to drought stress in Trial 1. WUE was calculated on a root basis. 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment. $LSD_{(p=0.05)}$ = 19.25; Each value was calculated by dividing the combined root mass of 18 plants by the soil surface area and the irrigation supplied for the selected treatment.

The water use efficiency for W-119 declined less in severe stress conditions, compared to the mild stress, although not significantly different from the other genotypes. Larger differences in WUE values were observed between the mild stress and the severe stress treatments compared to the difference between the control and the mild stress treatments. The cultivar Isondlo displayed the best WUE value, in control and mild stress conditions, compared to the other cultivars although not significantly different.

6.5.2.2. Trial 2

There were significant declines in the WUE of the genotypes planted in Trial 2 (Table 6.2) with 2005-11-3, 2005-12-2, 2005-16-1, 2005-3-10, 2005-4-1, 2006-15-1, Blesbok, Ndou, Phala, Resisto, Tanzania and Zapallo displaying large reductions in severe stress

Table 6.2 Water use efficiency (WUE) in kg/ha/mm of 35 sweet potato cultivars and breeding lines subjected to control and drought stress conditions.

	Treatment		
Variety	**Control**	**Stress**	**% decline**
Isondlo	68.6	45.8	33.2
Purple Sunset	72.2	39	45.9
2005-1-11	119	61.9	47.9
2005-11-3	62.2	5.0	91.9
2005-1-16	63.9	33.3	47.8
2005-12-2	53.3	13.3	75.0
2005-3-13	76.0	50.5	33.5
2005-16-1	101.1	28.6	71.7
2005-2-2	83.8	72.2	13.5
2005-3-10	80.8	21.5	73.3
2005-4-1	77.0	10.4	86.0
2005-5-5	69.0	35.5	48.5
2005-7-4	94.7	86.0	9.1
2006-14-4	56.8	34.0	40.1
2006-15-1	67.4	22.4	66.7
2006-2-4	57.0	33.8	40.7
2006-3-4	86.2	63.3	26.5
2006-4-4	80.1	68.6	14.3
2006-4-5	76.9	49.5	35.6
2006-6-2	45.3	37.0	18.3
2006-7-3	58.6	40.4	31.0
2006-7-7	65.5	55.5	15.2
2006-7-8	53.1	41.0	22.7
Beauregard	91.6	38.5	57.9
Blesbok	51.7	4.4	91.4
Bosbok	95.1	43.4	54.3
Impilo	95.5	56.5	40.8
Jewel	75.4	32.1	57.4
Lethlabula	107.2	82.8	22.7
Ndou	94.4	16.5	82.5
Phala	78.7	28.8	63.4
Resisto	61.2	8.4	86.2
Tanzania	44.4	3.5	92.1
W-119	51.7	23.2	55.1
Zapallo	109.9	22.2	79.7
mean	75.0	37.3	50.2

$LSD_{(p=0.05)}$=47.68 indicated significant differences between the control and stress. Control= control treatment, Stress = severe stress treatment. Each value was calculated by dividing the combined root mass of five plants by the soil surface area and the irrigation supplied for the specific treatment, control or severe stress.

$MSE_{(d.f.=68)}$=217.2

conditions. A large reduction in yield was also observed with these genotypes as displayed in Table 6.1. The reduction in WUE was in accordance with **(BLUM, 2005)** who observed a decline in WUE as yield was reduced. The cultivars and breeding lines Isondlo, 2005-3-13, 2005-2-2, 2005-7-4, 2006-14-4, 2006-3-4, 2006-4-4, 2006-6-2, 2006-7-3, 2006-7-7, 2006-7-8 and Lethlabula performed the best with less reduction in WUE in the 30% treatment (stress) while 2005-7-4, 2006-7-7 and 2006-7-8 should be considered for selection in a breeding programme where water usage is to be one of the important criteria.

It is interesting to note that amongst the best performers with regard to WUE Isondlo, 2005 -2-2, 2005-7-4, 2006-3-4, 2006-4-4, 2007-7-7 and Lethalabula also indicated the least reduction in yield in the severe stress treatment (Table 6.1). This observation is helpful in the process for making a decision on the selection of appropriate genotypes to be used in the breeding programme.

6.5.2.3. Trial 3

Significant difference in WUE values between cultivars and breeding lines were observed in the different treatments (Figure 6.4). The cultivar Resisto appeared to be an inefficient user of water, in the severe stress treatment, due to its low yield produced compared to the high value of Bophelo under the same growth conditions. The cultivars Bophelo and 199062.1 both displayed the highest WUE values resulting in speculation that these two cultivars use the water supplied very efficiently in the severe stress treatment while Hernandez displayed significantly lower values that might indicate wasteful use of water. In the control treatment Resisto also displayed a low WUE value while the cultivars Bophelo and 199062.1 still exhibited the highest WUE values.

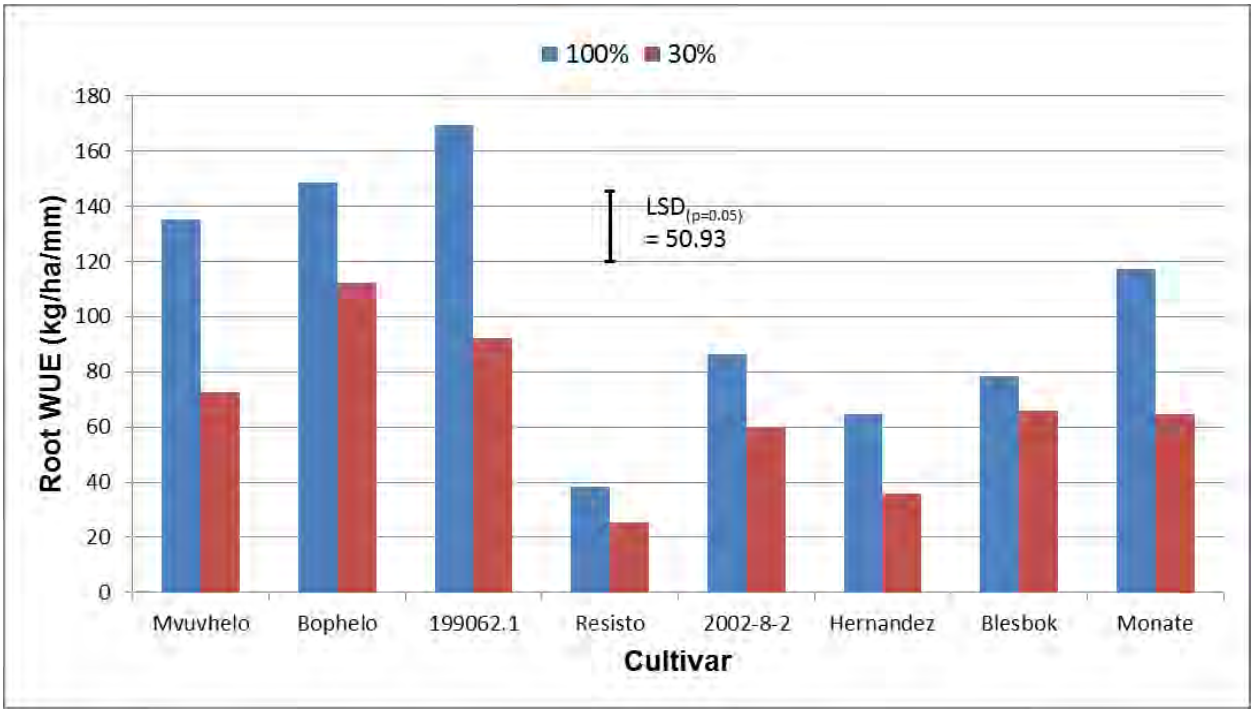

Figure 6.4 Water use efficiency (WUE) of eight sweet potato cultivars and breeding lines subjected to drought stress in Trial 3. $LSD_{(p=0.05)}$ = 50.93; 100% = control treatment, 30% = severe stress treatment. Each value was calculated by dividing the combined root mass of 18 plants by the soil surface area and the irrigation supplied for the selected treatment.

The assumption by **BLUM, 2005; MUNOZ, VOLTAS, ARAUS, IGARTUA and RAMAGOSA, (1998)** that high yield under water limited conditions are associated with reduced WUE were confirmed in these experiments with the contrary as well where the cultivar Bophelo has shown a low yield at reduced irrigation (Figure 6.2) while showing a high WUE (Figure 6.4). This then indicates that high yield combined with a high WUE cannot be used as a single criterion for screening sweet potatoes for drought tolerance but it is suggested that this criterion can be used as a tool in combination with other parameters for the successful selection of high yielding, water efficient growing sweet potato cultivars.

6.5.3 Carotenoid content

6.5.3.1 Trial 1

Significant increases in carotenoid content were observed in the roots of two sweet potato cultivars subjected to drought. (Figure 6.5).

