Supporting Emergence or Reference Drought Tolerance Phenotyping Centers - Drought Phenotyping Network

Generation
Cultivating Plant Diversity for the Resource-Poor

Embrapa

CIMMYT
International Maize and Wheat Improvement Center

FAPED

Organized at Embrapa Maize and Sorghum, Rodovia MG 424 km 45
35701-970 Sete Lagoas, MG, Brazil | 17 to 18th of June 2008

DROUGHT TOLERANCE PHENOTYPING IN MAIZE AND SORGHUM: PRELIMINARY, INTERMEDIATE AND ADVANCED EVALUATIONS

Elto E. Gomes e Gama[20], *Fredolino G. dos Santos*[20], *Frederico O. M. Durães*[21], *Reinaldo L. Gomide*[20], *Paulo Emílio P. de Albuquerque*[20], *Edson A. Bastos*[22], *Milton J. Cardoso*[22], *Luiz B. Morgado*[23], *Camilo de Lelis T. de Andrade*[20], *Jurandir V. Magalhães*[20], *Claúdia T. Magalhães*[20], *Flávio D. Tardin*[20], *Lauro J. M. Guimarães*[20], *Adelmo R. Da Silva*[20], *Paulo C. Magalhães*[20], *Newton P. Carneiro*[20], *Sidney N. Parentoni*[20], *Robert E. Schaffert*[20]

INTRODUCTION

Among the environmental stresses, drought is considered the main source of maize and sorghum grain yield instability in tropical areas. Genotypic selection for adaptation to differentiated water regimes is an important strategy in a breeding and molecular programs. Thus, under a water stress condition (drought) is desirable to evaluate and select genotypes of maize and sorghum for better adaptation and tolerance to water constrains environments, as well as high and stable productivity. In order to perform that it is necessary a good understanding and knowledge of environments effects (climate condition registration, irrigation water control, soil water status measurement) and the performance of plants genotypes in these environments which will result in grain yield, identifying the causes (genetic versus environmental) which will affect at the end a yield reduction of the genotypes maximum capacity to produce grains.

Maize and sorghum genotypes were phenotyped in well characterized sites for drought tolerance through appropriated screening techniques and defined protocols, techniques, and methods in order to evaluate and select drought tolerant genitors to provide material to be used in genetic breeding programs, focused on regions historically known as prone to water deficit during crop growing season. The improvement of maize and sorghum drought tolerance programmes are relieing on the manipulation of the traits that limit yield per each crop specie and their accurate phenotyping under the prevailing field environment conditions being targeted. These issues are particularly crucial for the breeding programs and identification of QTLs for traits categorized as adaptive as well as compared to the constitutive traits. The use of molecular markers has provided an important advance in genetic studies, generating new interest in their application in maize and

---

[20] Embrapa Maize and Sorghum, Rod. MG 424 km 45, 35701-970 Sete Lagoas, MG, email:gomide@cnpms.embrapa.br.

[21] Embrapa Agroenergy, 70770-901 Brasília, DF.

[22] Embrapa Mid-North, 64006-220 Teresina, PI.

[23] Embrapa Semi-Arid, 56302-970 Petrolina, PE.

sorghum breeding programs. The development of molecular markers has provided tools to assess important genes identifications in maize inbred lines. Overall, on these purposes there is no doubt that is necessary to have good infrastructure to allow plants exposure to water deficit pressure to be used for the evaluation of genotypes and characterization of plant physiological responses to these water stress conditions.

PHENOTYPING AND BREADING STRATEGIES IN MAIZE AND SORGHUM

Maize and sorghum genotypes characterization and selection for drought tolerance were phenotyped according to the following strategy:

1) ***Preliminary phenotyping*** – controlled and well characterized target environment (specific sites), including surface climatic conditions, irrigation water application, soil water status, crop water requeriments – must be monitored, controlled, and managed; genetic material characterization under selection for drought shoud be performed only during critical crop specie phenological phase, with only a few appropriated screening techniques per crop specie, and a few selected simple stress index indicators based on each specie phenotipic parameter, similar to a typical conventional breeding procedure, where a great number of access for each studied specie is emphasised;

2) ***Intermediate phenotyping*** - besides the preliminar phenotyping procedures already described, must consider the genotypes characterization by means of few auxiliary descriptors techniques established, and emphasis on smaller number of accesses selected material; and

3) ***Advanced phenotyping*** – must take into account the preliminar and intermediate phenotyping stated before, but with a specific genotypes characterization using especial plant organs (e.g., grains, roots, leaves, stems, etc.), based on mechanisms studies, and with emphasis on elite genetic material with very few accesses.

MAIZE AND SORGHUM GENETIC BACKGROUND (GENOTYPES) AND PLANTS BASIC TRAITS EVALUATED FOR DT PHENOTYPING

MAIZE GENOTYPES

F3 maize progenies evaluation: In Janaúba-MG site, two experiments were carried out to evaluate 100 F3 maize progenies, in June 2006. These progenies came from a F1 contrasting cross between two inbred lines tolerant and non tolerant to drought. The trials consisted of 100 maize F3 progenies including the two parental, using a lattice 10 x 10 design with three replications. A dripping irrigation system was used for better water application control in the two water regime treatments, with and without water stress, which were induced in the pre- flowering stage. These experiments were harvested by the end of October, 2006, and all the data were collected for several traits (Figure 1).

Figure 1. Drought phenotyping in maize for progenies population composed by 98 $F_{2:3}$ families with the two contrasting parental lines, evaluated with and without water deficit, controlled by means of a drip irrigation system at Janaúba, MG site, 2006.

Maize single crosses evaluation: **S**ingle cross (SC) maize hybrids were evaluated to enhance drought tolerance in inbred lines in the Janaúba-MG site. The experiment consisted of 176 SC plus five commercials hybrids as testers: BRS 1010, DKB 390, DKB 359, AG 9010 and P30F90. A dripping irrigation system was used for better water control in the two treatments, with and without water stress, applied in the pre-flowering stage. This experiment was harvested at the middle of December 2006 and all the collected data for several traits are available on the database.

Maize open pollinated variety (OPV) evaluation: Tropical maize populations are usually composite made up of crosses of several populations, whereas temperate populations are synthetics made up of few inbred lines. However, the narrow x broad genetic bases do not seem to be important because they display enough genetic variability for improvement in any breeding selection program. The results of these trials are being useful in identifying possible materials to be used in a breeding program for drought tolerance. We proposed for the next step a breeding scheme of reciprocal half-sib selection using the two best material selected in this previous study. This trial consisted of eight treatments.

Synthetic Jaíba NP: Synthetic Jaíba has been selected during 4 years (cycles) for drought tolerance; BAG-SE 029: selected for P use efficiency; BAG-BA 183: selected for P use efficiency; Synthetic multiple tolerance: 12 lines with tolerance to acid soils and Al toxicity and P use efficiency 4; BR 106: most OPV planted in Brazil, released by Embrapa Maize and Sorghum; BRS Sertanejo: variety develop for the Brazilian Northeast region with some tolerance to high temperature; BRS 1010- best single cross hybrid released by Embrapa Maize and Sorghum (Figure 2). A randomized complete block design with 5 replications was applied. Four types of experiments were set up in Janaúba-MG site in August/2006. Two of them were planted in areas with high (>20 ppm) and low P (3 ppm) levels. The others two experiments were planted in normal fertile soils. In addition, we have imposed in these areas, by the pre- flowering time, a controlled period of water stress. The experiments were harvested by middle December, 2006, and the data collected are being processed.

Figure 2. Drought phenotyping in maize for progenies population composed by Synthetic multiple tolerance (Al, P) families (Synthetic Jaíba), evaluated with and without water deficit, controlled by means of a drip irrigation system at Janaúba, MG site, 2006.

F3 maize progenies: One hundred (100) F3 maize progenies were evaluated again in two experiments at Janaúba-MG and Teresina-PI sites, 2007. The Janaúba trials were planted on May 22 and 23 2007. The Teresina experiments were sowed on July 2007. These progenies came from a F1 contrasting cross between two inbred lines tolerant and non tolerant to drought. In each site, the experiments consisted of 100 maize F3 progenies including the two parental, using a lattice 10 x 10 design with three replications, which were planted in two blocks, where the two water regime treatments (with and without water stress) were applied in the pre-flowering crop growth stage. A dripping and a conventional sprinkler irrigation systems were used in Janaúba-MG and Teresina-PI sites, respectively, for better water application control. All the field experiment data were collected and the results are available in the database. In the last year (2006), at Janaúba site, the maize genotypes were harvested on October, 2006, and, in Teresina site, they were harvested on Febrary, 2007, and all the data were collected for several traits in both sites. Some results of these experiments (2006 and 2007) were utilized to identify the presence of news QTL for drought tolerance in a suitable contrasting cross for drought tolerance through the use of molecular markers, which is providing an important advance in genetic studies, generating new interest in their application in maize breeding programs. The development of molecular markers has provided tools to assess important genes identifications in maize inbred lines.

Maize single crosses (SC) evaluation: Single cross hybrids (SCH) were evaluated again to enhance drought tolerance in inbred lines in 2007, Janaúba-MG site. The two experiments consisted of 176 SCH tested last year + 29 new SCH + five commercials hybrids as testers: BRS 1010, DKB 390, DKB 359, AG 9010 and P30F90. A dripping irrigation system was used for better water application control. The experiments were planted on May 23 and 24 2007, under two water regime treatments, with and without water stress, applied at the pre-flowering crop growth stage. The last year experiments (2006) were harvested at the middle of December 2006 and all the collected data for several traits are stored on the project database.

Maize progenies derived from two Synthetic: Synthetic drought tolerance (DT) has been selected during 4 years for N and P uptake. BAG-SE 029: selected for P use efficiency; BAG-BA 183: selected for P use efficiency; Synthetic multiple tolerance: 12 lines with tolerance to acid soils and Al toxicity and P use efficiency 4; BR 106: most OPV planted in Brazil, released by Embrapa Maize and Sorghum; BRS Sertanejo: variety develop for the Brazilian Northeast region with some tolerance to high temperature; BRS 1010- best single cross hybrid released by Embrapa Maize and Sorghum. In Janaúba-MG site, four types of experiments were installed in June/2007. Two of them were planted in areas with high (>20 ppm) and low P (3 ppm) levels. The others two experiments were planted in normal fertile soils. A randomized complete block design with 4 replications was utilized. In addition, it was imposed controlled periods of water stress in these experiments, which were initiated by the pre-flowering time. These experiments were harvested by middle December, 2007, and all the data collected are on the database.

