CHAPTER 1

Various Methods of Conducting Crop Experiments

Effective phenotyping should require a set of core setups in which plants are cultivated either under laboratory conditions or in experimental fields. Such experiments enable researchers to determine the phenotypic responses of plants to defined experimental treatments and evaluate the performance of different genotypes or species in a given environment. To enable generalizations across experiments, it is necessary that results are not only replicable, but also reproducible. Replication of results is achieved when the same researcher finds the same results when repeating an experiment in time. In plant biology, achieving a high degree of reliability and reproducibility is a challenge. This chapter provides information on different methods of conducting experiments for crop and data to be recorded on various abiotic environment parameters apart from regular plant biometric data.

1.1 Field Experiments

Field experiments are typically undertaken under conditions where some, but not all variables, can be controlled. These sometimes represent a particular stress (e.g. drought, nutrient or temperature), or under favorable conditions where the aim is to understand physiological and agronomic factors contributing to yield potential. Similarly, assessment of genotypes under a controlled stress requires an understanding and reporting of factors contributing to their differential performance in response to stress. If some of the observed

differences in yield relate to differences in plant height, flowering, or greater leaf area then the cataloguing of such variation must be undertaken.

Field experimentation with rainout shelter facility

Measuring and reporting of this variation can be varied among the researchers. This makes interpretation across multiple experiments difficult as one researcher may view and undertake sampling differently to another. It is critical that there is consistency in how measurements are undertaken and reported. Hence, standardizing procedures and phenotyping among individuals will provide data that is robust, reliable and repeatable. This will lead to more cost-efficient research where high quality data can be produced and re-used.

(i) ***Selection of site*****:** For critical planning and interpreting field response data, good knowledge of the site and expected seasonal conditions based on prior knowledge of long term weather trends are essential. Information such as soil conditions viz., soil type, soil texture, soil moisture, soil nutritional status, soil born pest and diseases etc. should be analyzed for the experiment area. Identification of uniform blocks with perfect leveling to reduce residual (error) variation in large size field experiments is essential. Long-term seasonal rainfall and temperatures are to be collected and should be used in planning for the need for sowing date, irrigation, and imposing abiotic stresses.

(ii) ***Plot type and size*****:** Phenotyping of complex physiological traits and particularly hose associated with canopy development,

biomass and yield is challenging when experiments comprise diverse genotypes. This is especially so when confounded with variation in traits such as height and maturity which are known to affect yield.

(iii) ***Implications in row and plot experiments***

(a) Row plantings

Limited seed and resources may encourage field-assessment in single, spaced rows or smaller, un-bordered plots. Competition for water, light and nutrients required for canopy growth is variable as adjacent rows are genetically different and competition is greatest particularly under stress conditions. Response to changes in resource availability varies among diverse genotypes, alters genotype ranking and thus reduces heritability. In turn, the relevance of such growing conditions to commercial field-grown crops is unclear.

(b) Plot experiments

The planting of multi-row plots and the simple exclusion of plot borders at harvest increases experimental precision and confidence in genotype response. Well-planned field studies and particularly those focusing on the dynamics of yield formation (canopy-related characteristics) must consider the use of multiple-row plots and with border rows to minimize the effects of inter plot competition. Plots should contain two outer rows ('edge' or 'border') and multiple inner rows to minimise inter-plot competition effect, e.g. edge effects due to shading, nutrients, water availability, or compaction.

(c) Phenotyping in the field

Assuming that both the type and the number of treatments (genotypes, irrigation volumes, etc) to be evaluated are adequate for the specific objectives of each experiment, the following general factors should be evaluated carefully to ensure the collection of meaningful phenotypic data in field experiments conducted under water-limited conditions:

- Experimental design
- Heterogeneity of experimental conditions between and within experimental units
- Size of the experimental unit and number of replicates
- Number of sampled plants within each experimental unit
- Genotype-by-environment-by-management interaction.

(iv) ***Weather measurements***

The weather has a huge impact on the crop growth and development, and the stress that the plants will experience. Recording accurately the main weather variable is thus crucial in success of any field experiment.

(a) Stable weather station

Generally, the daily weather data viz., Solar radiation, rain, maximum and minimum temperatures, wind speed, air humidity, pan evaporation are collected from research stations where experiment is conducted or nearby organizations which has stable weather station. The demerits of such data are

1. They can be far from the field trial, while environmental factors such as rain can vary within short distances.
2. They only deliver daily measurements that are not always accurate to evaluate stress events.

