PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE:

HEAT STRESS

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HEAT STRESS

A field manual

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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

Tolerance to heat stress was previously not a priority trait in tropical maize compared to tolerance to other abiotic stresses such as drought and low nitrogen. However, in recent years, heat has been identified as one of the key abiotic stresses for maize in the tropics. This is due to the fact that maize is grown year round, including during the summer and spring seasons, when it is likely to face heat stress. Also, climate change effects experienced during recent years in the tropics have resulted in increased temperatures, both maximum (Tmax) and minimum (Tmin) during key maize crop stages in the lowland tropics.

This manual was developed to help maize breeders and field technicians in South and Southeast Asia, Latin America and sub-Saharan Africa to perform heat screening/phenotyping and identify genotypic variability in tropical maize for use in breeding programs aimed at developing heat tolerant tropical maize germplasm. This manual will enable them to:

- Identify suitable field sites for heat stress phenotyping
- Screen for heat tolerance using key secondary traits along with grain yield
- Generate high quality phenotyping data by reducing experimental error through proper trial management

Heat stress in tropical maize

Heat stress can be defined as exposure to temperatures (maximum and/or minimum) above a threshold level for a period of time that causes irreversible damage to maize crop growth and development and is a function of the intensity, duration, and rate of increase in temperature. Maize can survive brief exposures to extreme temperatures (heat shocks); however, the growth limits are somewhat lower, from 8°C to nearly 30°C. Optimal temperatures for growth vary between day and night, as well as over the entire growing season; for example, during the daytime, the optimal temperatures range between 25-33°C, while night temperatures range between 17-23°C (Zaidi and Singh, 2005).

Apart from the air temperature, relative humidity (RH) plays a key role in defining the ultimate effect of heat stress on maize plants, as it contributes to a key climatic parameter called vapor pressure deficit (VPD). VPD is the difference (deficit) between the amount of moisture present in the air at a given air temperature and the amount of moisture the air can hold when it is fully saturated. Therefore, it is a measure of the drying power of the air around crop canopy, which eventually plays a key role in the overall effect of high temperatures on plant tissues. Consequently, the combination of high temperatures (Tmax >33°C and Tmin >23°C) with low RH (<40%) increases the probability of irreversible damage to various maize plant organs and functions, including visible heat stress symptoms such as leaf firing and tassel blast, and therefore considered heat stress for maize crops grown in tropics.

Different plant tissues and organs are affected by heat stress in different ways, depending on the susceptibility of the dominant metabolic processes that are active at the time of the stress. For example, the temperature threshold for heat stress damage is significantly lower in reproductive organs than in other organs (Stone, 2001). Accumulated high temperatures can cause an array of morphological, anatomical, physiological, and biochemical changes within maize plants. The most significant factors associated with maize yield reduction include shortened life cycle, reduced light interception, and increased sterility (Stone, 2001) in both male and female inflorescences. Though most growth stages of maize plants are sensitive to heat stress, sensitivity varies with the developmental stage. As for the maize crop cycle in the tropics the reproductive stage is relatively more sensitive and unfortunately more likely to be exposed to heat stress, which causes irreversible damage and eventually affects overall productivity.

Precision phenotyping for heat stress

Irrespective of the breeding approach (whether conventional or molecular), high-quality phenotyping data are the key for enabling efficient selection decisions and breeding progress. In conventional breeding, precision phenotyping allows reliable identification and selection of superior progenies for targeted traits, and 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 a must for realizing a crop improvement program's intended progress on a targeted trait, especially complex polygenic traits such as heat stress.

Some basic requirements for generating high-quality phenotyping data for heat stress under field conditions are described below.

1. Understanding the target environment

The target population of environments (TPE) are the agro-ecologies where the newly developed varieties will be cultivated. Usually, the TPE consists of many different types of farms and seasons; thus it is best not to consider it as a single environment but a variable set of future production environments (Cooper and Byth, 1996). A clear understanding of the TPE is essential for planning and selecting the best selection environment where the phenotyping site should ideally be established. The phenotyping site does not necessarily have to be in the

target environment, but should have a good and relevant relationship with the TPE. Therefore a minimum amount of information about the TPE is required:

- Historical daily weather data, preferably from the last 10 years, including maximum temperature, minimum temperature, relative humidity and rainfall, for understanding and defining the most relevant type of heat stress in TPE.
- Soil type, cropping season and cropping system, especially the window for planting maize in TPE.
- Other relevant information, such as major biotic stresses.