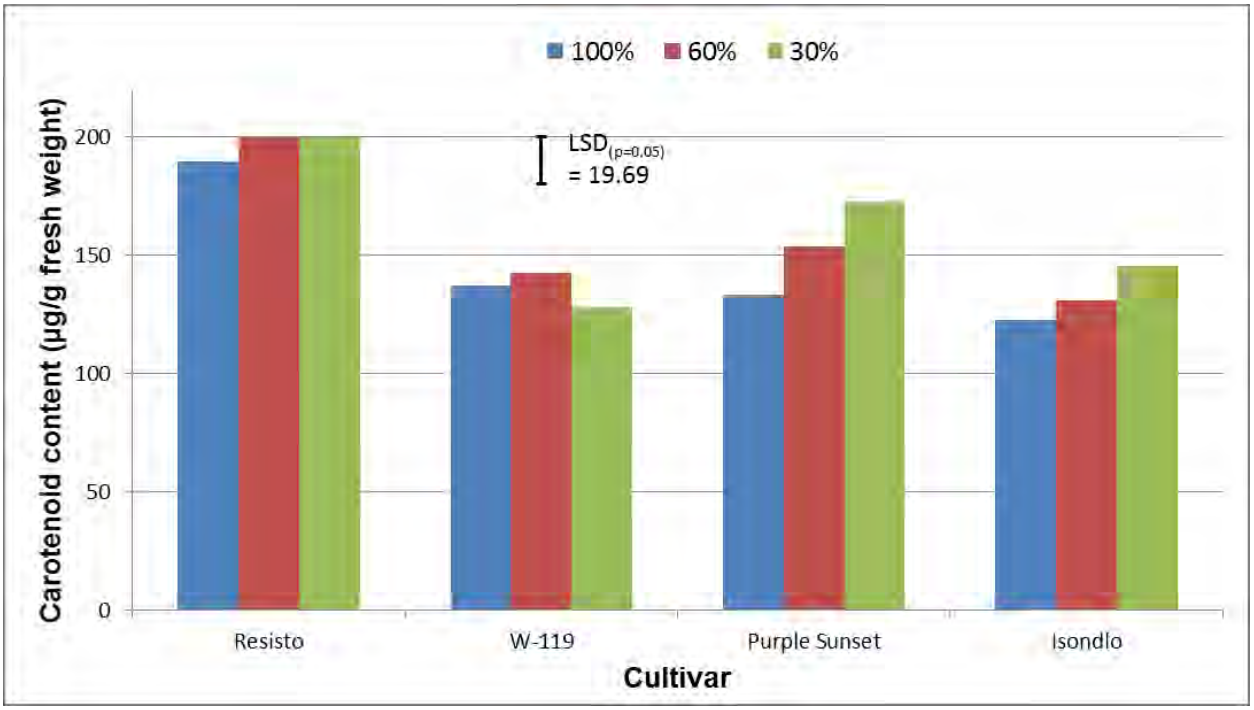

Figure 6.5. Carotenoid content of four sweet potato cultivars subjected to drought stress conditions in Trial 1. $LSD_{(p=0.05)}$ = 19.69; 100% = control treatment, 60% = mild stress treatment, 30% = severe stress treatment. Each value is the average of three repeats per cultivar for each treatment.

This observation correlated with results obtained by **(LAURIE, FABER, VAN JAARSVELD, LAURIE, DU PLOOY and MODISANE, 2012)**. Total carotenoid content in cultivar W-119 did increase with mild stress but not in severe stress, while the cultivars Purple Sunset and Isondlo displayed continued increases up to the severe stress treatment. Although not analyzed in different drought conditions **(BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO, 1999)** also found increases in the carotene content of wheat subjected to drought conditions. Increases with Purple Sunset seemed to be of the same level over the treatments while the cultivar Isondlo displayed a slightly larger increase in severe stress conditions compared to mild stress conditions.

6.5.3.2. Trial 2

Carotenoid levels for drought stressed genotypes in Trial 2 differed significantly from each other (Appendix 6-H). The majority of cultivars and breeding lines cultivated in Trial 2 exhibited high carotenoid levels (Table 6.3) which resulted in a dark yellow colour in the extraction medium. The higher carotenoid content in the drought stressed sweet potatoes correlate with findings of LAURIE, FABER, VAN JAARSVELD, LAURIE, DU PLOOY and MODISANE, (2012) and BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO, (1999) who found increases in carotenoid content in sweet potato and wheat respectively. Significant differences were observed between the control and stress in eight of the genotypes. Low carotenoid levels indicate lighter colored genotypes (TEOW, TRUONG, MCFEETERS, THOMPSON, PECOTA and YENCHO, 2007) which were originally included in the trial for the analysis of other characteristics. The carotenoid content of Resisto under stress conditions was the highest and did differ significantly from the majority of the other genotypes. If the aim is the production of genotypes that contain more carotenoids, this cultivar could be preferred in the selection process in the breeding programme. Other breeding lines to be considered could be 2005-1-11, 2005-11-3, 2006-4-5, 2006-7-7, 2006-7-3 and 2006-7-8 due to their ability to increase the carotene content. White fleshed cultivars like Bosbok, Lethlabula, Ndou and Phala indicated on average large increases in carotene content but the end product was negligible compared to the orange fleshed genotypes.

Table 6.3. Carotenoid content expressed as µg/g of 35 sweet potato cultivars and breeding lines subjected to drought.

	Treatment		
Varieties	**Control**	**Stress**	**% increase**
Isondlo	117.6	133.6	13.6
Purple Sunset	84.2	164.2	95.0
2005-1-11	163	185.5	13.8
2005-1-16	196.4	175.4	-10.6
2005-11-3	194.2	216.8	11.6
2005-12-2	208.2	175.9	-15.5
2005-16-1	159	144.8	-8.9
2005-2-2	103.3	99.1	-4.0
2005-3-10	123	178.8	45.3
2005-3-13	129.6	173.4	33.7
2005-4-1	108.8	95.5	-12.2
2005-5-5	76.1	105.3	38.3
2005-7-4	151.6	169.1	11.5
2006-14-4	152.4	156	2.3
2006-15-1	117.9	164.8	39.7
2006-2-4	90.9	147	61.7
2006-3-4	163.5	167.8	2.6
2006-4-4	129.4	149.9	15.8
2006-4-5	160.4	197.2	36.8
2006-6-2	111	124.8	12.4
2006-7-3	156.3	185.9	18.9
2006-7-7	123.7	195	57.6
2006-7-8	140	182.5	30.3
Beauregard	70.9	145.1	104.6
Blesbok	73.8	73.3	-0.6
Bosbok	1.6	2.6	0.6
Impilo	79.1	79.2	0.001
Jewel	84.9	64	-24.6
Lethlabula	0.9	1.9	111.1
Ndou	5.4	22.4	314.8
Phala	2.2	3.1	40.9
Resisto	130.9	227.2	73.5
Tanzania	5.3	27.8	424.5
W-119	75.9	130.5	71.2
Zapallo	28.6	45.1	57.6
mean	106.2	128.8	21.2

$LSD_{(p=0.05)}$ = 44.03 indicated significant differences between the control and stress. Control= control treatment; Stress = severe stress treatment.
Each value was calculated by dividing the combined root mass of five plants by the soil surface area and the irrigation supplied for the specific treatment, control or severe stress.
$MSE_{(df=63)}$=466.9

6.5.3.3. Trial 3

Increases in total carotenoid levels were observed in six of the eight cultivars subjected to drought stress of which two, Resisto and 2002-8-2, were significant (Figure 6.6). The higher total carotenoid levels in sweet potato in the severe stress treatment confirms the findings by **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)**, who indicated an increase in carotene content in wheat after drought stress.

LAURIE, FABER, VAN JAARSVELD, LAURIE, DU PLOOY and MODISANE (2012) also found increases in the carotenoid content of sweet potato subjected to drought stress. The white fleshed cultivars Blesbok, Monate and Mvuvhelo showed very low carotenoid levels (as expected) with 199062.1 showing a significantly higher content than the first three.

