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Crop Water Stress Management in the Tropics and Subtropics (380c)



Potential yield of Venezuelan maize varieties under variable water supply

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Abstract

Erratic rainfall patterns have caused severe drought conditions in Venezuela which directly affected white maize (*Zea mays* L.) production, increased the economic risk of smallholders and compromised food security. This project aimed to evaluate the differences in yield production based on morphological traits, gas exchange, root hydraulic properties and intrinsic water use efficiency (WUE) in five Venezuelan white maize hybrids (D-3273, Danac-842, D1B-718, D1B-283, Danac-223). At 55 days after sowing, plants were subjected to two contrasting water availability regimes; well watered plants under continuous irrigation (WW) and drought plants (DW), on which irrigation was withheld during five days followed by resumption of water supply. Under drought conditions, soil water content was sharply reduced due to a combination of leaf, root and shoot responses. Flowering length, biomass accumulation, and yield of the five hybrids were negatively affected. However, Danac-223 plants were less affected with no reduction of harvest index. Drought stress also caused a remarkable decline on the gas exchange parameters and, stomatal control was the major determinant affecting photosynthesis. Under variable water supply, intrinsic water use efficiency was improved in D1B-278 and Danac-223, whereas it decreased considerably in Danac-842. In all hybrids, water stress induced a decreased root hydraulic conductivity with a good correlation to leaf water potential. From the responses of Danac-223 plants, with a medium specific leaf area, smaller number of leaves per plant, a smaller reduction of the photosynthetic rate, a more efficient water transport through the roots, enhanced WUE, and yield production under drought stress, it may be considered as a very promising hybrid for cultivation in the western plains of Venezuela under variable water supply.

Key words: white maize, drought tolerance, secondary traits.

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Table of Content

List of Tables	vii
List of Figures	viii
List of abbreviations.....	x
Chapter I. Introduction	12
1.1 Hypotheses and objectives	15
Chapter II. Literature review	16
2.1 Tropical maize yield.....	16
2.1.1 Drought effect on maize during different growth stages.....	18
2.2 Root-Shoot signaling and responses	19
2.3 Development and photosynthesis affected by drought	20
2.4 The need for drought tolerance	22
2.5 Maize in Venezuela	24
2.5.1 Climate change and Venezuelan maize constraints	25
Chapter III. Material and Methods	27
3.1 Experimental site and greenhouse conditions	27
3.2 Hybrids, experimental design and growth conditions	28
3.2.1 Seeds germination.....	29
3.2.2 Irrigation treatments	30
3.3 Observations and measurements	30
3.4 Statistics	33
Chapter IV. Results	34
4.1 Crop phenological development	34
4.2 Drought stress imposition	35

4.2.1	Yield components.....	37
4.2.2	Root-shoot growth and relations.....	39
4.2.3	Physiological responses.....	42
Chapter V. Discussion.....		46
5.1	Drought.....	46
5.2	Yield, Harvest index and Drought index.....	48
5.3	Crop phenological development.....	50
5.4	Root-shoot responses.....	52
5.5	Physiological responses.....	55
5.6	Promising hybrids.....	57
Chapter VI. Conclusions and outlook.....		60
Appendices.....		72

List of Tables

Table 1: Number of yellow and white hybrids evaluated in experimental trials from 1999 to 2009, and comparison of the national and experimental yield average during those years. NA= Not available. Modified from Segovia and Alfaro, 2009.....	13
Table 2: Daily maximum and minimum temperature and minimum and mean relative air humidity in the greenhouse during the drought period (13 th August to 20 th August 2011).....	28
Table 3: Names, cycle of selection, origin and pedigree of hybrids used in the experiment.	29
Table 4: Number of green leaves, senescent leaves, height and specific leaf area at 55DAS. Numbers represent mean values (n=3).....	34
Table 5: Total plant biomass at harvest, grain yields, harvest index (HI) and index of drought stress tolerance (DSTI) and intensity (DII) of five white maize hybrids at harvest under well-watered (WW) and water deficit conditions (WD). Numbers represent mean values (n=3).	37
Table 6: Total plant biomass at vegetative stage, shoot-root ratio, root length of five white maize hybrids at SWC _{max} and SWC _{min} and Rolling Index (RI) under water deficit condition. Numbers represent mean values (n=3).	40
Table 7: Intrinsic water use efficiency of five white maize hybrids at SWC _{max} and SWC _{min} . Numbers represent mean values (n=3-5).....	44

List of Figures

- Figure 1: Summary of the main effects of water stress on the photosynthetic parameters of C₄ leaves. Stomatal and non-stomatal factors are indicated by dashed and continuous lines respectively. The (-) sign indicates an effect in the opposite direction. Modified from: Ghannoum, 2009. 22
- Figure 2: Germplasm varieties of Venezuela. From: Segovia and Alfaro, 2009 .. 24
- Figure 3: Yield and area harvested of maize and population of Venezuela from 1961 to 2009 (FAO, 2011). 25
- Figure 4: A) Root conductivity measurement with a pressure chamber. B) Shoots excised above the crown of the roots in Venezuelan maize varieties. 32
- Figure 5: The course of soil water content (SWC) during five days of water deficit plants, with its control (red column) and during re-watering (Day 7, green column) for five white maize hybrids. (Mean values \pm SE n=3-5). Statistical notation for each hybrids, SWC mean values with different letters were significantly different ($P= 0.05$) * shows significant differences between days, and capital letters between hybrids..... 35
- Figure 6: Leaf water potential (Ψ_w) during five days of water deficit, with its control (red column) and during re-watering (Day 7, green column) in five white maize hybrids. (Mean values \pm SE n=3-5). Statistical notation for each hybrids, mean values with ** were significantly different ($P= 0.05$). 36
- Figure 7: White maize hybrid under variable water supply at harvest. 38
- Figure 8: Schematic compare of the growth length periods for a standard maize crop and Venezuelan white maize under well-watered and water deficit conditions. Growth stages figure: <http://extension.entm.purdue.edu>..... 39
- Figure 9: Root hydraulic conductivity (L_p) as a function of leaf water potential Ψ , during SWC_{max} (filled symbols) and SWC_{min} (open symbols), in the five hybrids. A linear and non-linear regression, the derived equations, and the respective correlations coefficients were plotted..... 41
- Figure 10: Photosynthesis (P_N), transpiration (E), stomatal conductance (g_s) and intercellular CO₂ concentration during five days under water deficit, with its control (red column) and during re-watering (Day 7, green column) for five white maize hybrids. Mean values \pm ES (n=3-5).

Different letters were significantly different means within hybrids ($P=0.05$)..... 43

Figure 11: Net photosynthetic rate (P_N) as a function of stomatal conductance (g_s), during the drought pulse in five Venezuelan white maize hybrids. Means ($n=3-5$), non-linear regression (significant $P<0.01$), the derived equation and r^2 coefficient were plotted..... 45

List of abbreviations

AEZ	Agro-ecological zone
ASI	Interval between anthesis and silking in maize
Ci	Partial pressure of CO ₂ in the mesophyll intercellular space
CIMMYT	Centro Internacional de Mejoramiento del Maíz y Trigo
DAS	Days after sowing
DSTI	Drought stress tolerance index
DW	Drought stressed plants
E	Transpiration rate
FAO	Food and Agriculture Organization of the United Nations
g	gram
g _s	Stomatal conductance
hr	Hour
ha	Hectare
HI	Harvest Index
IVIC	Instituto Venezolano de Investigaciones Científicas
IRGA	Infrared gas analyzer
LAI	Leaf area index
Lp	Hydraulic conductivity of roots
m asl	Meters above sea level
min	Minutes
N:P:K	Nitrogen Phosphorus and Potassium fertilizer
P _N	Net photosynthetic rate
ppm	part per million

RH	Relative humidity
RI	Rolling Index
RWC	Relative water content of the leaves
SE	Specific error
SLA	Specific leaf area
SRPAC	Soil-root-plant-atmosphere continuum
SWC	Soil water content
SWC _{max}	Maximum soil water content
SWC _{min}	Minimum soil water content
UN	United Nations
WUE	Water use efficiency
WW	Well-watered plants
Ψ_w	Leaf water potential

Chapter I

1. Introduction

Worldwide maize (*Zea mays* L.) is sown in approximately 161 million ha with an annual grain production of 844 million tons, making it the second most important crop worldwide according to its production after wheat and followed by rice (FAO, 2011). Maize is used as food, feed or as ingredient in manufactured products that play an important role in the nutrition of the world's population (Hallauer *et al.* 1988), as well as biofuels. Therefore, the enormous economic importance of maize in the world is indisputable.

Depending on the color and taste of the kernel, maize grown around the world is generally categorized into two broad groups: yellow and white. Yellow maize constitutes the majority of the total world maize production and international trade; it is mostly temperate maize and used for animal feed. White maize, which requires more favorable climatic conditions for growing, is produced mostly as tropical maize, and its considered as a staple food crop (Abbassian, 2006).

In Venezuela maize is the most important cereal crop due to its high dietary value for the Venezuelan population, however the highest consumption is observed between poor people in urban and rural areas, provides 8.1 g of protein person⁻¹day⁻¹ and 393 calories person⁻¹day⁻¹ (11 percent of protein and 16 percent of energy of the daily intake for an adult) (INN, 2011). Maize production also contributes to the regional and national economy (Cabrera and Rodriguez, 1996)

In Venezuela the spreading of hybrid seeds has been performed for 35 years, specifically in 2008 resulting into 38.244 tons of grain harvested from 783.314 ha (FAO, 2011). Most of this area is located in the commercial production maize zones on the central and western plains of Venezuela (FEDEAGRO, 2011). Approximately 80 percent is white maize harvested for direct human consumption as precooked

maize flour (Segovia and Alfaro, 2009). Nevertheless, the production of white maize is low, and has become part of the national food security problem. On the one hand, according with the report of SENASEM-INIA (2008) not as many seeds as needed are available, although according to Segovia and Alfaro (2009) the national market was satisfied in 2007. On the other hand, the hybrids grown nowadays have had yield stagnations for ten years and a lower productivity, compared to yields obtained from the same hybrids under experimental optimal conditions by Venezuelan breeders (Table 1). This shows that environmental conditions and agricultural management play an important role in white maize production in Venezuela.

Table 1: Number of yellow and white hybrids evaluated in experimental trials from 1999 to 2009, and comparison of the national and experimental yield average during those years. NA= Not available. Modified from Segovia and Alfaro, 2009.

Year	N° of hybrids		Experimental Yield (kg ha ⁻¹)		National Yield (kg ha ⁻¹)
	White	Yellow	White	Yellow	
1999	29	18	7,297	7,350	3,132
2000	31	8	6,552	7,208	3,500
2001	28	11	6,445	5,734	3,449
2002	31	10	6,766	6,947	3,111
2003	23	10	6,705	7,125	3,433
2004	30	20	6,681	7,205	3,450
2005	26	26	6,326	6,017	3,262
2006	19	20	6,243	7,028	3,334
2007	17	25	7,376	7,276	3,472
2008	18	27	6,664	6,619	N A
	Average		6,706	6,871	3,291

Requirements on selection criteria of new Venezuelan maize hybrids change according to the traits involved: new maize hybrids must have an acceptable color, flavor, good mouth haptics and a fair price to satisfy consumers. They must

have a high milling yield and dough properties to satisfy processors. Additionally, smallholders as well as medium- and large-size farmers prefer hybrids with high grain yield, resistance to a broad range of diseases, pests, and tolerance to abiotic stresses. Therefore, the focus of research to meet all the requirements mentioned before needs to be sustainable as possible in the public and private sector in Venezuela.