Maize inbred lines morphological root system evaluation: This work was carried out in 2006 with the objective of studying growth characteristics of root and plant canopy of inbred lines selected for drought tolerance. Two inbred lines tolerant (L1 and L3) and two sensitive (L2 and L4) to drought were sowed, manually at space 0,20 m among plants and 0,20 m among lines, in seedling beds (0.8 m^2 - four lines of 1.2 linear m being five plants per linear m) with different levels of phosphorus, low (4 mg. dm^{-3}) and high (20 mg. dm^{-3}) at Sete Lagoas, MG site. The experimental design used was randomized complete blocks, with six replications. There were three evaluations of root system morphology by using the digital images system, by means of WinRhizo Pro 2007a (Regent Instruments Inc.) methodology and as well as evaluations of characteristics of growth roots and plant canopy at 14, 21, 28 (days after sowing). It was observed significant differences for root morphological attributes root and canopy growth. In general the inbred lines considered tolerant to drought showed root system different from inbred lines sensitive to drought. Those tolerant resulted in a larger root length, surface area, volume, and greater contribution of roots with diameter less than 0.5 mm in plants grown in the condition of low phosphorus availability.

SORGHUM GENOTYPES

Sorghum inbred lines: Two experiments were carried out in both Janaúba and Teresina sites, in 2006 and 2007, including 49 sorghum inbred lines using lattice 7 x 7, 3 replications, experimental parcel with two rows 5 m (useful parcel with central 2 rows 4m), and plant arrangements with 0.50m spacing and 200 thousands plants/ha population, under two water regimes, with and without water stress (plenty irrigation), applied in the pre-flowering stage (Figure 3). The irrigation system scheme in both sites was the conventional sprinkler, with the sprinklers spaced by 12 m x 12 m. In Janaúba-MG, two others experiments were carried out, with similar design, aiming the evaluation of 100 sorghum progenies to cross two contrasting inbred lines for drought tolerance (SC 283 and BR 007). The results objectives were to identify isogenics inbred lines (RILs) aiming molecular markers studies. All those experiments were harvested by middle of December 2006, and all the grain yield, yiel components, and traits data collected are available in the project database. All the Janaúba experiments were harvested by middle of December 2007 in the second year, and the Teresina experiments are scheduled to be harvested by February of 2008. The acquired data for the second year are stored on the project database.

Figure 3. Drought phenotyping in sorghum population composed by 49 inbred lines, evaluated with and without water deficit, controlled by means of a conventional sprinkler irrigation system at Janaúba, MG site, 2006.

Sorghum Experiments, in Petrolina-PE site: In 2006, three experiments were carried out with two water regimes, one with water stress applied at the pre- and pos-flowering, and other with plenty irrigation (no water stress). The water was applied and controlled by means of a drip irrigation system (Figure 4). A total of 30 sorghum genotypes (10 early, 10 medium cycle, and 10 late) was utilized on the trials, designed in a random blocks with 4 replications (parcels of 2 rows: 0.5 m spaced and 9 m long).

Figure 4. Drought phenotyping in sorghum population composed by 30 inbred lines, evaluated with and without water deficit, controlled by means of a drip irrigation system at Petrolina, PE, site, 2006.

The three experiments were installed again in Petrolina, PE, in 2007 with pre- and pos-flowering water stresses, and plenty irrigation water regimes, utilizing 36 to 49 sorghum genotypes, which were divided in early, medium, and late cycles, utilizing an experimental design with a random blocks with 4 replications (parcels of 2 rows: 0.5 m spaced and 9 m long). The following traits were measured in both year: flowering, plant height, leaf enrolment, waxy, leaf death percent (at flowering, dough grain, and physiological maturity), panicle weight, grain weight, grain number/panicle (at pre-flowering stress), 100-grain weight, grain weight/panicle weight relation. The results of the two years are available in the project database.

CONTROLLING AND MONITORING WATER STRESS

FOR DROUGHT TOLERANCE PHENOTYPING IN MAIZE AND SORGHUM

IRRIGATION WATER APPLICATION, CONTROLLING, AND MANAGEMENT

The irrigation systems installed in the Sete Lagoas and Janaúba-MG, Teresina-PI, and Petrolina-PE specific sites are: conventional sprinkler (low to medium service pressure) and localized (drip), which were tested and evaluated locally for water distribution uniformity (flow rate/discharge) and applied water depths in each site by means of measuring and controlling water pressure, flow rate, radius of throw, and emitters or sprinklers spacing. The water depths applied in the irrigations were measured in collectors or catch cans in each genotype field plot. These collectors were placed transversally to the crop rows following a rectangular grid or a transect layout in the plots. The uniformity of the water distribution in the irrigated plot was set to be equal or greater than 90 %, calculated by means of Christiansen Uniformity Coefficient equation. Some hydrometers were coupled to the irrigation systems main lines. The irrigation water application rate was set to be lower than basic soil saturated water infiltration rate in order to avoid surface runoff, which was not allowed in the site areas.

Conventional Sprinkler System

The conventional sprinkler system was used to deliver water to the crop through a network of mainlines, secondary lines and lateral lines with the sprinklers heads spaced along their lengths (Figure 1). The rotating or impact driven sprinklers type, with multiple nozzles, was selected on basis in the size and shape of the irrigated area of Janaúba-MG and Teresina-PI sites.

It is very important to obtain adequate uniform water coverage and distribution, that the area watered by each sprinkler must overlap substantially the area watered by the adjacent sprinklers. Thus the sprinklers spacing must be intentionally designed to require at least 100% overlap of watered areas to avoid great variation in the amount of water applied or even dry spots. That means each sprinkler should throw water all the way to the next sprinkler in each direction, which is known as "head-to-head coverage or spacing" or the distance between sprinklers equal to the sprinkler radius (Figure 5). The sprinklers irrigation spacing recommended and used in the sites were 12 m x 12 m (Teresina-PI) and 12 m x 18 m (Sete Lagoas-MG) (numbers means spacing between sprinklers on the lateral lines and between lateral lines, respectively). The sprinklers nozzle sizes range from 3.5 to 4.5 mm diameter, which must be operated with pressures of 2.5 to 3.5 $kgf.cm^{-2}$. These pressures provide sprinkler water flow rates from 1.14 to 1.75 $m^3.h^{-1}$ and average water application rates from 8.0 to 12.2 $mm.h^{-1}$. Those values can change slightly depending on nozzle size, pressure and sprinkler spacing. It is important to follow the sprinkler manufacturer recommendation regard its specific installation, design and operating requirement.

Figure 5. Conventional sprinkler irrigation system installed at Janaúba, MG - Embrapa Maize and Sorghum (left), and at Teresina, PI - Embrapa Mid-North (right) sites.

Improper installation and operation of a sprinkler system will result in poor uniformity and water waste. Water pressures higher than recommended tend to make small water drop size which are subject to evaporation and drift in wind conditions. Low water pressures decrease the radius of throw and do not break up the water stream properly, causing poor uniformity of application. The sprinklers lateral lines must be installed on level and positioned in a perpendicular direction (90^{o}) or forming 45^{o} (best position) in relation to the prevailing wind direction. As a lateral pipe line length is 6 m, it is important to set the plots size (length and wide) values, a number multiple of 6 in order to facilitate the experimental system layout in the field.

Localized Irrigation System

Localized irrigation system, sometimes called drip or trickle irrigation, delivers water to the crop using a network of mainlines, sub-mains and lateral lines with emission points spaced along their lengths (Figure 6). Each dripper/emitter, orifice must supply a measured, precisely controlled uniform application of water, nutrients, and other required growth substances directly into the root zone of the plants. The choice of an adequate emitter is based mainly on the flow rate, which must be uniform and constant along the lateral lines. These devices also must present sensibility to obstructions or clogging, and also resistance to insect and rodent animals attack. The flexible polyethylene pipes used presented internal diameter of 12.5 mm.

The water and nutrients application by drip irrigation system enter slowly the soil from the emitters, moving into the root zone of the plants through the combined forces of gravity and capillary. The high efficiency of drip irrigation results from the fact that the water soaks into the soil before it can evaporate or run off and it is only applied where it is needed (at the plant's roots), rather than sprayed everywhere. In this way, the plant's withdrawal of moisture and nutrients are replenished almost immediately, ensuring that the plant never suffers from water stress (unless if a water limitation regime is intentionally introduced), thus enhancing quality, its ability to achieve optimum growth and high yield.

Figure 6. Drip irrigation system installed at Janaúba, MG - Embrapa Maize and Sorghum (left), and at Petrolina, PE - Embrapa Semi-Arid (right) sites.

The water applied by the emitters penetrates and redistribute into the soil generating a wet bulb, whose size and shape depend upon the emitter type, applied flow rate, duration of irrigation, and soil type. The soil water infiltration rate occurs in all directions; however it is greater in the vertical direction for the sand soils.

Localized irrigation system was used because it is indicated on very “light” soil (sand) with high water infiltration rate (above 150 mm/h) and low moisture retention capacity. These soil conditions are not adequate for surface and even conventional sprinkler irrigation methods. In general, the total operating pressure of a localized system will be 50 to 70 % of a conventional sprinkler system. Thus, operating costs may be reduced (saving energy). These systems allow much easier and more efficient control of pests and weeds because the crop foliage and all the soil surface are not wetted, allowing access to the field at all times. The drip systems utilization implies in a high irrigation frequency when compared to other irrigation systems. The key principle of it is to maintain a moist segment of the root zone with relatively small applications of water applied continuously or intermittently.

Field Calibration and Evaluation of the Irrigation System

It is important to determine the application water depths uniformity when applying water to the genotypes plots. Collection containers distributed throughout the application area must be used to measure, calibrate, and evaluate water application uniformity. Many types of containers can be used such as standard rain gauges, pans, plastic buckets, jars, or anything with a uniform opening and cross section can be used, provided the container is deep enough (at least 10.0 cm) to prevent splash and excessive evaporation, and the liquid collected can be easily transferred to a scaled container for measuring. The rain gauges work best and are recommended because they already have a graduated scale from which to read the water application depth. All containers should have the same size and shape, and should be placed in the field at the same height relative to the height of sprinkler nozzle position (discharge elevation). As a general rule, the top of each container should be no more than 100 cm above the ground and no more than 90 cm below the sprinkler or nozzle discharge elevation. In addition, it is important to certify that there is no interference from the crop canopy when installing the collectors in order to avoid interference or water splash from the leaves into the collection container.

