Drought phenotyping in crops: from theory to practice

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Introduction

The development of drought-tolerant varieties of economically important crops represents a major challenge for the 21st century, when global agricultural growth will be limited by water availability. The first step to be taken in this direction is to use appropriate screening techniques to select germplasm adapted to conditions of water stress. The demanding objective is then to identify and characterise drought-resistant and tolerant parents to provide material for use in breeding programmes focused on regions historically known to be prone to drought during the crop growing seasons. The improvement of drought resistance and tolerance relies on manipulation of traits that limit yield in each crop and their accurate phenotyping under the prevailing field conditions in the target population of environments (TPE). This is particularly crucial for identification of quantitative trait loci (QTLs) and in breeding activities for traits categorised as adaptive (as opposed to constitutive). For this purpose, it is necessary to have an infrastructure allowing plants to be exposed to water deficit, to permit the evaluation of genotypes and their characterisation for plant physiological and morphological responses to water stress.

Among environmental stresses, drought is considered to be the main source of grain yield instability for cereals and legumes in tropical regions. Better adaptation and tolerance to water shortage is desirable for high and stable productivity. Thus, genotypic selection for adaptation to different water regimes is an important strategy in breeding programmes in these regions. The procedures for selection and adaptation to drought require an understanding of the TPE (climate, soil, and water regime and management) and of genotype-by-environment interactions (GEI). Experimental results from diverse field conditions allow evaluation of GEI that limit adaptation of cereals and legumes.

This chapter focuses on the description of procedures and practices for drought tolerance phenotyping in cereals and legumes under contrasting environments. It emphasises the selection, establishment and characterisation of the target environments according to their location, climatic conditions, and soil physical and chemical properties, as well as the choice of procedures, genetic materials and traits to be used. The use of trait databases and models to gain a better understanding of GEI for grain yield and of the causes of yield reduction under water stress selection pressure is also considered. GEI is largely the result of phenotypic plasticity in terms of adaptive morphogenesis and phenology, as a response to the water regime and soil–plant–water dynamics. Most of the research quoted here has focused on short-term physiological and morphological responses to water stress (eg, stomatal or osmotic adjustment).

Comparing target environment characteristics for drought tolerance phenotyping

Drought is considered the main environmental stress leading to grain yield instability in cereal and legume crops in tropical areas. A breeding programme should have a good understanding of the environmental characteristics for evaluating and selecting genotypes under conditions of water shortage. Abiotic stress research programmes use a standard methodology involving low fertility soils and imposing drought conditions in breeding and selecting genotypes, without performing a complete characterisation of the environment. Problems related to variations in soil water dynamics (at the plant's effective root depth in the soil profile) as well as some other physical and chemical properties of the soil, have hindered drought tolerance studies in different environments.

A number of factors including variations in soil and rainfall are responsible for threats to cereal and legume production in the tropics, such as in the northeast, southeast, mid-north, and centre-west regions of Brazil. Spatial soil variability in the field is one of the most important factors, because it influences the availability of nutrients and water. Variations in rainfall in the two distinct periods of the dry and rainy seasons also present great risks for agriculture.

The kind of study discussed here requires a good characterisation of the environment, involving the measurement and recording of several soil, water, plant, and atmospheric features, together with the use of differential global positioning system (DGPS) and geographic information system (GIS) technologies (Gomide et al, 2001b). Recognition of spatial variability in the field has been the reason for dividing the field into zones or specific sites and investigating specific sites more closely to look for uniform characteristics.

Studies of the drought process require a good knowledge of some factors in the TPE and how these factors interact with the performance of plant genotypes under water stress. The objective of this section is to describe procedures and practices for selecting, establishing, and characterising specific experimental sites in terms of geographical coordinates, elevation, climatic conditions, and physical and chemical properties of the soil. Variability of some soil and environmental characteristics is discussed for particular target environments, based on precision agriculture methods used for data acquisition, storage, treatment, analysis and visualisation.

Site selection criteria

The choice of the specific experimental sites in Brazil for drought tolerance phenotyping studies took into account the representativeness of the sites with regard to economic and social factors, information on agriculture and cropping systems, and characterisation of climatic conditions. Studies drew on at least 15 years of historical weather data and soil features including hydrology (soil water balance), physical properties (texture, structure, porosity, apparent and real density, soil moisture retention curves, and water infiltration rate) and chemical properties (fertility, organic matter, and macro- and micro-nutrient content). Variations in some key soil characteristics were evaluated by means of topographic survey, dividing the sites into a 25m x 25m grid, using an accurate survey laser total station, georeferencing according to the South American Datum (SAD-69) and the Universal Transverse Mercator (UTM) projection (south zone 23, 48° to 42°W), and DGPS.

Soil samples were collected at the grid intersections for analysis of soil physical and chemical properties. The results of these analyses were then used to generate contour maps of the specific sites by means of a geostatistic method using a kriging interpolation model. These maps were used to divide the sites into uniform zones for water stress studies in cereals and vegetables. In each selected site, the water table was deep in order to avoid soil water capillarity effects on the root system, and high and low points were identified in order to avoid drainage problems.

Table 1 shows the specific sites by Brazilian Federative Unit (State), along with their geographic coordinates, altitudes, crops species, and type of phenotyping strategy and genetic material for the drought tolerance studies. Most of the sites have been established and characterised, and they are being used in the Generation Challenge Programme (GCP) Subprogramme 1 (SP1) project 'Supporting emergence of reference drought tolerance phenotyping centres–Drought phenotyping network (DPN) Project No. 6'. Figure 1 shows the locations of the specific sites, each of which has direct administrative and technical research support from an Embrapa (Empresa Brasileira de Pesquisa Agropecuaria; Brazilian Agricultural Research Corporation) Research Unit or Centre. Thus,

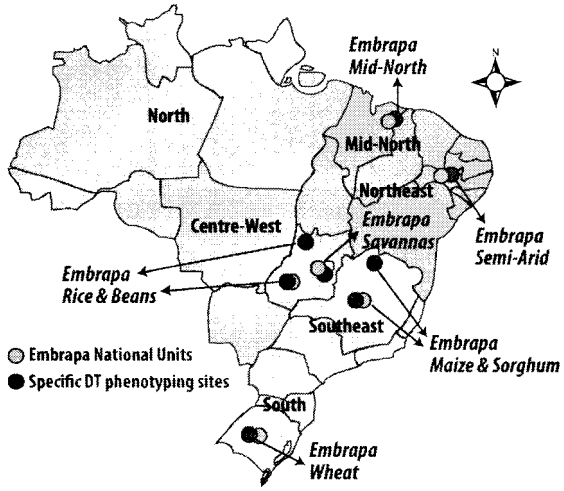

Figure 1. Location of the specific site areas for crop drought tolerance (DT) phenotyping in different Embrapa National Units and regions of Brazil.

Table 1. Location and features of the specific sites in Brazil.

State of Brazil	Specific site[1] location	Longitude (°W)	Latitude (°S)	Altitude (m)	Crop species	Phenotyping strategy[2]	Genetic material
MG	Sete Lagoas (E)	44.2467	19.4658	731	Maize, sorghum	P, I, A	Breeding lines, elite germplasm
MG	Janaúba (R)	43.3089	15.8025	533	Maize, sorghum	P, I	Breeding lines, elite germplasm
DF	Planaltina (R)	47.6142	15.4528	944	Wheat	P, I	Breeding lines, elite germplasm
GO	Santo Antônio de Goiás (E)	49.1711	16.2811	823	Rice, common beans, wheat	P, I, A	Breeding lines, elite germplasm, segregant lines
GO	Porangatu (R)	49.1486	13.4408	396	Rice	P, I	Breeding lines, elite germplasm
PI	Teresina (R)	42.8019	5.0892	72	Maize, sorghum, cowpeas	P, I	Breeding lines, elite germplasm
PI	Parnaíba (R)	41.7767	2.9047	5	Maize, sorghum	P	Breeding lines
PE	Petrolina (R)	40.5008	9.3986	376	Maize, sorghum, cowpeas	P, I	Breeding lines, elite germplasm
RS	Passo Fundo	52.4067	28.2628	687	Wheat	P, I	Breeding lines, elite germplasm

[1] E = Site of Excellence; R = Reference site.
[2] Type of phenotyping strategy: P = Preliminary; I = Intermediate; A = Advanced.

support is provided by Embrapa Maize and Sorghum for Sete Lagoas and Janaúba in Minas Gerais (MG) state, by Embrapa Rice and Beans for Santo Antônio de Goiás and Porangatu in Goiás (GO) state, by Embrapa Mid-North for Teresina and Parnaíba in Piauí (PI) state, by Embrapa Savannah for Planaltina in Federal District (DF) Brasília, by Embrapa Wheat for Passo Fundo in Rio Grande do Sul (RS) state, and by Embrapa Semi-Arid for Petrolina in Pernambuco (PE) state.

Soil characteristics for establishment of a specific site area

Spatial variability, a natural property of soils, is a function of the soil formation process, and is related to the complex interactions among natural environmental factors and human activities (Webster, 2000) that can act to both diminish and increase the variability (Couto et al, 1997; Nkedi-Kizza et al, 1994). This variability may be in the range of one metre or less (Solie et al, 2001), demanding accurate identification and mapping. It is important to emphasise that traditional soil maps are not produced on an adequate scale for the goals of a precise experimental site, where local microvariations may be a source of uncontrolled error that compromises results and their analysis. Moreover, some important agronomic characteristics, such as soil compaction and soil water availability, are not usually displayed in soil maps. It is important to take into account the influence of the past use and management of experimental areas, such as the residual effect of applying fertilizer as is common in experimental stations. Detailed information of such variability is still lacking for most locations (Dobermann et al, 1995).

In order to reduce experimental errors, the uniformity of abiotic factors should be maximised when locating the specific sites for breeding and phenotyping experiments. The selection of the specific site should take into account the actual situation in the field regarding uniformity, as well as the available resources and infrastructure. The specific site concept was developed for commercial crop production systems, and uses technological resources such as global positioning systems (GPS) and others electronic devices for data collection, transfer, and storage. Ultimately, all acquired data are combined and processed by means of GIS software.

The procedures described below provide the minimum information required for selecting and establishing specific sites in the field.

Main steps for a specific site selection and establishment in the field

1. *Choice of site:* Choose the site according to the availability of land and infrastructure, usually restricted to within an experimental station, or in an area where rigorous control can be achieved. As far as possible, this location should be representative of the agricultural areas where the genetic materials under examination would be cultivated.

2. *Bibliographic review of local knowledge:* Use as sources all available thematic maps, aerial photos, images, scientific papers, theses, dissertations, and technical reports referring to that region. In compiling the bibliography, include soil and land use maps, geologic and geomorphologic surveys, topographic maps, and photographs or images of the local area.
3. *Preparation of a historical area use report:* Include experimental data and activities conducted on the site, related works and any other change resulting from use. If possible include in the report information dating back to when the area was first occupied. Use oral reports from past inhabitants or workers from the area, although well-documented registers are preferable.
4. *Preliminary mapping of the area:* Carry out this step using available images or photographs, trying to identify patterns on the ground and targets for sampling.
5. *Planning and organisation of the field work:* Prepare a workplan, a work schedule and a check list of the materials and equipment required.
6. *Planimetric and altimetric surveying of the site area:* Draw contour lines in the topographic map with a vertical distance accuracy of one metre or less. Appoint a specialised team to perform this work, using geodesic GPS and a total laser station. Place permanent ground control points as a reference for future sampling work.
7. *Preparation of a highly detailed soil survey:* Include description and sampling of representative trenches, as necessary, to provide the best description of all the soil classes present, according to an up-to-date version of the classification system in use and current or best soil survey methods (eg Soil Survey Division Staff, 1993). Assist the mapping by auger sampling between the trenches, to check for the borders of soil classes and transitions to differing soils.
8. *Detailed survey:* Describe important agronomic or experimental properties not included in the previous survey, such as soil strength, water infiltration rate curves in the soil profile, average thickness of horizons, electrical conductivity, etc. As described earlier, use a sampling grid 25m x 25m as an initial estimate for the soil sampling. Use DGPS for the georeferencing of the sampling points as recommended, but if not available or for short distances, use a tape measure. In this case, refer the measurements to a control point with known coordinates, preferably a geodesic reference point. For each point in the sampling grid, collect a compound sample with at least three simple samples for each, in a maximum radius of one metre from the grid node.
9. *Soil chemical analyses:* Include standard fertility status (pH, Ca^{2+}, Mg^{2+}, Al^{3+}, N, P, K macronutrients, organic matter, electrical conductivity, cation exchange capacity) and micronutrients content (Fe, B, Zn, Cu), according to current methods (Embrapa–CNPS (Centro Nacional de Pesquisa de Solos), 1997; Page et al, 1982). In acid soils with aluminium toxicity problem in the soil profile, determine aluminium saturation to correct the pH through lime application.
10. *Determination of soil physical properties:* Include granulometry (texture) and structure, particle density (apparent and real), macro- and micro-porosity, water infiltration rate and soil moisture retention curves (field capacity, permanent wilting points, and total soil water availability).
11. *Export and compilation of all data into a GIS:* Produce the thematic maps and digital terrain models on an adequate scale for the site. Choose the software to match the needs for data analysis, processing and data export and for compatibility with other systems in use.

A specific site selection and establishment case study: Sete Lagoas (Minas Gerais), Janaúba (Minas Gerais) and Teresina (Piauí), Brazil

Site selection and establishment

The specific site at Sete Lagoas was selected and established in the Embrapa Maize and Sorghum experimental fields for the GCP DPN and Whole plant physiology modelling (WPM) projects. The selected area was surveyed and mapped with a Leica TC 805L total laser station system, which provided a precision of 00°00'01", by means of topography software. Contour lines were drawn at 0.5m vertical distance, and three geodesic reference benchmarks were placed in the area using a Topcon Hiper L1L2 GPS and a Topcon datalogger, model FC–100 and the software TopSURV. The differential signal was processed by the Instituto Brasileiro de Geografia e Estatística (IBGE) bases at Uberlândia and Varginha, using Topcon Tools Software (Table 2).

Characterisation of soil homogeneity and characteristics

A regular square grid was placed in the field, with grid nodes at a distance of 25m. Soil samples were collected in triplicate, in a radius of 1m from each node point, at depths of 0–10cm and 10–30cm. These samples were analysed for soil fertility, according to the Embrapa (1997) procedure. The interpolated maps of phosphorus, potassium and pH results for the upper layer (0–10cm) are presented in Figure 2. In one of the areas, where a corn experiment was under way, a sampling grid for non-deformed soil samples was established for soil density characterisation. The samples were collected in triplicate at depths of 0–5cm, 10–15cm and 25–30cm, using 50mm diameter and 50mm height rings. The results for the 10–15cm depth are shown in Figure 3.

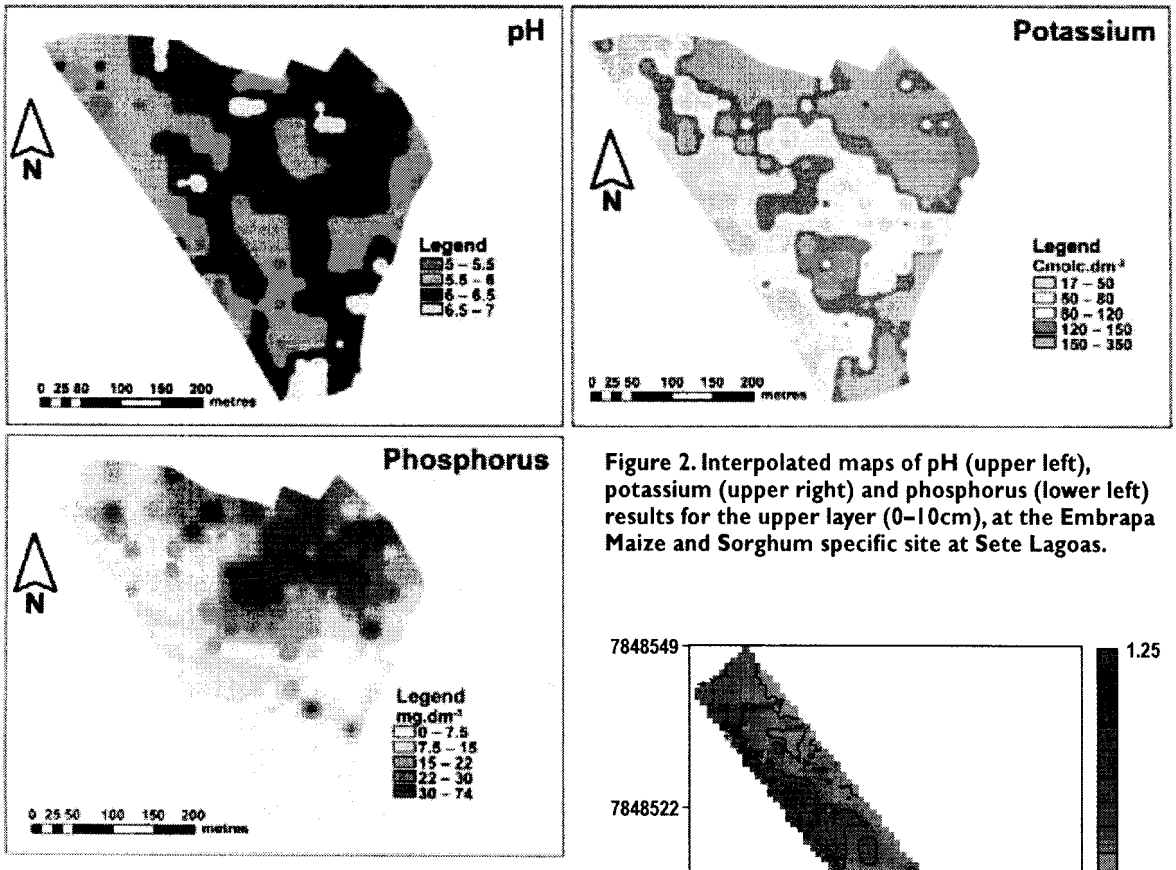

Figure 2. Interpolated maps of pH (upper left), potassium (upper right) and phosphorus (lower left) results for the upper layer (0–10cm), at the Embrapa Maize and Sorghum specific site at Sete Lagoas.

