PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE:

DROUGHT STRESS

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CIMMYT®

PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE
DROUGHT STRESS

A field manual

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Acknowledgements

Breeding for drought tolerance is among the topmost priorities for CIMMYT's Global Maize Program. Drought tolerant maize is one of the flagship products of CIMMYT. Research on drought stress in maize at CIMMYT started in 1970s, and continued as one of the priority areas. This has been further intensified in the last ten years to cope-up with the climate change effects in the tropics. A series of drought tolerant pools, populations and synthetics were developed at CIMMYT over the decades. Besides open-pollinated drought tolerant maize varieties, an array of drought tolerant donor inbred lines and hybrids have been developed and deployed for sub-Saharan Africa, Latin America and Asia.

CIMMYT has been a pioneer in developing and deploying protocols for drought stress phenotyping, selection strategy and breeding for drought tolerance (Banziger *et al*., 2000). The present field manual for precision phenotyping for drought tolerance builds on the work undertaken by several scientists and experiences gained over decades at CIMMYT.

The information presented in this manual is based on the work on drought stress phenotyping and breeding for drought tolerance that received strong and consistent support from several donor agencies, especially the USAID, BMGF, DFID, GIZ, and the MAIZE CGIAR Research Program, as well as public and private sector partners in sub-Saharan Africa, Asia and Latin America.

CIMMYT – the International Maize and Wheat Improvement Center – is the global leader in publicly-funded maize and wheat research-for-development. Headquartered near Mexico City, CIMMYT works with hundreds of partners worldwide to sustainably increase the productivity of maize and wheat cropping systems, thus improving global food security and reducing poverty. CIMMYT is a member of the CGIAR Consortium and leads the CGIAR Research Programs on MAIZE and WHEAT. The Center receives support from national governments, foundations, development banks and other public and private agencies.

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Preface

In agriculture, the term *drought* refers to a meteorological condition in which the amount of water available through rainfall and/or irrigation is insufficient to meet the crop needs for optimal growth and development; this eventually affects overall productivity. Rainfed maize crops grown during the rainy summer season in the tropics occasionally face extreme weather conditions. These extreme weather conditions translate into various abiotic stresses, such as drought, which constitutes one of the key abiotic constraints for maize production in many parts of the world, particularly the tropics. The erratic rain distribution pattern in the tropics due to climate variability occasionally causes prolonged dry spells at different crop growth stages; this has been identified as one of the major factors responsible for year-to-year fluctuations of rainfed maize.

Crop breeding programs using conventional and/or molecular breeding approaches rely heavily on high-quality phenotypic data generated by evaluating genotypes in different environmental conditions, such as drought. In this manual, the traits of interest for phenotyping are those that mitigate yield losses rather than those involved in the survival or escape of plants exposed to drought stress.

This manual is targeted at maize breeders and field technicians in tropical environments who are working on improving maize tolerance to drought stress. It covers aspects related to:

- Selecting locations/fields suitable for phenotyping for drought stress,
- Factors affecting the intensity and severity of drought stress
- Maintaining uniform stress in drought trials
- Precision and accuracy when collecting phenotypic data

Drought stress in tropical maize

Strategies that allow plants to mitigate the negative effects of water deficits often referred to as drought can be classified into two broad categories (Levitt, 1972): (i) *dehydration avoidance*, which encompasses morpho-physiological features (e.g., deep roots, early flowering, etc.) that enable the plant, or parts thereof, to maintain hydration; and (ii) *dehydration tolerance* involving features that allow the plant to maintain, at least partially, proper functionality even in a dehydrated state.

Maize plants may respond differently to drought stress at different crop stages. Poor establishment and bad plant stand are usually the result of soil drying during or after germination. Drought effects on maize include reduced leaf area resulting in incomplete ground cover as well as reduced stem and root expansion due to reduced assimilate fluxes to growing organs. In general, the root/shoot ratio increases slightly under drought stress; however, as the stress becomes more severe, root growth decreases and nutrient uptake from dry soil is sharply reduced (Bänziger et al., 2000).

Among various crop stages, the reproductive stage—especially 3-4 weeks bracketing male flowering (anthesis)—is the maize crop's most susceptible phase (Claassen and Shaw, 1970; Grant et al., 1989). Female reproductive structures are more seriously affected than the male flowers (tassels). Extreme sensitivity seems confined to the period -2 to 22 days after anthesis, with a peak at 7 days, and almost complete barrenness can occur if maize plants are stressed in the period from just before tassel emergence to the lag-phase of grain-filling (Grant et al., 1989). Unlike other cereals, in maize the male and female flowers are

separated by as much distance as one meter; therefore, pollen and fragile stigmatic tissue have to be exposed to a dry and hostile atmosphere for pollination to occur. As silk growth and early kernel development appear to depend directly on the flow of current photosynthetic products drought-induced decrease in rate of photosynthesis at this stage significantly enhances the sensitivity to the stress (Schussler and Westgate, 1995).

When photosynthesis per plant at flowering is reduced by drought, silk growth is delayed, leading to an increase in the anthesis-silking interval (ASI), and kernel and ear abortion (Bolaños and Edmeades, 1996; NeSmith and Ritchie, 1992). Leaf senescence begins from the bottom of the plant (older leaves affected first) and proceeds towards the top of the plant. However, in conditions of high evapotranspiration due to combined heat and drought stress, leaf senescence may also occur at the top of the plant. Known as leaf firing, this further reduces the leaf area for radiation interception.

