RESPONSE OF PEARL MILLET TO WATER STRESS

DURING VEGETATIVE GROWTH

CINISANI MFAN'FIKILE TFWALA

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by

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BLOEMFONTEIN

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DECLARATION

I declare that the dissertation hereby submitted by me for the Master of Science in Agriculture degree at the University of the Free State is my own independent work and has not been previously submitted by me for a qualification to another University/Faculty.

I further cede copyright of the dissertation in favour of the University of the Free State.

Cinisani Mfan'fikile Tfwala

Signature:

Date: May 2010

Place**:** Bloemfontein

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DEDICATIONS

To my grandmother

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This dissertation is dedicated to my grandmother who departed during the course of this work (November 2009). I miss her caring, guidance and love. I very much appreciate the role she played in my upbringing and her contribution to my educational life.

To my father

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How I wish I was celebrating this moment with both of you Manyamandze.
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ABBREVIATIONS AND SYMBOLS

Abbreviations

ANOVA	-	Analysis of Variance
ARC – GCI	-	Agricultural Research Council – Grain Crops Institute
ARC – ISCW	-	Agricultural Research Council – Institute for Soil, Climate and Weather
CV	-	Coefficient of variation
DM	-	Dry mass
DUL	-	Drained upper limit
ETo	-	Evapotranspiration
FM	-	Fresh mass
IR	-	Irrigation treatment
LAI	-	Leaf Area Index
NMR	-	Nuclear Magnetic Resonance
OA	-	Osmotic adjustment
OA_0	-	Osmotic adjustment at turgor pressure equal to zero
OA_{100}	-	Osmotic adjustment at 100% RWC or 100% turgor pressure
P_c	-	Pressure measured by pressure chamber
P-V	-	Pressure – Volume
Rs	-	Solar radiation
RWC	-	Relative Water Content

SAWS	-	South African Weather Service
SEM	-	Scanning Electric Microscopy
TM	-	Turgid mass
WC	-	Water content
WRC	-	Water Research Commission
WSD	-	Water saturation deficit

Symbols

Ψ_t	-	Total water potential
Ψ_π	-	Osmotic potential
Ψ_p	-	Turgor pressure
Ψ_m	-	Matric potential
Ψ_g	-	Gravitational potential
$\Psi_\pi^{p=0}$	-	Osmotic potential at turgor pressure equal to zero
Ψ_π^{100}	-	Osmotic potential at 100% RWC or 100% turgor pressure
π	-	Pi

ABSTRACT

RESPONSE OF PEARL MILLET TO WATER STRESS DURING VEGETATIVE GROWTH

by
CINISANI M. TFWALA (M.Sc. Irrigation Science), University of the Free State
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Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a drought tolerant cereal crop planted mainly in arid and semi-arid regions of the world. Water stress still remains one of the challenges facing agriculture. Crops face water stress at various stages due to low and erratic rainfall in arid and semi-arid regions. The response of two pearl millet lines (GCI 17 and Monyaloti) to water stress during vegetative growth was investigated at University of Free State, Department of Soil, Crop and Climate Sciences experimental farm at Kenilworth during the 2009/2010 growing season. The two pearl millet lines were grown under three irrigation treatment levels, namely full (IR3) moderate stress (IR2) and rainfed (IR1). A line source sprinkler system was used to irrigate the experiment.

Stressed plants of GCI 17 were about 30% shorter than irrigated plants. For Monyaloti, the stressed plants were 25% shorter than irrigated plants. The highest leaf area index (LAI) of 7.9 was found in IR2 plants of GCI 17 at 7 weeks after planting while the stressed plants of this line attained a highest LAI of 3.6 at 8 weeks after planting. The highest LAI attained by Monyaloti was 9.5 in IR2 plants at 8 weeks after planting and the stressed plants attained a highest LAI of 4.7 during the 9^{th} week after planting thus showing that mild water stress caused a delay in canopy development and limited the size to about half. However, the number of tillers and leaves on the main shoot were not affected by water deficit conditions.

The leaf water potential measured by the pressure chamber showed some difference between irrigated and stressed plants after 3 days of withholding rain of 5.6mm from stressed plots. The differences in water potentials of stressed plants and irrigated plants were increasing simultaneously with water stress progression. The water potential of GCI 17 dropped to as low as -1.83 MPa on water stressed plants after 11 days of withholding rain. The leaf water potential for Monyaloti remained significantly higher in the corresponding irrigation treatments. The diurnal changes of leaf water potential showed well watered GCI 17 plants to have water potential of -1.08 MPa around midday while the stressed plants had lower potential of -1.75

MPa. Well-watered plants of Monyaloti had leaf water potential of -0.76 MPa while their stressed counterparts had -1.05 MPa.

The seasonal stomatal conductance did not show differences between the pearl millet lines. Stressed plants had lower stomatal conductance values than the irrigated plants, which was also more pronounced as water stress progressed. The stomata of GCI 17 were partly closed for the whole day as revealed by diurnal stomatal conductance. For Monyaloti even the stressed plants had their stomata wide open in the morning and became partly closed by 1300hrs and during the rest of the afternoon.

On day 16 after withholding rain (17th February 2010) from water stressed plots, GCI 17 plants had relative water content (RWC) of 72.7% while the well watered plants had 90.3%. Water stressed Monyaloti plants were at 82.8% RWC while the well-watered plants had a RWC of 92.9%. The RWC of stressed plants was continuously decreasing with progress in water stress.

The osmotic potential at full turgor was -1.62 MPa for well-watered plants of GCI 17 while -1.83 MPa was measured in the water stressed plants of this line. For Monyaloti, well-watered plants had osmotic potential of -1.11 MPa compared to -1.47 MPa for water stressed plants. At turgor pressure equal to zero, GCI 17 plants from stressed and well-watered plots did not show any adjustments as they were about similar (-2.22 and -2.27 MPa respectively). For Monyaloti water stressed plants had potential of -1.72 MPa and well-watered plants had -1.61 MPa at turgor pressure equal to zero showing an osmotic adjustment of 0.11 MPa.

The density of stomata was found to be lower on water stressed plant leaves than on well-watered plants. The abaxial surfaces of pearl millet leaves were found to have lower densities than the adaxial surfaces. The stomata areas calculated from the length and width of the stomata were larger on the adaxial surfaces of GCI 17 plants than those found on the abaxial surfaces. The opposite of this was observed in Monyaloti.

The plant height, LAI and biomass accumulation for the two pearl millet lines were found to be lower in water stressed plants when compared with irrigated plants. Monyaloti plants were taller, had higher LAI and accumulated more biomass than GCI 17 plants at corresponding water treatment levels, showing that Monyaloti was less affected by water stress. It was also observed that water stressed plants have lower leaf water potential when compared to irrigated plants. The leaf water potential was maintained higher in Monyaloti plants compared to GCI 17

plants and the same effect was seen with the stomatal conductance which was also lower in water stressed plants than irrigated plants in the pearl millet lines. The highest growth was observed for IR2 plants. Thus from all of growth and physiological field measurements it can be seen that Monyaloti is better adapted to the water stress conditions. It will continue to grow and produce a crop despite the mild water stress due to maintenance of leaf water potential and through osmotic adjustment. Further investigation of the effects of age on the leaf water potential, stomatal conductance, RWC and stomatal characteristics in relation to photosynthesis was recommended.

Key words: Pearl millet, water stress, vegetative growth, leaf water potential, stomatal conductance, relative water content, osmotic potential, stomatal characteristics, drought adaptability.

UITTREKSEL

REAKSIE VAN MANNA OP WATERSTREMMING GEDURENDE VEGETATIEWE GROEI

deur

CINISANI M. TFWALA (M.Sc. Besproeiingswetenskap), Universiteit van die Vrystaat

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Manna (*Pennisetum glaucum* [L.] R. Br.) is ‘n droogte verdraagsame graangewas wat meestal in ariede en semiariede streke van die wêreld aangeplant word. Waterspanning bly maar een van die uitdagings in die landbou. Gewasse word blootgestel aan waterspanning op verskillede stadiums as gevolg van lae en wisselvallige reënval in ariede en semiariede gebiede. Die gedrag van twee manna lyne (GCI 17 en Monyaloti) t.o.v. waterspanning tydens vegetatiewe groei is by die Universiteit van die Vrystaat, Department Grond, Gewas en Klimaatwetenskappe te eksperimentele proefplaas by Kenilworth tydens die 2009/2010 groeiseisoen ondersoek. Die twee manna lyne is onder drie besproeiings behandelingsvlakke, naamlik volle spanning (IR3), middelmatige spanning (IR2) en droëland (IR1) verbou. ‘n Sisteemlynbron sproeier is vir proefdoeleindes gebruik.

Gespanne GCI 17 plante is ongeveer 30% korter as besproeide plante. By Monyaloti, is gespanne plante omtrent 25% korter as besproeide plante. Hoogste blaarareaindeks (BAI) is 7.9 by IR2 plante van GCI 17 op 7 weke na aanplanting, terwyl gespanne plante uit hierdie lyn hoogste BAI van 3.6 by 8 weke na aanplanting behaal het. Die hoogste BAI deur Monyaloti behaal is 9.5 in IR2 plante by 8 weke na aanplanting en die gespanne plante het ‘n hoogste BAI van 4.7 tydens die 9de week na aanplanting getoon. Hieruit word aangetoon dat gematigde waterspanning ‘n agterstand in blaardak ontwikkeling wat lei tot beperkte grootte van tot die helfte. Nietemin is die aantal uitspruitsels en blare op die hoofuitspruitsel nie deur waterterkorte beinvloed nie.

Die blaarwaterpotensiaal deur die drukbom gemeet het verskille tussen besproeide en gespanne plante na 3 dae van reënweerhouding van 5.6 mm by gespanne akkers aangetoon. Die verskille in water potensiale van gespanne plante en besproeide plante het gelyktydig toegeneem met waterspanning voortuitgang. Na 11 dae van reënweerhouding het die waterpotensiaal van GCI 17 tot so laag as -1.83 MPa in watergespanne plante geval. Die blaarwaterpotensiaal vir Monyaloti het beduidend hoër in ooreenstemmende besproeiingsbehandelings gebly. By die daaglikse veranderinge van blaarwaterpotensiaal is

waterryke GCI 17 plante waterpotensiaal van -1.08 MPa op die middaguur waargeneem, terwyl gespanne plante laer potensiaal van -1.75 MPa toon. Waterrye plante van Monyaloti het blaarwaterpotensiaal van -0.76 MPa terwyl hul gespanne ewebeelde -1.05 MPa getoon het.

Die seisoenale huidmondjie geleibaarheid het nie verskille tussen die manna lyne uitgewys nie. Gespanne plante het laer huidmondjie geleibaarheidswaardes teenoor besproeide plante getoon wat ook meer beklemtoon is soos waterspanning vooruitgang gemaak het. Die huidmondjies van GCI 17 is gedeeltelik toe heeldag lank soos deur daaglikse huidmondjie geleibaarheid aangetoon. By Monyaloti het selfs die gespanne plante hul stomata heeltemal oop in die oggend en dan gedeeltelik toe teen 1300 en die res van die namiddag.

Die GCI 17 plante op watergespanne akkers het op dag 16 na weerhouding van reën (17 Februarie 2010) RWC van 72.7% terwyl die waterryke plante 90.3% getoon het. Water gespanne Monyaloti plante is by 82.8% RWC terwyl die waterryke plante ‘n RWC van 92.9% getoon het. Die RWC van gespanne plante het deurlopend verminder met voortuigang in waterspanning.

Die osmotiese potensiaal by volle opswelling is -1.62 MPa vir waterrke plante van GCI 17 terwyl -1.83 MPa gemeet is by watergespanne plante van hierdie lyn. Waterryke Monyaloti plante het ‘n osmotiese potensiaal van -1.11 MPa vergelyke met -1.47 MPa vir watergespanne plante. By opswellingsdruk gelyk aan nul, het GCI 17 plante van gespanne- tot watteryyk akkers geen verstelling getoon nie omdat hulle redelik gelyk (-2.22 and -2.27 MPa, respektiewelik) voorgekom het. Monyaloti watergespanne plante het ‘n potensiaal van -1.72 MPa terwyl waterryke plante -1.61 MPa by P=0, wat osmotiese verstelling van 0.11 MPa beteken.

Digtheid van huidmondjies is gevind om laer te wees op watergespanne plantblare vergeleke met waterryke plante. Daar is ook gevind dat abaksiale opervlaktes van manna blare laer digthede as adaksiale oppervlaktes toon. Die huidmondjie oppervlaktes vanaf lengtes en breedtes van huidmondjies bereken is langer op adaksiale oppervlaktes van GCI 17 plante in vergelyking met díe op abaksiale oppervlaktes. Die teenoorgestelde verskynsel is by Monyaloti waargeneem.

Planthoogte, BAI en biomassa akkumulasie vir die twee manna lyne is gevind as laer in watergespanne plante vergeleke met besproeide plante. Monyaloti plante is langer met hoer BAI en het meer biomassa geakkumuleer as GCI 17 plante met ooreenstemmende

waterbehandelingsvlakke en dui aan dat Monyaloti minder beinvloed is deur waterspanning. Daar is ook waargeneem dat watergespanne plante laer blaarwaterpotensiaal toon vergeleke met besproeide plante. Die blaarwaterpotensiaal is in Monyaloti plante behou in vergelyking met GCI 17 plante en dieselfde effek met huidmondjie geleibaarheid wat deurgangs laer in watergespanne plante teenoor besproeide plante in die manna lyne voorgekom het. Die hoogste groei is in IR2 plante waargeneem. Dus kan daar waargeneem word vanuit alle groei en fisiologiese proeflesings dat Monyaloti beter aangepas is tot waterspanning toestande. Dit sal voorgaan met groei en ‘n opbrengs lewer ten spyte van matige waterspanning as gevolg van onderhouding van blaarwaterpotnsiaal en osmotiese verstelling. Daar is aanbeveel dat die effekte van ouderdom op blaarwaterpotensiaal, huidmondjie geleibaarheid, RWC en huidmondjie kenmerke in verhouding tot fotosintese verder ondersoek behoort te word.

1. INTRODUCTION

The biggest challenge for agriculture in the present and future is to meet the food and fiber needs of the ever increasing world population. Increasing the production of cereals is therefore of paramount importance. Pearl millet (*Pennisetum glaucum* [L.] R. Br. formerly known as *P. americanum* [L] Leeke) is a variety of the millet family also known as pearl, bulrush, spiked or cat-tail millet. It is the staple food for drier parts of Africa, particularly the arid and semi-arid regions of West Africa. It is also grown as a fodder crop in some areas such as America. It is generally grown under rainfed conditions in arid and semi-arid regions of the world.

Due to the low and erratic rainfall in these regions, the crop can face water stress at various stages of development (Manga & Yadav, 1993). More often the deleterious effects of drought are exaggerated by high temperature and low nutrient status of soils (Yadav & Bhatnagar, 2001). Pearl millet is more adapted to dry and hot areas than maize or sorghum (Do *et al.*, 1996; Baryeh, 2002) and is characterized as a short-day plant (Maiti & Wesche-Ebeling, 1997; van Oosterom *et al.*, 2001). The crop is also reported to have some significant tolerance to acid soils (Kennedy, 2002) and to salinity (Kusaka *et al.*, 2005) though it is most adaptable to pH values between 6.2 and 7.7 (Maiti & Wesche-Ebeling, 1997).

Alam (1999) defined the onset of water stress as the point when the efflux of water from a plant is greater than the influx of water into the plant. This point of onset of water stress was previously defined by Meyer & Green (1981) as the point at which the rate of water loss declines below that of a well-watered crop in the same locality. Kusaka *et al*. (2005) and Moussa & Abdel-Aziz (2008) defined water stress as the absence of adequate moisture necessary for a normal plant to grow and complete its life cycle. Water stress has different impacts on different stages of crops, which result in different losses in yield. For example, sensitive stages are during flowering and boll formation in cotton; during vegetative growth in soybean; flowering and grain filling stages of wheat; and vegetative and reproductive stages of sunflower and sugar beet (Istanbulluoglu *et al*., 2009).

Under water shortage conditions, water could be reserved for irrigation during the most critical growth stages (Seghatoleslami *et al*., 2008b) and where climate permits, the growing season can be shifted towards times of low evaporative demand such as winter (López-Urrea, 2009). Water shortage has been described by many authors as a major limiting factor to growth and yield of crops around the world (Umar, 2006; Seghatoleslami *et al*., 2008a; Garcia *et al*., 2009;

Puangbut *et al*., 2009; Payero *et al*., 2009; Yousfi *et al*., 2010). Therefore studies of the responses of plants to water deficits are of great interest, from the cellular level to the whole plant and crop community level (van der Weerd *et al*., 2001; Yadav & Bhatnagar, 2001; Yousfi *et al*., 2010).

Water stress has effects on several morphological and biological aspects of plants. It leads to a reduction in the efficiency of important plant processes, including protein synthesis, photosynthesis, respiration and nucleic acid synthesis (Porporato *et al*., 2001). With limited water the solubility of plant nutrients in the soil solution, their diffusion towards the root surfaces and generally their uptake by plants is reduced (Alam, 1999). These changes result in a reduction in biomass production and subsequently the yield (Payero *et al*., 2009).

Plants adapt to dry environments through adjusting their growth habits such as the continued growth of the roots while shoot growth has slowed or ceased. This behavior is under the influence of abscisic acid (Hartung *et al*., 1999), thus plants under water stress will close their stomata and hence reduce the amount of water lost through transpiration (Jackson *et al*., 1988; Hartung *et al*., 1999).

Even though the adaptation of millet to the driest environments is realized, its vegetative response to water deficits has not been clearly described. The generation of information on the response of the crop to drought will be of great benefit to determine the adaptability of the crop to the arid and semi-arid areas of South Africa as well as representation of this information in a crop growth model. This study ran parallel with a PhD study entitled “Characterization of Water Relations of Amaranth and Pearl Millet” by Mr. Z.A. Bello and was funded by the Water Research Commission (WRC) under project number: KS/1771/4.

Main Objective

The main objective of the study was to understand how the vegetative phase of pearl millet responds to water stress.

Specific Objectives

1. To determine the effect of water stress on vegetative growth of two pearl millet lines.
2. To study the physiological plant water status of pearl millet at a range of water deficits.

3. To determine the density and size of stomata as influenced by water deficit using microscopic methods on pearl millet leaves.

Null Hypothesis

The vegetative growth, physiological plant water status and development of stomata of pearl millet do not differ under well-watered and water stress conditions.

2. LITERATURE REVIEW

2.1 General Importance of Water to Plants

Water is undoubtedly the most important constituent of all life forms. In living plants, 60 to 95% of their mass is water (James, 1988). As an example, a maize plant weighing 800 g at tasseling stage contains 700 g of water. This large involvement of water in plants makes it necessary to understand how water is used in plant development because it is the largest input in agriculture (Boyer, 1995).

Water is important for the solubility and availability of nutrients in the soil (Alam, 1999). Within the plant, water is important for the maintenance of turgor pressure to keep cell and organ shape and hence for cell growth (Acevedo *et al*., 1971; Hsiao *et al*., 1976). It is also important as a reactant, serving as a medium for the ionization of metabolites and stabilization of biomembranes (Hsiao *et al*., 1976). Water is also required for plant processes including photosynthesis, transport of minerals, carbohydrates and photosynthates as well as meeting the transpiration requirement of plants (James, 1988).

2.2 Water and Plant Growth

According to Hsiao *et al*. (1976), growth is “irreversible cell enlargement”. Growth is described by several authors to be the most sensitive among plant processes that are affected by water stress (Acevedo *et al*., 1971; Hsiao *et al*., 1976; Van Volkenburgh & Boyer 1985; Hsiao, 1990). This makes the measurement of growth parameters to be of paramount importance in monitoring plant responses to water stress. Decline in growth rate, particularly of leaves, results in large reductions in the rate of photosynthesis since leaves are the main photosynthetic surfaces of most plants (Boyer, 1976). A speedy development of the leaf canopy is important in crop production because it means a quick increase in the photosynthesis factory size (Hsiao, 1990).

Plant growth occurs in two ways: cell division and cell expansion. Cell division creates additional cells while cell expansion is an increase in the size of an existing individual cell. Water uptake provides the physical driving force for cell enlargement (Acevedo *et al*., 1971). If water is limited, the final size of the cell is limited and hence growth is also limited. Well-watered plants keep their shape because of the internal pressure created by water in the cells, called turgor pressure

(Hsiao *et al*., 1976). When water is insufficient, the turgor pressure drops and the growth rate of plants declines (Acevedo *et al*., 1971).

During the development of water stress, one of the symptoms shown by plants is the restriction of leaf expansion. Water stressed plants restrict total leaf area by producing fewer and smaller leaves and shedding the older ones (Kirkham, 1990; Sarkar *et al*., 2009). Huda *et al*. (1987) as cited by Kirkham (1990) studied two sorghum varieties (CSH 8 and M35-1) and found that the final number of leaves per plant were one leaf less under non-irrigated conditions compared to irrigated conditions for both varieties. In another study for millet, reported by Do *et al*. (1996), it was observed that under drought conditions there was a lower leaf area index (LAI) caused by the restriction of growth and enhancing leaf senescence. They reported that stressed plants had leaf area 30 to 50% lower than that of the control plants after 15 days without irrigation. They also stated that this response was noticeable within 4 to 5 days after the last irrigation. Acevedo *et al*. (1971) in a study of maize also reported lower LAI under drought conditions.

Alam (1999) stated that drought affected above ground biomass production more than dry matter below the ground. Hartung *et al*. (1999), Kusaka *et al*. (2005), Kirnak & Dogan (2009), Payero *et al*. (2009) and Yousfi *et al*. (2010) also reported lower total biomass production when plants are subjected to drought conditions compared to their well-watered counterparts.

2.3 Physiological Plant Water Status

Living cells need to be hydrated for normal functioning i.e. more or less saturated with water (Slavik, 1974; Turner, 1981). Plants are however not completely saturated with water. There is often a certain hydration deficiency. This deficiency represents a driving force of water within the plant (Slavik, 1974; Jarvis, 1976), and also acts as a factor affecting its physiological activity (Slavik, 1974).

There are two basic parameters which describe the degree of unsaturation:

i) energy status of water in the cell, and

ii) cell water content.

The energy status is usually expressed as total water potential while the water content is usually expressed relative to that at full saturation. Even though these two parameters are linked in such a way that a decrease in the water content leads to a decrease in the total water potential, the relationship is not unique but varies with species, growth conditions and stress history

(Turner, 1981). Turner (1981) continues to say that this relationship is variously known as the “moisture release curve”, “water potential isotherm” or “water retention characteristic”. For completeness therefore, both the energy status and the water content of the plant tissue has to be measured so as to describe and react to the water status of crops.

The plant, positioned midway in the soil-plant-atmosphere continuum is the integrator of its own hydro environment (Hsiao, 1990). Knowledge of the plant water status provides inference of the soil water conditions as well as the current performance, health and well-being of the plant. The challenge is that the coupling of the plant with the evaporative demand of the atmosphere renders the plant water status exceedingly dynamic and not a simple static indicator of when the next irrigation is due in irrigated agriculture (Hsiao, 1990). Part of the challenge is the fact that plants vary in their response to water stress. Deep-rooted plants may succeed when shallow rooted individuals may fail to grow (Boyer, 1995).

The total water potential (Ψ_t) has various components: the osmotic potential (Ψ_π), turgor pressure (Ψ_p), matric potential (Ψ_m) and gravitational potential (Ψ_g). Together with these components, the water potential is expressed as pressure units, with the chemical potential of pure water at atmospheric pressure and the same temperature as the reference point. These component potentials have multiple roles which affect different aspects of plant growth and water regulation (Hsiao, 1973; Slavik, 1974; Passioura, 1980). Equation 1 is a representation of the total water potential and its components (Boyer, 1967; Turner, 1981; Kirkham, 1990; Porporato *et al*., 2001).

$$\Psi_t = \Psi_\pi + \Psi_p + \Psi_m + \Psi_g \quad \text{.....(1)}$$

The last component, Ψ_g, is only 0.01 MPa m^{-1}, the earth surface being a reference point (0.1 MPa = 1 bar), it can therefore be neglected except in tall trees (Turner, 1981). In well-watered plants and fleshy tissue, the Ψ_m component is also negligible (Boyer, 1967, Hsiao, 1973; Passioura, 1980). Passioura (1980) argues that the particles in the cytoplasm are mobile and as such can still be treated as a single phase with the pressure potential as hydrostatic pressure. This takes into consideration that the particles are not always in contact with each other; but the argument is that they therefore cannot develop the Newtonian forces necessary to alter the general hydrostatic pressure of the water. Turner (1981) stated that, in practice, the matric potential in the cytoplasm forms part of either a pressure term or osmotic term. This renders the total water potential inside the plasmallema to essentially be from only the osmotic and pressure

components. The final equation of the total water potential in the plant tissue is therefore (Slavik, 1974; Ritcher, 1978; Kusaka *et al*., 2005):

$$\Psi_t = \Psi_\pi + \Psi_p \quad \ldots\ldots(2)$$

The total water potential and the osmotic potential can both be measured by the use of the Scholander pressure chamber among other methods such as psychrometers and test solutions, and then the pressure potential obtained by difference of the two (Hsiao, 1990).

Hoffler (1920) as cited by (Slavik, 1974; Ritcher, 1978; Turner, 1981) initially proposed a linear dependence of the pressure potential on the cell water content (dotted line of Ψ_p in Figure 2.1). However he was aware that the linear relationship cannot hold in all cases. He then improved his diagram to show a deviation of the pressure potential from the linearity to a curve (Figure 2.1) which is due to an influence of the tissue counter-pressure near saturation.

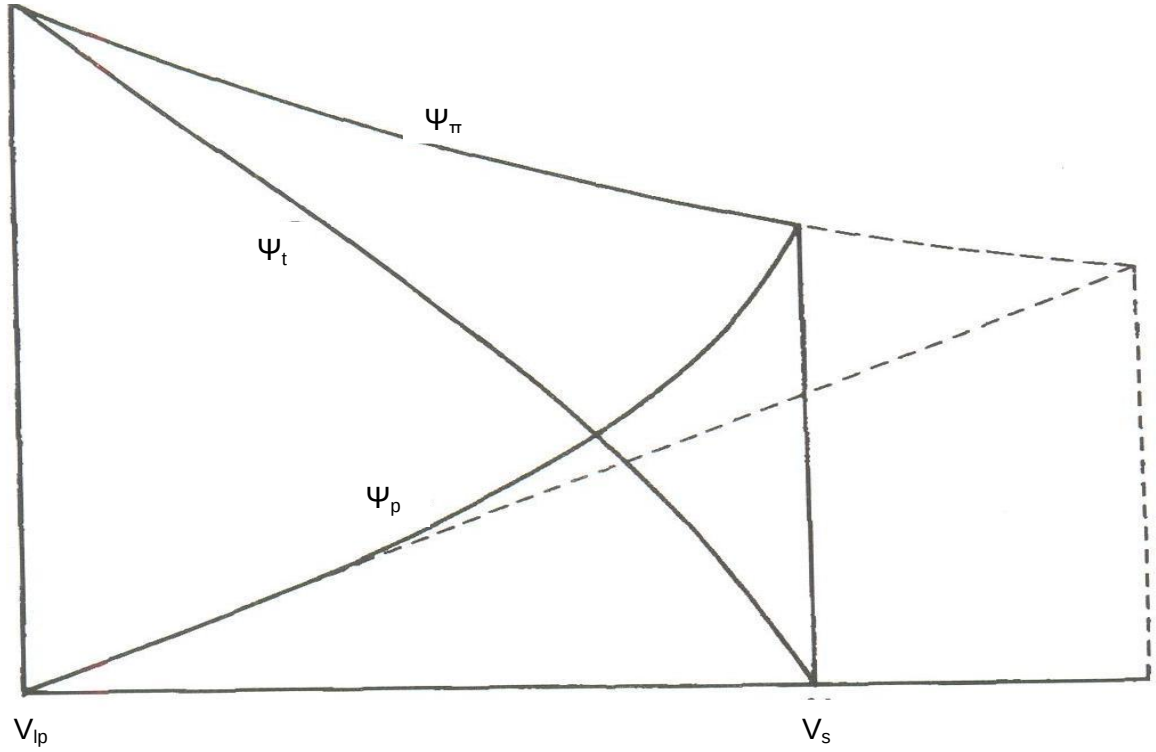

Figure 2.1: Schematic Hoffler diagram showing relationship between water potential and volume of cell. V_{lp}: volume of cell at limiting plasmolysis and V_s: volume of cell at full saturation (Ritcher, 1978)

Total water potential

The total leaf water potential has gained prominence as the one measurement of plant water status that can be measured consistently and represent the water status of the plant. According to Turner (1981) this has been partly due to the importance of the total water potential as the driving force of water movement through the plant and partly because of its relative ease of measurement. A large number of methods have been described for the determination of water potential (Slavik, 1974). This large number of methods shows that it has been difficult to obtain a perfect and universal method.

