Durum wheat adaptation and sustainability: ensuring accurate phenotyping for improving drought tolerance and yield stability

Monneveux P.

in

Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110
2014
pages 244-278

Article available on line / Article disponible en ligne à l’adresse :

http://om.ciheam.org/article.php?IDPDF=00007078

To cite this article / Pour citer cet article


http://www.ciheam.org/
http://om.ciheam.org/
Durum wheat adaptation and sustainability: ensuring accurate phenotyping for improving drought tolerance and yield stability

Philippe Monneveux
International Potato Center, Lima, Peru

Abstract. In most of its area of cultivation, durum wheat is facing water shortage. Climate change is expected to produce more frequent drought events. As a consequence, durum wheat breeders are now considering drought tolerance as an essential breeding objective. However, phenotyping still represents a major bottleneck in selecting for abiotic tolerance traits. Efficient phenotyping implies accurate i) definition of target populations of environments based on the performance of known varieties, ii) choice and characterization of the managed stress environment, iii) stress monitoring and iv) “secondary” (drought tolerance related) traits measurement. Improving drought phenotyping in durum wheat should take advantage of the new technologies developed to refine target populations of environments definition, precisely describe managed stress environments and efficiently monitor drought stress. This will permit establishment of a precise typology of target populations of environments based on drought scenarios, better predict adaptation of the tested germplasm, and finally increase response to selection. The utilization of geographic information system (GIS) tools and more integrative drought tolerance related traits assessment methods should be encouraged. The development of research networks among different partners and establishment of phenotyping platforms in the main durum wheat cultivation areas could simulate sharing of knowledge and experience and quicker evaluation of germplasm in diverse environments and facilitate dissemination and germplasm products, thus ensuring larger impact of breeding efforts.


Adaptation et durabilité du blé dur : assurer un phénotypage précis pour améliorer la tolérance à la sécheresse et la stabilité du rendement


Durum wheat is cultivated on 17 million ha worldwide and represent around 8% of the total wheat area and 6% of the wheat production (Belaid, 2000). It is mainly grown in West Africa (4.5 million ha) and North Africa (3.3 million ha). In Europe, where the crop covers around 2.5 million ha, durum wheat is cultivated in the southern part of the continent (Italy, France, Spain and Greece). In North America (2.9 million ha), it is mainly found in the Saskatchewan province in Canada, in North Dakota, Montana, Minnesota, South Dakota and California States in the USA, and in the States of Sonora, Baja California, Sinaloa, and Baja California Sur in Mexico. In South America, its cultivation is limited to the central part of Chile and the southern part of the Buenos-Aires Province in Argentina. Durum wheat cultivation is also significant in Australia (New South Wales and Queensland), Russia and India. In most of these regions, environmental stresses as well as pests and diseases drastically limit crop production and reduce the commercial and utilization value of the grain (Morancho, 2000). Climate change is expected to increase the effects of these constraints and to move durum wheat cultivation toward higher latitude areas where it will experience unfamiliar pests, diseases, weeds, and soil constraints.

The socio-economic impact of environmental stresses on yield and quality is of particular importance in the marginal areas of West Asia and North Africa (WANA). In this region durum wheat, an important component of cropping systems, is a main staple food crop that is critical to food security, and income generation for resources-limited farmers. It is mainly grown under rainfed conditions (Nachit and Ouassou, 1988) and rainfall explains 75% of its yield variation (Blum and Pnuel, 1990). Finally, these regions are also predicted to face the most dramatic and negative changes in climate predicted for any part of the world, particularly more frequent droughts, increased evapo-transpiration, and changes in rainfall patterns (Thomas, 2008). Crop yields are expected to decrease by as much as 10–30% by the 2080 if no efforts are made to mitigate climate change effects (IPCC, 2001).

