Water stress characterization in contrasting environments for drought tolerance phenotyping[1]

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Introduction

The drought process studies require knowledge of some environmental factors and how these factors interact with plants genotypes performance under water constraint conditions. It is necessary to understand the environmental effects which result in grain yield, identifying the causes (genetic versus environmental) which will result ultimately in a yield reduction of the genotype maximum capacity to produce grain due to limited water condition in the soil. Most of the research has been largely focused on short-term physiological (e.g. stomatal or osmotic adjustment) and general morphology responses, while ignoring the importance of phenotypic plasticity (in terms of adaptive morphogenesis and phenology) which governs the access and utilization of resources, including water. Genotypic versus environmental interactions are largely a result of phenotypic plasticity.

One of the main technicians' problems in the phenotyping environment sites for maize and sorghum 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 environmental specific sites (ESS) for drought tolerance phenotyping (DTP) should be necessary a training program to the technicians on irrigation water management, field evaluation of the used irrigation systems, and soil water status evaluation and measurements, since in most cases, new technicians are unfamiliar with the basic principles on operate and manage the irrigation systems and soil water content 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 ESS for DTP, 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, microclimate factors and soils water content data, which should be collected on a continuous basis in order to have control and register of their changes along the crops growing season.

The main objective of this work was to describe the procedures of controlling and monitoring water stress in contrasting environments for drought tolerance phenotyping of cereals and legumes to better understand the effects of plants genetic and environmental (GxE) interactions for grain yield, identifying and characterizing the causes which will result in genotypes yield reduction due to water shortage.

Environmental Specific Site

Each selected environmental specific site (ESS) must have determined its geographical coordinates and altitude (by means of a differential global position system -DGPS), hydrological soil water balance, soil physical (texture, structure, porosity, apparent and real density, soil moisture retention curves, water infiltration rate) and chemical (fertility, organic matter, micro nutrients) properties characterization (at different soil layers) for cereals and legumes genotypes drought tolerance phenotyping investigation.

Irrigation Water Application, Control and Management

The irrigation schemes designed and installed in the ESS of Embrapa were the following: conventional sprinkler (low to medium service pressure), localized (drip), and continuously moving straight lateral or linear-move systems. These schemes were tested and evaluated for water distribution uniformity (flow rate/discharge) and applied water depths 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 trial. 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 plots was set to be equal or greater than 95 %, calculated by means of Christiansen Uniformity Coefficient equation. Some hydrometers were coupled to the irrigation systems main lines. All irrigation water application rates were set to be lower than basic soil saturated water infiltration rate in order to avoid surface runoff, which was not allowed in the ESS areas.

In the conventional sprinkler schemes, 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. The sprinklers irrigation spacing recommended and used in the ESS were 12 m x 12 m (Teresina, PI) and 12 m x 18 m (Janaúba, MG). The used sprinklers nozzle sizes ranged from 3.5 to 4.5 mm diameter, which were 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}. As a lateral pipe line length is 6 m, it is important to set the genotypes plots size (length and wide) values, a number multiple of 6 in order to facilitate the experimental system layout in the field.

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.

Irrigation water management for the different genotypes was carried out by means of reference evapotranspiration (ETo) and crop evapotranspiration (ETc) computation, using both 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 respective 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.

After sowing, the irrigation was uniform to assure good genotype germination and stand formation, with 100% replacement of the ETc and soil water availability (SWA) - non water stressed condition. Afterwards, the water stress treatments were induced or initiated with different replacement levels 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 (levels).

Climatic Condition Characterization and Measurements

Climatic condition was characterized and hydrological water balance (Thornthwaite & Mather) was determined, with 15 to 50 years historical data series, obtained from standard weather stations (Brazilian National Institute of Meteorology – INMET), for each ESS.

A standard procedure was established for each ESS to install locally the equipments and sensors of automatic weather stations, configured to measure with intervals of half to one hour the following microclimatic surface parameters: temperature and relative humidity of the air, global solar radiation (net radiation in some station), precipitation, speed and direction of the wind, class A pan water evaporation (in some station) for drought tolerance phenotyping purposes.

Soil Water Status Evaluation and Measurements

Measurements of the soils capacity to store water, in different layers within the root system, is important for differentiate precisely the irrigation water regime (water stress) in order to quantify the soil water availability (SWA) to the maize and sorghum genotypes. In the ESS 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 *moisture storage capacity of the soil*. Fortunately, some of the specific sites established present sand soils (Janaúba-MG and Teresina-PI). 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. For crops grown in the second harvest (“safrinha”), the stress may be applied after flowering (Sorghum genotypes). 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 die. It is crucial, though that some sort of monitoring been done in the soil, plant and weather. Monitoring soil-water status allows stress level quantification and helps making the decision on when to interrupt water stress. In fact, the level of stress a plant genotype will suffer depends on the interaction between plant, soil and weather.

