Management of drought stress in field phenotyping

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Acknowledgements

Breeding for drought tolerance is among the top 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.

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; Zaman-Allah *et al*., 2016). The information presented in this manual is based on the work on quantitative management of drought stress phenotyping under field conditions that received strong and consistent support from several donor agencies, especially the BMZ/GIZ, Germany and the MAIZE CGIAR Research Program. The financial support for compilation and publishing this field manual from CGIAR Excellence in Breeding (EiB) platform is duly acknowledged.

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, WHEAT and Excellence-In-Breeding (EIB) platform.

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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 crops grown during the summer-rainy season in the tropics occasionally face extreme weather conditions. These extreme weather conditions translate into various abiotic stresses, such as intermittent/contingent drought, which constitutes one of the key abiotic constraints for crop production in many parts of the world. The erratic distribution pattern of rain in the tropics due to inter-annual variability occasionally causes prolonged dry spells at different crop growth stages, which results in poor crop growth and development and eventually poor yields.

Crop breeding programs targeting drought tolerance using conventional and/or molecular breeding approaches rely heavily on high-quality phenotypic data generated under drought stress. One of the key pre-requisites for generating a quality drought phenotyping data is accuracy and precision in managing drought stress in field phenotyping, so that genotypic variability could be expressed and precisely identified. Applying drought stress at required intensity with uniformity and precisely at targeted crop stage has been a major challenge, especially under field conditions, as several weather factors interact and affects the stress development and its intensity. Occasionally, the managed drought stress trials ended with either low or too severe stress, which is often realized after completing the trial. In this manual, the quantitative approach of managing drought stress with required intensity at targeted crop stage (for example – flowering stage), and with uniformity over space (across plot) and duration is discussed. This manual is developed for field crop breeders, crop physiologists, agronomists, masters or Ph.D. students and field technicians who are working on phenotyping and/or selection for drought stress tolerance in field crops.

Management of drought stress in field phenotyping

Crops grown under rainfed conditions in tropics occasionally face contingent/intermittent drought largely due to erratic distribution pattern of rains. Drought is identified as one of the major factors responsible for year-to-year variation and instability in crop productivity and production in tropics. Crop breeding programmes targeting drought tolerance using conventional or molecular breeding approaches rely heavily on high quality phenotyping data generated from drought screening trials.

Timing, intensity and uniformity of imposed stress in a drought trial is key for precision phenotyping and identifying available genotypic variability among test entries for drought tolerance.

The key aspects for conducting a managed drought stress trials are described as follows:

1. Understanding the target population environment:

A clear understanding about target population environment (TPE) is essential for planning and selecting the best suitable selection environment where the phenotyping site should be established. The phenotyping site does not necessarily have to be in the target environment, but should have a good representation of the TPE. Therefore, a minimum set of information about the TPE is required for establishing phenotyping site, such as:

- Daily weather data, at least for past 5 years, including maximum temperature (Tmax), minimum temperature (Tmin), relative humidity (RH) and rainfall.
- Soil type, cropping season and cropping system, especially the cropping window for the targeted crop (e.g. maize) in the TPE.
- Other relevant information, such as major biotic stresses and socio-economic constraints.

Analysing these information will help in defining the most relevant type of drought stress and understanding the requirements for establishing a phenotyping site that is significantly related to the TPE.

2. Selection of precision phenotyping site:

Though drought is a meteorological phenomenon that occurs due to prolonged dry-spell during rainy season, it is almost impossible to precisely predict such dry-spells at targeted crop stage during rainy season. Therefore, a managed drought stress screen is conducted during rain-free dry season, where stress is imposed by managing irrigation schedule in such a way that test entries are exposed to desired level of drought at targeted crop stage. On the basis of a careful analysis of past 5 years weather data including T_{max}, T_{min} and rainfall for all potential locations, a suitable site with rain-free period during targeted crop stage could be identified. Apart from rain-free period, other weather conditions should be suitable for growing the crop at the selected location in the planting window identified

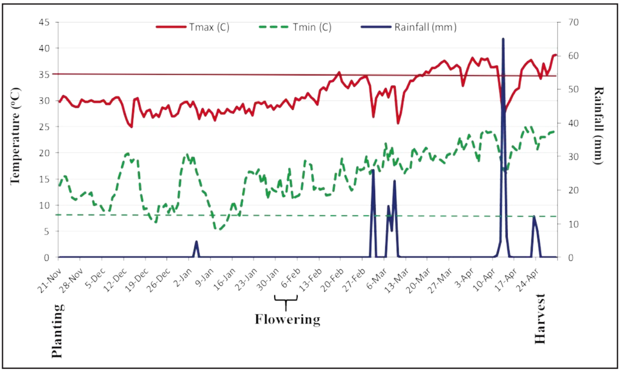

Fig. 1: Usual weather condition, including Tmax, Tmin and rainfall at Hyderabad, India during dry season (November to April).