In many countries of the WANA region, farmers still traditionally grow durum landraces that are well adapted to severe moisture stress conditions but give a poor yield in more rainy years relative to modern cultivars. Those landraces still cover more than 20% of the area (Heisey et al., 2002). Over several decades, breeders have attempted to produce wheat cultivars adapted to these semi-arid environments with limited success in earlier years. Breeding work for drought-prone environments was largely empirical, with grain yield being the primary trait for selection. Then, with the use of indirect selection, modern cultivars have been developed that yield the same as the traditional cultivars in dry years while showing a better response to more favourable conditions of moisture and nutrient supply (Osmanzai et al., 1987). Due to their improved yield stability, these modern cultivars are increasingly grown in dry regions, with rates of adoption approaching those attained in irrigated and high rainfall areas (Heisey et al., 2002).

Further progress in developing drought tolerant germplasm depends on the efficiency of breeding methodologies. Despite the huge amount of information provided by molecular biology in the past few decades, the application of these techniques in the development of improved germplasm has been quite disappointing, largely because the present phenotyping approaches and methods still limit our ability to capitalize on plant functional genomics and modern breeding technologies (Tuberosa, 2012). An improvement of approaches and tools and a more rigorous application of the proposed methods are required to accurately address complex traits and generate the high-quality quantitative data that are needed for genetic analysis and gene identification and transfer. This may allow information from molecular experiments to be more efficiently translated into plant performance in farmers’ fields.
II – Phenotyping, the main bottleneck in breeding for abiotic stress tolerance

Plant phenotyping (from the Greek phainein, to show) is the comprehensive assessment of plant complex traits such as growth, development, tolerance, resistance, architecture, yield, and the basic measurement of individual quantitative parameters that form the basis for the more complex traits. Plant phenotyping has been performed by farmers, since humans started to select plants, to increase yield or enhance other desirable traits, and during the last century by breeders. It was at that time mostly based on experience and intuition. Over the last two decades, some progress has been done in the development of more reproducible measurements reducing the individual subjectivity factor of the phenotyping person. However, the basic attributes of a good phenotyping approach are not just the accuracy and precision of measurements, but also the relevancy of experimental conditions. Efficient phenotyping implies accurate i) definition of target population of environments, ii) characterization of the testing environment or managed stress environments (MSE), iii) stress monitoring and characterization and iv) measurement of secondary traits.

III – Identification of target populations of environments

Any variety is adapted to several environments. Fischer et al. (2003) refer to this group of environments as the target population of environments (TPE). Deploying different cultivars for different TPEs is the only way to reduce genotype by environment interactions. A TPE, also called yield stability target by Annicchiarico (2002), can be defined as the set of all environments in which an improved variety is expected to perform well. An important objective for breeders is to clearly define the TPE for which each variety is developed. The environments constituting a TPE must be sufficiently similar for one genotype to perform well in all of them.

There are several complementary ways to define the TPE. A first step is the definition of mega-environments, based on information about environmental constraints, mainly derived from breeder’s experience (Rajaram et al., 1995). The provided information can be refined through an analysis of the performance of known varieties and the genotype by environment interaction (Nachit et al., 1992). More recently, new tools provided by spatial analysis can also help defining TPE and target genotypes (Hyman et al., 2013).

1. Definition of mega-environments

The definition of mega-environments is mainly based on spatial information (mainly provided by breeder’s experience) about environmental constraints (including water availability) at the ecosystem or sub-ecosystem level. A total of 12 wheat mega-environments have been defined by Rajaram et al. (1995) (Table 1). These mega-environments are broad, often non-contiguous or trans-continental areas with similar biotic or abiotic stresses and cropping systems (Braun et al., 1996).