About 2-3 weeks after pollination, once kernels enter the linear phase of grain-filling, they develop the sink strength needed to attract reserve assimilates stored in the stem and husk, apart from accessing current assimilates. If kernels reach this stage, they normally grow to at least 30% of the weight of kernels of unstressed plants, even if drought becomes more severe (Bolaños and Edmeades, 1996).

Breeding approaches for drought-prone environments

Selection for high yield potential

High yield potential is a constitutive trait that often gives increased yield under moderate levels of drought. In such conditions, the likelihood of spill-overs from one environment to another can be estimated through the genetic correlation between yields of the same cultivars grown in those two environments (Bänziger et al., 2000). Spill-overs can be expected when the genetic correlation (rG) between yields in stressed and well-watered sites is positive and significant. If rG is weak/non-significant, selection for yield potential alone does not contribute much to drought tolerance.

Selection for earliness

A major goal of breeding is to develop cultivars that can escape drought by being sufficiently early maturing so as to complete their life cycle within a given favorable season length. Selection for earliness matches the phenology of the crop to the pattern of water availability/rainfall. Since the time from sowing to flowering or physiological maturity is a highly heritable trait, selection for earliness can be easily accomplished. However, earliness carries a yield "penalty," especially when moisture availability is optimal. Under those circumstances, the yield of an early maturing cultivar is limited by the amount of radiation the cultivar can capture—normally less than the radiation captured by later maturing cultivars (Bänziger et al., 2000).

Selection for drought tolerance

In the tropics, a successful maize cultivar must be able to withstand year-to-year variation in rainfall and associated moisture deficits at critical growth stages. Drought tolerant cultivars are characterized by minimal yield losses when soil moisture availability is significantly reduced. Except at the seedling stage, traits that increase plant survival but not production are of little value in selection (Bänziger et al., 2000). To minimize yield losses under drought without significantly compromising yield under optimal moisture conditions, selection for grain yield along with stress-adaptive secondary traits under drought stress should be coupled with selection for high yield potential and desirable agronomic traits.

Precision phenotyping for drought stress

A step-wise selection procedure to identify genotypic variability and the best performing progenies is often used by breeding programs. During the first stage, often called screening, large numbers of early generation progenies with only few (or no) replicates (although check entries are replicated to estimate trial repeatability) are evaluated at a few sites, while the second stage (referred to as phenotyping) deals with selected genotypes with more replicates and sites for detailed characterization. Progress in conventional or molecular breeding is highly dependent on phenotypic data quality. In conventional breeding, precision phenotyping allows reliable identification and selection of superior progenies for use in breeding for the targeted traits; in molecular breeding, it is the base for establishing genotype-phenotype associations and identifying potential genomic regions for use in forward breeding. Therefore, precision phenotyping is essential for efficiency and progress in crop improvement programs targeting a particular trait, especially complex polygenic traits such as drought stress.

Basic requirements for generating high-quality phenotyping data for drought stress under field conditions are described below.

1. Establishing the phenotyping site

A suitable location should be selected for establishing a site dedicated to phenotyping for drought stress. There are some basic requirements that must be met by the selected field, such as:

- Soil with medium texture and good moisture-holding capacity (FC between 35-40% and PWP 15-20%), in order to avoid frequent irrigation or fast depletion of soil moisture after imposing drought stress.

- A well-leveled field to facilitate irrigation and avoid water runoff and stagnation in patches.
- Good irrigation (and drainage) facility available to avoid random drought stress or excessive moisture/waterlogging.
- Field located away from large bodies of water (such as rivers, lakes, and ponds) as these could influence the micro-climate of the experimental field by increasing relative humidity (or reducing VPD) which could interfere with drought stress development. Bodies of water in the vicinity of the drought phenotyping site may also interfere through underground lateral movement of water to compensate for moisture depletion in the field due to the imposed drought stress. This may significantly affect the intensity/severity of the intended level of stress in the trial.
- No part of the field should be exposed to shading by trees or any other structures in the vicinity of the site.
- Spatial field variability should be mapped. Knowledge of soil physical and chemical properties that affect plant growth and stress development, and their uniformity within a field, is essential for selecting a suitable phenotyping field. A spatial field variability map can help establish a suitable experimental design and trial layout, so that no part of the trial is located in a very bad patch of the field. While initial characterization of potential phenotyping sites increases phenotypic accuracy by eliminating sites with unwanted variability and confounding factors, soil mapping can also be used to improve the precision of field experiments.

There are several options for mapping spatial field variability:

(i) Ideally, it should be carried out by growing a single crop variety (preferably maize, because other crops may vary significantly in their sensitivity to soil physical and chemical problems) to be able to identify existing field variability and bad patches, if any.

(ii) Soil variability can be directly assessed through destructive soil sampling at 30-cm soil depth intervals (to a depth of 90 or 120 cm) and analysis of key soil physical and chemical properties, which can provide information on the suitability of a site for phenotyping. Ideally soil samples should be taken across a field using a square grid basis with a minimum of five sampling points per hectare (Masuka et al., 2012).

(iii) Many high-throughput techniques are now available for mapping spatial field variability based on soil electrical conductivity sensors, penetrometers, spectral reflectance, thermal imagery of plant canopies and measurements of plant growth as surrogates for variability (Prasanna et al., 2013).

