

**Conclusions of the workshop  
Generation Challenge Programme  
*"Phenotyping and water deficit"***

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## Overview

*Phenotype analysis is now a major limiting factor in genomics. However, the parallel between "phenotyping" and "genotyping" is only apparent, because a phenotype is a simplified representation of a complex reality under a series of pedoclimatic scenarios. "Phenotyping", viewed as a set of methods to associate a phenotype to a combination of a genotype and of environmental conditions, is therefore a scientific process per se. The workshop aimed to progress in this topic around three series of questions.*

*-What set of environmental variables is necessary to interpret jointly different experiments on the genetic variability of the response to water deficit and carry out breeding programmes ? How representative are the conditions in a given experiment in relation to most likely pedoclimatic scenarios (target populations of environments) ? The objective was here to allow joint and/or retrospective analyses of multi-environmental experiments carried out over several years and countries, by using covariables or a modelling approach. Common sets of environmental variables should also help in the communication between disciplines, between field and laboratory studies and between field studies in different countries. Consequences for the GCP database are proposed, together with some possibilities for capacity building.*

*- Which traits for which genetic strategies of drought tolerance ? This is an old debate which began with the first studies of drought tolerance and has no reason to stop. Rather than proposing an unrealistic choice of relevant traits, the objectives here were (i) to identify partial consensus and points of discussion about the criteria to choose traits, (ii) to design a method for continuing a fruitful discussion on this topic between disciplines, between species and between environments over the whole duration of the GCP, (iii) to propose elements for evaluating the "quality" of a trait in a programme of drought tolerance.*

*- Does association genetics require particular phenotyping designs ? What is possible and what is not ? Again, the objective here was to compare views and design strategies rather than obtaining a consensus view.*

*More direct questions such as "when should water deficit be imposed" or "which degree of water deficit is preferable", which directly depend on the objectives and goals of each project, are addressed in the sections "environment" (for characterising deficits) and "traits" (for building an experimental strategy).*

## Control of environmental conditions

*These conclusions have been drawn from 5 invited conferences, two on environmental conditions (H. Jones and G. Mc Laren) and three on modelling (F. Tardieu, M. Dingkuhn and S. Chapman). A session of discussions in small groups (about 10 persons) allowed collecting individual views, and was followed by a general discussion in which a rapporteur reported the debates and conclusions of each group to the whole workshop. A general discussion followed and final conclusions were drawn on the 9th July.*

### **I Objective, categories of measurements.**

The measurement of environmental conditions is understood here at the field (or greenhouse or growth chamber) level. It has three essential objectives.

- Characterise each experiment in such a way that the levels of water deficit can be compared (i) between "environments", i.e. combinations site x treatment x year (plus within an experimental plot due to spatial variability), and (ii) to most likely pedoclimatic scenarios in the same site and in other sites (TPEs).
- Reduce the Genetic x Environment interaction by taking into account external variables that contribute to the trait.
- Enable groups of the GCP to carry out a retrospective analysis of several multi-environmental experiments over several years and locations. This can be either *via* a modelling approach (analysis of the relationships of traits with environmental variables over the whole set of data ; calculation of indices), or with the use of environmental covariables in the genetic analyses (*e.g.* GxE interaction, QTL x E detection).

These objectives need a database which stores environmental data at a daily timestep (e.g. spreadsheets), and the main characteristics of the experiment. In some cases, experiments on more accurate physiological traits also require environmental data on an hourly base. However, a daily timestep usually allows (i) retrospective interpretations which were not envisaged in a first analysis, (ii) recording extreme events which can be very important for data interpretation but have a limited effect on averages (*e.g.* extreme temperatures, "Sirocco" events with an extreme evaporative demand etc. ). Two levels are envisaged.

A first level is a characterisation of environmental conditions during the experiment at the plot level (i.e. that are not affected by the individual behaviours of genotypes). This has three consequences. (i) The set of environmental variables to be collected is common to the whole GCP database, regardless of species and including experiments in the field as well as in controlled conditions. (ii) An experiment with 3000 genotypes has the same structure of environmental dataset as another one with 10 genotypes only. (iii) The level of stress sensed by individual genotypes is not characterised *per se*, genotypes with deep root systems or low transpiration would therefore have the same basic environmental characterisation as other genotypes. This first level involves an amount of information which has been kept to a minimum. Only this information would be recorded in the database. It allows determination of water deficit typology (TPE) and calculation of one or several stress indexes.

A second level is genotype dependent : for the same global conditions as defined at the first level, genotypic differences in growing patterns affect the soil water status and the environment as sensed by plants (*e.g.* canopy temperature, soil evaporation vs plant transpiration), which in turn affect plant functions. This second level aims at (i) comparing individual genotypes, especially for levels of water deficit, access to water, light

interception... (ii) comparing the time courses of crop phenology with the time course of environmental conditions, (iii) characterise the coupled conditions x phenological stages at which samples are taken for detailed analyses (transcripts or protein contents, metabolite concentrations etc). (iv) allowing a modelling approach which avoids potentially unrealistic hypotheses. To the difference of the first level, it is specific to species, categories of environments and objectives of the experiments. It would therefore be decided at the level of each individual project of the GCP and usually not recorded in the general database. The strategy consists to concentrate measurements on test genotypes.

## **II "First level", minimum dataset.**

It comprises information collected in two files, valid for both field, greenhouse and growth chamber experiments.

- A text form which records basic information on the experiment.
- A numeric file (e.g. spreadsheet) which records daily environmental information.

The text file is mainly descriptive and gives information on the type of experiment, species and genotypes used, design and protocols, management practices and all other information of interest. Some data are quantitative such as geographical situation (longitude, latitude, altitude, used for energy balance) or soil texture (% clay and loam, for rough calculation of soil water retention and hydraulic properties). A form is proposed in annexe 1.

The numeric file provides basic information for calculations of (i) a "climatic" soil water balance or stress indices for all genotypes, (ii) thermal time and (iii) potential biomass accumulation in the considered site. No measurement of soil water status is required in this section because of numerous methodological and practical difficulties. Of course, extra information provided by tensiometers, neutron probes or TDR sensors are also extremely useful to check the behaviour of the water balance model in the experimental site. The calculation of the water balance is available on different websites, in particular :

- FAO : <http://www.fao.org/docrep/X0490E/X0490E00.htm>
- APSRU : <http://www.apsru.gov.au/apsru/Documents/products.htm>

It remains to be evaluated if any of these methods can be used without any change, or if the GCP should generate its own tools such as a special spreadsheet. This can be decided after a period of tests. If special tools are to be designed, this should be considered in the "commissioned research" programme of the GCP.