A review of the strategy of maize production needs to be considered, in the light of the yearly and more often occurring situation of erratic rainfall patterns and increasing temperature (Ovalles *et al.* 2008); exacerbating the problem that current production is 49% lower than the yield for the same hybrids obtained in experimental optimal conditions. This can increase the food insecurity not only for rural people but also for poor people in urban areas where over 93 percent of Venezuela's population live (FAO, 2011), and are highly depending on natural resources from rural areas. The country's population is estimated to reach 35 million by 2030 (U.S Census Bureau, 2010). Hence, the future pressure on environmental services in rural areas will increase in order to meet food security and economic development, but also will depend on policy makers at national and regional level that those modifications should be assess based on sustainable strategies.

Breeding programs focusing not only on morphological traits, but also on the selection of drought tolerant hybrids and therefore specifically adapted to each cropping region in Venezuela, may be the only affordable option for many Venezuelan smallholders, under unexpected, but lately more frequent, negative weather conditions. Especially, where possibilities for irrigation do not exist (i.e. low crop input and water accessibility). These breeding programs can be a sustainable alternative to mitigate the impact of yield losses due to climate change and reduce the risk of food insecurity for both rural and urban population, which are large consumers on daily basis of the precooked maize flour obtained with white maize.

1.1 Hypotheses and objectives

Erratic rainfall patterns have directly affected crop production in Venezuela (Ovalles *et al.* 2008). In addition, a progressive decrease of potential yield in white maize hybrids highlights the importance to develop a breeding program, aiming at offering plants tolerant to variable water supply that can be used by smallholders in each region. Hence, it is necessary to study the drought tolerance of the principal white maize hybrids grown in Venezuela. It should be possible to offer several sowing material options, and therefore enhance food security for rural and urban population in a drought scenario.

Based on the above ideas the following hypotheses are the basis for the objectives and greenhouse experiments:

1. Drought resistance index, total biomass, harvest index and potential yield are likely to be different among Venezuelan white maize genotypes under variable water supply.
2. The formation of grain-yield on each hybrid under stress is related to the expression of particular basic components of root-shoot relations, plant growth or crop yield, during the physiologically active period under drought conditions or in the absence of stress.

Based on these hypotheses the following objectives have been formulated for research under greenhouse conditions.

- I. To identify one or more specific yield traits, which are evaluated as selection criteria for drought tolerance, with the purpose to assess changes in yield under variable water supply.
- II. To separate the traits that are correlated to grain yield under drought stress conditions, into expressions of the effects of yield potential, and drought tolerance by hybrids.

Chapter II

2. Literature review

The classification of maize is made according to the latitude and environment where it is cultivated. Its ability to adapt to a wide range of environmental conditions, is higher than that of other crops, leading to a wide range of growth from 58° of north latitude in Canada and Russia to 40° latitude south in Chile and Argentina, although the majority is growing in middle latitudes (Mosser, 2004). The maize grown between the equatorial line and 30° south and north latitude is defined as *tropical maize*, while *temperate maize* is cultivated above 34° in both hemispheres. Tropical maize is cultivated in 66 countries and is considered as a crop of economic importance in 61 of them (Paliwal *et al.* 2001). In 2007 approximately 100 million ha were planted with maize and 11 million tons of grains were harvested (Vagno de Souza *et al.* 2008).

Lack of current statistical records in white maize production is not a recent problem. Since 1997 a report on white maize production made by CIMMYT and FAO emphasized the problem with an accurate estimation of the total area in the world used to white maize grown, and the subsequent calculation of average yields. Thus considering that white maize is mostly used for direct consumption as staple food in many developing countries (Segovia and Alfaro, 2009); it would be advantageous to have recent production figures necessary to develop strategies related to breeding programs for food security.

2.1 Tropical maize yield

The tropical agro-ecological zone (AEZ) is characterized by humidity range from 300 to 1800 mm, temperatures between 15 and 20°C and differences in photoperiod smaller than 30 min. In addition, a bi-modal pattern of rainfall brings a rainy and a dry season. Annual climatic variability may constitute a major

constrain related to plant growth, and nutrient, and water availability in tropical agroecosystems in developing countries (Ziervogel and Ericksen, 2010). Exacerbated by a significant change in climate on a global scale it will impact agriculture and consequently affect the world's food supply (de la Peña and Hughes, 2007).

Under optimal tropical conditions, temperature 20-40°C, water availability higher than -0.35 Mpa, maize has a reported growth rate of 250 and 350 kg ha⁻¹ day⁻¹ (Fischer and Palmer, 1984). Specifically, conditions such as average temperatures of 20 to 24°C during the day and above 14°C at night (Brouwer and Heibloem, 1986), precipitation between 500 and 800mm (Mosser, 2004), and preferable sandy loam soils at pH 5.5 to 8 (Rehm and Espig, 1991) are required for maximum production of medium maturity grain to reach an average yield of 3.1 ton ha⁻¹. Considerably low compare with the yield potential of maize reported of 20 ton ha⁻¹ (Bänzinger *et al.* 2004).

A quantitative understanding of the change patterns of the relative amount of biomass during developmental stages, across environments and among genotypes, and how could be improved and used is of fundamental importance to agricultural practices (Reich, 2002). Moreover, grain yield of tropical maize is mainly determined by the final number of kernels per plant or per unit area that reach maturity (Rossini *et al.* 2011). In addition, yields depend directly on the ratio of total biomass to kernels; this is reflected in the harvest index (HI), defined as the ratio between kernel biomass and total above ground plant biomass. Under favorable conditions in tropical maize, this parameter can vary from 0.2 in regional genotypes to 0.3—0.4 in improved genotypes (Lafitte, 2001).

Grain yield in crops, HI and total plant biomass represents the expression of a complex network of genes whose functioning is integrated in response to soil conditions, crop management and the weather. Tropics are regions have been historically affected by unpredictable negative weather conditions (i.e. increase

on the rainfall intensity, droughts, gradual increase of the atmospheric temperature) (Ziervogel and Ericksen, 2010). Avoidance strategies through management options (i.e. change in the sowing date, introduction of genotypes with early maturity) might not be enough to avoid tropical maize losses due to variable water supply episodes, thus food insecurity (de la Peña and Hughes, 2007). Breeding programs aiming the reduction of the negative impact of stresses such as drought, which is common even on most productive regions, through the incorporation of resistant genotypes (i.e. controlling water loss from transpiration surfaces, sink-source adjustment) (Sunkar, 2010). But this adds further complexity to the change and management for smallholders on the growth and development of tropical maize (Richards *et al.* 2010).

2.1.1 Drought effect on maize during different growth stages

Drought stress is a major cause of variation in the grain yield of maize, which is grown in tropical and subtropical lowland areas (Mosser, 2004). Nevertheless, despite its importance, drought stress operational definition varies greatly (Mosser, 2004; Sunkar, 2010). These definition discrepancies are mostly due to variation of the occurrence, intensity, and duration that limits water supply (Abarshahr *et al.* 2011). In addition to the events being considered of the plant development i.e. water uptake, water use efficiency and HI (Passioura, 1996; Passioura, 2007). From an agronomic point of view, drought stress in crops occurs when leaf water potential decreases to a level where physiological damage occurs and yield is reduced (Sunkar, 2010). In this study, drought stress and drought tolerance are used in terms of yield variation in relation to a limited water supply.

Tropical and temperate maize requires adequate water in all stages of its development to attain optimum productivity (Rehm and Espig, 1991). But white maize tends to be more susceptible to negative environmental conditions compared to yellow maize (CIMMYT and FAO, 1997). Likewise, the amount of yield

loss on maize that occurs during drought depends on growth stage, stress intensity, and the environmental changes during water scarcity (Passioura, 2007).

Under variable water supply (mainly due to differences on soil moisture) according with the development stage of the plant, the response of maize are: (i) in the vegetative period: maize appears relatively tolerant to water deficits (Paliwal *et al.* 2001), although Nilson and Orcutt (1996) highlight the effect of pre-anthesis drought in the modifications in carbon gain, since effective leaf area might be reduced as a result of leaf rolling. Hence, carbon gain under drought can be reduced (Blum, 1997). (ii) During the flowering period including tasselling, silking, and pollination (from one week before tasseling to two weeks after silking): low water potential during anthesis does not prevent pollination (Westgate and Boyer, 1986), but a decrease in grain yield should be expected by a reduction in grain number per cob (Fischer and Palmer, 1984). Decreases in the water available during this stage hinder the development of embryos due to low assimilate supply (Schussler and Westgate, 1991) below the necessary threshold for ovules to develop (Edmeades *et al.* 1993, Saini and Westgate, 2000). (iii) Water deficits during the yield formation period (Anthesis-silking interval) may lead to a considerably delayed of kernel formation and reduced yield due to a decrease in grain size rather than grain number (Boyer and Westgate, 2004; Mosser, 2004); and water deficit during the ripening period (iv) has little effect on grain yield (Paliwal *et al.* 2001).

2.2 Root-Shoot signaling and responses

Plant growth is a function of a complex interplay between source and sink limitations of the root system and the shoot, establishing a functional equilibrium (Brouwer, 1983) between leaves that fix carbon, stems that provide mechanical support and a hydraulic pathway, and roots that absorb nutrients and water, and provide anchorage (Poorter *et al.* 2012). Roots also have the capacity to sense parameters such as water and nutrient status of the soil, and adjust their growth

and water transport properties accordingly to maintaining the water status of the plant in a changing environment (Maurel *et al.* 2010).

Several environmental and hormonal signals are known to trigger short-term (minutes to hours), as well as, mid- and long term (days) adjustments of the root hydraulic properties such as water permeability or hydraulic conductivity (L_p) (Steule, 2000; Ehlert *et al.* 2009). In particular, reduced water availability in soils usually induces a marked drop in L_p at mid- and long term (Yang and Tyree, 1994).

A reduction in L_p may primarily impact on water potential gradients along the soil-root-plant-atmosphere continuum (SRPAC); hence, the root-shoot communication within plants, transfers the roots perception of a drying soil to the shoot as an hydraulic signal (Steule, 2000; Christmann *et al.* 2007; Maurel *et al.* 2010), which induce water-saving reactions in the leaves by stomatal regulation of leaf diffusive conductance, and thereby influence water loss and carbon gain (Buckley, 2005).

In temperate maize Asch *et al.* (2009) investigated early signals of root-shoot communication of field-grown maize under conditions of progressive drought, but the knowledge of how abiotic factors influence water supply in different plant stage of tropical white maize are not yet adequately understood.

2.3 Development and photosynthesis affected by drought

Traditionally maize breeding has been focused on morphological traits such as: (i) general appearance (size and shape of ear, straightness of rows, and uniformity of kernels), (ii) productiveness (maturity, vitality, shelling percentage), (iii) breed type (size and shape of ear and kernel, dent of kernel, color grain, color of cob) (Hallauer *et al.* 1988). An alternative approach to improve plant breeding in maize, for better stress tolerance is the introduction of physiological selection markers (Holá *et al.* 2010). Breeding strategies should use physiological traits for plant stress

tolerance with a good relation to both, tolerance to the target stress factor (e.g. variable water supply) and to relevant yield parameters (Sayar *et al.* 2008).

Net photosynthetic rate (P_N) measured with a portable gas analysis systems (IRGAs) has been determined together with the stomatal conductance (g_s) and transpiration rate (E) as potential selection marker for the detection of drought tolerance in maize (Zarco-Perelló *et al.* 2005; Cruz de Carvalho *et al.* 2011). Due to their potential to provide information to the understanding of growth, productivity and water use efficiency (WUE) of this crop. The number of environmental factors influencing how these quantitative traits are expressed, such as the timing and duration of variable water supply, together with early plant responses to the stress can be seen as a first line of defense allowing survival in a short term (Cruz de Carvalho *et al.* 2011), whereas acclimation processes (Sharp *et al.* 2004) are considered survival strategies to cope more persistent drought stress periods, in addition to soil type, heat, and humidity.