for drought phenotyping trial. For example – in maize breeding for drought tolerance the targeted crop stage is flowering and early grain-filling stage. At Hyderabad location in India (17.3850° N, 78.4867° E, 545 masl), November to February months are usually a dry season as most parts during this period is almost rain-free (Fig. 1). Also, T_{max} is <35^{0}C and T_{min} is >8^{0}C in most part of this period, which is suitable for growing maize crop. Therefore, this site is identified as a suitable location for drought phenotyping, where planting can be taken-up during last week of November, a trial with medium maturity group of entries reaches to peak flowering stage around 1st week of February, and most critical stages of reproductive phase complete within month of February. At this site drought stress could be imposed at a desired intensity (and duration) and timing (targeted crop stage) with good uniformity by managing irrigation schedule.

3. Establishing the drought phenotyping site:

Based on TPE analysis followed by suitable location identification, a field phenotyping plot need to be selected for establishing a dedicated site for drought stress phenotyping. It is important to ensure that the selected field satisfies following basic requirements:

- A field with medium textured soil (Fig. 2) and good water holding capacity in order to avoid frequent irrigation and/or very fast drought stress development after imposing stress by withdrawing irrigation.

Fig. 2: Soil type requirement for a suitable field plot for drought phenotyping.

- Soil physical properties of the phenotyping site should be characterized to determine the field capacity (FC) and permanent wilting point (PWP). This need to be done only once as it is a site-specific physical property of the soil and does not change over several years.
- Well-levelled field to facilitate smooth water-flow during irrigation to avoid water run-off and stagnation in patches.
- Good irrigation (and drainage) facility to avoid random drought stress or excessive moisture/ waterlogging during trial.
- Field located away from large water bodies water (such as rivers, lakes, ponds etc.) as these could influence ground water-table as well as the micro-climate of the experimental field by increasing relative humidity (RH) or reducing vapour pressure deficit (VPD) that can interfere with stress development and intensity.
- No part of field should be exposed to shading effect by trees or other high-rising structures in vicinity of the field.
- *Spatial field variability*: knowledge about soil physical and chemical properties that affects plant growth and stress development, and within field variability date of key soil parameters is essential in selection of suitable phenotyping field. A spatial field variability mapping can help in establishing a suitable experimental design and trial layout, so that no part

of the trial is located in bad patches. While initial site characterisation increases phenotypic accuracy by eliminating sites with high field variability and confounding factors, soil mapping can also be used as covariate to further improve the precision in field phenotyping data quality.

There are various options for mapping spatial field variability:

- Ideally, it should be carried-out by growing a single crop variety (preferably the same crop for which drought phenotyping site is to be developed, as different crop may vary significantly in their sensitivity to soil physical and chemical properties) to be able to identify existing field variability and bad patches, if any.
- Direct assessment of soil variability is possible through destructive soil sampling at different depth intervals (up to a depth of 90 or 120 cm) and analysis of key soil physical and chemical properties. This analysis can provide information on the suitability of a site for drought phenotyping. Soil samples should be taken across field using a square grid basis with a minimum of five sampling points per hectare (Masuka *et al*., 2012).
- 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 of variability (Prasanna *et al*., 2013).

4. Managing variation in phenology:

Drought phenotyping trials should be carried out with more phenologically homogeneous entries in a trial. Genotypes should, therefore, be grouped in trials based on similarity in their flowering-time. Such grouping should be preferably based on using growing degree-days (GDD) units. This is crucial for avoiding different levels of stress within a trial, as entries with different maturity group will reach the targeted crop stage (for example, flowering/early grain-filling) at different times. Ideally, all entries within a trial should have comparable flowering days, for example- in case of maize, a difference of 2-3 days (or equivalent GGD units) is acceptable, but more than 5.0 days difference in

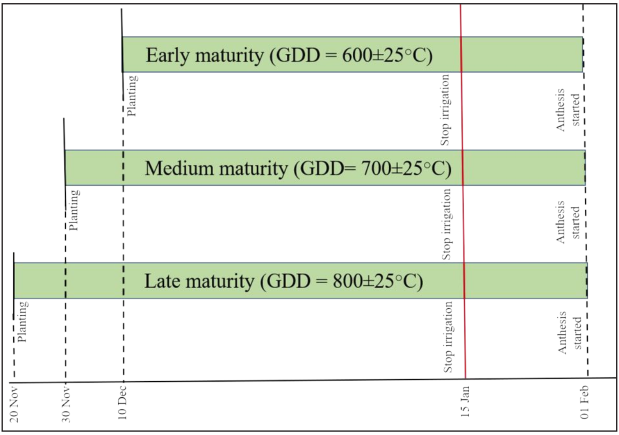

Fig. 3: Staggered planting of different maturity group of trials to facilitate imposing drought stress (applying last irrigation same time in all the trials) to achieve desired level of drought stress at targeted crop stage (e.g.- flowering and early grain filling stage); GDD = growing degree days.

flowering days within a trial should be strictly avoided. In case there is no option but to take-up more than one maturity group trials at same site, then staggered planting (late maturity trials first and early at last, Fig. 3) can be used so that last irrigation is applied at same time in all trials with significant variation in flowering time.