Analyzing this information will help in understanding the requirements for establishing a phenotyping site that is significantly related to the TPE.

2. Establishing the phenotyping site

Based on TPE, a suitable field phenotyping location should be selected for establishing a dedicated site for heat stress phenotyping. Once the location is identified, it is necessary to ensure that satisfies the basic requirements of a phenotyping site, such as:

- Medium-to-heavy textured soil with good moisture holding capacity, in order to avoid frequent irrigation.
- Well-leveled field to facilitate irrigation and avoid water stagnating in patches.
- Good irrigation (and drainage) facility to avoid 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 RH (or reducing VPD).
- No part of the field should be exposed to shading by trees or other structures in the vicinity of the site.
- Mapping soil variability: knowledge of soil physical and chemical properties that will affect plant growth and stress development, and their uniformity within a

site is essential in the selection of suitable phenotyping. There are various option available for mapping field variability, as follows:

i) Ideally should be carried-out by growing a single variety of a crop (preferably maize because different crops may vary significantly in their sensitivity to soil physical and chemical problems) to be able to identify bad patches.

ii) Direct assessment of soil variability can be made through destructive soil sampling at 30 cm depth intervals (to a depth of 90 or 120 cm soil depth) 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 variability within field sites 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).

While initial site characterisation of potential phenotyping sites increases phenotypic accuracy by eliminating sites with high unwanted variability and confounding factors, soil mapping can be used to further improve the precision of field experiments.

3. Grouping test entries by maturity

This is one of the basic requirements for phenotyping most abiotic stresses, including heat stress. Test entries need to be grouped based on their anthesis date (preferably using growing degree days, GDD units). GDD, also known as heat degree units (HDUs), are a measure of temperature accumulation over a period of time, which could be used to predict plant development rates and time when crop

will reach to various phenological stages, such as flowering, crop maturity etc. The method of calculating GDD is described below in section-4. Grouping of test entries 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 for heat stress) 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 20-40°C GDD units, is acceptable (but more than 5.0 days of variation in days to anthesis within a trial should be strictly avoided). After grouping the entries based on their anthesis time (in a previous season in an optimal trial), groups of entries with comparable days to anthesis should be included in the same trial. To avoid complicating crop management operations, ideally a maximum of two trials with entries having different maturities should be planted at one trial site. This can be done by staggering the planting in such a way that the late flowering group of trial(s) is planted first and the next group of trial(s) is planted after a number of days equal to the difference in their anthesis days. In this way, all the trials within a site will reach the targeted crop stage for heat stress at almost same time and will be exposed to relatively the same level of stress.

4. Crop management

Except for manipulating the planting time to make the reproductive growth stage coincide with heat stress, all other recommended crop management practices should be followed in heat stress phenotyping trials. Adequate crop management, including timely application of recommended inputs and agronomic operations, is a pre-requisite for quality phenotyping.

Therefore, a few reminders about key crop management practices are described as below:

- **Planting time:** Given that the duration of the desired level of heat stress at a particular location occurs only during a particular time period, planting time is key

for successful field-based phenotyping under natural heat stress. Therefore, the planting time needs to be chosen carefully, so that the targeted crop stages (i.e., flowering and early grain-filling stage) are exposed to natural heat stress.