Field calibration and evaluation helps ensure that water depths are applied uniformly and at proper rates in the plots. The calibration of the irrigation systems involves setting out collection containers, operating the system, measuring the operating pressure, water flow rate, and amount of water collected in each container, then computing the average application volume or rate and, finally, the application uniformity (Figure 3). Generally, an in-line flow meter installed in the mainline or sub-mainline provides a good estimate of the total water volume pumped from the water source during each irrigation cycle. Overall, the average application depth in the whole field can be determined by dividing the pumped volume by the application irrigated area.

Field calibration or evaluation should be performed during periods of low water evaporation and when wind speed is not strong. Suggested times are before 10:00 hours (morning) or after 16:00 hours (afternoon). The readings of the volume (depth) collected should take place as soon as the evaluation process ends or when the irrigation system (linear moving) completely passes over the row of collection containers in order to minimize water evaporation from the containers.

Conventional sprinkler irrigation system is evaluated and calibrated by setting up a 2 m x 2 m grid of collector container among four sprinkler heads of two adjacent lateral lines (Figure 7). It is important to place containers equally spaced in the field. Application water volumes should be read as soon as all gauges stop being wetted.

Among the main coefficients used to express and to determine the variability of distribution of the water depths applied by the conventional and drip irrigation systems, the two most utilized are those recommended by Christiansen, which adopts the absolute medium deviation of the water depths values as dispersion measurement, and is known as Coefficient of Uniformity of Christiansen (CUC); and by Criddle et al. (1956), which considers the ratio between the average of the smallest 25 % water depths values and the average of all water depths values collected, and is defined as Coefficient of Uniformity of Distribution (CUD).

Localized irrigation system is evaluated and calibrated in the field by measuring the flow rate of the tubes or pipes (mainline and secondary lines) and emitters with direct volumetric method, using a graduated gauge of 100 or 500 cm^3 and a chronometer. It is necessary to have at least three replications. The uniformity of water application along the lateral line (emitters) is directly related to the flow rate variation of the emitters, due to the friction losses along the tubes, the insertion of the emitters, the gains and losses of position energy (elevation), the quality of the tubes, the obstructions and clogging, and the water temperature effects on the flow regime and geometry of the emitters (Howell & Hiller, 1974). The pressure in the beginning of the mainline, secondary lines, and irrigation lines (emitters) is measured with a Bourdon manometer of 100 kPa, with 2 kPa accuracy. It is necessary to execute five flow rate tests with time variation of seven days.

Uniformity in amount of water emitted or applied (from emitters - drip) is measured by the emission uniformity (EU), and may be expressed as a percentage. Non-uniformity can be caused by: i) variability in distribution characteristics due to quality control in the manufacturing processes; ii) faulty or incompetent system design and management; iii) operational pressures outside those suggested for the distribution system being used; iv) physical changes in the system that may have occurred with time.

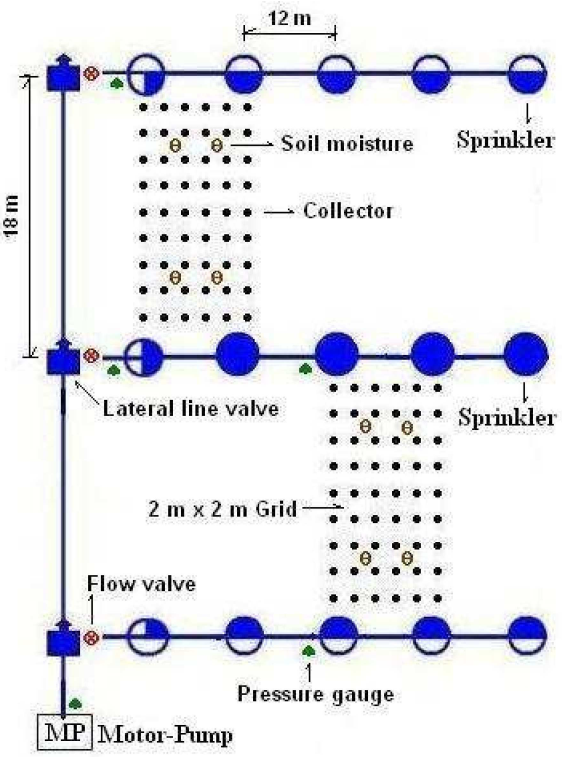

Figure 7. Collection containers placement and soil moisture points samplings throughout a conventional sprinkler scheme irrigated area for measure water depths application and uniformity.

Field evaluation and calibration of the EU can be easily carried out by following the procedure outlined:

a) Select an operational unit representative of average operating conditions in all operational units of the system.
b) Locate 4 lateral lines along an operating sub-main line within an operational unit; one lateral near the inlet end, one lateral near the far end, two laterals evenly spaced in the middle section.
c) Measure, under normal operating conditions, the pressures at the inlet and at the far end of each lateral line. This will produce 8 pressure readings.
d) On each lateral line select 2 adjacent distributors at 4 different plant locations (at the inlet, 1/3rd of the way down the lateral, 2/3rds and at the end points of the lateral). In the case of a multi-outlet distributor any two emission points can be chosen.
e) Measure the discharge from the distribution points selected according to item 4 above. Collect the flow for a full number of minutes (1, 2, or 3 min, etc.) to obtain a reasonable volume of water for each distributor (something between 100 and 250 ml). This will produce 32 discharges measured at 16 locations.
f) Enter the information collected into a proper data sheet.
g) Compute the average discharge for each pair of distributors. This will result 16 average discharge values.
h) Use the average of the lowest 4 discharge values of all the readings as the minimum rate of discharge. This average of the lowest fourth of all the emission readings is used as the minimum

discharge to avoid the effect that one blocked or bearly blocked distributor might have on the total evaluation.

i) The average of all the readings is the average rate of discharge per distributor.

j) Calculate the field emission uniformity (EU) by the following equation:

$$EU = \frac{q_{\min}}{q_{avg}} x100$$

In that, EU is the emission uniformity coefficient, in %, q_{min} is the minimum rate of discharge, in $cm^3.s^{-1}$, and q_{avg} is the average rate of discharge per distributor or emitter, in $cm^3.s^{-1}$.

A typical field-determined EU values will range from 85 to 95 % for localized schemes, but in the crops drought tolerance studies phenotyping environment sites must be carefully considered to ensure that this EU ≥ 95 % in order to assure optimum irrigation water control and to quantify and differentiate very well the irrigation water deficit regime in the crop root system zone.

IRRIGATION WATER MANAGEMENT AND CROP WATER REQUIREMENTS

One of the main technicians' problems in the phenotyping environment sites for crops drought tolerance studies is the lack of training and knowledge to manage adequately their irrigation schemes to assure optimum irrigation water control in order to quantify and differentiate precisely the irrigation water regime. In the established specific sites areas should be necessary a training program to the technicians on irrigation water management and field evaluation of the used irrigation systems, since in most cases, new technicians are unfamiliar with the basic principles on operate and manage the irrigation systems equipments efficiently.

Correct timing of irrigation is essential mainly when water is in short supply or evaporative demand of the atmosphere is high. This situation is the case of the phenotyping environment sites, where the genotypes are submitted to a controlled field water deficit. Thus, decisions must be made regarding the irrigation timing criteria, involving information and knowledge on irrigated cropping systems, such as establishing the crop growth stage, anticipated yield reduction due to water stress induced, climate and soils data, which should be collected on a continuous basis in order to have control and register of their changes along the crops growing season.

Basically, the irrigation water management (scheduling) involves three decisions which are related to three questions: i) How to apply the irrigation water (the design of the right irrigation scheme)? ii) When to irrigate (Timing)? iii) How much water to apply in the irrigation (Quantity of applied water depths)? The answers to these questions are critical for the correct management decision of any irrigation system (conventional sprinkler, linear moving, localized-drip, etc). The purpose of optimum irrigation scheduling is to ensure an adequate supply of soil moisture to minimize plant water stress during critical growth stages, resulting in water and energy savings with no yield loss.

Irrigation water management at Sete Lagoas-MG, Janaúba-MG and Teresina-PI sites was carried out by means of reference evapotranspiration (ETo) and crop evapotranspiration (ETc) computation, using either class A pan and modified Penman-Monteith equation methods, with the crop (kc) and pan (kp) coefficients. The ETc was determined by multiplying ETo for each genotype crop coefficient (Kc). Irrigation management strategy and irrigation timing criteria were performed based on spread sheet (Excell) for ETo and ETc computation and soil water balance within the root system depth determination, associated with the measurements of soil water content in different layers. The irrigation was uniform after sowing, germinating, and stand formation with 100% replacement of the ETc and soil water availability (SWA) - non water stressed condition. Afterwards, the water stress treatments were obtained with different replacement level of the ETc, generating different application of water depths in the plots, and consequently different SWA, at pre-defined crop growth phases, defined for each genotype, according to breeder and physiologist indication in order to establish the water stress intensity.

The maize and sorghum irrigation scheduling followed the variation of crop water needs in the different environment (all sites) during the crop growing season in order to achieve high water use efficiency and high crop production. Irrigation timing (when to irrigate?) and quantity of water depths application (how much water to apply?) were directly related in most cases.

The crop water requirements under localized irrigation systems were determined in different way when compared with ETc computed for other pressurized systems (overhead sprinklers, center pivot, linear moving), primarily because the land area wetted is reduced resulting in less water evaporation from the soil surface.

WATER STATUS AND WATER STRESS LEVELS EVALUATION AND MEASUREMENTS

SOIL WATER STATUS EVALUATION AND MEASUREMENTS

Measurements of the capacity of the soils to store water, in different layers within the crop root system, is important for differentiate precisely the irrigation water regime (water stress) and irrigation water control in order to quantify the soil water availability (SWA) to the genotypes root system. In the specific sites regions of Brazil where the maize and sorghum were cultivated, the capacity of soils to store available water to be used for the growing crops changes a lot. This is good and important for the drought tolerance studies, because the depth of water to apply in the irrigations and the interval between irrigations are both influenced by the *storage capacity of the soil*. Fortunately, some of the specific sites established present sand soils (Janaúba-MG, Teresina-PI, and Petrolina-PE). Thus, these sites do not have large water-storage capacity, and the irrigation interval must be necessary as frequently as would be desirable in order to avoid high water stress levels and great reduction on crops grain yield.