All the test entries should be first grown in the main crop season. GDD of each test entry can be calculated using the equation given below, and entries with similar maturity group should be grouped in one trial for managed drought stress phenotyping trials.

GDD = (Tmax+Tmin)/2) – base temperature or absolute minimum temperature

where Tmax = hourly maximum temperature

if Tmax >absolute maximum then Tmax = absolute maximum

Tmin = daily minimum temperature

if Tmin < absolute minimum then Tmin = absolute minimum.

Note*: There are four temperature thresholds, called the cardinal temperatures that define the growth of a crop, including the absolute minimum, the optimum minimum, the optimum maximum, and the absolute maximum. The absolute minimum and maximum temperatures define the coldest and hottest temperatures beyond which a crop will stop to grow. Temperatures between the optimum minimum and maximum define the best suitable range of temperature for the crop growth. These cardinal temperatures vary for different crops; maize (Zea mays L.), for example, has an absolute minimum temperature of 8*°C, an optimum minimum of 18°C*, an optimum maximum of 33°C, and an absolute maximum of 44°C.*

5. Crop management

Except irrigation management 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 high quality phenotyping data.

- **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 is not a limitation, planting extra seeds per hill (or double density) and thinning-out extra seedlings, once seedlings are fully established is recommended.
- **Border effect**: Border rows should be planted (in double-density spacing) all around the trials in order to avoid border effects on test-entries and any physical damage. In addition, to maintain the same level of competition, no space should be left empty (un-planted) inside the trial. Any empty space in the trial whether border row or free space due to non-germination should be planted using some bulk seeds and clearly indicated in the field map.
- **Moisture management:** Before imposing drought stress, irrigation intervals need to be well defined so that the crop has optimal moisture conditions for good establishment and growth. If possible, method of irrigation should be combination of furrow/flood

and sprinkler system to ensure uniformity in moisture availability across the field, which eventually helps in achieving a uniform drought stress treatment in a field trial. In case of furrow irrigation, it should be ensured that no water stagnated in any part of the field, as this may affect the microclimate or may cause excessive moisture stress. However, drip irrigation is preferred in drought phenotyping trials because of its high precision in achieving uniform moisture level across the field, and therefore uniformity in stress treatment as well.

- **Application of recommended inputs**: Recommendations regarding inputs including fertilizers (their time of application and doses), weed, insect-pest and disease control measures are usually location-specific and depend on soil physical and chemical properties and common biotic pressures. Therefore, it is essential to have updated information on the recommended package-of-practices for the phenotyping site and ensure that they are implemented on time, in order to keep the crop free from nutrient stress and any biotic stresses such as weeds, insects or diseases.
- **Interactions with other stresses:** Presence of other biotic or abiotic stress agents that influence plant growth and functions can limit the accuracy of drought phenotyping. These agents may 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., presence of weeds,

salt stress) and source capacity (e.g. foliar diseases, insect damage to the canopy). Similarly, interactions may occur when evaluation for drought stress is done in presence of other unintended abiotic factors (e.g. low-nitrogen fertility, high or low temperatures etc.).

6. Weather data

In field-based drought phenotyping trials, it is essential to record weather data (including Tmax, Tmin, relative humidity and rainfall) that could significantly alter the overall effects of drought stress experienced by the crop. A portable weather data recorder should be installed within or in vicinity of the phenotyping field for regular recording of these weather parameters. Apart from directly recorded parameters, vapour pressure deficit (VPD) can be calculated at given air temperature and respective humidity value using the formula given below, and expressed in kPa (kilo Pascal).

V.P.D. = ((100 - RH)/100)*SVP,

where R.H. = relative humidity and S.V.P. = saturated vapor pressure.

S.V.P. can be calculated as follows:

S.V.P. = 0.6108 * exp(17.27 * T / (T + 237.3)), where T = temperature (in °C)

7. Management of drought stress

Timing, intensity, and uniformity of the stress are the key factors to consider in managing drought stress in field conditions.