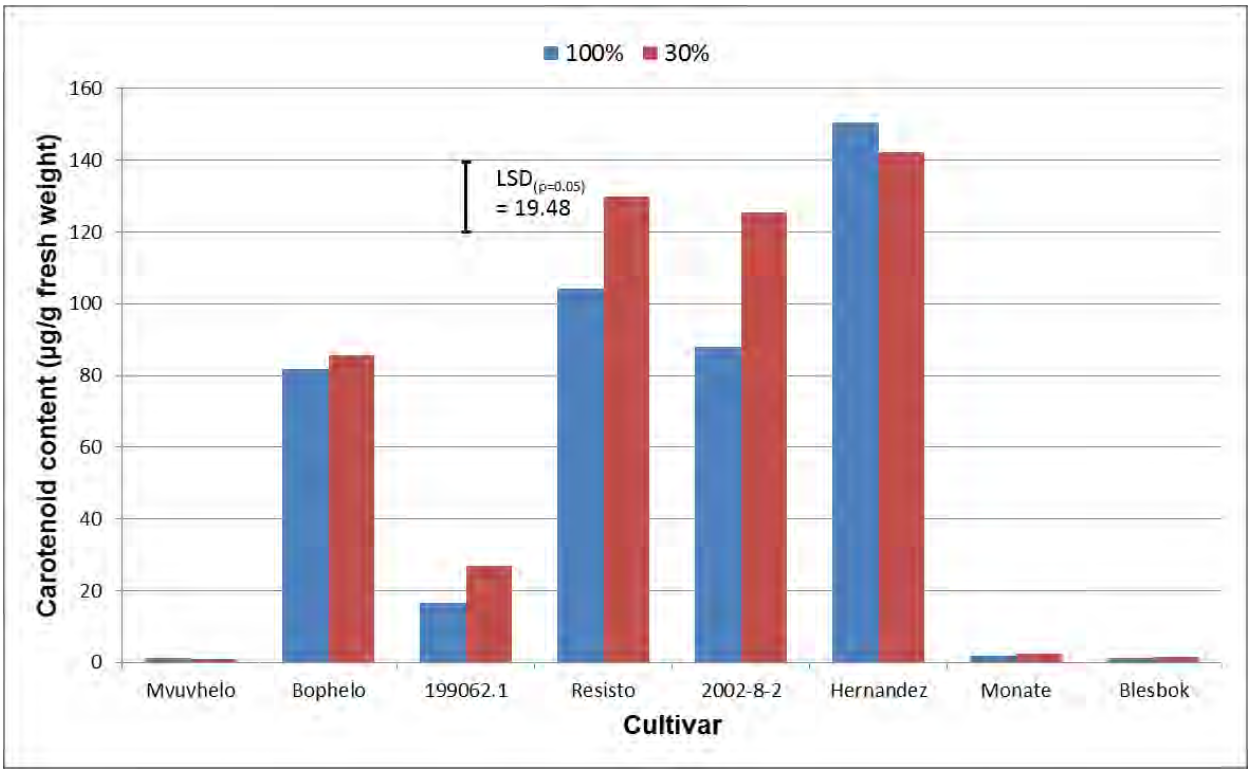

Figure 6.6. Carotenoid content of eight sweet potato cultivars subjected to drought stress conditions in Trial 3. $LSD_{(p=0.05)}$ = 19.48; Each value is the average of three repeats per cultivar for each treatment. 100% = control treatment, 30% = severe stress treatment

This correlates with the findings of **TEOW, TRUONG, MCFEETERS, THOMPSON, PECOTA and YENCHO (2007)** who also found less carotenoids in lighter fleshed sweet potatoes. The genotypes Resisto and 2008-8-2 showed significant increases in the carotene content with the drought treatment while Hernandez featured a slight decrease. All 3 genotypes displayed significantly higher carotenoid concentrations than Bophelo which indicates possible preference as choices for selection.

6.6. CONCLUSIONS

The present study confirms significant reduction in yield of all the genotypes in the three trials especially in severe stress conditions treatment. The severe stress treatment seemed to be too severe to possibly aid in the classification of drought tolerant sweet potato genotypes with regard to yield, while the mild stress treatment displayed clearer yield differences between the genotypes although not significant.

WUE values followed the trend of decline as yield is reduced in all three trials and enabled the classification of some genotypes with regard to their ability to grow in drought conditions.

Carotenoid concentrations increased during drought stress in both the orange colored and “white” colored sweet potato root flesh in all three trials. The mild stress treatment can be recommended for achieving an appreciable amount of carotenoid while the genotype still supplied a good yield. Although the severe stress treatment indicated the highest carotenoid content in the root material the yield, in severe stress conditions, is so low that it does not make it economically viable.

6.7. APPENDICES

Appendix 6-A: ANOVA of Trial 1 for total yield.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2520.4	1260.2	0.70	
REP.Wplot stratum					
TMT	2	35141.0	17570.5	9.71	0.029
Residual	4	7235.1	1808.8	3.98	
REP.Wplot.Splot stratum					
VARIETY	3	4085.4	1361.8	2.99	0.058
TMT.VARIETY	6	3818.1	636.4	1.40	0.268
Residual	18	8185.7	454.8		

Appendix 6-B: ANOVA of Trial 2 for total yield.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	62014.03	62014.03	212.02	0.005
Residual	2	584.97	292.49	15.16	
REP.Wplot.VARIETY stratum					
VARIETY	34	7874.67	231.61	12.01	<.001
VARIETY.TMT	34	4271.69	125.64	6.51	<.001
Residual	68	1311.65	19.29		

Appendix 6-C: ANOVA of Trial 3 for yield.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	62.2	31.1	0.22	
REP.Wplot stratum					
WATER	1	43027.9	43027.9	299.31	0.003
Residual	2	287.5	143.8	0.59	
REP.Wplot.Splot stratum					
VARIETY	7	9536.5	1362.4	5.62	<.001
WATER.VARIETY	7	3963.0	566.1	2.34	0.052
Residual	28	6787.9	242.4		

Appendix 6-D: ANOVA of Trial 1 for WUE.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	941.30	470.65	2.39	
REP.Wplot stratum					
WATER	2	21963.40	10981.70	55.79	0.001
Residual	4	787.31	196.83	2.47	
REP.Wplot.Splot stratum					
VARIETY	3	226.81	75.60	0.95	0.437
WATER.VARIETY	6	233.16	38.86	0.49	0.809
Residual	18	1432.54	79.59		

Appendix 6-E: ANOVA of Trial 2 for WUE.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum					
TMT	1	49527.6	49527.6	6.68	0.123
Residual	2	14818.0	7409.0	34.11	
REP.Wplot.VARIETY stratum					
VARIETY	34	42316.4	1244.6	5.73	<.001
VARIETY.TMT	34	14732.0	433.3	1.99	0.008
Residual	68	14772.3	217.2		

Appendix 6-F: ANOVA or Trial 3 for WUE.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	152.	76.	0.18	
REP.Wplot stratum					
WATER	1	17983.	17983.	42.23	0.023
Residual	2	852.	426.	0.42	
REP.Wplot.Splot stratum					
VARIETY	7	53145.	7592.	7.55	<.001
WATER.VARIETY	7	5827.	832.	0.83	0.573
Residual	28	28149.	1005.		

Appendix 6-G: ANOVA of Trial 1 for carotenoid content.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	31.9	15.9	0.12	
REP.Wplot stratum					
TMT	2	1576.3	788.2	5.78	0.066
Residual	4	545.3	136.3	1.02	
REP.Wplot.Splot stratum					
VARIETY	3	23167.2	7722.4	57.57	<.001
TMT.VARIETY	6	2061.5	343.6	2.56	0.057
Residual	18	2414.6	134.1		

Appendix 6-H: ANOVA of Trial 2 for carotenoid content.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP.Wplot stratum						
TMT	1		17857.3	17857.3	16.07	0.057
Residual	2		2222.0	1111.0	2.38	
REP.Wplot.VARIETY stratum						
VARIETY	34		478440.1	14071.8	30.14	<.001
VARIETY.TMT	31	(3)	32256.2	1040.5	2.23	0.004
Residual	63	(5)	29415.2	466.9		

Appendix 6-I: ANOVA of Trial 3 for carotenoid content.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
REP stratum	2		256.7	128.4	0.61	
REP.Wplot stratum						
WATER	1		917.0	917.0	4.35	0.172
Residual	2		421.9	211.0	1.75	
REP.Wplot.Splot stratum						
VARIETY	7		151170.6	21595.8	179.00	<.001
WATER.VARIETY	7		2465.7	352.2	2.92	0.023
Residual	24	(4)	2895.6	120.6		

CHAPTER 7

POSSIBLE RELATIONSHIPS BETWEEN MEASURED PARAMETERS FOR SCREENING SWEET POTATO IN DIFFERENT TRIALS.

7.1. INTRODUCTION

Reports of the usage of correlations and relationships between different parameters are abundant for a variety of crops such as sugar beet (**MONTI, AMADUCCI, PRITONI and VENTURI, 2006**; **BLOCH, HOFFMANN and MÄRLÄNDER, 2006),** coffee (**DA MATTA, 2004),** potato (**DEBLONDE, HAVERKORT and LEDENT, 1999)** and wheat (**MONNEVEUX, REYNOLDS, TRETHOWAN, GONZÁLEZ-SANTOYO, PEÑA and ZAPATA, 2005). AKHTER, SABIR, LATEEF, ASHRAF and HAQ (2008)** reported significant positive correlations between carbon isotope discrimination (Δ) and grain yield $WUE_{(grain)}$ in bread wheat genotypes. These results indicated that Δ can be used as a reliable indirect tool for the selection of high grain yield and WUE genotypes. **DEBLONDE, HAVERKORT and LEDENT (1999)** however, did not find a good relationship between Δ and WUE although the relationship between Δ and dry matter of potato was very positive. **CANAVAR, GÖTZ, ELLMER, CHMIELEWSKI and KAYNAK (2014)** found significant correlations between leaf area, chlorophyll, WUE, RWC, dry weight, proline and Δ in sunflower. This suggests that since a successful correlation was obtained between morphological parameters in sweet potato (**MAQUIA, MUOCHA, NAICO, MARTINS, GOUVEIA, ANDRADE, GOULAO and RIBEIRO, 2013)** the correlation between morphological and physiological traits is an option to investigate to assist in the selection process for the ideal genotype for drought tolerance.