On drought tolerance field trials, crops are subjected to water stress which can be imposed along the whole cycle or in some of its cycle stages. The stress application period, duration and intensity depends on the crop susceptibility and the objectives of the study. In Brazil, for crops grown in the second harvest ("safrinha"), such as sorghum, the stress is applied after flowering. For crops that might be subjected to dry spells in early stages, the stress is applied prior to flowering (Maize genotypes).

By simply cutting water supply via irrigation does not mean that a crop will suffer water stress. Soil-water retention capacity is different from soil to soil and this must be taken in consideration when planning a drought tolerance filed trial. On the other hand, it is not desirable that a crop undergoes a permanent wilting and dye. It is crucial, though that some sort of monitoring been done in the soil, plant and weather.

Monitoring soil-water and plant-water status allows stress level quantification and helps making the decision on when to interrupt water stress. In fact, the level of stress a crop will suffer depends on the interaction between plant, soil and weather.

Soil Moisture Content Evaluation and Measurements Methods

Soil moisture content, in different soil layers, was monitored by gravimetric method and other equipments and sensors; such as the gypsum blocks sensors, which were calibrated by means of the gravimetric method by taking measurements of the electrodes (inside the porous blocks) electrical resistance (electrodes involved in known soil reservoir and embedded in water until saturation) against its water content by weighing. The wetter is a porous block, than the lower is the resistance measured across two embedded electrodes. This type of sensor is suited to various irrigation applications mainly with soil water stress condition. These sensors were left in field to automatically monitor continuously soil moisture, allowing many replications. The soil sampling and sensors installation to register soil water content were made in at least four soil depths (15, 30, 50, and 80 cm). The time domain reflectometers (TDR) equipment combines the knowledge of the waves signal propagation velocities in the presence of water in the soil medium, which affects the speed of these electromagnetic waves (slows them down slightly). The accuracy of TDR measurements depends on precise measurement of time and precise calibration with the relative volumetric content of water around the probe. The hand-held capacitance probe (model Diviner 2000, Sentek Pty Ltd.) was used for monitoring the soil water content in the soil profile too. This is a portable soil moisture-monitoring device consisting of a portable display/logger unit, connected by a cable to an automatic depth-sensing probe that moved up and down into an access tube. The Capacitance method includes a probe with a pair of electrodes or electrical plates that work as a capacitor. When activated, the soil-water-air matrix works as a dielectric of capacitor and completes an oscillating circuit. Readings of this device were made for every 0.1 m until 1.0 m depth.

Gravimetric (Standard), Time Domain Reflectometry, Soil-Water Potential Methods

Gravimetric: A disturbed or undisturbed sample is collect at the desired depth of the soil profile, using an auger. The sample is stored in a metallic can with lid and kept closed and sealed with a ribbon adhesive of paper until it is taken to the laboratory to be weighted. After weighting the sample is put to dry in an oven with temperature between 105 e 110 ^{o}C and kept in there until constant weight. This might take 24 to 48 h for soils with sandy or loamy texture. Heavy textured soils might take longer time to reach constant weight. With those weights and weight of the aluminum cans, one can calculate soil-water content. If the sample is from disturbed soils, water content is expressed in kg kg^{-1}. If the sample is from undisturbed soil, with a known volume, water content is expressed in m^{3} m^{-3}.

The gravimetric method has the advantage of being simple, accurate and dependent on relatively simple equipments such as auger, cans, precision scale and oven. However, spatial variability of soil physical properties and of soil-water in the field can affect the results. Some sampling criteria, such as replication and composed samples must be used to minimize that problem. One drawback of the gravimetric method is that it is destructive. Every time a sample is collected a hole is left in the soil profile. By the end of a season the experimental area might be full of holes.

Specifically on the drought tolerance trials, sampling have to be done on both fully irrigated and on under-stress plots. In some soils, it might be very difficult to auger by hand after it dries. It might be necessary a mechanical or motorized sampling device.

Time Domain Reflectometry (TDR): This is one the most promising methods for soil-water content measurement since it does not poses any risk to human health and can be as accurate as the neutron probe. According to the manufacturers, TDR need not be calibrated for common mineral soils. However, for research purposes, this is desirable. It can detect small variation in soil-water content and can also be used to take measurements in small volumes when required. Large soil volumes can be sampled depending on the way the wave-guides are installed. When detailed measurements of the soil profile are required, a trench need to be opened into the soil profile in order to allow the installation of wave guides at different depths of the soil profile. The instrument can be fully automated by using data loggers and multiplexers. The major disadvantage is the high cost, especially when automation is required. TDR measures the soil bulk dielectric constant that varies with soil-water content. The dielectric constant for water is about 81, while for the remaining soil components, is smaller (3 to 5 for mineral soils and 1 for the air). The average error on the soil-water content measurements with TDR is around 3% but it can be lower if the equipment is locally calibrated.

Different sizes and shapes of wave-guides allow soil-water monitoring from the very top layer up to a depth of about 1,5 m of the soil profile. A soil-water content profile can be obtained by installing wave-guides at many soil profile depths. Some manufacturers build segmented wave-guides and others provide access tubes similar to neutron probes, what allows measurements at the same point. The major disadvantage of the models with access tubes or segmented wave-guides is the difficulty on the installation. It is required a perfect fitting between wave-guide or access tube and the soil material. Any gap left can lead to wrong readings at that point.

Soil-Water Potential : Another way of quantifying the stress a crop is suffering in drought tolerance trials is by estimating or measuring the soil-water potential. In general, the simplest instruments available in the market measure only the matric potential. Basically two methods are available, the tensiometers and the resistance blocks. ***Tensiometers:*** It is composed of a ceramic tip connected by one side to a tube and by the other side to a pressure meter. The device, filled with water, is inserted into the soil at the desired depth. The soil-water gets in equilibrium with the tensiometer's ceramic cup water, generating a pressure inside the ceramic tip, which can be positive or negative. When the soil is saturated the pressure is positive, when it is dry the pressure is negative. The pressure is measured by the meter connected to the other tube extremity and it represents the soil-water matric potential. Nowadays, the pressure meters are built with electronic transducers, assembled as portable meters or individually connected to data loggers, allowing fully automation. The operation range of tensiometers is from +20 to –80 kPa. Therefore, they are only useful to monitor soil-water potential of fully irrigated treatments or prior to onset of water stress. ***Resistance blocks:*** They have been used for many years to correlate the electrical resistance between two electrodes inserted into the soil, to its matric potential. They are manufactured with different types of materials: gypsum, nylon, fiberglass and a combination of materials that built the porous media involving the par of electrodes. All those devices are sensible to the presence of salts in the soil, but those made out of fiberglass are the most ones.

SOIL MOISTURE INSTRUMENTS INSTALLATION PROCEDURES

Soil moisture instruments were used to determine the soil moisture content and soil matric potential. Minimum typical procedures for installation of the soil moisture instruments in irrigated fields with relatively uniform soil conditions and crops must be followed:

a) Number of stations to measure soil water status profile - 3 to 4 per irrigation water regime treatment;

b) Number of depths in each station and depth placement of the instruments - 3 to 4 (20, 40, 80, and 100 cm), with the following soil profile distribution: on the top of maximum root activity zone, near the bottom of active root zone, and midway between top and bottom positions (intermediate). Temporarily, a shallow depth might be needed where seedlings are being established;

c) Location to place the instruments – the ideal is within the crop root zone system. Tensiometers might be located near tree lateral line, at least 60 cm away from distributors (emitters);

d) Site conditions - representative soil in the plots and vigorous and non-disease crop area might be selected.

PLANT WATER STATUS MEASUREMENTS: MAIZE AND SORGHUM

Plant based methods and techniques for estimating plant water status should more accurately reflect irrigation needed to replace soil water, instead of estimating soil moisture directly or by some method that estimates soil water balance (Hydrological techniques), since the plant integrate its total environment. In Brazil, there are several points that need to be investigated which affect directly whether irrigation scheduling is optimum for increasing yield efficiency. Additional information that quantifies such factors as the level of plant water status at which irrigation is needed, the quantity of water to apply, the effect of plant growth stage on sensitivity to water stress, and the effect of irrigation application frequency on plant response is needed.

The partial replacement of the plants water requirements and the selection of better genotypes adapted to the water shortage conditions contributed to the increase of water availability in the agriculture. Although the effects of water stresses on the plant development are known, few reliable methodologies used for its characterizations based on parameters directly related with the plants exist, aiming to maintain good productivity levels and to increase the tolerance to the water deficiency, mainly due to the difficulties of environmental control of the water factor.

Remote Sensing Canopy Temperature Methods

Remote sensing of a vegetated surface was accomplished with infrared thermometry methods. Thermal infrared radiation data provided a unique input into energy balance models. Canopy temperatures was utilized to estimate crop water stress (CWS) directly and water availability to a crop. Automatic remote sensing methods applied to the water status of a crop are a relatively new field of investigation in Brazil. Clawson et al. (1989) showed that a significant water savings could be achieved with no yield loss when irrigation scheduling for bean was based on a canopy temperature variability method. Gomide and Sediyama (1998) also suggest that remotely sensed canopy temperatures, when taken into account with other environmental variables, could provide a good indicator of crop water demands and result in water savings by the crop. Canopy temperatures can be incorporated into the crop water stress index (CWSI). Jackson (1982) discussed in depth how remotely sensed information could be used in irrigation management and concluded that these techniques had promise as irrigation guides.

The attractiveness of the remote-sensing methods is that the application of a microcomputer based system could provide important tools in irrigation management by means of an automatic data processing. In addition, large areas can be quickly surveyed, with an entire field sampled rather than only select points within a field, as for soil the soil water status available instruments situation.

Infrared Thermometry

The use of canopy temperatures to detect water stress in plants is based upon the assumption that transpired water evaporates and cools the leaves below the temperature of the surrounding air. As water becomes limiting, transpiration is reduced and the leaf temperature increases. If little water is transpired, leaves will warm above air temperature because of absorbed radiation (Jackson, 1982). Further refinements are necessary to improve the utility of the crop canopy-air temperature difference measurements in plant water stress detection (Idso et al. 1981, Jackson et al. 1981). Later authors investigation suggests that the inclusion of net radiation (R_n) and water vapor pressure deficit (VPD) should improve the capability of the (T_c - T_a) measurement to detect plant water stress.