(b) Portable weather station

A better alternative is to install a portable weather station in the field trial, to record climatic data more frequently (e.g. measurements every min). Typically these weather stations have a solar radiation sensor, a tipped-bucket rainfall gauge and an air temperature and relative humidity probe mounted in a Stevenson screen. In additional, many other sensors can also be included, such as:

- Thermistors to measure soil temperature,
- Thermocouples to measure soil, leaf temperatures

- Infra-red sensors to measure canopy temperature continuously
- Solarimeter tubes to measure light interception
- An anemometer and a wind vane to measure wind speed and direction.

(v) ***Merits of field experiments***

1. Field conditions are relatively close to the natural environment that crops experience in the field
2. It provides an opportunity to compare plants under conditions in which spatial heterogeneity is relatively small.

(vi) ***Demerits of field experiments***

1. Uncontrolled variations in light, temperature and water supply.
2. Various environmental conditions may change in concert i.e., a period of high irradiance may come with high temperatures and low precipitations.

1.2 Experiments under Green Houses

Glasshouses and polyhouses are good alternative and provide more buffered conditions for growing plants. They offer better control of water supply and protection against too low temperatures. Additional lighting in the glasshouse may ensure a minimal daily irradiance and a fixed photoperiod while shade screens can protect against high light intensities in summer (Max et al. 2012).

Demerits

1. In practical terms, plants grown in glasshouses will usually experience higher-than-outdoor air temperatures during nights and winters and lower irradiance because of shading.
2. Most glasshouses or polyhouses without humidity control have limited possibilities to reduce temperatures during periods of strong solar irradiance in summer.
3. In many greenhouses where there is no artificial lighting, significant spatial heterogeneities in irradiance, due to shading by the greenhouse structure itself is observed.

1.3 Experiments in Growth Chambers

These are climate-controlled growth chambers and are expensive in terms of investments as well as running costs. They offer the most sophisticated possibilities for environmental control and thereby good reliability of experiments.

Climate-Controlled Growth Chamber

Demerits

1. Conditions in growth chambers are generally the furthest away from those in the field, not only because environmental values are often programmed within a relatively small diurnal range, but also with regard to the absolute values of, for example, light and temperature, at which they operate (Garnier and Freijsen, 1994).
2. Although growth chambers enable a strong temporal control over conditions, spatial variability is often larger than anticipated and higher than those measured in experimental fields. For example, light intensity may vary from place to place in the growth chamber (Granier et al. 2006) and can be especially lower close to the walls.
3. Gradients in air velocity may go unnoticed in growth chambers, although they can affect evaporative demand. Variation in air circulation may be especially large when plant density is high or plants are placed in trays, which may block air circulation around the plants. Both too high and too low wind speeds are undesirable.
4. A factor that may strongly vary in a temporal manner is the local atmospheric CO_2 concentration: generally, CO_2 levels in a building are higher than outside.

5. Under greenhouses as well as growth chambers crops are experimented either through hydroponics or pot culture method of growing crops.

1.4 Hydroponics

Roots provide nearly all the water and nutrients that a plant requires. If the aim is to design an experiment in which these two factors have the least limiting effect on growth, then hydroponics or aeroponics is the preferred choice (Gorbe and Calatayud, 2010). Hydroponics systems can be either based on roots suspended in a water solution or in some solid medium such as sand, rockwool, or another relatively inert medium, which is continuously replenished with nutrient solution (Cooper, 1979). Frequently used nutrient solutions are described by Hoagland and Snijder (1933) and Hewitt (1966), although the truly optimal composition is species specific. Preparation of macro, micro nutrients (Appendix VI) and Hoagland solution (Appendix VII) were given in appendices as ready recoknoire.

Hydroponics Experiment in Glasshouse

Precautions

1. Water-based systems have the advantage that they allow easy experimental access to the roots for physiological or biomass measurements. However, care has to be taken while roots are transferred from one solution to another, as breakage of roots may easily occur.
2. There is also a need to take into account the composition of tap water when setting for the final composition. Because of the

much higher mixing rate in soilless systems and the direct access of plant roots to the nutrients, the concentrations of nutrients that are needed to sustain supply are 5-10 times lower than those required for plants growing on sand where there is an absence of continuous flow through.