7.2. AIM

The aim was to establish possible relations and correlations between different parameters during the measurement of sweet potato performance under normal and drought stress conditions which might give insight into the reactions of certain genotypes under these conditions and aid in possible selection into a breeding programme.

7.3. MATERIALS AND METHODS

Correlation of data from the different parameters measured in the trials were combined to establish how well they were related. A Pearson correlation approach was used to establish the possible linear relationship between two sets of data.

7.4. RESULTS AND DISCUSSION

7.4.1. Possible relationships between measured parameters for screening sweet potato for the different trials.

7.4.1.1. Trial 1

A strong significant correlation was observed between various measured parameters in Trial 1 (Table 7.1). Yield (t/ha) was positively correlated with WUE and g_s but poorly correlated with Δ (Figure 7.1). The poor correlation of yield with Δ correlates well with the findings of **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1998)** showing poor correlation between yield and Δ in cotton. The correlation between yield and g_s (Figure 7.1) confirms the expectations that yield will be reduced when the plant is experiencing a decline in stomatal conductance. The results of **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** confirmed this finding although the correlation was not as strong. This is also true with WUE where a decline in yield will be accompanied by a decline in WUE **(BLUM, 2005)**. Poor correlation between Δ and g_s contradicts the assumption that a reduction in g_s will result in a decline in Δ due to the fact that the stomata closes and therefor reduces the abundance of ^{12}C in relation to ^{13}C and thereby reducing the discrimination factor. This finding is consistent with results observed by **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** although the correlation was less pronounced. A significant, although not very strong, correlation was found between yield and stem length which agrees with the findings of **SAYRE, ACEVEDO and AUSTIN (1995)** who also observed less reduction in

canopy cover with higher yield in wheat. Positive relationships (significant) were also observed between proline, GR and AP confirming the argument that proline might act as a stabilizing factor during stress to provide support for optimum enzyme activity during the stress **(PEDERSEN, FELDNER and ROSENDAHL, 1996)**.

7.4.1.2. Trial 2

A strong correlation between yield and LAI was observed indicating the effect that leaf coverage has on the root production of the plant. These results correlate with the findings of **SAYRE, ACEVEDO and AUSTIN (1995)** who also found significant correlation between yield and % reduction in plant height in wheat. Strong correlation was observed between yield (t/ha) and nitrate reductase (NR) activity as well as yield and CCI values (Table 7.2). This agrees with the finding of **GARKAR, BHARUD and MATE (2011)** who found that in sugar cane a parallel reduction in total chlorophyll content, nitrate reductase activity and yield when subjected to drought. NR is responsible for the formation of ammonia to be used for the synthesis of chlorophyll. Reduction in NR activity will therefor decreases the supply of N for the formation of chlorophyll **(GARKAR, BHARUD and MATE, 2011)** and will cause a decline in chlorophyll content. This explains the relationship between NR and CCI as shown in Table 7.2. Since the decline in NR will have a negative effect on the chlorophyll content the photosynthetic processes should be affected which will in turn affect the formation of storage roots in the plant.

Table 7.1. Pearson correlation coefficients among 14 parameters of four sweet potato genotypes subjected to three water treatments in Trial 1.

Variables	LAI	cm	GR	SOD	AP	CCI	Δ	g_s	RWC	NR	proline	t/ha	WUE	carotene
LAI	**1**	-	**-**	-	-	-	**-**	**-**	-	-	**-**	-	**-**	-
cm	0.251	**1**	**-**	-	**-**	-	-	**-**	**-**	**-**	**-**	**-**	**-**	-
GR	**0.469**	**0.548**	**1**	**-**	**-**	-	-	**-**	**-**	**-**	**-**	**-**	**-**	-
SOD	0.218	0.121	**0.405**	**1**	-	-	-	**-**	-	**-**	-	**-**	**-**	-
AP	0.308	**0.472**	**0.392**	0.150	**1**	-	-	-	**-**	**-**	**-**	**-**	**-**	-
CCI	0.263	0.037	0.320	0.334	0.053	**1**	-	-	-	**-**	-	-	-	**-**
Δ	**0.388**	0.213	0.092	0.231	0.117	0.140	**1**	**-**	-	-	-	-	-	-
g_s	**0.690**	**0.414**	**0.552**	**0.479**	0.264	0.272	**0.382**	**1**	-	**-**	**-**	**-**	**-**	-
RWC	0.245	**0.578**	**0.647**	0.224	**0.408**	0.249	0.130	0.251	**1**	**-**	**-**	**-**	**-**	-
NR	0.239	**0.465**	**0.578**	**0.425**	**0.483**	**0.420**	0.027	**0.365**	**0.644**	**1**	**-**	**-**	**-**	-
proline	**0.423**	**0.598**	**0.621**	0.178	**0.448**	0.184	0.032	**0.434**	**0.558**	**-0.440**	**1**	**-**	**-**	-
t/ha	0.326	**0.526**	**0.633**	**0.374**	**0.417**	0.169	0.017	**0.501**	**0.512**	**0.633**	**0.503**	**1**	**-**	-
WUE	**0.487**	**0.587**	**0.728**	**0.395**	**0.445**	0.308	0.076	**0.608**	**0.664**	**0.611**	**0.680**	**0.698**	**1**	-
carotene	0.238	0.101	0.300	0.073	0.021	**0.658**	0.145	0.112	0.249	-0.126	0.295	0.082	0.247	**1**

Values in bold are different from 0 with a significance level alpha=0.05. Figures in red indicate strong relationships.

Leaf area index (LAI), stem length (cm), glutathione reductase (GR), super oxide reductase (SOD), ascorbate peroxidase (AP), chlorophyll content index (CCI), carbon isotope discrimination (Δ), nitrate reductase (NR), free proline, stomatal conductance (g_s), relative water content (RWC), root yield (t/ha), water use efficiency (WUE) and carotenoid concentration (carotene)

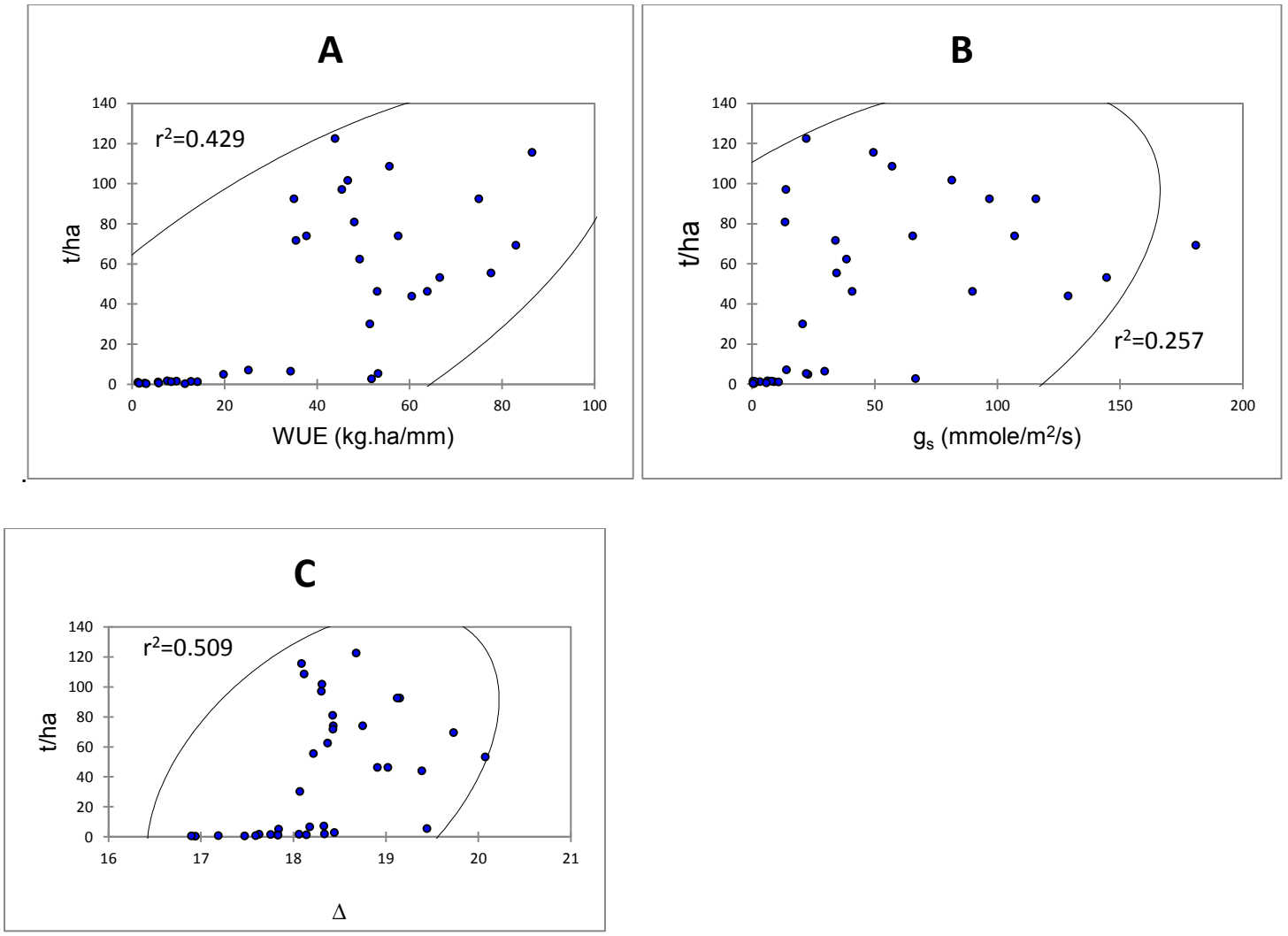

Figure 7.1. Scatter plots with confidence ellipses of linear relationship between t/ha, WUE (A), g_s (B) and Δ (C).