- For example, at Hyderabad and Raichur sites in South India, the intended level of heat stress occurs during the first fortnight of May. Therefore, planting at these sites must be completed within the third week of March for intermediate maturing germplasm with anthesis of about 750°C GDD (Fig. 1).
- **Plant population**: The 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, we recommend planting two seeds per hill (or double density) and thinning out the extra seedlings at the V_{2-3} stage, once seedlings are properly established. Depending on the water-holding capacity of the soil and irrigation facility, plant populations in heat stress phenotyping trials should be between 53,000 and 80,000 per hectare.
- **Border and filler rows**: It is essential to plant border rows (in double density spacing), at least three rows (or 3.0 meters at both ends of the rows) all around phenotyping trials in order to avoid border effects on test entries and any physical damage. Also, to maintain the same level of competition, no row should be left empty (un-planted). If some rows are empty in the trial map, they should be planted with bulk seed.
- **Moisture management:** Special care should be taken when managing irrigation, as heat stress phenotyping trials are usually planted during the (hot) dry season. This includes:
  - Timely application of irrigation in order to avoid the compound effect of drought and heat stress (unless the targeted stress is combined drought + heat stress).

- When doing combined drought + heat stress phenotyping, irrigation should be stopped about one week before anthesis in order to achieve the desired level of drought stress at the time of anthesis and silk emergence. However, for precision in the time for stopping irrigation, it is best 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. Method of calculating GDD and using them for managing drought stress is described below.

  GDD = ((Tmax-Tmin)/2) – Base temperature (8°C)

  Heat accumulation should be recorded on per day basis from planting till anthesis, and total accumulated heat during this period is taken as GDD for anthesis. Depending upon prevailing temperature regime (per day heat accumulation) estimate an approximate time of applying last irrigation for imposing drought stress at flowering stage; for example – if per day average heat accumulation at a heat stress phenotyping site is 15°C, the last irrigation should be applied about a week before the time of anthesis.
- The method of irrigation used should be furrow/flood (preferably drip irrigation for achieving uniform moisture level across the field); sprinkler irrigation should be strictly avoided (except maybe when applying the first irrigation for seed germination), as it will temporarily change the micro-climate and break the continuum of heat stress.
- In case of furrow irrigation, make sure that no water stagnates in furrows in any part of the field, as this may affect the micro-climate or cause excessive moisture stress.

• **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, depending on soil physical and chemical properties and

common biotic pressures. It is therefore 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.

In essence, the phenotyping trials must be grown under optimal conditions so that the crop is not exposed to any other stress, except the intended stress (i.e., heat or heat + drought stress). If these precision practices are followed, and the compound effects of other stress(s) with the intended stress are avoided, high quality phenotyping data on the effects and genotypic variability for heat stress within trials could be successfully captured.

5. Weather data

In field-based phenotyping trials, it is indispensable to record hourly weather data (including Tmax, Tmin, relative humidity, rainfall, dew, and wind velocity), which could significantly alter the overall effects of the heat stress actually experienced. A portable weather data recorder should be installed within the phenotyping field for recording weather parameters. The 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 observed parameters, vapor pressure deficit (VPD) can be calculated for a given temperature and the respective humidity value using the formula given below, and expressed in terms of kilo Pascal, kPa (Abtew and Melesse, 2013).

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)

This information will help define heat stress intensity and duration at a particular site, i.e. actual duration of heat stress (in hours) at each phenotyping site, and cluster different sites according to stress level before performing across-site data analysis.

6. Managed heat stress

In field-based phenotyping, heat stress can be applied by carefully selecting a suitable cropping season and manipulating planting time in such a way that the reproductive growth stage, from tassel emergence to early grain-filling (lag-phase), is exposed to heat stress. A suitable cropping season for heat stress phenotyping should include a rain-free window of at least one month around flowering and grain-filling. During the same period, Tmax should rise >35°C, Tmin >23°C, and RH should be <40% for at least two weeks during tassel emergence until one week after pollination. To identify this period at potential sites, weather data from at least past ten years should be carefully analyzed. For example, in many parts of South Asia, the most suitable cropping season for applying heat stress is spring season (February to June), because it is, for the most part, rain-free. To meet the temperature and humidity requirements for applying heat stress, spring planting should be delayed about 3-4 weeks. The optimal planting window for spring maize in most of South Asia is during second fortnight of January up to the second fortnight of February. However, intermediate maturity trials for heat stress phenotyping should be planted during the second/third week of March so that the reproductive stage starts the first/second week of May. During the first fortnight of May, Tmax invariably remains >35°C, Tmin >23°C, and RH <40% (Fig. 1, page 11).