2. Variation in phenology

Large variation in genotypes' flowering time will bias the interpretation of the effect of drought-adaptive traits on yield under drought stress. Therefore, drought phenotyping trials should be carried out with more phenologically homogeneous materials. Before establishing the trial, genotypes should be grouped based on the similarity of their maturity (in days to anthesis). Genotypes should be grouped based on their anthesis date (preferably using growing degree days, GDD). This is crucial for avoiding different levels of stress within a trial, as entries with different maturities will reach the targeted crop stage (for example, flowering/early grain filling) at different times. Ideally, all entries within a trial should have comparable days to anthesis, though a difference of 2-3 days (which may be equal to around 30-50°C GDD) is acceptable; however, more than 5.0 days' difference within a trial should be strictly avoided. GDD can be calculated using the equation below (Kiniry, 1991):

$$GDD = \sum((Tmax+Tmin)/2) - \text{Base temperature } (8°C)$$

where Tmax = maximum temperature, and Tmin = minimum temperature.

However, if during some time periods Tmax is >34 or Tmin is <8°C, then the Tmax and Tmin values for those particular time periods need to be adjusted, as below, before using them in the GDD equation.

If Tmax >34, then Tmax = 34 - 2.6*(Tmax - 34).
If Tmax >44, then Tmax = 34 - 2.6*(44-34) = 8.
If Tmin <8, then Tmin = 8.

Staggered planting can help stop irrigation at the same time for trials of different maturity groups with significant variation in flowering time (Fig. 1).

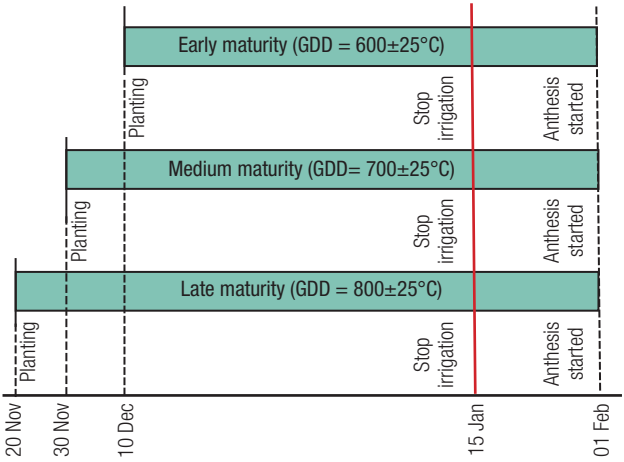

Figure 1. Staggered planting of trials of different maturity groups at the same location to facilitate imposing drought stress (and stop irrigation at the same time) and achieving the desired level of drought stress at the targeted crop stage (e.g., the reproductive stage).

3. Crop management

Except for managing irrigation during the reproductive growth stage to impose drought stress, all other recommended crop management practices should be followed in drought stress phenotyping trials. Adequate crop management, including timely application of recommended inputs and agronomic operations, is a pre-requisite for quality phenotyping.

A few reminders about key crop management practices are as follows:

- **Planting time:** Planting time is key for successful field-based phenotyping under managed drought stress. Therefore, planting time needs to be chosen carefully, so that the targeted crop stages (i.e., flowering and early grain-filling stage) coincide with a rain-free period to avoid early relief of the stress. This is done based on long-term weather data (for at least the past 5 years), including Tmax, Tmin and rainfall, which should be used to identify a suitable planting window.

  For example, at a location in Hyderabad, India (17.3850° N, 78.4867° E, 545 masl), the period from November to February is usually dry, i.e., almost rain free (Fig. 2). During most of this period, Tmax is < 35°C and Tmin is >8°C. Such a site is very suitable for drought phenotyping, because planting can be done during the last week of November, so that a trial with intermediate maturity entries will reach flowering sometime during the 1st week of February. The critical reproductive stage will be completed within the month of February, which is usually completely dry.

- **Plant population**: Number of plants per unit area is one of the components of final grain yield; therefore, this needs to be given due attention to ensure that the required plant population is maintained in the field. If seed availability is not a limitation, planting two seeds per hill (or double density) and thinning-out the extra seedlings at the 2- to 3-leaf stage (V_{2-3} stage) are recommended. Depending on

the soil's water-holding capacity and the irrigation facility, plant populations in drought stress phenotyping trials should be between 53,000 and 80,000 per hectare.

- **Border effect**: Fields and/or trials with different drought stress levels should be far enough apart (at least 10 m) to prevent border effects, especially when using sprinkler or furrow irrigation. Border rows (in double-density spacing) should be planted all around the trials in order to avoid border effects and any physical damage to test entries. To maintain the same level of competition, no row should be left empty (unplanted). Any empty row in the trial, whether a border row or a non-germinated entry, should be planted with bulk seeds and clearly indicated on the field map.
- **Moisture management:** Before imposing drought stress, irrigation intervals are designed so that the crop receives optimal moisture for good establishment and growth. If possible, the method of irrigation should be a combination of furrow/flood and sprinkler irrigation to ensure moisture is uniformly available across the field, which will later