A strong correlation between the CCI and yield was observed which could explain why yield declines as the CCI values drop. Drought causes radicals to form and should the plant's antioxidant system not cope with the stress conditions the chlorophyll will be broken down and cause the photosynthetic apparatus of the plant to be damaged or destroyed **(MAFAKHERI, SIOSEMARDEH, BAHRAMNEJAD, STRUIK and SOHRABI, 2010)**. The rather weak correlation between yield and carbon isotope discrimination (Δ)(Figure 7.2), which agrees with the findings of **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** who found poor correlation in cotton, is disappointing since a strong correlation would provide a means of possibly assisting the selection for high yielding genotypes. The same could also be said where the correlation between WUE and Δ was also average although it could be used together with other parameters in the selection process for the ideal genotype to be cultivated in drought conditions. The correlation between yield and g_s was not as strong as in Trial 1 and might be due to the difference in growth conditions of the trial. Since the control of Trial 1 was planted in the open field, and also received rain, and the control of Trial 2 was planted in a rainout shelter, this could cause a difference in the reaction of the plants in the control treatment of both trials. The strong negative correlation between SOD and CCI (Figure 7.3) indicated that SOD activity is strongly enhanced when the CCI values decreases. This agrees with the findings of **KIM, KIM, LEE and KWAK (2009)** who found strong correlation between SOD levels and CCI values in aging sweet potato leaves. This could be due to the high concentration of oxygen radicals being formed during the stress period causing the degradation of chlorophyll but also the increase of SOD activity to combat radical formation **(LEI, YIN and LI, 2006).**

Table 7.2. Pearson correlation coefficients among 13 parameters on 35 sweet potato genotypes subjected to two water treatments in Trial 2.

Variables	LAI	GR	SOD	AP	CCI	Δ	g_s	NR	proline	t/ha	WUE	carotene
LAI	**1**	-	-	-	-	-	-	-	-	-	-	-
GR	**-0.640**	**1**	-	-	-	-	-	-	-	-	-	-
SOD	**-0.678**	**0.673**	**1**	-	-	-	-	-	-	-	-	-
AP	**-0.344**	**0.356**	**0.531**	**1**	-	-	-	-	-	-	-	-
CCI	**0.662**	**-0.779**	**-0.832**	**-0.467**	**1**	-	-	-	-	-	-	-
Δ	**0.538**	**-0.484**	**-0.519**	**-0.268**	**0.542**	**1**	-	-	-	-	-	-
g_s	**0.457**	**-0.481**	**-0.578**	**-0.336**	**0.535**	**0.330**	**1**	-	-	-	-	-
NR	**0.713**	**-0.793**	**-0.826**	**-0.534**	**0.897**	**0.560**	**0.639**	**1**	-	-	-	-
prolien	**-0.720**	**0.760**	**0.822**	**0.420**	**-0.875**	**-0.552**	**-0.627**	**-0.887**	**1**	-	-	-
t/ha	**0.711**	**-0.740**	**-0.812**	**-0.467**	**0.840**	**0.595**	**0.580**	**0.884**	**-0.851**	**1**	-	-
WUE	**0.643**	**-0.532**	**-0.624**	**-0.247**	**0.566**	**0.551**	**0.461**	**0.592**	**-0.639**	**0.835**	**1**	-
carotene	-0.109	**0.344**	0.107	-0.144	**-0.194**	0.014	-0.106	-0.151	**0.236**	**-0.220**	**-0.206**	**1**

Values in bold are different from 0 with a significance level alpha=0.05. Figures in red indicate strong relationships.

Leaf area index (LAI), glutathione reductase (GR), super oxide reductase (SOD), ascorbate peroxidase (AP), chlorophyll content index (CCI), carbon isotope discrimination (Δ), nitrate reductase (NR), free proline, stomatal conductance (g_s), root yield (t/ha), water use efficiency (WUE) and carotenoid concentration (carotene)

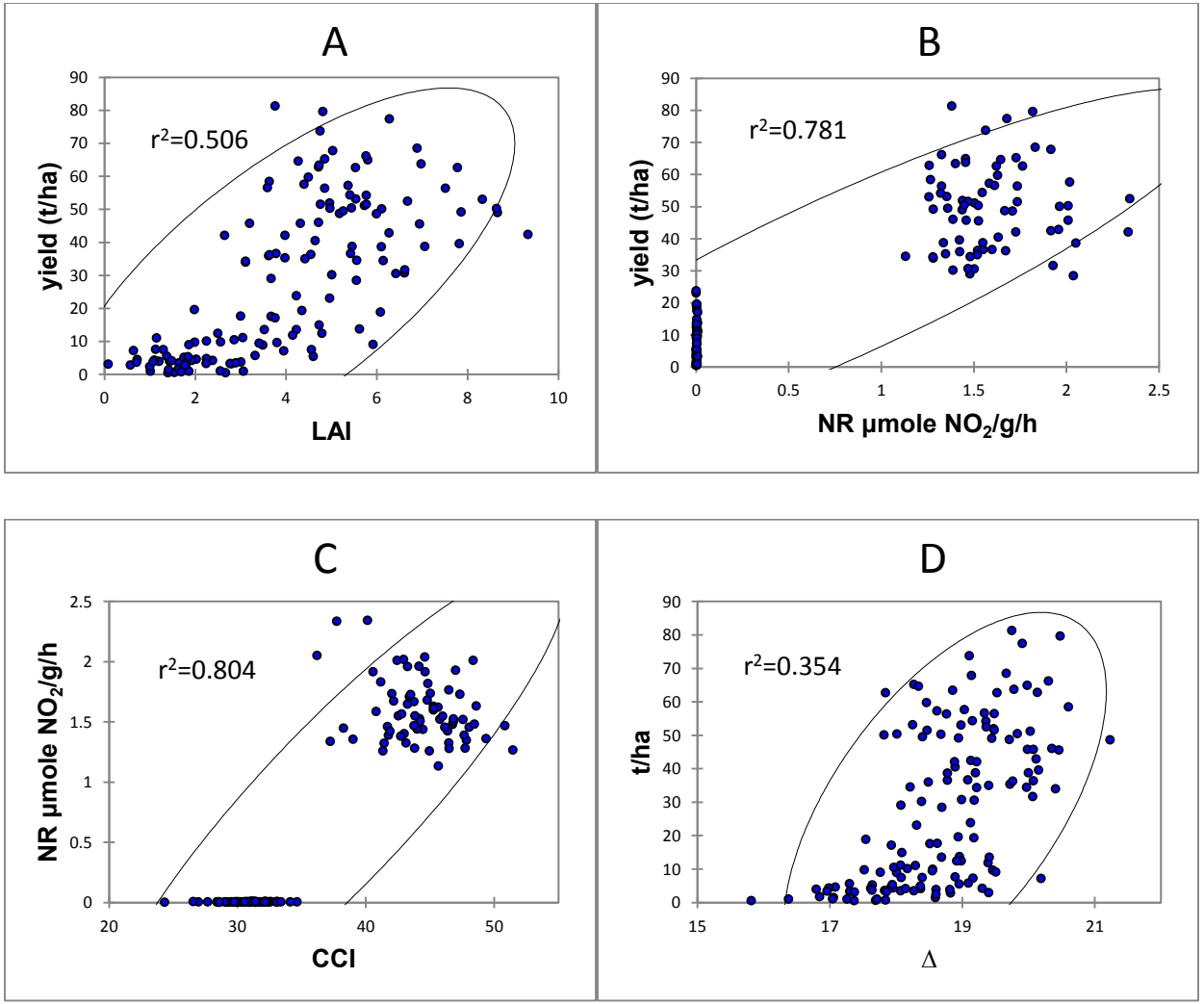

Figure 7.2. Scatter plots with confidence ellipses of linear relationship between t/ha and LAI (A), t/ha and NR (B), NR and CCI (C) and t/ha and Δ (D).