Slavik (1974) categorizes the methods of water potential determination into three kinds:

i) compensation methods, for example test solutions,
ii) direct methods measuring water vapour pressure above the tissue, for example psychrometers, and
iii) the pressure chamber method.

Turner (1981) refers to the thermocouple psychrometers and the pressure chamber as the two basic methods for the measurement of water potential. He however states that the thermocouple psychrometer has limited use in field studies because of the long time required for calibration and the limited number of samples that can be measured over time. The pressure chamber will be discussed briefly as it is the method that was used in this study.

The pressure chamber is one method that is convenient for rapid field use to measure leaf water potential (Hsiao, 1990). Because of its ease of use, its speed and reliability, the pressure chamber technique has been used throughout the world for the measurement of total water potential (Turner, 1981). To measure the potential of a leaf or a small branch, the sample is enclosed in a transparent plastic bag prior to cutting to minimize water loss through transpiration. The sample is then excised from the plant and quickly sealed in the chamber with the cut end protruding from the chamber through a rubber stopper. Pressure in the chamber is then raised by compressed air (but not oxygen), nitrogen or any other inert gas from a cylinder until sap appears at the cut end. This balance pressure is the opposite of the total water potential of the sample as it is a suction or negative pressure (Boyer, 1967; Turner, 1981; Hsiao, 1990).

Accuracy of the results primarily depends on factors such as:

i) accuracy of the pressure gauge,
ii) handling of samples, and

iii) rate of increasing pressure.

The rate of increase also influences the temperature in the chamber (Slavik, 1974). The effects of rate of increase can also be explained with the aid of the Ideal gas law:

$$PV = nRT \quad \ldots\ldots (3)$$

where P is pressure of the gas, V is the volume, n is the amount of substance, R is the universal gas constant and T is the absolute temperature. With all the other parameters fixed as is the case within the pressure chamber, an increase in the pressure will result in an increase in the temperature.

Rapid rates of pressurization lead to more negative values of leaf water potential than slower increasing pressure (Turner, 1981). Hsiao (1990) recommends an increase rate of less than 0.1 MPa s^{-1} initially and less than 0.02 MPa s^{-1} as the balancing pressure is approached. This is almost in line with a suggestion of an increase rate of 0.025 MPa s^{-1} by Turner (1981). Transpiration from the excised leaf must also be minimized. This is done by ensuring that the interior of the chamber is humidified by a moist cloth and by covering the leaf sample with clear plastic (Slavik, 1974; Turner, 1981).

Since the water potential is a suction of water from the plant tissue by the atmosphere, it is therefore a negative value. Studies have shown that water potential falls from high values (close to zero) in the morning to lower values (more negative) during the middle of the day and recover in the afternoon and evening (Hsiao, 1976; Jarvis, 1976; Acevedo *et al*., 1979; Fiscus & Kaufmann, 1990). This therefore dictates that the time for measuring leaf water potential should be consistent during the midday plateau for comparable results over time.

Osmotic water potential

Osmotic potential is generally lower in plants growing in dry habitats, it is also lower in woody plants than herbaceous plants, and it decreases as water stress increases (Kramer, 1988). Research on osmotic adjustment has been done for several species, mung beans (Zhao *et al*., 1983) pear (Larher *et al*., 2009), pearl millet (Henson, 1982, 1983), rice (Lilley & Ludlow, 1996), sorghum (Walker, 1988), sunflower (Chimenti *et al*., 2002) and wheat (Bajji *et al*., 2001).

The lowering of osmotic potential is achieved as a result of an accumulation of solutes by plants under water stress conditions. This accumulation of solutes minimizes the reduction in cell turgor (Henson, 1982, 1983). The concentration of solutes may also increase due to the limited

expansive growth during water stress conditions rather than an accumulation of solutes (Walker, 1988). Osmotic adjustment has been recognized as an important adaptive response to water stress by enhancing plant function and survival during dry conditions by preventing stomatal closure and cessation of growth as well as other physiological activities (Henson, 1982; Meinzer *et al*., 1986). According to Slavik (1974), osmotic potential ranges within the values from -0.4 to -3.0 MPa for most plants. He however states that higher and/or considerably lower values may also be found.

A number of techniques are widely used to measure osmotic potential, viz refractometric, cryoscopic, psychrometric and pressure chamber techniques. The psychrometric and pressure chamber techniques can be used to measure both the total water potential and the osmotic water potential (Turner, 1981). The pressure chamber method, which employs the pressure-volume (P-V) curve method will be considered briefly as it is the technique that was used in this particular study.

A P-V curve is a graphical representation of the reciprocal of the balance pressure and the volume of expressed sap (Roberts & Knoerr, 1977; Meinzer *et al*., 1986). In the pressure chamber, the turgor pressure is reduced to zero by applying pressure to the leaves thus the osmotic potential can be obtained from the pressure volume relationship of the intact cells. Once the turgor pressure reaches zero, the cell water volume and the applied pressure are related as follows (Tyree & Hammel, 1972; Turner, 1981)

$$\frac{1}{P_c} = \frac{(V_s - V)}{RTN} \quad \ldots\ldots(4)$$

Where P_c is the pressure in the chamber, V_s is volume of symplastic water in the turgid leaf, V is volume of the expressed symplastic water, R is the universal gas constant, T is the absolute temperature (in Kelvin) and N is the number of moles of solute in the sap. The P-V curve thus becomes linear as the turgor pressure becomes zero.

In a study of pearl millet (*P. americanum*), cultivar BJ 104, Henson (1982) found an adjustment of 0.36 MPa in osmotic potential of stressed plants (-1.17 MPa) compared to an osmotic potential of (-0.81 MPa) for control plants. In that study the turgor pressure of stressed plants was 0.3 to 0.4 MPa higher than that of well-watered plants. Different crop plants and even different cultivars adjust differently. Under similar stress conditions, Henson (1982) found that

cultivar Serere 39 adjusted less than cultivar BJ 104. Variation in osmotic adjustment of the locally available pearl millet genotypes has not been reported, yet its exploitation in breeding programmes may be of major importance where increased drought tolerance is a consideration.

Studying the spatial pattern of leaf growth of sorghum as affected by water stress and its implication for canopy development, Walker (1988) found that osmotic potential of water-stressed sorghum leaves, ranging from -1.2 to -1.8 MPa was lower than that of well-watered leaves. The reduction was in the range of 34% to 78% compared to the control over a period of 4 to 5 days. The decline in growth for these plants was 40% to 60% over the same period. The reduction in osmotic potential was also found to be larger in the growth zone than in the mature region of these monocot leaves (Walker, 1988).

Water content

Changes in water content (WC) were the first quantitative measurements of plant water stress made on a routine basis (Kramer, 1988). These measurements are expressed either on a fresh mass (FM) or dry mass (DM) basis (Turner, 1981; Kramer, 1988) as follows:

$$\mathrm{WC}_{\mathrm{DM\ basis}} = \frac{\mathrm{FM}-\mathrm{DM}}{\mathrm{DM}} \times 100 \quad \text{.....(5)}$$

$$\mathrm{WC}_{\mathrm{FM\ basis}} = \frac{\mathrm{FM}-\mathrm{DM}}{\mathrm{FM}} \times 100 \quad \text{.....(6)}$$

Water content expressed on a fresh mass basis is however a poor indicator of plant water stress because of the large diurnal changes in water content of leaves and stems of transpiring plants due to the through flow of water. The dry mass basis expression of water content is also limited by diurnal and seasonal changes because of carbohydrate accumulation in the sunlight and increase in cell wall thickness with age (Kramer, 1988). To overcome these problems, water content is expressed by many researchers as a percentage of turgid mass (TM) (Turner, 1981; Henson, 1982; Clayton-Greene, 1983; Luo & Strain, 1992; Kinark & Dogan, 2009; Lenzi, *et al*., 2009). This expression is referred to as the relative water content (RWC) or the difference from 100% as water saturation deficit (WSD):

$$\mathrm{RWC} = \frac{\mathrm{FM}-\mathrm{DM}}{\mathrm{TM}-\mathrm{DM}} \times 100 \quad \text{.....(7)}$$

$$\text{WSD} = \frac{\text{TM}-\text{FM}}{\text{TM}-\text{DM}} \text{ x } 100 \text{.....(8)}$$

$$\text{WSD} = 100 - \text{RWC} \text{.....(9)}$$

The measurement of RWC or WSD therefore needs an additional step, namely the measurement of the saturated or turgid mass of the tissue. This is achieved by placing the tissue in contact with water in a humid chamber at constant temperature and allowing it to absorb the water until it is fully turgid. The time required to reach saturation varies with species and condition of the plant tissue. For instance, Turner (1981) reported that water uptake is rapid and greater in young tissue compared to mature tissue. Hsiao (1990) generalized RWC to be 88% or higher for well-watered plants during midday. He further highlighted that when RWC is reduced to 50 to 60% for several hours, cells in the leaf will die and thus the damage becomes irreversible.

RWC unfortunately suffers from inaccuracy due to the uncertainties involved in the determination of the saturation water content of the sample. The TM which is supposedly the maximum mass of the sample as it becomes saturated with water is elusive as it has no clear stopping point (Hsiao, 1990). The mass gain of plant tissue upon floating is rapid at first and slows after some hours (Kramer, 1988; Hsiao, 1990). Hsiao (1990) revealed that in the case of growing leaves, growth will continue even after excision, provided that water is available for the plant tissue. This was found to be due to infiltration of water through the cut edges of the tissue. This problem can also be encountered even with mature tissue.

Studying the effect of water stress on watermelon, Kirnak & Dogan (2009) found that stressed plants had significantly lower RWC compared to fully irrigated plants. Similar findings were reported by Umar (2006) in studies of mustard, sorghum and groundnut, and Lenzi *et al*. (2009) in their studies with some oleander cultivars suitable for pot plant production. In the same study by Umar (2006), dry biomass accumulation was also significantly lower in water stressed plants together with the RWC.

Studying the response of drought tolerant and drought sensitive maize genotypes to water stress, Moussa & Abdel-Aziz (2008) also found significantly lower RWC in water stressed plants compared to the control plants. The drought tolerant genotype had significantly higher RWC in both non-stressed and water stressed conditions. It was further suggested that physio-biochemical processes could be performed much more efficiently in the tolerant genotype than

in the susceptible one due to the high RWC (Umar, 2006). Significantly lower RWC in stressed plants compared to their controls have also recently been reported by Yousfi *et al*. (2010) during his studies with *Medicago truncatula* and *M. laciniata* populations.

Different species respond differently to water stress in terms of water loss and hence RWC. Studying two species of eucalyptus (*E. melliodora* and *E. microcapa*) and *Callitris columellaris*, Clayton-Greene (1983) found the eucalyptus species to be losing less water for a given decline in total water potential than the *C. columellaris* at moderate water stress (0 to approximately -4 MPa). Below this potential *C. columellaris* showed a greater resistance to water loss compared to both eucalypt species. This suggested a greater ability of *C. columellaris* to tolerate severe stress than the eucalypt species. The study also highlighted that young tissue loses water more readily than mature tissue. This was observed where adult shoots of *C. columellaris* had RWC of 81% compared to 59% for juvenile material. A similar trend was observed with *E. melliodora* (Clayton-Greene, 1983).

Stomatal conductance

It is well known that water stress effects stomatal closure, therefore the degree of stomatal opening can be an indication of the plant water status (Hsiao, 1990). However, Jarvis (1976) highlighted five variables which affect the stomatal conductance: quantum flux density, ambient CO_2 concentration, leaf-air vapour pressure difference, leaf temperature and leaf water status. This review was mainly focused on the leaf water status due to the nature and objectives of this study.

Umar (2006) stated that through evolution, a hydraulic stomatal optimization mechanism has developed to ensure that water loss does not exceed uptake by the roots. The concentration of potassium ions moving through the xylem was cited to be behind this mechanism by influencing the hydraulic conductivity of the transport pathway, and perhaps by affecting the nature of pit membranes within the xylem vessels. The resultant reaction can be a root-sourced chemical signal that can influence the hydraulic signaling between the root and the shoot. ABA has also been cited as being responsible for the chemical signaling between roots and shoots and hence the closure of the stomata during water stress (Hsiao, 1973; Hsiao *et al*., 1976; Kirkham, 1990; Hartung *et al*., 1999).

Several researchers have highlighted that stomatal conductance is reduced when plants are under drought conditions. This is through closure of the stomata as a strategy to minimize

further water loss through transpiration (Henson, 1982; Johnson & Ferrell, 1983; Hsiao, 1990; Do *et al*., 1996). This inhibition of stomatal opening and photosynthesis by water stress has been long established (Hsiao, 1973). The stomata are not affected until the leaf water potential drops to or below a threshold level which differs across species, growing conditions and the history of the plant with respect to water stress (Hsiao *et al*., 1976). At the same threshold level for the leaf water potential, the inhibition of photosynthesis sets in due to lower CO_2 inflow into the sub-stomatal cavity (Boyer, 1976; Hsiao *et al*., 1976; Canova *et al*., 2008; Moussa & Abdel-Aziz, 2008; Cui *et al*., 2009). Conductance was also reported to decline simultaneously with age of potato leaves (Vos & Oyarzun, 1987).

Hsiao *et al*. (1976) also stated that the threshold potential for stomatal closure may be lower for upper leaves than lower leaves in the canopy. This can be a setback for CO_2 assimilation since the upper leaves are the ones that receive most of the radiation but would not be able to continue assimilation because of the water stress induced stomatal closure (Hsiao *et al*. 1976; *Lenzi, et al*., 2009). Studying the effect of water deficit at different stages of pear-jujube tree, Cui *et al*. (2009) found that stomatal conductance was significantly lower after 5 days of water stress, and the percentage reduction was increased with the degree of water deficit. Lower stomatal conductance values under limited soil water conditions have also been reported by Blonquist *et al*. (2009) in their studies with turfgrass and alfalfa. Similar findings have been reported for pearl millet planted in a greenhouse (Henson, 1983), and greenhouse planted rose-scented geranium (Eiasu, 2009).

Liu *et al*. (2008) studied diurnal changes of stomatal conductance of cucumber leaves in a solar greenhouse in northeast China over a period of four months (October to January). He discovered that the conductance was higher early in the season and decreased with time. The diurnal variation in October and November was bimodal with the first peak late in the morning and the second late in the afternoon. Similar findings were reported by Reich & Hinckley (1989) in two oak species. In December and January, a unimodal curve of diurnal variations was observed with a peak between 1200 to 1300hrs (Liu *et al*., 2008).

2.4 Stomatal Distribution and Size

Water movement from plants to the atmosphere is through the stomatal openings found on leaf surfaces (Jackson *et al*., 1988; Hartung *et al*., 1999; Mehri *et al*., 2009). Under water stress conditions the stomata will close partially or completely depending on the extent of water stress.

Developing smaller but more densely populated stomata has been viewed as a means of adaptation in leaves growing under water stress conditions. This allows the leaf to rapidly regulate stomatal closure and hence reduce transpiration (Hsiao, 1973; Ozyigit & Akinci, 2009).

Different techniques have been devised to quantify the size of stomatal complexes and their frequency on leaf surfaces. These range from using Nuclear Magnetic Resonance (NMR) (van der Weerd, 2001), light microscopy of epidermal strips, silicone rubber impressions with fluorescence microscopy and Scanning Electron Microscopy (SEM) (Karabourniotis *et al*., 2001). It was also revealed by Karabourniotis *et al*. (2001) that the SEM is the most accurate device even though it has disadvantages due to it requiring expensive equipment as well as the fact that observation of a large number of samples is not possible. Despite these disadvantages the SEM was used in the present study as the microscopic facility are available at the University of the Free State (UFS).

In addition to the regulation through opening and closing of the stomata, the frequency and size of the stomata can be used as a water stress adaptation strategy by plants. In a study to determine variation of stomata dimensions and densities of tolerant and susceptible wheat varieties to drought stress, Mehri *et al*. (2009) found that stomata length and area were found to be significantly smaller in water stressed plants than in the controls on both surfaces of leaves. They also reported that stress tolerant varieties had fewer stomata than drought sensitive varieties. Smaller stomatal perimeters and areas have been reported with water stressed plants of Roman nettle (*Utrica pilufera* L.) when compared with well-watered plants (Ozyigit & Akinci, 2009).

In a study to evaluate the influence of genotype on stomatal characteristics and chlorophyll fluorescence parameters in the course of leaf development of European beech cultivars, Canova *et al*. (2008) found significant differences in stomatal length measured on the lower (abaxial) surfaces of the leaves of the different cultivars. The stomatal length was also found to be increasing gradually with phenological growth stages. Stomatal densities also differed significantly on the leaf surfaces of different cultivars. Maghsoudi & Maghsoudi (2008) also found significant differences in stomatal densities on the flag leaves of different wheat cultivars. They also observed that cultivars with more stomata had smaller guard cells.

Genotype also appears to be the controlling agent of the distribution of stomata between the upper (adaxial) and the abaxial surfaces of leaves. “Perfect” hyperstomaty occurs when all of

the stomata are located on the adaxial leaf surface and “perfect” hypostomaty occurs when all of the stomata are located on the abaxial surface of the leaf. When the stomata are more or less equally distributed on both surfaces the leaf is amphistomatic (Hardy *et al*., 1995). Different wheat cultivars have been observed to have differences in the distribution and size of stomata between the adaxial and abaxial surfaces (Maghsoudi & Maghsoudi, 2008).

Studying drought induced leaf modification of semi-arid grassland species; Hardy *et al*. (1995) found that C_3 meadow grass species had more stomata than C_3 range grass species on both leaf surfaces. C_4 range grass species were found to have greater stomatal densities on both surfaces when compared to C_3 range grasses. C_3 grasses displayed a pronounced tendency toward hyperstomaty, while most C_4 species were amphistomatic with exceptions towards hypostomaty.

2.5 Summary and Way Forward

Several studies on growth, and plant water relations have been done on various plant species as revealed by the literature reviewed. Several aspects of pearl millet were studied but most work was done either under controlled environments or in the field but covering different aspects from the objectives of this particular study. In our present work the focus was on the response to water stress during vegetative growth of two pearl millet lines under semi-arid conditions of South Africa. Specifically, growth parameters, physiological water status and characteristics and distribution of stomata were studied under three irrigation treatment levels for the two pearl millet lines.

The research will investigate a detailed comparison of well-watered and water stressed performance of two pearl millet lines in terms of growth, physiological plant water status and stomatal development. This information will in turn be used to describe the adaptability of the two pearl millet varieties to the semi-arid conditions in South Africa.

3. MATERIALS AND METHODS

3.1 General Materials and Methods

Study area

The study was carried out at the Department of Soil, Crop and Climate Sciences experimental farm at Kenilworth during the 2009/2010 summer growing season. Kenilworth is located at a latitude of 29° 01' S and longitude 26° 09' E and is 1354 m above sea level. The soil is loamy aridic ustothents (Bainsvlei Amalia 2300). It is reddish brown in colour with a fine sandy texture and with 8 - 14% clay and 2 - 4% silt (Soil Classification Working Group, 1991). According to M. Hensley (personal communication)[1] the drained upper limit (DUL) for the upper 1.8 m profile is 475 mm. From DULs for profile layers of different depths at the same site pre-determined by Chimungu (2009), the DULs for each 30 cm profile layer to the depth of 1.8 m were calculated.

Agronomic practices

Seedbed preparation was done conventionally using a plough and rotavator to achieve a fine tilth for a more effective seed and soil contact since millet is a small seeded crop. Irrigation was supplied by a line source sprinkler system and a neutron probe was used to monitor the soil water content through access tubes which were installed at the centre of the plots in each of the water treatments. The neutron probe measurements were done at 30 cm intervals to a depth of 1.8 m and this was done at least once a week. Rain gauges were also installed in all the water treatments to measure the amount of irrigation applied and rainfall received. Fertilizer was applied a day before planting at the rates of 40 kg N ha^{-1}, 30 kg P ha^{-1} and 20 kg K ha^{-1}. There were four rows planted for each plot at a spacing of 0.9 m while the spacing within the row was 0.2 m. Three to five seeds per planting hole were sown by hand on the 16th December 2009 and thinned to two plants two weeks later. Weeding was done regularly by hand or hoes to maintain the trial weed free.

Seeds

Two lines of pearl millet, GCI 17 and local race Monyaloti, which were sourced from the Agricultural Research Council – Grain Crops Institute (ARC – GCI) in Potchefstroom were used

[1] Prof M. Hensley, 2010. University of the Free State, P.O. Box 339, Bloemfontein, 9300

in the study. To check that the seeds are viable, a germination test was done prior to planting (Tfwala, 2009).

Irrigation

Three irrigation treatments, namely full (IR3), moderate stress (IR2) and water stress (IR1) were envisaged. Sprinkler heads spaced at 6 m intervals on the lateral (line source) provided linearly decreasing water amounts with distance away from the lateral and applying to a radius of 14 m. Irrigation was done when the available water in the soil in IR3 treatments was reduced to 70%, and water was applied to bring soil water content back to DUL for the whole 1.8 m soil profile.

The distance of the irrigation level plots from the lateral and relative water application are presented in Table 3.1. As IR1 plots are representing rainfed treatments, these plots were deliberately placed a few metres away from the irrigated plots to avoid lateral flow of water in the soil to the rainfed plots (IR1).

Table 3.1: Distance of water treatment plots from lateral and relative water application

Water treatment	**Distance from lateral (m)**	**Relative water application**
IR3 (full irrigation)	0 - 3.6	1
IR2 (Moderate stress)	7.2 – 10.8	0.52
IR1 (rainfed)	18.2 – 21.8	0

Field lay-out

Four replications were planted for this trial. Each line of pearl millet had three plots per each replication, which represented the three different irrigation treatments from fully irrigated to rainfed. Each plot was 3.6 m wide and 7 m long. The fully irrigated plots were situated closer to the line source and irrigation water application decreased to 0 mm as the distance away from the line source increases as shown in the upper part of Figure 3.1. This ensured that each pearl millet line is represented in each water regime. The spaces between the marked plots (IR1 to IR3) were used for other treatments for the PhD study (Bello, 2011).

Weather data

Weather data was measured hourly and recorded daily by the automatic weather station at the experimental site and obtained courtesy of Agricultural Research Council - Institute for Soil, Climate and Water (ARC – ISCW).

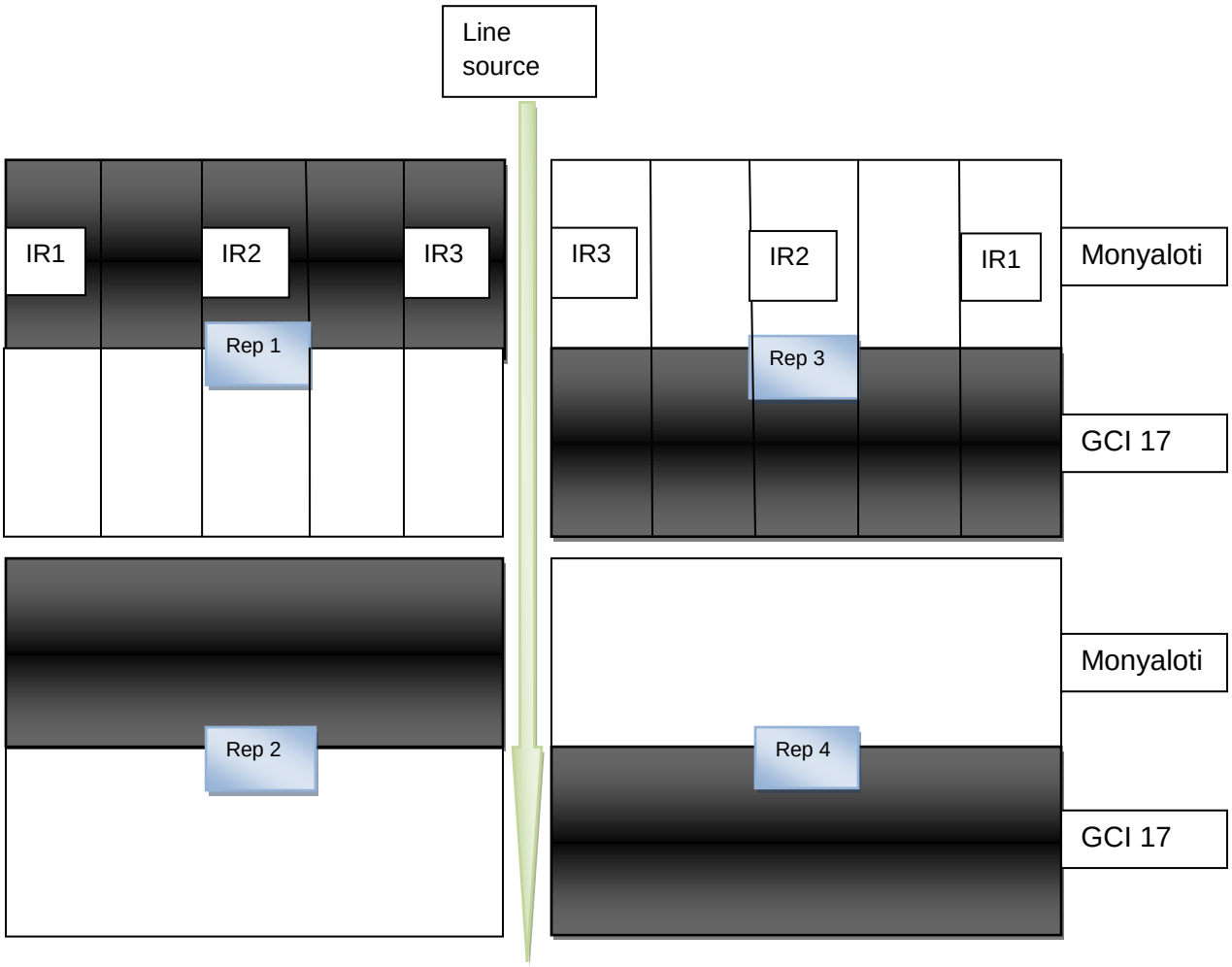

Figure 3.1: A sketch of the plot lay-out in the field showing irrigation level plots (IR1 to IR3), pearl millet line (□; Monyaloti and ■; GCI 17) and replications (Rep)

3.2 Growth Measurements

Growth measurements included plant height, number of tillers, number of leaves on the main shoot, leaf area and biomass accumulation. These parameters were measured weekly from three weeks after planting up to nine weeks after planting. Four plants were randomly selected from each treatment to monitor the plant height, number of tillers and number of leaves on the main shoot. Four plant samples per treatment from each of the four replications were cut at the soil surface every week. From these samples the leaves were removed and a LI3000 leaf area meter (LI-COR Inc., Lincoln, Nebraska, USA) was used to determine the green leaf surface area from the tillers and the main shoot and hence the whole plant sample. All the leaves together with the stems were then dried in an oven at 70°C for 72 hours to a constant dry mass, which represented the dry matter accumulated up to that particular week.