Table 2. Geodesic benchmark coordinates of the Sete Lagoas specific site (datum SAD-69, UTM projection S zone 23, 48° to 42°W)

Name	Latitude	Longitude	Altitude Ellipsoid (m)
BU9-BaseM-1	19° 27′ 17.20515″	44° 10′ 19.34919″	730.702
BU9-BaseM-2	19° 27′ 18.30578″	44° 10′ 27.55812″	741.035
BU9-BaseM-3	19° 27′ 17.04167″	44° 10′ 22.71995″	731.214

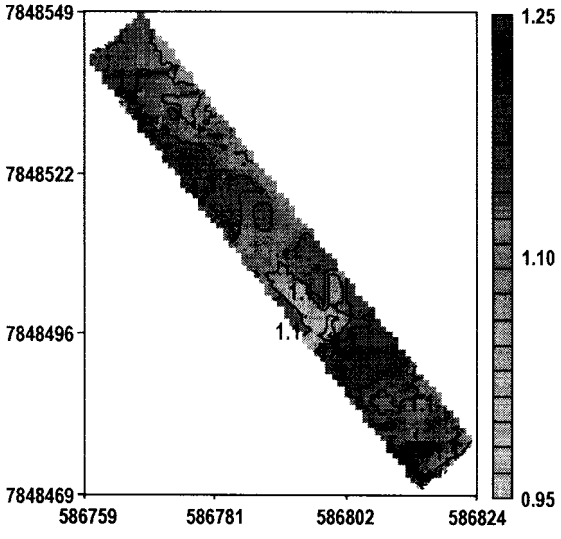

Figure 3. Interpolated maps of soil density results for the 10–15cm layer, at the Embrapa Maize and Sorghum specific site at Sete Lagoas.

Climatic characterisation

Environmental climatic characterisation and recording are essential in drought tolerance phenotyping investigations, to determine and quantify the crop water requirement or evapotranspiration (ET_c), for irrigation water management, and to control different water regime treatments and crop water stress levels. The main atmospheric parameters which must be registered close to the vegetation surface are air temperature, global solar radiation, air relative humidity (RH), wind speed, air water vapour pressure deficit (VPD) and precipitation. These parameters, in association with other plant and soil water content characteristics, directly affect a genotype's adaptation to water deficit (Gomide et al, 2005; Merva, 1995).

Acquisition of weather data should be done by means of an automatic or a standard weather station. The use of an automatic weather station (AWS) is preferable, since it can easily be configured and programmed to acquire data at short time intervals (eg, one hour). Direct evaluation of plant parameters, combined with some measurement of soil water content and microclimatic characteristics at the soil surface level, are always recommended to provide better quantification of the crop water deficit. This procedure is the key to explaining how certain genotypes adjust to water shortage condition better than others (Gomide et al, 2005). Climatic characterisation must be done with a time climatic series database of at least 15 years duration acquired from standards or from an AWS.

Description of specific sites

Sete Lagoas specific site

The climatic classification of the specific site at Sete Lagoas, MG, is *Cwa* according to Köppen (McKnight and Hess, 2000). This means that it is a savannah type of climate with a dry winter and wet rainy summer. The annual average air temperature is 21.1°C, with a thermal amplitude of ca 6°C. The lowest air temperature values fluctuate close to 11.5°C, registered in June and July. The highest values are in the range of 28.5–30°C, observed from January to March and from October to December. August and September are the driest months of the year with air RH of ca 58 percent, and January to March and December are the wettest months (RH ca 75 percent). The dry season begins in May and ends in September. During this period monthly rainfalls are in the range 9–40mm. The rainy season extends from November to March with precipitation rates of 150–290mm. The average annual precipitation is 1384mm. March to May show low values of wind speed, which starts to increase in June, but September shows the strongest winds (about 2m s^{-1}).

At the Sete Lagoas site, a climatic time series database of 45 years (from 1960 to 2005) was used to characterise the local climate. This database was provided by the 5th District of the National Institute of Meteorology (INMET), located at Belo Horizonte, MG. The soil water balance was determined using the recommended method of Thornthwaite and Mather (Sentelhas et al, 1999) in order to obtain information monthly throughout the year for ET_c, water deficit, water excess, and water storage in the soil. Figure 4 shows the hydrological soil water balance results obtained at the Sete Lagoas site. The calculations assume a total water availability in the soil profile of 100mm, to a depth of 1m (according to the soil type, this is the total amount of water retained between field capacity and permanent wilting). A deficit period was observed from May to September, during the winter or dry season, when the lowest average values of air temperature and total precipitation were registered. The greatest water deficits occurred in August and September (–42.4mm and –32.2mm, respectively). From November to March the deficiencies were zero, and at the beginning of April they resumed very low values (around –3.8mm). However, beginning in May, the deficiencies started to increase again (–13.6mm). The greatest values of excess water were noted in December and January (about 180mm).

Janaúba specific site

The annual averages of the main climatic elements at the specific site at Janaúba, MG, are the following: precipitation of 873.5mm, air temperature of 24.7°C and relative humidity of 65 percent. Again, according to Köppen's classification, the typical Janaúba climate is *Aw*, that is, savannah with dry winters and medium air temperatures of 18°C in the coldest month. The Penman–Monteith reference evapotranspiration (ET_o; Allen et al, 1998) shows the lowest values in June with an average of ca 3.4mm day^{-1}, and highest values in October with an average of about 5.0mm day^{-1}. An AWS was installed at Janaúba (Albuquerque et al, 2005).

The representative soil is a red-yellowish Latosoil, having a clay-sandy texture in the 0–20cm layer. The total available water in the soil was about 130mm m^{-1} of soil; this is the amount of water retained between the potentials of –10 and –1500kPa. The soil densities in the 0–20cm and 20–40cm layers were 1590kg m^{-3} and 1650kg m^{-3}, respectively.

A series of climatic data from 1977 to 1990 (ie, over 14 years) was used to compute the hydrological soil water balance, again according to the Thornthwaite and Matter method (Sentelhas et al, 1999; Figure 4). A very distinct period of water deficit was observed from March to November, coinciding with the winter season and even beyond, continuing throughout the summer season. The largest water deficit (above 70mm) in Janaúba was recorded from July to October. In February and March, the water deficits showed their lowest values (–2 to –5mm). However, starting again in April, the deficit began to rise sharply until November. A water excess was observed only January (ca 45mm).

Teresina specific site

At the specific site at Teresina, PI, a 25-year series of climatic data covering 1980–2005 was used to compute the annual averages of the main climatic elements and the hydrological soil water balance, according to the Thornthwaite and Matter method (Sentelhas et al, 1999; Figure 5). The annual average of precipitation is 1291mm, the air temperature 28.1°C and the relative humidity 71.9 percent. According to Köppen's classification, the typical Teresina climate is *Aw*, that is, tropical with a dry winter and a medium air temperature in the coldest month of 23.1°C. The ET_o was about 5.0mm day^{-1}. An AWS was installed at Teresina.

Automatic weather station

An AWS should be established at each environment target for drought tolerance phenotyping studies. The main purpose of the AWS is to provide climatic data for adequate control and management of the cropping systems and agricultural practices. Thus, it will facilitate decision-making on when to irrigate and how much water to apply, on evaluation and control of the water deficit, on pest and disease control, and on selection of the best planting dates and evaluation of the risks of

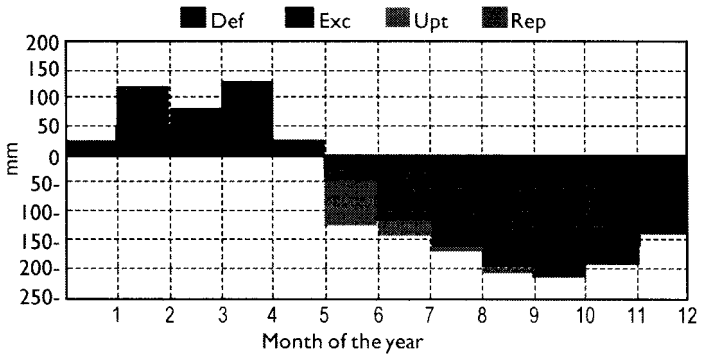

Figure 5. Monthly hydrological soil water balance variation at the specific site at the Embrapa Mid-North experimental fields at Teresina, based on a climatic time series database covering 25 years (1980–2005), and assuming a total soil water availability of 100mm to a depth of 1m.

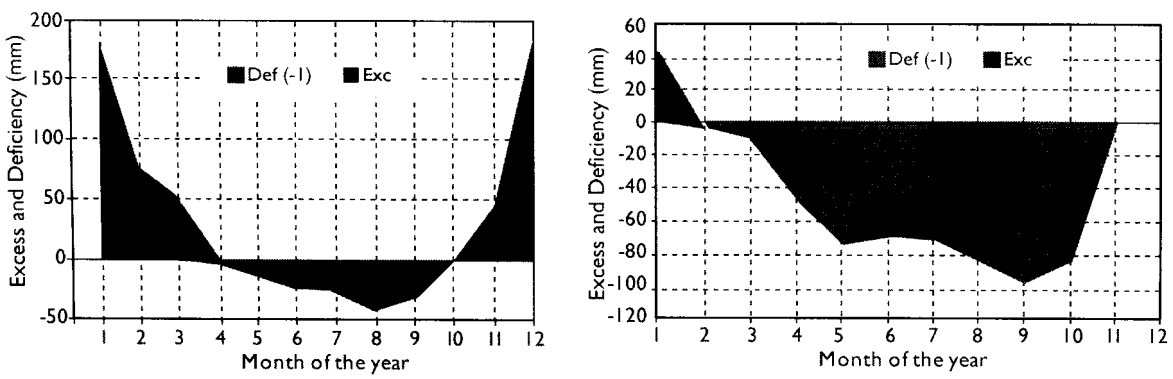

Figure 4. Monthly hydrological soil water balance variation at the specific sites at the Embrapa Maize and Sorghum experimental fields at Sete Lagoas (left), and Janaúba (right), based on climatic time series databases covering 45 years (1960–2005) and 14 years (1977–1990) respectively, and assuming a total soil water availability of 100mm to a depth of 1m.

unfavourable climatic conditions, etc. The station should be placed inside each specific site area, in a representative part of the field far away from obstacles such as buildings or trees. The distance from an obstacle should be at least 100 times its height, and the AWS should be surrounded by an irrigated grass field of at least 50m x 50m. The AWS should occupy an area of at least 12m x 18m with short grass as ground cover.

Minimum climatic parameters recorded at the AWS should include air temperature (maximum and minimum), relative air humidity, precipitation (by rain gauge), wind (speed and direction), solar radiation (global and net radiation if possible) and water evaporation from a US Weather Bureau Class A pan. Installation should follow the general layout of a weather station, with the correct location of instruments, equipment and sensors, according to manufacturer recommendation. The services of a technician will be required for equipment and sensor installation, calibration, and downloading of recorded data.

The Class A pan used to measure the water evaporation rate is 1.21m in diameter, 0.25m in depth, and is mounted about 0.15m above the surface of the ground with a water depth of 0.18–0.20m inside the pan. Pans are usually constructed of 20-gauge galvanised metal, and the water evaporation loss is measured automatically with a transducer sensor, or manually using a hook gauge in a stilling well.

An AWS will measure and, very precisely and accurately, record climatic parameters at ground level, at programmed time intervals by means of totally integrated digital electronic systems. These systems include sensors to provide electronic signals of the climatic parameters and dataloggers to process, store and transfer collected data (Gomide, 1998). Portable computers (notebooks) should be used with a direct datalogger interface for transfer of programs and data. The source of energy used to power an AWS in the field comes from solar panels and rechargeable batteries. In operation, these stations should be scheduled to scan all the sensors every 30–60 seconds, and to calculate and store the averages of the registered parameters every 30–60 minutes.

Figure 6 shows the AWS installed in the specific site at the Embrapa Maize and Sorghum experimental fields at Sete Lagoas, MG (latitude 19° 27′ 17.04167″ S, longitude 44° 10′ 22.71995″ W, altitude 731,214 m).

Air temperature (T_a in °C) and air RH (percentage) are registered at 1.5–1.8m height above the ground. The sensor used to measure T_a is a platinum resistance thermometer (TRP, 1000Ω), with ceramic sheathing. It is stable, reliable, and responsive, assuring a precise transduction of the T_a signal. For RH, a capacitive sensor is used which, like the resistance thermometer, produces an output signal from 0–1000mV. Both are mounted in a single probe placed inside a meteorological shelter (Vaisala, HMP 35C model; Figure 6).

The wind speed and direction sensors are made of aluminium for low weight and high corrosion resistance, and both are installed at 2–2.5m height above the ground. The wind speed, in m s^{-1} is measured with a three cup anemometer, which produces a signal, the frequency of which is detected by an optical chopper switch. The wind direction is measured in degrees from the true magnetic north in a clockwise direction, using a precise potentiometer sensor (Climatronics CS800-L model; Figure 6). Solar

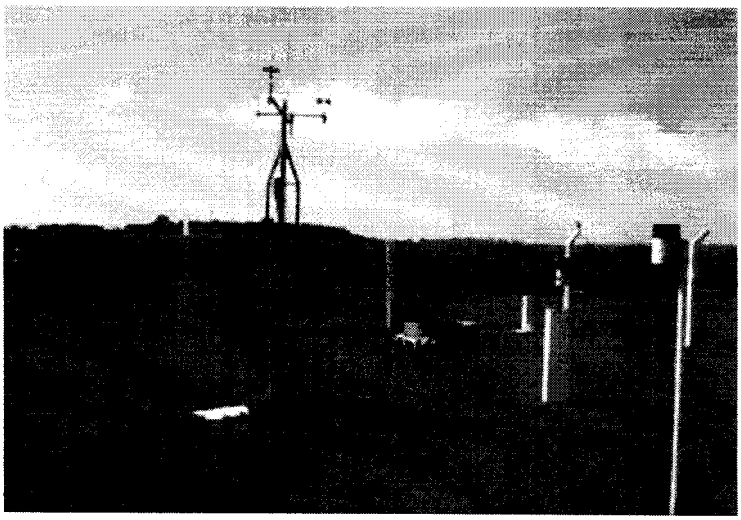

Figure 6. Automatic weather station installed in the Sete Lagoas specific site for local microclimate characterisation, ET_o determination, and irrigation water management.

radiation is registered in W m^{-2}, at 1.5–2.0m height above the ground, with a precise silicon photovoltaic sensor (LI-COR model; Figure 6), providing an output signal of 0.2kW m^{-2} mV^{-1}.

Precipitation is measured in mm h^{-1} using a tipping bucket rain gauge (Texas TE525 model; Figure 6), installed at a height of 1.5m above the ground. The unit is made of aluminium and consists of a funnel, a base and a bucket. The top cross section of the rain gauge is 158.8mm in diameter, and the sensor is calibrated to record precipitation with 0.25mm resolution through generating an electromagnetic pulse delivered to the datalogger. The water level variation in the Class A pan is registered with a sensor that provides an electrical signal proportional to the water level. The unit is made up of a floating device, a counter weight, a small metal chain, and a cogwheel that is attached to a precision potentiometer of 1kΩ (NOVALYNX model). This is all installed inside a polyvinyl chloride (PVC) column 20.3cm in diameter and 66.0cm high, with levelling screws at its base. The column works as a stabiliser well and is connected laterally to the Class A pan (Figure 6). The system is calibrated to give water level readings in mm.

Figure 7 shows the results of the daily variation of ET_o, in mm day^{-1}, determined by the Penman–Monteith method, and of average wind speed (U), in m s^{-1}, for the Sete Lagoas specific site. All of the data used in the calculations came from the AWS. The highest and lowest daily values of ET_o were in the range 4.8–6.6mm day^{-1} and 1.2–2.8mm day^{-1}, respectively. The average daily variation of the wind speed values were in the range 0.6–2.4m s^{-1}. The greatest and lowest ET_o monthly values were obtained from September to March (130–141mm) and from May to July (82–95mm). June showed the lowest daily average ET_o values (2.7mm) and October the highest (4.7mm).

Procedures for monitoring and controlling water stress for drought tolerance phenotyping

The procedures for monitoring and controlling water stress in plants and soils are recorded in consensus documents, developed and adopted by a team of Embrapa researchers to meet the requirements for identifying, characterising and selecting drought-tolerant genotypes of cereals and legumes in contrasting target environments. These documents form the basis for the following sections. They describe field and laboratory equipment including structures, recording instruments and irrigation facilities for the precise control of parameters such as surface climatic conditions, irrigation water application, soil water status, plant water status and evapotranspiration. Coverage includes the design, installation, calibration, evaluation, measurement, recording, storage, and transfer of data at each specific site.

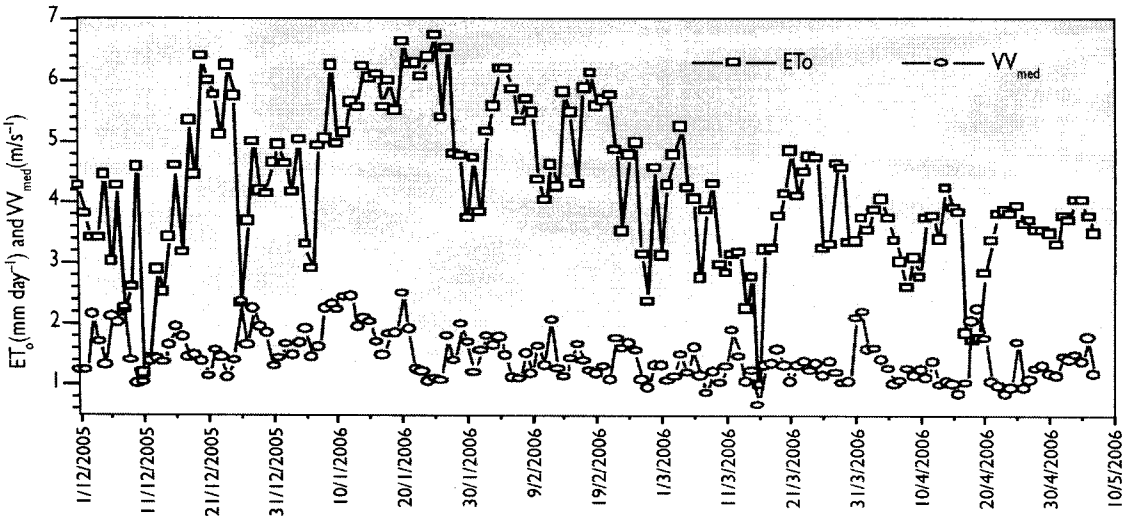

Figure 7. Daily variation of reference evapotranspiration (ET_o), in mm day^{-1}, and of average wind speed (VV_{med}), in m s^{-1}, during December 2005 to May 2006, at the AWS at the Sete Lagoas specific site.