Durum wheat is mainly cultivated in Mediterranean-type climates (i.e. the Mediterranean Basin which represent 60% of the total area under Mediterranean climate, Central Chile, Western and Southern Coast of Australia and California). Durum wheat growth and yield are limited, in these environments, by low temperatures shortly after the crop establishment and water deficit often associated with high temperatures during the reproductive phase of the growth cycle. This situation corresponds in Rajaram’s classification of mega-environments to ME4A (winter rain or Mediterranean-type drought mega-environment) which cover half of the total durum wheat cultivated area and in which durum wheat is more cultivated than bread wheat (Table 2). Durum wheat is also cultivated in ME1 (Nile Valley, Egypt and Yaqui Valley, Mexico), ME2A (Ethiopia), ME4B (southern Cone of Latin America), ME4C (India), ME11 (Russia) and ME12 (Turkey). The
high yields obtained in ME1 and ME11, compared to other MEs highlight the impact of water limitations on grain yield (Heisey et al., 2002).

Table 1. Wheat mega-environments with their main features (according to Rajaram et al., 1995).

<table>
<thead>
<tr>
<th>ME\sub ME</th>
<th>Moisture regime</th>
<th>Temperature</th>
<th>Wheat type</th>
<th>Area (%)</th>
<th>Production (Ml t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME1 Irrigated</td>
<td>Irrigated</td>
<td>Temperate</td>
<td>Spring</td>
<td>36.1</td>
<td>83</td>
</tr>
<tr>
<td>ME2 High Rainfall (&gt;500 mm)</td>
<td>High Rainfall (&gt;500 mm)</td>
<td>Temperate</td>
<td>Spring</td>
<td>8.5</td>
<td>25</td>
</tr>
<tr>
<td>ME3 Acid Soil</td>
<td>Acid Soil</td>
<td>Temperate</td>
<td>Spring</td>
<td>1.9</td>
<td>3</td>
</tr>
<tr>
<td>ME4 Low Rainfall (&lt;500 mm)</td>
<td>Low Rainfall (&lt;500 mm)</td>
<td>Temperate/hot</td>
<td>Spring</td>
<td>14.6</td>
<td>20</td>
</tr>
<tr>
<td>ME4A Winter rain or Mediter.-type drought</td>
<td>Winter rain or Mediter.-type drought</td>
<td>Hot</td>
<td>Spring</td>
<td>7.1</td>
<td>12</td>
</tr>
<tr>
<td>ME4B Winter drought or Southern Cone-type rainfall</td>
<td>Winter drought or Southern Cone-type rainfall</td>
<td>Temperate</td>
<td>Spring</td>
<td>6.2</td>
<td>13</td>
</tr>
<tr>
<td>ME5 Tropical</td>
<td>Tropical</td>
<td>Hot</td>
<td>Spring</td>
<td>7.1</td>
<td>12</td>
</tr>
<tr>
<td>ME5A Low-humidity tropics</td>
<td>Low-humidity tropics</td>
<td>Hot</td>
<td>Spring</td>
<td>7.1</td>
<td>12</td>
</tr>
<tr>
<td>ME5B High humidity tropics</td>
<td>High humidity tropics</td>
<td>Hot</td>
<td>Spring</td>
<td>7.1</td>
<td>12</td>
</tr>
<tr>
<td>ME6 Semi-arid</td>
<td>Semi-arid</td>
<td>Temperate</td>
<td>Spring</td>
<td>6.2</td>
<td>13</td>
</tr>
<tr>
<td>ME6A High rainfall</td>
<td>High rainfall</td>
<td>Temperate</td>
<td>Spring</td>
<td>6.2</td>
<td>13</td>
</tr>
<tr>
<td>ME6B Semi-arid</td>
<td>Semi-arid</td>
<td>Temperate</td>
<td>Spring</td>
<td>6.2</td>
<td>13</td>
</tr>
<tr>
<td>ME7 Irrigated</td>
<td>Irrigated</td>
<td>Cool</td>
<td>Facult.</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td>ME8 High Rainfall</td>
<td>High Rainfall</td>
<td>Cool</td>
<td>Facult.</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td>ME9 Semi-arid</td>
<td>Semi-arid</td>
<td>Cool</td>
<td>Facult.</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td>ME10 Irrigated</td>
<td>Irrigated</td>
<td>Cold</td>
<td>Winter</td>
<td>15.0</td>
<td>30</td>
</tr>
<tr>
<td>ME11 High Rainfall</td>
<td>High Rainfall</td>
<td>Cold</td>
<td>Winter</td>
<td>15.0</td>
<td>30</td>
</tr>
<tr>
<td>ME12 Semi-arid</td>
<td>Semi-arid</td>
<td>Cold</td>
<td>Winter</td>
<td>15.0</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Wheat mega-environments in which durum wheat is significantly cultivated (from Heisey et al., 2002).