3.3 Plant Water Status Measurements

Upon realizing that there were no differences in the water status measurements between the irrigated and rainfed treatment plots due to high rainfall during the vegetative phase, a rain prevention shelter (Figure 3.2) was erected over the rainfed plots 46 days after planting (1st February 2010). The plots were covered just before each rainfall event based on weather forecasts or personal observations. The clear plastic roofing was removed after every rainfall event until the next one. The plot under this structure is labeled IR1* when reporting parameters such as growth and soil water content while IR1 is a mean of the other three replicates of the rainfed treatment.

Figure 3.2: Rain-out shelter on the water stress treatment plots erected 46 days after planting (Tfwala, 02/02/2010)

Leaf water potential

A pressure chamber was used to determine the water potential of selected pearl millet leaves in all the water treatments. The procedure that was followed is the one outlined by Ritchie *&* Hinckley (1971), Henson (1982) and Hsiao (1990). Five leaf samples per water treatment level were randomly selected from fully expanded and fully exposed leaves of the plants to avoid age effects. The leaves were enclosed in a transparent plastic bag prior to cutting to minimize loss of

water through transpiration. A sharp razor blade was used to cut the leaves just above the ligules. The samples were sealed in the chamber with only a minimum amount of the cut end protruding. With the sample in the chamber, pressure was applied slowly until sap appeared at the protruding cut end. This end-point was observed carefully with the aid of a magnifying glass. The negative pressure value (P) at this end point was noted as the total water potential of the leaves. Water potential measurements were done on three days in a week between midday and 1400 hrs, beginning from week four up until 50% flowering in both pearl millet lines. Exactly eight days after the rainfall prevention structure was erected on the water stress plots, water potential was measured every 2 hours from pre-dawn (0500hrs) to dusk (1900hrs) to establish diurnal changes in the plant water status.

Osmotic potential

Two leaf samples per variety were taken for osmotic potential analysis from the well-watered and rainfed treatments on the 10th February (9 days of stress). The pressure chamber was used, employing the pressure - volume (P-V) curve method (Tyree & Hammel, 1972; Roberts & Knoerr, 1977; Turner, 1981) to determine the osmotic potential and hence osmotic adjustment in water stressed plants relative to well-watered for the two lines of pearl millet.

After sampling, leaves were put in distilled water and left overnight (12 - 14 hours) to rehydrate to full turgor before establishing the P-V relationship. The pressure chamber was used to force the sap to return to the cut end. The leaf was over-pressurized by -0.2 to -0.3 MPa for 10 minutes and the water which was forced from the leaf was collected into a vial and weighed with a scale recording up to four decimal places in grams. This value was then converted into volume of expressed sap, V_e, using 1000 kg m^{-3} as density of water. Since small volumes of water were collected, filter paper in the vial was used to efficiently collect the water.

The pressure in the chamber was reduced (from the over-pressure value) to the previous P, and then slowly increased to obtain a new P value. These steps were repeated 10 times or until enough points to obtain the linear portion of the P-V curve have been plotted. The P-V curve is constructed by plotting $\frac{1}{P}$ against the cumulative V_e. A linear least-squares fit was made through the points on the linear part of the curve and used to extrapolate to $\frac{1}{P}$ when $V_e = 0$ (100% RWC). These gave the estimates of the inverse of the initial osmotic water potential.

Relative water content

The procedure outlined by Henson (1982), Kramer (1988) and Kirnak & Dogan (2009) was followed to determine the RWC. Three leaves per treatment were sampled from the fully expanded, fully exposed section of the plants every week. Sub-samples were taken from the middle portion of the leaves. These sub-samples were weighed to obtain the fresh mass (FM). They were then floated in distilled water in Petri-dishes to full hydration. After re-hydration, the leaves were weighed to obtain the turgid mass (TM). At the end, the imbibed leaves were oven dried at 70^{o}C for 48 to 72 hours to a constant dry mass (DM). A scale with precision to four decimal places was employed for the weighing in grams. The RWC was calculated using Equation 7.

Stomatal conductance

A Decagon Leaf Porometer was used to measure the stomatal conductance on the abaxial surfaces of fully expanded and fully exposed leaves. Conductance was measured for five leaves randomly sampled every three days in a week. This was from week four after planting to 50% flowering in all the different water treatments for the two lines of pearl millet. The measurements were taken between midday and 1400hrs.

3.4 Microscopic Study of Stomatal Characteristics

Two leaf samples from each of the irrigation treatments were taken on the 16th February (15 days after withholding rainfall from water stress plots (IR1*)) Samples were taken just after midday. A sub-sample from the middle of each leaf was selected to determine the effect of water stress on the number of stomata per unit leaf surface area and measure their size (length and width) on both abaxial and adaxial surfaces. The percentage of open stomata at the various irrigation levels was also counted on both surfaces for the two lines of pearl millet.

Leaves from the fully expanded and fully exposed section of the plants were sampled 15 days (16th February) after imposition of the rain-out structure. The leaves were put into a 2.5% gluter (di)aldehyde (for more than 4 hours) and then to 2% osmium tetroxide (OsO_4) (1 hour) for fixation. Dehydration was done in an ethanol of acetone series of 30%, 50%, 70% and 95% for ten minutes at each stage.

A critical point drier was used to dry the samples using liquid CO_2 (to replace the ethanol) at 37°C. The leaf samples were then mounted on metal stubs prior to coating with thin gold using a

BIO-RAD sputter coater. Finally they were analyzed under a JSM 6400 Orion scanning electron microscope operated at 5 kV at UFS, Centre for Microscopy, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.

The density (number of stomata mm^{-2}) and percentage of open stomata were calculated on five pictures per treatment taken at X 450 magnification. The length and width of stomata were measured using a ruler from the picture printouts and converted to actual dimensions using the scale on each picture (Figure 3.3). All data were means of four observations expressed in micrometres (µm). The stomatal area (A) was calculated using equation (10) assuming that the stomata resemble the shape of a perfect ellipse:

$$A = \pi(\tfrac{1}{2}L)(\tfrac{1}{2}W) \qquad (10)$$

where L is the length and W is the width.

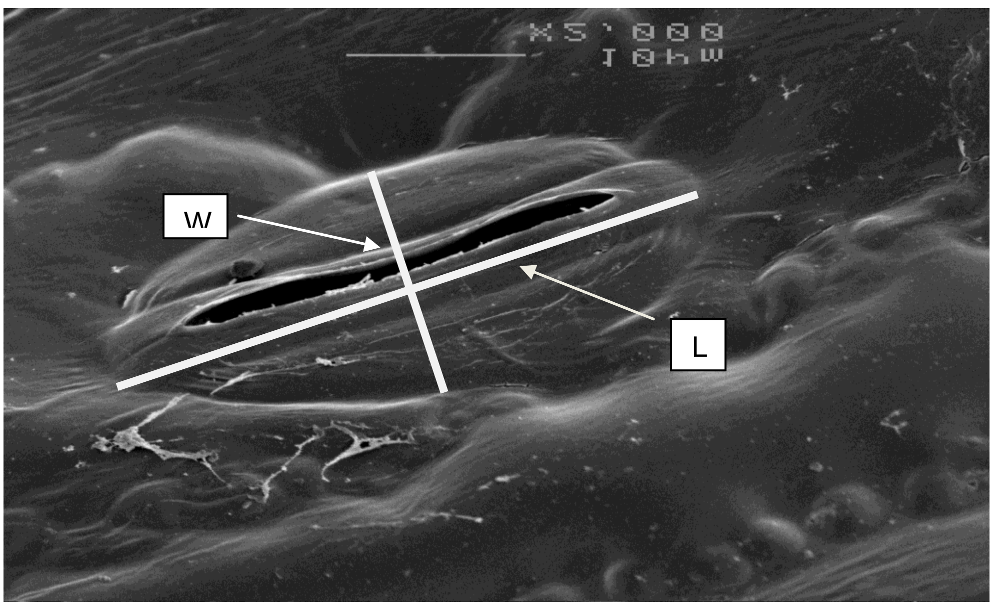

Figure 3.3: Illustration of stomatal length (L) and width (W) measurements (magnification X 5000)

3.5 Data Analysis

Data for growth parameters, physiological plant water relations and stomatal characteristics were analyzed using SAS 9.1 statistical package. The significance of differences were determined using Fisher's LSD because of its simplicity and power to separate means compared to other post-hoc methods such as Tukey test method (Kemp, 1973; Gomez & Gomez, 1984).

4. RESULTS AND DISCUSSION

This chapter will be presented in four sections: weather and soil water conditions, growth measurements, plant water relations and stomatal characteristics and distribution. Some tables of analysis of variances (ANOVA) are included in the appendices section to support statistical discussion in some parts of the latter three sections of the chapter.

4.1 Weather and Soil Water Conditions

The 2009/10 growing season was characterized as hot and very wet as the monthly average temperatures and precipitation were higher than the long-term figures of these variables at the experimental site (Table 4.1). Solar radiation recorded during the study ranged from 15.35 to 28.63 MJ m^{-2} d^{-1}, 4.78 to 27.57 MJ m^{-2} d^{-1} and 19.93 to 25.83 MJ m^{-2} d^{-1} during the months of December, January and February respectively (Figure 4.1).

Table 4.1: Comparison of air temperature and precipitation for 2009/10 growing season at Kenilworth experimental farm (ARC – ISCW) with long-term weather data for Bloemfontein airport (SAWS, 2002)

Month	**Weather parameter**					
	Air temperature (°C)				Rainfall (mm)	
	Long-term		Current season		Long-term	Current season
	Average min.	Average max.	Average min.	Average max.		
December	13.8	30.1	15.0	32.8	60	58
January	15.3	30.8	16.9	28.4	83	133
February	14.7	28.8	16.8	29.9	111	120

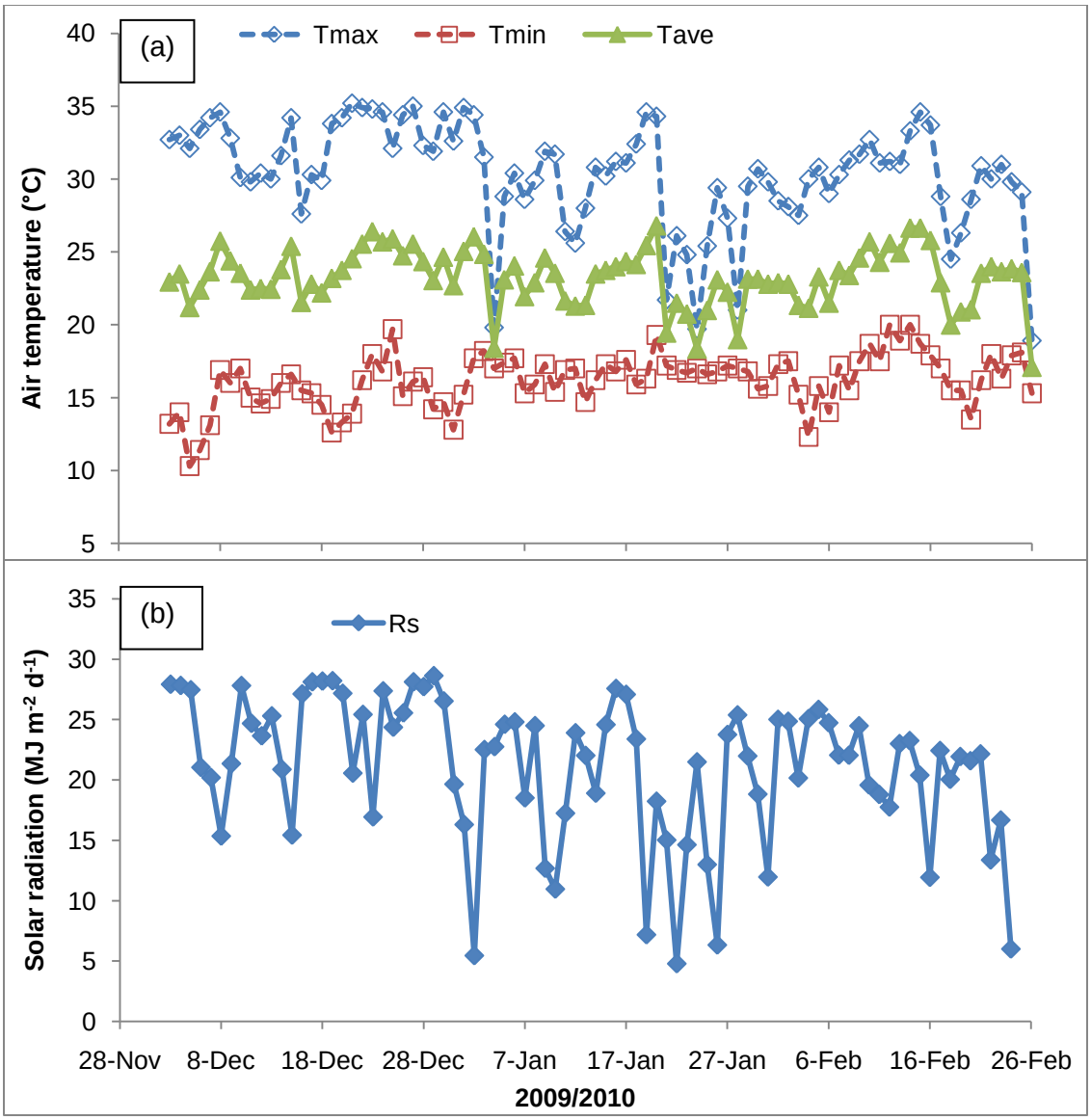

Figure 4.1: (a) Air temperature, T_{max}, T_{min} and T_{ave} are the daily maximum, minimum and average air temperatures and (b) solar radiation (Rs) at the University of the Free State - Kenilworth experimental farm during the 2009/10 growing season

The daily precipitation was recorded and reference evapotranspiration (ETo) was calculated using data obtained from the automatic weather station at the experimental site and show the variability through the summer season (Figure 4.2). The precipitation was distributed throughout the season but more events occurred during January and resulted in a total of 133 mm which is 60% more than the long-term mean value.

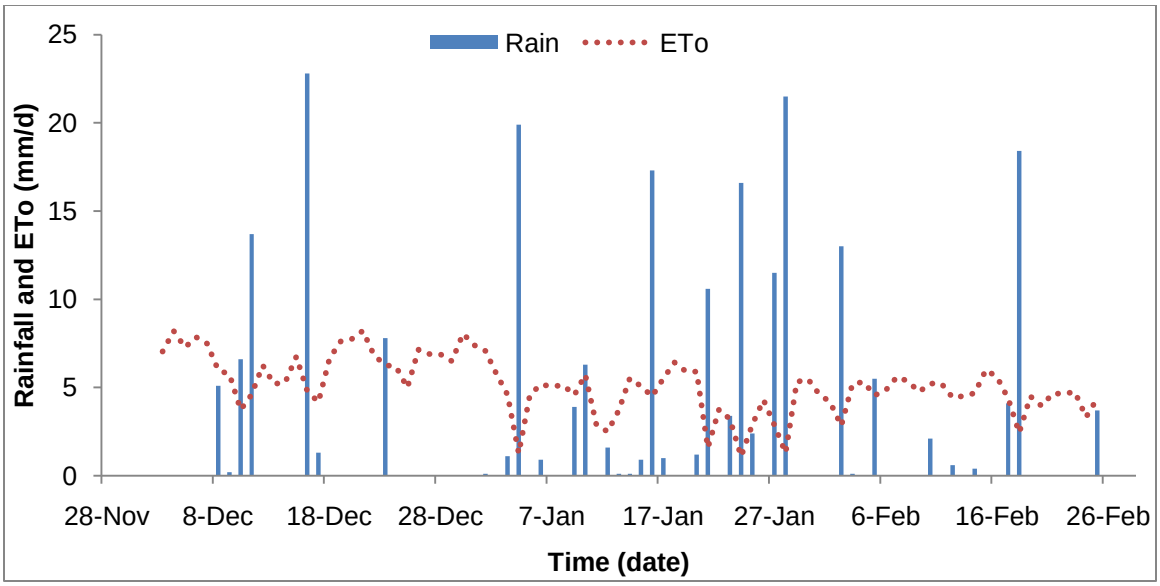

Figure 4.2: Rainfall recorded and reference evapotranspiration (ETo) calculated by the automatic weather station at Kenilworth experimental farm during the months of December, January and February 2009/10

The few dry spells in late December and early February were the only opportunities which allowed for implementation of three irrigation events for the water treatments (Table 4.2).

Table 4.2: Irrigation dates and irrigation amounts applied in the three irrigation levels

Date	**Irrigation amount (mm)**		
	IR3	IR2	IR1
30 Dec	20	11.5	0
13 Jan	5.5	2.65	0
8 Feb	42	16.5	0

The soil water content measured at 30 cm depth intervals for the three water treatment levels is presented in Figure 4.3. The top 30 cm soil layer generally had varying water content according to water treatment levels. The same trend was observed in the second layer (30 – 60 cm) but the differences in soil water content for the irrigated plots (IR3 and IR2) were smaller throughout the experiment. In the third layer (60 – 90 cm) even the rainfed plots had high water content until late January 2010 for GCI17.

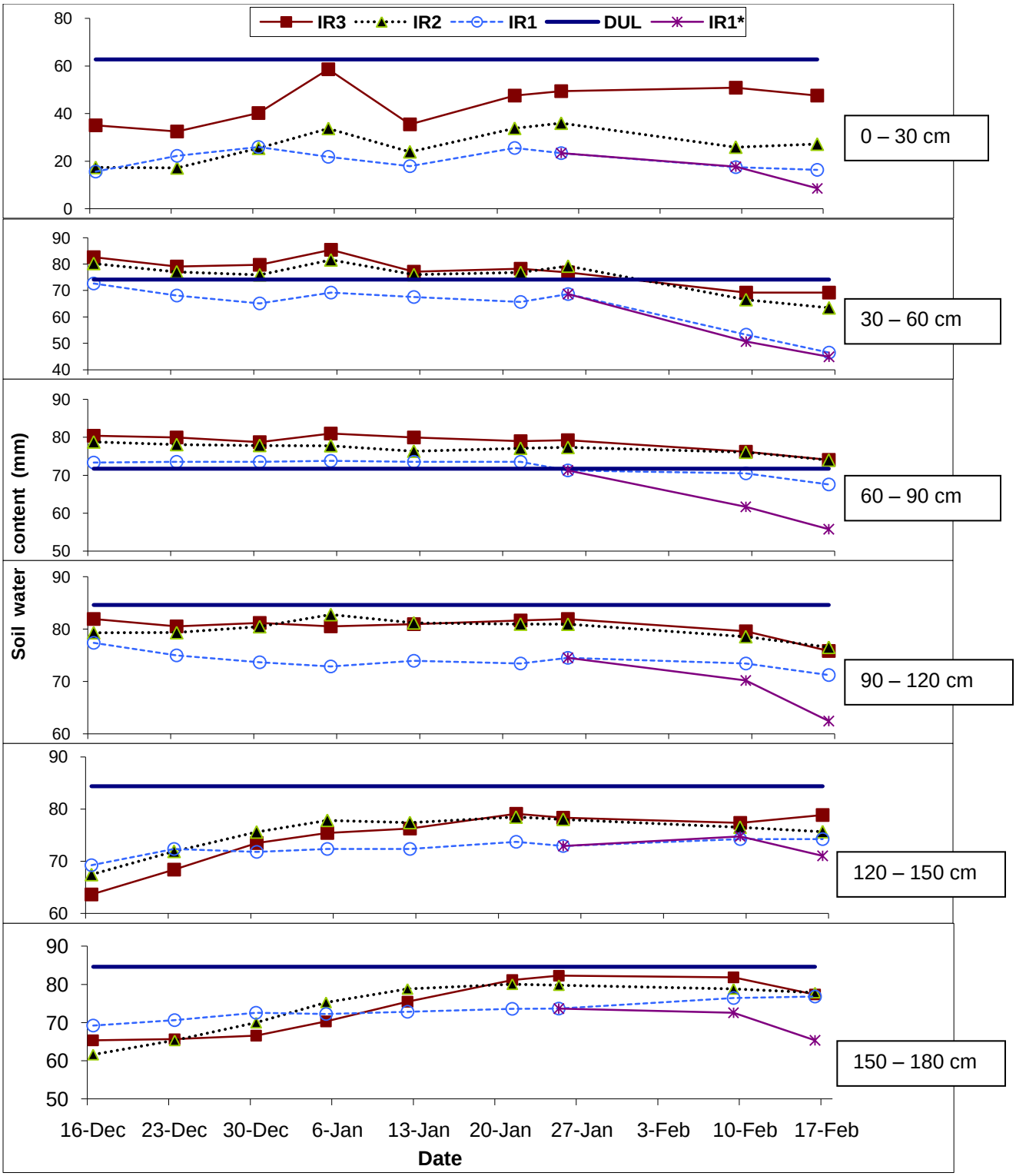

Figure 4.3a: Soil water contents of profile layers for GCI 17 planted plots at depths as shown in blocks at Kenilworth during 2009/10 growing season according to irrigation treatments, IR3 is well-watered, IR2 is moderately stressed, IR1 is rainfed and IR1* is rain-out plots between 1st and 17th February 2010

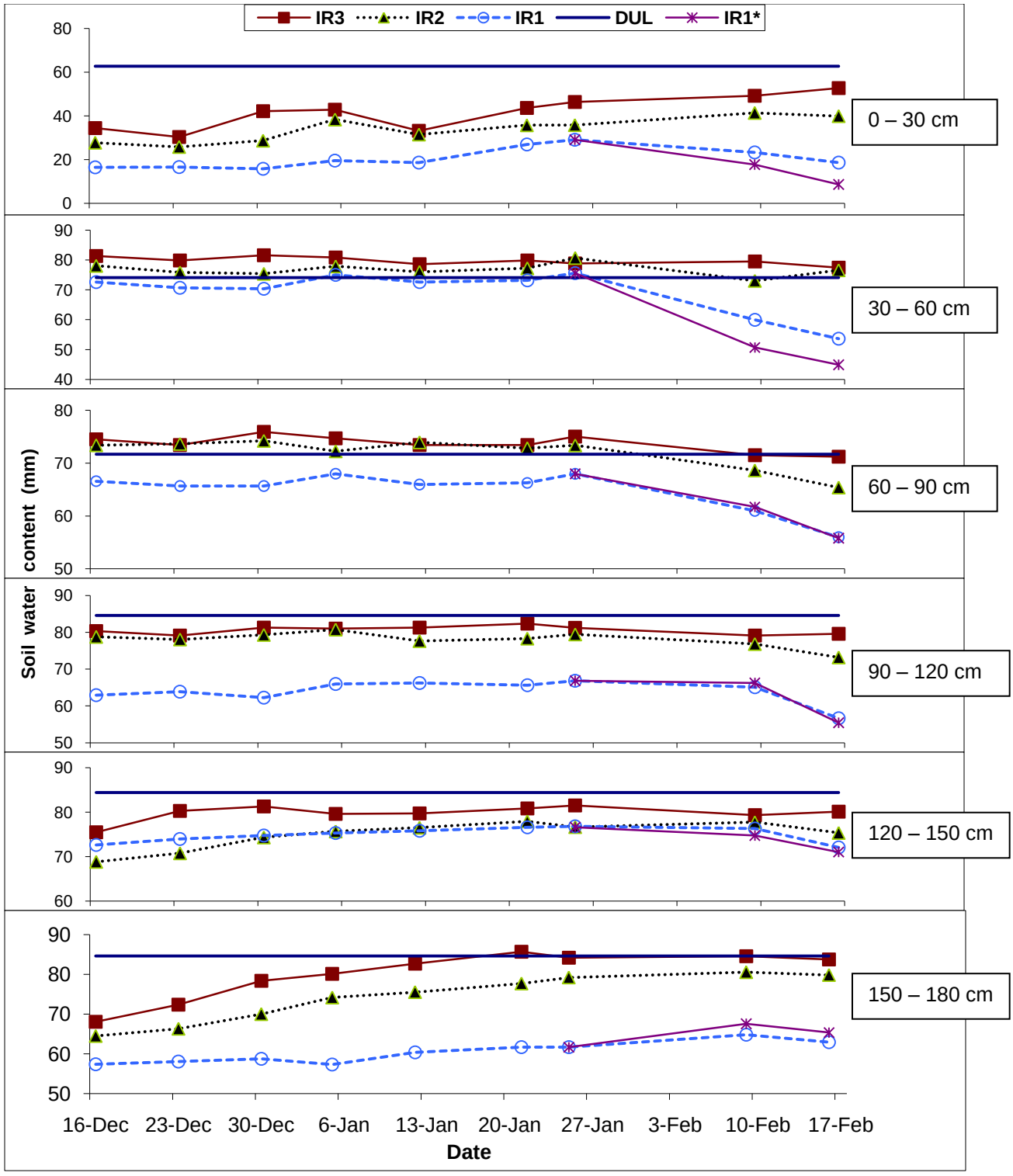

Figure 4.3b: Soil water contents of profile layers for Monyaloti planted plots at depths as shown in blocks at Kenilworth during 2009/10 growing season according to irrgation treatments, IR3 is well-watered, IR2 is moderately stressed, IR1 is rainfed and IR1* is rain-out plots between 1st and 17th February 2010

In the top layer (30 cm) IR3 plots were always wettest throughout the vegetative growth season. At the end of this period, GCI IR1 plots were drier than Monyaloti IR1 plots by about 10 mm. In the 60-90 cm profile layer the soil water content for Monyaloti IR1 was less than that of GCI 17 plots by about 7 mm. At 90-120 cm both lines showed that IR1 had almost 20 mm less water than irrigated soils. At 150-180 mm, Monyaloti plots were drier than GCI 17 plots throughout the season.

In GCI 17, the rain-out plots (IR1*) were observed to be less deviating from the IR1 particularly in the top 60 cm of the soil profile. In the underlying soil profile layers the IR1 plots were wetter than the IR1* plots. In Monyaloti, much more deviation was seen in the top 60 cm as the IR1 plots were wetter than the IR1* plots. For the rest of the profile layers, the soil water content of the IR1 and IR1* plots were relatively the same.

It seemed that there is an impeding layer just below this profile layer (60 – 90 cm) as the water content from 30 cm to 90 cm was equal or slightly above the soil drained upper limit (DUL). In the deeper profile layers (90 – 180 cm) the soil water content of the irrigated plots were generally similar and higher than that of the rainfed plots. The lower levels of the profiles began with low soil water (after dry season) and improved as the season progressed except for the deepest layer (150-180 cm) in the rainfed treatment.

The soil water content for the plots under the rain-out structure (IR1*) are shown by the lines deviating from IR1 plots in Figures 4.3 and 4.4. In Monyaloti, the downward deviation of soil water was mainly in the top 60 cm of the soil profile only and the total soil water presented in Figure 4.4 reveals that there was a smaller difference between the IR1 plots of Monyaloti and IR1*. A relatively bigger difference was observed between total soil water content of IR1 plots of GCI 17 and the IR1* plots. It was again revealed that the irrigated treatments had generally higher soil water contents than the IR1 plots which were on the lower side throughout the season.