Geiser et al. (1982) and Slack et al. (1981), in research with corn in Minnesota, found that the addition of R_n and VPD parameters improved the ability of the (T_c - T_a) measurement to detect plant stress and found that water savings would result if this method were used to determine when to irrigate. Idso et al. (1981) proposed that VPD might be a sufficient normalizing criterion and showed that for alfalfa the relationships between (T_c - T_a) and VPD were the same for several locations. Also, they proposed that the plant water stress index (PWSI) be calculated from the later mentioned relationship. Overall it appears feasible that plant water stress can be evaluated by means of canopy temperatures which are measured remotely. However, additional environmental inputs are required in order to adequately assess the crop water status.

Remotely sensed canopy temperature was utilized to measure maize evapotranspiration and plant water status by means of an infrared thermometer. The procedures for field measurements involved registering the difference in temperature between the crop canopy (Tc) and the air (Ta) with a infrared thermometer. The Penman-Monteith equation in terms of crop canopy-air temperature difference, aerodynamic resistance and canopy resistance may be expressed as (Monteith 1973, Jackson 1982):

$$\frac{\rho c_p}{r_a}(T_c - T_a) = R_n + G - \frac{\rho c_p}{\gamma(r_a + r_c)}(e_c^* - e_a)$$

where T_c is the crop canopy temperature (^oC), T_a the air temperature (^oC), R_n the net radiation (W/m^2), G the heat flux to or from the soil below the canopy (W/m^2), e^* the saturated vapor pressure at T_c (Pa), e_a the actual vapor pressure at the point of measurement of T_a (Pa), ρ the density of air (Kg/m^3), c_p the heat capacity of air at constant pressure (J/Kg ^oC), γ the psychrometric constant (Pa/ ^oC), r_a the aerodynamic resistance to heat and mass transfer (s/m), and r_c the canopy resistance to vapor transfer (s/m).

The sensible heat transfer (W/m^2) from the canopy to the air (H) is given by the left hand side of above equation and the latent heat transfer to the air or heat transfer through evapotranspiration (λE) is given by the third term on the right hand side of the same equation. The above equation can be rewritten as

$$\rho_w \lambda E = R_n + G - \frac{\rho c_p}{r_a}(T_c - T_a)$$

where λE was denoted as $\rho_w\lambda E$, E is the rate of evapotranspiration (m^3/m^2 s), λ the latent heat of vaporization (J/Kg), and ρ_w the density of water (Kg/m^3).

Maize crop water requirements or evapotranspiration (ETc) were evaluated from measurements of the last equation terms. Crop canopy-air temperature differences (T_c - T_a) were monitored with an infrared thermometer (Figure 4), and a net radiometer registered R_n, and a soil heat flux plates measured G.

Over the relatively narrow range of temperature and pressure, under most field environment conditions, the parameters λ, c_p, γ, and ρ_w can generally be considered to be constant. However, these parameters can be determined as functions of temperature and pressure if so required.

The portable infrared thermometer should be configured to measure both air and surface temperature or the difference between the two. Canopy-air temperature difference measurements can be taken with one of the infrared thermometer (IRT) transducer model commonly used in the market (Apogee, Everest, and Everest are the IRT manufacturers, Figure 8). But it is important that these transducers models be selected to operate with crop condition. This means that they should present the following specification: low temperature range readings (ideal up to 80^{o}C), 8 to 14 μm band wave length (thermal portion of electromagnetic spectrum), and emissivity set to 0.98 (Figure 8).

Plant temperature registration were obtained for selected crop stages using this IRT that was held at about 45^o angle from ground surface, just above the canopy, and pointed toward four predetermined direction (N, S, E and W) to determine average canopy temperatures. Average wind speed, air vapor density deficit (saturated minus actual air vapor density), total solar radiation, and net radiation were recorded hourly near the crops field plots.

Equations relating the actual difference T_c-T_a (dT) to the lower and upper limits of T_c-T_a (dT_l and dT_u, respectively) were used in the crop water stress index (CWSI) values computations as follow:

- Lower limit (dT_l): non water stress crop condition, then theoretically r_c= 0

$$dT_l = T_c - T_a = \left(\frac{r_a R_n}{\rho c_p}\right)\left(\frac{\gamma(1 + r_c/r_a)}{\Delta + \gamma(1 + r_c/r_a)}\right) - \frac{e_a^* - e_a}{\Delta + \gamma(1 + r_c/r_a)}$$

- Upper limit (dT_u): non transpiring or water very stressed crop condition, thus r_c tend to infinite (∞)

$$dT_u = T_c - T_a = \left(\frac{r_a (R_n - G)}{\rho c_p} \right)$$

The CWSI equation is given by

$$CWSI = \frac{dT - dT_l}{dT_u - dT_l} \quad \text{or} \quad CWSI = 1 - \frac{ETc_{actual}}{ETc_{potential}}$$

Figure 8. Infrared thermometer transducer models commonly used in the canopy-air temperature difference measurements (from left to right: Apogee IRTS-P5, Telatemp AG510b, and Everest Intersciences 4000.3ZL).

A crop water stress index value of about 0.15 (CWSI ~ 0.15) may be used as a limit to differentiate the irrigated crop from a non water stress to a water stressed condition, as a criteria to avoid significant yield loss for the main grain crops. In maize and sorghum phenotyping for drought tolerance trials the CWSI values are in the range of 0.6 to 0.7 (this means replacement of approximately 40 to 30 % of ETc).

Sap Flow Probes

The maize crop transpiration rate and water stress were measured and registered with the sap flow probes water stress technique in the advanced Phenotyping at greenhouse work, Sete Lagoas, MG, site. Two maize (*Zea mays L.*) inbred lines water stress levels were characterized by means of an automatic sap flow (F) measurements with a set of energy balance probes, installed on segments of plants stems. The probes are composed of heating electric resistance (thermal jacket) and registering sensors of heat and temperature flow. The thermal jacket supplied a constant rate of heat to the stem segment. Thermocouples of copper-constantan were used as sensors to detect the losses of heat from the thermal jacket to the air surrounding the stem and the temperature differences in the stem segment studied. The automatic acquisition data system, manufactured by Dynamax Inc., Dynagage probes model SGB19, involved a datalogger, sensors, a portable computer, a solar panel and rechargeable batteries (Figure 9).

A program was used for readings in the probes sensors and calculations of the sap flow rates. An equation, expressing the plants water stress index (PWSI) and involving terms of the measured F rates under two water regime conditions (non stressed and stressed), was used in the water stress characterization. The probe is flexible and adjustable to the maize stem diameter. The eight plants stems diameters varied from 1.53 to 1.75 cm.

Figure 9. Energy balance probe (left) and automatic sap flow measurement system, installed on segments of eight maize (*Zea mays L.*) inbred lines plants stems for transpiration rate and water stress levels measurements at Sete Lagoas, MG, site (Embrapa Maize and Sorghum, Gomide et al., 2006).

Figure 10 shows the variation of sap flow (F) per unit leaves area ($g.h^{-1}$ m^{-2}) and plant water stress index (PWSI) of two inbred lines maize (*Zea mays L.*) (L 1170 and L 13.1.2) as a function of time for two soil water regime (non water stressed - NWS and water stressed - WS) (Gomide et al., 2006). The results indicated that the probes were sensible to detect variation of sap flow and the PWSI was an appropriate methodology for water stress characterization of the two maize inbred lines investigated. The total leaf area of the two studied inbred lines was reduced by the water stress condition.

The sap flow (F) data were converted in each maize inbred lines to unit of leaves area and expressed in $g\ h^{-1}m^{-2}$, on total leaves area basis. This conversion allowed characterizing the PWSI, which was calculated by the equation:

$$PWSI = 1 – F_{WS} / F_{NWS}$$

In which, F_{NWS} and F_{WS} are the F rates obtained under the two water regime studied, non water stressed (NWS) and water stressed (WS), respectively.

The maize inbred line L1170 presented smaller values of sap flow and was more sensible (larger values) to the PWSI studied, the maize inbred line L13.1.2 presented larger values of sap flow and was more tolerant to PWSI (smaller values).

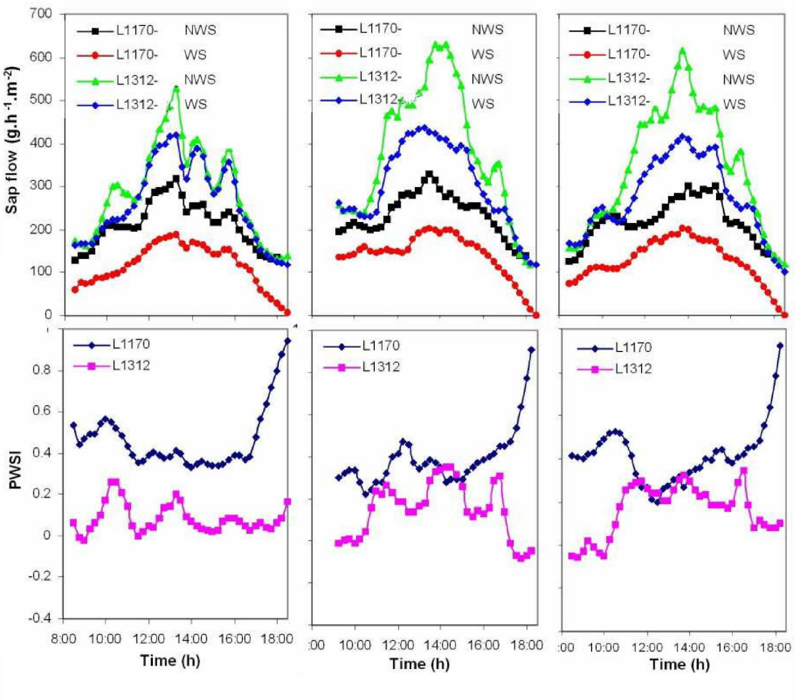

Figure 10. Sap flow (F) per unit leaves area (g.h^{-1} m^{-2}) and plant water stress index (PWSI) variation of two inbred lines maize (*Zea mays L.*) (L 1170 and L 13.1.2) as a function of time for two soil water regime (non water stressed - NWS and water stressed - WS) at Sete Lagoas, MG, site (Embrapa Maize and Sorghum, Gomide et al., 2006).

Overall, the registration of water requirements, stress indexes, and irrigation timing criteria of the crops can be accomplished with microcomputer based systems through automatic data processing, providing important tools in water stress and irrigation water management measurements and offering a way of coupling the plant-based measurements with the soil-atmosphere system.