Despite that C4 plants make a significant contribution to the global carbon budget, and C4 crops, such as maize are strategic staple food crops to current and future global food security (Pingali, 2001) the response of C4 photosynthesis to drought stress has not been studied intensively (Ghannoum, 2009).

When drought occurs, i.e. by a decrease in soil water availability, often measured as the soil water content (SWC) plants experience a number of combined responses. The decline in SWC results in a larger differences of the gradient between the water potential (Ψ) of the soil and the Ψ_w of the plant (SRPAC) and thus limits the ability of the plant to take up water (Boyer, 1995). Nevertheless, stomatal and non stomatal factors are likely to be responsible for inhibiting C4 photosynthesis (Figure 1). The stomatal factors refer to the downstream effect of CO₂ diffusion at the early phase of water stress. When Ψ_w is higher than -0.4 Mpa, the decline in CO₂ assimilation rates (P_N) is largely the result

of reduced intercellular CO₂ concentration (C_i) due to decreased stomatal conductance (g_s) (Carmo-Silva *et al.* 2008).

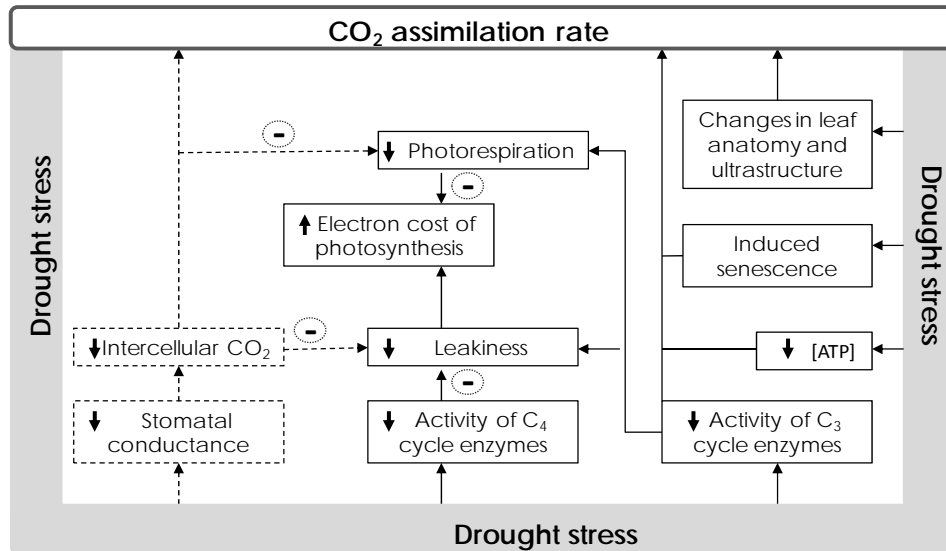


Figure 1: Summary of the main effects of water stress on the photosynthetic parameters of C₄ leaves. Stomatal and non-stomatal factors are indicated by dashed and continuous lines respectively. The (-) sign indicates an effect in the opposite direction. Modified from: Ghannoum, 2009.

The non-stomatal factors encompass everything else, at values of Ψ_w between -0.4 and -0.85 Mpa (Saliendra, 1996), including the direct effects of reduced leaf and cellular water status on the activity of enzymes involved in the CO₂ fixation reactions, induction of early senescence, and change to leaf anatomy and ultrastructure (Ghannoum, 2009).

2.4 The need for drought tolerance

The increase of the world population holds exceptionally difficult challenges for the planet like exceeded ecological footprints (Ewing *et al.* 2010) and degraded ecosystem services (Millennium Ecosystem Assessment, 2005). This scenario points to the fact that the global pattern of food consumption is unsustainable at long term (Fitter, 2012).

Today 1.02 billion people suffer from hunger (FAO, 2011) although a high effort has been made to halve the proportion of people who suffer from hunger between 1990 and 2015 (UN Millenium Development Goals). Over the next 30 to 50 years with an estimated population of 9–10 billion people, the biggest problem that arises is how to feed this population, especially in the least developed countries (Beddington, 2010). The problem is increased by the inevitable loss of productive farmland to urbanization, the high current rate of degradation of soils, and the negative effect of climate change (Lal, 2007).

There is a real concern that one of the biggest impacts of climate change will be to increase the frequency, severity and global scale of drought periods (Romm, 2011). Drought is an especially challenging problem because of the absolute scarcity of water in many parts of the world and the use of the 80% of the intercept run-off precipitation in agriculture (Fitter, 2012).

No crop will survive to a severe drought in general, but climate change not only intensifies prolonged dry conditions given by weather phenomena such as El Niño, which mostly affect crop areas in the southern hemisphere (Abbassian, 2006). Water variable supply is also playing an important role in combination with other factors such as nutrient availability and soil quality, which tend to be more frequent but less severe than droughts in plant's resilience (Tollefson, 2011).

Maize is widely cultivated throughout the world. Most of the world production is hybrid maize, but there is a limit to hybrid maize breeding in the identification and abundance of suitable genotypes for generating heterosis (Hadi, 2007). Due to this limitation, there is a growing interest in local cultivars as a source of genetic variability (Hallauer *et al.* 1988). Breeding programs in developing countries may have objectives of developing, evaluating, and improving populations and testing population hybrids. These objectives may have been initiated in more advanced programs several years ago and may have little interest for breeders emphasizing inbred line and hybrid development unless unique inbred lines are to be

developed (Paliwal *et al.* 2001). Turner (2002) concludes that, in environments with varying drought stress, it may be preferable to breed for phenological adaptability to enable genotypes to profit from favorable growth periods.

2.5 Maize in Venezuela

Since colonial time (16th to 17th century) to early 40's of the 20th century, "creolized" (regional) maize varieties were sown in Venezuela (Figure 2) (Grant *et al.* 1965). Modernization of the crop was started approximately 70 years ago and it began in 1939 with the incorporation of improved cultivars from Colombia, Mexico, Central America and the Caribbean (Segovia and Alfaro, 2009).



Figure 2: Germplasm varieties of Venezuela. From: Segovia and Alfaro, 2009

During 1940's, the first cultivars were obtained from open pollination, being the beginning of seed production. In the 1950's, hybrids productions started and the results were commercially marketed as double hybrids or from four lines. In the 60's, the governmental project "Maize's Plan" ("Plan del Maiz") was introduced. The goal was to improve the agronomical management through high quality seeds, fertilization and mechanization of the production, the latter being the principal aim (Segovia *et al.* 2006). Positive results of this plan were evident only 10 years later, in the 80's due to a slow adoption process. In the late 90's the incorporation of new

cultivars was stopped as consequence of the commercialization the hybrid CENIAP PB-8, sowed on 80% of the land area in Venezuela (Segovia and Alfaro, 2009), increasing effectively the national yield (Figure 3). Nowadays, such a decision can be questioned considering the importance of regional germplasm in breeding programs, especially for producing lines being adapted to marginal areas and region specific problems like droughts, floods and unfertile soils.

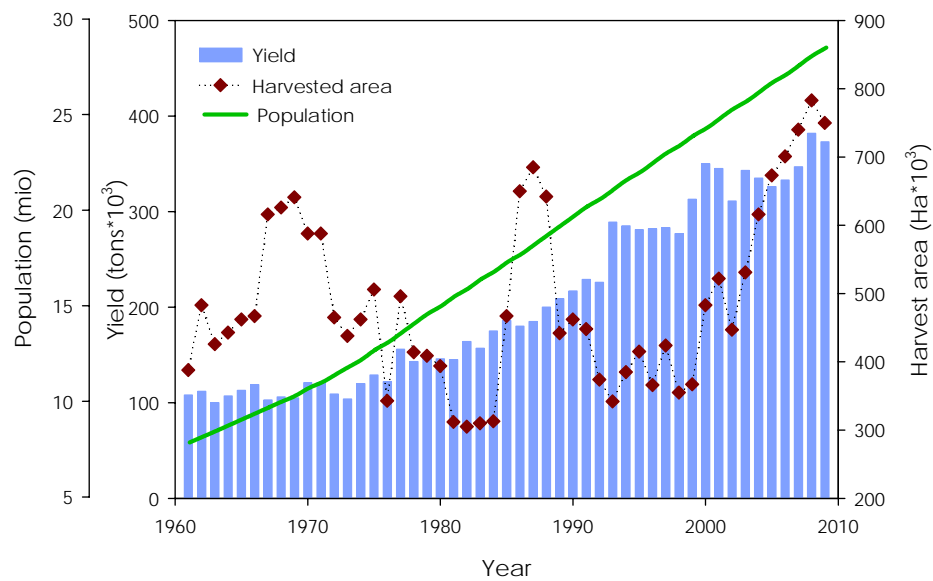


Figure 3: Yield and area harvested of maize and population of Venezuela from 1961 to 2009 (FAO, 2011).

2.5.1 Climate change and Venezuelan maize constraints

From a historical perspective, the most striking improvement in maize production in the last century in Venezuela was the introduction of hybrids. Even if it is considered the stagnation of the production level and, the low performance of hybrids under normal conditions compared to experimental yield assessments. According to the International Research Center on “El Niño”, Venezuela is one of the South American countries likely to be affected by this weather phenomenon. In detail, in those years with “El Niño” episodes, the national production of white maize decreased between 10 and 15% in relation to previous and following years.

The western plains of Venezuela, with an area of 106,736 km² are one of the most important regions of agricultural production in Venezuela. With annual homogeneous temperature, in average between 24 and 28°C, it is influenced by winds from the Venezuelan Gulf and the Alisios. A regular rainy season is expected between May and November, given an annual precipitation between 1,400mm and 2,000mm decreasing from the mountainous region to the plains (INE, 2011). Due to changes in the rainfall pattern in the last two years, probably attributed to the global climate change, a severe drought was recorded in 2008 and 2009, where accumulated rainfall was 30 to 60% lower than previous years (INAMEH, 2010).

Problems caused by drought during 2008 and 2009 were at regional and national level. In order to maintain crop grain yield, irrigation was needed. Both, the economic risk due yield losses of those small producers without access to proper irrigation systems under agro-ecological marginal conditions and the input needed per ha for larger producers. Nevertheless, the offset given by irrigation was not enough to supply the demand in the market and the import of maize for those years was 60% higher than usual (USDA, 2011).

So far, Venezuela has avoidance management strategies to reduce yield losses in white maize production through selection of genotypes with intermediate life cycles but with some tolerance under variable water supply. This enables not only to escape late drought stress but provides also additional revenue instead of losses (Ing. J. Alezones-DANAC, Personal Communication).

Studies have started in Venezuela to breed white maize for phenological adaptability to enable genotypes to profit from favorable growth periods. This should be complemented with studies to elucidate the physiology of the response to variable water supply conditions. In particular, physiological-based traits are needed for the evaluation of water variability tolerance for applied plant breeding programs.

Chapter III

3. Material and Methods

3.1 Experimental site and greenhouse conditions

The greenhouse experiment was conducted at Venezuelan Scientific Research Institute (IVIC), 11km south-west of Caracas, Venezuela (10° 24' N; 66° 59' W; 1730 m asl) during the rainy season. Caracas is a valley located in the northern part of the country at the Venezuelan coastal mountain range (Cordillera de la Costa) separated from the Caribbean Sea by a steep 2200 m high.

The experiment was conducted during the rainy season of 2011, starting on June 20th, 2011, under controlled conditions (12h of photoperiod). The daily temperature and relative air humidity from June until the end of the experiment (6th December, 2011) in the greenhouse were recorded every hour with an Onset HOBO Micro Station Data Logger, Bourne, Massachusset USA (30°C day/16°C night and RH of approximately 81%). The daily maximum and minimum temperature and minimum and mean relative air humidity in the greenhouse during the drought period are shown in the table 2.