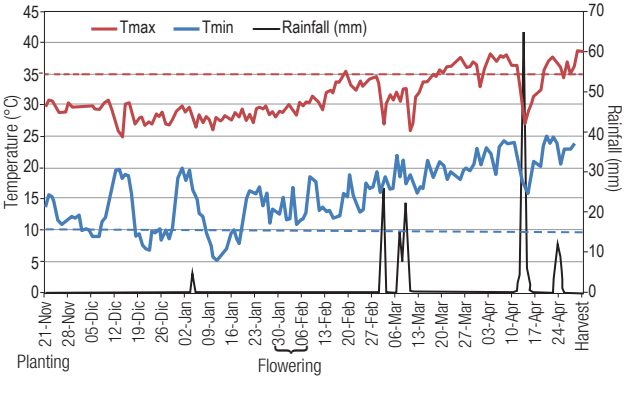

Figure 2: Usual weather conditions, including Tmax, Tmin and rainfall at Hyderabad, India (17.3850° N, 78.4867° E, 545 masl) during dry season (November to April).

also help to achieve uniform drought stress in the field trial. When using furrow irrigation, care should be taken to ensure that no water stagnates in furrows in any part of the field, as this may affect the micro-climate or cause excessive moisture stress. Though, drip irrigation is preferred for drought phenotyping trials because of its high precision in achieving a uniform moisture level across the field and, therefore, a uniform stress treatment as well.

- **Application of recommended inputs**: Recommendations regarding inputs including fertilizers (and their time of application and doses) and weed, insect pest and disease control measures are usually location-specific and depend on soil physical and chemical properties, and common biotic pressures. It is therefore essential to have updated information on the package of practices recommended for a particular phenotyping site and ensure that they are implemented on time, in order to keep the crop free from nutrient stress and biotic stresses such as weeds, insects or diseases.
- **Interactions with other stresses:** The presence of other biotic or abiotic stresses that influence plant growth and functions can limit the accuracy of drought phenotyping. These stresses can cause mechanical damage to roots (e.g., nematodes, root-worms), impairment of root growth (e.g., soil acidity, boron toxicity, salt stress) and/or reduce water availability to the crop (e.g., weeds, salt stress) and source capacity (e.g., foliar diseases, insect damage to the canopy). Similarly, interactions may occur when the effects of water deficit are evaluated in the presence of other abiotic stress factors (e.g., low-N fertility, high temperatures) that accelerate leaf senescence and/or enhance the role of specific adaptive mechanisms, such as the relocation of stem water-soluble carbohydrates in cereals, which normally play a less predominant role in determining yield (Tuberosa et al., 2012).

4. Weather data

In field-based phenotyping trials, it is essential to record weather data (including Tmax, Tmin, relative humidity, rainfall, dew, and wind velocity), which could significantly alter the overall effects of drought stress experienced by the crop. A portable weather data recorder should be installed within the phenotyping field for recording these weather parameters. Frequency of data recording is set at one-hour intervals, so that all critical weather data are captured on an hourly basis. Apart from directly recorded parameters, vapor pressure deficit (VPD) can be calculated for a given air temperature and the respective humidity value using the formula given below, and expressed in kPa (kilo Pascal).

VPD = ((100 - RH)/100)*SVP,where RH = relative humidity and SVP = saturated vapor pressure.

SVP can be calculated as follows:

SVP = 0.6108 * exp(17.27 * T / (T + 237.3)), where T = temperature (in °C)

This information will help to define how fast and severe the drought stress occurred at a particular site and later to cluster different sites according to the stress pattern before performing across-site data analysis.

5. Management of drought stress

Timing, intensity, and uniformity of the stress are the key factors to consider in stress management.

(i) *Stress timing* should be managed so that the crop is exposed to stress at the targeted growth stage(s).

(ii) *Stress intensity* should be severe enough so that traits that become important for yield are distinctly different from those which affect yield under non-stressed conditions. Drought tolerance per se is expected to play a progressively more important role than yield potential as the severity of drought escalates, with genotype ranking for yield changing considerably once

the mean yield falls below 20-30% of yield under optimal moisture (Blum, 2006) as a result of water scarcity. Consequently, germplasm evaluation in areas where drought severity fluctuates widely should be carried out preferably under well-watered conditions and at different levels of drought stress. In general, drought stress is considered intermediate when mean yield of the drought trial ranges between 40-50% of yield under optimal moisture, and severe when it goes below 30%.

(iii) Stress uniformity over space and time needs to be achieved so that genetic differences within a trial can be observed with ease.

When to stop irrigation to impose drought stress?

There are various methods that can be used to determine the date of the last irrigation (Bänziger et al., 2000). Generally, for the reproductive growth stage (from tassel emergence to early grain filling) to be exposed to drought stress, depends upon soil type (for example – in a medium texture soils), irrigation should be stopped about two weeks before anthesis in order to achieve the desired level of drought stress at anthesis and silk emergence. However, for improved accuracy, the recommendation is to apply the last irrigation and impose drought stress based on growing degree days (GDD), rather than days before anthesis, which may vary significantly with prevailing weather conditions (the formula for calculating GDD is given in section 3).

For example, in a trial with intermediate maturing genotypes having approximately 750±25°C GDD for anthesis and a site with about 10-12°C GDD per day during the period just before anthesis, a value of 550°C ∑GDD is recommended for applying the last irrigation before the drought treatment (Table 1). However, the GDD value for applying the last irrigation is site- and maturity group-specific; therefore, it needs to be calibrated once for a particular phenotyping site and maturity group.