MAIZE AND SORGHUM RESULTS: TOLERANT AND SENSIBLE GENOTYPES, MAIN TRAITS, YIELD AND YIELD COMPONENTS

Table 1 shows some maize drought phenotyping field results, with and without water deficit, for the traits: anthesis silk interval (ASI), plant height (PH), ear height (EH), final stand, proportion of dead leaves (DL), prolificity (Prol) in number of ears per plant-EN/p, and grain yield (GY) in kg/ha for a population composed by 98 $F_{2:3}$ families with the two contrasting parental lines, evaluated at Janaúba, MG site in 2006. The complete data set of this experiment was inserted in the Morpho database.

The mean yield averages during the 2006 year were 2100 k g ha^{-1} and 3820 kg ha^{-1}, with and without water deficit, respectively, which corresponded an 45 % in yield reduction. The other evaluated traits anthesis silk interval, plant height, ear height, final stand, proportion of dead leaves, and prolificity averages values were of -0.69 and -0.45, 129.37 and 138.63, 66.47 and 67.45, 0.41 and 0.19, and 0.80 and 1.09 for the treatments with and without water deficit, respectively.

Table 1. Some maize drought phenotyping field results with and without water deficit for the traits: anthesis silk interval (ASI), plant height (PH), ear height (EH), final stand, proportion of dead leaves (DL), prolificity (Prol) in number of ears per plant-EN/p, and grain yield (GY) in kg/ha for a population composed by 98 $F_{2:3}$ families with the two contrasting parental lines, evaluated at Janaúba, MG site in 2006. (Complete data set inserted in the Morpho database).

		ASI (days)	PH (cm)	EH (cm)	Final stand	EN	DL (%)	Prol (EN/p)	GY (kg/ha)	ASI (days)	PH (cm)	EH (cm)	Final stand	EN	DL (%)	Prol (EN/p)	GY (kg/ha)
T	R	With water deficit								Without water deficit							
1	1	0	85	45	7	6	0.33	0.86	341.3	-2	95	30	1	2	0.2	2	153.2
2	1	-1	110	50	11	10	0.25	0.91	992.87	0	130	60	13	11	0.2	0.85	1274.78
3	1	-1	85	40	10	7	0.25	0.7	822.22	0	115	50	11	12	0.2	1.09	1934.81
4	1	-2	135	50	13	13	0.33	1	2218.45	-1	130	60	17	21	0.2	1.24	4391.06
5	1	-1	135	60	15	13	0.33	0.87	3288.89	0	140	65	10	17	0.2	1.7	3668.86
6	1	-1	115	60	7	8	0.25	1.14	1241.09	-1	125	70	8	12	0.25	1.5	1196.21
7	1	0	135	70	16	17	0.25	1.06	4467.93	0	150	75	14	20	0.25	1.43	4083.51
8	1	-1	120	70	15	11	0.25	0.73	1489.31	0	120	65	3	4	0.25	1.33	714.62
9	1	-1	120	50	17	15	0.25	0.88	1722.02	0	125	50	14	20	0.25	1.43	2324.89
10	1	0	100	40	11	5	0.33	0.45	341.3	-2	110	30	18	14	0.25	0.78	1056.39
…										…							
91	1	-2	120	50	16	10	0.33	0.63	757.07	-1	130	55	11	14	0.2	1.27	2776.94
92	1	0	100	40	13	16	0.25	1.23	1116.98	1	135	60	16	15	0.2	0.94	1675.72
93	1	-2	110	55	14	8	0.25	0.57	1520.34	-1	115	45	18	19	0.2	1.06	4929.81
94	1	-1	130	75	15	15	0.33	1	1911.28	0	150	85	15	18	0.25	1.2	3583.33
95	1	0	135	55	15	14	0.33	0.93	3444.03	-1	140	60	18	18	0.2	1	3636.64
96	1	0	130	65	19	17	0.33	0.89	2870.03	-1	140	70	12	19	0.2	1.58	4117.28
97	1	-2	115	70	14	10	0.66	0.71	2016.77	0	125	55	8	10	0.25	1.25	1831.9
98	1	0	110	45	15	14	0.33	0.93	1861.64	0	120	55	11	14	0.25	1.27	1660.34
99	1	0	140	65	20	17	0.33	0.85	2761.43	-1	135	60	16	11	0.2	0.69	1977.01
100	1	-2	140	70	16	9	0.33	0.56	791.2	0	140	60	15	16	0.2	1.07	3386.42
1	2	-1	110	55	12	10	0.25	0.83	1213.15	-1	130	60	14	12	0.15	0.86	1321.55
2	2	-1	125	60	15	10	0.33	0.67	2422.84	0	140	65	19	15	0.15	0.79	2652.01
3	2	1	115	55	13	10	0.33	0.77	1337.57	0	130	55	17	17	0.15	1	3614.22
4	2	0	125	60	16	18	0.4	1.13	3066.15	-1	150	70	18	21	0.2	1.17	5223.33
5	2	-2	130	75	14	10	0.4	0.71	1833.15	1	160	80	19	24	0.15	1.26	6056.03
6	2	0	130	60	15	16	0.25	1.07	2578.13	1	130	60	13	15	0.2	1.15	2506.54
7	2	0	140	75	19	20	0.33	1.05	4120.11	0	150	60	15	18	0.15	1.2	5021.28
8	2	1	140	70	20	13	0.33	0.65	1797.92	-1	155	75	19	20	0.2	1.05	3488.79
9	2	-1	120	55	16	13	0.25	0.81	1426.54	0	140	70	14	15	0.2	1.07	2534.12
10	2	0	110	40	14	4	0.25	0.29	124.11	-1	110	40	19	11	0.15	0.58	873.22
…										…							
91	2	-4	130	75	18	11	0.4	0.61	1312.86	-2	0	65	19	18	0.15	0.95	3935.24
92	2	2	110	40	9	8	0.33	0.89	744.83	2	120	50	12	12	0.15	1	1737.93
93	2	-2	135	70	15	11	0.33	0.73	2779.17	-1	135	65	15	15	0.15	1	3664.19

94	2	-2	150	80	19	18	0.33	0.95	2465.52	0	155	80	18	16	0.2	0.89	3595.83
95	2	1	135	65	19	19	0.33	1	3297.52	0	135	60	18	19	0.15	1.06	4386.49
96	2	-2	135	70	20	15	0.33	0.75	2366.81	0	135	60	19	20	0.15	1.05	4000.74
97	2	0	130	60	16	12	0.25	0.75	2243.64	-1	145	75	18	18	0.25	1	4915.59
98	2	-1	125	65	18	12	0.33	0.67	1590.09	-1	150	60	18	16	0.15	0.89	2861.42
99	2	0	130	60	18	13	0.33	0.72	1691.38	1	150	80	18	18	0.2	1	3521.55
100	2	-2	155	80	17	13	0.25	0.76	2129.44	-1	170	85	19	21	0.2	1.11	4897.95
1	3	0	115	60	12	11	0.33	0.92	1239.94	0	120	55	18	12	0.15	0.67	1225.57
2	3	1	140	75	18	11	0.33	0.61	1879.96	-1	125	50	16	15	0.15	0.94	2068.16
3	3	0	120	60	16	10	0.33	0.63	1034.81	0	125	60	20	9	0.15	0.45	1777.08
4	3	1	140	70	20	20	0.33	1	2916.27	1	145	80	16	21	0.15	1.31	4917.62
5	3	-1	140	70	13	14	0.33	1.08	2439.03	0	145	80	18	21	0.15	1.17	4366.11
6	3	2	120	60	14	14	1	1	1910.63	-1	120	70	10	12	0.2	1.2	2190.71
7	3	1	135	65	18	20	0.33	1.11	5054.78	-1	150	85	12	13	0.2	1.08	3125.22
8	3	-1	125	70	15	11	0.33	0.73	1642.92	-1	120	55	16	13	0.15	0.81	1945.6
9	3	-3	115	60	13	15	0.33	1.15	1016.79	-1	115	60	17	19	0.2	1.12	2910.74
10	3	-4	100	35	16	7	0.4	0.44	46.54	-1	115	50	17	10	0.15	0.59	306.39
…										…							
91	3	-2	120	60	17	10	0.25	0.59	550.22	-1	150	75	15	15	0.15	1	3171.17
92	3	2	105	55	18	10	0.25	0.56	1363.83	1	125	60	17	18	0.25	1.06	2634.99
93	3	0	135	75	15	12	0.25	0.8	2628.48	0	175	100	20	16	0.15	0.8	4366.11
94	3	0	140	60	20	11	0.33	0.55	1590.09	1	150	85	18	16	0.15	0.89	3906.52
95	3	-1	130	65	16	18	0.33	1.13	3286.1	-1	145	70	19	24	0.15	1.26	5499.77
96	3	1	130	70	14	14	0.33	1	1972.41	1	135	65	19	16	0.15	0.84	3339.69
97	3	0	130	75	19	12	0.33	0.63	2088.94	-1	140	70	17	15	0.15	0.88	3263.09
98	3	-1	100	60	12	16	0.25	1.33	1743.97	-1	135	70	17	16	0.2	0.94	2726.9
99	3	0	130	55	12	13	0.25	1.08	1601.98	0	150	65	19	20	0.2	1.05	3676.72
100	3	-1	150	80	15	15	0.33	1	1528.38	0	170	80	17	20	0.15	1.18	3738

These 98 $F_{2:3}$ families + two contrasting parental lines accesses revealed the existence of significant genetic variability for all phenotypic traits evaluated [anthesis silk inverval (ASI), plant height (PH), ear height (EH), proportion of dead leaves (DL), prolificity (Prol), and grain yield (GY)] (Tables 2 and 3). It can be verified that most of the coefficient of variance can be considered from low to medium values. In addition, these analysis reveals the draught stress was also enough to reduce around 45% of the grain yield, which can be considered adequate for this kind of study.

The results are allowing identifying genomic regions associated with drought tolerance in maize accesses. In order to accomplish this study, a second year trial, using 200 segregating families at the same site and with the same protocols as used previously, was carried out in the year of 2007. This second year trial was very important to identify QTLs conserved in different field experiments.

Table 2. Variance analysis of maize drought phenotyping field results with (WD) and without (NWD) water deficit for the traits: anthesis silk interval (ASI), plant height (PH), and ear height (EH) for a population composed by 98 $F_{2:3}$ families with the two contrasting parental lines, evaluated at Janaúba, MG site in 2006.