Table 2: Daily maximum and minimum temperature and minimum and mean relative air humidity in the greenhouse during the drought period (13th August to 20th August 2011).

Days	DAS	Temperature(°C)		Relative humidity (%)	
		Minimum	Maximum	Minimum	Mean
13 August	55	19.5	24.8	75.8	75.8
14 August	56	19.4	24.8	73.0	77.1
15 August	57	18.0	29.3	65.6	80.9
16 August	58	15.1	31.6	51.4	75.0
17 August	59	17.4	33.5	50.8	76.4
18 August	60	18.6	29.5	67.3	79.9
19 August	61	16.1	31.1	65.7	79.3
20 August	62	18.2	26.4	56.7	76.8

3.2 Hybrids, experimental design and growth conditions

The seeds of five white maize hybrids used in this study, characterized by grain type dent and late maturity were obtained from Fundación para la Investigación Agrícola- DANAC. D-3273, Danac-842 and Danac-223 are commercialized hybrids, whilst D1B-718 and D1B-278 are experimental hybrids under different cycles of selection and pedigree (Table 3).

Table 3: Names, cycle of selection, origin and pedigree of hybrids used in the experiment.

Hybrids	Cycle of selection	Origin	Pedigree
D-3273	-	Commercial	D-3273
Danac-842	-	Commercial	Danac-842
D1B-718	C4	10A6A-10A-X2AW-27/28	03Mn7B-7-1-#-#-#// F655-1-1-2-#
D1B-278	C6	11A6A-X3AW-110/111	03Mn7B-7-1-#-#-#-# #// F618-4-2-2-#-#-#
Danac-223	-	Commercial	Danac- 223

3.2.1 Seeds germination

Seeds were pretreated with an antifungicide mixture (Gaucho¹ and Maxim² 1:10; [¹:Gaucho 60® (Nitromidazolidin-2ilideamin); ²:Maxim XL® (Fluoxonil and Metalaxil-M)], thereafter were germinated and grown for five days in dark on sterile wet filter paper in Petri dishes, under controlled growth conditions (23°C and RH of 73%). The seeds were sown in mid June, been consider as the initial day of sowing (0DAS), and kept during 15 days in the seedbed. Then they were selected, at the very late V1 growth stage to very early V2 seedling, and placed one per pot (4 kg) in soil. The characteristics of the soil: a sand-loam soil with 60% sand, 33% silt and 10% clay. At transplant day a surface fertilizer was added (0.3 kg.m⁻², Nitrogen Phosphorus and Potassium (N:P:K) 10:20:20, El productivo, Pequiven, Venezuela). A second dose of nitrogen was applied at 35DAS (0.1 kg m⁻², Urea, Pequiven, Venezuela). The pots were randomly distributed among treatments in a greenhouse under controlled conditions and watered daily to field capacity (approximately 20% of soil water content).

3.2.2 Irrigation treatments

Fifty-five days after the germination, plants were divided in two contrasting water regimes treatments: (i) non irrigated, (drought plants, DW) on which water stress was imposed by withholding water for five days, and after 24 hours were evaluated the recovery response, and (ii) watered plants (WW). During this experimental period of seven days, plants were sampled on the day 0, 3 and 5 as well as, day 1 of recovery (day 7). During each sampling day, 4–5 leaves from different plants (true replicates) were used per cultivar (five cultivars, four sampling dates and five replicates). Control plants (well-watered) were measured at day 5 in three leaves from different plants per hybrid. Physiological measurements were made on the mid segment of the last fully expanded leaf using the same replicates along the study per sampling date. Experiments started at the same time each day to eliminate possible confounding diurnal effects.

3.3 Observations and measurements

After 55DAS, data was collected on plant biomass, plant height, number of fully expanded leaves, number of dead leaves, and leaf area in order to assess specific leaf area (SLA) as the ratio of the leaf area between plant dry biomass.

Assessment of biomass was done at (i) the beginning of the imposition of water stress, (ii) at day 5 of drought pulse and (iii) at harvest period on three plants per hybrid cut and separated into stems, leaves and roots. The plant materials were chopped and oven-dried at 80°C to constant weight. At the beginning of the imposition of drought stress was determined and the root-shoot ratio on three plants per variety. The grain yield of the plants was also measured as grain weight of sampled plants. Harvest index was calculated as the proportion of the grain weight to total plant biomass. Drought stress tolerance index (DSTI) was calculated based on grain yield data under well-watered and water deficit conditions using

the index of Fisher and Maurer (1978), and the drought stress intensity (DII) was calculated according to Fernandez (1993):

$$DSI = (1-(Y/Y_p)) / (1-(X/X_p)) \text{ (Equation 1)}$$

$$DII = 1-(X/X_p) \text{ (Equation 2)}$$

where Y is the hybrid mean under stressed condition, Y_p is the grain yield of hybrid under non-stress condition; X is the mean yield of all hybrids under stress, and X_p is the mean yield of all hybrids under non-stress condition.

At 55DAS the drought treatment was imposed during five days, on which water status of the plants and soil, as well gas exchange of the leaves were measured.

The soil water status was evaluated as the SWC, expressed as a weight fraction (Coombs *et al.* 1987). The following equation was used:

$$SWC (\%) = 100*(FW-DW)/DW \text{ (Equation 3)}$$

where, FW was the fresh weight of a soil portion of the internal area of each pot and DW was the dry weight of the same soil portion after oven-dried at 80°C for three days.

Plant water status was evaluated by the leaf water potential (Ψ_w), using the psychrometric method and a thermocouple psychrometer (Wescor HR-33T Dew point Microvoltmeter, C-52 sample chamber; Wescor, Inc., Logan, Utah, USA).

The L_p of whole root systems was measured with the pressure chamber technique in three plants per hybrid at maximum SWC (SWC_{max}; day 0) and minimum SWC (SWC_{min}; day 5). Before every measurement the pot was watered

and drained on absorbent paper, afterwards the shoots were excised 5 to 10cm above de root crown (Figure 4b), then the cut mesocotyl protrude was sealed in the pressure chamber through the lid (Figure 4a).



Figure 4: A) Root conductivity measurement with a pressure chamber. B) Shoots excised above the crown of the roots in Venezuelan maize varieties.

Pressurizing the chamber induced water flow through the root system. Preliminary measurements indicated that the flux-pressure relationship was linear between 0.2 and 0.9Mpa. Thereafter, all root systems were initially pressurized between 0.2 and 0.8Mpa for three to five minutes, before collecting exudates, to ensure steady-state conditions.

The water flux was determined by collecting the xylem sap accumulated every 3 minutes during 30 minutes in a tightly fitted silicon tube to the cut end of the mesocotyl and weighed. After the initial equilibration period, thorough the collection of the exudates of the root was calculated conductivity from the linear regression of water flux against applied pressure. The total length (Tennant, 1975) and biomass of the root systems were determined after washing the roots, drying them at 80°C during five days and weighed.

Gas exchange was measured with an infrared gas analyzer (IRGA) (CIRAS 2 PP System Co., Ltd Amesbury, Massachusetts USA). Photosynthetic (P_N) and transpiration (E) rates, partial pressure of CO_2 in the mesophyll intercellular space

(C_i) and stomatal conductance (g_s) were calculated using the von Caemmerer and Farquhar (1981) equation. Photosynthetically active radiation was provided by a LED array placed on top of the leaf clip, and a photosynthetic photon flux density of approximately 1,000 μmol of photons $\text{m}^{-2}\text{s}^{-1}$. Leaves were illuminated under normal atmospheric CO_2 content (approximately, 370ppm), a relative humidity of 50–60% and a temperature of about 25°C. At day 5 of the drought period (60DAS) the Rolling Index (RI) of the leaves was determined quantitatively as:

$$\text{RI (\%)} = 100 * (W_{ri} / W_{fel}) \text{ (Equation 3)}$$

where, W_{ri} was the rolled width of a leaf under drought conditions, and W_{fel} was the fully expanded width at the same leaf, measured with a vernier caliper.

3.4 Statistics

Data were analyzed with the SAS (version 9.2 SAS Institute North Carolina, USA) program. The experiment was a completely randomized design, forming a 5×2×4 factorial (five hybrids, two watering regimes, and fourth sampling periods) with five plants in individual containers per treatment-combination as replication. The experimental plot was one plant per pot. For yield and control data, each replicate represented the mean of three determinations. Data were statistically examined using analysis of variance and tested for significant ($P \leq 0.05$) hybrid, sampling period and irrigation treatment differences using Tukey tests.

A Pearson correlation analysis including all the parameters determined was also performed. Possible differences between cultivars in the correlation between two parameters were analyzed by comparison of regression lines.

Chapter IV

4. Results

4.1 Crop phenological development

Treatments were imposed at 55DAS (during the late vegetative phase, before flowering), when morphological parameters such as number of leaves, number of dead leaves, height and specific leaf area (SLA) were evaluated (Table 4). No significant differences among hybrids were found in the number of green or senescent leaves. Minimum and maximum heights were observed in D-3273 and D1B-718 respectively. SLA showed considerable variations between hybrids, D1B-278 recorded the highest value, 171.8 cm²g⁻¹ and, D1B-718 the lowest SLA with 48.7 cm²g⁻¹ together a higher height.

Table 4: Number of green leaves, senescent leaves, height and specific leaf area at 55DAS. Numbers represent mean values (n=3).

Hybrid	N° of leaves	N° of senescent leaves	Height (cm)	SLA (cm ² g ⁻¹)
D-3273	9.6	2.3	45.9 ^b	55.3 ^b
Danac-842	10.3	3.0	50.1 ^{ab}	105.9 ^{ab}
D1B-718	9.1	1.4	53.4 ^a	48.7 ^b
D1B-278	11.2	2.5	48.8 ^{ab}	171.8 ^a
Danac-223	9.2	2.4	47.6 ^{ab}	72.5 ^{ab}
Mean	9.6	2.2	49.0	90.8

Mean values with different letters were significantly different according to Tukey test at ($P=0.05$).

4.2 Drought stress imposition

Soil water content

Soil water content was determined as an indicator of water availability in pots for each plant. Soil water content progressively decreased in the five hybrids, since day 3 significant differences ($P<0.01$) were recorder. Effects were showed between hybrids ($P<0.006$) and among days ($P<0.001$). At day 5, minimum values of SWC were reached (Figure 5). After 24 hours of re-irrigation to field soil capacity, SWC increased to similar values as in irrigated plants.

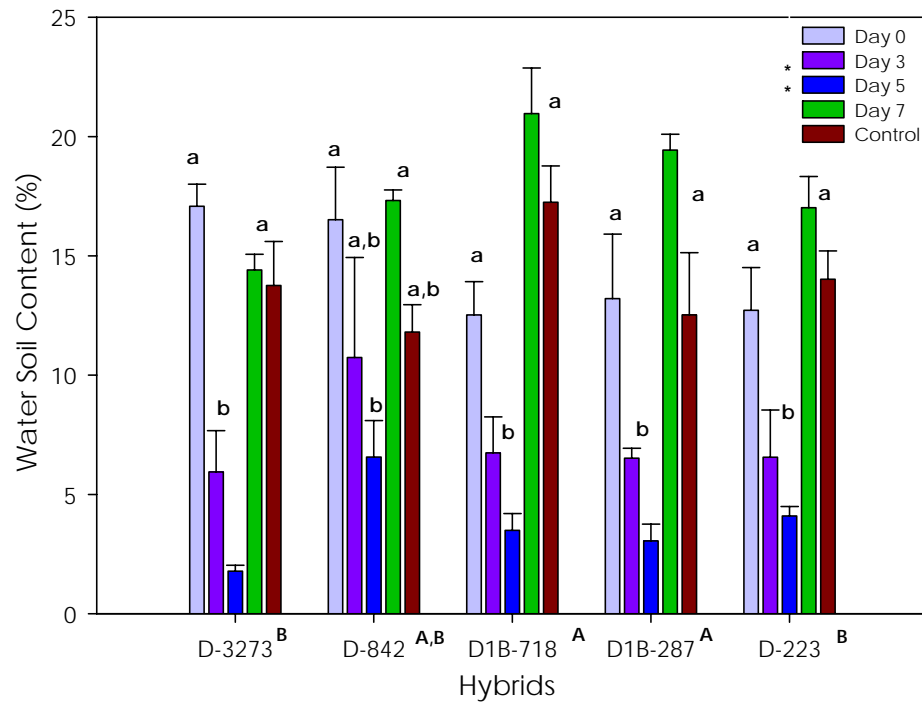


Figure 5: The course of soil water content (SWC) during five days of water deficit plants, with its control (red column) and during re-watering (Day 7, green column) for five white maize hybrids. (Mean values \pm SE $n=3-5$). Statistical notation for each hybrids, SWC mean values with different letters were significantly different ($P=0.05$) * shows significant differences between days, and capital letters between hybrids.