<table>
<thead>
<tr>
<th>Mega-environment</th>
<th>Area (million ha)</th>
<th>Percentage of the total durum wheat cultivated area</th>
<th>Percentage of the total wheat area</th>
<th>Average durum wheat yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME1</td>
<td>0.6</td>
<td>7</td>
<td>1.6</td>
<td>4.15</td>
</tr>
<tr>
<td>ME2A</td>
<td>2.1</td>
<td>26</td>
<td>29.6</td>
<td>1.99</td>
</tr>
<tr>
<td>ME4A</td>
<td>4.0</td>
<td>50</td>
<td>67.8</td>
<td>1.19</td>
</tr>
<tr>
<td>ME4B</td>
<td>0.1</td>
<td>1</td>
<td>3.1</td>
<td>2.06</td>
</tr>
<tr>
<td>ME4C</td>
<td>0.1</td>
<td>1</td>
<td>1.5</td>
<td>0.97</td>
</tr>
<tr>
<td>ME11</td>
<td>0.1</td>
<td>1</td>
<td>2.8</td>
<td>4.80</td>
</tr>
<tr>
<td>ME12</td>
<td>1.1</td>
<td>14</td>
<td>19.3</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Within the Mediterranean region, Nachit (1998) identified three main agro-ecological zones (continental areas with low winter temperatures, temperate areas with mild winters and high altitude areas with severe cold winters). Similarly, Eser (1998) defined three environments for durum wheat cultivation in Turkey, the spring zone, the central plateau and transitional zone (winter and facultative wheat), and the southeast (spring and facultative).

However, the mega-environments and agro-ecological zones do not always offer a sufficient level of resolution in the definition of TPEs. This is particularly true for the Mediterranean region where rainfall and temperatures markedly differ due to differences in topography, nearness of regions with temperate or arid climates and maritime and continental influences (Ryan et al., 2006). Genotype by environment interaction analysis and spatial analysis are useful tools to refine the TPE definition.
2. Use of genotype by environment interaction analysis

A. Implementation of multi-local trials

An important objective, by implementing multi-local trials and analyzing genotype by environment interaction (GEI) is, besides describing the behaviour of genotypes across different environments, to define groups of locations that share the same best cultivar(s), i.e., that show little or no crossover (Yan and Rajcan, 2002). As there is a large non-predictable component of GEI associated with year-to-year variation, particularly in the Mediterranean climate considered as the most variable of the world (Ward et al., 1999) and characterized by a high fluctuation of precipitations (Keatinge et al., 1986), it is sometimes difficult to define consistent patterns for the grouping on the basis of locations (Cooper et al., 1999). Substantial datasets are consequently required to accurately estimate frequencies of environmental types based on variable water conditions.

If the TPE is too narrowly defined, few trials will be conducted within each TPE and least significant difference values will be very large, preventing accurate evaluations and reducing progress from selection. The TPE might include three to five evaluation sites. Evaluation of the GEI helps to decide on the number of TPEs for the breeding program. In rain-fed environments, GEI may be large and a high number of TPEs, each served by different varieties, may be optimal. Since each new TPE will need additional breeding and testing resources, there is however a practical limit to the number of TPEs used in a breeding program. Moreover, in some TPEs, the size of the target area can be insufficient to justify the resources required for a separate effort, and the breeders should rely on the spill-over of a variety from another TPE. A compromise should be consequently searched between precisely defining the TPE and achieving enough replication within it. The biplot analysis and the AMMI (additive main effects and multiplicative interaction) and GGE (genotype main effects and genotype × environment interaction effects) models are the most commonly used for clustering location and defining TPEs (Yan et al., 2007). Table 3 provides a list of attempts to define TPEs for durum wheat in the Mediterranean region.