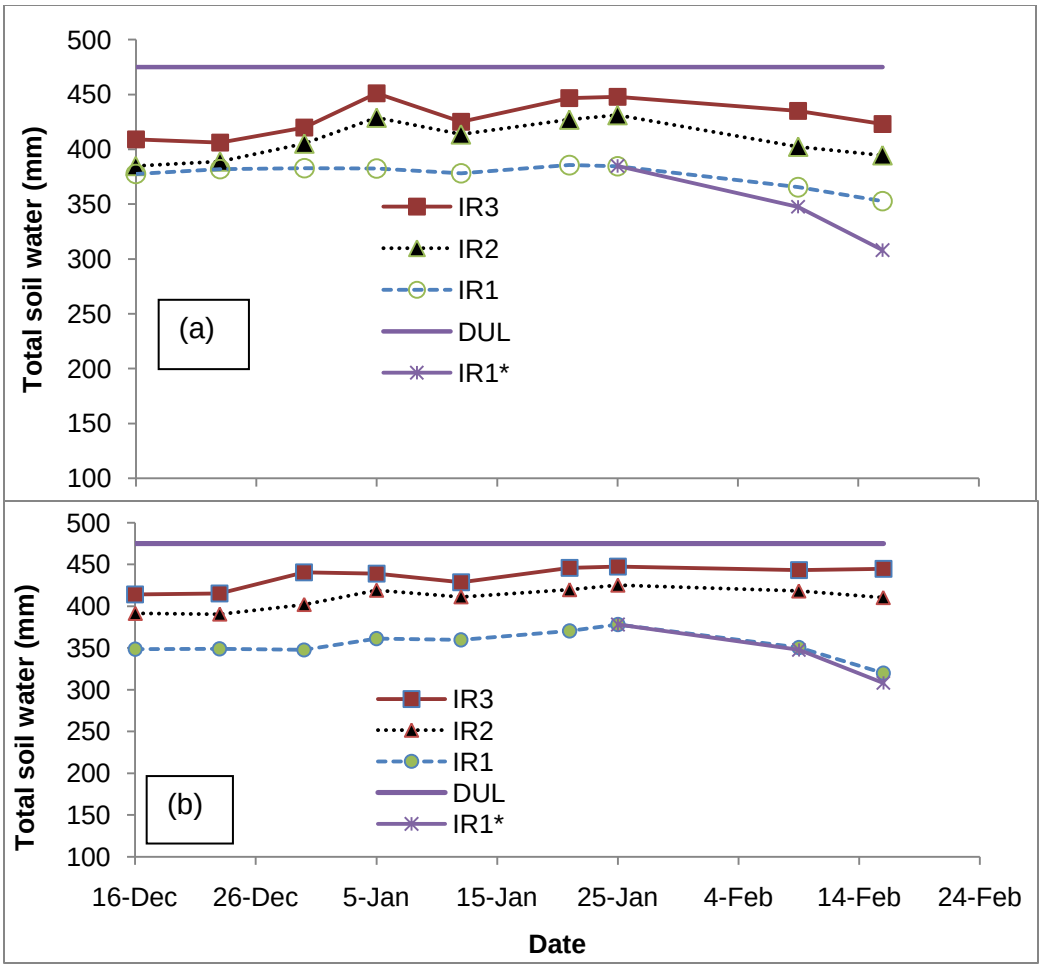

Figure 4.4: Soil water content for the profile (0 – 1.8 m) at Kenilworth during 2009/10 growing season according to irrigation treatments, IR3 is well-watered, IR2 is moderately stressed, IR1 is rainfed and IR1* is rain-out plots between 1st and 17th February 2010, for (a) GCI 17 and (b) Monyaloti

4.2 Growth Measurements

All growth parameters were measured once a week beginning from the third week after planting until the ninth week of this study. This was the time when both pearl millet lines had reached a point where at least 50% of main shoots were flowering in all the treatments. The irrigated plots of GCI 17 reached 50% flowering a week earlier than its own non-irrigated plots. Water treatments did not have any effect on the time to 50% flowering on the local race, Monyaloti; however it attained 50% flowering a week later than the irrigated GCI 17. The discussion of growth parameters will include: plant height, number of tillers, number of leaves, leaf area development and biomass accumulation.

Plant height

Plant height was not different among the treatments (both lines and irrigation levels) until 6 weeks after planting (Figure 4.5). It was only during the seventh week that differences were observed between both pearl millet lines and irrigation levels (Figure 4.6) even though the standard error bars still overlapped. At this stage, GCI 17, an early maturing variety was taller than Monyaloti which is a late maturing variety. The irrigated plots also began to have taller plants compared to their water stress counterparts in both pearl millet lines.

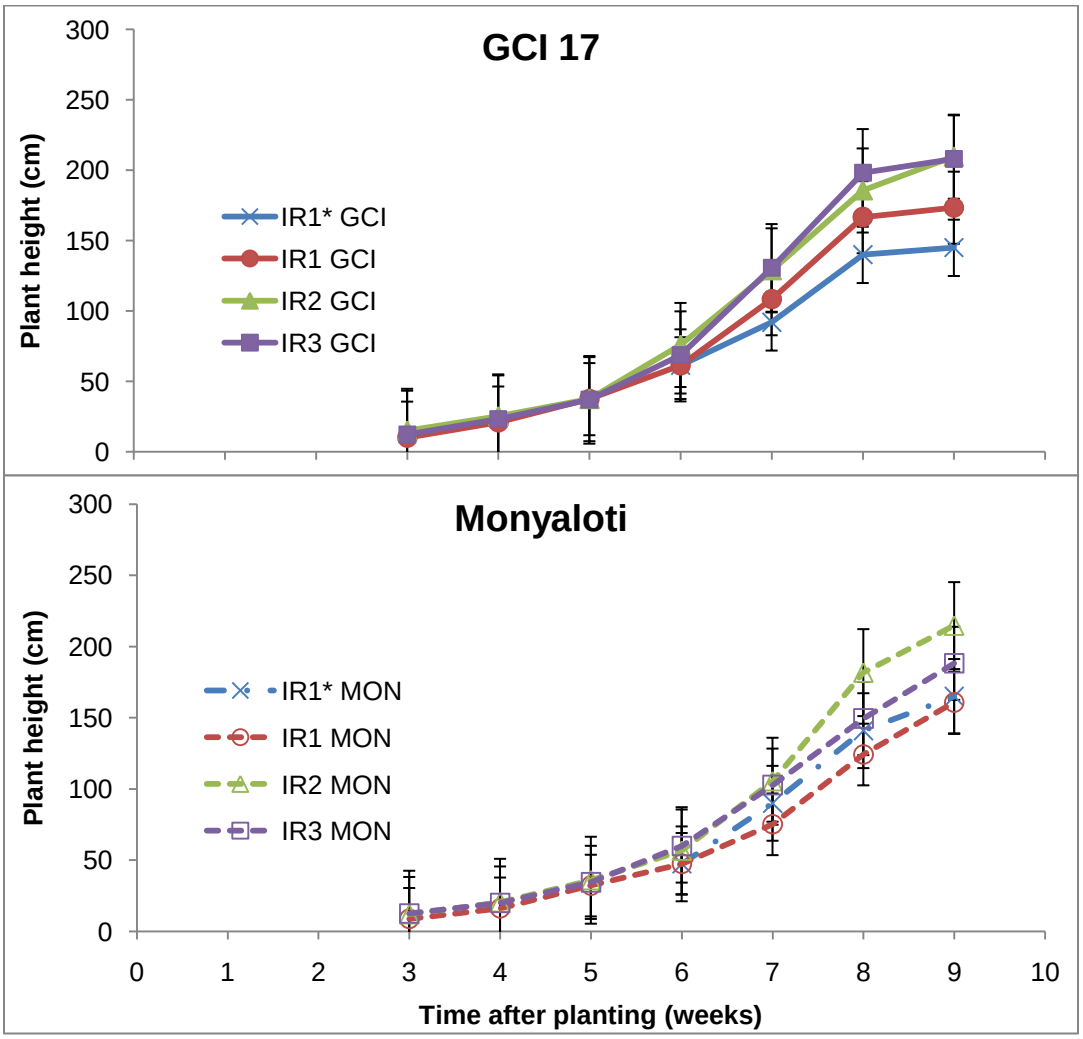

Figure 4.5: Plant height for two pearl millet lines subjected to three irrigation levels at Kenilworth during the 2009/10 growing season, IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

The difference between the lines was significant (Appendix 1A) during week 7 which was within a week after withholding water (rain) on the IR1* plots ($P<0.05$) had started. During week 8, GCI 17 which was already flowering, particularly on the irrigated plots, was still significantly taller

than Monyaloti. Again on week 9 there were no significant differences between lines since Monyaloti was still vigorously growing while vegetative growth had generally stopped for GCI 17.

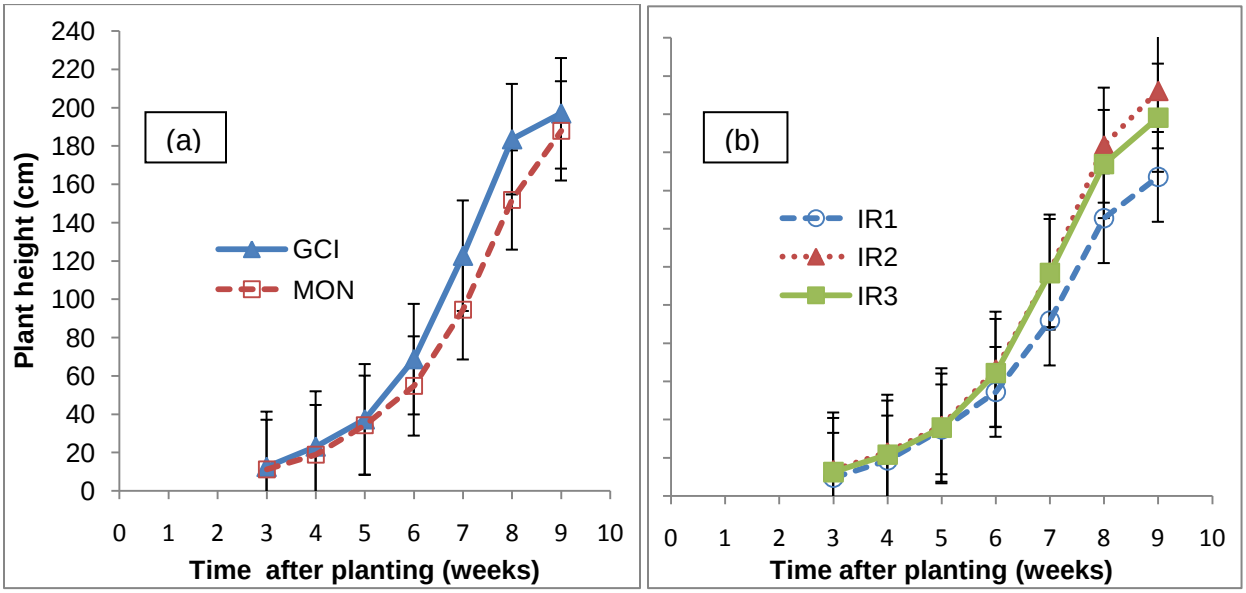

Figure 4.6: Plant height according to (a) pearl millet line and (b) irrigation level

The fully irrigated and moderately stressed plants were never significantly different in plant height in the respective lines throughout the study (Appendix 1A). It was noticiable that the moderately stressed plants were taller than the control plants. On the third week (week 1 after application of irrigation treatments) the irrigated plots had significantly taller plants than IR1 plots. This difference was however short lived as it lasted only this particular week with the control plants and one more week with moderately stressed plants (IR2).

Because of the rains in January, even the rainfed plants grew fast and caught up with the irrigated plants such that differences in plant height were only realised on week 8 between moderately stressed and stressed plants. The control plants were intermediate and significantly different from both the IR2 and IR1 plants. During week 9 water stressed plants were significantly shorter than irrigated treatments.

Number of tillers

The irrigation treatments did not induce any significant effects on the number of tillers (Appendix 1B), and likewise neither did the line of pearl millet. GCI 17 had noticeable higher number of tillers compared to Monyaloti plots (Figure 4.7 and Figure 4.8 (a)) until between week 6 and 7

when both lines had reached their peaks which was about 13 tillers per plant. It was only during week 9 that the number of tillers for Monyaloti was higher than for GCI 17. This was the time when tillers which had not flowered were dying off with most of assimilates directed towards the reproduction organs especially in GCI 17 which had flowered earlier than Monyaloti.

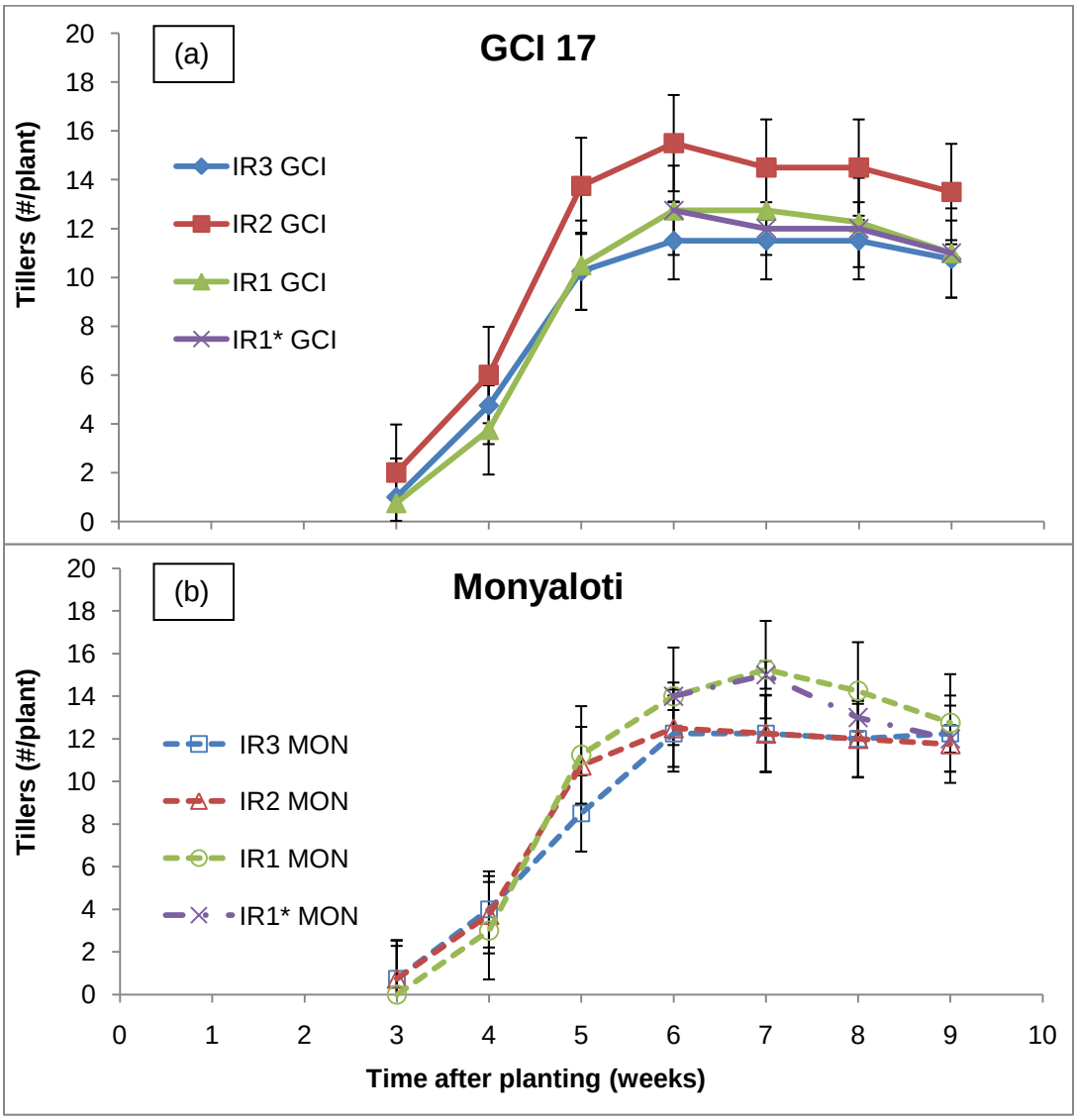

Figure 4.7: Number of tillers per plant for two pearl millet lines subjected to three irrigation levels at Kenilworth during the 2009/10 growing season, IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. (a) GCI is GCI 17 and (b) MON is Monyaloti

The moderately stressed plants seemed to develop tillers faster than the fully irrigated and water stressed plants (weeks 3 to 6 on Figure 4.7b). After week 7 a decline in the number of tillers was observed, particularly in the water stressed plants of Monyaloti (IR1) and all GCI 17 treatments (Figure 4.7).

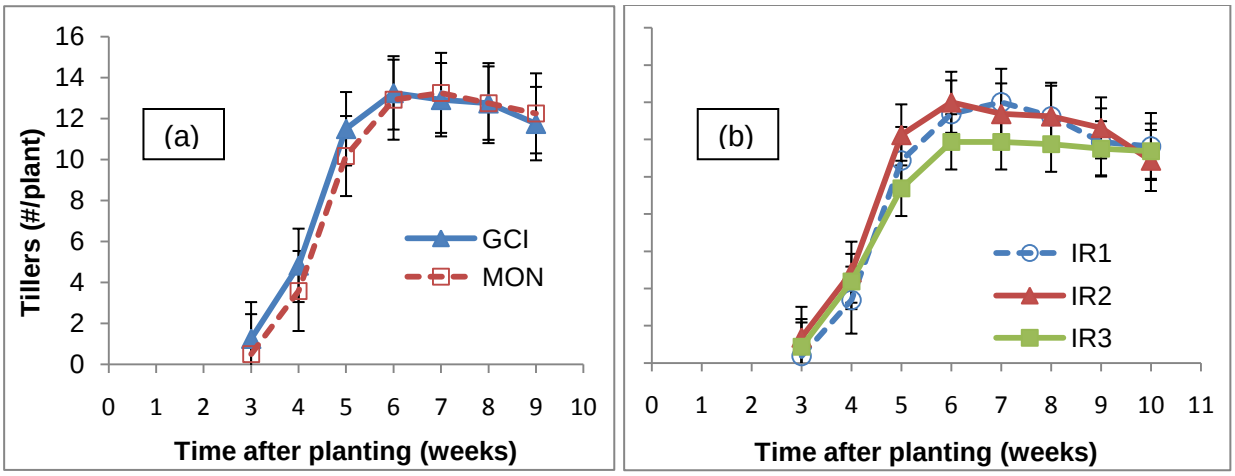

Figure 4.8: Number of tillers per stand according to (a) pearl millet line and (b) irrigation level

Number of leaves on main shoots

The irrigation treatments did not result in differences (P>0.05) in the number of leaves (Figure 4.9) on the main shoots throughout the experiment. The number of leaves started to differ after week 7 (Appendix 1C) and the difference was due to genetic variation between the two pearl millet lines. From week 8, Monyaloti had significantly (P<0.05) more leaves (10.9) compared to GCI 17 (9.9).

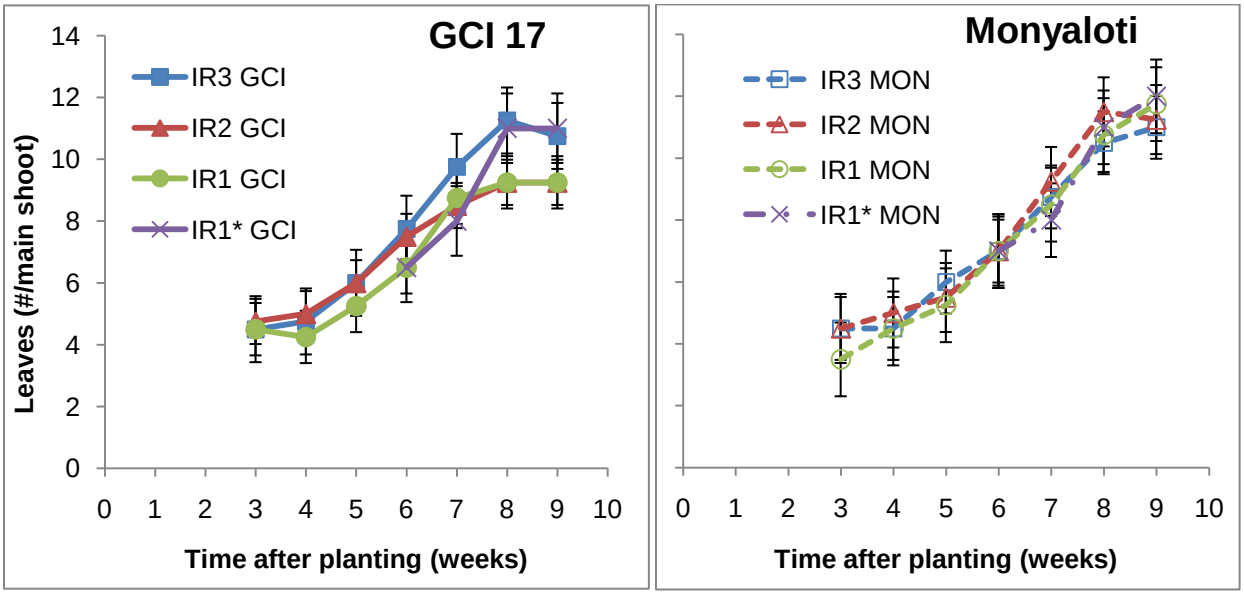

Figure 4.9: Number of green fully expanded leaves per main shoot for two pearl millet lines subjected to three irrigation levels at Kenilworth during the 2009/10 growing season, IR1, 2 & 3 are irrigation levels from rainfed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

Highly significant (P<0.01) differences in the number of leaves between pearl millet lines were observed during week 9 (Figure 4.10a). By this time GCI 17 was concentrating its assimilates towards reproduction and hence the lower leaves were drying. Similar observations were reported by Do *et al*. (1996).

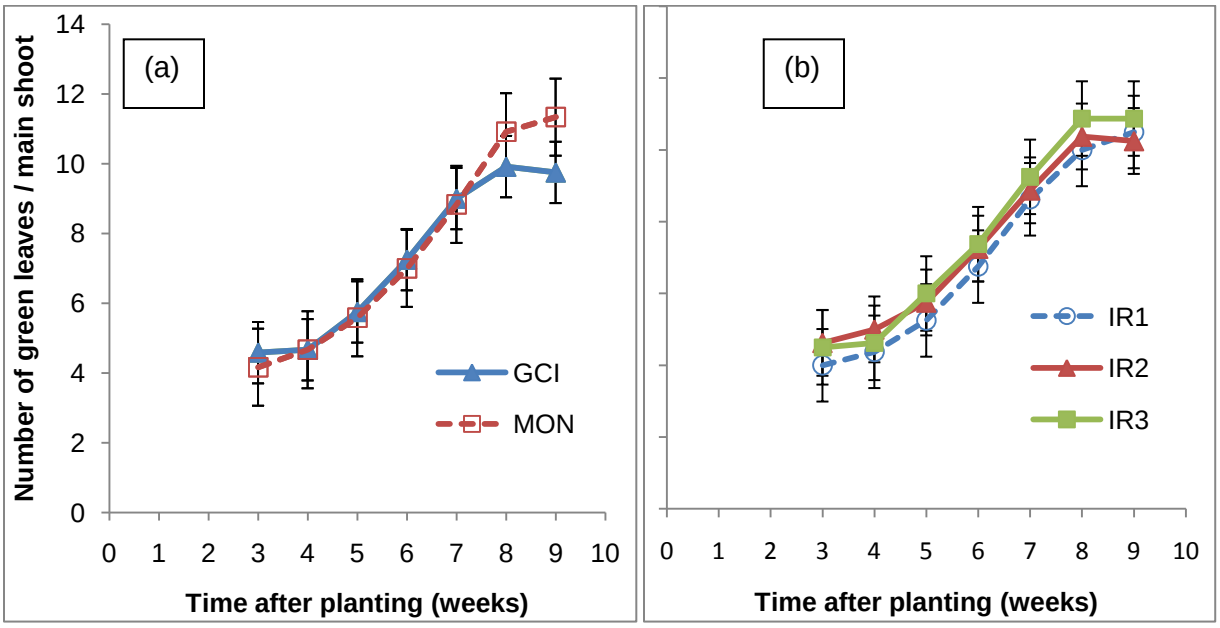

Figure 4.10: Number of green leaves per main shoot according to (a) pearl millet line and (b) irrigation level

Leaf area development

The leaf area development, expressed in the form of leaf area index (LAI) for the two pearl millet lines subjected to the various water treatments are presented in Figure 4.11. For GCI 17, the leaf area index showed differences as from the fourth week after planting. It was on the fifth week that the LAI of moderately stressed plants (IR2) was already significantly higher than that of the water stressed plants (Appendix 1D). The well-watered plants (IR3) consistently had lower LAI values than the IR2 plants throughout the growing season. The highest LAI values, attained 7 weeks after planting were 7.9 and 7.2 for IR2 and IR3 plants respectively. The growth was noticeable delayed in the stressed plants (IR1 and IR1*) as they attained their highest LAI values of 5.1 and 3.6 a week later (week 8). These two values were significantly different from each other and were both significantly lower than the highest LAI values attained by the irrigated plants.

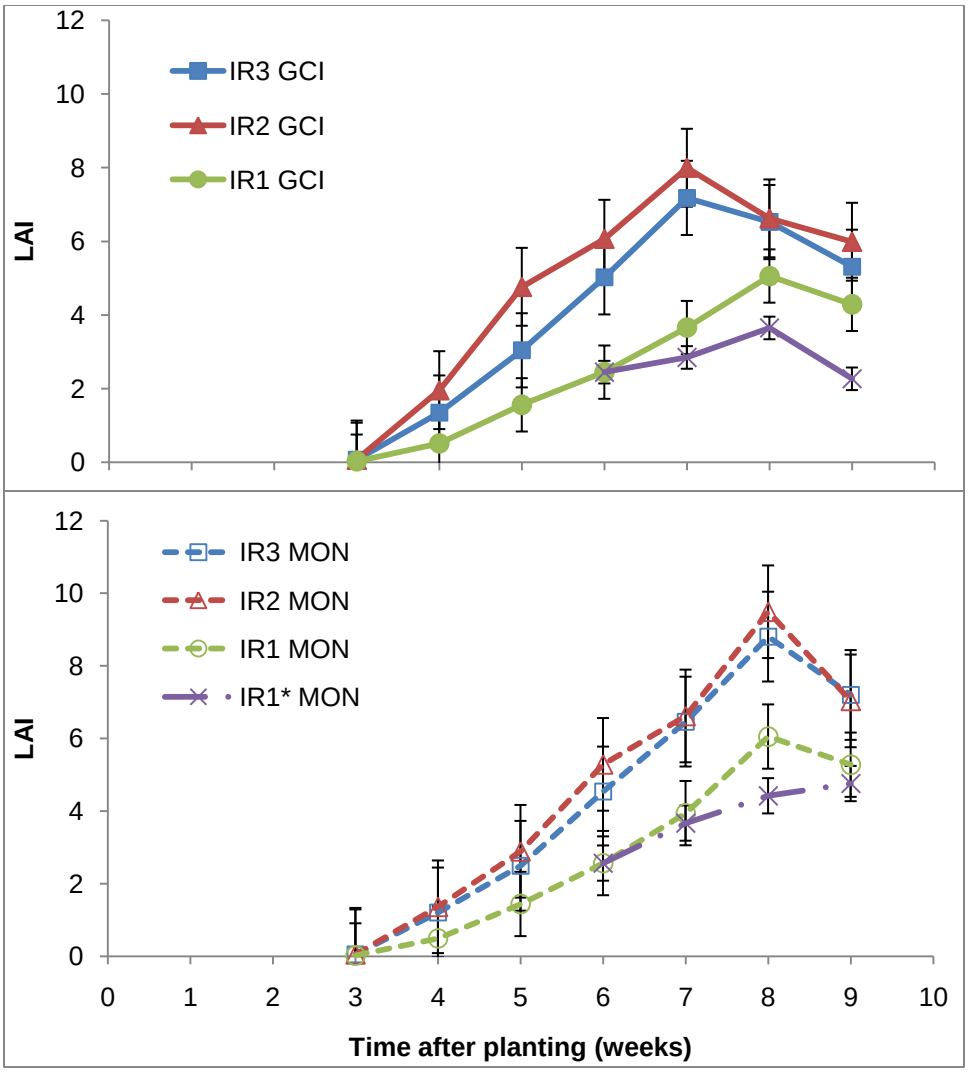

Figure 4.11: Leaf Area Index (LAI) for two pearl millet lines subjected to three water treatment levels, IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

A similar trend of LAI development as in GCI 17 was observed for Monyaloti. IR2 plants had the highest LAI values throughout the growing season, but almost equal values were observed in the IR3 plants. These two treatments had greater LAI values than the stressed plants throughout the season. Highest values of 9.5 and 8.8 were attained by IR2 and IR3 plants of Monyaloti. IR1 plants attained a highest LAI of 6.1 while IR1* plants which were seemingly still growing (Figure 4.11) attained a highest LAI of 4.7 in week 9 after planting. Monyaloti was seen to have higher LAI values than GCI 17. This also implied a higher capability of Monyaloti to capture solar radiation for photosynthesis and thus growth.