- *Stress timing* should be managed such that the targeted growth stage(s) are exposed to the desired level of drought stress.
- *Stress intensity* should be severe enough so that important traits for yield under stress become distinct 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, as genotype ranking for yield changes considerably once the mean yield falls below 20–30 percent of yields under optimal moisture as a result of water scarcity. In general, a drought stress is considered intermediate when mean yield of the drought trial ranges between 40-50 percent of yield under optimal moisture, and severe when it goes down below 30 percent.
- *Stress uniformity* over space and time is necessary for expression of genotypic variability within a trial and that could be clearly observed and recorded.

There are some key factors that contribute in achieving a desired level of drought stress at targeted crop stage with reasonable uniformity.

(a) When to apply last irrigation to impose drought stress?

Various methods are available to determine the day of last irrigation for imposing drought stress at targeted crop growth stage, For example - in case of flowering stage drought stress in maize, depends upon soil type (for example – in a medium texture soils), irrigation should be stopped about two weeks before anthesis (Banziger *et al*., 2000). However, the criteria based on days or weeks might not always be accurate because it is largely dependent on prevailing weather conditions in a particular cropping season/year. Therefore, for improved accuracy there is need of a quantitative criteria such as growing degree-days (GDD) from planting to identify the day of last irrigation for imposing drought stress.

For example – in case of a reproductive stage drought trial with medium maturing group of genotypes having GDD for anthesis of approximately 750±25^{0}C, and a site with about 10-12^{0}C GDD per day during pre-flowering stage, the day when about 550^{0}C ∑GDD is reached, is identified appropriate time for applying the last irrigation (Table-1). However, the GDD value for last irrigation is a site and maturity group specific, therefore, this need to be calibrated at least once for a particular phenotyping site and maturity group. An example of how to calculate GDD is given in *Annexure-I*.

Table 1 : Accumulation of growing degree days (GDD) and time to apply last irrigation for imposing drought in a medium maturity group of maize hybrid trial at Hyderabad location in India.

Activities	Month	Day	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**

Uniformity of last irrigation before imposing drought stress is critical for uniform moisture regime across field, and therefore development of uniform drought stress.The best option for achieving this uniformity is to use drip irrigation system until full saturation. The second best option is sprinkler irrigation system, applied in two instalments; for example: first round of 3-4 hours and after a gap of few hours second round until full saturation. It is difficult to achieve a uniform moisture across field using furrow/flood irrigation at least at this critical stage for drought trials. After applying last irrigation uniformly up to full saturation level all possible sources of water heading towards drought trials should be properly closed, and as a precaution, install a board displaying '*Drought trial or No irrigation*' at all possible sides of drought plot (Fig. 4).

Fig. 4: Last irrigation at GDD = 556.4°C in a drought phenotyping trial using sprinkler system at a drought phenotyping in Hyderabad, India.

Some basic precautions for ensuring uniform irrigation using sprinkler system are as below:

- Irrigation should be done at a time of the day when there is little or no wind.
- Sprinkler system should be cleaned and checked for leakages.
- Catch cans should be used to measure the amount of irrigation at places in the field where sprinkler water is expected to be relatively low. If placed systematically in the field, the volume of water collected in the catch cans can be used to adjust the sprinklers for uniformity.

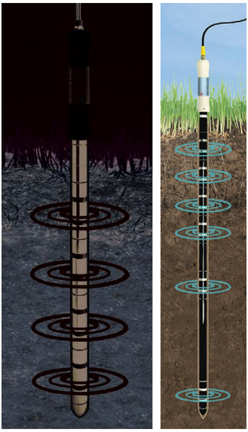

Fig. 5: Vertical soil moisture probes for moisture profiling at different soil depth.

(b) Soil moisture profiling:

Recording soil moisture data after imposing drought stress helps in regular monitoring of drought development in field and achieving desired level of stress, which eventually helps in generating high quality phenotyping data.

The key steps involved in the process are as follows:

Installation of soil moisture profile probes: Vertical profiling of soil moisture can be done using a suitable soil moisture profile probes depending on root depth of the crop (For example: Short and long versions of the PR2, with 4 or 6 sensors along the length, Fig. 5, https://www.delta-t.co.uk/product/pr2/). Porous access tubes (Fig. 6a) need to be installed in field once all mechanical field operations are completed, at least one week prior to applying last irrigation. These tubes are fibre glass

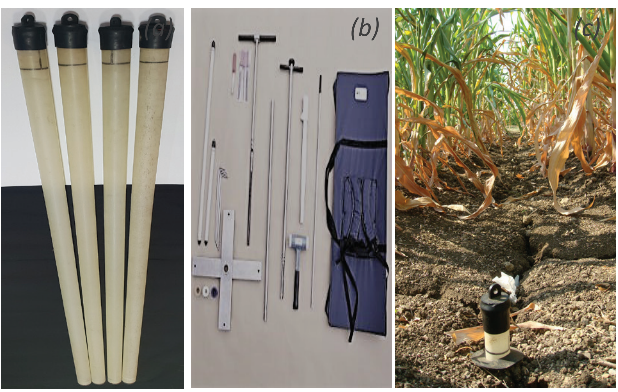

Fig. 6: (a) Porous access tubes, (b) accessories for installation of the tubes in field and (c) a tube properly installed in field.