Irrigation water application and control

Irrigation systems must be carefully selected to ensure optimum control of the irrigation water for crops in drought tolerance studies. A number of factors affect decisions about irrigation system selection in a given situation, including characteristics of the fields, soils, crops, cropping systems, available water resources, managerial and field labour, economic factors, and local support infrastructure. Guidelines are provided covering basic rules for irrigation system design, layout, and management.

An appropriate irrigation scheme design and layout at a specific experimental site are essential for the selection of drought-tolerant genotypes, in order to facilitate irrigation water control and management. The standard methodology of many drought research programmes does not take into account environmental variation in agroclimatic conditions, soil physical and chemical properties or cropping systems. Such consideration is, however, essential for the design of a suitable and economically viable irrigation system that is able to deliver a measured quantity of water at the root zone of each crop at regular intervals.

Irrigation scheme selection

An inventory of the resources available at a site, such as the water supply, the type of soil(s), climate and topographical conditions, and available labour and energy supply, is a prerequisite. Where water is in short supply and the evaporative demand of the atmosphere is high, a decision must be made at an early stage regarding the times at which water can be saved and allocated to irrigated plots. Wherever the water supply is limited and/or expensive, a localised irrigation scheme is indicated, because the amount of water and the frequency of application can be controlled very precisely, supplying water directly to the root zone through a closed-circuit system (Jensen, 1983; Keller and Bliesner, 1990). This approach saves water and it could be expected to use about 30 percent less water than a well-managed overhead sprinkler system. The low level of discharge required within a localised system will allow the use of low-yielding water sources such as springs, small dams and shallow wells. It may be necessary to measure river and groundwater table fluctuations and reservoir operations, together with periodic measurement of water quality and sediment content. Soil salinity should also be analysed at regular intervals.

Conventional sprinkler system

A conventional sprinkler system is commonly used for water application to annual crops. It delivers water to the crop through a network of main lines, secondary lines and lateral lines with sprinkler heads spaced along their length (Figure 8). Selection of sprinkler type depends on the shape and size of the area being irrigated, and the flow rate and pressure of the water supply. The most commonly used are rotating or impact-driven sprinklers with single or multiple nozzles. Full and part circle rotating sprinklers are used to irrigate large plot areas in the field. As water sprays from a sprinkler it breaks up into small drops, the drop size depending on the sprinkler operating pressure and the size of the nozzle. The uniformity of

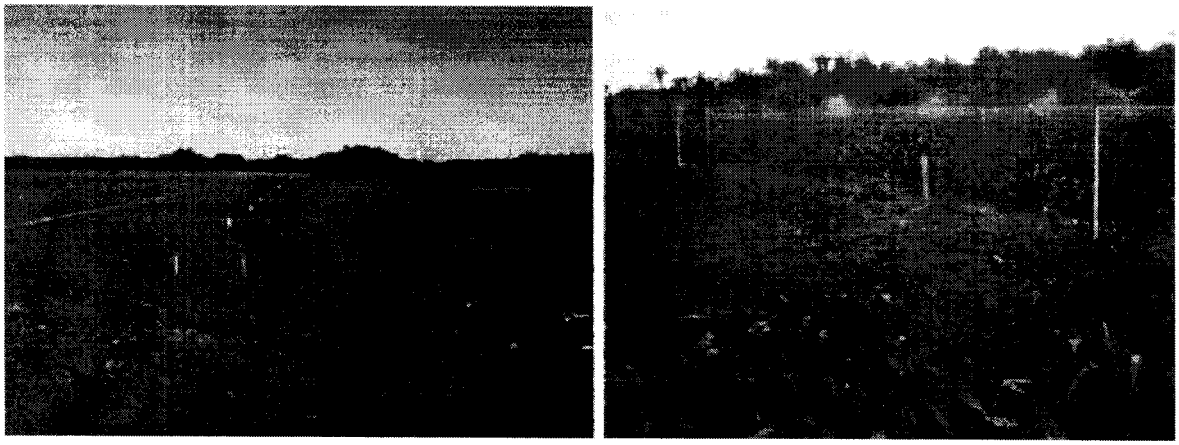

Figure 8. Conventional sprinkler irrigation system installed at the Embrapa Maize and Sorghum site at Janaúba (left) and at the Embrapa Mid-North site at Teresina (right).

application of water by rotating sprinklers depends on the geometry of the sprinkler, its angle of trajectory, nozzle size and operating pressure, as well as the spacing between sprinklers and the wind speed.

It is very important to obtain adequately uniform water distribution, such that the area watered by each sprinkler overlaps substantially with the area watered by the adjacent sprinkler. Accordingly, the sprinkler spacing must be designed to allow at least 100 percent overlap of watered areas to avoid great variation in the amount of water applied or even dry spots. Thus, each sprinkler should throw water all the way to the next sprinkler in each direction, known as 'head-to-head coverage or spacing' (Figure 8). The recommended spacing is 12m x 12m (spacing between sprinklers on the lateral lines and between lateral lines, respectively) or 12m x 18m. Sprinklers nozzle sizes of 3.5–4.5mm must be operated with pressures of 3.5–4.0kgf cm^{-2}. This provides sprinkler water flow rates of 1.14–1.75m^3 h^{-1} and average water application rates of 8.0–12.2mm h^{-1}. The values can change slightly depending on nozzle size, pressure and sprinkler spacing. It is important to follow the sprinkler manufacturer's recommendations regard specific installation, design and operating requirements.

Improper installation and operation of a sprinkler system will result in a lack of uniformity of coverage and will waste water. Water pressures higher than recommended tend to produce small-sized water drops, which are subject to evaporation and drift on the wind. Low water pressure decreases the radius of throw and fails to break up the water stream properly, causing poor uniformity of coverage. The sprinkler lateral lines must be installed on the level and positioned in a perpendicular direction (90°) or at 45° to the main line, the latter being the best position in relation to the prevailing wind direction. Since the lateral pipe line length is 6m, it is important to make the plot size length and width multiples of 6m in order to facilitate the layout of the irrigation system.

Localised irrigation system

A localised irrigation system, sometimes called 'drip irrigation' or 'trickle irrigation', delivers water to the crop using a network of main lines, sub-mains and lateral lines with emission points/drippers spaced along their length (Figure 9). Each dripper orifice supplies a precisely measured uniform application of water, nutrients, and other growth substances required directly to the root zone of the plant. The choice of emitter is based mainly on the flow rate, which must be uniform and constant along the lateral lines, sensitivity to obstructions or clogging, and also resistance to insect and rodent attack. Flexible polyethylene pipes are available with internal diameters of 10, 12.5 and 15mm. Water and nutrients enter the soil slowly from the emitters, moving into the root zone of the plants through the combined forces of gravity and capillarity. 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, moisture and nutrients taken up by the plant are replenished almost immediately, ensuring that the plant never suffers from water stress (unless a water limitation regime is intentionally applied), thereby enhancing the plant's quality and its ability to achieve optimum growth and high yield.

Figure 9. Drip irrigation system installed at the Janaúba and Petrolina sites.

The water applied by the emitters penetrates into the soil generating a wet 'bulb', the shape and size of which depend upon the type of emitter, the flow rate, the duration of irrigation, and the soil type. The infiltration of the water into the soil occurs in all directions but is more rapid in the vertical direction for sandy soils. A localised irrigation system is indicated on very light sandy soils with a high water infiltration rate (above 150mm h^{-1}) and low moisture retention capacity. These soil conditions are not adequate for surface or conventional sprinkler irrigation methods.

In general, the total operating pressure of a localised system will be 50–70 percent of a conventional sprinkler system. Thus, energy can be saved and operating costs reduced. Localised systems allow much easier and more efficient control of pests and weeds because the crop foliage is not wetted, nor is the entire soil surface, allowing access to the field at all times. The use of drip systems involves a higher frequency of irrigation when compared to other systems. This reflects its key principle, which is to maintain a moist sector in the root zone with relatively small applications of water applied continuously or intermittently.

Linear moving system

A linear moving irrigation system, sometimes called a 'lateral moving system', is built in the same way as one with a central pivot, with moving towers and spans of pipe connecting the towers. The main difference is that all of the towers move at the same speed and in the same direction following a straight line path. The water supply can normally be supplied to the linear moving system by connecting a flexible 'drag hose' (a special polyethylene material, 100 or 150mm in diameter and 150–400m in length) in turn to a series of hydrants attached to riser points from underground piping as the system moves along the field. The primary advantage of this system is that it can irrigate rectangular fields. The main component is a command unit, which can be located in the centre or at the side of the moving lateral line carrying the sprayers (Figure 10). This cart is responsible for the movement of the whole system which is designed to provide a water application rate of 2.5–40mm h^{-1}. The cart consists of a command tower housing the control panel governing the system speed and, consequently, the water application rate.

The most important rule is that the irrigation scheme selected must be designed to provide water application rates that are always lower than the basic soil water infiltration rates, in order to prevent soil surface ponding and runoff. The wind is the main climatic factor that directly affects the pattern of water application in sprinkler and linear moving irrigation systems, due to distortion and shift of smaller drops to outside of the irrigated plot. Wind speeds greater than 0.8m s^{-1} affect the water distribution in the field, mainly in conventional sprinkler systems. This wind effect can be reduced

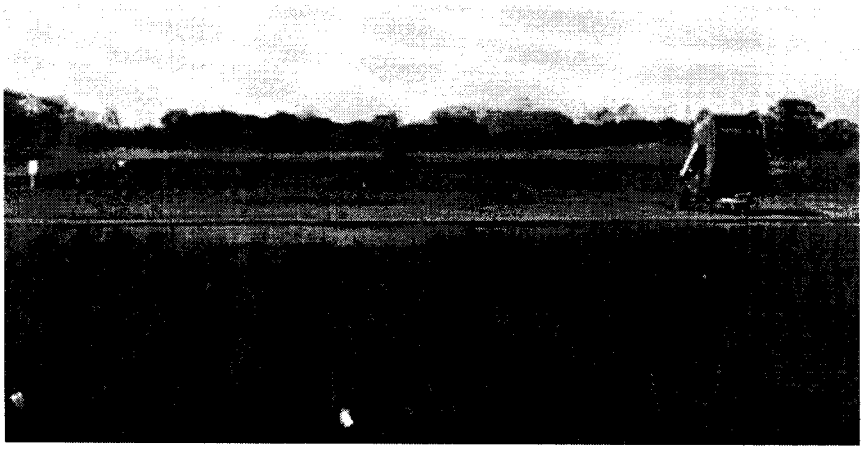

Figure 10. Linear moving irrigation system installed for rice at the Porangatu site.

by orienting the irrigation lateral or travel linear lines, ideally, at 45° or at 90° to the prevailing wind direction. It is recommended to irrigate at night if possible, since the wind speed decreases at night time in most environments. This is particularly the case for strong winds (> 1.5m s^{-1}) when irrigation should definitely not be conducted during the day time.

In all irrigation systems, clogging of nozzles (emitters) or crystal deposits in main and secondary lines can result in increased pump pressure but reduced flow at the sprinkler and emitter. Blocked intakes will reduce the operating pressure. An operating pressure below the design pressure greatly reduces the diameter covered, wet bulb formation, and uniformity of application.

Field calibration procedures for water application and distribution

The site-specific irrigation system equipment used in a drought phenotyping network must be calibrated in the field, or evaluated in accordance with existing design charts and tables following manufacturer recommendations. The specialists selecting and providing genotypes tolerant to water deficit in the field must also ensure that operators have been provided with guidance on calibration and adjustment for all field irrigation system equipment. Information presented in manufacturers' charts is based on average operating field conditions for relatively new equipment, and does not take into account environment parameters which affect changes over time in discharge rates and application rates as equipment is operated in the field (Merriam and Keller, 1978). As a result, the equipment should be field calibrated or evaluated regularly to ensure that water application rates and uniformity are consistent with values used in the system design and as given in manufacturers' specifications. Field calibration and evaluation involves collection and measurement of the water applied at several locations in the irrigated area. Step-by-step guidelines are provided here for field calibration of conventional sprinkler, linear moving, and drip irrigation systems.

The calibration of an irrigation system involves setting out collection containers distributed throughout the application area, operating the system, measuring the operating pressure, water flow rate and amount of water collected in each container, then computing the average application volume and rate and, finally, the uniformity of application. Generally, an in-line flow meter installed in the main line or sub-main line provides a good estimate of the total water volume pumped from the water source during each irrigation cycle. The average application depth in the whole field can be determined by dividing the pumped volume by the irrigated application area.

It is important to determine the uniformity of the depth of application when applying water to plots. Many types of container can be used for this such as standard rain gauges, pans, plastic buckets, jars, or anything with a uniform opening and cross section, provided that the container is deep enough (at least 10cm) to prevent splash and excessive evaporation, and that the liquid collected can easily be transferred to a graduated container for measuring. 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 the sprinkler nozzle (ie, the discharge elevation). As a general rule, the top of each container should be no more than 100cm above the ground and no more than 90cm below the sprinkler or nozzle discharge elevation. In addition, when positioning the containers, it is important to ensure that there is no interference from the crop canopy in order to avoid shielding by or water splash from the leaves into the collection container.

Field calibration and evaluation should be performed during periods of low water evaporation and when the wind speed is not strong. Suggested times are before 10:00 hours or after 16:00 hours. In order to minimise evaporation, readings of the depth or volume of the water collected should be taken as soon as the evaluation process ends or, for a linear moving system, when the system has completely passed over the row of collection containers.

Container setup for conventional sprinkler and linear moving irrigation systems

A conventional sprinkler irrigation system is evaluated by setting up a 2m x 2m grid of containers between four sprinkler heads of two adjacent lateral lines (Figure 11). A linear moving system is evaluated by placing at least three rows (transects) of water collection containers perpendicular to the straight line path of the linear movement of the system as

shown in Figure 12. These rows should be positioned at the beginning, middle, and end of the path, thus covering the entire length of the irrigated field. Placing two or more rows of collection containers at each position increases the accuracy of the calibration. It is important to space containers equally in the field. For lateral moving systems, place containers throughout. In the case of the linear moving system, it should be operated so that the minimum travel distance exceeds the diameter of the spray wetted area for the container rows closest to both extremities. The water depth or volume should be read as soon as all gauges are no longer being wetted.

Calculation of the uniformity of water distribution

Among the coefficients available to determine variation in the water depth applied by the irrigation systems, two are most commonly used. One is that recommended by Christiansen (1942), which adopts the absolute medium deviation of the water depth values as dispersion measurement, known as the 'coefficient of uniformity of Christiansen' (CUC). The other is recommended by Criddle et al (1956), and considers the ratio between the average of the smallest 25 percent water depth values and the average of all water depths values collected. It is defined as the 'coefficient of uniformity of distribution' (CUD).

The uniformity coefficients CUC and CUD are calculated by the following equations:

$$CUC = 100\left\{1 - \frac{\sum_{i=1}^{n} |x_i - x_{med}|}{n.x_{med}}\right\}$$

$$CUD = 100\left\{\frac{x_{25}}{x_{med}}\right\}$$

For CUC, expressed as a percentage, x_i represents the values of applied water depth, x_{med} is the general average of all values, and n is the total number of points sampled (collectors). For CUD, again expressed as a percentage, x_{25} is the average of the smallest 25 percent of water depths collected and x_{med} is the general average of all values.

In general, the acceptable minimum value of CUC is 80 percent. However, for the conventional sprinkler and linear moving irrigation systems used in the phenotyping environment sites for crop drought tolerance studies, a value of CUC of at least 90 percent should be ensured in order to have optimum irrigation water control and to quantify accurately the water deficit regime in use.

Several authors (Gomes, 1994; Gomide et al, 1978; Rocha et al, 1999; Rodrigues et al, 1997) have evaluated the sensitivity of different uniformity coefficients to changes in the irrigation system operational factors and the environment. They concluded that the spacing between lateral lines and sprinklers or emitters, the nozzle or emitter diameter, the operational water pressure and the wind speed, were most influential. In contrast, the duration of the water distribution tests and the height of the sprinkler heads were the factors that least influenced the coefficients.

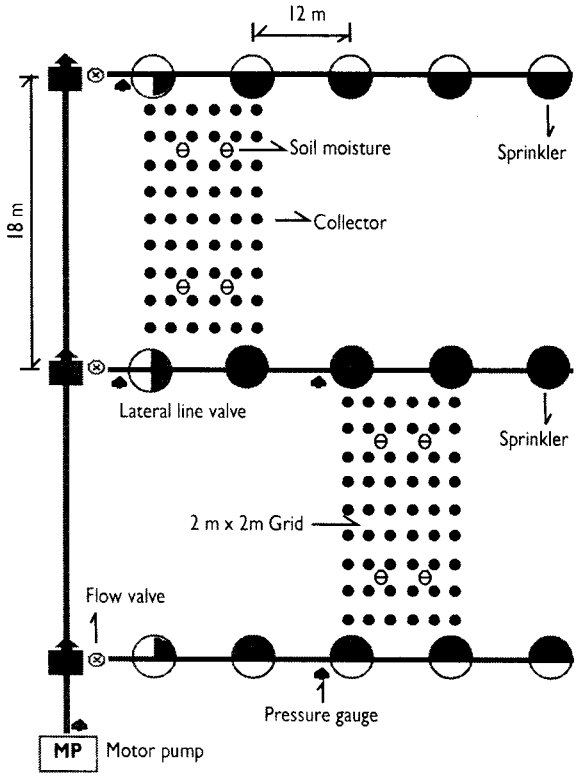

Figure 11. Location of collection containers and soil moisture sampling points to measure water depth of application and uniformity in an area irrigated by a conventional sprinkler system.