The protocol for soil water content measurements in the ESS of Embrapa, considering different soil layers, consists of selecting the soil moisture places to be evaluated, deciding upon the sampling frequency and strategy, assembling the necessary instruments and materials. The gravimetric method of soil water content evaluation was utilized as standard, which consisted of collecting soil samples with an auger, weighing, drying overnight in an oven, and then reweighing the soil samples. Also the following equipments and sensors were used to register the water content in the soil profile: gypsum blocks, tensiometer, time domain reflectometers (TDR), Diviner, neutron probe, and other electrical resistance methods.

Minimum typical procedures for installation of the described soil moisture instruments in the ESS, with relatively uniform soil conditions and crops, were taking into account as follow: 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, with sensors/ access tubes be located near crop row lateral line (at least 60 cm away from emitters (for drip irrigation)); d) Site conditions: representative soil in the plots and vigorous and non-disease genotypes area might be selected.

The hand-held capacitance probe (model Diviner 2000, Sentek Pty Ltd.) and the neutron probe were used for monitoring the soil water content in the soil profile. These are portable soil moisture-monitoring devices, 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 these devices were made for every 0.1 to 0.2 m until 1.0 m soil depth.

Plant Water Status Evaluation and Measurements: Canopy Temperature

Remotely-sensed infrared canopy temperature (T_c) measurements provide an efficient method for rapid, non-destructive monitoring of the whole-plant response to water stress. With the advent of portable infrared thermometer (IRT), the concept of using T_c measurements to infer water stress and transpirational rates of genotypes has been advanced and received some interest as a drought-tolerance screening technique for research purposes. Canopy–air temperature difference ($T_c - T_a$) measurements can be taken with one of the IRT transducer models available, which should be configured to measure both the air (Ta) and surface temperatures (T_c), or the difference between the two. However, it is important that the transducer models be selected to operate under crop condition. This means that they should meet the following specifications: low temperature range readings (ideally up to 80°C); 8–14µm band wave length (the thermal portion of the electromagnetic spectrum); and emissivity set to 0.98 (for most crops genotypes).

The water stress treatments were obtained with different ETc replacement, generating different water depth applications in the plots at pre-defined crop growth phases, defined for each genotype, in order to establish the water stress level.

The procedures for field measurements involved registering the difference $T_c - T_a$ with an infrared thermometer. The Penman-Monteith equation in terms of crop canopy-air temperature difference, aerodynamic resistance and canopy resistance may be expressed as (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 (°C), T_a the air temperature (°C), 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 °C), γ the psychrometric constant (Pa/ °C), 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 and sorghum crop water requirements or evapotranspiration (ETc) should be evaluated from measurements of the last equation terms. 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.

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) should be 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 should be computed by

$$CWSI = \frac{dT - dT_l}{dT_u - dT_l} \quad \text{or} \quad CWSI = 1 - (ETc / ETp)$$

In which, ETc and ETp represent the actual or real and the potential or maximum crop evapotranspiration, respectively. A CWSI value of about 0.20 should be used as a limit to differentiate the irrigated crop from a non water stressed to a water stressed condition, as a criteria to avoid significant yield loss for the main grain crops. In the drought tolerance phenotyping trials in maize and sorghum, the CWSI values should be in the range of 0.40 to 0.70 to assure yield reduction above 30 %. This CWSI range magnitude means replacement of approximately 60 to 30 % of ETc (water stressed condition).

Remarks and Recommendation

Collecting environment data on water (irrigation & rainfall), microclimate surface parameters, soil moisture profile content, and cropping systems (traits related to genotypes phenology & physiology) is essential when water is in short supply (drought) and evaporative demand of the atmosphere is high in order to better understand the effects of genotypes and environmental (GxE) interactions.

Wherever environment specific site conditions are short in water supply and evaporative demand of the atmosphere is high, a localized scheme (microsprinkler or drip) is indicated because the amount of water and the frequency of application can be controlled very finely, direct to the crops root zone.

For drought tolerance phenotyping of cereals and legumes studies at the ESSs of Embrapa, the CWSI values were in the range of 0.4 to 0.7. It is recommended values of CWSI of 0.20 as a limit for irrigation water management to differentiate the irrigated crop from a non-stressed to a stressed condition, as criteria to avoid significant yield loss.

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.

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