Biomass accumulation

The dry matter accumulated (total above ground biomass) by the two pearl millet lines in the various irrigation treatments is presented in Figure 4.12. Higher accumulation rates were observed in IR2 plants, followed by IR3 plants and water stressed plants were producing the least biomass.

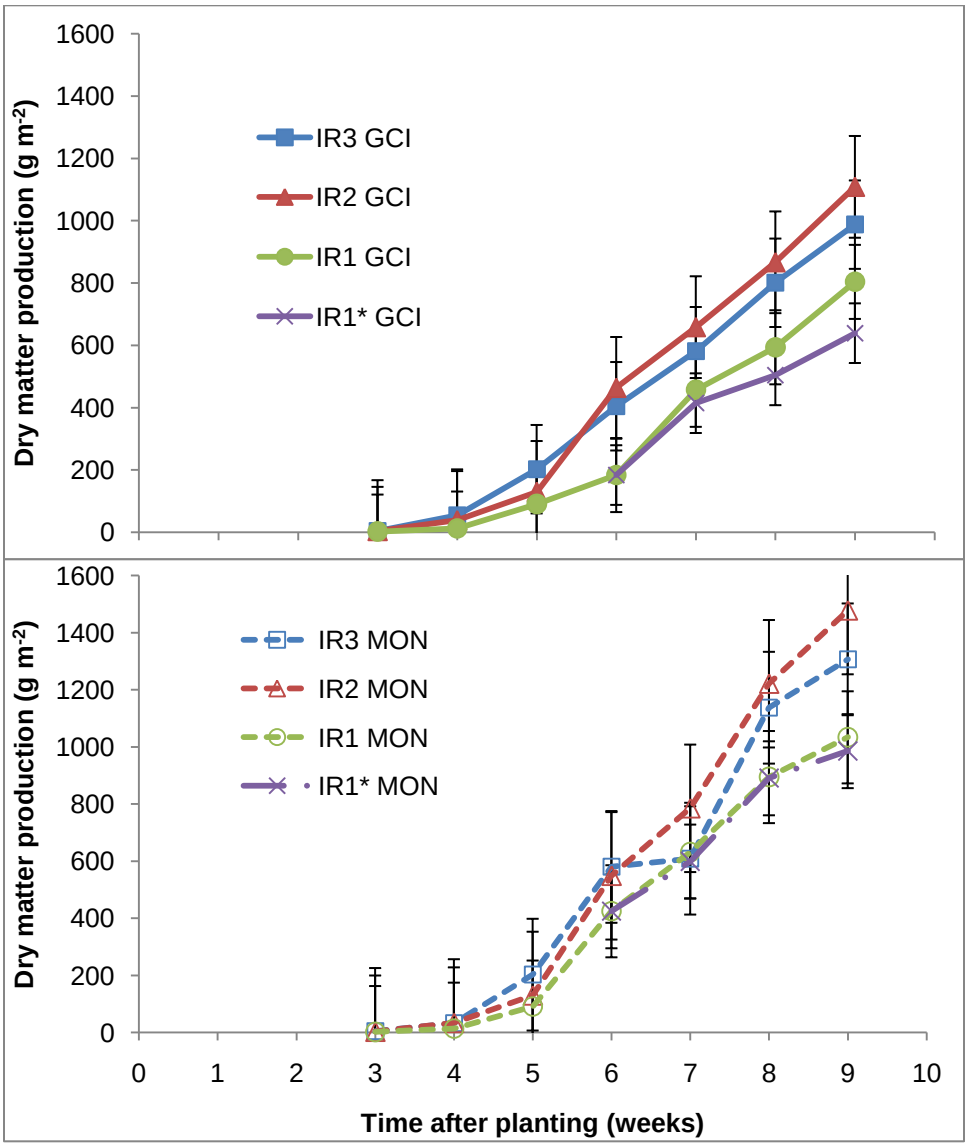

Figure 4.12: Seasonal dry matter accumulation for two pearl millet lines subjected to three water treatment levels, IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

The dry matter accumulation was even much lower for IR1* plants as it was evidently less than that produced in the rainfed plots (IR1) particularly for GCI 17. During week 9 after planting, dry

matter production values were 1109 g, 988 g, 804 g and 639 g for IR2, IR3, IR1 and IR1* plants of GCI 17. A similar trend was realized in Monyaloti where 1478 g were produced by IR2 plants, 1307 g by IR3 plants, 1034 g by IR1 plants and 985 g by IR1* plants. The difference in dry matter production between IR1 and IR1* plants (<50 g) in Monyaloti was smaller than that realized in the corresponding treatments of GCI 17 which was 165 g. This observation implied that the impact of withholding rain for the period of two weeks was greater in GCI 17 than in Monyaloti. Significant differences between pearl millet lines were observed as from week 8 (Appendix 1E).

Considering only the period of withholding rain on the water stressed plants (last two weeks), the accumulation of biomass for the two lines of pearl millet were analyzed (Figure 4.13). These were then compared to the well-watered plants.

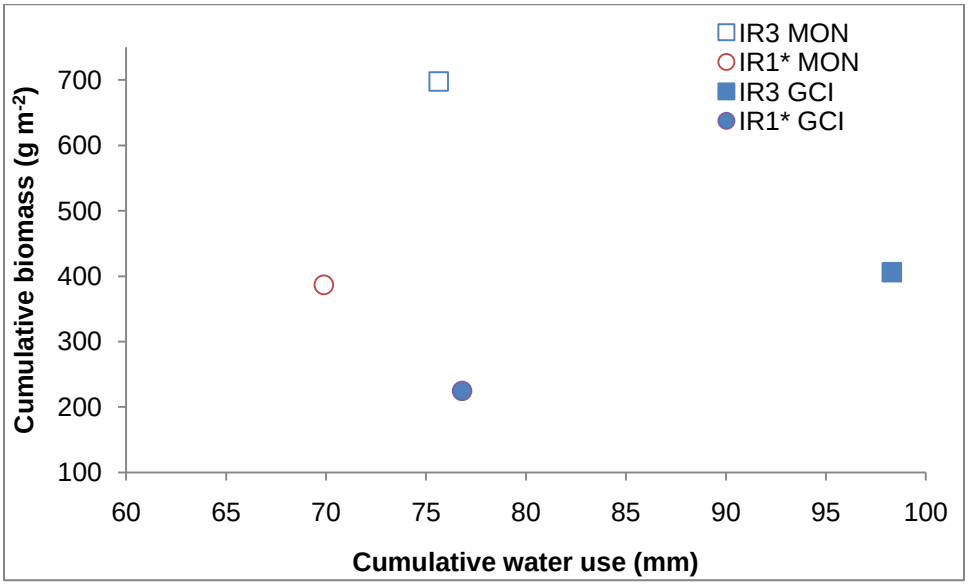

Figure 4.13: Relationship of cumulative water use and cumulative biomass production in control plants (■□; IR3) and stressed plants (●○; IR1*) of GCI 17 (GCI) and Monyaloti (MON) measured on selected days during a period of withholding rain (1st to 17th February 2010) on water stress plots

The cumulative water use of well-watered GCI 17 plants was 98.3 mm within the last two weeks and the biomass produced was 406 g m^{-2}. The stressed plants produced 225 g m^{-2} of biomass from a cumulative water use of 76.8 mm. Within this period the water use efficiency (WUE) of the well-watered plants was higher (4 g mm^{-1}) than that of the stressed plants (3 g mm^{-1}). A similar trend was observed in Monyaloti, where the well-watered plants had a cumulative biomass of 697 g produced from 75.6 mm of soil water (9 g mm^{-1}). The water stressed plants

produced 387 g from 69.9 mm of soil water (6 g mm^{-1}). The WUE was lower in the water stressed plants than in the well-watered plants for both pearl millet lines. It was however noticed that under both the stressed and well-watered conditions, the WUE of Monyaloti for biomass production was higher than that of GCI 17. The lower WUE of GCI 17 was postulated to be due a high contribution of the evaporation component on the evapotranspiration since the LAI was also found to be relatively lower in this pearl millet line.

4.3 Plant Water Relations

Total leaf water potential

Before the rainfall prevention shelter was installed on the water stressed plots, the heavy and frequent rainfall events throughout January prevented the implementation of water treatments. On day 3 (4th February) after the structure was erected on the 1st February 2010, a reduction of total leaf water potential was measured on the water stressed plants (Figure 4.14). Despite that only 5.6 mm of precipitation was being prevented over this period. The total leaf water potential was -0.69 MPa for GCI 17 and -0.76 MPa for Monyaloti under water stress conditions. The irrigated plants had leaf water potential ranging from -0.58 MPa to -0.42 MPa for both lines of pearl millet. The difference in total leaf water potential between water stressed and irrigated plants was increasing with the degree of water stress caused by soil water depletion.

On day 5 (6th February), some variations even between the pearl millet lines were observed (Appendix 2A), with Monyaloti having a higher total leaf water potential (-1.01 MPa) compared to GCI 17 (-1.22 MPa). On day 7 (8th February), the differences between varieties became significant (Figure 4.14). Following an irrigation event at the end of day 7, day 8 (9th February) showed greater differences with irrigated treatments ranging from -0.67 MPa in GCI 17 IR3 to -0.81 MPa in Monyaloti IR2.

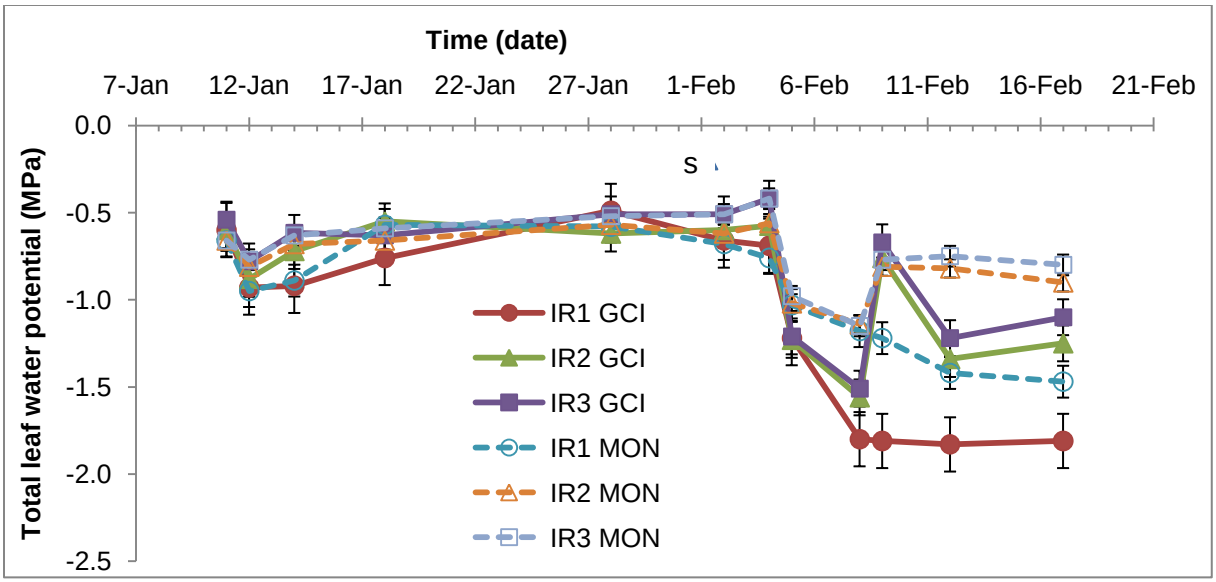

Figure 4.14: Seasonal changes in total leaf water potential for two pearl millet lines subjected to three water treatment levels IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti. S indicates date when rainfall was withheld on water stressed plots

From day 7, 8 and 11 (8th, 9th and 12th February) after water withholding, the stressed plants of GCI 17 had total leaf water potentials of -1.80, -1.81 and -1.83 MPa respectively. The irrigated counterparts of GCI 17 increased in total leaf water potentials after irrigation but this improvement was relatively short-lived (less than 3 days) compared to Monyaloti. The rapid response of GCI 17 (short maturing and physically shorter variety) as revealed in Figure 4.14 was attributed to a supposedly shallow root system as it was earlier reported by McIntyre *et al*. (1995) and Kusaka *et al*. (2005) since shoot growth and root development are linked (Payne, 2000). Early maturing pearl millet varieties have also been reported to have shallower root systems when compared to long maturing varieties (Bruck *et al*., 2003).

The water potential of water stressed Monyaloti (MON IR1) on the other hand was gradually declining as shown in Figure 4.14. The irrigated plots of Monyaloti maintained higher total leaf water potential for a longer period. On the 4th day after irrigation event, both irrigation treatments of Monyaloti had significantly higher leaf water potentials than irrigated GCI 17 plants. The irrigated GCI 17 plants had total leaf water potentials just higher than water stressed Monyaloti. The water stressed GCI plants' total leaf water potential was significantly lower than in all the treatments on this day (4th after irrigation).

Figure 4.15 provides a summarised comparison of the pearl millet lines and the water treament levels. In Figure 4.15(a) there is a more rapid decline in total leaf water potetial for GCI 17 compared to Monyaloti. Monyaloti seemed to maintain a higher potential for prolonged periods towards the end of the season. The irrigated plots had consistently higher potentials than the water stressed ones (Figure 4.15b). It was occasionally increased by either irrigation (8th February) or rainfall events (16th February) as seen by measurements on the 9th and 17th of the same month following wetting.

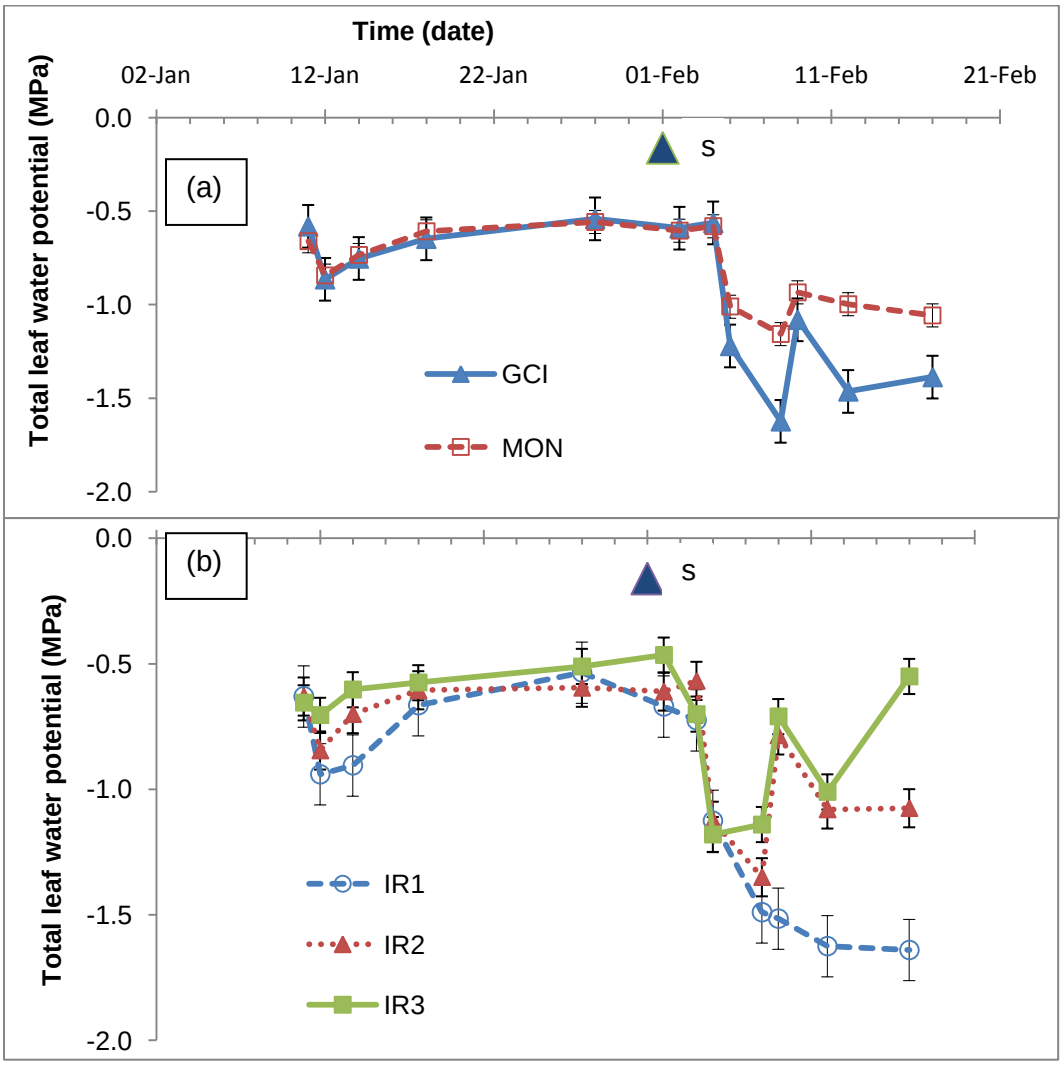

Figure 4.15: Mean values of seasonal total leaf water potential according to (a) pearl millet line and (b) irrigation level. S indicates date when rainfall was withheld on water stress plots

The diurnal changes of total leaf water potential for the two lines under the well-watered and stressed conditions were monitored on day 8 (9th February) after withholding water. High total leaf water potential values were obtained in the morning (predawn) and late in the afternoon

while minimum values were obtained around midday (Figure 4.16), which concurs with other previous reports (Hsiao, *et al*., 1976; Jarvis, 1976; Fiscus & Kaufmann, 1990). It was also observed that GCI 17 which had a few more tillers (Figure 4.8(a)) compared to Monyaloti, hence was more stressed during drought conditions as earlier reported by Do *et al*. (1996) in their studies with pearl millet that more tillering pearl millet lines were more affected by droght.

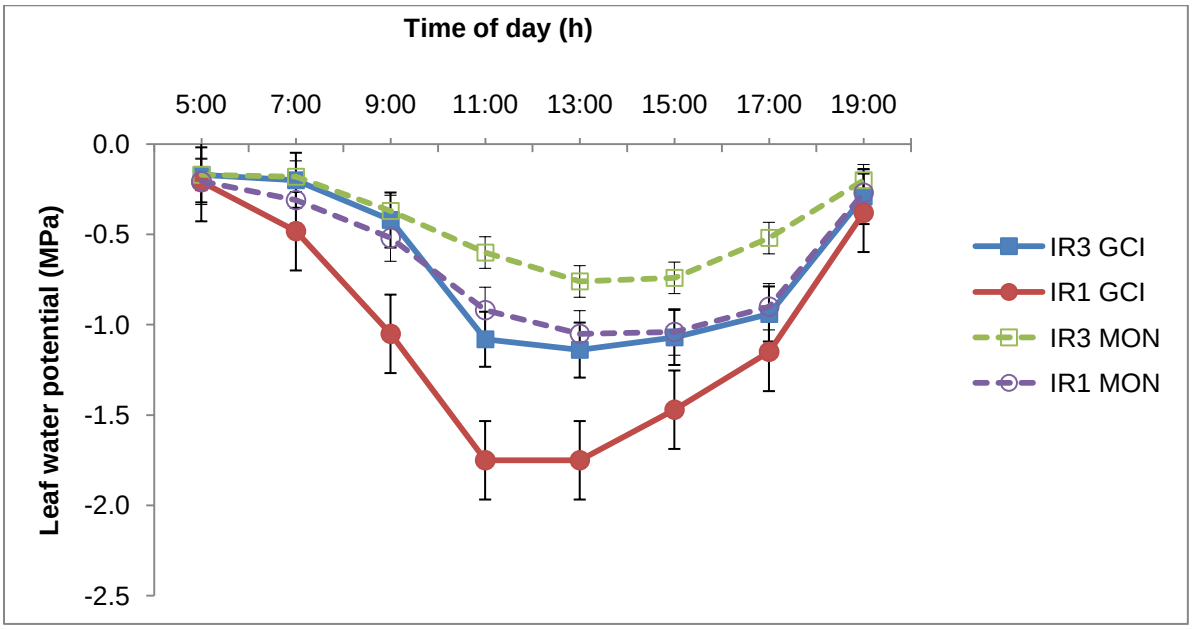

Figure 4.16: Diurnal chances of total leaf water potential for well-watered (IR3) and water stressed (IR1) plants of two pearl millet lines

Stomatal conductance

The results for stomatal conductance are presented in Figure 4.17. There were complications caused by a very high instantaneous variation on the readings taken. This challenge was also encountered by Do *et al*. (1996) in his studies with pearl millet planted in the field at Institut des Radio-Isotopes of the University of Niamey, in Niger. This was perhaps due to the fact that the regulation of stomata is affected by many environmental factors (Jarvis, 1976; Maruyama & Kuwagata, 2008). The stomatal conductance increased gradually and reached its seasonal peak sometime around peak tillering stage and then decreased gradually as the crop was moving into the reproductive stage. Such findings have recently been reported by Takai *et al.* (2010) in their study for rice and previously by Do *et al*. (1996) for pearl millet.

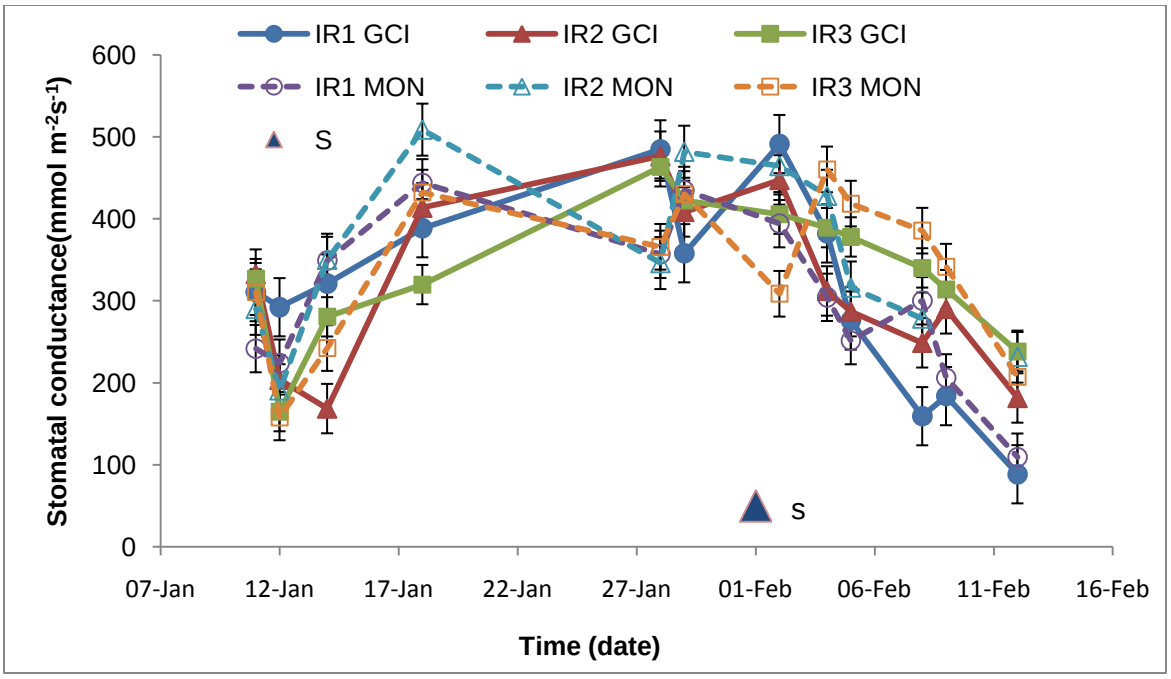

Figure 4.17: Seasonal changes in stomatal conductance for two pearl millet lines subjected to three water treatment levels, S indicates date when rainfall was withheld on water stress plots. IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

Varietal differences were not realized throughout the study (Figure 4.18a) and (Appendix 2B). On day 4 (5th February) after withholding rainfall on water stress plots, lower stomatal conductance values were observed when compared to irrigated plots (Figure 4.17 and Figure 4.18b) as previously reported by Hsiao (1990) and Eiasu (2009). This difference between the irrigated and water stressed plots became more pronounced with increase in the degree of water stress (Figure 4.17 and Figure 4.18b).

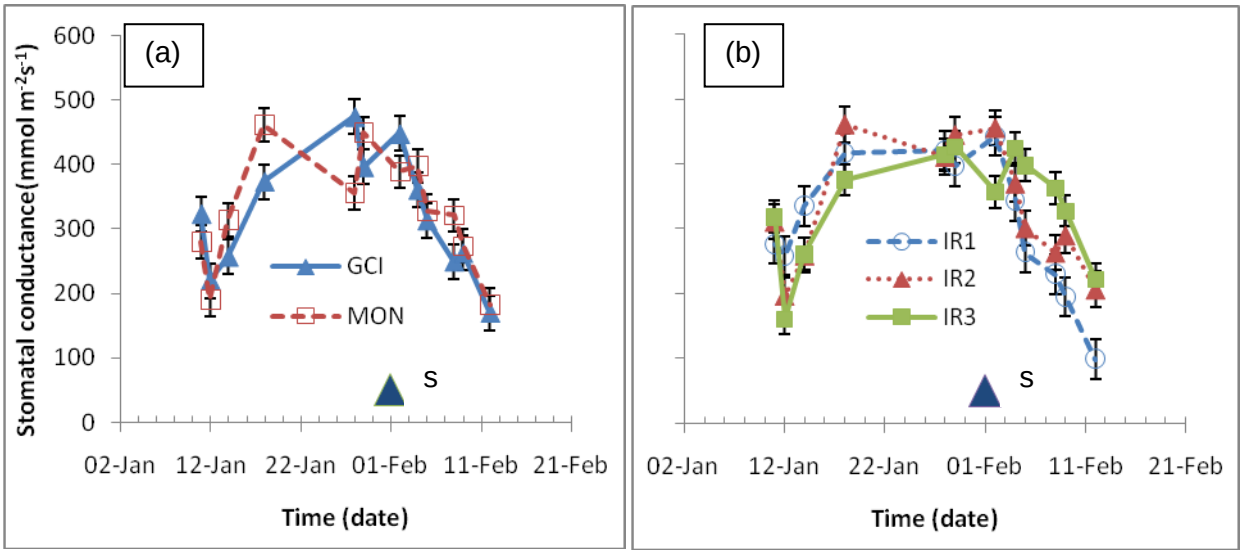

Figure 4.18: Seasonal changes in stomatal conductance according to (a) pearl millet line and (b) irrigation level. S indicates date when rainfall was withheld on water stress plots

Diurnal changes of stomatal conductance for the control plants (IR3) and the water stress treatments (IR1) were done on the same day (9th February) and alongside the total leaf water potential (Figure 4.19). Both stressed and control plants for Monyaloti followed a unimodal mode behavior with the peak values obtained around midday, similar findings were reported by Jarvis, (1976) and Cui *et al,* (2009). With GCI 17, the stressed plants started with higher values in the early morning hours and went down around midday. The peak for this treatment was obtained 1300hrs. Water stressed plants of GCI 17 had lower stomatal conductance compared to the control plants.