Procedures for the evaluation of applied water depth and distribution

Conventional sprinkler system

1. Determine the operational pressure of the irrigation system with a Bourdon manometer immersed in glycerin and graduated from 0 to 600kPa, with a resolution of 10kPa. Regulate this pressure by means of valves or regulators installed at the beginning of the lines of lateral sprinklers or emitters.
2. Carry out the test in a smooth area with no more than a 2.0 percent slope.
3. For a spacing between sprinklers of 18m x 12m, set up at least 54 (9 x 6) collectors between four sprinklers, spaced in the form of a 2m x 2m grid, and with four access points for measurement of the soil moisture content in the soil profile at a minimum of four depths (Figure 11).
4. Record the temperature and relative humidity of the air in an automatic weather station, located close to the area under calibration (no more than 100m away). Record the wind speed and direction at 10min intervals, with the sensor installed at 2.0m above the soil surface.
5. Determine water evaporation measurements using two collectors filled with a known volume of water and placed close to the test area at the beginning of the test. Place these two collectors out of the range of the irrigation system under evaluation.
6. Measure the soil moisture in at least four layers, either gravimetrically or by the use of sensors (neutron probe, time domain reflectometry, electric resistance blocks, Diviner 2000).
7. Calculate the water depth necessary to bring the soil moisture content up to the field capacity point (at the soil depth equivalent to the root zone).
8. Base the duration of the test (irrigation time period) on the water flow rate and the application rate of the irrigation system as calculated from the sprinkler or emitter manufacturer's catalogue.
9. Place the water collectors on level ground and facing upwards.

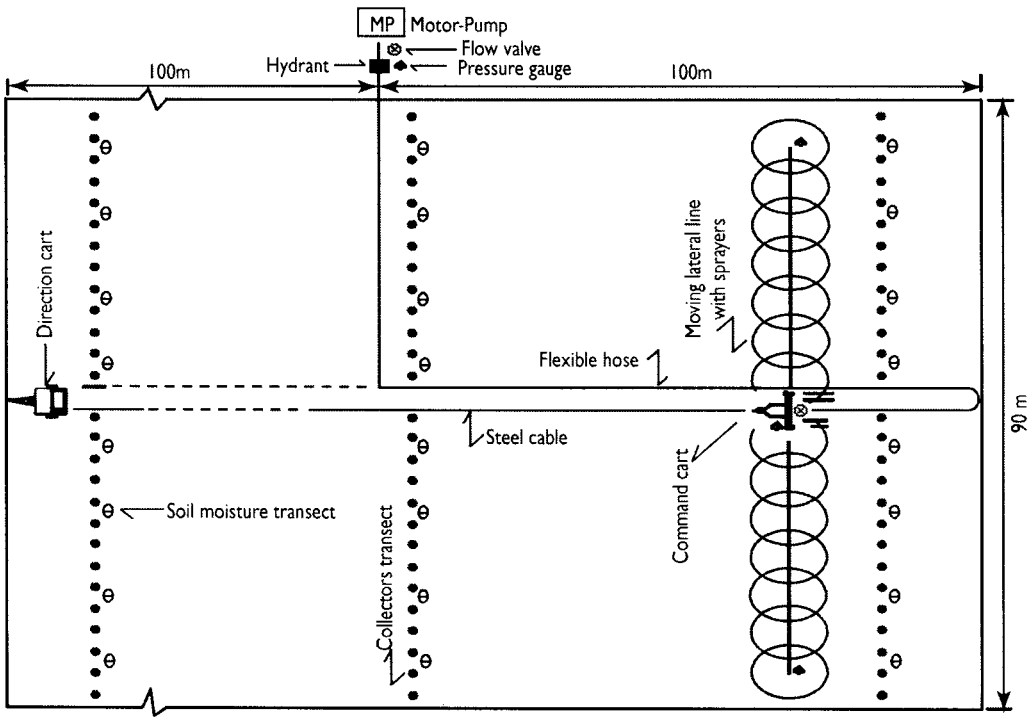

Figure 12. Location of collection containers and soil moisture samplings point transects to measure water depth of application and uniformity in an area irrigated by a linear moving system.

10. Turn on the pump to initiate the evaluation and calibration of the irrigation system. At the very beginning of the test, adjust the operational pressure of the system by means of valves or regulators, checking the pressure readings on the manometer.

11. Turn off the pump at the end of the irrigation period.

12. Measure the water volume accumulated in each collector using graduated 100 and 500ml gauges, and use the data to determine the coefficient of water distribution uniformity in the test area.

13. Finally, determine once more the average soil water content in the test area soil profile as in step 6 above.

Localised irrigation system

The uniformity of water application along the lateral line emitters in a localised irrigation system is directly related to the flow rate of the emitters. This flow rate can vary due to frictional losses along the tubes, the connection of the emitters, the gains and losses of energy due to elevation, the quality of the tubes, any obstructions and clogging, and effects of water temperature on the flow regime and geometry of the emitters (Howell and Hiller, 1974). The field calibration and evaluation procedure for the flow rate of the tubes and emitters should be done using a direct volumetric method, by means of a 100 or 500ml graduated gauge and a chronometer, using at least three replications. The pressure at the beginning of the main line, secondary lines, and irrigation lines (emitters) should be measured with a Bourdon manometer reading up to 100kPa, with 2kPa accuracy. It is necessary to carry out five flow rate tests at seven-day intervals.

Practical procedures for the field evaluation of the uniformity and the efficiency of existing localised irrigation systems should be used at sites. Uniformity in the amount of water applied is measured by the emission uniformity (EU), and expressed as a percentage. Non-uniformity can be caused by: (i) variability in distribution characteristics due to deficiencies in quality control in the manufacturing process; (ii) faulty or incompetent system design and management; (iii) use of operational pressures outside those suggested for the distribution system in question; and (iv) physical changes in the system that may have occurred with time.

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

1. Select an operational unit representative of average operating conditions in all operational units of the system.

2. Locate four lateral lines along an operating sub-main line within an operational unit: one lateral near the inlet end, one lateral near the far end, and two laterals evenly spaced in the middle section.

3. Measure, under normal operating conditions, the pressures at the inlet and at the far end of each lateral line. This will produce eight pressure readings.

4. On each lateral line, select two adjacent distributors at four different locations: at the inlet, one third and two thirds of the way down the lateral, and at its end point. In the case of a multi-outlet distributor, any two emission points can be chosen.

5. 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 3min, etc) to obtain a reasonable volume of water for each distributor (between 100 and 250ml). This will produce 32 discharge volumes measured at 16 locations.

6. Enter the information collected into a data sheet.

7. Calculate the average discharge for each pair of distributors. This will result in 16 average discharge values.

8. Use the average of the lowest four values of all of the readings as the minimum rate of discharge. This figure is used as the minimum to avoid the potential effect of one blocked or nearly blocked distributor on the total evaluation.

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

10. Calculate the field EU using the following equation:

$$EU = \frac{q_{min}}{q_{avg}} \times 100$$

where EU is expressed as a percentage and q_{min} is the minimum rate of discharge and q_{avg} the average rate of discharge per distributor or emitter (both in ml s^{-1}).

A typical field-determined EU value will range from 85–95 percent for localised systems but, in the phenotyping environment for crop drought tolerance studies, sites must be carefully considered to ensure that EU is at least 95 percent. This is in order to ensure optimum control of irrigation and to quantify and differentiate the irrigation water deficit regime in the region of the crop root system.

Application storage efficiency

The application storage efficiency (K_s) is the ratio between the average water stored in the root zone and the average amount of water applied. It is a coefficient that expresses the water storage efficiency of the soil, and takes into account unavoidable deep percolation as well as other losses. K_s values depend on soil management. Suggested values of K_s for different soils are 87, 91, 95, and 100 percent for coarse sand, sand, silts, and loam/clay soil types, respectively.

Application efficiency

The overall application efficiency (E_a) of localised irrigation systems may be calculated as follows:

$$E_a = K_s \cdot EU$$

Percentage of wetted soil

Normally, the percentage of wetted soil (P) is expressed in area or volume. The field evaluation of P involves monitoring the percentage of soil brought into active exchange with water and nutrients. The percentage of wetted soil as compared to the whole irrigated area depends on the water distributor discharge, spacing and soil type. In the field, the value of P should be checked at one location (ie, an emission point) on each of the lateral lines tested. It is best to select a different relative location on each lateral.

The probe of a soil auger or even a shovel can be used to estimate the extent of the wetted zone, penetrating about 15–30cm below the soil surface, around each location. The percentage value of P is determined by dividing the wetted volume by the surface area between adjacent emission points. In the case of an orchard, it will correspond to the area between four trees. A proper minimum value of P has not yet been established but it can be concluded that systems with a high P provide more insurance in case of systems failures, due to the higher soil water reserve, and allow easier scheduling. It seems that the minimum value of P is around 30–35 percent for arid regions. In the case of supplementary irrigation, a P of 15–20 percent can be used.

Irrigation water management

One of the main problems in the phenotyping environment sites used for crop drought tolerance studies is technicians' lack of knowledge and training to manage irrigation schemes adequately in order to ensure optimum control of irrigation water and precise characterisation of the irrigation water regime. A training programme on irrigation water management and field evaluation of the irrigation systems in use is likely to be necessary for the technicians since, in most cases, new technicians will be unfamiliar with the basic principles for operating and managing the equipment efficiently.

Correct timing of irrigation is essential mainly when water is in short supply or the evaporative demand of the atmosphere is high. This is the case for phenotyping environment sites where genotypes are submitted to a controlled field water deficit. Thus, decisions must be made regarding the timing of irrigation, involving information on and knowledge of irrigated cropping systems, including establishing the crop growth stage, the anticipated yield reduction due to induced water stress, and collection of climate and soil data. Data collection should be carried out on a continuous basis in order to have a record of changes through the growing season (Jensen et al, 1990).

The purpose of optimum irrigation scheduling is to ensure an adequate supply of soil moisture to minimise plant water stress during critical growth stages, resulting in water and energy savings with no yield loss. Basically, decisions on irrigation water management scheduling involve the following three questions:

1. How should the irrigation water be applied (the design of the right irrigation scheme)?
2. When should irrigate be carried out (timing)?
3. How much water should be applied (the depth of water)?

The answers to these questions are critical for the correct management decisions to be made on any irrigation system, whether it be conventional sprinkler, linear moving, localised-drip, etc.

Soil moisture measurements on drought tolerance field trials

Measurement of the water stored in the soil profile and the capacity of a given soil to store water are both important for precise definition of the irrigation regime (water stress) and control of soil water availability (SWA) to the root system. In the specific sites in Brazil, the capacity of soils to store available water to be used by the growing crops changes a lot. These changes are important for the drought tolerance studies, because the depth of water to apply through irrigation and the interval between irrigations are both influenced by the storage capacity of the soil. Some of the specific sites have sandy soils. These sites do not have a large water-storage capacity. Consequently, the irrigation must be frequent in order to avoid high water stress levels and resultant large reductions in yield.

In drought tolerance field trials, crops are subjected to water stress, which can be imposed throughout the whole cycle or at certain stages. The stress application period, duration and intensity depend on the susceptibility of the crop to stress and the objectives of the study. In Brazil, for crops such as sorghum grown for the second harvest (*'safrinha'*), the stress is applied after flowering. For crops that might be subjected to dry spells at early stages, the stress is applied prior to flowering.

Simply cutting the water supply via irrigation does not mean that a crop will suffer water stress. Soil water retention capacity differs from soil to soil and this must be taken into consideration when planning a drought tolerance field trial. On the other hand, it is not desirable that a crop undergoes permanent wilting and dies. The level of stress that a crop will suffer depends on the interaction between plant, soil and weather. It is crucial, therefore, that some sort of monitoring be done on these three elements. Monitoring the soil water and plant water status allows stress level quantification, and helps make the decision on when to interrupt water stress.

Evaluation and measurements of soil moisture content

The soil water status can be quantified in terms of feel or appearance and actual water content, expressed as the volume of water per volume of soil, or as matric potential. Soil water matric potential values are normally negative, indicating that the larger the absolute value, the smaller is the potential. The higher the soil water content, the larger is the potential. The relationship between those two quantities is called the 'soil water characteristic curve' or 'soil water retention curve' and can be used to convert one quantity to another. In drought tolerance trials, either soil water content or soil water potential can be measured, and there are many methods to measure or estimate both. One of the oldest and yet considered as the standard is the gravimetric method. Other procedures have also been developed and tested, among them the neutron probe, time domain reflectometry and frequency domain reflectometry. A complete description can be found in specific texts (Andrade et al, 2007; Hillel, 1982). A brief description follows of the methods more appropriate to drought tolerance trials.

Feel and appearance

If there is no equipment or there are no sensors to evaluate the soil moisture content, an old and widely used method is to look at the soil and to feel it. Using the soil auger, samples can be obtained readily throughout the root zone of the crop. Tables published by Hansen et al (1980) should be used as a guide for judging how much available moisture has been removed from the soil by means of feeling and evaluating its texture.

Gravimetric determination of soil moisture content

In this method, 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 a lid, and kept closed and sealed with adhesive tape until taken to the laboratory to be weighed. After weighing, the sample is dried in an oven at 105–110°C until a constant weight is reached. This might take 24–48h for soils with a sandy or loamy texture. Heavy textured soils might take longer to reach constant weight. The differential weights can be used to calculate the soil water content. If the sample is from disturbed soil, the water content is expressed in kg kg^{-1}. If the sample is from undisturbed soil, with a known volume, it is expressed in m^3 m^{-3}.

The gravimetric method has the advantage of being simple, accurate and dependent on relatively simple equipment such as an auger, cans, a precision balance and an 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 taking composite samples must be used to minimise such problems. One drawback of the gravimetric method is that it is destructive, and every time a sample is collected, a hole is left in the soil profile. By the end of a season the experimental area could be full of holes. Specifically in drought tolerance trials, sampling has to be done on both fully irrigated and under-stress plots. In some soils, it might be very difficult to auger dried soil by hand, making it necessary to use a mechanical or motorised sampling device.

Some care must be taken to minimise errors when using the gravimetric method:

1. Plan the soil sampling. Set aside an area for augering. It is desirable that the soil sampling area coincide with the irrigation depth measurement area.
2. Prepare all apparatus for soil sampling and laboratory analysis. Identify the cans and their lids. Prepare a sheet for taking notes.
3. If undisturbed samples are to be collected, prepare the volumetric rings or cylinders. Check the containers one by one after labelling them.
4. Clean the cans, lids and rings. In particular, remove the glue residue from sealing tapes.
5. Weigh cans with their respective lids or the volumetric rings. This information is necessary to calculate the soil net weight. Never use an average weight for cans or rings, and recheck the weight from time to time.
6. Mark depths on the auger handle in order to check sampling depths; sampling at the wrong depth is a common error.
7. Auger at about 10cm from the crop row, so that the active rooting system soil is sampled. Remove weeds, mulching or anything that might contaminate the soil sample. Clean the auger by hand every time the soil is going to be used to put in the cans. Before taking a sample that will be put into a can, clean the hole by augering and eject the soil. This avoids contamination of lower layers by soil or other material from the surface.
8. The weight of the sample must be between 200 and 400g. Smaller samples are not desirable unless a composite sample is to be used. Larger samples take up too much space in the oven.
9. Put each soil sample in a metallic can, fit the lid and seal it immediately. Volumetric rings have their own lids. Use paper adhesive tape to seal the cans or rings. Plastic tape is not good for this purpose. Never keep samples in plastic bags.
10. If possible, keep cans in a thermal insulated container or keep them in the shade until they can be taken to the laboratory.
11. To minimise the effects of spatial variability and of non-uniform soil water distribution, use sampling replication and/or composite samples.
12. Take the samples as soon as possible to the laboratory, remove the tapes and weight them with the lids on. Never leave unsealed cans to be weighed later.
13. Remove the lid from each can and put it on the bottom of the can. Put the cans in the oven. Weigh the cans with their respective lids after 24–48 hours. Dispose of the soil and clean the cans for use in the next sampling. Use steel wool to clean the cans and take care to remove any remaining glue from the tape seals.
14. When working with a certain soil type for the first time, weigh the cans after 24, 48 and 72 hours in the oven. This will allow definition of the drying time based on reaching a constant weight.
15. Use a good resolution, precision laboratory balance to weigh the cans, ideally a balance with three decimal place resolution and with the weighing platform shielded from air movement.

Neutron probe

The neutron probe employs a radioactive technique to measure the soil water content (Andrade et al, 2006). An access tube is inserted into the soil profile up to the depth where the soil water content is to be measured. The top 20cm of the access tube is left outside the soil surface. The neutron probe is fitted in the top of the access tube allowing the probe to be lowered inside it. An access tube made of aluminium, steel or PVC can be used. Readings are taken in 30–60 seconds at each soil depth. After taking a reading at one depth, the probe is lowered to the new depth and a new reading taken.

The major advantage of the neutron probe is that it measures soil water content at the same point every time. Another advantage is that the neutron

probe element reads a large soil sphere ranging from 20–60cm in diameter. This minimises the influence of the soil's physical properties or of spatial variability in the soil water content.

One disadvantage of the neutron probe is that it can not be automated to monitor the whole soil profile and is not appropriate to measure water content at depths less than 20cm. For some crops, especially under no-tillage conditions it is desirable at times to take measurements at the soil surface. Special types of probe have been produced for this purpose. The major disadvantage of the neutron probe, however, is the use of radioactive materials (americium and beryllium) to emit the neutrons. This presents a health risk to the operator if care is not taken when using the equipment. A nuclear techniques permit is usually required from the local or national authority for the use of neutron probes. Strict safety rules need to be followed including operator training, use of radiation monitoring devices, and provision of a special place to store the equipment.