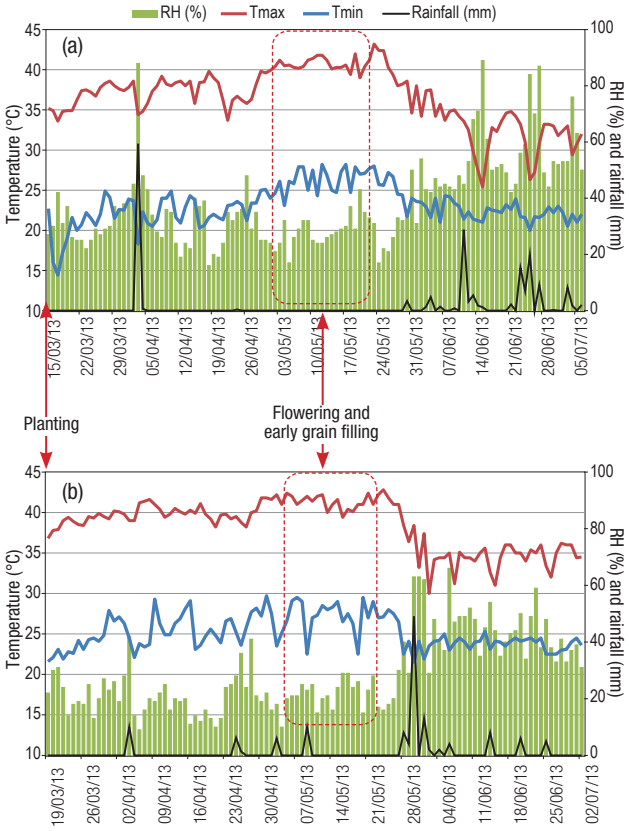

Fig. 1. Weather conditions at two heat stress phenotyping during Spring season sites in India, (a) Hyderabad and (b) Raichur.

Phenotyping criteria

Phenotyping may be defined as *the measurement under particular conditions (for example, heat stress) of observable characteristics and functions related to crop phenology that are determined by both the crop's genetic makeup and the environment*. Yield is a trait of primary interest; however, dissecting it into its components (secondary traits associated with yield) gives better understanding, especially in molecular breeding. In genomic region discovery efforts it helps in establishing trait-marker association, which could be effectively used in forward breeding for genetic enhancement for the targeted traits (such as heat stress). Even when using a conventional breeding approach, recording key secondary traits along with yield helps to keep track of stress intensity for mid-term correction, if needed. 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 heat stress under field conditions and thus should be recorded in heat stress phenotyping trials are:

((A) Priority traits (must record)		(B) Additional traits	
1	Days to 50% anthesis and silking	13	Days to emergence
2	Anthesis-silking interval (ASI)	14	Seedling vigor
3	Leaf firing	15	Pollen shedding (duration)
4	Tassel blast	16	Pollen viability
5	Tassel sterility	17	Silk receptivity
6	Plant and ear height		
7	Plant lodging		
8	Plant population		
9	Ears per plant		
10	Physiological maturity		
11	Grain yield		
12	Grain moisture		

Descriptions of each trait, along with the suitable stage and method of data collection are given below.

(A) Priority traits

1. **Days to 50% anthesis and silking:** These male (anthesis) and female (silking) flowering traits demonstrate the effect heat stress on reproductive behavior, and eventually affect overall reproductive success.

   When to record: Starting from first tassel emergence in the field, observe each plot on a daily basis until all the entries in the trials have completed anthesis and silking. If entries are properly grouped by maturity, it should take approximately one week to finish recording data on days to anthesis and silking, except in case of highly susceptible entries where anthesis or silking may be delayed due to stress.

   How to record: Both anthesis and silking are recorded on a plot basis (not on the basis just 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. 2). Convert them into days after planting date, which indicates how many days it took to reach 50% anthesis or silking.

   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 change in anthesis or silking under heat stress.