Source of variation	DF	ASI (days)		PH (cm)		EH (cm)	
		NWD	WD	NWD	WD	NWD	WD
Blocks	2	1,00	2,73	1890,08	792,58	1023,25	717,58
Genotypes	99	1,28**	1,64*	293,33**	312,93**	179,37**	192,30**
Error	198	0,73	0,73	137,22	65,73	83,69	54,45
Total	299						
Mean		-0,45	-0,69	138,63	129,37	67,45	66,47
C.V. (%)		-189,51	-160,40	8,45	6,27	13,56	11,10
Heritability (%)		42,97	25,22	53,22	78,99	53,34	71,69
Ratio CVg/CVe		0,50	0,34	0,62	1,12	0,62	0,92

** and * effects significant by the F test at 1% and 5% respectively, ns = non significant.

Another strategy to identify genes associated with drought tolerance is to develop subtractive cDNA libraries. For this experiment, the same contrasting maize lines were planted under well controlled conditions at green house submitted to drought stress and well watered.

Sorghum drought phenotyping field results with and without water deficit for the traits: flowering (50 % of the plants), plant height, proportion of dead leaves, and stem diameter for a population composed by 48 cultivars, evaluated at Janaúba, MG site, in 2006, are presented in Table 4. For each evaluated trait was computed a hydric stress index (IEH). Complete sorghum data set was inserted in the Morpho database. Table 5 shows Sorghum drought phenotyping field results with and without water deficit for the traits: 100 grains weight, grain and panicle ratio, panicle grains weight, and grain yield for the same population composed by 48 cultivars, evaluated also at Janaúba, MG site, in 2006.

The mean sorghum accesses yield averages during the 2006 year were 4909 k g ha^{-1} and 6346 kg ha^{-1}, with and without water deficit, respectively, which corresponded an 22.6 % in yield reduction. The other evaluated traits flowering (50 % of the plants), plant height, proportion of dead leaves, stem diameter, 100 grains weight, grain and panicle ratio, panicle grains weight, and grain yield averages values were of 68.8 and 67.4, 133 and 126, 8 and 63, 21 and 18, 2.86 and 2.36, 0.72 and 0.76, 32.16 and 26.70, and 6.35 and 4.90 for the treatments without and with water deficit, respectively (Tables 4 and 5).

Table 3. Variance analysis of maize drought phenotyping field results with (WD) and without (NWD) water deficit for the traits: proportion of dead leaves (DL), prolificity (Prol) in number of ears per plant, and grain yield (GY) for a population composed by 98 $F_{2:3}$ families with the two contrasting parental lines, evaluated at Janaúba, MG site in 2006.

Source of variation	DF	DL		Prol		Grain Yield (kg/ha)	
		NWD	WD	NWD	WD	NWD	WD
Blocks	2	0,11	0,05	0,30	0,19	2689673,35	1087584,6
Genotypes	99	0,0009ns	0,07**	0,11**	0,12**	3720343**	2657023**
Error	198	0,0008	0,03	0,04	0,03	562810,65	360427,57
Total	299						
Mean		0,19	0,41	1,09	0,80	3820,29	2100,13
C.V. (%)		15,09	45,01	19,38	22,75	19,64	28,58
Heritability (%)		13,07	53,39	57,64	73,08	84,87	86,43
Ratio CVg/CVe		0,22	0,62	0,67	0,95	1,37	1,4574

** and * effects significant by the F test at 1% and 5% respectively, ns = non significant.

Table 4. Sorghum drought phenotyping field results with (C/E) and without (S/E) water deficit for the traits: flowering (50 % of the plants), plant height, proportion of dead leaves, and stem diameter for a population composed by 48 cultivars, evaluated at Janaúba, MG site in 2006. Hydric stress index (IEH). Complete data set inserted in the Morpho database.

Cult. Nº	Flowering (days)			Plant height (cm)			Dead leaves (%)			Stem diameter (cm)		
	S/E	C/E	IEH	S/E	C/E	IEH	S/E	C/E	IEH	S/E	C/E	IEH
48	65	64	0.71	150	148	0.29	5	23	0.33	23	21	0.82
14	71	71	0.00	155	145	1.45	8	47	0.71	17	13	1.65
4	64	63	0.71	173	165	1.16	10	75	1.18	24	23	0.41
25	69	67	1.43	135	133	0.29	12	52	0.73	22	17	2.06
3	65	63	1.43	173	168	0.72	10	47	0.67	22	27	-2.06
22	66	64	1.43	153	150	0.43	10	32	0.40	23	22	0.41
19	69	68	0.71	165	153	1.73	7	43	0.66	22	15	2.88
20	69	68	0.71	157	147	1.45	10	50	0.73	17	20	-1.23
31	70	69	0.71	128	125	0.43	10	48	0.69	18	13	2.06
43	70	69	0.71	118	112	0.87	5	27	0.40	18	13	2.06
35	67	64	2.14	118	117	0.14	7	33	0.47	20	24	-1.65
40	73	70	2.14	122	113	1.30	5	30	0.45	23	20	1.23
12	70	68	1.43	122	120	0.29	10	52	0.76	15	15	0.00
26	69	68	0.71	117	122	-0.72	8	32	0.44	19	16	1.23
42	69	65	2.86	132	115	2.46	12	20	0.15	16	15	0.41
21	66	63	2.14	165	152	1.88	7	55	0.87	22	21	0.41
27	61	64	-2.14	168	155	1.88	10	55	0.82	19	16	1.23
37	72	71	0.71	113	110	0.43	7	33	0.47	23	17	2.47

30	73	72	0.71	112	85	3.90	7	22	0.27	17	10	2.88
2	61	60	0.71	160	173	-1.88	8	67	1.07	28	26	0.82
34	72	72	0.00	140	132	1.16	7	27	0.36	26	22	1.65
47	72	69	2.14	122	113	1.30	13	67	0.98	22	18	1.65
17	70	71	-0.71	140	132	1.16	7	37	0.55	23	23	0.00
33	69	65	2.86	143	127	2.31	8	25	0.31	19	22	-1.23
1	58	60	-1.43	185	180	0.72	7	38	0.56	25	31	-2.47
41	71	70	0.71	118	105	1.88	5	23	0.33	19	9	4.12
24	67	65	1.43	142	143	-0.14	7	48	0.75	22	21	0.41
13	70	68	1.43	122	117	0.72	8	60	0.95	17	16	0.41
6	70	68	1.43	117	108	1.30	12	58	0.84	17	20	-1.23
46	72	71	0.71	122	107	2.17	8	58	0.91	16	12	1.65
11	69	69	0.00	108	105	0.43	8	60	0.95	19	18	0.41
28	71	70	0.71	140	120	2.89	10	22	0.22	30	28	0.82
38	76	72	2.86	107	100	1.01	10	45	0.64	24	15	3.70
45	63	62	0.71	147	138	1.30	10	63	0.96	22	23	-0.41
5	61	57	2.86	183	182	0.14	8	37	0.53	29	27	0.82
18	71	70	0.71	118	113	0.72	12	43	0.56	24	20	1.65
36	70	70	0.00	125	107	2.60	7	42	0.64	23	16	2.88
10	61	62	-0.71	147	153	-0.87	10	47	0.67	20	21	-0.41
23	68	66	1.43	135	123	1.73	12	27	0.27	24	22	0.82
9	69	68	0.71	115	103	1.73	7	63	1.02	21	15	2.47
44	70	70	0.00	115	112	0.43	10	55	0.82	19	15	1.65
15	70	68	1.43	107	98	1.30	5	25	0.36	15	12	1.23
39	75	71	2.86	98	100	-0.29	5	50	0.82	17	12	2.06
29	73	72	0.71	97	87	1.45	7	28	0.38	18	12	2.47
16	71	71	0.00	135	127	1.16	8	42	0.62	20	20	0.00
8	70	69	0.71	135	128	1.01	10	60	0.91	16	13	1.23
7	69	67	1.43	102	103	-0.14	8	80	1.31	16	16	0.00
32	74	71	2.14	90	87	0.43	7	38	0.56	19	14	2.06
Média	68.8	67.4	0.97	133	126	1.00	8	63	1.00	21	18	1.00
Difer.		1.4			6.9			55			2.4	

Table 5. Sorghum drought phenotyping field results with (C/E) and without (S/E) water deficit for the traits: 100 grains weight, grain and panicle ratio, panicle grains weight, and grain yield for a population composed by 48 cultivars, evaluated at Janaúba, MG site in 2006. Hydric stress index (IEH). Complete data set inserted in the Morpho database.

Cult. Nº	100 grains weight (g)			Grain/ Panicle ratio			Panicle grains weight (g)			Grain yield (t/ha)		
	S/E	C/E	IEH	S/E	C/E	IEH	S/E	C/E	IEH	S/E	C/E	IEH
48	4.030	3.153	1.765	0.76	0.84	2.00	35.92	31.78	0.758	**7.200**	**7.142**	**0.040**
14	3.612	2.949	1.334	0.63	0.69	1.50	41.33	35.00	1.159	**9.233**	**6.950**	**1.589**
4	2.522	2.193	0.662	0.77	0.78	0.25	39.41	28.99	1.908	**8.667**	**6.800**	**1.299**
25	2.441	1.939	1.010	0.81	0.78	-0.75	43.06	30.72	2.260	8.667	6.608	1.433
3	2.308	2.183	0.252	0.65	0.82	4.25	29.18	30.87	-0.310	**6.025**	**6.592**	**-0.395**
22	2.891	2.472	0.843	0.68	0.81	3.25	30.29	31.48	-0.218	**6.642**	**6.275**	**0.255**
19	3.234	2.536	1.404	0.74	0.81	1.75	41.10	38.57	0.463	**6.984**	**6.133**	**0.592**
20	2.775	2.159	1.239	0.73	0.78	1.25	36.09	26.65	1.729	8.317	6.075	1.560
31	3.639	3.189	0.905	0.72	0.78	1.50	38.49	29.24	1.694	**7.492**	**5.983**	**1.050**
43	3.330	2.481	1.708	0.74	0.82	2.00	40.10	35.66	0.813	**7.792**	**5.825**	**1.369**