The following recommendations are offered to guide use of the neutron probe:

1. Calibrate the neutron probe for each soil type on which it is going to be used in the field, preferably in the area where the trial will be conducted in order to produce consistent results.
2. Never take readings when the internal battery is low in charge.
3. Check the depth markers of the probe cable; readings at wrong depths are a common error.
4. Use proper access tubes; aluminium tubes are preferable, although rigid PVC tubes can be used. Calibration is necessary for each tube type. The probe must fit snugly into the access tube but must not get stuck in it.
5. Leave 20cm of tube above the soil surface. Put a rubber stopper at the bottom and a lid at the top of the tube to avoid moisture accumulating inside it. Clean the inside of the tube periodically.
6. Care must be taken during installation of the access tube. It must fit tightly into the soil profile. Use an auger of the right diameter; some users prefer hammering the tube into the soil using a rubber hammer and augering it from inside. If necessary, wet the soil to facilitate augering and access tub installation. Raise the soil close to the tube at the surface to avoid preferential water infiltration.
7. Calibration must be done for a wide range of soil water contents. Wet the soil if necessary, but avoid abrupt transitions between wet and dry soils and the wetting fronts. In wet soils, take 60 second readings and immediately collect undisturbed samples.
8. If there are differences in the physical properties of the soil within the soil profile, calibration must be done for the different layers.
9. During calibration, the soil water content must be determined by the gravimetric method, using volumetric rings or cylinders to get results directly in $m^3\ m^{-3}$.
10. Install an access tube with at least one metre outside the soil surface near the trial area, so that the neutron probe standard reading can be taken. Such readings are required in order to account for radioactive decay and also for the effect of air temperature.
11. Keep the neutron probe locked in an appropriate place when not in use.

Despite the disadvantages and the necessary precautions, the neutron probe is still considered to be one of the best instruments for soil water monitoring, producing consistent results over time.

Time domain reflectometry

Time domain reflectometry (TDR) is one of the most promising methods for soil water content measurement since it does not pose any risk to human health, and can be as accurate as the neutron probe. Although, according to the manufacturers, TDR need not be calibrated for common mineral soils, for research purposes it is desirable. TDR can detect small variations in soil water content and can also be used to take measurements in small volumes when necessary. Large soil volumes can be sampled depending on the way the TDR probes (termed 'wave guides') are installed.

The instrument can be fully automated by using data loggers and multiplexers. The major disadvantage of TDR is the high cost, especially when automation is required (Andrade et al, 1998). 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 air). The average error on soil water content measurements using TDR is around 3 percent but can be lower if the equipment is calibrated locally.

Different sizes and shapes of wave guide allow soil water monitoring from the very top layer up to a depth of about 1.5m into the soil profile. A soil water content profile can be obtained by opening a trench and installing wave guides at many soil profile depths. The major disadvantage of models with access tubes or segmented wave guides is difficulty in installation. A perfect fit is required between the wave-guide or access tube and the soil. Any gap left can lead to inaccurate readings.

Recommendations for using TDR are:

1. Calibrate the equipment locally to increase accuracy.
2. During calibration, sample close to the wave guide rods since the distance the electromagnetic wave penetrates into the soil is only of the order of centimetres.
3. Use longer wave guides if the average soil water content to be monitored is low, as is the case in drought tolerance trials.
4. Use coated wave guides if the soil is saline.
5. Install the wave guides vertically if the average water content of a thicker soil layer is desired. Horizontal installation allows a detailed sampling (in thin layers) of the soil profile.
6. Follow manufacturer's instructions regarding cable lengths and multiplexing; long cables can be a source of error.

Frequency domain reflectometry and capacitive sensors

These types of sensor use an oscillator to generate an alternating current field, which is applied to the soil to detect changes in its dielectric properties, in a way similar to TDR. Capacitive sensors are built with a pair or a set of stainless steel rods or concentric rings which, when inserted into the soil, form a capacitor, having the soil as the dielectric material. The capacitor works in synchrony with an oscillator. Changes on the soil water content affect the dielectric of the capacitor, in turn altering the oscillator's operating frequency.

Frequency domain reflectometry (FDR) sensors work similarly to capacitive sensors. They employ a set of different frequencies to detect the soil water content. The resonant frequency is related to the soil water content. Some sensors use access tubes and do not get into contact directly with the soil. By using various sensors assembled in a rod, the soil water content of different depths of the profile can be measured simultaneously. In such an assembly, full automation is possible by using data loggers. Portable devices can be built and this makes the instrument competitive in terms of cost with TDR, the latter being less portable for hand readings. The accuracy of FDR and capacitive sensors is less than that of TDR sensors, although local calibration can improve accuracy.

One of the major disadvantages of FDR and capacitive sensors is the influence of soil air pockets on the water content readings, especially those that operate within access tubes. Access tube installation is crucial in this case. Also, for devices that operate with frequencies less than 20MHz, errors due to soil salinity can be large (Murphy, 1996).

Recommendations for using FDR and capacitive sensors are:

1. For sensors that must be pushed into the soil to get a reading, make sure the rods are not bent; this would affect the readings.
2. For dry soils, as is the case of drought tolerance trials, it is quite difficult to insert the sensor rods into the soil.
3. Any disturbance in the soil that produces a void close to a rod will affect the water content determination.
4. A great deal of experience and care is required when dealing with sensors that use access tubes. The efficacy of access tube installation will, in general, dictate the quality of the data set that will be collected.
5. Do not auger a hole to insert the access tube in it; auger from inside the tube and push it into the soil. A special apparatus is required for this type of installation, which is costly, time consuming and requires a lot of patience.
6. Calibration of these sensors requires extra care. As in TDR, the soil volume sampled by the sensor is small. Undisturbed soil samples must be taken close to the rods or to the access tube.

Methods for monitoring 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. The simplest instruments available on the market measure only the soil matric potential. Two methods are available, the tensiometer and the resistance block.

Tensiometers

The tensiometer, a device that is still useful, consists of a ceramic tip connected on one side to a tube and on the other side to a pressure gauge. The device, filled with water, is inserted into the soil at the desired depth. The soil water equilibrates with the water in the tensiometer's ceramic cup, generating pressure – positive or negative – inside the ceramic tip. When the soil is saturated the pressure is positive, when it is dry the pressure is negative. The pressure recorded by the pressure gauge represents the soil water matric potential. Nowadays, pressure meters are built with electronic transducers, assembled as portable meters or individually connected to data loggers, allowing full automation.

The basic tensiometer is a simple and relatively cheap device but, for fully automated models, the cost can rise quickly. When properly tested, installed and operated, the tensiometer can generate consistent data sets. Measurement is rapid, although the response time can be limiting for some applications. The operational range of tensiometers is from +20 to –80kPa. Therefore, they are only useful for monitoring the soil water potential of fully irrigated treatments or prior to the onset of water stress.

Recommendations for using tensiometers are:

1. Let the tensiometers soak for at least 24h before testing and installing in the field. Use a syringe to remove air bubbles from the ceramic cup.
2. Check each tensiometer for leakage with a pressure pump before installing in the field. In addition, fill with water and allow the water to evaporate from the ceramic cup. Check if a vacuum is developing within the tensiometer.
3. Using distilled water is desirable. If no distilled water is available, use tap water that has been boiled for at least 30min.
4. Properly mark the desired depths in the tensiometer tubes. Install them using a screw auger. Make sure that there is good contact between the ceramic cup and the soil.
5. Service a tensiometer when the water level within it drops more than 1cm bellow the base of the rubber stopper. This is a requirement for most tensiometers.
6. Tensiometers with a mechanical Bourdon type of vacuum gauge are not suitable for research data collection because they are not sufficiently accurate.

Resistance blocks

Resistance blocks have been used for many years to relate the electrical resistance between two electrodes inserted into the soil to its matric potential. Resistance blocks are manufactured from different types of material: gypsum, nylon, fibreglass and a combination of materials in the porous media used for the construction of the electrodes. All materials allow sensitivity to the presence of salts in the soil, but those made out of fibreglass are the most sensitive.

The advantages of resistance blocks are their simplicity, the possibility of being 'home-made' and the ease of automating data collection. The major drawbacks are their inaccuracy, especially at high soil water potential/wet soil, large variation among blocks particularly when locally made, and the effect of soil temperature and soil solutes on readings. In addition, when made of pure gypsum, blocks might loose material by reacting with the soil with which they are in contact (Andrade et al, 1998). Resistance blocks need to be calibrated and, in some cases, calibration in groups is required.

Recommendations when using resistance blocks are:

1. Soak the blocks for at least 24h before testing and installing them.
2. Check each one for failure of the electric circuitry by using an electronic resistance meter.
3. Properly mark depths on the wires.
4. To facilitate installation and removal, fix the blocks to a piece of PVC pipe using epoxy glue.
5. Install the blocks the same way as for tensiometers. Make sure there is good contact between the blocks and the soil.

6. Some resistance blocks are made out of a mix of gypsum and sand. They are good for accessing high soil water potential/wet soil but do not allow readings lower than –200kPa. For the level of stress normally applied in drought tolerance trials, those types of block are useful only for fully irrigated plots and prior to reducing irrigation on stressed plots.

Available soil moisture – upper and lower limits

Field capacity

When gravitational water has been removed from the soil, the moisture content is called 'field capacity' (FC). This is the upper limit of moisture available to the crop. In practice, FC is usually determined over a few hours to one day for light (sandy) soils and over about two days for heavy soils. Normally, the soil moisture tension at FC is 0.1 and 0.3atm for light and heavy soils, respectively.

Permanent wilting point

The soil moisture content when plants are permanently wilted is called the 'permanent wilting point' (PWP). This point will occur at the lower end of the moisture range available to the crop. A plant will wilt when it is no longer able to extract sufficient moisture from the soil to meet its water needs. Permanent as well as temporary wilting depends upon the rate of water use by the plant (ie, the crop water requirement), the depth of the root zone, and the water holding capacity of the soil. Normally, the soil moisture tension is 15atm at PWP. The total soil moisture available to the crop is the difference in moisture content of the soil between the FC and the PWP.

Soil moisture release curve

It is recommended that the soil moisture release curve be determined in a soil physics laboratory where the standard procedure is available. This curve will provide technicians with details of the changes in soil moisture content for different soil moisture tensions. The soil moisture content should be determined at the following tensions: 0.1, 0.3, 0.7, 1.0, 8.0 and 15.0atm. It is also necessary to know the soil bulk density (D_{sb}) to obtain the soil moisture content on a volume basis (θ_{vol}).

Soil moisture instrument installation procedures

Soil moisture instruments are used to determine the soil moisture content, the water movement pattern in the soil, and in some cases, to initiate the irrigation cycle. Tensiometers and Buoyoucos scale hydrometers have been used to measure soil moisture content.

Typical minimum procedures for installing the instruments in irrigated fields with relatively uniform soil conditions and crops are:

1. Install three to four stations per irrigation treatment.
2. Monitor three to four depths covering the range 20–100cm at each station and depth placement, with the following soil profile distribution: above the maximum active root zone; near the bottom of the active root zone; and midway between the top and bottom positions. In addition, a shallow depth might be needed temporarily where seedlings are being established.
3. Ideally, locate tensiometers within the crop root zone. If near an irrigation lateral line, locate at least 60cm away from emitters.
4. Select conditions to be representative of the soil in the plots and with vigorous, disease-free crops.

Calculation of soil water availability

The following SWA parameters are important for adequate management of the irrigation schemes in different drought tolerance phenotyping sites, in order to ensure optimum control of irrigation water and for precise quantification and differentiation of the irrigation regimes. These parameters should be computed as follows:

Total soil water availability

The total soil water availability (SWA_t) can be calculated as follow:

$$SWA_t = [(FC - PWP) / 10] \cdot D_{sb}$$

where SWA_t is the total soil water availability (in mm cm^{-1}), FC and PWP are the field capacity and the permanent wilting point (as a percentage on a dry weight basis), and D_{sb} is the bulk density of the soil (in g cm^{-3}).

Real soil water availability

In irrigated plots, the soil moisture content should never be allowed to reach the PWP, in order that between two successive irrigations only a fraction of the real soil water availability SWA_t would be used. Thus:

$$SWA_r = f \cdot SWA_t$$

where f is the soil moisture availability factor. Normally, f values cover the range 0.2–0.8 for most crops. Water stress sensitive crops genotypes usually have small f values. Within each crop group, the f value to be selected will depend on the crop's sensitivity, the water deficit level established, and the crop evapotranspiration rate of the location.

Determination of crop water requirement

The crop water requirement or evapotranspiration (ET_c) is a combined process of transfer of water, as vapour, from the vegetated surface to the atmosphere, including direct evaporation from the soil surface and the plant's direct transpiration. In the water vapour transfer process, the climatic elements of the atmosphere control the water demand, which acts as a drain in the soil–plant–atmosphere continuum. The scheduling of the irrigation should follow the variation in crop water requirements in the different environments during the crop growing season, in order to achieve high water-use efficiency (WUE) and high crop production (Doorenbos and Kassam, 1979). The timing of irrigation and the quantity of water to apply are directly related in most cases. Thus, the important issue is to have a method to determine the ET_c.

The ET_c under a localised irrigation system may be different from that under surface and other pressurised systems (overhead sprinklers, central pivot or linear moving systems), primarily because the land area wetted is reduced, resulting in less water evaporation from the soil surface. ET_c values are influenced mainly by climate and plant properties, and are usually expressed in units of water volume per unit land area (ie, depth) per unit time.

As a first step, it is necessary to calculate the ET_c value for each genotype at the actual study sites, in order to determine precisely the irrigation water regime and ensure optimum control of irrigation water application. ET_c can be calculated most accurately using weighing lysimeters to directly measure the amount of water used by the crop under field conditions. However, such procedures are expensive, laborious and time-consuming.

The potential evapotranspiration (ET_p) or reference evapotranspiration (ET_o) should be calculated by the modified and combined Penman–Monteith method. This considers the case of a hypothetical reference crop, and is in agreement with both the original ET_p concept of Penman and the FAO ET_o concept (Allen et al, 1998; Doorenbos and Pruitt, 1977; Smith et al, 1991). The ET_o should be determined by the following equation:

$$ET_o = \frac{\delta}{\delta + \gamma^*}(R_n - G)\frac{1}{\lambda} + \frac{\gamma}{\delta + \gamma^*}\frac{900}{T + 275}U_2(e_a - e_d)$$

where ET_o is the reference evapotranspiration of a hypothetic crop (in mm d^{-1}), R_n is the net radiation (in MJ m^{-2} d^{-1}), G is the soil heat flux (in MJ m^{-2} d^{-1}), T is the air temperature (in °C), U_2 is the wind velocity at 2m in height (in m s^{-1}), $(e_a - e_d)$ is the air VPD (in kPa), δ is the slope of the saturation vapour pressure curve (in kPa $°C^{-1}$), λ is the latent heat of evaporation of water (in MJ kg^{-1}), γ^* is the modified psychometric constant (= 1 + 0,33 U_2, in kPa $°C^{-1}$), and 900 is the unit conversion factor. The climatic measurements can be obtained from an AWS or an automatic data acquisition platform in real time, and are used to calculate ET from existing methods to determine ET_o. The calculation of crop ET or ET_c is done by multiplying the ET_o value by the crop coefficient value (K_c). The whole process can be performed automatically, since the climatic data acquisition up to the point of calculating ET_c is directly available to users. This procedure supports decision making on when to irrigate and how much water to apply. Care should be taken to use appropriate K_c values for different crops and specific sites (Gomide, 1998; Gomide et al, 2001a).

The measurement of water evaporation (E_p) from a Class A pan can also be used to determine ET_o by means of the formula:

$$ET_o = K_p \cdot E_p$$

where K_p is the pan correction coefficient of E_p and is taken from a table that describes variation in K_p as a function of the size and type of the pan location, wind speed, and air RH (Doorenbos and Pruitt, 1977). The table was created using experimental data from several regions of the world, involving different climatic and plant conditions (albedo, rugosity, stomatal resistance).

Later, Snyder (1992) developed the following equation to allow interpolation of the K_p values from the table in order to facilitate their use in an automatic data acquisition weather station:

$$K_p = 0.482 + 0.024 \text{ Ln } (D_b) - 0.000376 \text{ U} + 0.0045 \text{ RH}$$

where D_b is the distance of the fetch (size) of the area where the pan is installed (in m), U is the wind velocity (in km d^{-1}), and RH is the average relative humidity of the air during the day (in percent). This equation must be used only within the limits of D_b, U and RH according to the original table.

In brief, the procedure to calculate ET_c is divided into three steps as follows:

1. Select a method to calculate ET_o (modified Penman–Monteith, Class A Pan) based on available climatic data and using mean data, analysing the magnitude and frequency of extreme values of ET_o and presenting the frequency distribution of ET_o. For Class A pan data: $ET_o = K_p \cdot E_p$.
2. Select the crop coefficient K_c which is defined as the ratio between ET_c and ET_o when both apply to large fields under optimum growing conditions. It is necessary to know the crop growth period and to determine some crop characteristics, such as time of planting or sowing and growing period. Thus, K_c should be extracted from the table in the FAO Irrigation and Drainage Papers 24 and 33 (Doorenbos and Pruitt, 1977; and Doorenbos and Kassam, 1979 respectively) for a given crop and stage of crop development under prevailing climatic conditions.
3. Calculate ET_c values using: $ET_c = K_c \cdot ET_o$ or $ET_c = K_p \cdot K_c \cdot E_p$. This ET_c calculation refers to irrigation schemes applying water over the whole irrigated area.

Crop water requirements in localised systems

Localised irrigation is used mainly for orchard crops (mango, citrus, guava and avocado) and row crops (cereals and legumes), where only part of the soil surface is occupied by the crop. The ET_c under localised irrigation systems is different from that under surface and other pressurised irrigation systems (overhead sprinklers, central pivot or linear moving systems) primarily because the land area wetted is reduced, resulting in less water evaporation from the soil surface. Consequently, a reduction factor should be applied to the conventional ET_c calculations. Freeman and Garzoli (see Vermeiren and Jobling, 1984) suggest the following expression to determine the reduction factor (K_r):

$$K_r = GC + 0.5 (1 - GC)$$

where GC is the ground cover (the percentage of the total surface area actually covered by the foliage of the plants when viewed from directly above). By using this relationship, the K_r values given in Table 3 can be obtained.