3. Ensure that the concentration of macro and especially micro-nutrients in a hydroponics system is not too high, as this will negatively affect plant growth or may even cause leaf senescence (Munns and James, 2003). On the other hand, nutrient concentrations should not become too low either, as plants will otherwise deplete the available minerals. Hence, regular replacement of nutrient solution is necessary.
4. Bigger plants usually need more nutrients and so the rate of replenishment must increase with plant size, unless the nutrient concentration itself is continuously monitored and adjusted.
5. Good mixing of aerated nutrient solution is vital to avoid depletion zones around the roots and anaerobic patches, but should not be too vigorous to avoid strong mechanical strains. In addition, specific uptake mechanisms like the release of chelating agents to increase iron availability (Romheld, 1991) or the release of organic acids by the root may be affected.
6. The pH of the hydroponic solution may increase or decrease, depending on whether nitrate or ammonium is present in the solution and the specific preference of a given species. For most plant species a pH of 6 seems to be optimal, although specific species may deviate significantly. Monitoring and adjusting the pH of the solution at a regular basis is highly recommended, keeping in mind that pH changes are stronger in small volumes of nutrient solution and for roots with faster nitrogen uptake rates.
7. It should also be checked that there is no accumulation of salts at the root: shoot junction over time, as this can damage the seedlings of some plant species.

1.5 Pot Culture

An alternative to hydroponics is to grow plants in pots filled with an inert solid medium (e.g. sand, perlite) or soil and to water them regularly or on demand. Use of pots with a solid substrate may at

least mimic the higher mechanical impedance to root growth that plants experience in soils and allows for a higher homogeneity and control of the nutrient and water conditions than in soil. Pot culture allows more freedom in the choice of the location of the experiment and ensures easy handling and manipulation of the shoots of individual plants. Most overlooked factors in pot culture is pot size and the fact that nutrients and water supply strongly interact with plant size.

(i) ***Pot size*****:** The size of the rooting volume also requires careful attention. The smaller the pot, the more plants fit into a growth chamber or glasshouse, an advantage for nearly all laboratories where demand for space is high. At the same time, in most experiments smaller pots will also imply a lower availability of below-ground resources and if pots are closely spaced, also a comparatively lower amount of irradiance available for each plant. Moreover, the smaller the pot the stronger roots become pot-bound, leading to undesirable secondary effects. In experiments in which rooting volume is varied, there is almost invariably a strong positive correlation between plant growth and pot volume reported. Conditions obviously differ from experiment-to-experiment, but as a rule of thumb, pot size is certainly small if the total plant dry mass per unit rooting volume exceeds 2 g/L (Poorter et al. 2012).

Pot culture Experiment

(ii) ***Precautions*:**

1. Demands for water and nutrients increase strongly with the size of the plants, so the water and nutrient availability that are amply sufficient for small plants at an early phase may become limiting at later developmental stages.
2. Nutrient availability of commercially provided soil will vary among suppliers and even over time from soil batch to soil batch. Mixing of slow-release fertilizer with the soil or regular addition of nutrient solution may mitigate this problem to some extent.
3. Root damage may occur if pots are black and warm up under direct solar radiation. Moreover, soil temperature per se and even gradients in soil temperature within single pots can affect plant growth and allocation (Fullner et al., 2012).

Phenotyping experiments with plants require careful planning. The most controlled growth environment is not necessarily always the best one. Growing crop plants for experimental purposes remain an art, requiring in-depth knowledge of physiological responses to the environment together with proper gauging of environmental parameters. Hence it is advocated to adopt a practical check list (Table 1.1) to document and report a asset of information concerning the abiotic environment, plants experienced during experiments. Similarly advantages and disadvantages of field verses controlled environments in relation to some physiological traits are given in Table 1.2.

Table 1.1 Checklist with the recommended basic and additional data to be collected in all methods of experimentation.

(Adapted from Poorter et al., 2012).