Water Potential

During the first three days of water deficit, Ψ_w in the hybrids decreased but not significantly different from the previous days, except for Danac-223 which had a decrease of 55% in leaf water potential since day 3. At day 5, the minimum value was reached by all hybrids; specifically the lowest value was -7.66 Mpa by D-3273, whereas Danac-842 showed no significant differences among days (Figure 6). After 24 hours of re-watering Ψ_w was fully recovery compared to the values of day 0 and control in the five hybrids. The effect of the hybrid and the interaction between hybrids and water availability per day were non-significant.

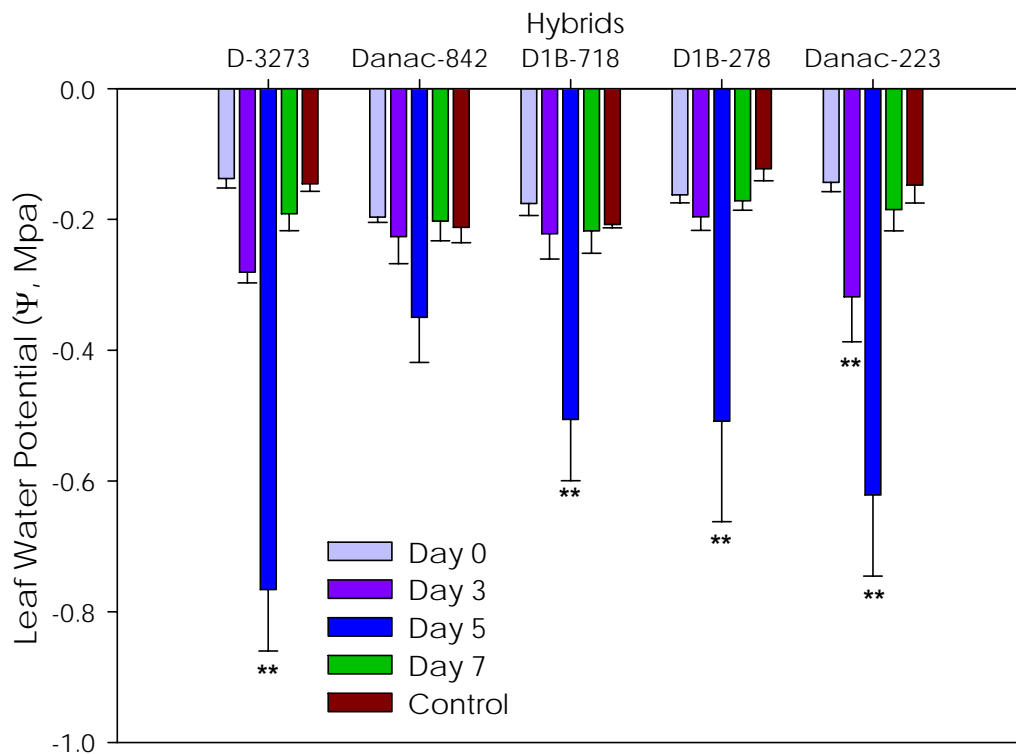


Figure 6: Leaf water potential (Ψ_w) during five days of water deficit, with its control (red column) and during re-watering (Day 7, green column) in five white maize hybrids. (Mean values \pm SE n=3-5). Statistical notation for each hybrids, mean values with ** were significantly different ($P= 0.05$).

4.2.1 Yield components

The effect of water deficit and hybrid and their interaction, were significant in plant biomass ($P<0.008$) and grain yield ($P<0.02$). HI showed significant differences between hybrids ($P<0.014$). In WD plant of D-3273 and Danac-842, no grain yield was recorder due to the absence of kernel in the cob. Multiple comparisons revealed that total biomass of Danac-223 was significantly lower than D-3273. On the contrary, Danac-223 with highest harvest index (HI) on day 5 was the hybrid with less drought impact index (Table 5).

Table 5: Total plant biomass at harvest, grain yields, harvest index (HI) and index of drought stress tolerance (DSTI) and intensity (DII) of five white maize hybrids at harvest under well-watered (WW) and water deficit conditions (WD). Numbers represent mean values (n=3).

Hybrid	Biomass (g plant ⁻¹)		Grain yield (g plant ⁻¹)		Harvest Index		DII	DSTI
	WW	WD	WW	WD	WW	WD		
D-3273	70.8	67.7*	9.9	-	0.05	-	0	0
Danac -842	80.8*	34.9	0.2*	-	<0.00	-	0	0
D1B-718	70.0	35.2	7.2	2.2	0.12	0.04	0.69	0.84
D1B-278	66.5	33.4	2.8	0.4	0.05	0.01	0.85	1.04
Danac-223	47.3	31.4	10.0	7.4	0.23	0.23	0.26	0.32
Mean	67.1 ^A	41.8 ^B	6.0 ^A	2.0 ^B	0.10	0.07		

Mean values with asterisk (*) were hybrids significant different; capital letters were significantly different treatments between in hybrids ($P= 0.05$).

The number of cobs per plant were constant in all plants (n=1) in both treatments. In DW plants was observed characteristic symptoms of drought during harvest (Figure 7), cobs with reduced number or even absence of kernels, and highly susceptible to diseases.



Figure 7: White maize hybrid under variable water supply at harvest.

4.2.2 Root-shoot growth and relations

Greenhouse growth conditions extended by thirty days the vegetative period of the growth length in both treatments in all the hybrids under study, as well as drought stress caused delayed flowering (Figure 8).

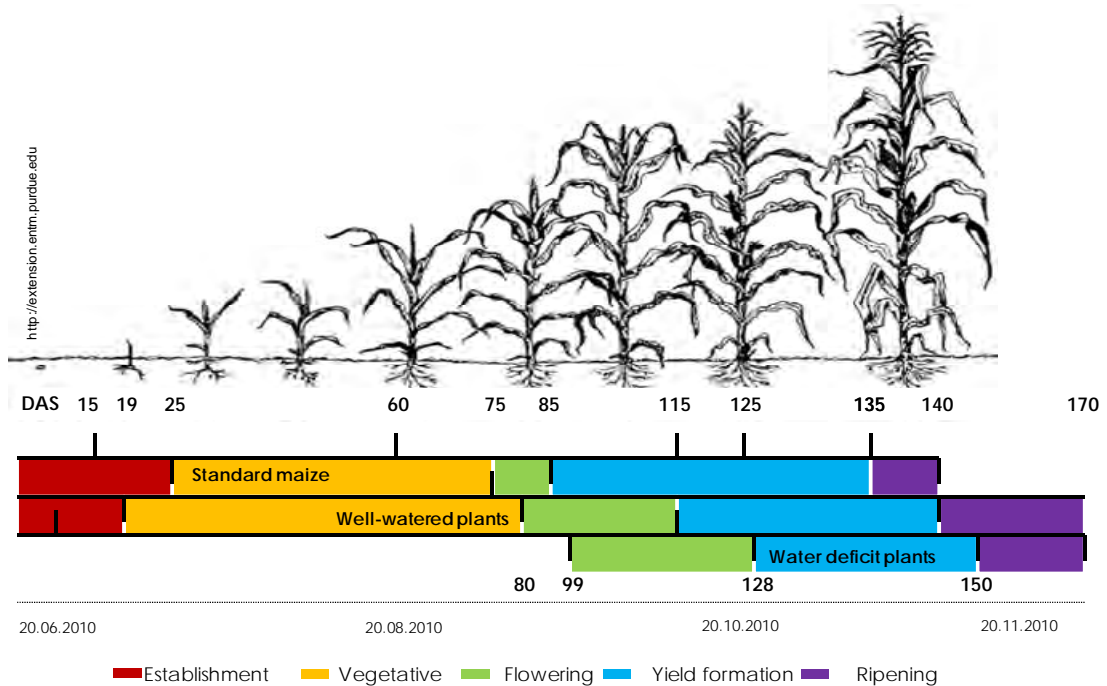


Figure 8: Schematic compare of the growth length periods for a standard maize crop and Venezuelan white maize under well-watered and water deficit conditions. Growth stages figure: <http://extension.entm.purdue.edu>.

Drought stress not only delayed flowering, but also decreased the number of flowering plants from 53% in WW plants to 35% under low water supply (DW plants). At 128DAS approximately 47 percent of the plants from both treatments were under yield formation.

The responses of the WW and DW plants with respect to biomass, root-shoot relation, root length and rolling index of the hybrids under study are shown in Table 7. The water deficit at SWC_{min} (day 5), with a general reduction in average of SWC

for all hybrids of 73 percent, led an increase on the root-shoot ratio (R/S) in the hybrids, only D1B-273 decreased, although total biomass remain almost similar in relation with the value obtained at SWC_{max} (day 0).

Table 6: Total plant biomass at vegetative stage, shoot-root ratio, root length of five white maize hybrids at SWC_{max} and SWC_{min} and Rolling Index (RI) under water deficit condition. Numbers represent mean values (n=3).

Hybrid	Biomass (g)		Root-shoot		Root Length (m)		RI (%)
	SWC _{max}	SWC _{min}	SWC _{max}	SWC _{min}	SWC _{max}	SWC _{min}	
D-3273	35.7 ^{ab}	13.8 ^b	0.7 ^B	1.4 ^A	52.7	23.6	28.3
Danac -842	25.4 ^{ab}	22.2 ^{ab}	0.3	1.1	40.3	68.1	25.3
D1B-718	49.3 ^a	40.6 ^a	0.3 ^B	1.9 ^A	34.7	32.8	25.0
D1B-278	20.8 ^b	19.8 ^{ab}	0.9	1.7	94.3	28.0	12.1
Danac-223	21.5 ^b	31.2 ^{ab}	0.4 ^B	2.2 ^A	40.6	28.8	18.0
Mean	30.6	25.6	0.5 ^B	1.7 ^A	52.5	36.2	22.2

Mean values (n=3) with different letters were significantly different hybrids within SWC and capital letters were significantly different SWC ($P= 0.05$).

The minimum value of rolling index was observed in D1B-278, and the maximum rolling in D-3273. Rolling Index had no significant difference between varieties.

Root hydraulic conductivity decreased at maximum water deficit conditions, strongly in D-3273 and D1B-718, and slightly on D1B-278 and Danac-842 (about 0.2 mg s⁻¹m⁻¹ Mpa⁻¹) and kept steady in Danac-223 (1.09 to 0.94 mg s⁻¹m⁻¹ Mpa⁻¹ (Figure 9)).

In the hybrids, a linear regression was obtained between L_p and Ψ_w at SWC_{max} and a non-linear regression at SWC_{min}, respectively. A higher correlation

was obtained in SWC_{max} ($r=0.84$) compare with SWC_{min} ($r=0.69$), where leaf water potential showed a higher variation between hybrids (Figure 9).