### **a. Daily irradiance ( $I_r$ ) or photosynthetic photon flux density (PPFD).**

This information about available light is essential because (i) it is an input for calculating the soil water balance, (ii) it allows estimating the potential biomass accumulation in the considered environment (Eq. 2 in chapter "traits"). Irradiance ( $\text{W m}^{-2}$ ) is better suited for the first use and is provided by pyranometers, PPFD ( $\text{mol m}^{-2} \text{s}^{-1}$ ) is better suited for the second use and is provided by PAR sensors. Because both variables can be translated to the other one in field conditions, both are acceptable. It can also be acceptable to have an inexpensive sensor of daily sunlight duration which, combined with constants linked to the latitude, allow a rough calculation of  $I_r$  and PPFD. This possibility is not encouraged but can be helpful.

Light intensity has a relatively low site-specificity. It is acceptable to record data from a weather station located at several km provided that (i) the weather station is in the same geographical situation as the experimental field (altitude...), (ii) one can have a reasonable

confidence in the data (especially missing data, quality of sensors). In contrast, special care has to be taken in greenhouse and growth chamber experiments, because of the high spatial variability of light in these ambients (both horizontal and vertical). A map of light intensity, or at least the use of several sensors, are recommended.

### **b. Air temperature (T)**

Together with irradiance, this information is necessary for calculating the soil water balance. It also allows estimating thermal time if plant temperature is close to air temperature (usually acceptable for well watered adult plants, prone to large errors during early phases in monocot species and in plants subjected to water deficit). This allows estimating the occurrence of high temperature stresses (e.g.  $T > 40^{\circ}\text{C}$ ), risks of oxidative stresses (e.g.  $T < 3^{\circ}\text{C}$  and  $\text{PPFD} > 1000 \text{ mol m}^{-2} \text{ s}^{-1}$ ) and an estimate of phenological stages with the use of thermal time.

This information must be recorded in the experimental field with the use of a local weather station or a data logger with thermocouples. Data are kept at a daily timestep as minimum and maximum temperatures. This information needs to be measured at plant height in greenhouse or growth chamber experiments.

### **c. Air relative humidity (HR), vapour pressure deficit (VPD), reference evapotranspiration ( $\text{ET}_0$ ).**

These three variables quantify the evaporative demand, essential for stress level characterisation and calculation of the soil water balance. They provide essentially the same information, but with different time scale and usefulness. HR (%) and VPD (kPa) are calculated on short timescales (minute to hour),  $\text{ET}_0$  ( $\text{mm day}^{-1}$ ) is at a daily timescale. The variable recorded in the database would be  $\text{ET}_0$ , either calculated from other climatic data (Ir, VPD, T windspeed) recorded in a datalogger (see above) or directly calculated by the weather station.  $\text{ET}_0$  is species- independent and calculated by energy balance.

RH and windspeed have relatively low site-specificities. As in the case of air temperature, it is acceptable to record these data from a weather station located at several km. RH must be recorded in greenhouse and growth chamber experiments, preferably with replications because of the large spatial variability. A method for calculation of  $\text{ET}_0$  is available on the site

[http://www.fao.org/docrep/X0490E/x0490e04.htm#reference%20crop%20evapotranspiration%20\(eto\)](http://www.fao.org/docrep/X0490E/x0490e04.htm#reference%20crop%20evapotranspiration%20(eto))

It might be useful to insist here on two frequent errors

- HR should not be interpreted *per se* because it does not characterise the evaporative demand when air temperature is fluctuating. The use of both HR and air temperature allow a very simple calculation of VPD which is the driving force for transpiration. Extreme events such as sirocco should be recorded as daily maximum VPD over a period of 3 or 4 h.
- Mean daily air VPD or HR are not acceptable for characterising the daily evaporative demand. Use  $\text{ET}_0$ .

### **d. Rainfall - irrigation.**

This must be recorded near the field (< 300 m) because of a very high spatial variability. Rain-gauges are efficient and inexpensive but require frequent visits, automatic rain gauges connected to a datalogger are more expensive but are useful in distant experiments.

### **e. Initial soil water content in the field**

The water balance begins at a given date (e.g. emergence) at which the soil water content must be recorded. This can be done with augers over a depth similar to the final rooting depth,

with a special care of spatial variability in the field. This measurement is of particular importance in experiments where the rainfall is null or negligible. Some "shortcuts" can be acceptable either when the rainfall or irrigation before the experiment is sufficient to guarantee that the soil is at retention (or field) capacity, or in other special cases.

#### **f. Presence / depth of a water table**

This information is necessary when a water table is within or close to the rooting volume. In this case, the soil water balance may be largely irrelevant because roots directly take up water from the water table. This information should be recorded, when relevant, with the use of piezometers. When no close water table is present, the information "no" is sufficient.

#### **g. Other measurements**

The set of "compulsory" environmental data would stop here, but other measurements can be extremely useful in some environments. They characterise (i) limiting factors other than water deficit, which complicate the interpretation of experiments. It is the responsibility of each project to decide whether participants wish to interpret experiments with such limiting factors by using appropriate statistical or modelling methods, or if it is better to discard them. (ii) physical measurements which can help to interpret data, or operate shortcuts.

- *Soil chemical analyses.* The objective of those is not to have indications on the nutrient status of plants, which would need repeated soil analyses (typically every week for N). It is rather to characterise a toxicity or major problem which may hamper the plant development, especially the root growth. Among them : soil pH, Al toxicity, salinity, other metal toxicity etc.

- *Soil physical and hydrodynamic properties.* These are essentially the variables which allow calculating soil water potential from soil water content, namely the profile of soil bulk density and a water release curve at different depths. They usually can be inferred from the soil texture recorded in the text file but other procedures can be useful.

- *Biotic stresses* A minimum visual characterisation should be recorded in the text form, to record the occurrence of obvious biotic stresses. The use of probe genotypes, for instance very sensitive lines or couple of near isogenic lines, is very useful to detect non-visible stresses such as nematodes which can largely interact with the onset of water deficit.

- *Nutrient status.* It is recommended to avoid nutrient deficiencies at least in experiments which aim to evaluate the ability of genotypes to cope with water deficit. The measurement of nutrient status is usually not feasible on all genotypes, but it is recommended to carry it out at key phenological stages on test genotypes (see below).

#### **h. Spatial variability.**

As a rule, fields with a large spatial variability are not suited to experiments with water deficit because of the numerous interactions and confounding effects which may occur.