2. **Anthesis-silking interval (ASI):** This is a key secondary trait that is significantly affected by most of the abiotic stresses, including heat stress. It is the difference between days to anthesis and days to silking, and demonstrates the synchrony between male and female flowering, which is essential for a maize plant's reproductive

success. Under optimal conditions, male and female flowering is usually well synchronized (occurring within 2 or 3 days). However, under stress conditions ASI may be prolonged, mainly due to a delay in days to silking (and, in some cases, due to a delay in days to anthesis as well), which results in poor synchrony and, eventually, reproductive failure.

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

What to select: Genotypes with ASI less than five days.

***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).*

Fig. 2. Recording days to 50% anthesis and silking in field.

3. **Leaf firing:** Leaf firing is a typical symptom of heat stress. *It starts from the top of the plant and progresses downward, where new/younger leaves are burned (immature drying) due to heat stress* (Fig. 3). This is in contrast to leaf senescence, where leaf drying starts at the base of the plant (older leaves first) and progresses upward due to assimilate and nutrient remobilization towards younger plant parts under stress, and also under optimal conditions, as the plant progresses towards maturity.

   When to record: Leaf firing is quite stable, and once symptoms appear, they remain for a long period. However, the best time to observe is 1-2 weeks after anthesis.

   How to record: Leaf firing is recorded as the percentage of plants in a plot with leaf firing symptoms. Count the number of plants in a plot with leaf firing symptoms, and calculate the percentage based on the total number of plants in the plot.

   What to select: Genotypes with no leaf firing symptoms under heat stress.

Fig. 3. Leaf firing symptom under heat stress.

4. **Tassel blast:** Like leaf firing, tassel blast is a typical symptom of heat stress in maize plants; these two symptoms often appear together. Tassel blast refers to *drying of the complete tassel (or most of it) without pollen extrusion* (Fig. 4). In field trials, tassel blast often may not seem to be directly related to grain yield, as genotypes with severe tassel blasting may still produce good yields, as they may get pollen from other genotypes in the trials. However, when a genotype with this trait is planted in isolation, where no other source of pollen is available, it will end up with severe yield losses. Therefore, all genotypes with tassel blasting symptoms under heat stress should be rejected, irrespective of their yields.

   When to record: Tassel blast is quite stable, and once symptoms appear, they remain for a long period. However, the best time to observe it is 1-2 weeks after tassel emergence.

Fig. 4. Tassel-blasting under heat stress.

How to record: Tassel blast is recorded as the percentage of plants in a plot with tassel blast symptoms. Count the number of plants in a plot with dry tassels (without pollen dehiscence), and calculate the percentage based on of the total number of plants in the plot.

What to select: Genotypes with no tassel blast symptoms under heat stress.

5. **Tassel sterility:** This is typically a heat stress-induced symptom, given that a genotype that is usually fertile under optimal temperature conditions may produce sterile tassels under heat stress conditions. Tassel sterility refers to *tassels with very poor or no pollen shedding, even though they may remain green/alive for a long time before natural senescence* (Fig. 5). This is in contrast to tassel blast, where tassels dry out without shedding pollen. Similar to tassel blasting, all genotypes with severe tassel sterility symptoms under heat stress should be rejected, irrespective of their yield.

Fig. 5. Maize plants with sterile tassel under heat stress.

When to record: Usually a tassel starts shedding pollen the first week after its emergence. Therefore, tassel sterility should be recorded one week after tassel emergence and should be completed within a week.

How to record: Tassel sterility is recorded as the percentage of plants in a plot with sterile tassels. Count the number of plants with sterile tassels, and calculate the percentage based on the total number of plants in the plot.

What to select: Genotypes with no tassel sterility symptoms under heat stress.

6. **Plant and ear heights:** Heat stress reduces internodal elongation, which results in reduced plant height (and also ear height in the same proportion). However, there is genotypic variability for these traits, which can be recorded and used in the selection process.

   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 (Fig. 6). Observations should be recorded on at least five representative plants within each plot, avoiding the plants near the alley, and noted as average.

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

7. **Root and stem lodging:** Due to competition for 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 heat stress.

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

Fig. 6. Measuring (a) plant and (b) ear height in a heat stress trial.