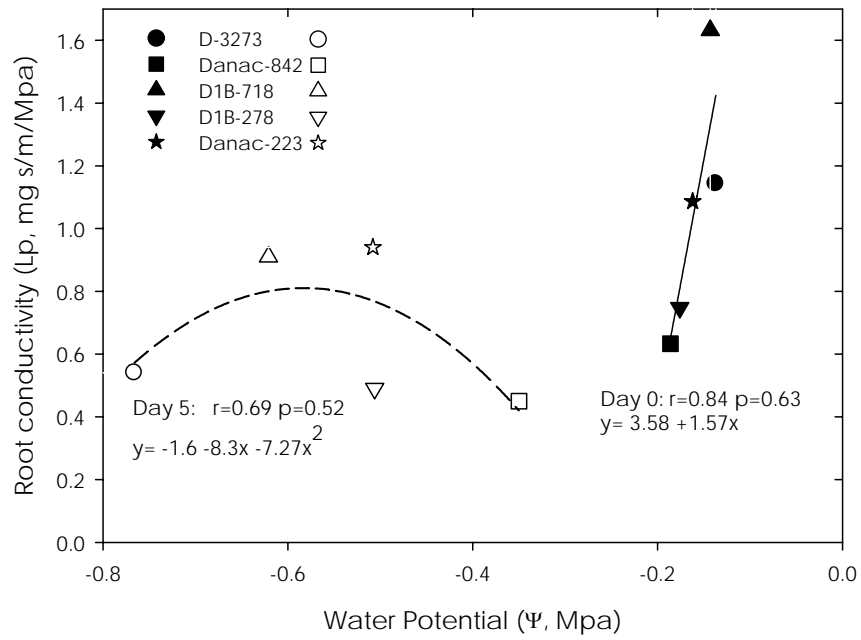


Figure 9: Root hydraulic conductivity (L_p) as a function of leaf water potential Ψ_w , during SWC_{max} (filled symbols) and SWC_{min} (open symbols), in the five hybrids. A linear and non-linear regression, the derived equations, and the respective correlations coefficients were plotted.

4.2.3 Physiological responses

Gas exchange

Gas exchange parameters were strongly negatively affected by the water deficit imposed; photosynthesis rate, transpiration rate and stomatal conductance of all hybrids decreased with increasing water deficit (Figure 10). The effect of the hybrids and the interaction between hybrid and water deficit among days were non-significant for all parameters measured.

On average photosynthesis rate in WW treatment plants (day 0) was 23.8 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$; the maximum value recorded in the hybrid Danac-223 was 26.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Increasing the severity of stress by day 5 ($\Psi_w = -5.5 \text{ Mpa}$, on average), further decreased. P_N to its minimum values (0.85 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ by D1B-718), with reductions higher than 90 percent in D-3273, Danac-842 and D1B-718. After re-watering, P_N recovered in all hybrids except Danac-223, to similar values to those of WW plants (Figure 10, P_N). Also, the hybrids registered minimum values for transpiration rate and stomatal conductance at day 5, showing reductions of 90 to 96 percent; in relation to the maximum values reached at the beginning of the drought period.

Internal CO_2 partial pressure was slightly decreased at maximum water deficit in D1B-278 and D-223 (from 208.7 to 217.4 $\mu\text{mol mol}^{-1}$ and 158.4 to 185.2 $\mu\text{mol mol}^{-1}$, respectively), but no significant differences ($P < 0.001$) were found between days. Only D1B-718, Danac-842, D-3273 had significant increases at day 5.

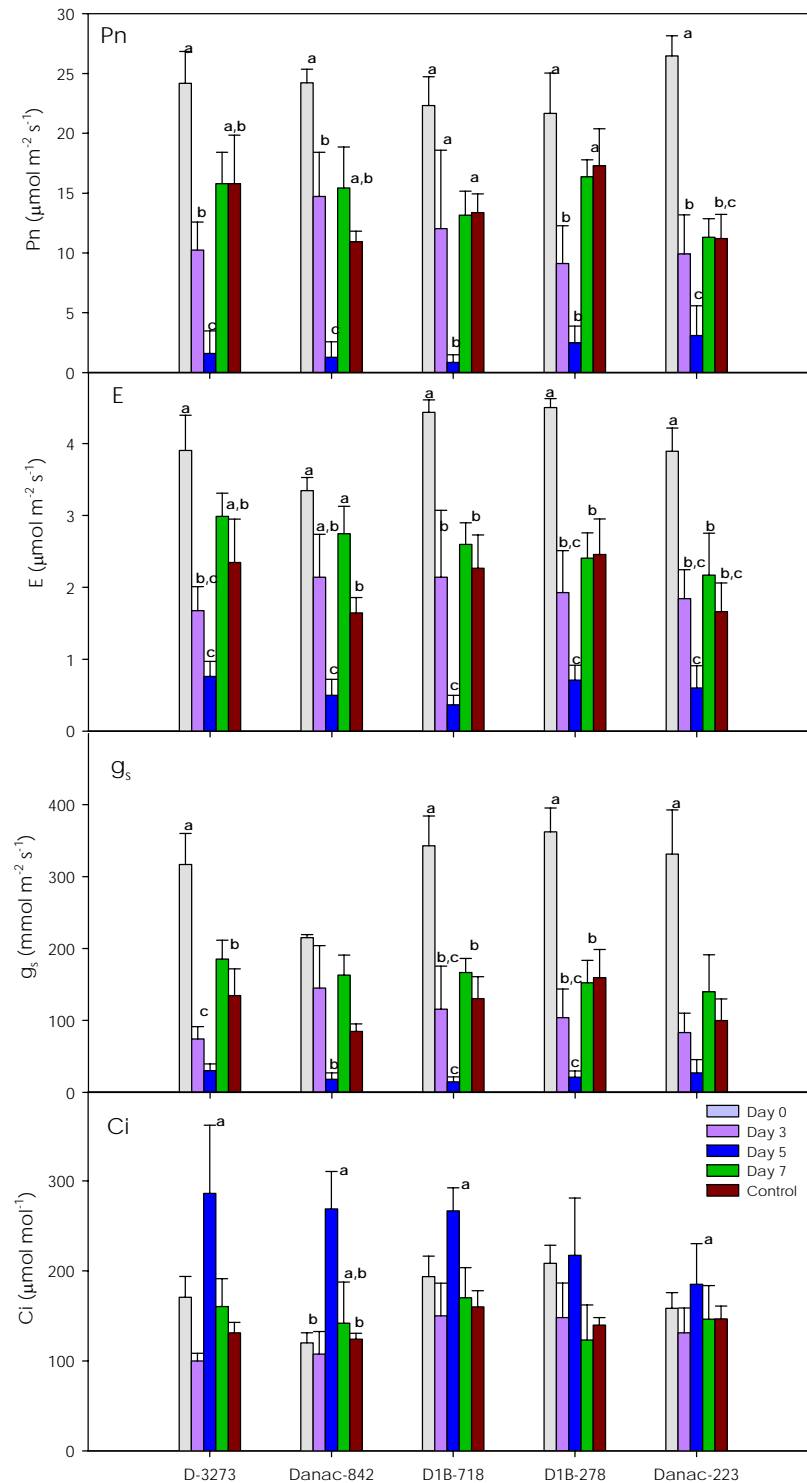


Figure 10: Photosynthesis (P_n), transpiration (E), stomatal conductance (g_s) and intercellular CO_2 concentration during five days under water deficit, with its control (red column) and during re-watering (Day 7, green column) for five white maize hybrids. Mean values \pm ES ($n=3-5$). Different letters were significantly different means within hybrids ($P=0.05$).

The intrinsic water use efficiency (P_N/g_s) had no significant differences between days with variable water availability (Table 7). However, the hybrid effect was significant ($P<0.039$). Danac-842 and Danac-223 registered the maximum values on days 0 and 5, respectively. In addition, WUE in D1B-718 and D1B-278 was similar between days.

Table 7: Intrinsic water use efficiency of five white maize hybrids at SWC_{max} and SWC_{min} . Numbers represent mean values (n=3-5).

Hybrid	Water Use Efficiency ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$)	
	SWC_{max}	SWC_{min}
D-3273	0.08 ^{ab}	0.03 ^b
Danac -842	0.11 ^a	0.05 ^{ab}
D1B-718	0.09 ^{ab}	0.06 ^{ab}
D1B-278	0.06 ^b	0.09 ^{ab}
Danac-223	0.08 ^{ab}	0.14 ^a
Mean	0.08	0.07

Mean values with different letters were significantly different between hybrids ($P= 0.05$).

A significant ($P>0.01$) positive non linear regression was obtained between P_N and g_s across the seven days of treatment in all hybrids with a correlation coefficient (r) of 0.85(Figure 11).

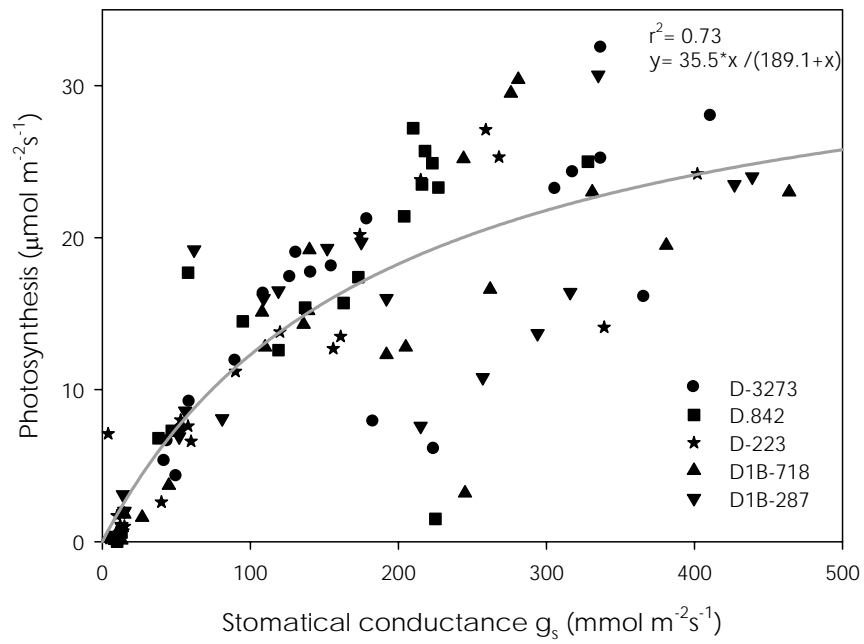


Figure 11: Net photosynthetic rate (P_N) as a function of stomatal conductance (g_s), during the drought pulse in five Venezuelan white maize hybrids. Means ($n=3-5$), non-linear regression (significant $P < 0.01$), the derived equation and r^2 coefficient were plotted.

Chapter V

5. Discussion

5.1 Drought

The last decades have been important for research in maize breeding in terms of number, effort, and input studies to address critical yield and productivity constraints as a consequence of drought stress (Cattivelli *et al.* 2008). As a result, it is well known at the moment that, at the plant level in tropical maize, water stress in form of drought has repercussions in plant phenology, growth, carbon assimilation, partitioning assimilation and plant reproduction processes (Bolaños and Edmeades, 1996; Edmeades *et al.* 1993, 2000; Campos *et al.* 2004; Cruz de Carvalho *et al.* 2011).

Nevertheless, the extension of land sowed with drought-tolerant maize hybrids in the world is still small; some releases into the crop market come from the private sector, mainly from developed countries like the USA (Tollefson, 2011). In general, the use of hybrids (without even being considered as a tolerant genotype) in drier areas has been much lower, and their impact on yields far weaker than in favorable areas (Evans, 2002). Thereby, farmers in developing countries are likely to be more vulnerable, thus increasing the economical risk due to the exposure to erratic rainfalls. At the moment, only a few breeding programs in developing countries study drought tolerant hybrids of white maize at regional level to pursue not only bio-diversity maintenance of the specie, but also sustainable yields during dry periods (e.g. Zarco-Perelló *et al.* 2005 in Mexico, and Oyekale *et al.* 2008 in Nigeria).