The control plants of GCI 17 had lower conductance values in the morning, as the sun rose and air temperature increased, the conductance also increased to peak values at 1100hrs. At this time the plants could not continue to meet the demand of the atmosphere for water and then the stomata began to close hence lower conductance values were measured in the afternoon. The same trend was observed for well-watered plants of Monyaloti even though slightly higher values were measured on this line. The water stressed plants of GCI had their stomata partly closed throughout the day, as revealed by the continuously lower stomatal conductance (Figure 4.19). All the treatments had lower stomatal conductance in the morning and late afternoon with their peaks around midday, similar findings were reported by Blounquist *et al*. (2009).

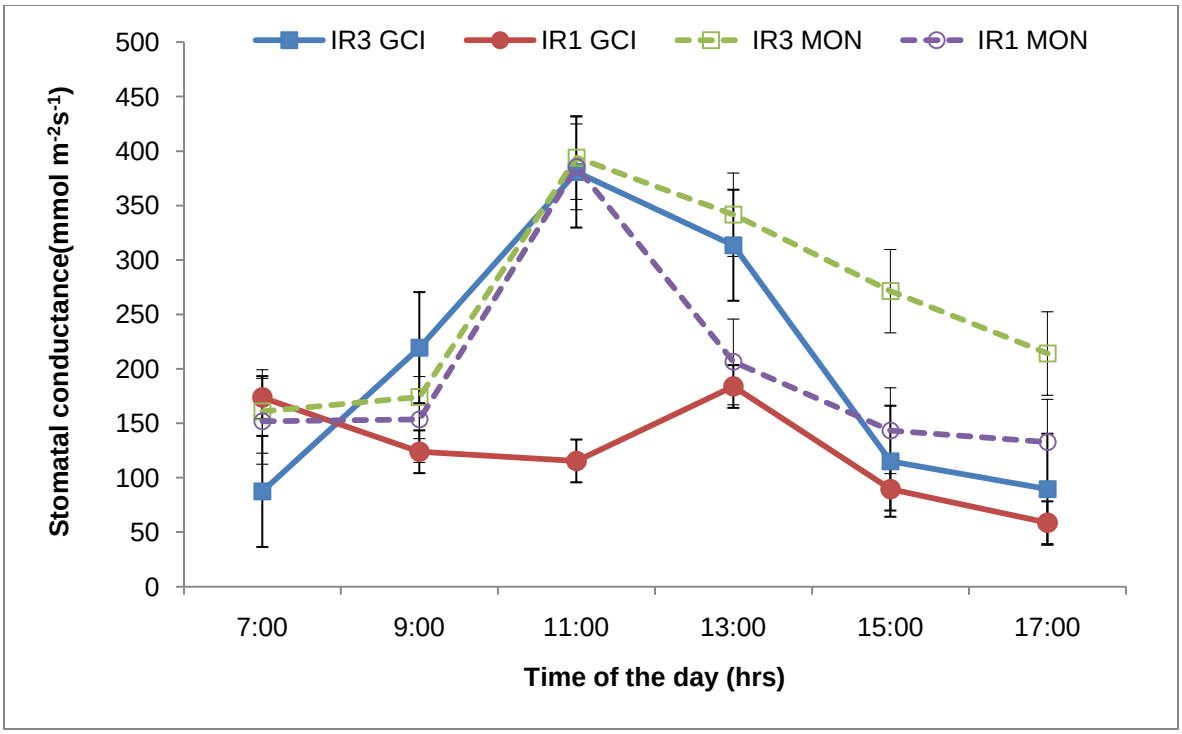

Figure 4.19: Diurnal chances of stomatal conductance for well-watered (IR3) and water stressed (IR1) plants of two pearl millet lines

Relative water content

The results of RWC are presented in Figure 4.20. On day 1 of the rainfall-withholding period, the RWC was generally the same in all the treatments regardless of the irrigation level or pearl millet line. It ranged from averages of 92.7% to 94.3%. Differences between the stressed and well-watered plants were realized three days after erecting the shelter (5.6 mm withheld) particularly for the stressed plants of GGI which then had RWC of 88.0% while stressed plants of Monyaloti had RWC of 91.1%. In the rest of the treatments RWC remained high and still ranged from 92.7% to 94.2% on this day.

It was only on day 9 (10th February) after withholding rainfall that Monyaloti had significantly higher (Appendix, 2C) RWC than GCI 17 in all corresponding water treaments (IR1 versus IR1 and also on the irrigated plots) (Figure 4.20). For GCI 17, IR3 and IR2 had RWC of 89.7% and 87.2% respectively while IR1 was considerably lower at 75.0%. On the other hand Monyaloti had 89.7% for IR3, 90.4% for IR2 and 83% for IR1. The RWC of GCI 17 plants under stressed was noticeably lower (75.0%) than the stressed plants of Monyaloti (83%). This trend was even more pronounced on the last day of these measurements (17th February) as it was after a rainfall event of 18.4 mm had occurred the previous day.

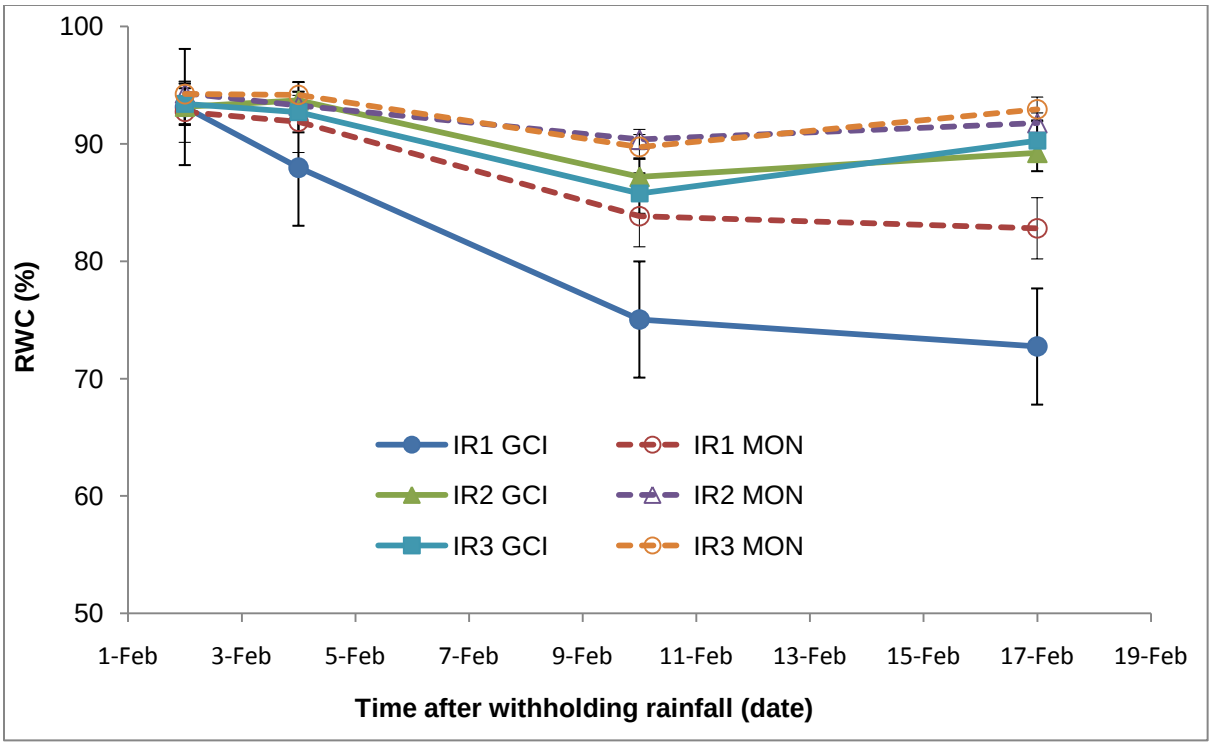

Figure 4.20: Seasonal changes in leaf relative water content (RWC) for two pearl millet lines subjected to three water treatment levels, measured on selected days during a period of rain withholding (1st to 17th February 2010) on water stress plots. IR1, 2 & 3 are irrigation levels from water stressed to well-watered respectively. GCI is GCI 17 and MON is Monyaloti

The rainfall increased the RWC on the irrigated treatments (open plots) while the decline in RWC of water stressed treatments was continuing as soil water depletion progressed on the plots under the shelter. Moussa & Abdel-Aziz (2008) also found significantly lower RWC in water stressed plants compared to the control plants in both drought tolerant and drought sensitive maize genotypes. These findings also concurred with several other reports (Do *et al*., 1996; Eiasu, 2009; Kirnak & Dogan, 2009; Lenzi *et al*., 2009).

It was seen that as the crop was maturing, the RWC was gradually declining as reported by Kramer (1988), with the RWC for well-watered plants being higher on day 1, 93.4% for GCI and 94.2% for Monyaloti. Despite continuous irrigation and rainfall, the RWC was 90.3% and 92.9% for the respective pearl millet lines on day 16 (17th February). This can be attributed to the effect of age on leaf tissue and physiological processes.

Osmotic potential

The P-V curve volume curve method was employed for osmotic potential determination of the well-watered and water stressed pearl millet leaves for the two lines (Figure 4.21).

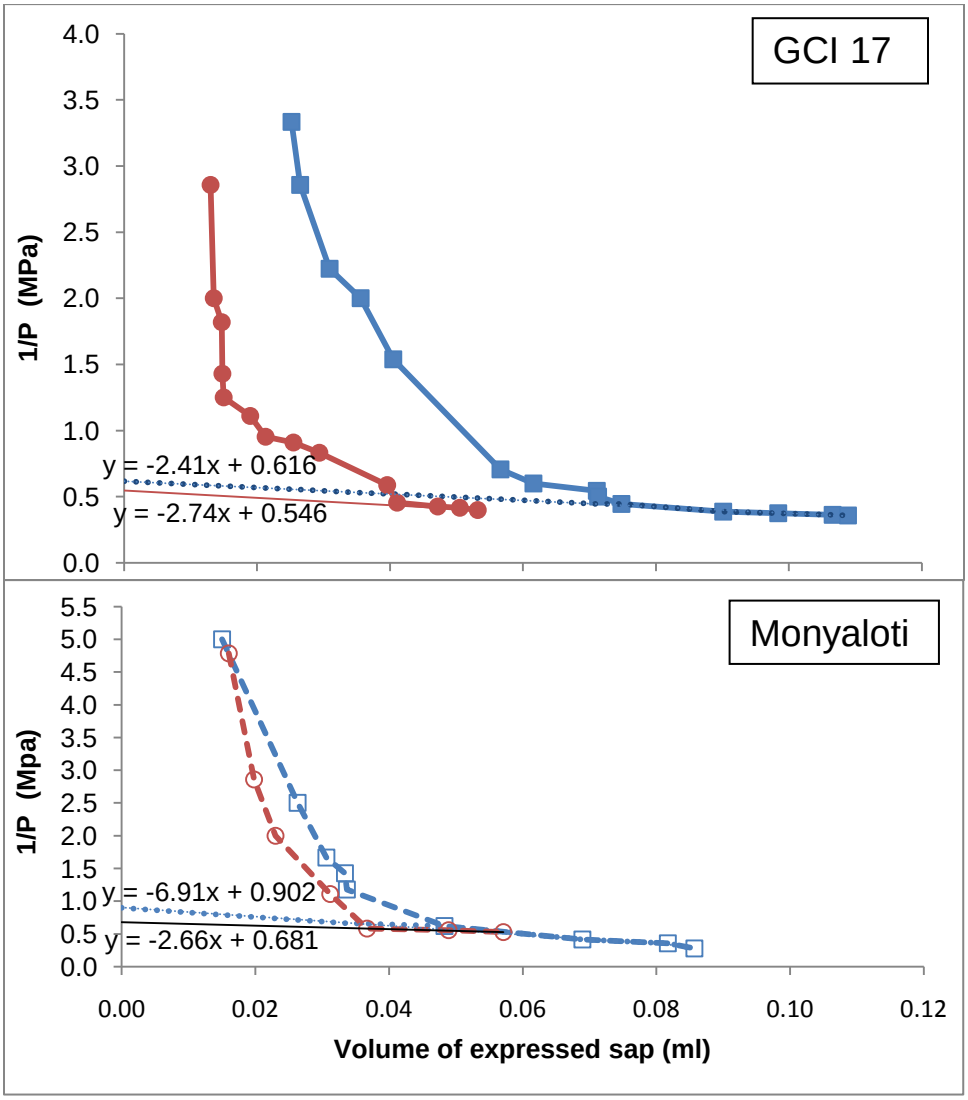

Figure 4.21: Pressure-volume (P-V) curves for osmotic potential (Ψ π) determination for control plants (■□; IR3) and stressed plants (●○; IR1) of GCI 17 (GCI) and Monyaloti (MON) measured on the 17th February 2010

The osmotic potential values at 100% RWC (zero expressed sap) for the two pearl millet lines, determined as the reciprocal of the y-intercept of the lines extended from the linear sections of the P-V curves are presented in Table 4.3.

Table 4.3: Osmotic potential (Ψ_π) and adjustment (OA) for two pearl millet lines subjected to water stress (IR1) and well-watered conditions (IR3), measured after 16 days of withholding rain (17th February 2010) on water stress plots

Pearl millet line	Water treatment	Ψ_π^{100} (MPa)	OA_{100} (MPa)	$\Psi_\pi^{p=0}$ MPa	OA_0
GCI 17	IR1	-1.83	0.21	-2.22	0.05
	IR3	-1.62		-2.27	
Monyaloti	IR1	-1.47	0.36	-1.72	0.11
	IR3	-1.11		-1.61	

Control plants of GCI 17 had osmotic potential of -1.62 MPa compared to -1.83 MPa for their stressed counterparts. For Monyaloti osmotic potential values had larger differences between the different water treatment levels, -1.11 MPa for control plants versus -1.47 MPa for stressed plants. The two lines of pearl millet were found to have lower osmotic potential at full turgor in the water stressed leaves than well-watered leaves. These differences also displayed some differences in the osmotic adjustment (OA) between the pearl millet lines. The reduction was less pronounced in GCI 17 (0.21 MPa representing 13% adjustment) than in Monyaloti (0.36 MPa). An osmotic adjustment of 0.36 MPa which was an adjustment of 32.3% in this study at full turgor, was also reported by Henson (1982) in his study of pearl millet.

At the point where the P-V curve turns to be linear (P=0), the osmotic potentials for the water stressed and well-watered plants of GCI 17 were relatively the same. This resulted to a minimal OA of 0.05 MPa at P=0, Monyaloti had osmotic potentials of -1.61 MPa and -1.72 MPa. This showed an adjustment of 0.11 MPa.

Osmotic adjustment was observed in Monyaloti and hence it was less affected by the water deficit. GCI 17 on the other hand showed minimal osmotic adjustment and as expected the effects of water stress were observed to be greater on this pearl millet line when compared to Monyaloti. Therefore, Monyaloti which is more drought tolerant had adapted more to the drought conditions than GCI 17.

Integrated analysis of physiological measurements

In order to understand the physiology of the plants and their reactions to water stress conditions, one needs to consider the effect of stress on the combined reaction and interaction of the various plant water relations parameters. These comparisons will be made both with the series of data collected through the seasonal soil water depletion cycle as well as through the series of diurnal measurements from sunrise to sunset on the 9th February 2010. Analysis of the stomatal conductance versus total leaf water potential at 1300hrs during day 8 without rain in water stressed plants is compared with the well-watered plants and presented in Figure 4.22.

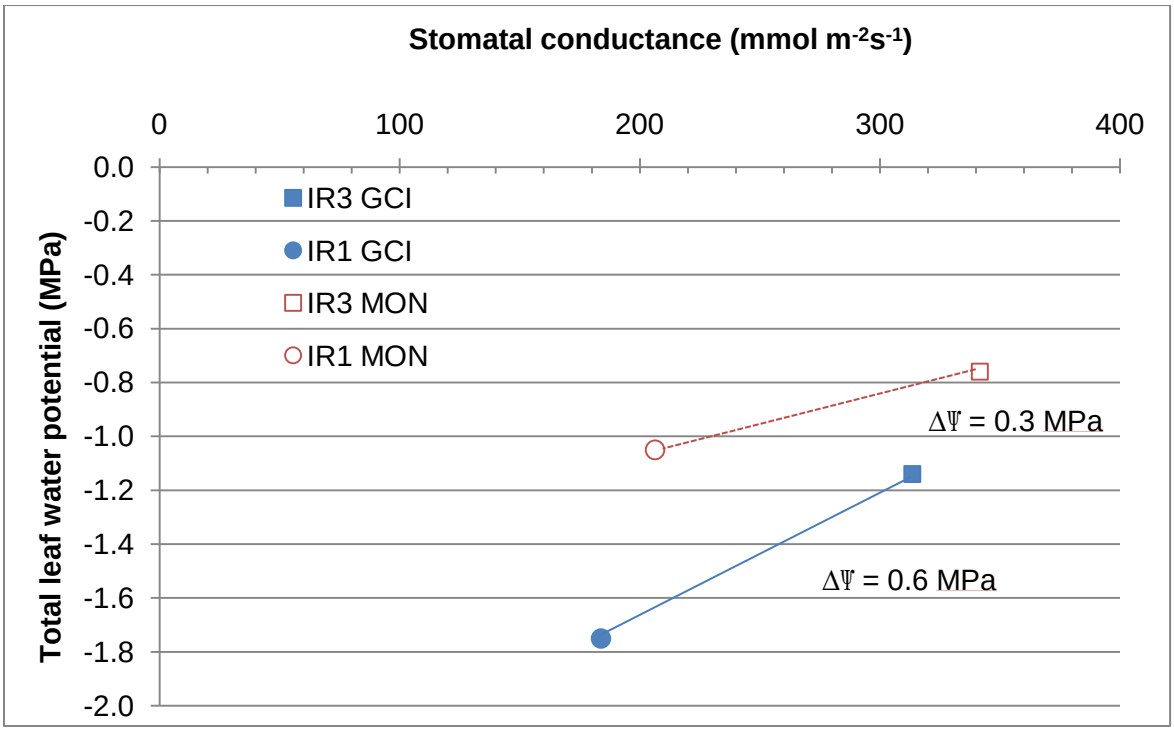

Figure 4.22: Stomatal conductance versus total leaf water potential in control plants (■□; IR3) and stressed plants (●○; IR1) of GCI 17 (GCI) and Monyaloti (MON) measured at 1300hrs on 9th February 2010

The difference in stomatal conductance of stressed plants relative to well-watered was generally the same for both lines (±130), from 313.5 to 183.8 mmol $m^{-2}s^{-1}$ for GCI 17 and from 341.6 to 206.4 mmol $m^{-2}s^{-1}$ for Monyaloti. The leaf water potential at 1300hrs was -1.75 MPa for stressed GCI 17 plants compared to -1.14 MPa of well-watered plants. A relatively smaller difference was realized between the stressed and well-watered plants of Monyaloti, being -1.05 MPa and -0.76 MPa respectively. These changes are also revealed by the slopes of the lines joining the stressed and well-watered plants of the respective pearl millet lines. The line for GCI 17 is

steeper showing a greater effect of water stress on the physiology of this line compared to the local race, Monyaloti.

The maintenance of a higher total leaf water potential with minimal change in Monyaloti suggests a better adaptability of Monyaloti to dry conditions than for GCI 17. The same suggestion could also be derived from the maintenance of a relatively higher conductance of Monyaloti when compared to GCI 17, particularly because they were subjected to similar soil water conditions.

The stomatal conductance relative to changes in total leaf water potential of the two pearl millet lines were also analyzed from the two hourly diurnal measurements on 9th February. As the stomata closed overnight (in the dark), the first conductance measurements in the early morning (after sunrise) were at the lowest values for both pearl millet lines. As the day became brighter, solar radiation and air temperature increased, the stomata opened and hence a high conductance was measured around midday (Figure 4.23).

Since the atmospheric demand for water drops at night, the plant gets the opportunity to equilibrate its water potential with the soil water. As such, water potential measurements in the leaves are higher in the morning and decline as the day progresses with higher solar radiation and higher temperatures which in turn increases the evaporative demand of the atmosphere. These conditions, since they cause the stomata to open, therefore enhance transpiration which then leads to the decline in the water potential of the leaf around midday.

The peak stomatal conductance of well-watered GCI 17 was much higher (380.7 mmol $m^{-2}s^{-1}$) than its stressed counterparts (183.8 mmol $m^{-2}s^{-1}$) (Figure 4.23(a)). These stomatal conductance values were measured at the same times as the lowest total leaf water potential values. This shows that as water potential drops due to the atmospheric demand, the leaf cannot continue to meet the demand and supply sufficient water. Therefore the water potential drops and the stomata begin to close in the stressed treatment.

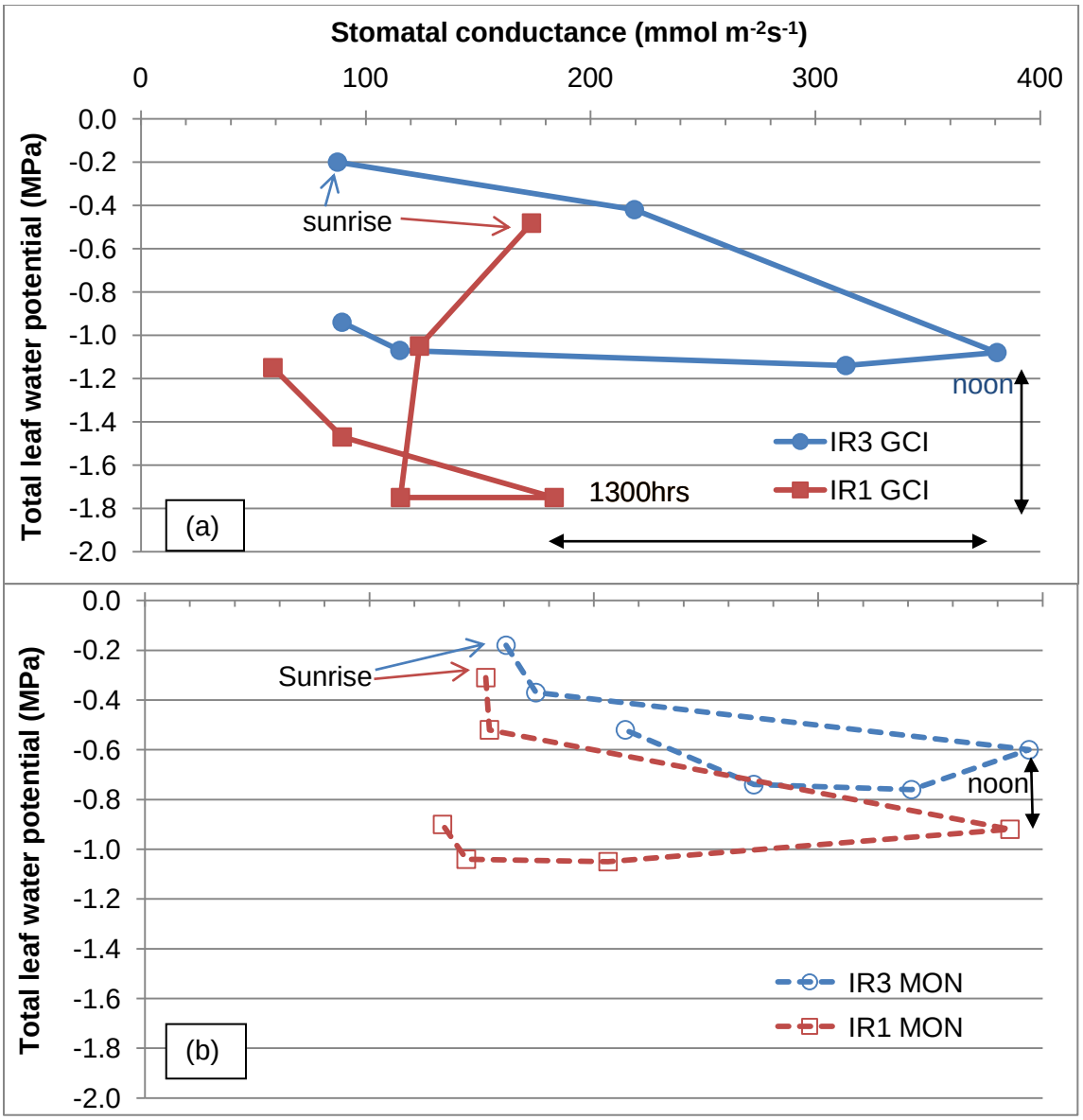

Figure 4.23: From diurnal measurements, stomatal conductance in relation to total leaf water potential in control plants (■□; IR3) and stressed plants (●○; IR1) of GCI 17 (a) (GCI) and Monyaloti (b) (MON) measured at intervals of two hours between 0700hrs and 1700hrs on the 9th of February 2010

Some recovery of GCI 17 plants overnight was evident as the stressed plants had a high water potential (-0.48 MPa), though slightly lower than in well-watered plants (-0.20 MPa), early in the morning. However, soon after sunrise a rapid drop in the water potential of stressed plants was realized such that it reached -1.75 MPa around midday. The well watered plants on the other hand only dropped to -1.08 MPa at the same time (midday) and had open stomata. This shows

that there was minimal soil water in the stressed plots to enable the plants to meet the atmospheric demand while relatively speaking the well-watered plants had sufficient soil water.

Overnight the potential of Monyaloti leaves in the stressed and well-watered plants had recovered to a similar value, namely -0.1 to -0.3 MPa. This shows that there was still some soil water accessible even by the water stressed plants. However, as soon as the sun had risen and the temperature increased and the evaporative demand was felt by the plants, the stressed plants could not sustain the supply and the potential immediately dropped and reached -0.92 MPa at midday. The potential of well-watered plants only dropped to a lowest value of -0.76 MPa at midday.

The total leaf water potential values where these peak conductance values were obtained were -1.08 MPa for well-watered and -1.75 PMa for stressed plants of GCI 17. For Monyaloti it was observed that even at low leaf water potential, a higher stomatal conductance was still maintained. This also suggested better adaptability characteristics of the cultivar (Monyaloti) to dry conditions compared to GCI 17 which seemed to be poorly adapted. Hsiao *et al*. (1976) stated that the stomatal aperture is not affected until the leaf water potential drops to or below some threshold level which can differ across species, growing conditions and the water stress history of the plant. Unfortunately, the effects of recurring drying cycles could not be monitored in this study due to the large amount of rainfall received throughout the season. It was also not possible to monitor the recovery and effects of previous stress.