- Spatial variability can be checked by carrying out measurements (e.g. soil water content, rooting depth) during the crop cycle. However, the spatial structure of this variability (cm to hm scales) is often complex.

- Placing a test genotype in several locations of the experiment, and/or carrying out a "blank" experiment with only one genotype previous to the experiment, are healthy precautions. In this case, periodic and spatialised measurements of plant water status (e.g. predawn leaf water potential), phenology and yield allow characterisation of the spatial variability.

- Statistical designs (e.g. blocks, alpha lattices, spatial autocorrelations etc) can take into account part of the spatial variability in the data analysis.

- It is useful to insist here on the large spatial variability of microclimatic conditions in greenhouse or growth chamber experiments (see above).

### **III Second level : characterisation of individual accessions or genotypes. ; test genotypes.**

The above-mentioned dataset provides no information on the water / nutrient status of individual genotypes, as modified by root system characteristics, stomatal behaviour or leaf growth, nor on the synchrony of water deficit with critical phenological stages. It is therefore important to characterise environmental conditions as sensed by individual genotypes, taking into account that those are part of genotype strategies such as escape (*e.g.* adjusting the phenology to avoid most likely stressing periods) and avoidance (*e.g.* deep root systems which can take up a greater water volume, thereby avoiding water deficit). It is generally impossible to carry out detailed measurements on all genotypes, so the use of test (or check or reference) genotypes on which measurements are concentrated is required. Information collected on test genotypes can be combined with a very limited number of inexpensive or quick measurements collected on all studied genotypes to infer the behaviour of all genotypes.

#### **a. Estimation of the crop phenology / synchronisation with the timing of water deficit.**

A precise record of the time of emergence (whole trial) and of the flowering date of all genotypes is essential at least for grain crops. Estimation of intermediate stages of the crop phenology for detection of the "critical periods" of individual genotypes can be done :

- Either with the use of thermal time ( $^{\circ}\text{C day}$ ), a better predictor of the progression of phenology than calendar time (days). This may require measurement of apex temperature in test genotypes, especially for monocots in early stage. In this case, the plant apex is in the soil and may have a temperature which differs from air temperature by more than  $6^{\circ}\text{C}$ .
- Or by recording weekly leaf number of 1 or 2 test genotypes, if possible common to several experiments. This provides an estimate of the progression of phenology of all genotypes. The time from emergence to flowering of each genotype is then split into a number of phyllochrons of the test genotypes.

Some experiments carried out in the GCP require a more precise record of the phenology of all studied genotypes, especially when samples are taken for analyses of transcripts, proteins or metabolites.

#### **b. Estimation of individual plant water status or water relations.**

Measurements usually cannot be carried out on all accessions (although automated experimental set ups can solve this problem in some cases). A possibility is

- to measure a time course of predawn time leaf water potential or stomatal conductance in one or a few number of test genotypes. Predawn leaf water potential provides an estimate of soil water status as sensed by plants, independent of other environmental conditions but which depends on the root system characteristics.
- and to assess difference between genotypes with rapid measurements such as leaf temperature. Precautions and modus operandi are provided in Jones et al. 2003 - 2004 (see annex).
- In C3 species, a measurement of the discrimination for  $^{13}\text{C}$  ( $\Delta^{13}\text{C}$ ) of all accessions can also be very useful, as it gives an integrated view of stomatal conductance over the developmental period (see Condon's presentation).

It should be noted that indirect measurements of plant water status are prone to errors of interpretation, because of the conflicting effects of several variables (see presentations of H. Jones and F. Tardieu).

### **c. Estimation of plant nutrient status**

In the same way, an estimation of plant nutrient status can be carried out at key phenological stages on test genotypes, to check whether deficiencies are observed.

It is noteworthy that a side effect of water deficit can be nitrogen deficiency, because the decrease in water flux mechanically decreases the nitrogen flux, to an extent which depends on species and environmental conditions.

## **IV Conclusion**

We believe that the minimum dataset described in paragraph II may considerably improve the interpretation of experiments of the GCP, especially in multi environment trials. It should also be an instrument of communication between experiments carried out in the field and in controlled conditions, between field experiments carried out in different countries, and between disciplines represented in the GCP.

One of the conclusions of the workshop was that access to equipment should not be an obstacle, especially in NARS : the considered equipment is largely inexpensive if compared either to the equipment needed in genomics, or to the cumulated salaries involved in the phenotyping process. It is therefore recommended that projects take this cost into account, or that the "capacity building" programme take this need into account.

An effort of training groups for the proper use of sensors (calibration, maintenance and follow-up) and for the use of simple models (water balance, energy conversion into biomass, crop models for TPE evaluation), may be envisaged in the future either by short stays in trained labs and/or special training courses.

## Which traits for which genetic strategies ?

*These conclusions have been drawn from 6 invited conferences, three on modelling (F. Tardieu, M. Dingkuhn and S. Chapman) and three on the linking of phenotypic traits, genetic analyses and physiological analyses (T. Condon, JM Ribaut and T. Setter). A session of discussions in small groups with a common interest on a category of crops (about 10 persons) allowed collecting individual views, and was followed by a general discussion in which a rapporteur reported to the whole workshop the debates and conclusions of each group. A general discussion followed and the conclusion session of 9th July was dedicated to identify partial consensus and a working method to continue. Conclusions are also based on (i) a survey in which each participant could rate the degree of priority of several traits in his/her research programme, (ii) partial conclusions for each crop, (iii) a description / characterisation of traits, carried out in a standardised form discussed during the workshop. Those elements are provided in annexes of the present text.*

### **I A first consensus : it is not desirable that the GCP chooses a uniform set of measured traits for each species.**

The two surveys of traits of interest according to the participant's perceptions are presented in Annexes 3 and 4. Although several traits are frequently cited and common to several species, it would be dangerous to decide that "consensus" traits are those to be considered in the GCP.