Thus, the crop water requirement for localised systems (ET_{cloc}) is given by:

$$ET_{cloc} = K_r \cdot ET_c \text{ or } ET_{cloc} = K_r \cdot K_p \cdot K_c \cdot E_p$$

Irrigation depth

Irrigation depth (ID) is the amount of irrigation water required by a crop genotype to ensure that it receives its full water needs or a predetermined proportion thereof (ie, under water stress). ID may often have to be greater to allow for possible losses in the irrigation system, such as leaching, deep percolation, or uneven distribution of water. On the other hand, if the plants are receiving some of their water from other sources such as rainfall, ID may be considerably less than ET_c. The net irrigation depth (ID_n) is defined as the depth of irrigation water required for normal crop production over the whole cultivated area, minus contributions from other sources. The gross irrigation depth (ID_g) is defined as the depth of irrigation water required for normal crop production over the whole cultivated area, minus contributions from other sources, plus water losses and/or operational wastes. Thus, $ID_g = ID_n / E_a$, where E_a is the application efficiency of the irrigation system.

Table 3. Ground cover (GC) and reduction factor (K_r) values as a fraction of the total surface area actually covered by the foliage of the plants when viewed from directly above.

GC %	10	20	30	40	50	60	70	80	90	100
Kr	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00

For good irrigation water management, it is recommended that the irrigator knows as accurately as possible the ID_n or the effective ID applied. Accordingly, all irrigation systems should have metering devices to measure flow volume or rates, so that the total amount of water applied can be verified easily.

The value of E_a depends on the performance of the system, its management, and environmental conditions (air temperature, relative humidity, wind, etc). Since localised irrigation does not, ideally, involve loss of water by deep percolation, it is generally unnecessary to provide water in excess of that calculated to meet the crop water needs. However, on sandy soils with poor water holding capacity and where there is a risk of using sources of saline water (mainly underground water), it may be a good idea to make some allowance for water loss by deep percolation. Usually, the minimum value utilised for E_a is 80 percent in conventional overhead sprinkler systems and 90 percent in the central pivot, linear moving and localised systems.

Once the irrigation water management parameters for a given crop are available, various ways can be used to put these parameters into practice in order to achieve an adequate and rational scheduling of the different irrigation schemes. The supply to individual fields can be scheduled using soil water indicators, plant indicators and/or climatic parameter indicators.

Measurement of crop water stress

Physiological irrigation scheduling techniques assess the need for water application through direct or indirect measurement of plant water status. Since the plant integrates its total environment, plant-based estimates of water status should accurately reflect the irrigation needed to replace soil water instead of estimating soil moisture directly or by some method that estimates soil water balance (ie, hydrological techniques). There are several points that need to be investigated that directly affect whether irrigation scheduling is optimum for increasing yield efficiency. There is a need for additional information that quantifies such factors as the plant water status at which irrigation is required, the quantity of water to apply, the effect of plant growth stage on sensitivity to water stress, and the effect of the frequency of application of irrigation on the plant's response.

The partial replacement of the plant's water requirements and the selection of genotypes better adapted to water stress can contribute to an increase in water availability in agriculture. Although the effects of water stress on crop development are known, few reliable methodologies exist that can be used for its characterisation that are based on parameters directly related to the plant, and that aim to maintain good productivity levels while increasing tolerance of a water deficit.

Remote sensing canopy temperature methods

Remote sensing of a vegetated surface can be accomplished with infrared thermometry methods. Thermal infrared radiation data can provide unique inputs into energy balance models. Canopy temperatures can be used to estimate crop water stress directly and estimate the water availability to a crop. Automatic remote sensing applied to the water status of a crop is a relatively new field of investigation in Brazil. Clawson et al (1989) showed that significant water saving could be achieved with no loss of yield when scheduling of irrigation for common beans was based on a canopy temperature variability method. Gomide (1998) has also suggested 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 saving. 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 remote sensing is that the application of a microcomputer-based system could provide important tools in irrigation management by means of automatic data processing. In addition, large areas can be surveyed rapidly, with an entire field's being sampled rather than only selected points within the field (Inoue, 1991; Sediyama et al, 2000).

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 (T_a). As water becomes limiting, transpiration is reduced and the leaf temperature (T_c) increases. If little water is transpired, leaves will warm to above the 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 investigations by these authors suggest that the inclusion of R_n and water VPD should improve the ability of the $(T_c - T_a)$ measurement to detect plant water stress.

In research on corn in Minnesota, Geiser et al (1982) and Slack et al (1981) found that the addition of R_n and VPD parameters did indeed improve 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 normalising criterion and showed that, for alfalfa, the relationship between $(T_c - T_a)$ and VPD was the same for several locations. Also, they proposed that the CWSI be calculated from the latter relationship. Overall, it appears feasible that plant water stress can be evaluated by means of canopy temperatures measured remotely. However, additional environmental inputs are required in order to assess the crop water status adequately.

Procedures for using remotely-sensed canopy temperature in the field to measure the crop's evapotranspiration and plant water status are:

1. Register the difference in temperature between the crop canopy (T_c) and the air (T_a) using an infrared thermometer (IRT; Figure 13).
2. Calibrate and use a portable instrument package to measure the major components of the Penman–Monteith energy balance equation for evapotranspiration.
3. Evaluate ways of computing aerodynamic resistance over a wide range of plant water regime conditions.
4. Evaluate the crop canopy energy budget over a wide range of plant water regime conditions.

The Penman–Monteith equation (Jackson, 1982; Monteith, 1973) may be expressed as follows:

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

where T_c is the crop canopy temperature (in °C), T_a is the air temperature (in °C), R_n is the net radiation (in W m^{-2}), G is the heat flux to or from the soil below the canopy (in W m^{-2}), e_c^* is the saturated vapour pressure at T_c (in Pa), e_a is the actual vapour pressure at the point of measurement of T_a (in Pa), ρ is the density of air (in kg m^{-3}), c_p is the heat capacity of air at constant pressure (in J kg^{-1} °C), γ is the psychrometric constant (in Pa $°C^{-1}$), r_a is the aerodynamic resistance to heat and mass transfer (s m^{-1}), and r_c is the canopy resistance to vapour transfer (s m^{-1}).

The sensible heat transfer (H, in W m^{-2}) from the canopy to the air 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 equation can then be rewritten as:

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

with λE denoted as $\rho_w \lambda E$, where E is the rate of evapotranspiration (m^3 m^{-2} s^{-1}), λ the latent heat of vaporisation (J kg^{-1}), and ρ_w the density

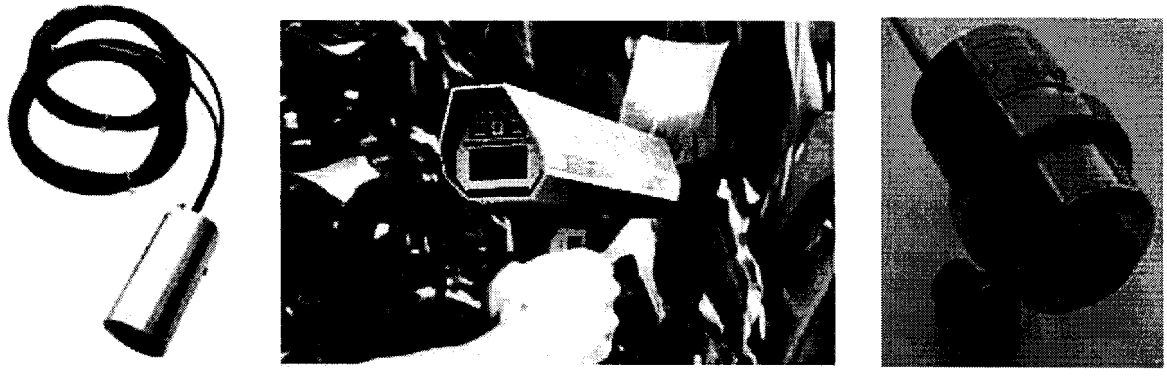

Figure 13. Infrared thermometer transducer models commonly used in the canopy-air temperature difference measurements (from left to right: Apogee IRTS-P5, Telatemp AG510b, and Everest Intersciences 4000.3ZL).

of water (kg m^{-3}). Crop water requirements or evapotranspiration (ET_c) should be evaluated from measurements of the terms in the last equation. As before, $T_c - T_a$ should be monitored with an IRT, a net radiometer should register R_n, and soil heat flux plates should measure G. Over the relatively narrow range of temperature and pressure found under most field environment conditions, the parameters λ, c_p, γ, and ρ_w can generally be considered to be constant. However, they can also be determined as functions of temperature and pressure if so required.

The portable IRT 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 IRT transducer models in common use and as manufactured by Apogee, Telatemp or Everest (Figure 13). 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 (ie, the thermal portion of the electromagnetic spectrum), and emissivity set to 0.98.

Plant temperature recordings should be obtained daily using the IRT held at about a 45° angle to the surface of the ground, just above the canopy, and pointed towards four predetermined directions (north, south, east and west) to determine the average canopy temperature. Average wind speed, air VPD (saturated minus actual air vapour density), total solar radiation, and net radiation should be recorded hourly near the crop 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) are used in the CWSI calculation as follow:

Lower limit (dT_l): non water stressed conditions (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 very water stressed conditions (thus r_c tends to infinity):

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

The CWSI equation is given by:

$$CWSI = \frac{dT - dT_l}{dT_u - dT_l} \text{ or } CWSI = 1 - \frac{ET_c}{ET_p}$$

For the main grain crops, a CWSI value of about 0.15 may be used as a limit to differentiate when an irrigated crop passes from non-water-stressed to water-stressed conditions, to avoid significant yield losses. In phenotyping for drought tolerance trials in maize and sorghum, the CWSI values are in the range 0.6–0.7 (this means replacement of approximately 40–30 percent of ET_c).

Sap flow probes

The crop transpiration rate and water stress can be measured and recorded with a sap flow probe. In an advanced phenotyping greenhouse at the Sete Lagoas site, water stress levels in two maize inbred lines were characterised by means of automatic sap flow (F) measurement using a set of energy balance probes installed on sections of the plant stem. The probes are flexible and adjustable to the diameter of the maize stem which ranges from 1.53–1.75cm. Each probe consists of an electric resistance heater (thermal jacket) and recording sensor for heat and temperature flow. The thermal jacket supplies a constant rate of heat input to the stem section. Copper-constantan thermocouple sensors detect the loss of heat from the thermal jacket to the air surrounding the stem and temperature differences in the stem section. The automatic data acquisition system manufactured by Dynamax Inc, using the Dynagage probe model SGB19, consists of a datalogger, sensors, a portable computer, a solar panel and rechargeable batteries (Figure 14).

A program was used to manage probe sensor readings and calculate the sap flow rate. An equation, expressing CWSI and involving the F rates measured under two water regimes (non-stressed and stressed), was used for water stress characterisation.

Figure 15 shows the variation of F per unit leaf area (g h^{-1} m^{-2}) and CWSI of two maize inbred lines (L1170 and L1312) as a function of time for two soil water regimes (non-water-stressed and water-stressed; Gomide et al, 2005). The results indicate that the probes were sensitive to variations in sap flow and that the measurement of CWSI was an appropriate means of water stress characterisation of

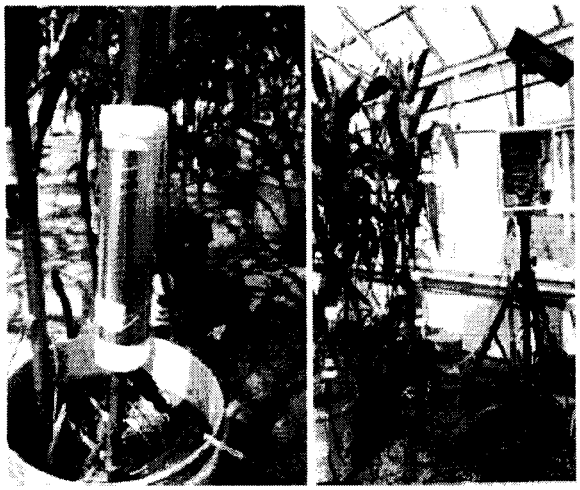

Figure 14. Energy balance probe (left) and automatic sap flow measurement system, installed on stem sections of eight maize inbred line plants to measure the transpiration rate and water stress levels at the Sete Lagoas site (Source: Gomide et al, 2005).

the two maize inbred lines investigated. The total leaf area of the two lines was reduced by the water stress conditions.

The F data should be converted in each maize inbred line to unit leaf area and expressed in g h^{-1} m^{-2}, on a total leaf area basis. This conversion allows characterisation of CWSI as calculated by the following equation:

$$CWSI = 1 - (F_{WS} / F_{NWS})$$

where F_{NWS} and F_{WS} are the F rates obtained under non-water-stressed and water-stressed conditions, respectively.

The maize inbred line L1170 presented smaller values of sap flow and a more sensitive CWSI (ie, larger values), whereas the line L1312 presented larger values of sap flow and a more tolerant CWSI (ie, smaller values).

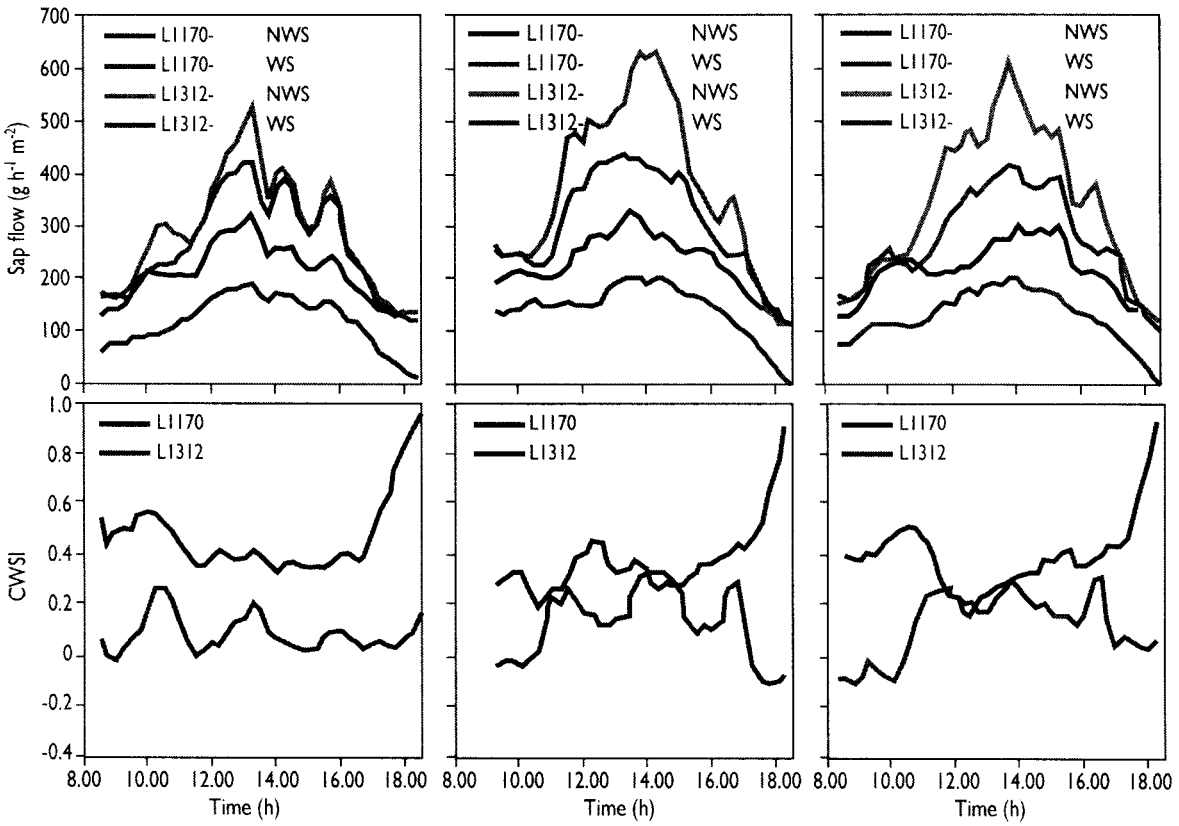

Figure 15. Sap flow (F) per unit leaf area (g h^{-1} m^{-2}) and plant water stress index (CWSI) variation in two maize inbred lines (L1170 and L1312) as a function of time for two soil water regimes: non-water-stressed (NWS) and water-stressed (WS), at the Sete Lagoas site (Redrawn from: Gomide et al, 2005).

Overall, the recording of water requirements, stress indices, and irrigation timing criteria for the crops can be achieved with microcomputer-based systems through automatic data processing. This provides important tools in the measurement of water stress and in irrigation water management, and offers a way of coupling plant-based measurements with the soil–atmosphere system.

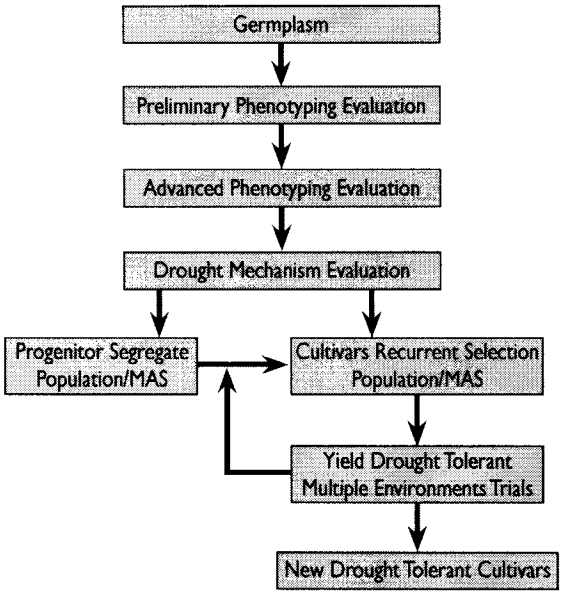

Figure 16. Flow chart of the Embrapa Rice and Beans drought tolerance evaluation programme conducted in conjunction with the upland rice and common bean breeding programmes. (MAS = marker-assisted selection).

Phenotyping cereals and legumes for drought tolerance

Drought at a low or high intensity generally occurs in the major upland cereal and legume producing regions of Brazil. Occasionally, this causes losses in rice, common beans and other crops. These crops are mainly grown in the savannah regions where Latosol soils, with good physical properties but low fertility, are dominant. In these regions, annual rainfall is about 1200–1500mm, occurring from October to April. However, from January to February, it is common to have dry periods with water shortages. These are mainly responsible for crop yield reductions. Therefore, it is recommended that new drought-tolerant cultivars be developed. Knowing the physiological variation in drought resistance can increase the efficiency of breeding such cultivars.