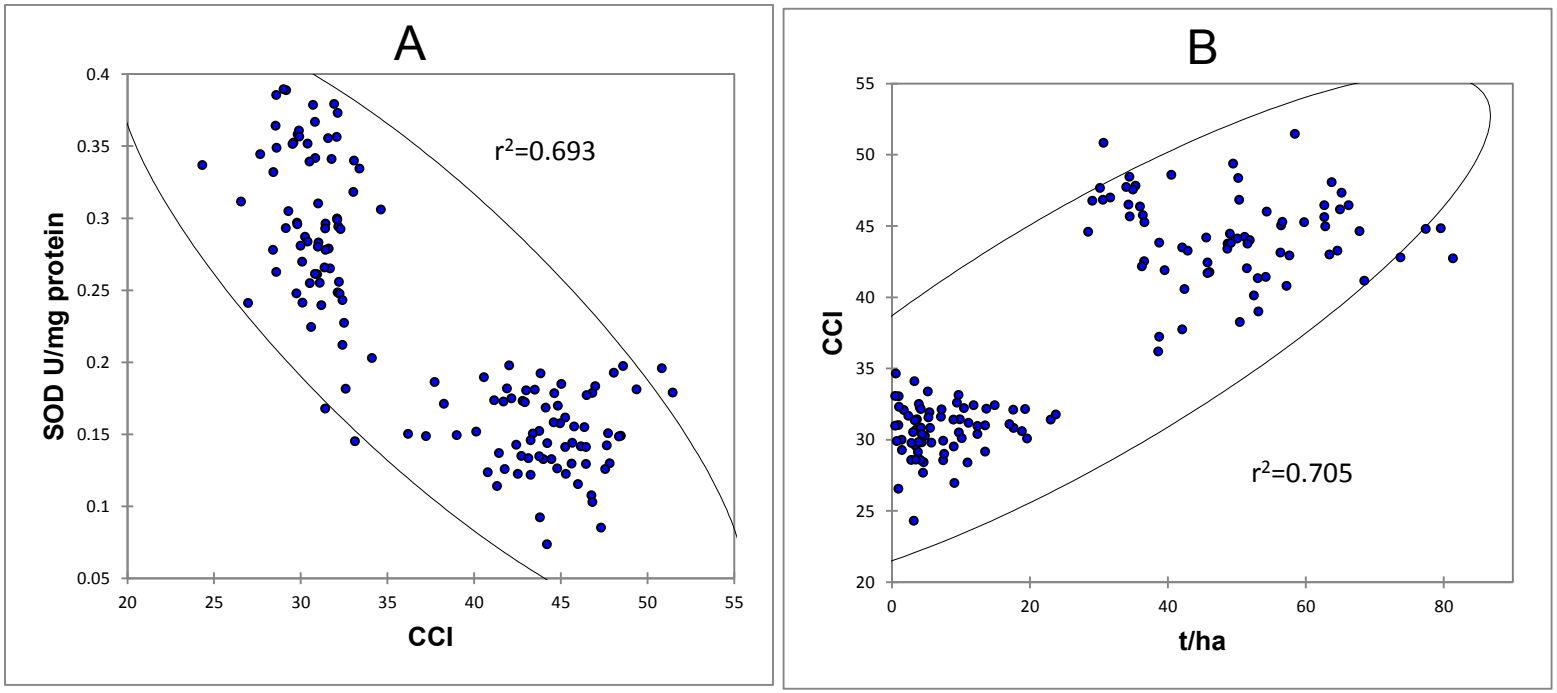

Figure 7.3. Scatter plots with confidence ellipses of linear relationship between SOD and CCI (A) and CCI and yield (B).

7.4.1.3. Trial 3

Strong positive and negative relationships between various parameters are illustrated in Table 7.3. A positive relationship was observed between free proline and the enzymes GR and AP (Figure 7.4). Although **LEI, YIN and LI (2006)** did not specifically do a correlation between the antioxidant enzymes and proline levels in poplar leaves they found parallel increases in concentrations and activity as drought progresses. It was shown in Chapter 3, that during drought stress the antoxidant enzyme activity of GR and AP will increase to combat the effects of the stress. This was confirmed by **LEI, YIN and LI (2006)** who indicated a rise in hydrogen peroxide levels in poplar leaves that led to an increase in AP levels. It was also shown in Chapter 5 that the free proline levels in the leaves increase in the case of drought stress, in sweet potato, to provide stability to the cell structure (**VAN RENSBURG, KRÜGER and KRÜGER, 1993** and **RUDOLPH, CROWE and CROWE, 1986)** as well as supporting the proteins i.e. enzymes. It is seen that in general with the sweet potatoes investigated in this trial that an increase in stomatal conductance, either displaying drought tolerance or irrigated conditions, resulted in an increase in Δ values with a possible increase in photosynthetic capacity. **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** also found positive correlations between stomatal conductance and Δ as well as photosynthetic rate which confirms the possible correlation with photosynthetic capacity. A fairly strong positive correlation (0.681) between yield and Δ was observed indicating that Δ can be used as a possible selection tool for high yielding sweet potato genotypes. The strong correlation is confirmed by the findings of **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** who showed a good relationship between yield and Δ in cotton subjected to drought. The determination of the carbon isotope could be time-saving for selection purposes. Although a good relationship was observed between WUE and yield (0.796) the relationship of WUE to Δ was poor and not significant. A significant strong correlation was observed between NR and g_s (Figure 7.5) was observed confirming the findings of **FERRARIO-MÉRY, VALADIER and FOYER (1998)** who indicated a strong relationship between nitrate reductase activity and stomatal conductance while studying the effect of drought stress on tobacco plants.

Table 7.3. Pearson correlation coefficients measured among the 14 parameters on eight sweet potato genotypes subjected to two water treatments in Trial 3.

Variables	LAI	cm	GR	SOD	AP	CCI	Δ	NR	proline	g_s	RWC	t/ha	WUE	carotene
LAI	**1**	-	-	-	-	-	-	-	-	-	-	-	-	-
cm	**0.382**	**1**	-	-	-	-	-	-	-	-	-	-	-	-
GR	**-0.289**	**-0.566**	**1**	-	-	-	-	-	-	-	-	-	-	-
SOD	-0.251	**-0.360**	**0.475**	**1**	-	-	-	-	-	-	-	-	-	-
AP	**-0.401**	**-0.519**	**0.478**	**0.451**	**1**	-	-	-	-	-	-	-	-	-
CCI	0.143	0.254	**-0.498**	-0.192	**-0.299**	**1**	-	-	-	-	-	-	-	-
Δ	**0.415**	**0.614**	**-0.758**	**-0.570**	**-0.703**	**0.441**	**1**	-	-	-	-	-	-	-
NR	**0.563**	**0.700**	**-0.707**	**-0.525**	**-0.726**	**0.554**	**0.841**	**1**	-	-	-	-	-	-
proline	**-0.502**	**-0.648**	**0.767**	**0.605**	**0.756**	**-0.478**	**-0.851**	**-0.917**	**1**	-	-	-	-	-
g_s	**0.388**	**0.585**	**-0.707**	**-0.511**	**-0.696**	**0.497**	**0.815**	**0.847**	**-0.867**	**1**	-	-	-	-
RWC	0.212	**0.523**	**-0.651**	**-0.327**	-0.250	0.268	**0.419**	**0.473**	**-0.583**	**0.532**	**1**	-	-	-
t/ha	**0.468**	**0.406**	**-0.563**	**-0.508**	**-0.611**	**0.521**	**0.681**	**0.764**	**-0.757**	**0.701**	**0.344**	**1**	-	-
WUE	0.263	0.153	-0.160	-0.194	-0.235	0.244	0.236	**0.352**	**-0.324**	**0.329**	0.129	**0.796**	**1**	-
carotene	-0.141	-0.282	0.114	0.092	0.223	0.132	-0.276	-0.177	0.173	-0.141	0.147	-0.259	**-0.307**	**1**

Values in bold are different from 0 with a significance level alpha=0.05. Figures in red indicate strong relationships.