Table 1: Accumulation of growing degree days (GDD) and time when the last irrigation is applied before imposing drought in a trial of an intermediate maturing group of maize hybrids at Hyderabad, India.

Activities	Month	Date	Day/Week	Tmax (°C)	Tmin (°C)	∑GDD (°C)
Planting	Dec	1	1	30.9	14.3	12.6
	Dec	7	Week-1	30.2	12.8	84.7
	Dec	14	Week-2	28.8	11.2	169.0
	Dec	21	Week-3	29.6	10.5	239.8
	Dec	28	Week-4	29.5	10.7	306.2
	Jan	4	Week-5	31.3	18.8	401.1
Install moisture probes	Jan	11	Week-6	27.8	15.1	502.5
Stop irrigation	Jan	18	49	25.4	10.2	556.4

When to resume irrigation to relieve drought stress ?

The time when irrigation is resumed is determined based on monitoring soil moisture depletion after imposing drought stress. The soil of the phenotyping site should be characterized to determine its field capacity (FC) and permanent wilting point (PWP). This can be done just once, as it is a location/site-specific physical property of the soil and does not vary much over years. Measuring soil water status makes it possible to repeat such experiments under comparable conditions and allows more rigorous assessment and sound interpretation of the results. It also allows quantifying and documenting the level of stress applied. Vertical profiling of soil moisture can be done using a suitable soil moisture profile probe with the following characteristics: a 1.0-m long moisture sensor that records soil moisture content at different depths (for example, 10, 20, 30, 40, 60 and 100 cm). Access tubes are placed in the field once all mechanical field operations are completed, at least 2 to 3 weeks prior to drought stress imposition. The number of access tubes depends on the spatial variability of the field, but ideally one tube in each block of the experimental design is recommended (if the number of available tubes is limited, then at least one tube per bed/range should be placed, depending on field variability/gradient). To keep track of moisture depletion at different soil depths and progress in drought stress development, soil moisture data are recorded

weekly, starting one week after applying the last irrigation in drought trials, until the stress is relieved. The appropriate time for resuming irrigation is when the soil moisture content at 30-40 cm soil depth approaches the PWP value (Fig. 3).

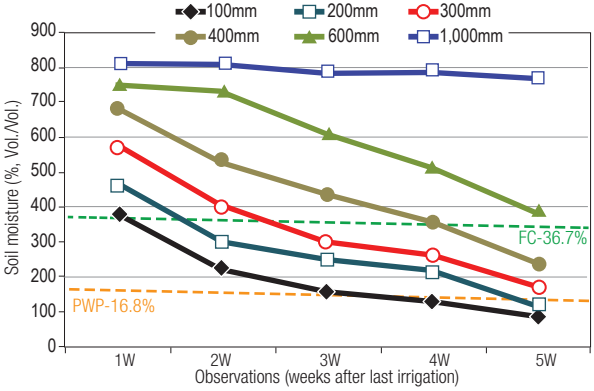

Fig. 3: Moisture depletion in a drought phenotyping trial after last irrigation to impose drought stress. (FC = field capacity and PWP = permanent wilting point of the soil at phenotyping site)

If a soil moisture monitoring system is not available, irrigation is resumed about two weeks after the end of male flowering in the trial to ensure grain-filling in the silks that were successfully pollinated and fertilized under drought stress. Apart from resuming irrigation to terminate drought stress, an additional irrigation may be necessary to ensure proper grain-filling. The following guidelines can help in the absence of (or in addition to) a moisture monitoring system:

Table 2: Guidelines for applying irrigation after flowering in a drought stress trial targeting the reproductive stage (Bänziger et al., 2000).

Average ASI (days) of the drought stress block	Irrigation after flowering
< 3	No
3-5	Yes, 2 weeks after male flowering
5-8	Yes, 1 week after male flowering
> 8	Yes, when 80-100% of the plots have completed male flowering

ASI = anthesis-silking interval.

6. Improving the uniformity of drought stress

After selecting a field or part of a field with low soil spatial variability, variation in the application of irrigation can introduce variation in drought stress intensity. It is therefore extremely important that the last irrigation before the stress period begins is applied as uniformly as possible, using at least a sprinkler system or, if available, a drip irrigation system until full saturation.

Some basic requirements for ensuring uniform irrigation using a sprinkler system:

- Irrigation should be done at a time of day when there is little or no wind.
- The sprinkler system should be cleaned and checked for leakages.Catch cans should be used to measure the amount of irrigation in places in the field where sprinkler water is expected to be relatively low. If the catch cans are placed systematically in the field, the volume of water collected in them can be used to adjust the sprinklers for uniformity.
- Field capacity in all parts of the field should be reached; the whole field normally remains at field capacity for one or two days after irrigation.

Increased uniformity of water application before the onset of stress will translate into more uniform drought stress and more uniform plant performance.

7. Data collection

Good phenotyping is critical for any kind of experimental activity. The challenges faced when attempting to collect good phenotypic data are often due to difficulties in standardizing, controlling and monitoring the environmental conditions under which plants are grown and the data are collected, especially in the field. The basic attributes of good phenotyping carried out with appropriate genetic materials are accurate and precise measurements, coupled with relevant experimental conditions that are representative of the target population environment (TPE). Accuracy involves

the degree of closeness of a measured or calculated quantity to its actual (true) value. Accuracy is closely related to precision (also termed reproducibility or repeatability), i.e., the degree to which further measurements or calculations show the same or similar results.