The choice of a trait or of a series of traits depends on the scientific objective of each individual project of the GCP. Phenotyping consists, finally, in a test of hypotheses. Typically, a person or a group first assumes that a given trait is worth including in a genetic strategy on several criteria, namely (i) its relationship with a characteristic of interest (mainly : yield, quality of products, environmental consequences of the crop), (ii) its heritability or reproducibility, (iii) its genetic variability (iv) its theoretical interest according to a framework of interpretation (see § II.1). The final judgement about the interest of the chosen trait will eventually be the ability of a breeding programme to reach the goals which were fixed in a given target population of environments. Presentations have shown successful comprehensive strategies which reached their goals. Other programmes presented in the literature over the last 20 years obviously did not. Several reasons make the choice of traits a crucial step of a breeding programme and of genetic studies, and make it unrealistic that common traits are chosen by the whole GCP. Traits can hardly be common to groups working on a different species with different objectives :

- Several presentations have suggested that a given trait has frequently contradictory effects on the final goal (e.g. transpiration efficiency vs photosynthesis in Condon's presentation), and the respective weight of positive vs negative consequences of the trait depend on environmental conditions in the target population of environments.
- The final goals are not necessarily the same in different regions or farmer groups for a given species (several participants mentioned quality and environmental consequences as important outputs). The "secondary" traits which help reaching this goal should therefore differ.
- Our collective understanding of the response of plants to water deficit changes with time, so our perception of relevant traits should also change. For instance, a survey carried out 10 years ago would have placed osmotic adjustment measured in mature tissues as a major trait, while this is more debated nowadays. Uniformity of traits is therefore not desirable from a scientific point of view.

The relatively recent "arrival" of ecophysiologicals and modellers in the topic of drought tolerance results in a new generation of traits, either new interpretations and procedures coming from a renewed theoretical background (e.g. IR canopy temperature or  $\Delta^{13}\text{C}$ ), or new categories of traits (e.g. parameters of response curves, or indices such as the ratio of biomass to incident light). This increases the "biodiversity" of the system, resulting in a more complicated picture, but biodiversity is a condition for species survival...

## **II Elements of consensus and points of discussion in the evaluation of traits**

### **1. Each trait should be placed in the process of yield formation or of other characters of interest.**

A general consensus is that the traits and genes which result in plant survival under extreme water deficits differ from those involved in maintenance of yield or of other characters of interest (mainly, yield quality or environmental consequences of the crop) under water deficits compatible with agriculture. This might cast doubt on the practical interest of studies in which the manipulation of one gene conferred an improved survival capacity of plants subjected to very severe water deficit.

Another consensus was that each trait should be placed in the process of yield formation, by using classical equations which split the yield into several components. Three families of equations have been used in the literature. None of them involves "drought resistance" *per se*. They rather express the processes by which crops achieve yield in a drought-prone environment, with a particular focus for each equation :

- The first one places the water used by the crop as the key process of yield formation (Passioura 1977, presented by T. Condon in the workshop)

$$\text{Yield} = \text{ET} \times \text{T} / \text{ET} \times \text{TE} \times \text{HI} \quad (1)$$

$$\text{Yield} = \Sigma (\text{ET} \times \text{T} / \text{ET} \times \text{TE}) \times \text{HI} \quad (1')$$

where ET ( $\text{Kg m}^{-2}$ ) is evapotranspiration (usually a fraction of  $\text{ET}_0$  defined above), T/ET ( $\text{Kg Kg}^{-1}$ ) is the fraction of evapotranspiration effectively transpired by plants and not evaporated from the soil, TE (transpiration efficiency,  $\text{Kg Kg}^{-1}$ ) is the ratio of accumulated biomass to transpired water and HI (harvest index,  $\text{Kg Kg}^{-1}$ ) is the ratio of harvested biomass to total biomass. Because several terms vary with time, this equation may be split into a summation over the duration of the crop cycle of the first 3 terms with a daily timestep, multiplied by HI (eq. 1').

- The second one, which applies more generally than Eq. 1, places the light available to the crop as the key process of biomass accumulation, transformed into yield by HI (Monteith 1977, presented by M. Dingkuhn in the workshop). In somewhat different forms, it is a central family of equations in all current crop models.

$$\text{Yield} = \Sigma (\text{PPFD} \times \epsilon_a \times \epsilon_b) \times \text{HI} \quad (2)$$

where PPFD ("light") is the photosynthetic photon flux density ( $\text{mol m}^{-2} \text{s}^{-1}$ ) as measured by a PAR sensor (see above),  $\epsilon_a$  (%) is the proportion of PPFD intercepted by leaves and  $\epsilon_b$  ( $\text{Kg mol}^{-1}$ ) is the conversion ratio of intercepted light into biomass. These terms are defined at a daily timestep and summed over the duration of the crop cycle. HI is defined as in Eq. 1.

- The third one, of general use among agronomists and breeders, is based on the identification of yield components which are determined at different periods of the crop cycle. It allows identification of "critical periods".

$$\text{Yield} = \text{grain (or tuber) number} \times \text{individual grain (tuber) weight} \quad (3)$$

in which grain number ( $\text{m}^{-2}$ ) can be split again into spike or ear number per  $\text{m}^2$  x grain number per spike, and each of these terms can be split again. Its main assumption is that yield is essentially sink-limited while Eqs. 1 and 2 assume that it is essentially source - limited.

It is not the place here for a thorough discussion of these 3 equations, already largely discussed in the literature. Each of them involves a simplified representation of the reality and of major traits involved in yield formation. The interest of the first one is to place water in the centre of the discussion, its main drawback is that individual terms are highly inter-dependent so it does not allow a modelling approach. The second equation has more independent terms at a daily timestep, and is a base of current crop modelling. It assigns a role to water quite different as that in Eq. 1, namely affecting the capacity of the crop to intercept light ( $\epsilon_a$ ) or to transform it into biomass ( $\epsilon_b$ ). The interest of the third equation is to phase the crop cycle into phenological periods with different sensitivities to water deficit.

The consensus of the workshop assembly is that each individual trait should be placed in relation to at least one of the terms of these equations, to check its relevance to yield. This exercise has been done for several traits. For instance :

- Traits related to the access to water (*e.g.* structure of the root system) are clearly identified in Eq. 1 via the term ET, and not explicitly in the other two equations.
- Traits related to early vigour and its determinants (*e.g.* embryo size, potential leaf expansion rate, maintenance of expansion rate under drought) are in two terms of Eq. 1 (T/ET and ET) and one term of Eq. 2 ( $\epsilon_a$ ).
- Traits related to stomatal functioning and photosynthetic capacity (*e.g.* stomatal conductance, leaf temperature,  $\Delta^{13}\text{C}$  etc) are related to two terms of Eq. 1 (ET and TE) and in one term of Eq. 2 ( $\epsilon_b$ ).
- Traits linked to the sensitivity of a particular phase of development are well identified in Eq. 3 (*e.g.* spike number, ASI), and less in Eq. 1 and 2 in which they participate to HI, but also to TE in Eq. 1 and to  $\epsilon_b$  in Eq. 2 via the sink limitation of photosynthesis.

It is noteworthy that most "physiological" traits cannot be placed in these equations in an unambiguous way. For instance, traits as ABA synthesis, osmotic adjustment or sugar accumulation may have an effect on all terms of Eqs 1 and 2.