Leaf area index (LAI), stem length (cm), glutathione reductase (GR), super oxide reductase (SOD), ascorbate peroxidase (AP), chlorophyll content index (CCI), carbon isotope discrimination (Δ), nitrate reductase (NR), free proline (proline), stomatal conductance (g_s), relative water content (RWC), root yield (t/ha), water use efficiency (WUE) and carotenoid concentration (carotene)

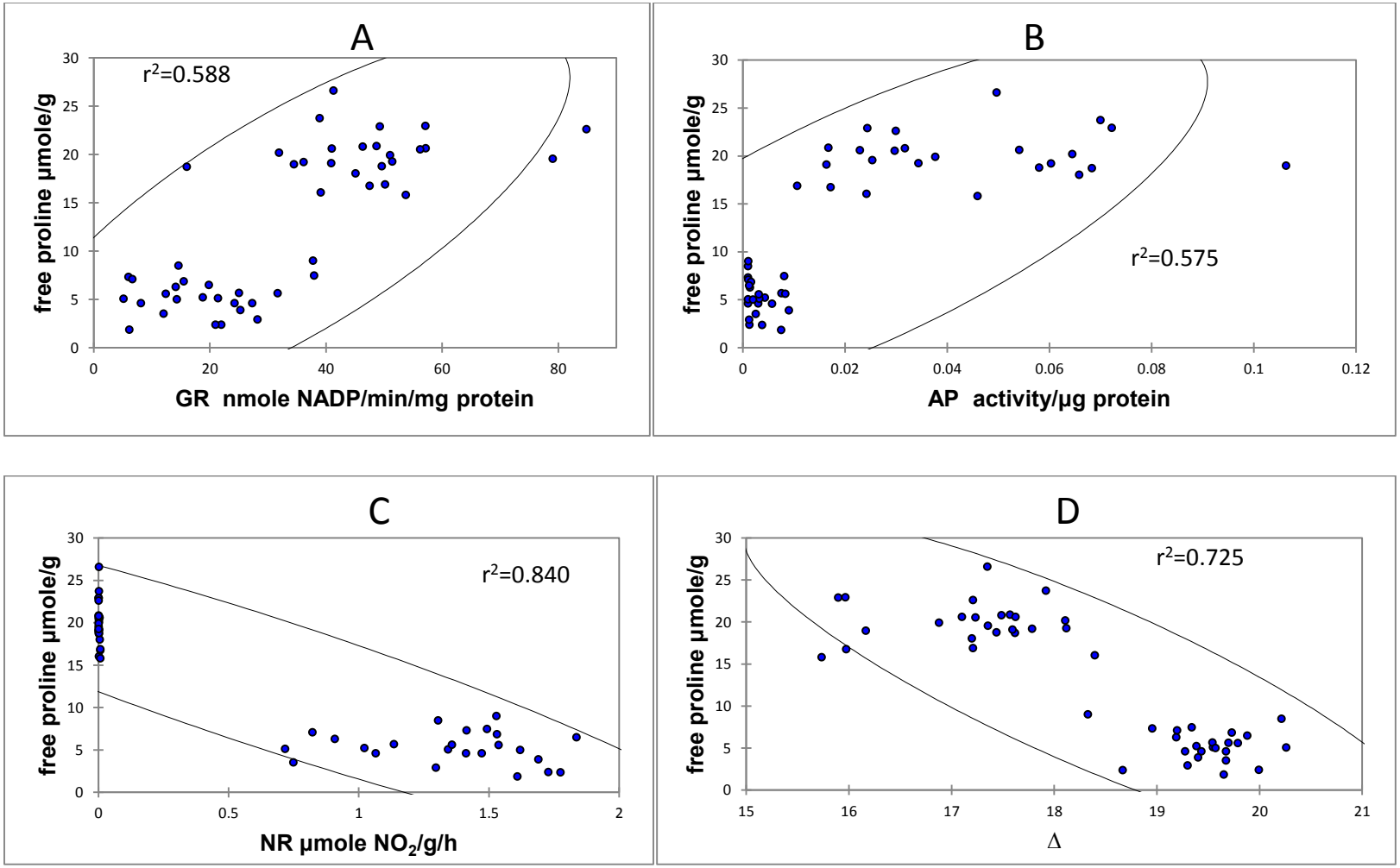

Figure 7.4. Scatter plots with confidence ellipses of linear relationships between free proline and GR (A), AP (B), NR (C) and Δ (D).

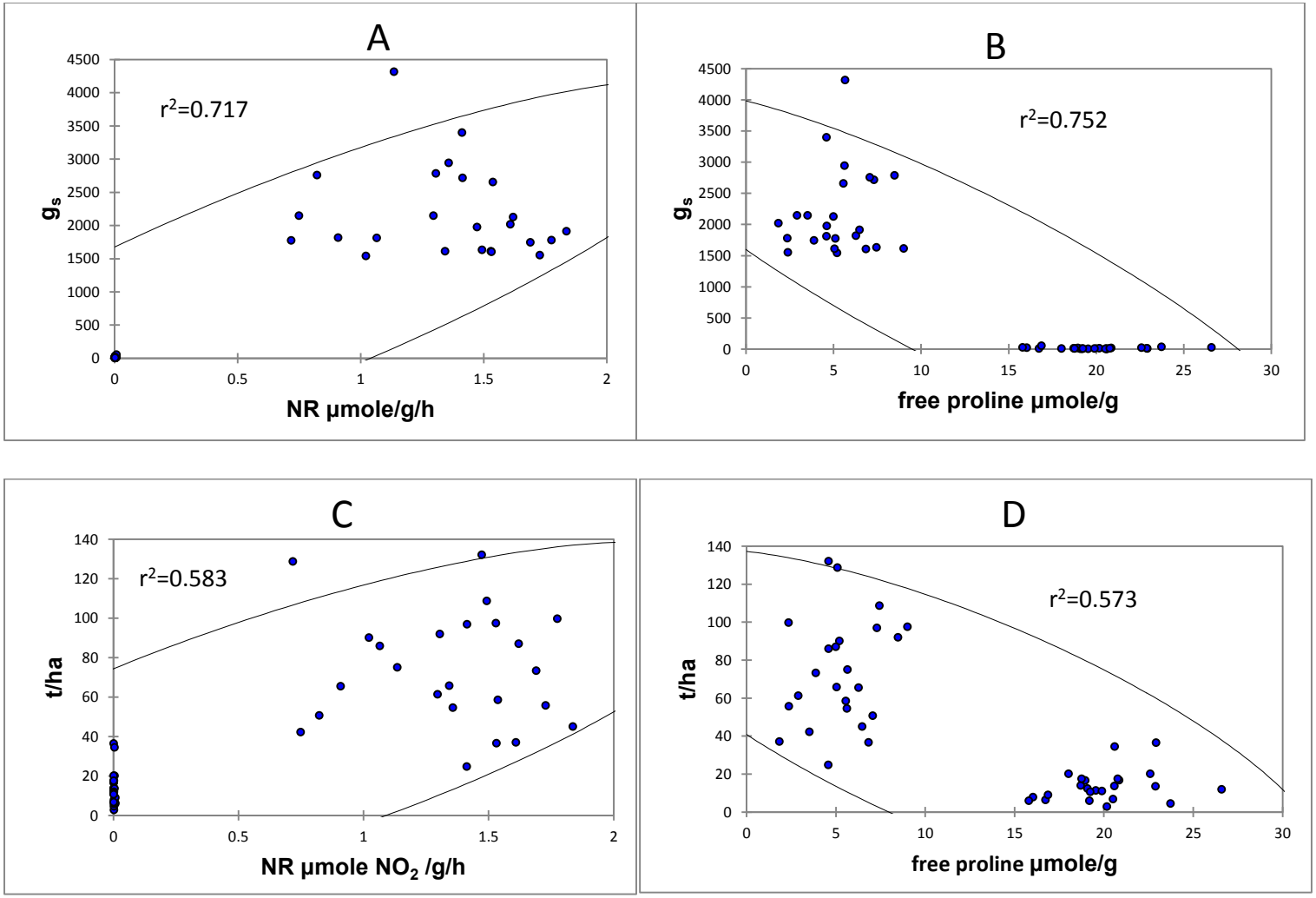

Figure 7.5. Scatter plots with confidence ellipses of linear relationships between g_s, NR and free proline (A,B) and yield, NR and free proline (C,D)

7.5. CONCLUSIONS

Good correlations were found between various parameters such as yield, stomatal conductance, Δ, chlorophyll content and nitrate reductase activity that indicated that such parameters could be used to identify promising genotypes in the breeding programme. Although a good indication was provided by correlation calculations of which parameters could be useful in the case of selecting possible genotypes for drought tolerance it was disappointing that the two/three repeats in the three trials were too few to allow the calculation of possible correlations that would distinguish between the different genotypes. Further investigations are to be pursued for possible relationships between parameters of the two times of measurement of all the trials which might give some insight on the physiological aspects regarding the reaction of the sweet potato plants during drought stress.

CHAPTER 8

FINAL CONCLUSIONS AND RECOMMENDATIONS

The study of the influence of drought on plants and the subsequent drought resistance present in some plants is a complex matter. Adding to this complexity is the fact that drought stress can seldom be separated from other abiotic stresses like heat or salinity **(VISSER, 1994).** Due to the fact that drought tolerance is a multiple gene, complex trait on its own **(VISSER, 1994)** the role of the environment x genotype combination can also play a prominent role in the selection for drought tolerance. Although sweet potato is a drought tolerant crop, variation occurs within the genotype and it is susceptible to drought especially in the early stages of growth. **VAN HEERDEN and LAURIE (2007)** have shown that the growth of the sweet potato plant is severely retarded during drought conditions, which affects yield. This also agrees with the findings of **MARTIN and JONES (1986)**.