Though yield is a trait of primary interest, dissecting it into its components (secondary traits significantly associated with yield under stress) gives a better understanding of the targeted trait, and helps to keep track of stress intensity for mid-term correction, if needed. In addition, dissecting complex traits such as grain yield under drought into components adds to efforts aimed at discovering genomic regions.

Secondary traits can also be used as preliminary selection criteria when the turn-around time between seasons is short.

Traits that are significantly affected by drought stress under field conditions and thus should be recorded in drought stress phenotyping trials are:

(A) Priority traits (must record)	(B) Additional traits
1 Days to 50% anthesis and silking	11 Days to seedling emergence
2 Anthesis-silking interval (ASI)	12 Seedling vigor
3 Plant and ear height	13 Number of kernels per ear
4 Root and stem lodging	14 Test weight (100-kernel weight)
5 Leaf senescence	15 Physiological maturity
6 Plant population	
7 Ears per plant	
8 Ear weight (field weight)	
9 Grain yield (grain weight)	
10 Grain moisture content	

A description of each trait, along with the suitable stage and method of observation, are given below.

(A) Priority traits

1. **Days to 50% anthesis and silking:** Male (anthesis) and female (silking) flowering traits can be affected by drought stress, especially regarding reproductive success.

   When to record: Each plot should be monitored daily from the emergence of the first tassel in the field until all the entries in the trial have completed anthesis and silking. If entries are properly grouped by maturity, it should take approximately one week to finish recording flowering data, except in the case of highly susceptible entries where silking may be delayed due to stress.

   How to record: both anthesis and silking dates are recorded on a plot basis (not on the basis of just a few plants in the plot). Record the date when at least half of the plants in a plot *extruded the first anther* (*pollen shedding begins*) as 50% anthesis, and when the *first silk is visible* on at least half the plants in the plot as 50% silking (Fig. 4 a & b). Convert them into days after planting date, which indicates how many days it took to reach 50% anthesis or silking. Both days to anthesis and days to silking can be expressed in GDD terms if daily maximum and minimum temperatures are recorded from the planting day.

   In some genotypes, pollen shedding may start when the tassel is still in the leaf whorl (tassel not visible outside). In such rare cases, the leaf whorl can be partially opened manually, so that the tassel is visible for recording days to anthesis.

   What to select: Genotypes with no significant changes in anthesis or silking under drought stress compared to optimal conditions.

2. **Anthesis-silking interval (ASI):** It is a key secondary trait that is significantly affected by most abiotic stresses, including drought stress. It is the difference between number of days to anthesis and number of days to silking, and illustrates the synchrony between male and female flowering, essential for reproductive success. Under optimal conditions, male and female flowering is usually well

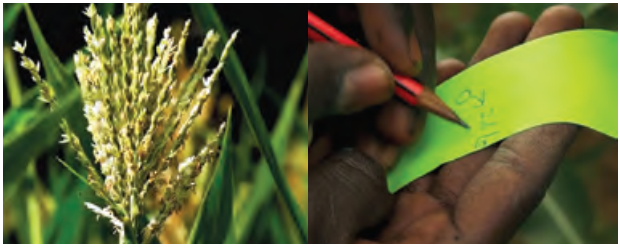

Fig. 4a. Recording anthesis date (50% of plants in a plot have started shading pollen)

Fig. 4b. Recording silking date (50% of plants in a plot have visible silk)

synchronized (occurring within 2 or 3 days). However, under stressful conditions, ASI may be prolonged, mainly due to a delay in days to silking (or, in rare cases, due to a delay in days to anthesis), which results in poor synchrony and, eventually, reproductive failure.

How to record: ASI is calculated as the difference between anthesis date (AD) and silking date (SD), as follows:

ASI = SD – AD.

What to select: Genotypes with short ASI (less than five days in the case of drought stress).

***Note**: In general, maize is a protandrous crop (male flowering happens first); therefore, ASI is mostly a positive value. However, in a few genotypes, ASI may be negative, given that some genotypes show protogyny (female flowering happens first).*

3. Plant and ear height:

When to record: Any time after anthesis and before harvest.

How to record: Plant height should be measured from the soil surface to the base of the tassel (excluding tassel length); ear height should be measured from the soil surface to the base of the ear, i.e., the node bearing the uppermost ear (Figs. 5 and 6). Observations should be recorded on five representative plants within each plot (avoiding plants near the alley) and noted as average.

What to select: Genotypes with the lowest reduction in plant and ear height due to drought stress compared to plant and ear height under an optimal water regime.

Fig. 5. Exact position for recording plant height (left) and plant height measurement in field (right).

4. **Root and stem lodging:** Due to competition for photo-assimilates and nutrients in favor of ear growth and grain development, there is a tendency towards increased plant lodging (stem or root lodging) under most abiotic stresses, including drought stress.

   When to record: Between physiological maturity (when most of the husk cover has dried) and harvest.

   How to record: Count the number of plants in a plot that lodged due to stem bending/breaking at an internode above the ground (*stem lodging*, Fig. 7a) or were uprooted from the base (*root lodging*, Fig. 7b). Calculate stem and root lodging percentages separately in relation to the total number of plants in the plot, including both lodged and un-lodged plants.

   What to select: Genotypes with no plant lodging under drought stress.

Fig. 6. Exact position for recording ear height (left) and ear height measurement in field (right).