specially constructed thin-wall tubes, which maximise the penetration of the electromagnetic field into the surrounding soil. A set of tools are supplied along with equipment for installation of porous tubes in field (Fig. 6b). The tools need to be used as per given guideline for proper installation of each porous tube in a way that full-length of the tube, up to black ring near the top, is inserted into soil (Fig. 6a). Each tube need to be immediately capped properly after installation using rubber cap supplied with tubes (Fig. 6c) so that any object including soil etc. should not fall inside the tubes. The number to be installed in a field depends on the spatial variability in field. Ideally, one tube in each block of experimental design is recommended, or at least one tube in each range (or bed) should be placed following spatial pattern in field (Fig. 7) to optimize moisture depletion data capture across field after imposing drought stress.

Fig. 7(a) Installation of tubes in field and (b) distribution of porous tube for soil moisture data recording using soil moisture profile probe in a drought trial field.

Fig. 8: PR2/6 profile probe with data-logger.

Recording soil moisture: In deep-rooted crops like maize vertical profiling of soil moisture is done using PR2/6 profile probes. The 1.0m long PR2 probe measures volumetric soil moisture at different depths within the soil profile including 10, 20, 30, 40, 60 and 100 cm depth. It consists of a sealed polycarbonate rod, ~25mm diameter, with electronic sensors as pairs of stainless steel rings arranged at fixed intervals along its length (Fig. 8). Each sensor produce an electromagnetic fields extend into the soil (Fig. 5) and a voltage output, which is converted into soil moisture using a general soil calibrations. The probe needs one-time calibration for specific soil type for a dedicated phenotyping site.

For recording soil moisture data the probe is fully inserted into the access tube after removing the cap and after 15-30 second volumetric soil moisture content ($m^3.m^{-3}$ or % volume) at different soil depth can be read in data-logger (Fig. 8), which can be manually noted or saved in the data-logger. The moisture reading process in each tube should be repeated at least twice, and

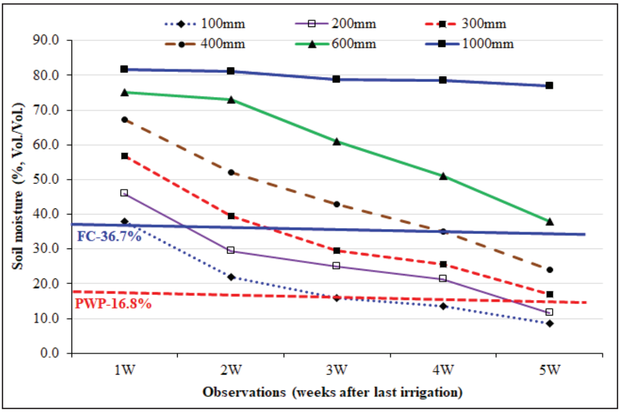

Fig. 9: Moisture depletion at different soil depth in a drought phenotyping trial after last irrigation. (FC = field capacity and PWP = permanent wilting point of the soil at phenotyping site).

average should be used as soil moisture content ($m^3.m^{-3}$ or percentage volume) for that particular tube. For second reading in the same tube, take-out the sensor and rotate it to about 90°, again insert it into access tube to repeat the data in same tube.

In a drought phenotyping trial moisture data need to be recorded at a regular interval, at least weekly, starting from one week after applying last irrigation, until the stress is relieved or irrigation is resumed. This helps in keeping track with moisture depletion at different soil depth (Fig. 9), progress in drought stress development, and deciding the threshold of drought intensity for terminating stress. Measuring soil water content allows the repetition of such experiment under similar conditions as well as more rigorous assessment and interpretation of the results. It also enables to quantify and document the level of stress applied.

(c) When to resume irrigation to terminate drought stress?

It is a critical decision to make in order to identify available genotypic variability for drought tolerance. The criteria such as days after imposing drought stress, visible drought stress symptoms in field etc. for terminating the stress are often misleading. A quantitative criteria based on monitoring soil moisture depletion in root zone after imposing drought stress is more reliable in taking decision on termination of stress in a drought trial. For example – stress should be terminated (irrigation resumed) in case of drought phenotyping trial for maize in a medium textured soil (clayey loam), once the soil moisture content at a depth of 30-40cm reached near permanent wiling point (PWP) of the soil (Fig. 9). However, this limit may vary for a particular site and crop, and therefore need to be calibrated at least for one season.