This section describes strategies to characterise and evaluate the crop response to drought, under controlled environment conditions with different water regimes. It covers preliminary and advanced phenotyping, study of drought resistance mechanisms, and evaluation of hybrids in addition to segregating and advanced populations. Figure 16 shows a flow chart of the Embrapa Rice and Beans drought tolerance evaluation programme conducted in conjunction with the upland rice and common bean breeding programme. Evaluation of specific aspects such as root quantification is being conducted under field and controlled environment conditions respectively at Porangatu and Santo Antônio de Goiás, both in Goiás state (Figure 17).

Figure 17. Root system evaluation at Embrapa Rice and Beans experimental sites under field (left) and controlled environment conditions (right).

Preliminary phenotyping

Preliminary phenotyping includes local and international cultivars and elite lines with high genetic diversity. The genotypes are exposed to adequate soil moisture at 15cm depth of –0.025MPa and –0.035MPa respectively for rice (Stone et al, 1986) and common beans (Silveira and Stone, 1994) until the crops are established. About 30 days after emergence for rice and 20 days for beans, two drought treatments are applied, namely maintenance of adequate soil moisture as in the early plant growth stage and induced drought up to the final growth stage. Irrigation is applied using a linear moving sprinkler irrigation system with electronic control of the irrigation water supply.

The discrimination of lines is based on the drought susceptibility index (DSI) defined by Fisher and Maurer (1978) as:

$$DSI = (Y_{w/ods} - Y_{w/ds}) / (Y_{w/ods} \cdot D)$$

where $D = 1 - YM_{cdh} / YM_{w/ods}$ and $Y_{w/ds}$ and Y_{cdh} are the yields of a given line without and with drought, respectively, and $YM_{w/ds}$ and $YM_{w/ods}$ are the yield averages of the experiments, without and with drought, respectively.

Advanced phenotyping

The preliminary evaluation provides a reduced number of improved lines for inclusion in advanced phenotyping. Phenotyping at this level can include lines from international cooperative programmes. The lines included in the phenotyping are maintained under good soil moisture conditions during the vegetative stage. Then, three water regimes are applied: severe water stress, moderate water stress, and no water stress. In the third treatment, irrigation is controlled using a tensiometer. Irrigation of approximately 25mm depth is applied whenever necessary. The moderate water stress and severe water stress treatments receive 2/3 and 1/3 respectively of the water applied in the non-water-stressed treatment. Lines are evaluated for yield potential as well as for agronomic and morphological characteristics, such as shoot dry matter, plant height and 100 seed weight. The DSI and canopy temperature are determined. In addition, yield components such as percentage of spikelet sterility, number of grains per panicle and tiller fertility are evaluated for rice, and flower abscission, number of pods per plant, and number of seeds per pods for common beans.

Drought resistance mechanisms

Drought resistance mechanisms are investigated on a reduced number of drought-resistant lines, evaluating them against the same number of susceptible lines under drought conditions similar to those applied in the advanced phenotyping. Once the major drought resistance mechanisms are known, they are used to choose lines for crossing purposes, with the objective of developing material for planting in drought-prone regions. Yield and yield components, leaf water potential, leaf diffusive resistance, leaf temperature, leaf area index (LAI) and plant growth analysis are evaluated. Soil moisture, root density and root efficiency in water absorption are determined at 20cm intervals up to 80cm soil depth.

Hybridisation and selection in segregating populations

Elite genitors for drought resistance are developed by backcrossing, incorporating alleles from donors in lines with commercial value. Improved F_1BC_2 plants are backcrossed to recurrent lines up to BC_4. Progeny are then evaluated, and the best ones incorporated by hybridisation into the Embrapa Rice and Beans breeding programme. Alternatively, when the allele donor source can be considered as an elite genitor, segregating generations are incorporated directly into the breeding programme.

Segregating families from backcrossing or selfing are subjected to drought stress and, at harvest time, the plants with potential superior yield as indicated by visual ratings are selected. In the advanced generation, F_2BC_4 (or F_5), 100 or more good plants are harvested individually from each family and planted in individual lines in the field in 5m rows without replication. Groups of 20 lines are separated from each other by two lines of, respectively, a tolerant and a susceptible genotype. Lines yielding more highly than the controls are selected and evaluated in field trials in drought-prone areas during the normal crop growth season. Drought-resistant lines are evaluated for specific characters such as commercial grain type and reaction to particular diseases, in order to define new cultivars which will be released for drought-affected regions. Efficiency of the process will be increased by identification of molecular markers and by adopting MAS.

Plant measurements

Plant water potential

The water potential of a cell, tissue, or organ can be described by the following equation:

$$\Psi_w = \Psi_s + \Psi_m + \Psi_p + \Psi_g$$

where Ψ_w is the total water potential of the system, Ψ_s is the osmotic potential, Ψ_m is the matric potential, Ψ_p is the turgidity or pressure potential, and Ψ_g is the gravitational potential (Boyer, 1967a; Turner, 1981). These authors consider gravitational effects as negligible in the excised shoot. Accordingly, the water in the system is affected only by osmotic, matric and pressure potential (Boyer, 1967b; Kramer, 1974; Turner, 1981).

The pressure chamber technique can be used for the measurement of plant water potential. Its widespread adoption since its reintroduction by Scholander et al (1965) arises from its ease of use, its speed and reliability, and the fact that it does not require fine control of temperature. In the technique, a leaf or branch is cut and placed in a pressure chamber, with the cut end of the petiole or stem just protruding from the chamber through a rubber gland that is used to seal the chamber. The pressure in the chamber is gradually increased by compressed air from a cylinder, until the sap just returns to the cut ends of the xylem vessels. The pressure inside the chamber is recorded, the pressure is released and the sample is removed. Detection of the end point can be aided by the use a microscope or hand lens. Although a hand lens is commonly used because of its practicality, the microscope is preferred since it also gives protection to the eyes should the seal break and the plant material be blown out of the chamber.

The pressure chamber is analogous to the pressure membrane apparatus used in soil physics. It measures the matric potential of the water in the apoplast or cell wall (Passioura, 1980), which will be similar to the total water potential of the leaf cells, provided that the osmotic potential of the apoplastic water is near to zero and the system is in equilibrium. In a transpiring plant, the apoplastic water may not be in equilibrium with the symplastic water, if there is a large resistance to flow between the two. Boyer (1967b) clearly demonstrated that any disequilibrium between the matric potential of the apoplastic water and the total water potential of the leaf cells disappears between the time of severing and the time of the first measurement in the pressure chamber. Hence the pressure chamber should give an accurate measurement of the total water potential of the leaf cells. This is attested by the acceptable degree of agreement between leaf water potential measurements by the pressure chamber technique and by thermocouple psychrometry (Ritchie and Hinckley, 1975). Turner and Long (1980) have recently suggested that when agreement is not good, this is probably the result of rapid water loss after excision.

Turner (1981) suggested several precautions to allow reliable results to be obtained, as follows:

1. Water loss between sampling and measurement must be prevented to avoid large errors. Protect the leaf to be sampled with a plastic cover or bag just before sampling (Turner and Long, 1980). Loss in the first 10 to 30 seconds can lower the water potential by –0.2 to –0.7MPa in rapidly transpiring leaves, but errors are negligible in leaves with stomata closed by low light or dehydration.
2. Humidify the air entering the chamber or line the inner walls of the chamber with wet paper towel to prevent water loss in the pressure chamber itself (Slavík, 1974). This is unnecessary if the sample is enclosed in a plastic bag or cover (Turner and Long, 1980).
3. Make only one cut on the leaf or petiole.
4. Leave outside the chamber only the minimum length of petiole or leaf necessary for satisfactory reading of the endpoint.
5. Pressurise the chamber slowly (Tyree et al, 1978). Rapid pressurisation leads to more negative values of leaf water potential than the slower pressurisation. A rate of 0.025MPa s^{-1} can be used for studies where errors of 0.005–0.1MPa are acceptable.
6. Prevent leakage of gas from the chamber, particularly if the leaf is not enclosed in a plastic cover. Even with a humidified chamber, gas leakage lowers the leaf water potential and introduces an error additional to that arising from rapid water loss.

7. Gas from the chamber can pass through the intercellular spaces of the leaf and escape from the cut surface of the leaf or petiole. This gas can force water from outside the xylem to the cut surface giving a false endpoint. Dry the cut surface with filter paper during pressurisation of the chamber to help detect the correct endpoint. If in doubt, over-pressurise by 0.1 or 0.02MPa. In most species, this will cause a flood of sap to be exuded if the endpoint has been reached, but little sap will exude if it has not been reached.

Measurements made using the pressure chamber may be partitioned into the following components:

$$\Psi_w = \Psi_s + P$$

where Ψ_w is the water potential of the leaf cells, P represents the negative components of the water potential of the xylem sap measured as a positive pressure in the pressure chamber, and Ψ_s is the osmotic effect of the solutes in the xylem sap. The terms P and Ψ_s represent the total force tending to remove water from the leaf cell, and Ψ_w represents the force tending to cause water to enter the leaf cells (Boyer, 1967b; Boyer and Ghorashy, 1971; Gee et al, 1974). For plant leaves, when xylem sap and cell sap are in equilibrium, and Ψ is small compared to P, then

$$\Psi_w \approx P = \Psi_{leaf}$$

and the pressure chamber can be used to obtain a direct measure of leaf water potential, Ψ_{leaf} (Boyer and Ghorashy, 1971; Gee et al, 1974). In soybean leaves, there is agreement between Ψ_w measured with the thermocouple psychrometer and P measured with the pressure chamber. The osmotic potential Ψ_s is negligible at less than –0.01MPa (Boyer and Ghorashy, 1971), which indicates that simple measurements of P with a pressure chamber are a useful means of estimating leaf water potential (Boyer, 1967b; Boyer and Ghorashy, 1971; Gee et al, 1974). To extend this measurement to total leaf water potential occurring in the intact plant, two assumptions must be made (Boyer, 1967b), namely that the water potential of the xylem sap is in equilibrium with that of the leaf cells at the time of measurement, and that water is arranged spatially in the same manner in the shoot under pressure as it is in the shoot on the intact plant.

Scientists have shown that in measurements made in a small fibreglass greenhouse, soybean leaf water potential varies linearly with soil water potential between 0 and –1.5MPa (Hiler et al, 1972). Brady et al (1974) found the same correlation with soil water potential between 0 and –1.0 MPa in the field during the vegetative and pod development stages. The soybean leaf water potential was slightly more responsive to soil water potential during the podding stage than during the vegetative stage. The slope of the curve describing the correlation between leaf water potential and soil water potential was 0.71 during pod development as opposed to 0.55 during the vegetative stage. Working with the same crop in a lysimeter, Clark and Hiler (1973) found a linear relationship for values lower than –0.07MPa leaf water potential and –0.20MPa soil water potential. They proposed that, in general, the correlation should be valid for most crops and soils.

Soil factors other than moisture affect leaf water potential. Elfving et al (1972) showed that, for soil moisture close to field capacity, leaf water potential fully recovered by sunrise at several soil temperatures. In contrast, leaf water potential did not recover by sunset when the soil temperature was a limiting factor (less than 15°C). Boyer (1968) found that leaf and soil water potential are approximately in equilibrium at the beginning of the day. Non-equilibrium is brought about by resistance to water flow. Brady et al (1974) reported higher leaf water potential than soil water potential before sunrise during the vegetative stage. It may be explained by the following hypotheses:

1. Leaf water potential measurements determined by a pressure chamber do not account for the xylem osmotic potential.
2. Determining a representative soil water potential as it affects the plant is extremely difficult.
3. Water may have been absorbed by the plant tissue from the atmosphere.

Guimarães et al (2006) also observed a total recovery of leaf water potential before sunrise in drought-resistant common bean varieties. Drought-susceptible cultivars presented a lower leaf water potential at midday and recovered later in the afternoon, compared to drought-resistant material.

Diurnal variation of the leaf water potential of two bean cultivars is shown in Figure 18. It can be verified that the Carioca and RAB 96 cultivars are, respectively, more and less drought-resistant.

Atmospheric evaporative demand is another factor affecting leaf water potential (Brady et al, 1974). The daily atmospheric demand may cause a decrease of 0.9–1.0MPa in stressed or non-stressed plants (Brady et al, 1974; Clark and Hiler, 1973).

Leaf water potential measurement has been shown to be a more sensitive indicator of changes in water deficit during the vegetative stage than during the pod development stage (Clark and Hiler, 1973). On the other hand, the studies of Brady et al (1974) indicated that in soybeans the minimum leaf water potential was slightly more responsive to soil water potential at the pod development stage than at the vegetative stage, with a slope of 0.71 during pod development as opposed to 0.55 during the vegetative stage. Begg and Turner (1976) and Clark and Hiler (1973) showed that leaf water potential, as measured by the pressure chamber method, was more responsive to changes in plant water status than leaf diffusive resistance. Finally, Brady et al (1974) proposed to estimate soil water potential from early morning values of soybean water potential, and Ritchie (1974) stated that leaf and soil water potential are approximately equal at the beginning of the day.

Water stress occurs whenever the loss of water in transpiration exceeds the rate of absorption (Kramer, 1963; 1969; Halevy, 1972). It is characterised by decreases in water content, growth, osmotic potential, and total water potential, accompanied by loss of turgidity and closure of stomata. On sunny days, lags in water absorption result in the development of temporary water deficits, even in plants growing in well-watered soil or diluted nutrient solutions (Kramer, 1963). Such stresses are eliminated overnight when water absorption exceeds water loss. However, as soil water potential decreases, water absorption becomes slower and slower, and the midday water deficits last longer and longer, until permanent wilting finally occurs (Boyer, 1968; Kramer, 1963).

The transpiration of southern peas (*Vigna sinensis* L (Endl), cv Burgundy) was essentially zero when the soil water potential dropped below –0.5MPa and/or when the leaf water potential fell below –1.1MPa (Hiler et al, 1972). At this point, the leaf diffusion resistance became very high because of stomatal closure. The soybean plant seems more tolerant to water stress. Boyer (1970) showed that photosynthesis, transpiration and diffusive resistance were unaffected by desiccation until the leaf water potential fell below –1.1MPa. Wilting has been observed at leaf water potentials varying from –1.2 to –1.6MPa (Sionit and Kramer, 1977). It occurred

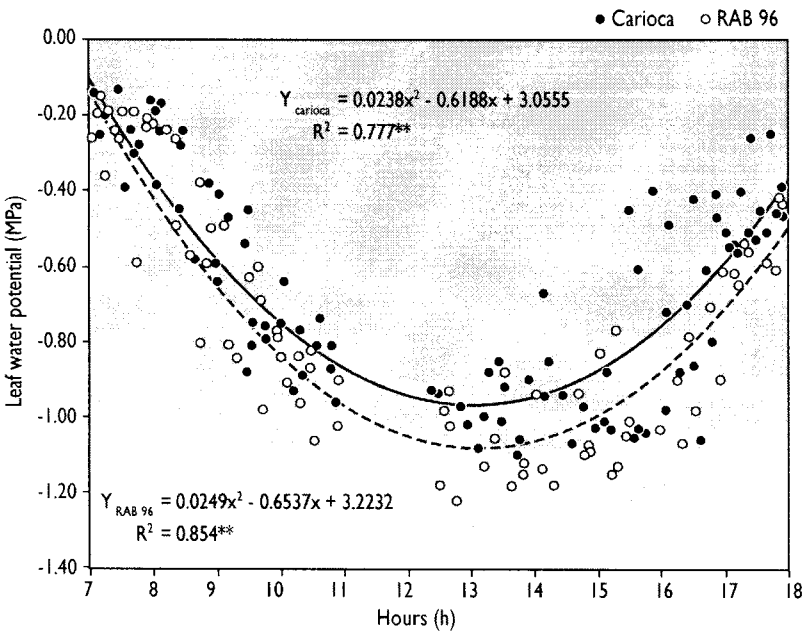

Figure 18. Diurnal variation of the leaf water potential for two common bean cultivars (Source: Guimarães et al, 2006).

at higher water potential (ie, less stress) in plants stressed at later stages of growth than in plants stressed before or at flowering (Sionit and Kramer, 1977). The imposition of water stress in corn, which lowered the leaf water potential to approximately –1.5 to –2.0MPa, caused a 30 percent reduction in the translocation of ^{14}C during the first two hours following labelling (Brevedan and Hodges, 1973).

Leaf position in the canopy is a further factor affecting leaf water potential. At noon, upper leaves had a lower water potential than lower leaves (Teare and Kanemasu, 1972). Hence, in the morning, a strong water potential gradient developed from the lower to upper leaves in the soybean canopy. In the afternoon, the gradient disappeared because the stomatal resistance of the upper leaves increased (Teare and Kanemasu, 1972). Upon rewatering, soybean plants stressed to –2.3MPa, gradually recovered and the total water potential returned to pre-stressed levels within 3–5 days (Sionit and Kramer, 1977). In contrast, Boyer (1971) did not find a complete recovery from desiccation. In severely desiccated tissue, the lack of return to pre-stressed growth rates was more pronounced than in the moderately desiccated tissue, and may have been due to additional factors induced by extreme desiccation rather than by factors associated with leaf growth per se (Boyer 1971).

Leaf diffusive resistance

The main pathway for gas exchange between plant and atmosphere is through the leaf stomata. Variation in the stomatal aperture markedly affects the transpiration rate and, thereby, the energy balance of individual plants and whole communities. These variations can also affect net rates of photosynthesis by their effect on CO_2 exchange (Morrow and Slatyer, 1971). The responsiveness of stomata to water deficits has been known for many years, and attempts have been made to measure stomatal behaviour using many different methods. These include direct observation with a microscope, differential permeability of fluids of various viscosities into the leaves, and use of a porometer to measure air flow through leaves.