How to record: Count the number of plants in a plot that lodged due to stem bending/break at an internode above the ground (*stem lodging*, Fig. 7a, page 20) or were uprooted from the base (*root lodging*, Fig. 7b, page 20). Calculate stem and root lodging percentage 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 heat stress.

8. **Plant population:** Though plant population may not be directly affected by heat stress, it is an important trait that should be recorded under various abiotic stresses and even in un-stressed trials. Plant population is directly related to yield per unit area, and also used in calculating various other stress related traits, such as plant lodging and ears per plant.

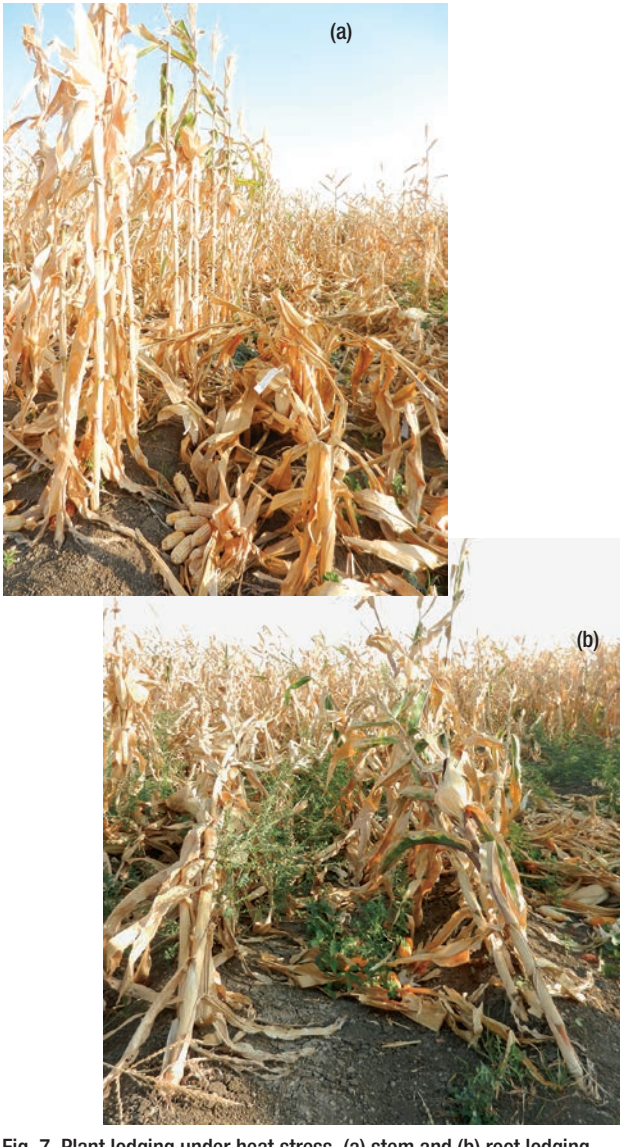

Fig. 7. Plant lodging under heat stress, (a) stem and (b) root lodging.

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.

9. **Physiological maturity**: At physiological maturity, a maize ear reaches its 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 black layer) is formed. At this stage, the crop could be harvested, as grains have fully matured (could be used as seed). However, at physiological maturity, grain moisture content is usually high (30-40%); therefore, the crop is left in field for a few more days so that grain moisture content is decreased to 20-25%, which is called *harvest maturity*. The period from physiological maturity to harvest maturity is called dry-down phase. A short dry-down phase is considered a desirable trait for a cultivar best-fit in cropping systems (such Maize-Rice system in South Asia) and genotypic variability is reported for this trait.

   When to record: About 4-6 weeks after anthesis, 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 the date when >70% of the husk cover leaves on the ears have dried.

   Before proceeding to the final harvest, please ensure that:

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

10. **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. 8). If an ear has at least one grain, it should be counted as one ear. Calculate the

Fig. 8. Ears arranged plot-wise for recording number of ears/plot.

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.