Along with soil moisture data, keeping track with accumulated VPD at Tmax after applying last irrigation helps in keeping track with severity of drought stress; *For example* – in case of maize drought trial at Hyderabad location during winter cycle, a total accumulated VPD ~ 120.0kPa from day after last irrigation to the day of terminating stress was calibrated and used along with moisture depletion data for taking decision on terminating stress. Moisture depletion data supported with VPD information is especially important in some unusual years, when crop encounters a spell of dry wind and a period of prolonged high VPD regime. In such unusual situation, even if moisture content is yet to be depleted to the targeted limit, trial may suffer with severe drought stress due to high VPD regime. Therefore, in such situation VPD criteria helps in taking decision of early termination of drought stress to avoid losing drought trial. VDP data also helps in clustering different sites according to stress pattern and severity before performing across-site data analysis. An example of how to calculate accumulated VPD is given in *Annexure-II*.

The three key steps, i.e.- time of imposing drought stress, monitoring/tracking moisture depletion and terminating the stress at appropriate time, are the key of a successful drought phenotyping trials. Once these steps are implemented based on quantitative criteria discussed in above section, the genotypic variability for drought stress is clearly expressed, which could be recorded in form of high quality data related to various agronomic and yield traits (Fig.10).

Fig. 10: Variability among maize genotypes for agronomic and yield traits under managed drought stress.

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Annexure-I

Calculation of growing Degree Days (GDD - degree C).							
Stage/key activities	Month	Date	Days	Tmax	Tmin	GDD value/day	∑GDD (0C)
				(A)	(B)	C = ((A+B)/2) - 8	∑ C
Planting	NOV	28	1	30.2	11.8	**13.0**	**13.0**
	NOV	29	2	29.8	12.4	13.1	26.1
	NOV	30	3	29.8	11.8	12.8	38.9
	DEC	1	4	30	12.4	13.2	52.1
	DEC	2	5	29.8	10	11.9	64.0
	DEC	3	6	29.8	10.2	12.0	76.0
	DEC	4	7	29.8	10	11.9	87.9
	DEC	5	8	30	9	11.5	99.4
	DEC	6	9	29.4	8.9	11.2	110.6
	DEC	7	10	29.4	8.9	11.2	121.7
	DEC	8	11	30.2	11.4	12.8	134.5
	DEC	9	12	30.6	12.2	13.4	147.9
	DEC	10	13	30.6	15	14.8	162.7
	DEC	11	14	29.4	17.2	15.3	178.0
	DEC	12	15	27	19.6	15.3	193.3
	DEC	13	16	25.6	19.8	14.7	208.0
	DEC	14	17	25	18.2	13.6	221.6
	DEC	15	18	30.2	18.8	16.5	238.1
	DEC	16	19	30.4	18.6	16.5	254.6
	DEC	17	20	28.2	12.6	12.4	267.0
	DEC	18	21	26.8	9.2	10.0	277.0
	DEC	19	22	28	9.4	10.7	287.7
	DEC	20	23	28.2	7.7	10.0	297.7
	DEC	21	24	26.7	6.9	8.8	306.5
	DEC	22	25	27.4	6.7	9.1	315.5

	DEC	23	26	26.8	9.8	10.3	325.8
	DEC	24	27	28.6	9.4	11.0	336.8
	DEC	25	28	28.4	10.2	11.3	348.1
	DEC	26	29	29	8.5	10.8	358.9
	DEC	27	30	27	10	10.5	369.4
	DEC	28	31	27	8.5	9.8	379.1
	DEC	29	32	27.6	10.2	10.9	390.0
	DEC	30	33	29.2	16	14.6	404.6
Install porous tubes	DEC	31	34	29.6	19	16.3	420.9
Install porous tubes	JAN	1	35	29.8	19.8	16.8	437.7
Install porous tubes	JAN	2	36	28.8	17.8	15.3	453.0
	JAN	3	37	29.8	19.6	16.7	469.7
	JAN	4	38	28.4	16.4	14.4	484.1
	JAN	5	39	26.4	15	12.7	496.8
	JAN	6	40	28.4	12.6	12.5	509.3
	JAN	7	41	27.2	12.2	11.7	521.0
	JAN	8	42	28.3	10.2	11.3	532.3
	JAN	9	43	27.6	8.4	10.0	542.3
Last irrigation	JAN	10	44	26.2	5.6	7.9	550.2
	JAN	11	45	28.2	5.2	8.7	558.9
Moisture data	JAN	12	46	27.6	5.5	8.6	567.4
	JAN	13	47	27.6	6.2	8.9	576.3
	JAN	14	48	28.4	7	9.7	586.0
	JAN	15	49	27.8	9	10.4	596.4
	JAN	16	50	27.7	10.8	11.3	607.7
	JAN	17	51	28.8	8.8	10.8	618.5
	JAN	18	52	28.2	8	10.1	628.6