B. Analysis of historical data

Most breeding programs routinely collect data from multi-environment trials (METs). From the 1960s to the 1980s, the Centers of the Consultative Group on International Agricultural Research (CGIAR) produced great networks of testing sites all over the world, particularly for wheat (e.g., Peterson and Pfeiffer, 1989). Many of the results are archived, and the analysis of these historical sets of data can contribute defining TPEs, by allowing clustering of environments, based on the correlation of variety means across trials. This method of grouping environments in the TPE should only be used if data from trials containing 20 or more varieties are available over several years.

3. Use of spatial analysis

Several advances over the last few decades have improved the capacity of spatial analysis to contribute to phenotyping and GEI analysis (Hyman et al., 2013). Advances in the development of computer hardware and software have permitted types of analysis that were impossible to carry out before and availability of climate data in digital formats has been key resource for spatial analysis in agriculture. These advances have led to the development of more precise agro-ecological zoning maps as the agro-climatic map developed for the Mediterranean region by UNESCO (1979) which includes 37 different zones (Ryan et al., 2006). They also allowed sophisticated statistical analysis of GEI (Crossa et al., 2004), improving our understanding of spatial and temporal aspects of the interactions (Löffler et al., 2005).

The grouping of trial sites provided by the GEI analysis does not tell us ultimately where genotypes can perform well because the sites only represent a limited number of point locations. By using soil and climate information on the trial sites it is possible to classify these point locations into more or less homogenous environment types (DeLacy et al., 1994; Roozeboom et al., 2008).
Linking individual trial sites to larger regions for which they are representative is very useful for develop maps of TPEs and, ultimately, for introducing varieties into environments where they are expected to perform well (Gauch and Zobel, 1997). In the case of durum wheat, spatial analysis combined with GEI has been for example used by Annicchiarico et al. (2002) to define durum wheat TPEs in Algeria and recommend cultivars for specific locations.

Table 3. Examples of contribution to the definition of durum wheat TPEs through GEI analysis in the Mediterranean region (the clusters and sub-clusters defined as a results of the analysis can be considered as TPEs).