## **2. Combining constitutive and adaptive traits, a role for modelling.**

An important point was raised during the conclusion session of the workshop, namely that geneticists are attracted by constitutive traits (*i.e.* those which are either under a small dependence on environmental conditions, or with a small genotype x environment interaction), while most analyses of transcript or protein levels in studies of water deficit deal with differences in gene expression between well-watered and water-deficient plants.

- The surveys presented in annexes 3 and 4 confirm the "popularity" of traits considered as constitutive (*e.g.* root system characteristics, early vigour) among the participants of the workshop. The rationale is here that breeding for these traits is more efficient than that of adaptive trait because of their low G x E interaction. Convincing arguments were presented in

the presentation of T. Condon, but a counter-example is the use of maize ASI in genetic and breeding programmes (Presentation of JM Ribaut).

- It is of clear theoretical and practical interest to identify which genes are under or over-expressed under water deficit, because this may allow molecular identification of adaptation processes (e.g. transcription factors, hormonal controls etc. ), especially those involved in the control of the extent of the resource capture system (leaves and roots) of the crop

Because the GCP aims to combine genetic and genomic approaches, two avenues can be considered to resolve this contradiction :

- That the GCP should consider, genomic studies which are not based only on the comparison of gene expression between well-watered and water-deficient plants. This involves the expression of key genes driving plant development and architecture (especially for the root system), but also in other processes (e.g. seed development for embryo size ; genes involved in the photosynthetic apparatus for  $\Delta^{13}\text{C}$ ).

- Design, *via* a modelling approach, a strategy to deal with adaptive traits in genetic analyses. This may consist in identifying stable and heritable characters of a genotype which drive the response of adaptive traits to environmental conditions. For example, parameters of the response curve of stomatal conductance or of leaf growth rate to quantified environmental conditions are stable for each genotype across sites and years. They can therefore be considered as constitutive traits in a genetic analysis (see F. Tardieu's presentation).

### **3. Linking genomic and macroscopic traits**

This is a key issue, in which all disciplines involved in the GCP should provide an input. Examples of integration of genetic, physiological and genomic studies were proposed in JM Ribaut's, T. Setter's and J. Bennet's presentations. This requires to find common ground in genomic and genetic studies of drought (see above), but also requires an important input of modellers at different scales of organisation (genetic or metabolic networks, individual plant modelling, crop modelling). Because this is a new challenge, it is not timely to suggest common approaches and several attempts with different views are required.

Three precautions or controversial points were proposed in T. Setter's presentation for the design of experimental procedures in the sampling of material for gene expression studies, which are worth citing in the conclusion :

- Respective importance of early and later molecular events after imposing a water deficit. The rationale for an early sampling is that gene expression is usually largest at that time, before any negative feedback, and that it helps identification of initiating factors and up-stream regulation. This is associated with major drawbacks : (i) It over-emphasises the "shock" effects, usually not realistic in natural conditions where stress imposition is slower than in controlled conditions. (ii) It leads to miss events which have a slow onset. (iii) It leads to miss longer effect mechanisms at plant level.

- An adequate characterisation of water stress environment is essential to avoid mis-interpretations. Stable, realistic and quantified levels of water deficit should be looked for.

- Elements have been provided for the optimisation of cost vs number of comparisons.

### **4. Timing and degree of water deficit**

- In experiments where the soil water status can be controlled, the experimental manipulation of water deficit is to be designed as a function of the studied trait. This involves the time at

which the trait is determined in relation to the plant phenology, but also the studies of the after-effects of a change in trait during later periods of the crop cycle.

- Reciprocally, relevant traits in a target population of environment (TPE) depend on the timing of most frequent water deficits in the TPE (G. Mc Laren's presentation). An appropriate way of checking whether a trait may or may not have large consequences on yield in a TPE is carrying out simulations with a crop model over a long climatic sequence (e.g. 20 years), as shown in S. Chapman and T Condon's presentations. S. Chapman's presentation showed, in addition, how the allelic composition of a population of genotypes in a breeding programme can be predicted under different climatic scenarios by the combined use of a genetic and of a crop model (Chapman et al. 2003).

### **III Conclusion : criteria for a "screening" of trait quality in relation to drought tolerance.**

Overall, the workshop agreed that the choice of relevant traits should be of the responsibility of individual projects of the GCP, rather than the object of a consensus which may prove counter-productive in the long run. The discussion inside the GCP should rather be focused on clarifying the hypotheses and the goals which lay behind each trait. The above paragraphs may provide shared criteria for evaluating the quality of traits :

- Genetic characteristics in a type of experimental design : heritability or reproducibility ; genetic variability, response to selection.
- Relationship with a characteristic of interest (mainly : yield, quality of products, environmental consequences of the crop). In particular, is the trait directly or indirectly associated to one or several terms of Eqs 1, 2 or 3 ? Can the trait be associated with a modelling approach (i.e. to identify a sub-trait that is stable for a genotype across environments) ?
- Ability to be associated with physiological or genomic studies : is the trait linked to identified physiological mechanisms or category of genes ?
- Relevance to a target population of environment : is the interest of the trait specifically associated with a pedoclimatic scenario ?
- Cost, ability to be measured in a high throughput analysis (e.g. via an automaton or robot) or in an imaging process (e.g. close remote sensing).

These criteria have been placed in a form which may help to evaluate the relevance of traits in a GCP project and propose a typology of traits (annexe 2).

#### How to continue the work on this topic after the workshop ?

Several steps are proposed for continuing the debate which begun in the workshop.

- Although this text essentially reflects the presentations and discussions, its organisation and emphases may result in new discussions. It is proposed to workshop participants to amend it via internet.
- Furthermore, a detailed analysis of individual views (see survey in annexe 3) and of the views of groups interested in a given crop (see annexe 4) reveals some opposed views. The synthesis presented here cannot reveal all individual views, which will probably evolve during the duration of the CPG. There is therefore room for discussion.
- The form in annexe 2 could be of great interest for a typology of traits in the future. Participants of the workshop can now validate and enrich their contributions, a synthesis will be provided later on.

- It is proposed that CGP meetings (e.g. Brisbane in September 2004) dedicate some time to this discussion, especially on the change with time of respective positions while the projects are progressing.

## Phenotyping for association genetics

*The discussion was responding to proposals/questions that had arisen at the SP1 "data analysis" workshop held two weeks earlier in Zaragoza and to a range of recommendations or warnings expressed in the presentation of JM Ribaut from experience on QTL analysis of drought tolerance related traits in maize. The synthesis is written by JC Glaszmann.*

Association studies require comparisons of molecular data (qualitative) and "phenotyping" data (generally quantitative) within a sample of, typically, two to four hundred accessions. The molecular data can be targeted to candidate genes whose involvement will be assessed and can be spanning the whole genome in case of linkage disequilibrium mapping in search for genome regions involved (comparable to a multidimensional QTL analysis).