Moisture data	JAN	19	53	29.4	11.7	12.6	641.1
	JAN	**20**	54	27.6	15	13.3	654.4
	JAN	21	55	28.4	16.4	14.4	668.8
	JAN	22	56	27.2	16.2	13.7	682.5
	JAN	23	57	29.5	15.6	14.6	697.1
	JAN	24	58	29.6	16.8	15.2	712.3
	JAN	25	59	29.4	14	13.7	726.0
Moisture data	JAN	26	60	29.8	16	14.9	740.9
Flowering	JAN	27	61	28.6	11.2	11.9	752.8
Flowering	JAN	28	62	29	13.4	13.2	766.0
Flowering	JAN	29	63	28.2	12.8	12.5	778.5
Flowering	JAN	30	64	29	12.5	12.8	791.2
Flowering	JAN	31	65	28.8	15.2	14.0	805.2
Flowering	FEB	1	66	29.6	11.7	12.7	817.9
Moisture data	FEB	2	67	30.2	11.8	13.0	830.9
	FEB	3	68	29.2	17	15.1	846.0
	FEB	4	69	28.4	11	11.7	857.7
	FEB	5	70	30.4	11.5	13.0	870.6
	FEB	6	71	30	11.8	**12.9**	**883.5**
	FEB	7	72	30.6	13	13.8	897.3
	FEB	8	73	30.5	18.5	16.5	913.8
Moisture data	FEB	9	74	31.4	18	16.7	930.5
	FEB	10	75	30.6	17.6	16.1	946.6
	FEB	11	76	30.2	13	13.6	960.2
	FEB	12	77	29.2	13.6	13.4	973.6
	FEB	13	78	32	13	14.5	988.1
	FEB	14	79	32.5	13.2	14.9	1003.0
	FEB	15	80	32	11.8	13.9	1016.9
Moisture data	FEB	16	81	33.6	12	14.8	1031.7
	FEB	17	82	33.7	12.2	15.0	1046.6

	FEB	18	83	34.5	16	17.3	1063.9
Terminate stress	FEB	19	84	35.4	15.4	17.4	1081.3
	FEB	20	85	33.6	18.8	18.2	1099.5
	FEB	21	86	32.8	15.6	16.2	1115.7
	FEB	22	87	32.4	14.2	15.3	1131.0
	FEB	23	88	33.8	12.8	15.3	1146.3
	FEB	24	89	32.8	13.4	15.1	1161.4
	FEB	25	90	33.5	17.5	17.5	1178.9
	FEB	26	91	34.2	16.6	17.4	1196.3
	FEB	27	92	34.4	17	17.7	1214.0
	FEB	28	93	34.6	19.4	19.0	1233.0
	MAR	1	94	32.8	16	16.4	1249.4
	MAR	2	95	26.8	17.4	14.1	1263.5
	MAR	3	96	30.4	18.6	16.5	1280.0
	MAR	4	97	31.7	16.5	16.1	1296.1
	MAR	5	98	31	16.6	15.8	1311.9
	MAR	6	99	32.2	21.8	19.0	1330.9
	MAR	7	100	30.6	18.4	16.5	1347.4
	MAR	8	101	32.6	21.2	18.9	1366.3
	MAR	9	102	32.6	17.2	16.9	1383.2
	MAR	10	103	25.6	18.8	14.2	1397.4
	MAR	11	104	27.5	17.5	14.5	1411.9
	MAR	11	105	31.5	16	15.8	1427.6
	MAR	12	106	32.2	17	16.6	1444.2
	MAR	13	107	33.7	16.7	17.2	1461.4
	MAR	14	108	33.7	21.1	19.4	1480.8
	MAR	15	109	34.6	20	19.3	1500.1
	MAR	16	110	34.8	18.4	18.6	1518.7
	MAR	17	111	34.7	20	19.4	1538.1
	MAR	18	112	35.6	21	20.3	1558.4
	MAR	19	113	35.2	20.4	19.8	1578.2
	MAR	20	114	36.2	18.2	19.2	1597.4