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

$$Barrenness = 1 - EPP$$

11. **Grain yield (kg/plot):** Grain yield is recorded in terms of ear weight per plot (also called *field weight per plot*) immediately after crop harvest. Ear weight per plot is converted into grain yield per hectare after adjusting for grain moisture content (to be recorded in the field, as described in section 12) and for shelling percentage (80%).

    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. 9, page 24). Please take the following precautions when taking ear weight in the field.

    - Calibrate the balance using a reference weight at the beginning of the season.
    - When weighing in the field, confirm the accuracy of the values displayed by 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 line/1st time testcross progenies with a large number of entries) and Stage 2 trials (advanced generation/2nd time testcross progenies with a high number of entries). However, direct grain yield of advanced stage lines, Stage 3 and Stage 4 hybrid trials 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.

12. **Grain moisture content:** In general, grain moisture content is high (about >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.

Fig. 9. Weighing ears for recording yields per plot in the field at harvest.

How to record: Prepare a sample by shelling grains 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. 10). Ideally, it should be recorded separately for each plot. However, in big trials with a large 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).

Fig. 10. Recording kernel moisture content in the field at harvest.

(B) Additional traits

13. **Days to seedling emergence:** This is a key trait often associated with seedling vigor. Significant genotypic variability is observed for this trait even under optimal conditions. It is measured as the time taken by seedlings to emerge from the soil surface (commonly referred 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 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.

14. **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 visually on a 1-5 scale (1 = high, 5 = low).

15. **Pollen shedding duration:** On average, pollen shedding in maize usually lasts 5-7 days under optimal temperature conditions. Heat stress significantly affects pollen shedding duration and, eventually affects reproductive success.

    When to record: Pollen shedding is recorded simultaneously with days to anthesis and days to silking, but continues until each genotype in the trial is finished shedding pollen.

    How to record: Once days to 50% anthesis in a plot are noted, continue monitoring until the anthers on the lower branches of the tassel stop shedding pollen. Note the date when most of the plants in a plot have stopped shedding pollen (Fig. 11). Pollen shedding duration can be calculated using the following formula:

    Pollen shedding duration (d) = Days to end of pollen shedding (E) – Days to 50% anthesis (A)

    ***Note**: As tassel blast is very common under heat stress, tassels must be observed carefully to determine whether they dried without shedding any pollen (tassel blast) or after shedding pollen for some time (end date of pollen shedding).*

Fig. 11. Recording pollen shedding duration by noting days to 50% anthesis and end of pollen shedding

16. **Pollen viability:** Lab studies on the viability of pollen collected from heat stress phenotyping trials have clearly shown that pollen viability is a key trait that is severely affected by heat stress. However, genotypic variability for this trait has been found in tropical maize (Fig. 12), though at low frequency (Zaidi *et al*., unpublished data).

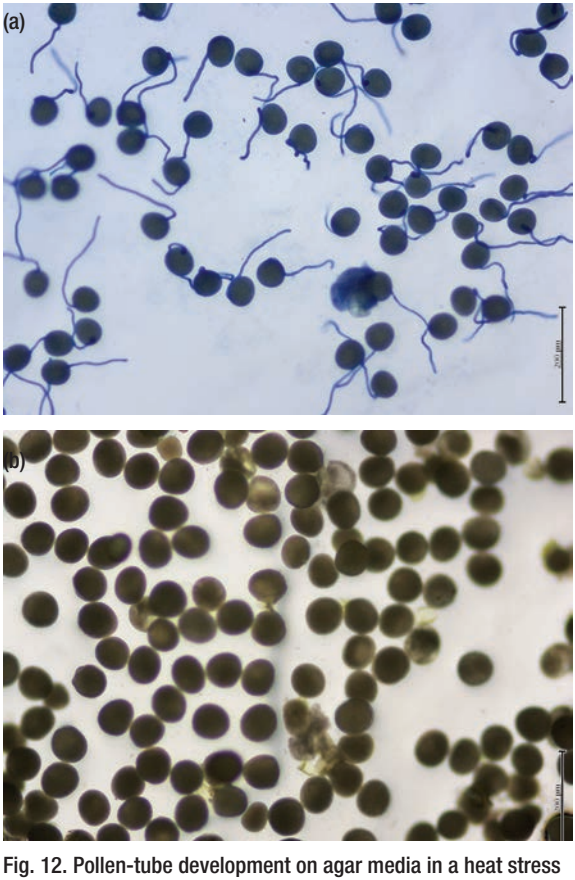

Fig. 12. Pollen-tube development on agar media in a heat stress tolerant genotype (a) and a heat susceptible genotype (b) with no pollen-tube.