The rationale of the Generation CP implies gene/allele discovery within the range of genetic resources available to the breeders. Within SP1, a survey of structural diversity of a global composite set representing the whole diversity is used to direct functional genotyping and phenotyping investigations to a manageable sample of materials. Such a reference sample would include:

- Representatives of the main components of the diversity ("Representation" accessions), to cover the range of allelic diversity; these may include a very wide range of "adaptations" or environmental specializations
- A large portion of accessions that would cover in a continuous manner the global range of genotypic diversity ("Star"-like sample), ideal for species-wide association studies, but also including diverse adaptation patterns
- Those sectors of the diversity that seem derived from recombination between two distinct components ("Interface" sections), ideal for LD mapping and probably including compatible adaptation patterns
- Some components with large continuous variation ("Compartments"), ideal for subspecific association studies and possibly including compatible adaptation patterns.

The value of concentrating phenotyping efforts on the same germplasm is recognised for its ability to enable integrated characterisation giving access to correlations between traits and the underlying pleiotropic and epistatic interactions. Plant and crop modelling should be considerably facilitated thereof. However, it can also be easier to undertake parallel analysis of the genetic basis of distinct traits on distinct populations by adapting the population choice to specific experimental constraints.

The users should be encouraged to using as much as possible of this reference sample but should also be allowed to include additional materials, such as their preferred checks, and to exclude those materials that are not adapted to the experimentation environment. The combination of all these criteria requires development of simple, easy to use softwares for elaborating the set of materials for any new experiment.

Genetic variation within the samples, e.g. in cases of (partial) outbreeders (not clonally propagated), is considered problematic for association studies. Simplification of the genetic constitution of the samples, with or without selection pressure, is considered a prerequisite for treating this kind of materials.

The rationale for undertaking characterisation experiments within the framework of drought tolerance phenotyping (*i.e.* a wide range of possible traits often complex) has to carefully take

account of three kinds of features: the expected type of genetic control (oligo- vs poly-genic) of the trait measured; the heritability, or interference of environmental conditions on the trait; the difficulty, or the cost of the measurement. Association studies will be most efficient on oligogenic, heritable and cheap-to-assess traits. Practically, they will be preferably undertaken in cases where two of the three features are favorable; this provides an effective decisional key.

The importance of specific characters to be homogenized among the materials that are to be compared in a phenotypic evaluation, such as phenology and gross morphology clearly emerged.

Most probably the typical extension of the first step in SP1, that is the identification of a reference sample, will be a season of gross field observation in the environment of future characterization in order to select those accessions that are most comparable and that will give the most meaningful phenotypic comparisons; this can also serve as seed increase to provide full planning autonomy to the scientists in charge of the phenotyping experiment.

## Overall conclusion and perspectives.

### 1. Several "operational" conclusions have been drawn

- a. The group has defined a minimum set of environmental data which should characterise the environment of all experiments of the GCP, in field, greenhouse or growth chamber experiments. It remains to be decided by the GCP direction whether and how this conclusion should be taken into account in individual projects, and in the construction of the GCP database.
- b. Another set of environmental data concerns (i) the perception of the environment by each studied individual genotype, (ii) the synchrony of the phenology of individual genotypes with environmental stressing events, (iii) the water holding characteristics of the soil. Methods have been proposed to combine detailed measurements on a very small number of test genotypes and a very small number of cheap and quick measurements on all genotypes.
- c. It is not desirable that the GCP selects a set of relevant traits which would be common to all projects. In contrast, elements for evaluating the "quality" of traits have been proposed. A database of trait characterisation is under way.
- d. Modelling should be involved at several steps of the phenotyping process. This involves, among several possibilities, the genetic analysis of adaptive traits, the determination and characterisation of TPEs and the integration of traits resulting in *in silico* breeding programmes.
- e. Further decisions, such as the choice of traits, the control and manipulation of environmental variables or the respective roles of genetic, modelling, physiology and transcript/protein analyses are of the responsibility of individual projects. They are an essential characteristic of the scientific quality of those. In this respect, the workshop aimed to help and propose, but not to fix general rules.

### 2. A community has been created, and wishes to continue working together.

The workshop assembly comprised well-balanced proportions of scientists coming from the CGs, ARIs and NARS (40, 30 and 30%). This community, with different disciplines, backgrounds and geographical origins, is probably useful to the GCP and wishes to continue working. An opportunity is given for that via a forum about the conclusions of the workshop and further discussions.

It may be useful to identify a corresponding "networking" task in the GCP.

### 3. An opportunity for capacity building.

One of the working hypotheses of the workshop was that phenotyping is not a series of repetitive trait measurements aimed to a standard characterisation of the genetic material, but a creative activity based on scientific hypotheses. It requires a combination of skills including physical and physiological concepts and methods, modelling approaches and genetic strategies. This working hypothesis has been confirmed by the workshop.

A consequence is that a considerable margin of progress exists in the different consortia of the GCP, to integrate modelling approaches and physical or physiological concepts in the phenotyping process. Possibilities for progressing include exchange of scientists, PhD theses elaborated in common, edition of a course and other possibilities.

**Annexe 1 :**  
**Proposed form for storing information about experiments**  
**(in addition to environmental data stored in a spreadsheet).**

*A form has been proposed by G. Mc Laren during the conclusion session and modified afterwards. It is similar to a short "material and methods" in a standardised form. A standard presentation can be proposed once this form has been checked and modified by participants.*

**1. Experiment management**

- Investigator (s)
- Type of Study – Field, greenhouse, growth chamber, survey etc
- Location, longitude, latitude, altitude
- Soil texture (% Clay and loam) for water content calculation)
- Species, genotypes
- Objective (s)
- Experimental Design (record layout, row/column – plot size). Whenever possible map of the treatments especially in greenhouse or growth chamber studies. Position of sensors in the map.
- Managed factors : property being manipulated, method of manipulation.

**2. Measurement protocol**

- Protocol for environmental measurements. Sensors used (type and make), periods of missing information if any (*e.g.* problems of sensors), method for data recovery if any (*e.g.* another distant weather station), timestep of observation (hour to day), units of observation.
- Property (trait) being measured : method, equipment if any (type and make). Periods of missing information if any (*e.g.* problems of sensors), timestep of observation, units of observation.