35	3.141	2.905	0.475	0.69	0.82	3.25	24.30	26.01	-0.313	**5.142**	**5.808**	**-0.463**
40	3.438	2.677	1.531	0.75	0.77	0.50	34.96	32.05	0.533	**6.106**	**5.642**	**0.323**
12	2.493	1.925	1.143	0.70	0.76	1.50	40.76	29.56	2.051	**7.025**	**5.558**	**1.021**
26	3.599	3.133	0.938	0.63	0.74	2.75	27.77	28.28	-0.093	**5.483**	**5.550**	**-0.047**
42	3.717	3.420	0.598	0.73	0.84	2.75	26.89	33.50	-1.211	**5.239**	**5.483**	**-0.170**
21	3.418	2.782	1.280	0.74	0.77	0.75	34.26	30.37	0.712	**6.792**	**5.400**	**0.969**
27	3.118	2.832	0.575	0.77	0.84	1.75	30.33	21.95	1.535	**6.775**	**5.383**	**0.969**
37	2.783	2.291	0.990	0.66	0.64	-0.50	30.63	26.93	0.678	**6.392**	**5.358**	**0.720**
30	1.977	2.131	(0.310)	0.76	0.73	-0.75	31.55	28.55	0.549	6.475	5.258	0.847
2	2.325	2.216	0.219	0.70	0.72	0.50	26.59	28.09	-0.275	6.392	5.242	0.800
34	2.692	2.107	1.177	0.72	0.78	1.50	35.32	30.88	0.813	6.508	5.058	1.009
47	3.438	2.566	1.755	0.70	0.69	-0.25	35.45	24.54	1.998	**6.758**	**5.000**	**1.223**
17	3.327	2.338	1.990	0.80	0.86	1.50	41.48	31.99	1.738	7.213	4.867	1.633
33	3.826	3.603	0.449	0.74	0.88	3.50	19.21	23.43	-0.773	4.158	4.792	-0.441
1	2.584	2.302	0.567	0.71	0.73	0.50	30.76	24.25	1.192	5.825	4.758	0.743
41	4.470	4.024	0.897	0.68	0.77	2.25	36.79	39.38	-0.474	6.936	4.750	1.521
24	2.947	2.286	1.330	0.75	0.81	1.50	31.07	24.19	1.260	7.292	4.675	1.821
13	2.425	1.947	0.962	0.74	0.77	0.75	35.09	25.22	1.808	6.284	4.650	1.137
6	2.285	1.750	1.076	0.72	0.70	-0.50	25.14	23.03	0.386	5.308	4.275	0.719
46	2.138	1.779	0.722	0.77	0.66	-2.75	33.87	22.98	1.995	7.275	4.208	2.134
11	2.594	2.179	0.835	0.70	0.74	1.00	29.87	31.16	-0.236	5.792	4.167	1.131
28	2.630	2.140	0.986	0.76	0.78	0.50	30.36	23.77	1.207	6.158	4.133	1.409
38	3.003	1.917	2.185	0.71	0.80	2.25	35.36	29.00	1.165	6.208	4.133	1.444
45	3.031	2.545	0.978	0.74	0.72	-0.50	24.86	21.94	0.535	5.467	4.125	0.934
5	2.821	2.641	0.362	0.64	0.62	-0.50	23.58	19.12	0.817	4.520	4.058	0.322
18	2.409	1.843	1.139	0.73	0.77	1.00	43.84	25.79	3.306	6.960	4.058	2.019
36	2.549	2.010	1.085	0.73	0.81	2.00	33.38	31.57	0.332	7.175	4.050	2.175
10	3.660	3.044	1.239	0.76	0.73	-0.75	32.19	22.28	1.815	6.767	4.025	1.908
23	2.746	2.288	0.922	0.79	0.85	1.50	25.41	19.90	1.009	5.242	4.000	0.864
9	2.116	1.533	1.173	0.71	0.82	2.75	28.14	22.20	1.088	6.250	3.983	1.578
44	2.349	1.910	0.883	0.75	0.71	-1.00	31.09	21.09	1.832	6.158	3.892	1.577
15	2.535	2.130	0.815	0.74	0.75	0.25	32.12	24.92	1.319	5.415	3.842	1.095
39	1.912	1.599	0.630	0.48	0.54	1.50	23.29	22.52	0.141	4.500	3.842	0.458
29	2.616	2.312	0.612	0.73	0.82	2.25	32.69	22.74	1.822	5.331	3.692	1.141
16	2.874	2.274	1.207	0.69	0.82	3.25	22.72	18.92	0.696	3.886	3.483	0.280
8	2.455	1.900	1.117	0.74	0.71	-0.75	28.05	17.22	1.984	5.383	3.400	1.380
7	2.262	1.596	1.340	0.77	0.64	-3.25	28.41	15.13	2.432	5.708	3.317	1.664
32	2.057	1.524	1.072	0.72	0.76	1.00	31.19	18.07	2.403	5.308	3.308	1.392
Média	2.865	2.368	1.000	0.72	0.76	1.00	32.16	26.70	1.000	6.346	4.909	1.000
	0.5			0.04			5.46			1.437		

BIBLIOGRAPHY

ALBUQUERQUE, P.E.P.; Durães, F.O.M.; Gomide, R.L.; Andrade, C.L.T. Estabelecimento de sítios-específicos experimentais visando imposição e monitoramento de estresse hídrico para fenotipagem de cereais. Embrapa Milho e Sorgo, ***Circular Técnica*** No. 61, Dezembro, 2005, 10 p.

Andrews, P.K., Chalmers, D.J. and Moremong, M. 1992. Canopy-air temperature differences and soil water as predictors of water stress of apple trees grown in a humid, temperate climate. *Journal American Society Horticultural Science*, Mount Vernon, Vol.117, pp.453-8.

BAVER, L. D.; GARDNER, W. H.; GARDNER, W. R. Soil physics. 4. ed. New York: John Wiley, 1972. 529 p.

Clawson, K.L., Jackson, R.D. and Pinter Jr., P.J. 1989. Evaluating plant water stress with canopy temperature differences. *Agronomy Journal.*, Madison, Vol.81, pp.858-63.

DURÃES, F. O. M.; Santos, M .X.; Gama, E. E. G.; Santos, F. G.; Guimarães, C. M.; Trindade, M. G.; Lopes, M. A. A phenotyping platform supporting breeding and genomics programs of cereals and legumes at Embrapa – Brazil. In: ***Drought Phenotyping Protocols Workshop***, 2004, Montpellier.

EMBRAPA/Serviço Nacional de Levantamento e Conservação de Solos. 1992/93. *Delineamento macroagroecológico do Brasil*. 1 mapa color. 85.5 ’ 113 cm. Escala 1:5.000.000. EMBRAPA/SNLCS, Rio de Janeiro, Brasil.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA. Serviço Nacional de Levantamento e Conservação de Solos. Manual de métodos de análise de solos. 2 ed., Rio de Janeiro, 1997. 212 p.

GENUCHTEN, M. Th. van. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Science Society of America Journal, Madison, v. 44, n. 5, p. 892-898, 1980.

Gomide, R.L.; Albuquerque, P.E.P.; Kobayashi, M.K.; Inamasu, R.Y. Microprocessor and Automation Techniques for Registration of Water Requirements, Stress Indexes, and Irrigation Timing Criteria of Corn and Beans Crops in the Brazilian South-East Irrigated Fields. *ASAE Annual International Meeting*, ASAE, Sacramento, California, USA, July 30-August 1, 2001. *Paper Number: 01-2049.*

GOMIDE, R.L.; Inamasu, R. Y.; Queiroz, D.M.; Mantovani, E.C.; Santos, W.F. An Automatic Data Acquisition and Control Mobile Laboratory Network for Crop Production Systems Data Management and Spatial Variability Studies in the Brazilian Center-West Region. ***ASAE Annual International Meeting***, Sacramento, California, USA, 2001, ***Paper Number: 01-1046***, 10 p.

GUPTA, S. C.; SHARMA, P. P.; DEFRANCHI, S. A. Compaction effects on soil structure. Advances in Agronomy, New York, v. 42, p. 311-338, 1989.

HAWTIN, G.; Iwanaga, M.; Hodgkin, T. Genetic resources in breeding for adaptation. Euphytica, Wageningen, v. 92, p. 255-266, 1996.

Idso, S.B., Jackson, R.D., Pinter Jr., P.J., Reginato, R.G. and Hatfield, J.L. 1981. Normalizing the stress-degree-day parameter for environmental variability. *Agricultural Meteorology*, Amsterdam, Vol.24, pp.45-55.

Inoue, Y. 1991. Remote and visualized sensing of physiological depression in crop plants with infrared thermal imagery. *Japan Agricultural Research Quarterly*, Ibaraki, Vol.25, pp.1-5.

Jackson, R.D. 1982. Canopy temperature and crop water stress. *Advances in irrigation.* New York, Vol.1, pp.43-85.

Jackson, R.D., Idso, S.B., Reginato, R.J. and Pinter Jr., P.J. 1981. Canopy temperature as a crop water stress indicator. *Water Resource Research*, Washington, Vol.17, pp.1133-8.

Jackson, R.D., Kustas, W.P. and Choudhury, B.J. 1988. A reexamination of the crop water stress index. *Irrigation Science*, Heidelberg, Vol.9, pp.309-17.

Jensen, M.E., Burman, R.D. and Allen, R.G. 1990. *Evapotranspiration and irrigation water requirements.* New York: ASCE, 332p. (ASCE - Manual and reports on engineering practice, 70).

KIEHL, E. J. Manual de edafologia: relação solo-água-planta. São Paulo: Agronômica Ceres, 1979. 262 p.

Monteith, J. L. 1965. Evaporation and environment. In: The state and movement of water in living organisms. New York Academic Press. Proc. Symp. Soc. Exp. Biol. 19: 205-234.

--------. 1973. Principles of environmental physics. Amer. Elsevier. N.Y. 243 pp.

RICHARDS, L.A. Methods of mounting porous plates used in soil moisture measurements. ***Agronomy Journal***, v. 41, p. 489-490, 1949.

Sediyama, G. C., kobayashi, M. K., Gomide, R. L., Magalhães, P. C., Durães, F. O. M. 2000. Dry bean evapotranspiration rates estimated by Penman-Monteith equation in ventilated greenhouse *In: 24th Conference on Agricultural and Forest Meteorology*, Davis, California, USA. p.18-21.

Smith, M., Segeren, A., Pereira, L.S., Perrier, A. and Allen, R. 1991. *Report on the expert consultation on procedures for revision of FAO guidelines for prediction of crop water requirements.* Rome: FAO, 45p.

van GENUCHTEN, M.Th. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci. Soc. Am. J.* v.44, p.892-898. 1980.

VOMOCIL, J. A.; FLOCKER, W. J. Effect of soil compaction on storage and movement of soil air and water. Transactions of the ASAE, St. Joseph, v. 4, p. 242-246, 1961.