Sr.No.		Basic data	Additional data
1	Light intensity (PAR)	• Average daily integrated PPFD measured at plant or canopy level (mol m^{-2} day^{-1}) • Average length of the light period (h)	• For GC: Light intensity (µ mol m^{-2} s^{-1}) • Range in peak light intensity (µ mol m^{-2} s^{-1}) • For GH: Fraction of outside light intercepted by growth facility components and surrounding structures

Table 1.1 *Contd..*

Sr.No.		Basic data	Additional data
2	Light quality	• For GC and GH: type of lamps used	• R/FR ratio (mol mol^{-1}) • Daily UV-B radiation (W m^{-2}) • Total daily irradiance (W m^{-2})
3	CO_2	• For GC and GH: controlled/ uncontrolled	• Average [CO_2] during the light and dark period (µ mol mol^{-1})
4	Rooting medium	• Water-based hydroponics/solid-based hydroponics including substrate used/soil type • Container volume (l) • Number of plants per container • For hydroponics and soil: pH • Frequency and volume of replenishment or addition	• Container height • For soil: soil penetration strength (Pam^{-2}); water retention capacity (g g^{-1} dry weight); organic matter content (%); porosity (%) • Rooting medium temperature
5	Nutrients	• For hydroponics: composition • For soil: total extractable N before fertilizer added • For soil: type and amount of fertilizer added per container or m^2	• For soil: concentration of P and other nutrients before start of the experiment • For soil: total extractable N at the end of the experiment
6	Air humidity	• Average VPD air during the light period (kPa) or average humidity during the light period (%)	• Average VPD air during the night (kPa) or average humidity during the night (%)
7	Water supply	• For pots: Volume (L) and frequency of water added per container or m^2 • Average day and night temperature (°C)	• For soil: range in water potential (MPa) • For soil: irrigation from top/bottom/drip irrigation • Changes over the course of the experiment
8	Salinity	• Composition of nutrient solutions used for irrigation	• For hydroponics: composition of the salts (mol l^{-1}) • For soils and hydroponics: electrical conductivity (dS m^{-1})

GH: Glass house, GC: Growth chamber

Table 1.2 Advantages and disadvantages of field verses controlled environments in relation to some physiological traits. (Adapted from Reynolds *et al.*, 2012).

	Field		Controlled facilities	
Traits to study	**Advantages**	**Disadvantages**	**Advantages**	**Disadvantages**
Treatments	Realistic	Less uniform Dependence on environmental/seasonal factors	Control of the intensity, uniformity, timing and repeatability of treatments. Out-of-season experiments are possible	Unrealistic
		Unpredicted interactions	Interactions between factors can be controlled Particular variables (radiation, ozone, etc.) can be manipulated and monitored	Variation in the glasshouse environment and handling of materials
Responses to drought	Realistic drying cycles	Co-occurrence of additional stresses (heat, low temperature)	Control of environmental factors	Unrealistic (rapid) drying cycles
	Realistic interactions with environmental factors	Less control over treatments	Control of water applied	Confounded by plant growth rate and differences in water status
	Realistic soil profile for root development	Confounding factors (toxicities, salinity)		Pot experiment limitations on root growth
Osmotic adjustment		Confounded by root depth and differences in soil water potential	Control of root depth Equal soil water potential by growing all genotypes in the same pot	Unrealistic (rapid) drying/rehydration cycles
Transpiration efficiency		Water fluxes can't be controlled	Precise control of water fluxes	

Table 1.2 *Contd..*

	Field		Controlled facilities	
Traits to study	**Advantages**	**Disadvantages**	**Advantages**	**Disadvantages**
Canopy temperature	Integrative measure-ment, scoring the entire canopy of many plants Related to the capacity of the plants to extract water from deeper soil profiles	Measurements must be taken when the sky is clear and there is little or no wind	Control of external factors	Only single plant/small groups of plants can be screened Not related to the capacity to extract water from deeper soil profiles -unless special pots are used
Root growth studies (biomass, length, growth rate, etc)	Realistic soil profile	Heterogeneity	Complete root systems are collected	Pot size, temperature, salinity, and hypoxia limiting root growth
		High sampling variance	Uniform sampling	
Adaptation to harsh soil	Realistic	Soil properties difficult to manipulate	Soil properties can be manipulated	Unrealistic
Phenotyping	Realistic	Risk of pollen flow	Low risk of pollen flow	Pot experiment limitations
Transgenic plants		Strict regulations and protocols	Less/easier regulations	

SECTION II