	MAR	21	115	36.2	19.4	19.8	1617.2
	MAR	22	116	36.7	18.8	19.8	1636.9
	MAR	23	117	37.2	18.6	19.9	1656.8
	MAR	24	118	37.6	18	19.8	1676.6
	MAR	25	119	37	19.6	20.3	1696.9
	MAR	26	120	36	19.8	19.9	1716.8
	MAR	27	121	36.2	19.4	19.8	1676.6
	MAR	28	122	36.8	20.6	20.7	1737.5
	MAR	29	123	36.2	23	21.6	1759.1
	MAR	30	124	32.8	20.4	18.6	1777.7
	MAR	31	125	35.2	21.6	20.4	1798.1
	APRIL	1	126	36.8	23.4	22.1	1820.2
	APRIL	2	127	38.2	22.2	22.2	1842.4
	APRIL	3	128	37.4	20.4	20.9	1863.3
	APRIL	4	129	36.7	18.2	19.5	1882.8
	APRIL	5	130	38	23.6	22.8	1905.6
	APRIL	6	131	37.7	24.4	23.1	1928.6
	APRIL	7	132	38	23.8	22.9	1951.5
	APRIL	8	133	36.4	24	22.2	1973.7
	APRIL	9	134	36.4	24	22.2	1995.9
	APRIL	10	135	36.5	22	21.3	2017.2
	APRIL	11	136	31.6	19	17.3	2034.5
	APRIL	12	137	26.8	17	13.9	2048.4
	APRIL	13	138	28.8	16	14.4	2062.8
	APRIL	14	139	29.7	18.4	16.1	2078.8
	APRIL	15	140	31.2	21.2	18.2	2097.0
	APRIL	16	141	32	20.6	18.3	2115.3
	APRIL	17	142	32.4	20.2	18.3	2133.6
	APRIL	18	143	35.8	23.2	21.5	2155.1
	APRIL	19	144	36.8	25	22.9	2178.0
Harvest	APRIL	20	145	37.4	20.4	20.9	2198.9

Annexure-II

Calculation of vapour presure deficit (VPD) after applying last irrigation								
	Temp	Saturated Vapor pressure (kPa)	Month	Date	Tmax	RH (%)	VPD (kPa)	∑VPD (kPa)
Last irrigation	0	0.611	JAN	10	26.2	28.0	2.42	2.42
	1	0.657	JAN	11	28.2	23.0	2.91	5.33
	2	0.706	JAN	12	27.6	23.0	2.75	8.08
	3	0.758	JAN	13	27.6	23.0	2.75	10.83
	4	0.814	JAN	14	28.4	24.0	2.87	13.70
	5	0.873	JAN	15	27.8	30.0	2.50	16.20
	6	0.936	JAN	16	27.7	35.0	2.32	18.52
	7	1.002	JAN	17	28.8	32.0	2.57	21.09
	8	1.073	JAN	18	28.2	28.0	2.72	23.81
	9	1.148	JAN	19	29.4	29.0	2.85	26.66
	10	1.228	JAN	20	27.6	36.0	2.28	28.94
	11	1.313	JAN	21	28.4	40.0	2.27	31.21
	12	1.403	JAN	22	27.2	44.0	2.00	33.21
	13	1.498	JAN	23	29.5	35.0	2.61	35.81
	14	1.599	JAN	24	29.6	39.0	2.44	38.26
	15	1.706	JAN	25	29.4	35.0	2.61	40.86
	16	1.818	JAN	26	29.8	35.0	2.61	43.47
	17	1.938	JAN	27	28.6	31.0	2.61	46.08
	18	2.064	JAN	28	29	32.0	2.73	48.80
	19	2.198	JAN	29	28.2	34.0	2.50	51.30
	20	2.339	JAN	30	29	35.0	2.61	53.90
	21	2.487	JAN	31	28.8	39.0	2.31	56.21
	22	2.644	FEB	1	29.6	41.0	2.36	58.57

	23	2.810	FEB	2	30.2	35.0	2.76	61.33
	24	2.985	FEB	3	29.2	41.0	2.36	63.70
	25	3.169	FEB	4	28.4	42.0	2.19	65.89
	26	3.363	FEB	5	30.4	29.0	3.01	68.91
	27	3.567	FEB	6	30	30.0	2.97	71.88
	28	3.782	FEB	7	30.6	19.0	3.44	75.32
	29	4.008	FEB	8	30.5	23.0	3.27	78.59
	30	4.246	FEB	9	31.4	29.0	3.19	81.78
	31	4.496	FEB	10	30.6	33.0	2.84	84.62
	32	4.759	FEB	11	30.2	39.0	2.59	87.21
	33	5.035	FEB	12	29.2	42.0	2.32	89.54
	34	5.325	FEB	13	32	39.0	2.90	92.44
	35	5.629	FEB	14	32.5	26.0	3.52	95.96
	36	5.948	FEB	15	32	27.0	3.47	99.44
	37	6.283	FEB	16	33.6	29.0	3.57	103.01
	38	6.634	FEB	17	33.7	20.0	4.03	107.04
	39	7.003	FEB	18	34.5	26.0	3.94	110.98
	40	7.388	FEB	19	35.4	23.0	4.33	115.31
	41	7.762	FEB	20	33.6	28.0	3.63	118.94
Terminate stress*	42	8.139	FEB	21	32.8	32.0	3.24	**122.17**

**This is maximum limit of accumulated VPD in case of maize at this location. Though 1st criteria of terminating stress is moisture content in root zone.*

Notes:

Notes:

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