**3. Management practice, cultivation techniques**

- Date of each relevant technique : planting or sowing, tillage, fertilisation, treatments for pest, disease and weed controls
- Type of tillage and mechanical weed control (apparatus, approximate depth)
- Sowing/planting method (technique or apparatus, geometry : distance between rows and between plants, approximate sowing depth)
- Type / amounts of inputs (fertilisers, treatments)
- Irrigation scheduling if any : technique, amount, dates.

**4. Events and observations**

- If relevant : timing and objective of visits – Trial diary
- Notation of incidents if any (*e.g.* "A cow ate part of plot 18 on 15th September", "Breakdown of irrigation equipment on 3rd August"...) )
- Observation on pest and diseases (dates / approximate phenological stage / type / semi-quantitative degree of attack if possible).
- Observation of obvious spatial variability in the experimental field.
- Pictures if useful and relevant.

**5. Name and format of the file of environmental data**

*e.g.* Huancayo\_2003.xls

These formats should be decided when constructing the database.

## Annexe 2 Proposed form for drought trait ontology

*This form has been built during the conclusion session of the workshop, from a proposal of G. Mc Laren, and revised afterwards.*

Column	Description	Example
Trait name		
Species		
Generic Trait	If traits are organized in a hierarchy of specificity, what would be the 'parent trait'	Xylem diameter is a specific trait of water transport.
Agronomical relevance	How the trait impacts on equations 1, 2 or 3, chapter "traits"	ASI is involved in HI, but also in TE (eq. 1) and $\epsilon_b$ (eq. 2)
Anatomy Class - Organ	What part of the plant is affected	
Growth stage/Timing of action	When is the trait expressed in terms of plant phenology ?	
Type of drought environment	In which type of drought environment is this trait most likely to be related to adaptation and performance ?	
Intensity of drought	What is the intensity of stress for which the trait is relevant ?	
Measurement method and units		
Description and impact		
Genetic variation	Is there genetic variation available for this trait	
Heritability/Repeatability	How high are $h^2$ in individual experiments and across experiments ?	
Mechanism/pathway	What are the physiological processes /gene categories / pathways involved ?	
Ability to be used in modelling ?	Can the trait be directly used in a modelling approach ? Type of model ?	
Cost / skill	How expensive is it to measure this trait ? Does this need special skill ?	
High throughput ability	Is the trait suitable for high throughput measurement ?	
Reference (literature)		
Comments		

### **Annexe 3 Survey of traits of interest**

*This survey was proposed and analysed by T. Setter. See attached Excel file.*

Participants were asked to indicate the percentage of resources they would devote to screening germplasm for performance under water deficit using various phenotyping traits if they were responsible for a breeding program for a selected crop. The intent of the survey was to determine the general opinion of workshop participants concerning which traits they value highly, and which less so, for germplasm evaluation. The survey might also indicate the extent to which there is agreement, disagreement, or the extent to which there is diversity of opinion depending on crop species, the target environment, and other considerations.

Some people indicated that their responses were for a program on QTL identification, phytotron screening, or other situations, rather than for a field program. These data are included with the others. These responses illustrate that selection of a short-list of traits is made difficult not only due to the diversity of crops and target environments, but also the wide range of phases in a genetic crop improvement program for which such selection might be applied.

Yield under drought was by far the highest-ranking trait among participants, with 76% of the respondents (32 out of 42) giving it some research resources, and 48% giving it more than 20% of their resources. Yield potential, evaluated under well-watered conditions, ranked second. Two other whole-plant traits that provide a time-integrated measure of plant performance also ranked near the top: biomass and harvest index.

Several root traits were given research resources by the participants (root depth, branching, biomass, and extraction of soil water), with 64% of the respondents including at least one root trait. Although root depth ranked highest among root traits (40% giving it some resources), none of the participants commented on the methods by which such assessment would be made. Seedling vigor ranked very high overall (third), with allocation of research resources especially high for cereals (7 out of 9) and rice (5 out of 7).

At least one trait related to water status was selected by 57% of respondents. Carbon isotope discrimination, which was included in 31% of the responses, was the highest-ranking in this category (note: it could be placed in several categories), and leaf osmotic adjustment was selected by 26% of the respondents.

It is unclear whether the low ranking of other measures of plant water status was due to low enthusiasm for these traits, or due to their not being included as listed items on the form. The same question applies to other categories such as timing of phasic development and leaf photosynthesis-related traits. Thus, a flaw of the present survey is its failure to include more listed items, because participants clearly responded to the suggestions of the list. Nevertheless, the survey generated many additional suggestions from the participants, and if the survey were repeated with these additional items, the results might be even more valuable.

Overall, it appears that participants are most confident of well established traits, with the exception of the recently-introduced delta-13C trait for which we heard evidence from Tony Condon of its successful use in a breeding program. It suggests that additional work is needed to develop suitable field-based methods of evaluating plant traits for which current methodologies are inadequate because they do not have a well-established relationship to critical stress-tolerance attributes, are too expensive, awkward for field use, imprecise, have low throughput, suffer from low heritability, or have other defects.

## Annexe 4 Conclusion of groups : traits for each species

*Each group involved scientists with a common interest for a group of species. It addressed several questions (i) usefulness of indirect indicators of ability to extract water ( $T$ , transpiration,  $G$ , stomatal conductance,  $\Delta$ , carbon isotope discrimination), (ii) characters directly involved in yield or quality maintenance in a range of drought environments, (iii) connection to transcript/proteins analyses where and when to collect samples, (iv) what timing and control of water deficit for which trait and, (v) which genetic material for which traits ?*

The group working on beans, roots, tubers crops and forage suggested a list of relevant target traits by species. The "rice" group encapsulated traits in a functional framework provided by Eq 1. The "maize and sorghum" group focused its analysis on the adaptation strategies that quantitative genetic or functional genomic would dissect. It provided criteria to fit genetic material, traits and design to allow future comparison. The group working on wheat, barley and durum wheat proposed to separate constitutive traits from adaptive traits then to adapt the selection conditions and to consider separately the characterisation of accessions from the analysis of elite material.

The results presented below represent a partial consensus for each crop which is of the responsibility of participants of each group. It was written according the oral presentations, and was accepted in the Brisbane meeting.

### ***- Beans, roots, tubers crops and forages***

The group selects traits expected to be of value in characterizing genetic variation for drought tolerance. Landraces and accessions have been mentioned as always available for such studies with specific adaptation as for *Brachiaria* where populations with Al-resistance and low P-adaptation are available. Development of mapping populations and establishment of core-collection allow genetic studies of drought tolerance in beans, roots and tubers crop-species.