Laboratory techniques are available to conduct pollen viability and pollen germination tests. However, the staining-based pollen viability test is often misleading, as pollen with a positive staining test (viable) may not produce a pollen tube (alive but non-functional). Therefore, the pollen germination test (pollen tube growth), rather than pollen viability, is a more reliable option for pollen viability phenotyping under heat stress.

Though the pollen germination test is quite precise for pollen viability studies, large-scale phenotyping using lab-based pollen-tube germination methods is not a very feasible option. This is because just a limited number of samples can be handled at a time, as pollen viability is not very stable over long periods after samples are collected from the field. Therefore, an indirect method of measuring pollen viability was developed for large-scale phenotyping. It is based on cross pollination of maize plants grown under optimal conditions (in the greenhouse under optimal temperature) with pollen collected from test entries grown under heat stress in the field. This is a surrogate for the lab-based pollen tube germination test, which is based on functional pollen viability. The steps involved in this method are as follows:

i) Select an elite maize hybrid that has the same maturity as the test entries to be phenotyped for heat stress in the field. Use the hybrid as the *female* plant and all the test entries as *male* plants.

ii) Plant hybrid seeds in pots outside the greenhouse and bring them inside about two weeks before tassel emergence, so that the entire reproductive phase takes place under optimal temperature and other growing conditions.

iii) The number of female plants in the greenhouse should be five times greater than the number of plots of test entries planted in the field for heat stress phenotyping. For example, if the total number of plots in the field is 100, then the number of female plants in the greenhouse should be 100 x 5 = 500 plants.

iv) Planting of hybrid in the greenhouse and test entries in the field should be done on same day, so that both reach flowering at almost the same time.

v) Tag five plants of the female parent in the greenhouse for each test plot in the field, so that at flowering, those female plants are crossed with pollen collected from the respective test plot in the field.

vi) At anthesis, collect pollen from each test plot and pollinate the respective tagged plants in the greenhouse.

vii) All five plants should be pollinated at one time, with enough pollen collected from the respective test plot in the field and bulked.

viii) At maturity, harvest the female plants in the greenhouse and

   a. Count the total number of kernels (both fully developed and rudimentary) on five ears and calculate the average number of kernels per plant.

   b. Count the number of potential kernels in each row (including developed, rudimentary and unfertilized ones), and the total kernels rows on each ear, and calculate the number of potential kernels per ear.

   c. Calculate pollen viability using the following formula:

$$\textit{Pollen viability (\%)} = \frac{\textit{Number of fertilized kernels per ear}}{\textit{Number of potential kernels per ear}} \times 100$$

17. **Silk receptivity:** Similar to pollen viability, silk receptivity is also significantly affected by heat stress in the field. Silk receptivity is estimated based on the number of fertilized kernels per ear (both fully developed and rudimentary). In this process, it is assumed that in a heat stress trial, where several different genotypes are planted in the same field, there is usually enough viable pollen available in the field for pollination, despite pollen viability issues. Therefore, kernel formation (at least rudimentary if not fully developed) is an indirect reflection of the silk receptivity of the test entries under heat stress.

    The steps involved in silk receptivity phenotyping are as follows:

    i) At harvest, count the total number of kernels, both fully developed and rudimentary, on at least five representative ears from each plot, and average to obtain the number of fertilized kernels per ear.

    ii) Count the number of potential kernels in each row (including developed, rudimentary and unfertilized ones) and total grain rows on each ear, and calculate the number of potential kernels per ear.

    iii) Calculate silk receptivity using the following formula:

$$\textit{Silk receptibility (\%)} = \frac{\textit{Total number of fertilized kernel per ear}}{\textit{Number of potential kernel per ear}} \times 100$$

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