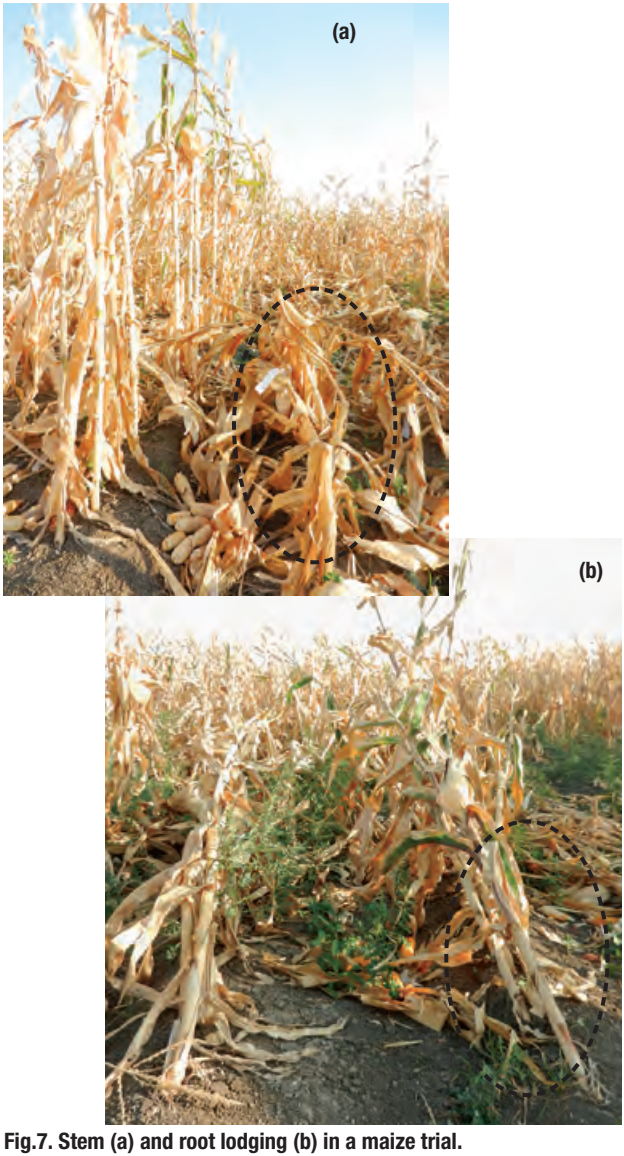

Fig.7. Stem (a) and root lodging (b) in a maize trial.

5. **Leaf senescence:** The speed at which a plant responds to drought stress by entering senescence reflects the ability of a plant to avoid or tolerate drought. This is particularly important during grain-filling, when resources are re-allocated within the plant. Initiation and rate of senescence are often correlated with yield under stress. Both the rate and degree can be assessed by visual scoring with heavy reliance on staff training and uniformity of drought stress application.

   When to record: Two weeks after flowering, on a weekly basis.

   How to record: score using a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead by 10: 1 = 10% dead leaf area; 2 = 20% dead leaf area; 3 = 30% dead leaf area; 4 = 40% dead leaf area; 5 = 50% dead leaf area; 6 = 60% dead leaf area; 7 = 70% dead leaf area; 8 = 80% dead leaf area; 9 = 90% dead leaf area; 10= 100% dead leaf area (Fig. 8).

   What to select: Genotypes with delayed senescence under drought stress.

6. **Plant population:** Though plant population may not be directly affected by drought stress, it is an important trait that should be recorded under various abiotic stresses and even in un-stressed trials. Plant population is directly

Fig. 8. Leaf senescence scoring on a scale of 0 (no dead leaf) to 10 (all leaves are dead).

related to yield per unit area, and also used in calculating various other stress related traits, such as plant lodging and ears per plant.

When to record: Between physiological maturity (when most of the husk cover has dried) and harvest.

How to record: Count the total number of plants in a plot (excluding one plant on each side of the alley), including both lodged and un-lodged plants.

7. **Ears per plant:** This is a key trait, as it is a yield attribute under both stress and non-stress conditions. It indicates the extent of barrenness among the genotypes under stress conditions.

   When to record: In the field, immediately after harvest.

   How to record: Count the total number of ears harvested in each plot (Fig. 9). If an ear has at least one grain, it should be counted as one ear. Calculate the number of ears per plant (EPP) using the formula given below. The purpose of making this observation is to assess stress-induced barrenness in the plot.

$$EPP = \frac{\text{Number ears per plot}}{\text{Total number of plants per plot}}$$

$$Barrenness = 1 - EPP$$

8. **Ear weight (kg/plot):** It is recorded in terms of ear weight per plot (also called *field weight per plot*) immediately after harvest.

   When to record: In the field, immediately after harvest.

   How to record: Measure total ear weight per plot using a suitable digital balance with a sensitivity of not less than 10 g (Fig. 10). Please take the following precautions when taking ear weight in the field.

   - Calibrate the balance at the beginning of the season using a reference weight.

Fig. 9. Recording number of ears per plot in field.

- When weighing in the field, confirm the accuracy of the values displayed on the balance at regular intervals (at least after every 100 plots) using a reference weight.
- Avoid using a hanging balance in windy conditions.