In the present study soil water content and leaf water potential were calculated as indicators for water availability and/or drought stress in five Venezuelan white maize hybrids; both are considered as important parameters in

the physiological processes during drought effects (Jones, 2007). The response to variable water supply was more versatile in the case of soil water content, where significant differences between variances distinguished three different groups across hybrids, and two between days (Figure 5). On the other hand, leaf water status, in form of Ψ_w , was relatively stable during the progressive decrease of available water over time (no significant differences) until day 5, after which it dropped in all hybrids ($P < 0.001$) (Figure 6). Although, Danac-842 could maintain its Ψ_w during the five days of stress pulse, at day 5 (minimum value of the SWC) its Ψ_w was on average 0.25 Mpa higher than in the other hybrids. The maintenance of leaf water status in Danac-842 might be explained by the low reduction of its SWC (60%).

After re-watering, the SWC of all hybrids was fully recovered in relation to either the initial or control values. Hence, the re-hydration of the leaf tissues upon re-watering was related to the increase of the SWC, showing a full recovery after a period of hours. The comparison of control and day 0 plants records (no significant differences), may suggest that no damages at cellular level occurred. In the study conducted by Cruz de Carvalho *et al.* (2011) the relative water content (RWC) of leaves was used as a water status indicator, and four out of the six temperate maize hybrids studied showed a full recovery of the RWC 24 hours after re-watering. The results obtained in the present study suggest that under lower values of SWC or Ψ_w the hybrids may still show a tolerant response to drought, but it is necessary to address that even if the plants are able to survive a strong drought stress period, potential yields could be negatively affected. Therefore, a longer exposure to water deficit in these crop plant hybrids is not recommended.

Based on the results obtained in this study, a Pearson correlation analysis (Appendix 1) was performed to relate the SWC and Ψ_w to all other variables analyzed. The SWC had a better correlation with most of the parameters in this study. Therefore, the SWC can be suggested as a better indicator to follow the effect of drought responses in white maize. However, it is necessary to consider

that the SWC is an estimated value of the soil. Thus, relations between shoot and roots were based on Ψ_w .

5.2 Yield, Harvest index and Drought index

White maize grain yield (g plant^{-1}) was strongly affected by the drought stress period imposed at 55DAS. In concordance with previous reports on field conditions, yields of tropical maize decreased under drought stress during flowering (Bolaños and Edmeades, 1996; Campos *et al.* 2004; Oyekale *et al.* 2008; Obeng-Bio *et al.* 2011). The results obtained in this study showed a strong dependency on water availability for both SWC and Ψ_w with kernel number per plant ($r>0.6$), yield ($r>0.55$) and ASI (77% of the WD plants).

In the present study, the higher sterility rate was observed in D-3273 and Danac-842, where no kernels per cob, thus no grain yield was achieved in DW plants. Significant differences were observed in grain yield in the hybrids and treatments (Table 5). The number of kernels per cobs was reduced in DW in all hybrids. These results showed that in tropical white maize, yield was more a sink than a source limited. D-3273 and Danac-842 were a clear confirmation of this. Both hybrids registered high biomass production throughout the experiment, medium to low SLA, in addition to no significant differences in height or number of photosynthetically active leaves (Table 4) in the period of drought stress imposition (55 to 60 DAS). However, grain yield was considerably hampered in DW plants of both hybrids. Therefore, despite having similar potential conditions to cope with the negative effects of water limitation during the late vegetative period; drought stress might more affect the assimilate supply below the necessary threshold for ovules to be developed. As a consequence an absence of kernels was observed in these commercial hybrids, in concordance to Fischer and Palmer (1984) and, Zinselmeier *et al.* (1995).

According to Lafitte (2001), under favorable conditions in tropical maize, HI had values of 0.3–0.4. In average, HI was 0.1 and 0.07 in WW and DW plants, respectively. Although, D-3273 and Danac-842 had high biomass productivity and a regular harvest index in well-watered plants (control treatment), in DW plants the opposite response was observed. Both D-3273 and Danac-842 registered the lowest HI (Table 5). Furthermore, the highest HI was observed in Danac-223, and no significant differences were found in HI and biomass production between treatments. Also, Danac-223 showed the higher grain yield in WW and WD plants (10.0 and 7.4 g plant⁻¹, respectively). Therefore, the performance of the hybrids under study (i.e. biomass production, grain yield and HI) under optimal irrigation conditions could not predict the performance under drought stress. These results are in concordance to Oyekale *et al.* (2008) and contradicting to the results of Duvick (1984) in temperate maize.

Significant differences in HI between hybrids were reflected in the drought intensity index (Table 5), where Danac-223 obtained the lowest value (0.26) and the experimental hybrid D1B-718 with a significant reduction of the grain yield between treatments showed an intermediate index (Obeng-Bio *et al.* 2011) of 0.69.

The drought stress tolerance index according to Fisher and Maurer (1978), originally a stress resistance indicator for wheat, has been used in other crops such as common bean (Porch *et al.* 2008) and tropical maize (Obeng-Bio *et al.* 2011). In the present study, the higher DSTI was observed in D1B-718 and D1B-278 with 0.84 and 1.04 respectively, which are considered high values in tropical maize (Oyekale *et al.* 2008 and Obeng-Bio *et al.* 2011). In contrast, Danac-223 had the lowest value (0.32). This value was different from than expected, since this hybrid showed both, high DII and grain yield. Blum (2005) concludes that when DSTI is calculated based of grain yield instead of biomass, it might be biased by genotypes with higher yield, in concordance with the results obtained in this study. Therefore DII is suggested as a better indicator than DSTI for the stress intensity in white maize hybrids under variable water supply.

5.3 Crop phenological development

From the establishment onwards until the vegetative stage, the development of the hybrids under study was similar to the growth length reported by maize under field (Bolaños and Edmeades, 1996) and greenhouse conditions (Cruz de Carvalho *et al.* 2011).

When the drought period was imposed at 55DAS, for five days during the late vegetative phase (V_{10} – V_{12}), no significant differences were observed between either the numbers of photosynthetically active (green) leaves or senescent leaves in the five hybrids studied. A slight difference in height was found ($P>0.047$), where D1B-718 reached a maximum value of 53.4 cm, and D-3273 a minimum value of 45.9 cm. Nevertheless, the SLA showed a considerable variation among the hybrids; D1B-278, with wide plant architecture recorded the highest SLA value 171.9 cm²g⁻¹. D1B-718 and D-223, hybrids with a narrow architecture, had a SLA of 48.7 and 55.3 cm²g⁻¹, respectively.

Theoretical considerations (Long *et al.* 2006) and studies in C₃ cereal crops (Araus *et al.* 2002) suggest that further increases in potential yield of the major crops will depend largely on increasing the use of water by the crop, through photosynthesis, i.e. molecular transformation (Gepts, 2002), and improvement or stomatal control of the water loss i.e. alteration of crop canopy architecture. Based on the results of this study, alterations of crop canopy architecture or enhancement of photosynthesis activity in tropical maize plants under drought conditions might not prove to be conclusive traits for drought tolerance breeding purposes. Due to the fact that no significant variation was observed in the number of photosynthetically active leaves or height; the hybrids under study may have similar light capture potential (source) during the late vegetative period. On the other hand, only SLA registered significant differences between hybrids, although it showed only a medium correlation ($r<0.51$) with grain yield or biomass production (Appendix 2). Nevertheless, under field conditions leaf area index (LAI) is largely

used in maize instead of SLA (Ellings, 2000), and the modern approaches to this trait it indicate that LAI can be assessed without destructive sampling by using techniques such as remote assessment (Viña *et al.* 2011).

Total crop duration was affected not only by the irrigation treatments, but also by growth conditions (greenhouse). An average delay of 30 days was observed between the hybrids in the present study and the standard time established for the same hybrids under field conditions which has an estimated harvest after 155DAS (J. Alezones, personal communication). A possible reason may have been the greenhouse-pot conditions, although temperature (maximum and minimum), light, nutrients and relative humidity were kept at optimal levels for this crop. The plants might have been affected by limitation of pot-size. In concordance with the conclusion of Poorter *et al.* (2012) who studied plants under similar conditions, the supply of nutrients and water may not be able to remain constant in concordance with the demand of developing, especially for faster growing high-light-treated plants.

Differences among irrigation treatments and hybrids were observed during flowering, yield formation and ripening stages. At 99DAS, 55% of the WW plants were in the flowering period whereas only 33% of the WD plants were flowering. D-3273 had the lowest percentage of flowering plants at 99DAS for both treatments (33% of well watered and 42% of drought stressed plants). In comparison, Danac-842 had 100% and 33% respectively, of flowering plants on the same day. In general terms, this stage was 20 to 23 days longer in well-watered plants and stressed plants than expected in the field.

Delay of flowering, as a consequence of water stress during the latest vegetative development (during flower induction), has been previously reported in tropical maize. Slow ear growth, and consequently silk emergence resulted in a widening of the interval between anthesis and silking (ASI) (Campos *et al.* 2004). Similar results have been reported in other C₄ crop plants such as millet and

sorghum (Mahalakshmi and Bidinger, 1985; Craufurd and Peacock, 1993). The response of ASI was variable in the hybrids studied, from no changes between WW and WD plants (e.g. Danac-223) to delays in WD plants compared to WW plants (e.g. D-3273 and Danac-842). Hence ASI might be considered as a representative trait for ear and plant growth rates during the flowering period in the studied hybrids, as was previously reported for tropical maize (Edmeades *et al.* 1993, 2000; Vega *et al.* 2001).

The five hybrids were characterized by DANAC as late maturing genotypes, but the hybrid Danac-223, presented shorter time spans of development stages as well as one of the lowest values for the number of leaves per plant (although not significantly lower than the others)(Table 4). Thus, it may be catalogued as a medium maturing hybrid in contrast to D1B-278, which developed a higher number of leaves at 55DAS, but also had a slower progress through each growth stage.

5.4 Root-shoot responses

No significant changes were found in the root-shoot ratio between treatments (in day 0 at highest SWC and, in day 5 at lowest SWC values), in contrast to Bramley *et al.* (2009), where changes in root-shoot ratio probably were observed, and related with the temporary accumulation of reserves in the stem. This result was expected since no changes in biomass were found (Table 4). A similar response was found in tropical maize under drought stress (Edmeades *et al.* 1993). However, significant differences within days were observed in biomass between hybrids ($P < 0.008$). D1B-718 had the highest biomass: 45.0 g in average (between day 0 and day 5), and D1B-278 the lowest, with 20.3 g. In concordance with the SLA, where high values of biomass were the lowest SLA values were observed, e.g. D-3273 and D1B-718. Probably the period of the drought pulse was not sufficient to alter carbohydrate reserves in the stems which in average represented 53.2% and 45.5% of the total biomass in day 0 and day 5, respectively. Although, was not clear the effect of SLA and Root-Shoot ratio to variable water supply in the specific

developmental stage studied (55 to 60 DAS). In the root-shoot ratio was observed a medium correlation ($r=0.37$) to grain yield (Appendix 1). In contrast, SLA showed a considerable coefficient dependence value ($r^2=0.60$) to grain yield in WW plants but not correlation with grain yield was observed in DW plants (Appendix 2). Therefore, root-shoot ratio might be an important secondary trait to consider.