From the analysis of data presented in Figure 4.23, it can be deduced that the threshold value of leaf water potential had been attained for GCI 17, a water potential of -1.75 MPa as the stomatal conductance also dropped to 183.8 mmol $m^{-2}s^{-1}$, a value less than half of the well-watered plants. The well-watered plants had relatively higher values as the water potential around midday was -1.08 MPa while the stomatal conductance was 380.7 mmol $m^{-2}s^{-1}$. On the contrary, Monyaloti had a higher water potential than the threshold as its stomatal conductance was not affected. Around midday, its conductance was still high (385.5 mmol $m^{-2}s^{-1}$) and almost equal to the conductance of well-watered plants (393.9 mmol $m^{-2}s^{-1}$). The lower conductance values for GCI 17, on stressed plants will result in a lower photosynthesis rate as the assimilation of CO_2 will be limited under such conditions with closed stomata.

Seasonal analysis showed that RWC was maintained relatively higher for well-watered plants than water stressed plants (Figure 4.24). This analysis was done over the period of withholding

rain on water stressed plots. RWC ranged from 85.8 to 93.4% in leaves of well-watered GCI 17 while it ranged from 89.7 to 94.2% in Monyaloti. The total leaf water potential range was also wider for well-watered GCI 17 (-0.42 to -1.10 MPa) than for well-watered Monyaloti (-0.42 to -0.8 MPa). The stressed counterparts for both lines had their RWC continuously decreasing simultaneously with decreasing total leaf water potential.

The trend lines in Figure 4.24 illustrate the development of water stress in the stressed plants of the two pearl millet lines. The line for stressed GCI 17 extends to a much lower water potential and much lower RWC compared to Monyaloti. The extent of RWC decrease was therefore larger for GCI 17 (down to 72.7%) than Monyaloti (down to 82.8%). The sustenance of relatively higher water content for Monyaloti leaves also suggested a better adaptability to dry conditions.

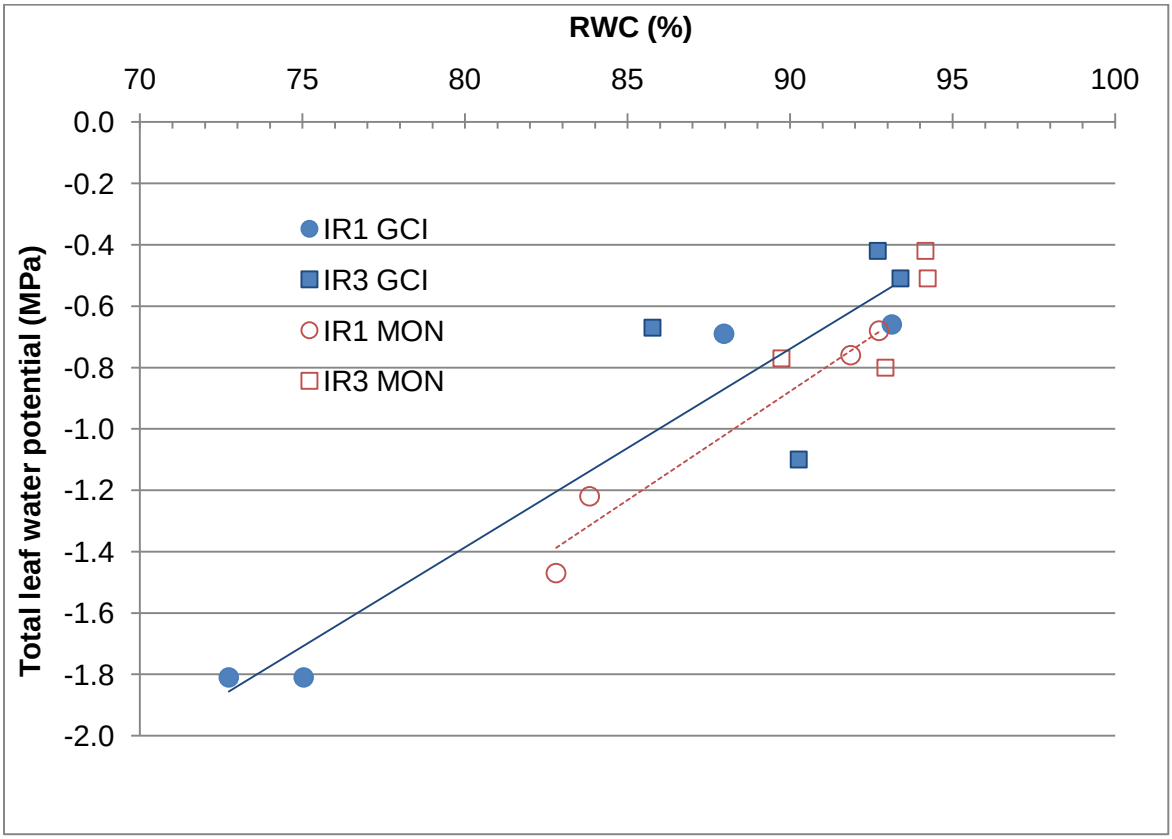

Figure 4.24: Leaf relative water content versus total leaf water potential in control plants (■□; IR3) and stressed plants (●○; IR1) of GCI 17 (GCI) and Monyaloti (MON) measured on selected days during a period of withholding rain (1st to 17th February 2010) on water stress plots

Since the effects of water stress were more prominent on GCI 17 than Monyaloti, the expectation is that photosynthesis was more affected in the former compared to the latter.

Growth is therefore expected to be limited in GCI 17. This idea was mainly deduced from the lower RWC in this variety. High RWC has been described to ensure maintenance of cell turgidity and thus also growth. If water is not sufficient, the turgor pressure drops and the growth rate of plants decline (Acevedo *et al*., 1971; Hsiao *et al*., 1976), therefore growth is favoured under depleting soil water in the pearl millet line (Monyaloti) which maintains higher water content.

The seasonal relationships of the stomatal conductance and total leaf water potential also exhibited some differences between the two pearl millet lines (Figure 4.25). The leaf water potential of GCI 17 was as high as -0.42 MPa in well-watered plants compared to a lowest value of -1.83 MPa in stressed plants. The total leaf water potential showed a noticeable decline with age; even after irrigation, the previous highest values were not attained. The conductance likewise was gradually declining as the water stress progressively developed. These observations were attributed to age effects as similar findings were reported by Vos & Oyarzun (1987).

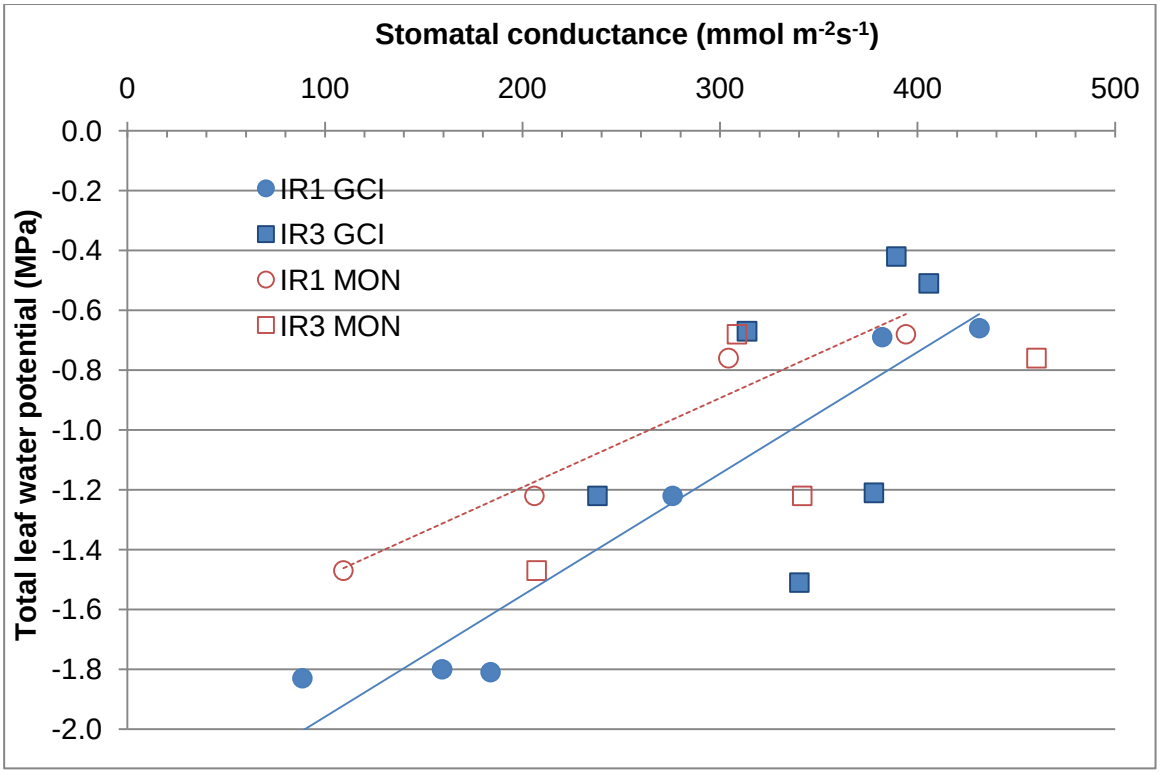

Figure 4.25: Seasonal values of stomatal conductance compared to total leaf water potential for control plants (■□; IR3) and stressed plants (●○; IR1) of GCI 17 (GCI) and Monyaloti (MON) measured on selected days during a period of withholding rain (1st to 17th February 2010) on water stress plots

The seasonal range of leaf water potential of Monyaloti between well-watered and stressed plants was narrower than that measured on GCI 17. An equal high value of -0.42 MPa was found in well-watered plants but the lowest in stressed plants was only -1.42 MPa for Monyaloti compared to the potential of -1.83 MPa attained in stressed GCI 17 plants. The stomatal conductance range was almost similar between the two pearl millet lines (±100 to <500 mmol $m^{-2}s^{-1}$).

The relationship between stomatal conductance and relative water content is presented in Figure 4.26. The trend of a similar range of stomatal conductance values between the two pearl millet lines is displayed as revealed in some earlier discussion (Figure 4.25). However the differences in RWC between lines are emphasized.

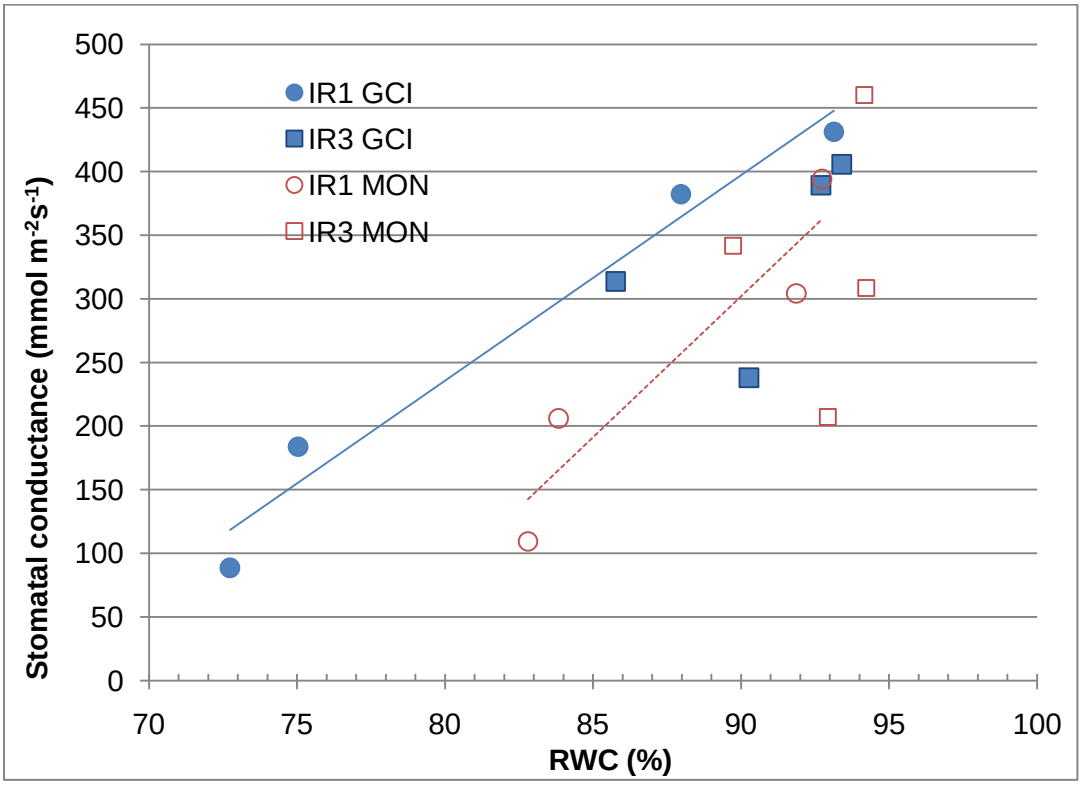

Figure 4.26: Leaf relative water content versus stomatal conductance for control plants (■□IR3) and stressed plants (●○IR1) of GCI 17 (GCI) and Monyaloti (MON) measured on selected days during a period of withholding rain (1st to 17th February 2010) on water stress plots

Even the relatively higher water content for Monyaloti than GCI 17 is again displayed in this relationship as it was the case in Figure 4.24. This shows that Monyaloti can maintain its turgidity better than GCI 17 when the stomata are closed. The well-watered plants also revealed some recovery in the water content after some irrigation for both lines of pearl millet. The

conductance on the other hand did not show any improvement even after irrigation. At 63 days after planting (day 16 of withholding rain), the water content (Figure 4.20) and conductance Figure 4.17) could not fully recover following rainfall or irrigation, this was similar to findings reported by Vos & Oyarzun (1987). The continuous decrease in conductance suggested a continuous reduction in the stomatal control opening with age as it did not improve even after replenishment of soil water by rainfall or irrigation.

4.4 Characteristics and Distribution of Stomata

Density

Both surfaces of pearl millet leaves were found to have some stomata on them rendering them amphistomatic. However, the crop is probably more classified as hyperstomaty as the difference between the stomatal density of the adaxial surfaces (162.7 mm^{-2}) and abaxial surfaces (141.7 mm^{-2}) was highly significant ($P<0.01$). In all the treatments (pearl millet lines and irrigation levels), the adaxial surfaces had higher densities than the abaxial surfaces. The only exception was observed in stressed plants of GCI 17, where the stomatal densities were equal on the two surfaces (Figure 4.27).

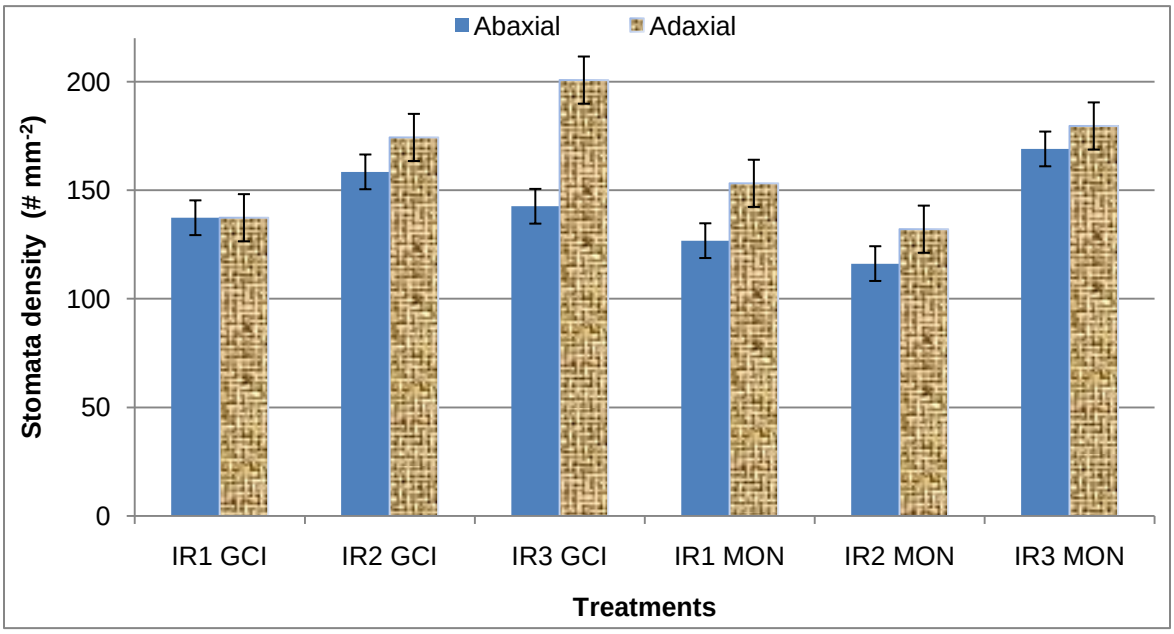

Figure 4.27: Stomatal density on leaf surfaces: Abaxial (solid blocks) and adaxial (speckled) for two pearl millet lines: GCI 17 (GCI) and Monyaloti (MON) subjected to three water treatment levels: water stressed (IR1) to well-watered (IR3)

Water stress affected number of stomata on the adaxial surfacec of GCI 17. Lower stomatal densities were observed on moderately stressed plants and even lower on severely stressed plants. In Monyaloti, the stomatal density was lower on moderately stressed plants. There was no further reduction in the stomatal density on stressed plants, instead IR2 plants had fewer stomata than IR1 plants. This means that the moderate stress was enough to affect the stomatal density.

For GCI 17, the highest density of 200.6 stomata mm^{-2} was found on the adaxial surface of well-watered plants. The lowest density (137.3 mm^{-2}) was on both surfaces of stressed plants. For Monyaloti, the maximum stomatal density of 179.5 mm^{-2} was measured on the adaxial surface of well-watered plants and the minimum of 116.2 stomata mm^{-2} was found on the abaxial surface of moderately stressed plants (IR2 MON). It was noticeable that the abaxial and adaxial surfaces of water stressed GCI 17 plants were equal. Under the well-watered conditions, the stomatal density on the adaxial surface was significantly higher than on the abaxial surface. Monyaloti seemed to use mainly stomatal density on the abaxial surface as an adjustment mechanism to water stress since the stomatal density on the abaxial surface was significantly lower than on the adaxial surface under the water stress conditions compared to well-watered conditions where both sides were almost equal. The stomatal density seem to have been influenced by the soil water deficits on both lines of pearl millet as lower densities were found on leaf surfaces of water stressed plants compared to well-watered plants (Figure 4.27).

The overall stomatal density was significantly different between pearl millet lines. However, the mean stomatal density was slightly higher in GCI 17 (158.4 mm^{-2}) compared to Monyaloti (146.0 mm^{-2}). Drought tolerant plants have been reported to have lower stomatal densities compared to sensitive ones (Mehri *et al*., 2009). Highly significant ($P<0.01$) effects of irrigation on stomatal densities were observed. Well-watered plants had higher overall stomatal density (172.9 mm^{-2}) than both stressed and moderately stressed plants which had stomatal densities of 138.6 mm^{-2} and 145.2 mm^{-2} respectively.

Size

The measured lengths and widths and calculated areas of the stomata are presented in Table 4.4. There was a significant difference ($P<0.01$) in stomatal lengths for the different pearl millets lines. Monyaloti had longer (37.1 µm) stomata compared to GCI 17 (32.84 µm). Table 4.4 shows that the stomata on the abaxial surface of Monyaloti were significantly longer than those on the abaxial surface of GCI 17. The stomatal lengths on the adaxial surfaces were however not signifantly different within the two pearl millet lines.

With regard to the widths of the stomata, the irrigated plants seemed to have wider stomata compared to the water stressed plants. Narrower abaxial stomata of average 14.4 µm were measured in water stressed GCI 17 plants compared to 16.7 and 15.4 µm on irrigated plants. A similar trend was observed in Monyaloti where the stressed plants had abaxial stomata of 17.2 µm on average compared to 19.8 and 20.8 µm on the abaxial surfaces of irrigated plants. Like in the case of stomatal lengths, the widths measured on the adaxial surfaces were not significantly different within the two pearl millet lines. This suggested a greater sensitivity of the abaxial surfaces in stomatal adjustment in response to water deficit conditions. Significant differences in width of stomata of abaxial surfaces were observed and the water stressed plants of both pearl millet lines were narower than those measured on the well-watered plants.

Table 4.4: Average stomatal lengths and widths on abaxial and adaxial leaf surfaces for two pearl millet lines subjected to three irrigation levels; Water stressed (IR1) to well-watered (IR3) measured 11 days (12th February 2010) after witholding rain on water stressed plots

Pearl millet line	**Water treatment**	**Stomatal Dimensions**					
		Length (L) (µm)		Width (W) (µm)		Area (A) (µm^2)	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
GCI 17	IR1	29.0±2.0^{c}	37.5±3.9^{a}	14.4±1.1^{c}	18.6±1.5^{a}	338.2^{b}	552.5^{a}
	IR2	29.7±2.2^{c}	35.6±1.8^{a}	16.7±0.8^{bc}	17.9±0.7^{a}	390.1^{b}	500.7^{ab}
	IR3	29.2±2.2^{c}	36.0±1.5^{a}	15.4±0.7^{bc}	18.4±1.1^{a}	352.9^{b}	521.6^{a}
Monyaloti	IR1	41.5±1.2^{a}	36.9±3.8^{a}	17.2±0.8^{bc}	18.2±1.1^{a}	559.9^{a}	527.8^{a}
	IR2	35.4±5.8^{b}	34.3±1.5^{a}	19.8±2.7^{ab}	15.0±1.5^{b}	555.7^{a}	405.7^{b}
	IR3	37.5±2.0^{ab}	36.4±2.5^{a}	20.8±3.9^{a}	17.2±2.3^{ab}	611.8^{a}	491.4^{ab}
$LSD_{0.05}$		4.4	4.0	3.1	2.2	106.5	97.0

The dimensions of stomata lengths and widths were used to estimate the size (area) of the stomata based on the assumption that the shape of the stomata resembles a perfect ellipse

using equation number 10. The stomata on the abaxial surfaces of Monyaloti leaves were larger than those on the same surfaces of GCI 17. It was also noticeable that the smallest sizes in each variety were found on the water stressed plants. The stomatal area on the adaxial surfaces were relatively similar as only moderately stressed Monyaloti had significantly smaller stomata than water stressed of both pearl millet lines as well as well-watered GCI 17. This also showed that moderate stress at a specific time is enough to affect the aperture of Monyaloti as seen with stomatal density (Figure 4.27). For the rest of the treatments stomatal areas were not significantly different. This also emphasizes the lower sensitivity of the adaxial surfaces to water deficits as reported by Wang *et al*. (1998) in their studies of broad bean plants.

More than 50% of the stomata on the leaf surfaces of water stressed GCI 17 plants were observed to be closed while their irrigated counterparts had all their stomata open (Figure 4.28). For Monyaloti, the stomata were observed to be open in all the water treatment levels on both the adaxial and abaxial leaf surfaces (Figure 4.29). This observation also emphasized the better adaptability of Monyaloti to water stress conditions when compared to GCI 17; it was able to maintain partly open stomata. This is confirmed by what was seen for stomatal conductance measurements during the diurnal study at 1100hrs (Figure 4.19).

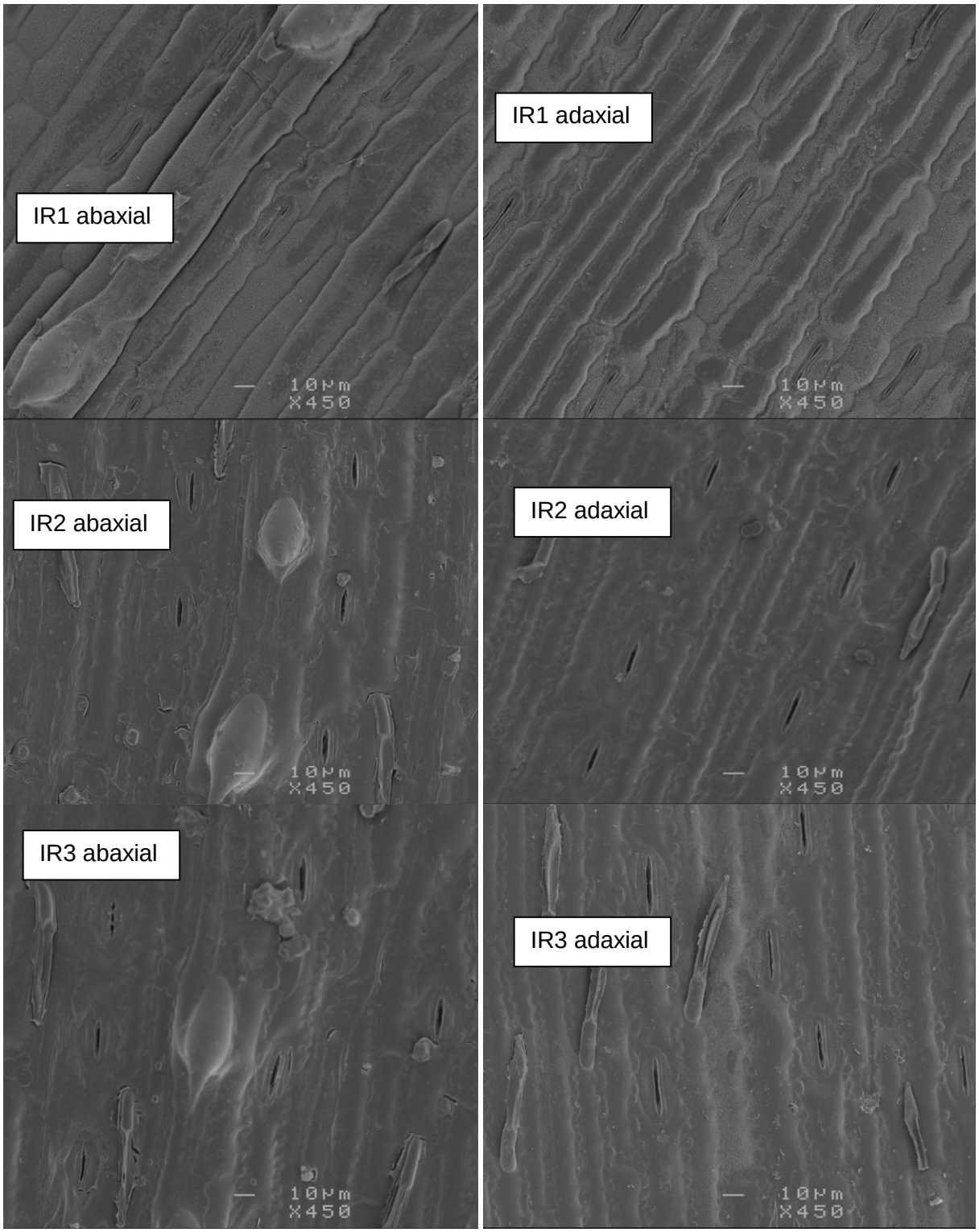

Figure 4.28: Sample microscopic pictures for abaxial and adaxial leaf surfaces of GCI 17 from three water treatment levels; water stressed (IR1) to well-watered (IR3)

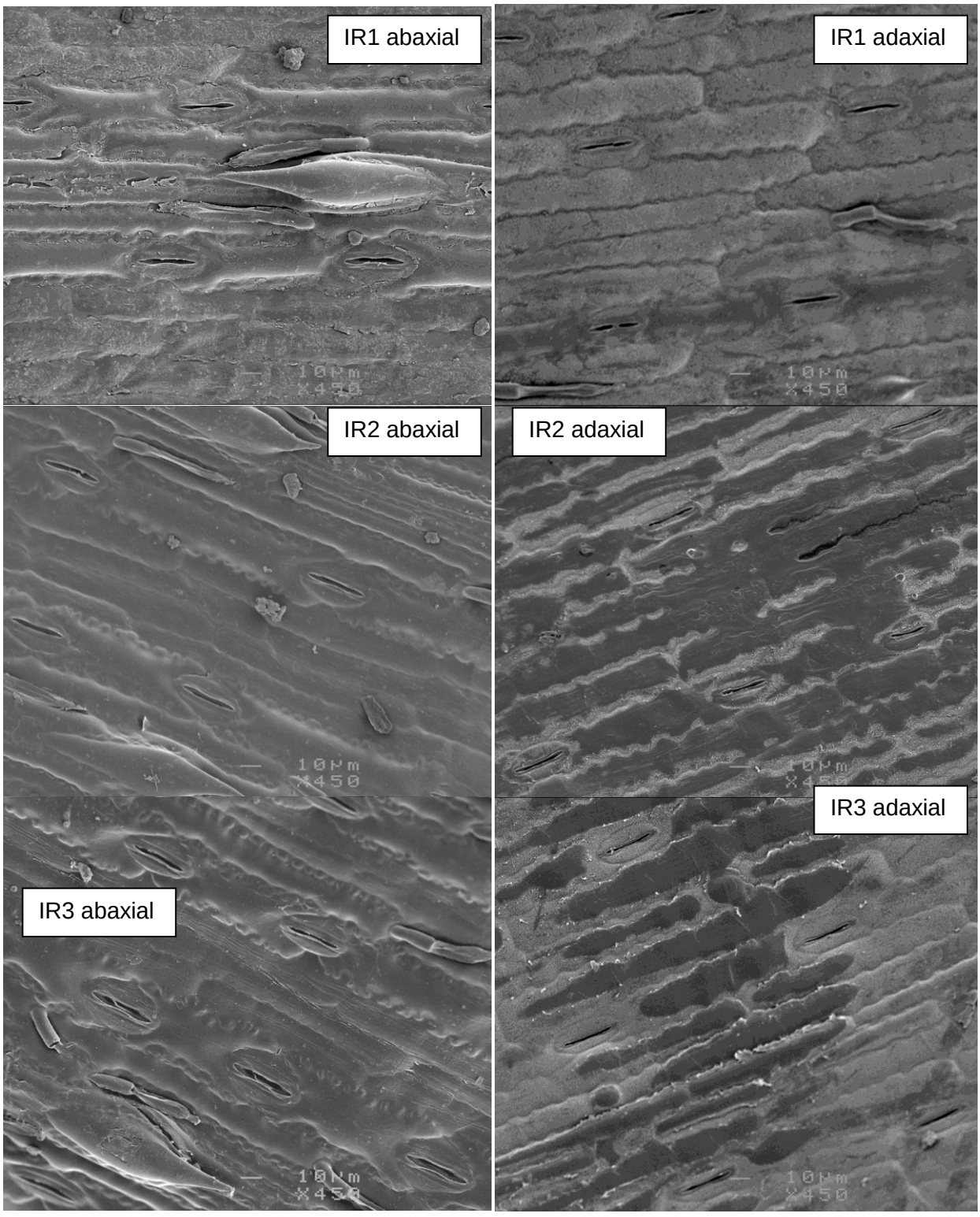

Figure 4.29: Sample microscopic pictures for abaxial and adaxial leaf surfaces of Monyaloti from three water treatment levels: water stressed (IR1) to well-watered (IR3)

Link between physiology and anatomy

On the same day when the stomatal analysis leaf samples were taken in the field, the stomatal conductance and leaf water potential measurements were also made. The relationship of the stomatal area to these measurements for well-watered and water stressed plants on that particular day show that the pearl millet lines different relationships (Figure 4.30).