In chickpea, terminal drought appears of importance but to capture genotypic differences screening in early growth stage is useful. Root traits are considered as relevant (deeper root distribution is expected). and C discrimination has been reported as positively correlated to WUE.

In Cowpea the water extracted by the root system and the efficiency of water use determine the amount of dry matter produced and thus grain yield in case of terminal drought. The pattern of roots distribution (root branching, root density), leaf area and leaf relative water content should be measured. Grain yield and yield components are estimated. C-discrimination, stomatal conductance, Osmotic Adjustment and canopy temperature have been recommended for application in a breeding program

In Common bean, the following traits have been recommended for discrimination of genotypes resistant against terminal and intermittent drought stress : C-discrimination, earliness, deep rooting, assimilate distribution (grain filling), P-concentration in grains, non-structural carbohydrates, ash content, leaf conductance, transpiration.

In Potato, several traits have been suggested for improving yield under mid-season (tuber onset) and terminal drought. It included yield *per se* (average tuber size and tuber number) and related traits such as root pulling resistance, leaf area index, recovery from drought, stomatal conductance

In Cassava, the group lists partial stomatal closure, root distribution, ABA accumulation, OA, leaf potassium and sugars, leaf retention, root distribution, net photosynthesis and stomatal conductance as relevant traits to screen genotype under early season drought.

In case of intermittent or terminal drought in Brachiaria forage, measurement of green foliage after 4-5 month drought, root distribution, green leaf nonstructural carbohydrates and leaf ash have been suggested. Deep root system, green foliage, leaf retention, stomatal conductance have been pointed out for Forage legumes (Cratylia, Arachis, Stylosanthes) in similar drought scenarios.

Putative traits have been identified for Ground nut : specific leaf area, C-discrimination before stress onset, for Pigeonpea : osmotic adjustment, deep rooting and for Soybeans : leaf/seed ash, leaf area.

#### - **Rice**

The group proposes to measure traits at a resolution level where GxE interaction is eliminated and to insert the traits within a functional frame work namely:  $GY = T \times TE \times HI$

##### Transpiration (T)

Traits should be related to processes such as access to water (total water reservoir and availability at the right time to maintain C/N supply) and control of water losses. They could included i) early vigor estimated with embryo size, 1st leaf width or/and SLA, ii) KdF (extinction coefficient), iii) soil volume exploration namely root depth and branching; root growth rate and iv) transpiring area : leaf area, leaf growth rate.

##### Transpiration efficiency (TE)

TE has been define as an intrinsic trait by the group and could be estimated via SLA, SLN and stomatal control.

##### Harvest index (HI)

C/N supply to establish reproductive structures and fill them as been considered of great importance; sink size should be dimensioned via number of spikelets or biomass and grains number

These target traits are relevant to water use and yield components but inter-dependant and some times contradictory. Use of modelling would allow to optimize combination of desired level of traits according to drought type.

#### - **Maize-Sorghum-Pearl Millet**

The group progresses around series of questions.

##### Characters directly involved in yield or quality maintenance in a range of environments

- Flowering time—record of phenology from emergence or 3-leaf, through 10-leaf, and flowering (also number of leaves at flowering) including panicle emergence and anthesis of each entry
- Tillering habit (ear number?) of each entry
- Meristem temperature of at least one accession in each environment (location × sowing date × stress regime) in the multi-environment field trial
- Other data depending upon the trait you wish to dissect, the type of experiment (multi-location full-season field trial vs more controlled and limited)

- Grain and biomass yield (in at least some studies) of each entry, provided that earlier measurements are non-destructive; it is desirable to close an experiment with collection of grain and biomass yield (and their components) for each genotype to provide a reference point for the trial. Plant height and ear height and stem diameter at a fixed point yield could be use as surrogates for biomass; ear number per plant and ear length as for grain yield.

Grouping by phenology and yield architecture based on an initial evaluation of a large set—  
based on flowering time, plant height within flowering time, and then tillering habit within flowering time × plant height subgroup

#### Soil types as environments

*AI, OM, low P, low N, sand vs clay, moisture availability*

#### Connection to transcript/proteins analyses where and when to collect samples

- Characterize accessions in control and stress
- Choosing candidate genes for drought-related traits:
  - Choice of candidate genes dependent upon expectation of a significant phenotypic effect in a knockout mutant
  - Pathway regulation and its implications require information from gene families, information on mutants within and across species, co-localization of QTLs within and across species (Positions of candidate genes within QTLs), profiling experiment results within and across species.
  - Carbohydrate pathways—fewer members of smaller gene families
  - Water channel regulation (aquaporins)
  - Oxidative stress

#### Which genetic material for which traits

Association mapping and QTL mapping studies are complementary, but might prefer to use one or the other depending upon the fineness or coarseness of the phenotypic variation being assessed. In association studies, it is recomman to set(s) of genetic accessions having no known relationships, genetically fixed materials with no population structure.

#### **- *Wheat, Barley and Durum***

According to this group, the type of trait determines whether phenotype must be evaluated under drought stress (adaptive traits) or not (constitutive traits). Differing from elite material, accessions may include non-adapted material with low yield due to inappropriate phenology and non-agronomic plant types (low plant height and severe sink limitation). Thus only only secondary traits should be considered. They are listed here:

#### Constitutive traits

##### ***Accessions:***

- Long coleoptile (for deep sowing, crop residues etc.)
- Early vigor (GC: leaf-width, tillering; NDVI)
- Delta (early leaves)
- Xylem diameter
- Pale leaves (photo-protection)
- Pubescence (photo-protection)

##### ***Elite lines:***

- Yield potential

Harvest index  
Phasic development pattern

Adaptive traits

**Accessions:** (best measured prior to grainfill to avoid sink limitation)

Biomass at flowering  
Stem carbohydrates (assume flowering)  
Peduncle length  
Solid stems  
Water extraction profile by roots  
CT (during peak stress period); CID-flag leaf  
Root pulling strength  
OA  
ABA, phaseic acid  
Wax (photo-protection)  
Anti-oxidants

**Elite materials**

CT & CID (at any stage)  
Yield  
Biomass  
HI (determined by grain filling characteristics)

But the group points out traits for which there is ambivalence: leaf rolling, anti-oxidants (which of many), ABA, OA (not by anyone here though!), CT (in windy & cloudy environments) and root pulling. In any case, the group recommends to use normal field screening environment where feasible and that it is preferable to use drip irrigation for precision and timing. Certain traits, e.g. OA, may require measurements to be made at a common plant water status ( $\emptyset$ leaf or RLWC); therefore pot studies enable easier control of water status.