Leaf water potential had a clear effect on the long-term (days) down-regulation of L_p in the hybrids studied (Figure 9). At the highest values of SWC (day 0) a linear regression with a high correlation ($r=0.84$) was found, whereas at day 5 the range of leaf water potential within hybrids showed a higher variation, and a non-linear function between parameters was observed with a lower correlation ($r=0.61$). Although good correlations were found at both low and high SWC (day 0 and day 5), they were not significant ($P<0.24$ and $P<0.52$, respectively); probably due to a high variability of the values obtained per plant and the small number of samples ($n=3$) per hybrid.

The result obtained for the root hydraulic conductivity under variable water supply suggested that, through changes in the SWC, from a simple hydraulic point of view taking a model developed from *Arabidopsis* (Iuchi *et al.* 2001), changes in the L_p of the hybrids under study could optimize soil water use and together with the stomatal regulation may be the driving forces (Jones, 1992) of the gradient among the observed soil-root-plant-atmosphere continuum (SRPAC) in tropical maize. This could be seen in D1B-278, where probably through hydraulic adjustments (low reduction of L_p) Ψ_w was maintained until day 5 despite of significant decreases of SWC.

Bramley (2002) concluded that increases in L_p in plants under water deficit can be explained by root extensions, i.e. changes in the root-shoot ratio of the plant, although a long-term response to severe drought condition is carbon depletion (Smith and Stitt, 2007). In the present study no significant changes in root extension or in the root-shoot ratio were observed (Table 6). Hence the results obtained in

white maize hybrids do not contradict their results, since they used water stressed *Arabidopsis* plants in an early development stage which were grown on a sandy soil. In addition, despite of a wide range of values in the root length (from 26.2 to 94.3 m) no significant differences were found either between hybrids or days. A high variability in the root length and root biomass may be explained by the experimental design used, where the hybrids were grown in pots with sand-loam soil (60% sand, 33% silt, and 10% clay) extracted from a maize plantation in the western plains of Venezuela. Typically in other experiments sandy soil it is used when root hydraulic conductivity is to be determined. Therefore, as it has been addressed by Poorter *et al.* (2012) difficulties in obtaining root biomass and root length may have hampered an objective analysis.

The values obtained for L_p were similar to those reported earlier in excised roots of maize (Steule *et al.* 1987). It was observed in concordance with Zhang and Mugo. (2002) that the response of the hydraulic root conductivity in maize is also related to the maintenance of grain yield under drought through increases in biomass, and not through changes in ASI and harvest index (Appendix 1), e.g. Danac-223.

When the Ψ_w reached the lowest values (from -0.35 to -0.78 Mpa, Figure 7), it was probable that leaf cells shrank and the cell walls relaxed, resulting in morphological changes such as leaf rolling. This response could be related to a strategy to avoid dehydration by reduction of the exposed area by leaf rolling. This behavior in turn may be related to root exploration and water extraction capacity (Barker *et al.* 2004). Thus, changes in RI in maize may be considered as an indicative trait of drought tolerance. D-3273 showed 28.3% of leaf rolling without significant changes in the root-shoot ratio. This suggested that the avoidance of dehydration was prioritized, rather than the translocation of the carbon assimilated from the stem or leaves to the roots, to increase the growth of the latest to maximized water uptake. In contrast, D1B-278 had the lowest rolling index (12.1%) despite of having the highest SLA and number of leaves, evidencing that the

maintenance of the plant water status was related with other processes instead of morphological changes.

5.5 Physiological responses

Daily courses of the gas exchange parameters analyzed reflected a clear response to the drought stress by five Venezuelan white maize hybrids to variable water supply (Figure 10).

Differences in response among hybrid to variable water supply were not significant. Maximum values of net photosynthetic rate (P_N) at day 0 ($26.5 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ by Danac-223), E ($4.5 \mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ by D1B-278) and g_s ($362 \text{ mmol m}^{-2}\text{s}^{-1}$ by D1B-278) were similar to others reported for temperate yellow maize under greenhouse (Cruz de Carvalho *et al.* 2011), and field conditions (Zarco-Perelló *et al.* 2005; Chun *et al.* 2011). A drop in rates of P_N , E and, g_s was observed after day 3 when the SWC dropped to 7.3%.

At day 7 (after re-watering) all hybrids, except Danac-223, showed similar values of P_N like the control and day 0 plants (well watered); a relatively rapid recovery (24 to 48 hours) has also been reported in temperate maize under water deficit after re-irrigation (Lal and Edwards, 1996; Foyer *et al.* 1998; Cruz de Carvalho *et al.* 2011). After 24 hours of re-irrigation the hybrids D1B-718, D1B-278 and Danac-223, showed no significant recovery in the parameters g_s and E , although their leaves were re-hydrated according to the recovery of their Ψ_w . Only Danac-223 was not able to recover P_N up to control values. This may suggest that non-stomatal factors were involved as well as down-regulation, and thus a longer period of time was needed to fully recover all the physiological responses. This was evident when at long term yield production was not hampered in this hybrid, showing that Danac-223 may be considered as a drought tolerant hybrid.

D-3273, Danac-842 and, D1B-718 showed a significant increase in the intercellular CO₂ partial pressure, a response often observed in maize (Becker and Fock, 1986) and other C₄ plants such as sugarcane (Du *et al.* 1996) and another grasses (Kalapos *et al.* 1996). This response was followed by a recovery similar to control values after re-hydration of the leaves, in correspondence to responses reported by Cruz de Carvalho *et al.* (2011) under similar conditions. However, a significant decrease of C_i was expected, due to reduced stomatal conductance and thus a limitation of P_N during early phases of water deficit (i.e. at day 3 with 7.3% of SWC and no significant differences in Ψ_w), which is in concordance to results in temperate maize (Lal and Edwards, 1986; Leakey *et al.* 2004). No significant changes of C_i in D1B-278 and Danac-223 were observed as in *Alloperopsis semialata*, a C₄ perennial grass, which reported a stable C_i value under water deficit. Therefore, P_N values at day 7 of D1B-718 and Danac-223, which were not related to the g_s response, might be attributed to the ability of using the CO₂ available for photosynthesis processes while there were coping stomatic and non-stomatic constraints.

Based on the results of the present study, in tropical white maize under variable water supply a variation in SWC might induce a tight stomatal closure, therefore plant status can be maintained within a range of 20 to 4% of SWC. Similar responses were observed in tropical maize (Edmeades *et al.* 1993; Campos *et al.* 2004; Zarco-Perelló *et al.* 2005). In all hybrids, a down-regulation of transpiration and photosynthesis during drought was observed, on the latter, mainly due to stomatical factors, i.e. dominated by stomatal closure (Buckley *et al.* 2005), since the response of P_N to SWC was highly correlated to g_s (r=0.85, non-linear) (Figure 11).

Differences in the control of the stomatal aperture during drought stress could be associated with variable intrinsic (P_N/g_s) water use on C₃ and C₄ plants (Cattivelli *et al.* 2008; Ghannoum, 2009). The hybrids studied showed an increase to even no significant changes on their intrinsic WUE, with the exception of Danac-842, which showed a significant decrease of g_s at day 5; and thus, its WUE was the

lowest registered at minimum values of SWC (Table 7) This suggests that in this hybrid the efficiency of stomatal control was very low, and thus was not enough to cope the drought pulse studied, or to hamper the assimilate production. The latter was confirmed during harvest when an absence of yield was recorded in this hybrid. In summary, intrinsic WUE by the hybrids under study may be used as a secondary indicator for higher tolerance to water deficit.

5.6 Promising hybrids

Limited knowledge of the physiological response of the detrimental effects of variable water supply on growth and yield of white maize in Venezuela, and the consequent lack of suitable screening traits may be two considerable reasons for the limited success of maize breeding in drought conditions. Identification and measurement of secondary traits associated with grain yield provide a guide to specific mechanisms that contribute to grain yield under drought (Campos *et al.* 2004).

The aim of the present study was to select one or more specific physiological traits as selection criteria for tolerance to drought stress, considering that relevant physiological traits are related to a wide range of processes along with the soil-root-plant-atmosphere continuum. Hence, in concordance with the objectives, it was expected that there was not as single response pattern or that not a specific trait would highly correlate with yield under all drought environments (Appendix 1).

Therefore, with the result obtained in the present study not only a sole trait response was considered for the selection of traits, i.e. higher biomass production or HI, but rather was made according to morphological characteristics, and responses of both root-shoot and gas exchange, in order to identify key processes and their effect on yield as secondary traits to guide strategies for sustainable breeding programs, focused on hybrids at regional level.

Furthermore, it was considered that the traits are associated with specific developmental stages. Thereby ASI, SLA and number of photosynthetically active leaves, were only considered as indicators during vegetative stage; while others, such as photosynthetic rate, stomatal conductance, intrinsic water use efficiency and drought stress index (DII and DSTI), were studied as indicators of plant growth throughout the life cycle of the crop. Based on the response and correlation of these secondary traits with grain yield under drought stress, the following combination of traits could be recommended as selection criteria for tolerance to variable water supply before flowering in white maize:

- Small rolling index and a small anthesis-silking interval.
- Low intensity drought index, with a smaller reduction in photosynthetic rate and low transpiration rate through an effective stomatal control.
- A more efficient water transport through the roots, and enhanced intrinsic water efficiency and grain yield production.

From the responses of the Danac-223 plants under greenhouse conditions, with a medium specific leaf area 72.5 grcm^{-2} , a smaller number of leaves per plant (9.2), a more efficient water transport through the roots under variable water supply, an enhanced water use efficiency, $0.10 \mu\text{molCO}_2 \text{ mmolH}_2\text{O}^{-1}$ under drought conditions vs. $0.06 \mu\text{molCO}_2 \text{ mmolH}_2\text{O}^{-1}$ in well watered plants, in addition to a low drought impact index and non detrimental effect on yield production in WD plants, it may be considered as the most promising hybrid from the five studied for cultivation under variable water supply.

Nevertheless, at the intensity of stress reached in this study the experimental hybrids D1B-718 and D1B-278 showed a medium drought impact index and a higher reduction of the grain yield. D1B-278 had an efficient water transport through the roots (a small reduction of L_p was observed), and the rolling index was

the lower observed. Whilst, D1B-718 had a considerable reduction of L_p , and a high RI. However, the stomatal control in both hybrids was very effective and they had a rapid recovery of the photosynthetic metabolism and Ψ_w . Therefore, the results obtained in the present study suggested that they may have an enhanced performance under medium SWC, or shorter drought periods.

Chapter VI

6. Conclusions and outlook

Differences in the effect to the potential yield, as well in the expression of secondary traits related not only to plant status but also to plant growth throughout the life cycle, suggest that Ψ_w , L_p , g_s , WUE, ASI and DII are effective indicators to drought tolerance in Venezuelan white maize hybrids under variable water supply.

Most of the hybrids responses to variable water supply were triggered by soil water availability, in addition to tissue water status.

Danac-223 is recommended for use in developing drought tolerance in white maize breeding programmes in Venezuela, due to consistent responses to morphological, growth, and both root-shoot and physiological traits to tolerate water variable supply.

To confirm the results of this study the following is recommended:

- Field research on the results obtained to have more clarification in the gene-to-phenotype associations for secondary traits in breeding populations at regional level to study heritable potential and stability over time.
- More detailed studies in the non-stomatal control on carbon assimilation under drought stress.

In order to decrease the Venezuelan vulnerability of crop production due to climatic hazards, based on the results obtained in the present study, it is evident

that solutions on short and medium term not only involve morphological traits in conventional white maize breeding programs for improved yield, but it was also successfully highlighted and therefore suggested, that secondary traits shall be considered as well as selection criteria for tolerance to drought stress in white maize. The incorporation and design of new breeding programs cannot be achieved by the agricultural sector alone, neither publicly nor privately. The interactions of different factors at institutional, social and ecological levels need to be considered as well and effective policies need to be developed to cope with future climate change effects.

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