Note: Though ear weight/plot may not give the exact grain yield, it is accurate enough for Stage 1 (early generation/1st time testcross progenies with a large number of entries) and Stage 2 trials (advanced generation/2nd time testcross progenies with a large number of entries). However, in Stage 3 and Stage 4 trials, direct grain yield should be recorded after shelling the ears from each plot. This will of course require collecting all the ears from each plot and carefully threshing them on a per-plot basis.

Fig. 10. Measurement of cob weight (field weight) per plot after harvest.

9. **Grain weight (kg/plot):** Grain weight is recorded after shelling the ears in each plot (also called *grain yield per plot*).

   When to record: After shelling the ears in each plot.

   How to record: Measure total grain weight per plot using a suitable digital balance with a sensitivity of not less than 10 g (Fig. 11). Please take the following precautions when taking grain weight in the field.

   - Calibrate the balance at the beginning of the season using a reference weight.
   - Avoid using a hanging balance in windy conditions.

Fig. 11. Measurement of grain weight per plot after ear shelling.

10. **Grain moisture content:** In general, grain moisture content is high (>20%) at the time of harvest. It may also vary significantly among the different entries in the trial. Therefore, it is important to record grain moisture content in order to calculate final grain yield at a uniform (15%) grain moisture content.

When to record: It should be recorded in the field immediately after harvest, when grain yield is calculated in the field based on ear weight per plot. However, when calculating grain yield directly, moisture content should be recorded immediately after shelling the ears.

How to record: Prepare a sample by shelling a few kernels from a few ears (not just from one ear) in a plot and bulking them. Using a grain moisture meter, record moisture content for the plot (Fig. 12). Ideally, it should be recorded separately for each plot. However, in big trials with a large

Fig. 12. Measuring grain moisture content.

number of plots, grain moisture content should be recorded on at least 20% of the total plots in a trial (i.e., this trait should be recorded on every 5th plot).

(B) Additional traits

11. **Days to seedling emergence:** This is a key trait often associated with seedling vigor. Significant genotypic variability is observed for this trait under different crop management conditions. It is measured as the time it takes seedlings to emerge from the soil surface (commonly referred to as *germination*), which indicates genotypic variability for seed germination and coleoptile elongation during the autotrophic phase of seedling establishment.

    When to record: Start from the 3rd day after the effective planting date until at least 50% of all seedlings have emerged above the soil surface. The (effective) planting date is the day when the 1st irrigation after planting is applied for germination, if sowing is done in dry soil. Otherwise, it is the sowing date, if planting is done in a pre-irrigated field with enough moisture for germination.

    How to record: Count the number of coleoptiles visible above the soil surface in a plot. Note the date when at least 50% of the coleoptiles (of all seeds planted) have emerged, and calculate the total number of days it took to reach 50% emergence.

    What to select: Genotypes with faster seedling emergence.

12. **Seedling vigor**: Early plant vigor is an important trait, as it contributes to the overall performance of a genotype, especially under stress conditions.

    When to record: During the second and third week after seedling emergence.

How to record: Record NDVI (Normalized Difference Vegetation Index) data using a GreenSeeker. If there is no GreenSeeker available, seedling vigor can be scored on a 1-5 scale (1 = low, 5 = high).

13. **Number of kernels per ear:** This is an important trait that may vary significantly under abiotic stress, including drought, due to scattered grain-filling in ears because of irregular pollination or kernel abortion due to stress at early grain-filling stage.

    When to record: It should be recorded after harvest by shelling all kernels from five representative ears from each plot.

    How to record: Count the total number of kernels on five ears (using a seed counter, if available) from each plot; record the average number of kernels/ear by dividing by five.

14. **Test weight (100-kernel weight):** This is an important trait that may vary significantly under abiotic stress, including drought due to scattered grain-filling in ears because of irregular pollination or kernel abortion due to stress at early grain-filling stage.

    When to record: It should be recorded after harvest by shelling an equal number of kernels from a few representative ears to make a 100-kernel sample for each plot.

    How to record: Prepare a sample by shelling kernels from a few ears (not just from one ear), count 100 kernels (using a seed counter, if available) separately for each plot, oven dry to achieve a constant moisture content (15%) and record 100-kernel weight as the test weight for each plot.

15. **Physiological maturity:** At physiological maturity, a maize ear reaches its technical maturity, as all grains have achieved their maximum dry matter weight and become disconnected (though not detached) from the ear, and an abscission layer (called the black layer) is formed. The crop could be harvested at this stage, as grains have fully matured (and could be used as seed). However, grain moisture content is usually high (30-40%) at physiological maturity; therefore, the crop is left in the field for a few more weeks so that grain moisture content will decrease to 20-25%, which is called *harvest maturity*. The period from physiological maturity to harvest maturity is the dry-down phase.

    When to record: About 3-6 weeks after anthesis (depending on the maturity group and prevailing weather conditions), when the crop is heading towards maturity.

    How to record: Physiological maturity is recorded on a plot basis (not just on a few plants per plot). At physiological maturity, the leaves wrapped around the ear (husk cover) start drying, beginning from the base and progressing towards the tip of the ear. Note down the date when >70% of the husk cover leaves on the ear have dried.

    Before proceeding to the final harvest, please make sure that:

    - *Data on plant and ear height, root and stem lodging, and plant population per plot are recorded.*
    - *If flowering data are recorded on field labels, they are transferred to a data sheet.*
    - *The first plant (along with the ear) in each row next to the alley is removed.*

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