The total diffusive resistance (R_f) is the sum of the stomatal and boundary layer resistances (Kanemasu et al, 1969). The stomatal resistance is affected by both the availability of water and the evaporative demand of the atmosphere (Brady et al, 1975). In studying the relative importance of the total resistance to CO_2 uptake under conditions of water stress, Redshaw and Meidner (1972) considered that it was made up of:

1. The air-phase resistance, consisting of: (i) the boundary layer resistance; (ii) the stomatal resistance; and (iii) the intercellular space resistance.
2. The liquid-phase resistance consisting of: (i) the chemical resistance, ie the resistance to CO_2 fixation within the chloroplasts; and (ii) the mesophyll resistance, ie the resistance to diffusion within the mesophyll cells.

The estimated liquid-phase resistance changed by a proportionately lower amount than the air-phase resistance and, consequently, represents a smaller proportion of the total resistance at wilting. The stomatal resistance can account for only 50 percent of the reduction in the rate of assimilation due to water stress (Redshaw and Meidner, 1972).

The studies of Boyer (1970) showed that the net photosynthetic rate of soybeans correlated well with stomatal behaviour. The net photosynthetic rate and R_f of leaves remained constant until the leaf water potential dropped to –1.1MPa. The mesophyll resistance remained constant to leaf water potentials of –1.6MPa. Below this water potential, the mesophyll resistance increased and ultimately doubled during severe desiccation (Boyer, 1970). The rate of photosynthesis in *Triticum aestivum* (cv Gabo) did not decrease prior to the onset of wilting in the leaves under water stress. However, from the onset of wilting, there was a progressive reduction in the rate of photosynthesis (Wardlaw, 1967). Grain growth was not affected by several days of leaf wilting, which was accompanied by a change in distribution of assimilates from the lower parts of the plant to the grain (Wardlaw, 1967).

Kramer (1969) suggested that stomatal resistance was the simplest and most direct approach to measuring the availability of water to a plant. Later, Halevy (1972) showed that the stomatal aperture was considered a reliable physiological indicator for timing the watering of gladioli (*Gladiolus* sp). It can also be used to discriminate lines for drought resistance. The leaf diffusive resistance (R_f) in common beans did not differ between cultivars early in the morning. In the

afternoon, the drought-susceptible cultivar RAB 96 presented a higher R_f than the drought-resistant cultivar Carioca (Figure 19).

Stomatal resistance is influenced by factors other than soil water potential and atmospheric demand (Brady et al, 1974 and 1975) such as: (i) location of measurement; (ii) physiological stage of growth of the plant; and (iii) wet leaves and recent irrigation.

Teare and Kanemasu (1972) observed that the upper leaves in soybeans had the lowest stomatal resistance in the morning, whereas in the afternoon the middle leaves had the lowest resistance. This was not true for sorghum, where the stomatal resistance of the upper leaves remained constantly low, but the resistance of the middle leaves increased significantly during the day. This indicated that the middle leaf stomata had closed to conserved water, while those on the upper leaves remained open allowing the inward diffusion of CO_2 for photosynthesis (Teare and Kanemasu, 1972). This may explain why Teare et al (1973) found that the WUE of sorghum was approximately three times that of soybeans. In contrast, Stevenson and Shaw (1971) showed that in sorghum, the diffusive resistance was consistently less for upper leaves than for middle leaves of plants growing on both dry and wet soil. Brun et al (1972), working with soybeans and sorghum under different levels of soil moisture and atmospheric demand, noted that the stomatal resistance of lower leaves was as much as 10 times greater than that of upper leaves in both crops under the same conditions.

The physiological stage of plant growth did not affect stomatal resistance in soybeans (Brady et al, 1975), but did in southern peas (*Vigna sinensis* L Endl, cv Burgundy), where the diffusive resistance of stomata became much more responsive during the later stage of growth (Clark and Hiler, 1973). No trend in the stomatal resistance of the irrigated plants was observed as a result of a particular growth stage. Brady et al (1975) also showed that stomatal resistance values of the irrigated plants increased linearly with potential evapotranspiration until a threshold of 4.0 s cm^{-1} was observed. Resistances values greater than 4.0 or 5.0s cm^{-1} resulted primarily from low soil water potential.

Leaf temperature

The leaf temperature of the plant is governed by both microclimate and soil environment. Under normal growth conditions without moisture restriction, the ambient temperature, solar radiation, humidity and wind speed are major climatic factors affecting the canopy temperature. Leaf temperatures are lower than ambient temperatures throughout the growth phases in rice, indicating an effective heat dissipating function of transpiration under conditions of unlimited water supply (Su and Yang, 1998). Temperature differences between the canopy and ambient air changed with time,

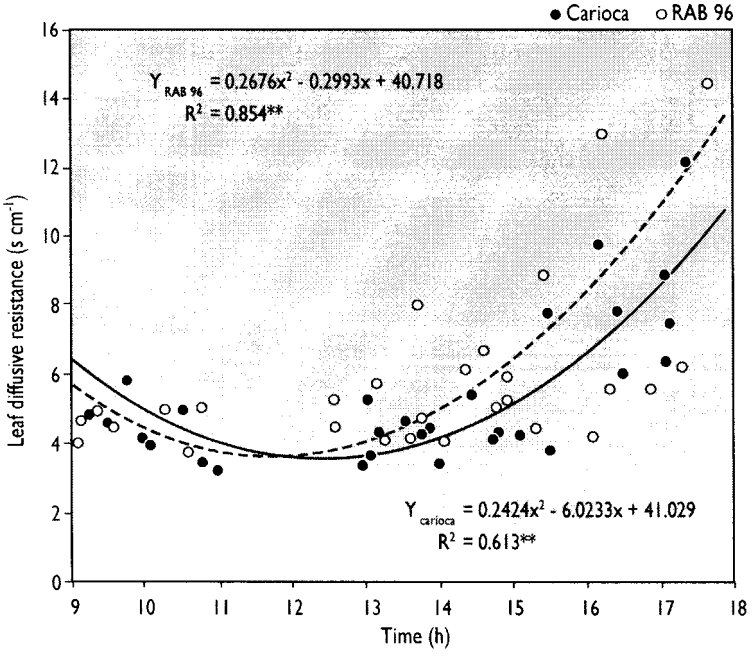

Figure 19. Diurnal variation of the leaf diffusive resistance of two common bean lines.

the higher the ambient temperature and the soil water deficit, the higher the leaf temperature. Again in rice, a positive correlation was observed between leaf temperature and spikelet sterility (Figure 20), and a negative one between leaf temperature and grain yield (Figure 21; Guimarães et al, 2010).

Hirayama et al (2006) also observed that transpiration and photosynthetic rates were highly correlated with leaf temperature, the latter also showing a significant relationship with grain yield. It was considered that rice varieties with a lower leaf temperature could maintain higher transpiration and photosynthetic rates under upland conditions. In addition, there was a significant positive correlation between leaf temperature and root growth. Upland rice varieties with deep rooting showed a lower leaf temperature than those with a shallower root system.

Analysis of the leaf temperature in different upland rice varietal groups showed that lines with medium-late maturity had the lowest temperature, followed by the early maturing lines and lines for cultivation with sprinkler irrigation (Hirayama et al, 2006). This tendency was in agreement with the general degree of drought tolerance of these varietal groups. To analyse the mode of inheritance of leaf temperature, the parent–offspring correlation of leaf temperature was examined in breeding materials. The leaf temperature was compared between the progeny lines (F_4 generation) of a cross between the upland rice variety Kantomochi168 (drought-tolerant) and the upland rice variety Norinmochi4 (intermediate drought tolerance). Kantomochi168 progeny showed a lower leaf temperature than Norinmochi4. A similar tendency was confirmed in the F_5 generation in the following year. A significant parent–offspring correlation ($r = 0.812^{**}$) was observed between the F_4 and F_5 generations. Since the

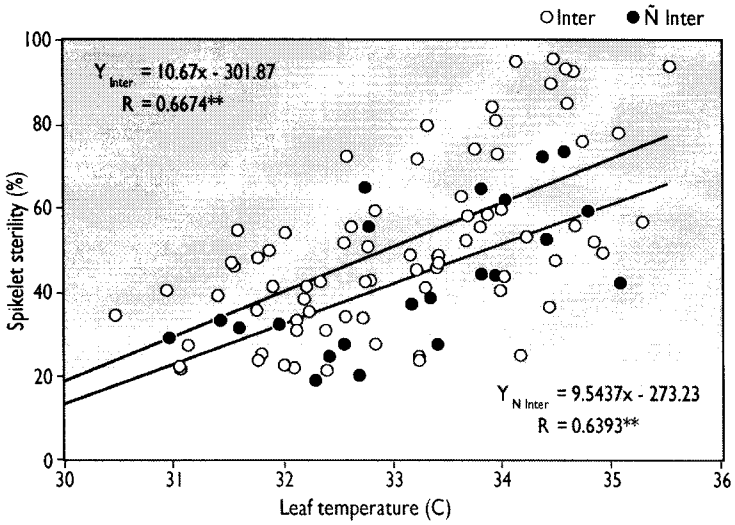

Figure 20. The relationship between spikelet sterility and leaf temperature in interspecific (inter) and non-interspecific (Ñ inter) rice lines under drought (Source: Guimarães et al, 2010).

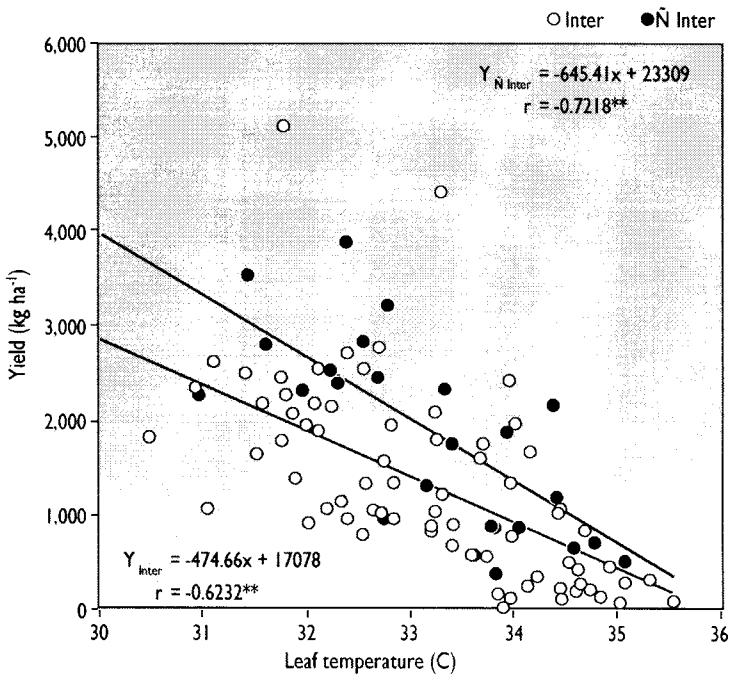

Figure 21. The relationship between yield and leaf temperature in interspecific (inter) and non-interspecific (Ñ inter) rice lines under drought (Source: Guimarães et al, 2010).

leaf temperature of upland rice progeny may display a relatively higher inheritance, the leaf temperature is considered to be an appropriate indicator to estimate drought tolerance for selecting lines in upland rice breeding.

Plant water content

The water content of a plant is determined by weighing the fresh plant material immediately after sampling, then placing this material in a preheated oven to dry at a high temperature (60–105°C), and reweighing later to obtain the dry weight. If it is not possible to weight the material immediately after sampling, the plant material should be placed in a metal container and hermetically sealed, to be weighed, dried and reweighed as soon as possible. The drying time depends on the size and compactness of the plant material, which is dried to constant weight. This process usually takes 12h for medium size samples. Also, discs samples (8mm in diameter) extracted from leaves can be dried for 2h in an oven at 70°C. Some authors point out that a fraction of the water remains bound in the tissue even after drying at 105°C. However, the error is negligible in routine determinations (Slavik, 1974). This method of determination the water content has one main disadvantage: the sample also usually loses some or all of the volatile compounds and protein nitrogen. However, the error is not usually significant (Slavik, 1974).

The water content (WC) can be expressed on a dry weight (DW) or fresh weight (FW) basis as follow:

$$WC_{(dw\ basis)} = \frac{(FW-DW)}{DW} \cdot 100$$

$$WC_{(fw\ basis)} = \frac{(FW-DW)}{FW} \cdot 100$$

However, because the dry weight can change diurnally and/or seasonally, comparisons of water content on a dry weight basis are unsatisfactory. When water content is expressed on a fresh weight basis, the problems of changing dry weight are still present (Turner, 1981). An alternative to overcome these problems is to express the water content on the basis of the water content at full turgidity weight (TW). Then, the water content is termed the relative water content (RWC) or water saturation deficit (WSD):

$$RWC = \frac{(FW - DW)}{(TW - DW)} \cdot 100$$

$$WSD = \frac{(TW - FW)}{(TW - DW)} \cdot 100$$

$$RWC = 100 - WSD$$

To determine the fully turgidity or fully saturated weight of the tissue, 10–15 leaf discs, about 1cm in diameter, are punched from a leaf or set of leaves and placed in a hermetically sealed, tared vial. After the fresh weight has been obtained, the leaf discs are floated for several hours on distilled water in a covered Petri dish until the discs become fully turgid. They are then surface dried, returned to the vial, weighed, oven dried at 85°C for 3h and the dry weight determined.

The length of time for the discs to reach saturation varies with species and conditions. Studies have shown that water uptake by leaf discs is initially rapid for several hours, followed by a slower rate of uptake that can persist as long as the floated discs remain healthy. The slow water uptake has been shown to be associated with growth (Barrs and Weatherley, 1962), and is greater in young tissue than mature tissue (Catský, 1965). The aim is to measure the weight of the discs at the completion of the rapid phase of water uptake associated with tissue rehydration. This usually occurs within 3–6h, but can take up to 20h in very dehydrated leaves.

Several precautions are necessary if reliable results are to be obtained:

1. Remove dew from leaves before sampling, using filter paper under the same standard conditions as those for surface drying the tissue after saturation.
2. Use a clean sharp cutter to minimise errors arising from infiltration and injection of water.
3. Float the discs in covered Petri dishes in a constant temperature room, since humidity and temperature will influence the measurement.
4. Float the discs under lights at a quantum flux density near to the compensation point (ca 10μE m^{-2} s^{-1}), to prevent dry weight losses due to respiration or gains due to photosynthesis.
5. After floating, surface dry the discs with filter paper under standard conditions of pressure that ensure removal of all surface water but do not force water from the leaf cells. Surface drying should be performed as quickly as possible, and preferably in a humid chamber (Turner, 1981).

Osmotic adjustment

The flow of water into or out of a plant cell is dependent on the ψ_w gradient between the cell and its surroundings. The ψ_w of a plant cell is governed by the equation:

$$\psi_w = \psi_s + \psi_p$$

where ψ_s is the osmotic potential and ψ_p is the pressure potential. A higher ψ_p can be achieved at a given ψ_w by accumulating solutes inside the cell, thus lowering ψ_p (Verslues et al, 2006). The accumulation of additional solutes in response to a low ψ_s is termed 'osmotic adjustment' (OA; Zhang et al, 1999). This refers to the accumulation of additional solutes in response to low ψ_w, excluding the effect of reduced water content on the concentration of existing solutes, which has been factored out. OA and accumulation of compatible solutes can be an important factor in drought tolerance in the field (Kramer and Boyer, 1995). In this case, the trade-off is that increased accumulation of compatible solutes can be energy and resource intensive for the plant and, in cases of severe stress where soil water content is largely depleted, may only have a small effect on water uptake (Kramer and Boyer, 1995). The osmotically active solutes do not interfere with cellular function. Thus, many plants accumulate one or more types of compatible solute, such as proline or glycine betaine in response to drought.

Immediately after the commencement of a dry period, field-grown rice osmotically adjusts rapidly during the day but loses its adjustment by dawn the following day. The OA is much higher at midday, particularly during the early stages of drought. Diurnal change in OA becomes smaller as water-stress develops further.

Soil water measurement

The amount of water to be applied to the soil with a rice or bean crop should be calculated using tensiometers and a soil water retention curve. Three sets of tensiometers should be installed in the experimental area at two soil depths, 15 and 30cm, in the middle of the plant rows. The 15cm tensiometers are called 'decision tensiometers', because they indicate the irrigation timing. The 30cm tensiometers are called 'control tensiometers', because they indicate if the irrigation is adequate, without excess water or a deficit. Irrigation should be done when the average measurement of the decision tensiometers is about –25kPa for rice and –35kPa for beans.

The procedure to determine the amount of water to be applied to the soil is as follows. Using the soil water retention curve, verify the soil water content expressed in cm^3 of water per cm^3 of soil corresponding to –25kPa. Then calculate the difference between soil water content at –10kPa (field capacity) and at –25kPa. This difference, multiplied by 30cm depth, will indicate the net irrigation amount. This is based on the fact that the 0–30cm soil layer encloses almost the totality of the root system of sprinkler irrigated rice, and that the decision tensiometer measurement represents the average soil water tension in this layer.

Structure, maintenance and management of a database and modelling for drought tolerance phenotyping

Phenotyping studies by Embrapa will generate data related to microclimatic condition, soil water status and soil water availability in the soil profile, crop water requirements and water stress, and soil physical and chemical properties. Data will also be generated on the selected number of genotypes studied for each crop species studied (maize, sorghum, rice, wheat, common beans, cowpeas) with evaluation of their traits, yields, and yield components. All of these data for each site studied are transferred into a database.

The data and information generated in the experiments at different sites, and for different species, are digitised into Microsoft Word documents and tables, and into Excel spreadsheets. Later these digitised data are inserted into 'Morpho', a data management tool for ecologists, agronomists, and others research scientists.

Morpho is a component of the Knowledge Network for Biocomplexity (KNB) and was created to provide an easy-to-use, cross-platform application for accessing and manipulating data and metadata (eg, documentation) both locally and on the network. Many types of data can be used with Morpho, including tables and images. The KNB is an international data repository dedicated to facilitating ecological and environmental research on biocomplexity. It enables the efficient discovery, access, and interpretation of data ranging from individual researcher's efforts to data from highly distributed field stations, research sites, and laboratories, providing the means to access network servers in order to query, view and retrieve relevant data. Morpho allows researchers to create metadata describing their data in a standardised format, and a catalogue of data and metadata against which to query, edit and view data collections. Embrapa is offering a specific server for managing and manipulating the data generated at its different phenotyping sites.

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