Testing the biochemical, physiological and agronomical responses of sweet potato genotypes entails the use of multiple screening techniques. The choice of these techniques is complex due to the multiple facets that need to be covered in such an investigation. Fortunately the metabolic processes in the plant are interconnected in such a way that certain key areas in the plant can be identified and investigated individually. The results from these individual areas can then be used to make assumptions and conclusions regarding certain reactions of the genotype in the areas not covered in the investigation. It is also important to decide what the location will be when the plants are tested. The easiest route to take would be to have them grown in the greenhouse in pots with controlled temperature, light and drought conditions. Here the problem comes in regarding the growth pattern of the plants. Due to the fact that plants have different growth patterns in the field due to their inheritance and since sweet potato is primarily a creeper it would be difficult to establish a proper result regarding the coverage of the upper growth. The choice was therefor made to have sweet potato grown through normal cultivation practices and control water usage by means of artificial water application. The planting in rainout shelters provided the ideal scenario to control water application but still allow the plants to grow in their growth season in a normal environment while measurements, on the plants, could also continue despite rainy conditions. The disadvantage of growing

sweet potato plants, for screening, under a rainout shelter is the issue of space. To get acceptable results during screening for drought tolerance, large populations with enough repeats need to planted and investigated.

Drought stress, in this study, proved to have a severe damaging effect on the growth of the sweet potato plants with regard to various phenotypical and biochemical traits. Canopy growth and stem length were severely retarded by the deficiency in water in this study (Chapter 2). This obviously inhibited the total area of exposure of the plant to sunlight and the air for respiration. Differences between the genotypes regarding LAI was in some cases nonsignificant especially where the stress was quite severe i.e. the severe stress treatment. The cultivar Purple Sunset featured in both the LAI and the stem length measurement as the best performer especially in the mild stress treatment. It can be argued that canopy cover and vine length would sustain each other during the growth cycle resulting in the probability of only using one parameter for testing but the reverse was also shown where Blesbok did not differ significantly from other genotypes with regard to canopy cover but showed a significant difference with regard to stem length measurement.

It is known that antioxidant systems, in sweet potato, react positively to drought stress (**KIM, KIM, LEE AND KWAK, 2009; RUMBAOA, CORNAGO and GERONIMO, 2009; TEOW, VAN-DEN TRUONG, MCFEETERS, THOMPSON, PECOTA and YENCHO, 2007)**. The effect of drought had the expected results with regard to the reaction of some antioxidant systems in the plants (Chapter 3). Results indicated that the enzyme systems reacted positively with regard to the drought stress. The majority of the genotypes experienced increased levels of antioxidant activity in all three antioxidant enzyme systems. No significant differences, in AP activity, between the genotypes, in control conditions, were detected in all three trials. The drought stress conditions, on the other hand, instigated significant differences in activity between the genotypes with regard to AP activity. Drought stress did result in significant increases between the different water treatments with regard to SOD, in the stress treatment, but differences between genotypes could only be seen in Trial 3. Significant differences were detected between genotypes in T2 of the control which possibly confirms the findings of **KIM, KIM, LEE**

AND KWAK (2009) where antioxidant levels will increase with leaf age and probably contribute to the genotypic differences. GR activities as a result of the drought stress did not indicate any significant differences between genotypes in Trial 1 although in both Trial 2 and Trial 3 significant differences were observed. It would seem that a mild stress did not have a detrimental effect on the chlorophyll content of the genotypes although a severe stress harsh (30%) treatment caused a significant decline specifically in Trials 1 and 3. This was in contrast with results obtained in Trial 2 where chlorophyll content values of some genotypes increased significantly which agrees with findings observed by **NIKOLAEVA, MAEVSKAYA, SHUGAEV and BUKHOV (2010)**.

Drought stress caused a decline in discrimination against ^{13}C in all three trials (Chapter 4). The decline was more pronounced in the severe stress treatment than in the mild stress treatment and was also more pronounced at T2 than T1 which indicated that the plants experienced less discrimination as the stress progressed. This was also confirmed by **DEBLONDE, HAVERKORT and LEDENT (1999),** who observed less discrimination with more drought stressed potato plants. The analysis in discrimination values resulted in the ability to distinguish between genotypes in all three trials. A decline in stomatal conductance seems to run parallel to the decline in discrimination values which was also observed by **LEIDI, LOPEZ, GORHAM and GUTIÉRREZ (1999)** in cotton grown in drought conditions. No significant differences could be detected between the genotypes in any of the trials in any of the drought conditions. The relative water content of the leaves declined significantly in both Trials 1 and 3. Levels as low as 70% were reached in Trial 3 but did not result in leaf wilting which indicates that the turgor is upheld well, even at low levels of water content, and could contribute to upholding cell viability.

Nitrate reductase (NR) activity was severely impeded by the drought stress conditions in all the trials (Chapter 5). The effect of the drought was to such a degree that no significant difference could be detected between the genotypes. Since nitrate reductase is a key enzyme in the nitrogen assimilation pathway, that is actively controlled during drought **(FRESNEAU, GHASHGHAIE and CORNIC, 2007; CARAVACA, ALGUACIL, HERNÁNDEZ and ROLDÁN, 2005; FOYER, VALADIER, MIGGE and BECKER, 1998),** it is assumed that a severe decline in enzyme activity in these trials will have a negative

effect on the growth of the plants. **PANDEY, BAIG and BHATT (2012)** have shown that a decline in photosynthesis also has a negative effect on nitrate reductase which might fit into our reasoning that stomatal conductance will interfere with photosynthetic rate and hence cause a decline in NR levels. The increase in proline levels in the majority of genotypes in all three trials confirmed the results obtained by a large number of researchers on numerous crops **(MONREAL, JIMÉNEZ, REMESAL, MORILLO-VELARDE, GARCÍA-MAURINO and ECHEVARRÍA, 2007)** sugar beet; **(VENDRUSCOLO, SCHUSTER, PILEGGI, SCAPIM, MOLINARI, MARUR and VIEIRA, 2007)** wheat; **(DE RONDE, VAN DER MESCHT and STEYN, 2000)** cotton and **(DE RONDE, CRESS, KRÜGER, STRASSER and VAN STADEN, 2004)** soybean. It was possible to distinguish and obtain significant differences between the genotypes making use of these results after screening for this trait.

Drought stress had a significant effect on the yield of the genotypes especially with regard to the severe stress treatment (Chapter 6). This correlates with the findings of **VAN HEERDEN and LAURIE (2008)** who also reported significant differences between two sweet potato genotypes subjected to drought stress. The mild stress treatment did not result in significant differences compared to the control. The severe stress treatment in general proved too severe to obtain data to be used in screening between the genotypes while the mild stress treatment might be too lenient to sufficiently create enough drought stress to distinguish between the genotypes in the case of sweet potato. This is illustrated by the findings of **VAN HEERDEN and LAURIE (2008)** who could not find significant differences between the genotypes in the mild stress treatment whereas at 50% irrigation treatment significant differences were obtained. It was possible to distinguish between genotypes on the bases of WUE in Trials 2 and 3. Although in the case of yield it was not possible to significantly distinguish between the genotypes in Trial 3 both treatments, control and severe stress, provided sufficient information, linked to other parameters, to be able to significantly differentiate between the genotypes. This clearly indicated that Bophelo and 199062.1 performed the best and Resisto and Hernandez the worst. Carotenoid concentrations increased in the majority of the orange fleshed sweet potato genotypes which confirms the findings of **BARTOLI, SIMONTACCHI, TAMBUSSI, BELTRANO, MONTALDI and PUNTARULO (1999)** and **LAURIE, FABER, VAN**

JAARSVELD, LAURIE, DU PLOOY and MODISANE (2012) who observed increases in carotenoid content in wheat and sweet potato respectively. In some instances the results was contradictory between the different trials where, for example, W-119 showed a non-significant increase in the mild and severe stress treatments but in Trial 2 indicated a significant increase compared to the control.

Significant positive relationships were obtained between yield and WUE and g_s (Chapter 7) in all the trials. The correlation between yield and g_s confirms the assumption that a decline in g_s will result in a reduction in yield in the case of sweet potato. The correlation between proline and the antioxidant enzymes GR and AP in Trials 1 and 3 might indicate that with drought stress the antioxidant enzyme activities will increase, as well as levels of proline to support cell structures during the stress. Significant correlation was observed between SOD activity and CCI which might indicate that due to the stress chlorophyll is damaged and SOD levels are enhanced to minimize damage.

The results of this study indicated that there are numerous relationships between different traits in the growth of the plant during its life cycle in control and drought stress conditions. Some relationships proved to be very strong while other were quite weak. It was also observed that some contradiction was seen, with regard to the relationships, between the different trials. In order to eliminate such contradictions and confirm either strong or weak relationships it is suggested that more genotypes are screened in a similar way. Due to the fact that relationships are much dependent on statistical analysis, a bigger number, of genotypes, will narrow the margin of error.

The results of the study also indicated that the severe stress treatment was too harsh to allow the investigator to discriminate between the genotypes and also to predict possible survival mechanisms of the plants of the genotypes. The mild stress treatment did allow for the discrimination between genotypes although for future studies it would be appropriate to induce a drought stress more severe than the mild stress treatment but less than the severe stress treatment. At this level plants will still be suffering the consequences of the stress but metabolic systems can be fruitfully investigated for the possible prediction of drought tolerance.

CHAPTER 9
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