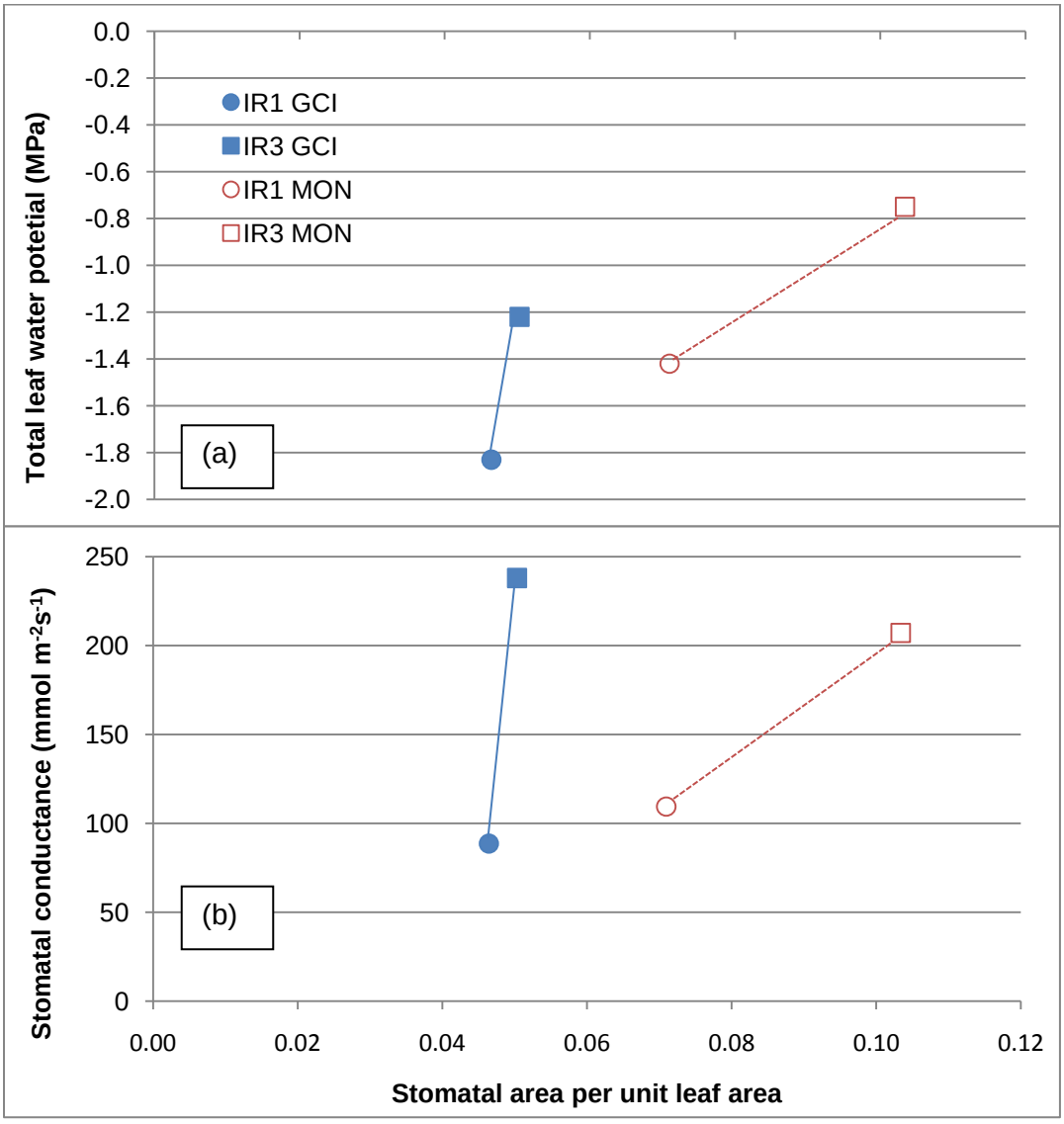

Figure 4.30: Relationship of (a) leaf water potential and (b) stomatal conductance to stomatal area on leaf surfaces of well-watered plants (■□IR3) and stressed plants (●○IR1) of GCI 17 (GCI) and Monyaloti (MON) measured 11 days after withholding rain (12th February 2010) on water stress plots

Even though the density of stomata was only slightly higher in GCI 17 compared to Monyaloti (Figure 4.27), the total area occupied by stomata was always higher in Monyaloti. The bigger

size of stomata in Monyaloti could counteract the higher density of stomata in GCI 17. The ratio of stomatal area to leaf surface area was lower (0.07) in stressed plants of Monyaloti compared to 0.10 for the control plants. This showed some adjustment of stomata during leaf initiation and developmental stages for Monyaloti since the area occupied by stomata in response to water deficit was lower. Due to this adjustment, stressed plants of Monyaloti did not lose water very quickly and as such could maintain higher leaf water potential and stomatal conductance even after a prolonged period of water stress (Figures 4.14 & 4.17). On the other hand, GCI 17 showed a very small reduction (<0.01) in stomatal area across the leaf surface in response to water deficits. The ratio of stomatal area to leaf area in well-watered plants was 0.05 and a ratio of just above 0.04 was calculated in water stressed plants. The small reduction (<0.01) in stomatal area in GCI 17 could be used to explain the severe water stress effects revealed by the lower leaf water potential and stomatal conductance in the stressed plants for GCI 17 (Figures 4.14 & 4.17).

The SEM anatomy investigation helps to explain the physiological findings of the two pearl millet lines. The importance of using different approaches to solve plant water stress problems in agriculture is revealed by the link between the physiology and anatomy investigations of the two lines.

5. CONCLUSIONS AND RECOMMENDATIONS

Field evidence shows that the vegetative growth phase of pearl millet was affected by water stress conditions as measured by plant height, leaf area index (LAI) and dry matter production. Plant height was lower in water stressed plants than in irrigated plants for both lines of pearl millet. Amount of supplied water is important considering that moderately stressed (IR2) plants were taller than plants in the other irrigation treatment (IR3). The number of tillers was neither significantly different between irrigation treatments nor in different pearl millet lines. Water treatments did not affect total numbers of leaves on the main shoot, but it shows that GCI 17 is an early maturing line considering that the leaves started senescing during the ninth week after planting while the Monyaloti line was still green and leaf number was increasing.

There was an effect of water stress on leaf area development as shown by the LAI. The peak LAI of the water stressed plants was lower (by almost half) than that of irrigated plants in both lines. Monyaloti produced higher LAI irrespective of the treatments than GCI 17. Moderately stressed plants (IR2) had the highest LAI values in both pearl millet lines and the two lines attained their peak of LAI at different times but both at the onset of the reproductive stage (7-8 weeks after planting). The same trend found for the plant height and development of leaf area was also observed for the production of biomass as it is dependent on the leaf area. Dry matter production was lower in water stressed crops than in the irrigated ones. IR2 plants produced the highest dry mass and Monyaloti accumulated more dry matter in all the corresponding irrigation treatments when compared to GCI 17.

Considering mainly the period of withholding rain on water stress plots, the following conclusions were made: Reduction in leaf water potential on the water stressed plants was observed 5 days after withholding rain in both pearl millet lines. Monyaloti plants were observed to maintain higher leaf water potentials than GCI 17 in the corresponding irrigation treatments. Stomatal conductance was observed to be much lower in water stressed plants of GCI 17 while Monyaloti could maintain relatively high conductance (open stomata) even under similar stress conditions. It was noted that there were high instantaneous variations in the measurements made of stomatal conductance. A thorough investigation of suitable strategies of using a leaf porometer for field measurements is therefore recommended. Leaf relative water content (RWC) was maintained higher in Monyaloti than GCI 17 in all the corresponding irrigation treatments. The reduction in RWC of stressed plants was less in Monyaloti than in GCI 17 with similar progression in soil water depletion. Greater osmotic adjustment was observed in

Monyaloti compared to GCI 17. A greater osmotic adjustment ability of Monyaloti was observed at 100% RWC, suggesting a better adaptability of this pearl millet line to water stress conditions than GCI 17. The osmotic adjustment due to water stress was greater in Monyaloti even at zero turgor pressure, where water stressed and well-watered plants of GCI 17 showed relatively equal osmotic potential values. This showed that Monyaloti could accumulate some solutes to better maintain cell turgor and hence growth than GCI 17 under water stress conditions.

The stomatal density was found to be higher on the adaxial surfaces than the abaxial surfaces for both lines of pearl millet. Adaxial surface stomata were observed to be larger in area than abaxial ones in GCI and the opposite was true in Monyaloti. The density of stomata was lower where less irrigation was applied (IR3 down to IR1) in GCI 17 while Monyaloti seemed to experience a difference between well-watered (IR3) and the least water stress (IR2) which was even lower than the IR1 plants. Under stress conditions in particular, Monyaloti was seen to have lower stomata density on the abaxial surface than the adaxial surface. GCI 17 maintained equal densities on both abaxial and adaxial surfaces under similar stress conditions.

Based on the conclusions from growth measurements, it is recommended that if dry matter production is the ultimate objective, Monyaloti is the better option between the two lines of pearl millet planted in this study. Moderate stress (IR2) used in this study was also revealed to be close to optimal water requirements. The plant water relations and stomatal characteristics also suggested that Monyaloti is the better adapted pearl millet line to arid and semi-arid conditions such as those of the Free State in South Africa.

A detailed analysis of the effects of leaf age on leaf water potential, stomatal conductance and RWC together with the stomatal characteristics and distribution is recommended as these parameters hinted to being dependent on the age of the plant tissue. The need to investigate the relationships of these parameters to photosynthesis on pearl millet cannot be over emphasized as this would help explain the feed through effect of water stress on leaf growth to production.

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APPENDICES

Appendix 1: ANOVA for Growth Measurements

Appendix 1A: Summarized ANOVA for plant height measured on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) measured from week 3 to week 9 after planting

Time (weeks)	Source of variation	F value	Probability > F	Significance	CV (%)
3	variety	1.61	0.2240	ns	21.0
	irrigation	5.71	0.0143	1b, 2a, 3a	
	var. x irrig.	0.79	0.4739	sn	
4	variety	9.99	0.0065	1a, 2b	15.5
	irrigation	3.70	0.0493	1b, 2a, 3ab	
	var. x irrig.	0.13	0.8789	ns	
5	variety	2.82	0.1137	ns	12.6
	irrigation	0.35	0.7115	ns	
	var. x irrig.	0.37	0.6949	ns	
6	variety	4.37	0.0539	ns	26.6
	irrigation	1.20	0.3278	ns	
	var. x irrig.	0.20	0.8169	ns	
7	variety	8.04	0.0125	1a, 2b	22.5
	irrigation	2.83	0.0907	ns	
	var. x irrig.	0.08	0.9235	ns	
8	variety	8.60	0.0103	1a, 2b	15.9
	irrigation	4.42	0.0310	1b, 2a, 3ab	
	var. x irrig.	1.68	0.2203	ns	
9	variety	0.99	0.3364	ns	11.7
	irrigation	8.34	0.0037	1b, 2a, 3a	
	var. x irrig.	0.64	0.5427	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti
Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered
Probability< 0.05 means significant difference
ns means not significant
Treatments followed by the same letter are not significantly different

Appendix 1B: Summarized ANOVA for number of tillers per plant counted on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) measured from week 3 to week 9 after planting

Time (weeks)	Source of variation	F value	Probability > F	Significance	CV (%)
3	variety	5.55	0.0325	1a, 2b	89.1
	irrigation	3.29	0.0655	1b, 2a, 3ab	
	var. x irrig.	0.82	0.4584	ns	
4	variety	13.03	0.0026	1a, 2b	20.2
	irrigation	6.49	0.0093	1b, 2b, 3b	
	var. x irrig.	2.08	0.1589	ns	
5	variety	1.26	0.2799	ns	26.89
	irrigation	1.95	0.1769	ns	
	var. x irrig.	0.86	0.4434	ns	
6	variety	0.04	0.8508	ns	32.6
	irrigation	0.52	0.6024	ns	
	var. x irrig.	0.59	0.5651	ns	
7	variety	0.04	0.8360	ns	29.6
	irrigation	0.63	0.5437	ns	
	var. x irrig.	0.77	0.4815	ns	
8	variety	0.00	1.0000	ns	29.1
	irrigation	0.44	0.6540	ns	
	var. x irrig.	0.76	0.4828	ns	
9	variety	0.14	0.7091	ns	26.8
	irrigation	0.25	0.7798	ns	
	var. x irrig.	0.73	0.4961	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different

Appendix 1C: Summarized ANOVA for number of leaves per plant counted on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) measured from week 3 to week 9 after planting

Time (weeks)	Source of variation	F value	Probability > F	Significance	CV (%)
3	variety	3.38	0.0859	ns	12.7
	irrigation	2.84	0.0901	ns	
	var. x irrig.	1.76	0.2063	ns	
4	variety	0.00	1.0000	ns	11.7
	irrigation	2.64	0.1042	ns	
	var. x irrig.	0.42	0.6666	ns	
5	variety	0.52	0.4831	ns	10.0
	irrigation	3.62	0.0521	ns	
	var. x irrig.	0.52	0.6064	ns	
6	variety	1.42	0.2517	ns	7.2
	irrigation	3.32	0.0642	ns	
	var. x irrig.	3.32	0.0642	ns	
7	variety	0.18	0.6804	ns	10.9
	irrigation	0.84	0.4518	ns	
	var. x irrig.	1.63	0.2283	ns	
8	variety	5.24	0.0370	1b, 2a	10.3
	irrigation	1.35	0.2897	ns	
	var. x irrig.	4.26	0.3430	ns	
9	variety	9.69	0.0071	1b, 2a	11.8
	irrigation	0.51	0.6106	ns	
	var. x irrig.	1.80	0.1996	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different

Appendix 1D: Summarized ANOVA for leaf area index (LAI) calculated for two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) measured from week 3 to week 9 after planting

Time (weeks)	Source of variation	F value	Probability > F	Significance	CV (%)
3	variety	1.80	0.1964	ns	43.1
	irrigation	7.59	0.0041	1b, 2a, 3a	
	var. x irrig.	0.07	0.9308	ns	
4	variety	1.09	0.3114	ns	51.6
	irrigation	7.87	0.0035	1b, 2a, 3a	
	var. x irrig.	0.52	0.6039	ns	
5	variety	5.59	0.0295	1a,	32.4
	irrigation	14.14	0.0002	1c, 2a, 3b	
	var. x irrig.	2.19	0.1409	ns	
6	variety	0.76	0.3950	ns	31.1
	irrigation	13.29	0.0003	1b, 2a, 3a	
	var. x irrig.	0.11	0.8973	ns	
7	variety	1.24	0.2816	ns	27.0
	irrigation	9.60	0.0007	1b, 2a, 3a	
	var. x irrig.	0.58	0.6345	ns	
8	variety	0.00	0.9794	ns	24.9
	irrigation	2.96	0.0637	ns	
	var. x irrig.	0.06	0.9806	ns	
9	variety	8.16	0.0114	1b, 2a	21.0
	irrigation	5.03	0.0121	1b, 2a, 3ab	
	var. x irrig.	0.33	0.8065	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different

Appendix 1E: Summarized ANOVA for biomass production for two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) measured from week 3 to week 9 after planting

Time (weeks)	Source of variation	F value	Probability > F	Significance	CV (%)
3	variety	3.34	0.0844	ns	38.1
	irrigation	5.47	0.0140	1b, 2a, 3a	
	var. x irrig.	0.35	0.7111	ns	
4	variety	3.22	0.0895	ns	38.5
	irrigation	14.47	0.0002	1b, 2a, 3a	
	var. x irrig.	2.07	0.1549	ns	
5	variety	0.0000	1.0000	ns	7.9
	irrigation	15.47	0.0001	1b, 2b, 3a	
	var. x irrig.	0.00	1.0000	ns	
6	variety	0.11	0.7403	ns	32.7
	irrigation	3.39	0.0562	ns	
	var. x irrig.	0.25	0.7797	ns	
7	variety	4.22	0.0568	ns	25.0
	irrigation	1.88	0.1737	ns	
	var. x irrig.	2.89	0.0681	ns	
8	variety	16.19	0.0010	1b, 2a	17.7
	irrigation	3.78	0.0319	1a, 2b, 3b	
	var. x irrig.	1.83	0.1816	ns	
9	variety	34.86	<.0001	1b, 2a	11.4
	irrigation	17.80	<.0001	1b, 2a, 3b	
	var. x irrig.	0.00	1.0000	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different

Appendix 2: ANOVA for Plant Water Relations Measurements

Appendix 2A: ANOVA for leaf water potential measured on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) on specified dates from the 11th January to 17th February 2010

Date (2010)	Source of variation	F value	Probability > F	Significance	CV (%)
11th January	variety	13.09	0.0014	1a, 2b	9.7
	irrigation	0.82	0.4532	ns	
	var. x irrig.	0.82	0.4532	ns	
12th January	variety	0.86	0.3638	ns	6.9
	irrigation	19.60	<.0001	1c, 2b, 3a	
	var. x irrig.	1.50	0.2433	ns	
14th January	variety	1.18	0.2880	ns	6.3
	irrigation	0.93	<.0001	1c, 2b, 3a	
	var. x irrig.	0.93	0.4075	ns	
18th January	variety	3.31	0.0813	ns	9.6
	irrigation	3.06	0.0656	ns	
	var. x irrig.	15.52	<.0001	significant	
28th January	variety	1.09	0.3075	ns	8.0
	irrigation	9.04	0.0012	1a, 2b, 3a	
	var. x irrig.	6.43	0.0058	significant	
2nd February	variety	0.30	0.5895	ns	11.2
	irrigation	14.65	<.0001	1b, 2b, 3a	
	var. x irrig.	0.07	0.9282	ns	
4th February	variety	0.94	0.3425	ns	9.1
	irrigation	86.57	<.0001	1c, 2b, 3a	
	var. x irrig.	1.91	0.1692	ns	
5th February	variety	14.00	0.0010	1b, 2a	13.8
	irrigation	0.13	0.8813	ns	
	var. x irrig.	0.04	0.9586	ns	
8th February	variety	183.18	<.0001	1b, 2a	6.8
	irrigation	8.52	0.0016	1b, 2a, 3a	
	var. x irrig.	5.20	0.0133	significant	
9th February	variety	20.93	0.0001	1b, 2a	8.7
	irrigation	252.79	<.0001	1b, 2a, 3a	
	var. x irrig.	48.01	<.0001	significant	
12th February	variety	231.95	<.0001	1b, 2a	6.8
	irrigation	169.38	<.0001	1c, 2b, 3a	
	var. x irrig.	1.08	0.3565	ns	
17th February	variety	47.13	<.0001	1b, 2a	12.3
	irrigation	62.51	<.0001	1c, 2b, 3a	
	var. x irrig.	0.59	0.5611	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti, rrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered, Probability< 0.05 means significant difference, ns means not significant and treatments followed by the same letter in same block (cell) are not significantly different

Appendix 2B: ANOVA for stomatal conductance measured on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) on specified dates from the 11th January to 17th February 2010

Date (2010)	Source of variation	F value	Probability > F	Significance	CV (%)
	variety	4.23	0.0508	ns	
	irrigation	1.90	0.1708	ns	
11th January	var. x irrig.	0.46	0.6338	ns	17.9
	variety	1.34	0.2577	ns	
	irrigation	0.98	0.3881	ns	
12th January	var. x irrig.	0.68	0.5172	ns	22.8
	variety	7.63	0.0109	1b, 2a	
	irrigation	2.82	0.0796	ns	
14th January	var. x irrig.	6.54	0.0054	significant	15.4
	variety	12.83	0.0015	1b, 2a	
	irrigation	4.90	0.0165	1ab, 2a, 3b	
18th January	var. x irrig.	0.23	0.8001	ns	16.5
	variety	67.40	<.0001	1a, 2b	
	irrigation	0.03	0.9740	ns	
28th January	var. x irrig.	0.49	0.6171	ns	9.7
	variety	1.38	1.38	ns	
	irrigation	0.40	0.40	1ab, 2a, 3b	
29th January	var. x irrig.	0.25	0.25	ns	29.0
	variety	3.46	0.0753	ns	
	irrigation	5.99	0.0078	1ab, 2a, 3b	
2nd February	var. x irrig.	2.14	0.1393	ns	14.6
	variety	3.44	0.0761	ns	
	irrigation	5.97	0.0079	1ab, 2a, 3b	
4th February	var. x irrig.	8.98	0.0012	significant	14.1
	variety	0.00	0.9673	ns	
	irrigation	10.21	0.0006	1ab, 2a, 3b	
5th February	var. x irrig.	0.58	0.5661	ns	18.3
	variety	6.32	0.0190	1b, 2a	
	irrigation	9.87	0.0007	1b, 2b, 3a	
8th February	var. x irrig.	1.25	0.3055	ns	25.7
	variety	0.82	0.3739	ns	
	irrigation	12.04	0.0002	1b, 2b, 3a	
9th February	var. x irrig.	0.08	0.9254	ns	23.2
	variety	0.65	0.4295	ns	
	irrigation	10.90	0.0004	1b, 2b, 3a	
12th February	var. x irrig.	0.27	0.7683	ns	38.1

Variety 1 is GCI 17 and variety 2 is Monyaloti, irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered, Probability< 0.05 means significant difference, ns means not significant and treatments followed by the same letter in same block (cell) are not significantly different

Appendix 2C: ANOVA for relative water content (RWC) measured on two pearl millet lines; GCI 17 and Monyaloti under three irrigation treatments; water stressed (IR1) to well-watered (IR3) on specified dates from the 11th January to 17th February 2010

Date (2010)	Source of variation	F value	Probability > F	Significance	CV (%)
2nd February	variety	0.21	0.7844	ns	1.7
	irrigation	0.08	0.8160	ns	
	var. x irrig.	0.15	0.8595	ns	
4th February	variety	1.36	0.2706	ns	1.5
	irrigation	2.39	0.2706	ns	
	var. x irrig.	1.34	0.3055	ns	
10th February	variety	14.26	0.0036	1b, 2a	1.9
	irrigation	21.89	0.0002	1b, 2a, 3a	
	var. x irrig.	6.11	0.0185	significant	
17th February	variety	16.72	0.0022	1b, 2a	2.2
	irrigation	39.44	<.0001	1b, 2a, 3a	
	var. x irrig.	3.44	0.0730	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different

Appendix 3: ANOVA for Stomatal Dimensions and Distribution

Parameter	Source of variation	F value	Probability > F	Significance	CV (%)
Density	variety	3.45	0.0795	ns	13.0
	irrigation	3.56	0.0499	1a, 2b, 3b	
	var. x irrig.	0.72	0.5006	ns	
Area on abaxial surface	variety	53.22	<0.0001	1a, 2b	15.3
	irrigation	0.49	0.6225	ns	
	var. x irrig.	1.16	0.3352	ns	
Area on adaxial surface	variety	53.22	<0.0001	1a, 2b	15.3
	irrigation	0.49	0.6225	ns	
	var. x irrig.	1.16	0.3352	ns	
Width abaxial surface	variety	18.18	0.0005	1a, 2b	11.9
	irrigation	2.96	0.0776	ns	
	var. x irrig.	1.20	0.3241	ns	
Width adaxial surface	variety	6.91	0.0171	1a, 2b	8
	irrigation	3.99	0.0367	1a, 2b, 3ab	
	var. x irrig.	1.35	0.2836	ns	
Length abaxial surface	variety	52.97	<0. 0001	1a, 2b, 3ab	8
	irrigation	1.81	0.1929	1b, 2a	
	var. x irrig.	2.66	0.0973	ns	
Length adaxial surface	variety	0.20	0.20	ns	7.5
	irrigation	1.37	1.37	ns	
	var. x irrig.	0.20	0.20	ns	

Variety 1 is GCI 17 and variety 2 is Monyaloti

Irrigation 1 is water stressed, 2 is moderately stressed and 3 is well-watered

Probability< 0.05 means significant difference

ns means not significant

Treatments followed by the same letter in same block (cell) are not significantly different