<table>
<thead>
<tr>
<th>Region</th>
<th>Design</th>
<th>Type of analysis</th>
<th>Clusters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean area</td>
<td>CIMMYT Elite Durum Wheat Yield Trial, 32 locations, 5 years</td>
<td>Pattern analysis</td>
<td>Two main clusters and six subclusters</td>
<td>Abdalla et al. (1996)</td>
</tr>
<tr>
<td>Algeria</td>
<td>24 genotypes, 18 locations, 2 years</td>
<td>Pattern analysis and AMMI</td>
<td>Two major clusters</td>
<td>Annicchiarico (2002)</td>
</tr>
<tr>
<td>Ethiopia, Bale Highlands</td>
<td>16 genotypes, 7 locations, 2 years</td>
<td>GGE</td>
<td>Two clusters: Selka, Gasser, Sinana, Sinja and Adaba, Robe, Agarfa.</td>
<td>Letta et al. (2008)</td>
</tr>
<tr>
<td>Iran</td>
<td>20 genotypes, 4 locations, 3 years</td>
<td>GGE</td>
<td>Two clusters: cold (Maragheh, Shirvan and Kermanzah) and warm (Ilam) environments</td>
<td>Mohammadi et al. (2009)</td>
</tr>
<tr>
<td>Italy</td>
<td>65 genotypes, 3 locations, 4 years</td>
<td>AMMI</td>
<td>3 clusters, one comprising locations from South Italy and Sicily</td>
<td>De Vita et al. (2010)</td>
</tr>
<tr>
<td>Iran</td>
<td>20 genotypes, 19 locations, 3 years</td>
<td>Pattern analysis and AMMI</td>
<td>Three clusters: cold (Maragheh, Shirvan), mild (Kermanzah) and warm (Ilam) environments</td>
<td>Mohammadi et al. (2011)</td>
</tr>
<tr>
<td>Morocco</td>
<td>23 genotypes, 6 sites, 4 years</td>
<td>AMMI</td>
<td>Two clusters: Deroua, Marchouch; and Tassaout, Jemaat –Shaim, Khmis-Zemama, Sidi-El-Aydi, mainly based on temperatures</td>
<td>Nsarellah et al. (2011)</td>
</tr>
<tr>
<td>South Portugal</td>
<td>9 genotypes, 11 locations, 2 years</td>
<td>AMMI</td>
<td>A small cluster (Elva) and a larger cluster with the remaining ten environments</td>
<td>Rodrigues et al. (2011)</td>
</tr>
<tr>
<td>Algeria</td>
<td>12 genotypes, 5 locations, 1 year</td>
<td>AMMI and GGE</td>
<td>No clustering among the five locations (Harrouch, Khroub, Setif, Sidi Bel Abbes and Saïda)</td>
<td>Nouar et al. (2012)</td>
</tr>
<tr>
<td>Iran</td>
<td>20 genotypes, 5 locations, 3 years</td>
<td>GGE</td>
<td>Three clusters: (1) Moghan, Gorgan, Sabaghnia et al. (2) Gachsaran and (3) Ilam</td>
<td>(2012)</td>
</tr>
</tbody>
</table>

IV – Choice and characterization of managed stress environments

1. Choice of managed stress environments

The major concerns in germplasm evaluation are: i) the choice and further characterization of the sites where to test the genetic material and ii) the capacity of this evaluation to predict the performance of genotypes in the range of target environments under which the released varieties will be grown.

The choice of the specific experimental sites for drought tolerance phenotyping studies should take into account their representativeness with regard to economic and social factors, information on agriculture, cropping systems, and edaphic and climatic conditions (based on historical
weather data and soil features including hydrology, physical properties, soil moisture retention
curves and chemical properties (Gomide et al., 2011).

In the past, plant breeders in rainfed systems have been quite reluctant to select under drought
stress and preferred to screen for traits such as height, maturity, plant type, pest tolerance, and
grain quality under optimal conditions on research stations. They evaluated under the stress
conditions of farmers’ fields only at the advanced testing stage, when relatively few genotypes
remained. The result was often a variety performing well under well-watered conditions but poorly
under stress. Growing evidence indicates that varieties developed for improved yield under drought
stress may respond to well-watered conditions if there is an early selection in both environments
and if the choice of stressed environments effectively takes into account the previously described
TPEs. Once the TPEs have been defined, a breeding strategy can then be developed for each
one, based on the adaptation to the prevalent water supply and type of drought.

The choice and monitoring of the managed stress environments (MSE) directly determine the
potential genetic gains in the TPE. Ideally, the MSE should mimic the TPE for water distribution,
profiles, potential evapo-transpiration rates, and physical and chemical soil properties. Any
deviations may result in significant GEI between TPEs and MSEs, and genetic gains achieved
in the MSE may not be expressed in the TPE. Geographic information system (GIS) tools can
help considerably in describing the relationships between TPEs and MSEs through establishing
homology maps that show the degree of similarity between any set of stations or a continuous
surface through spatial interpolation of climate data (Hyman et al., 2013).

2. Characterization of managed stress environments

   A. Documentation of climate and soil characteristics

For planning a drought phenotyping experiment, information is required on weather conditions
(rainfall events and evapotranspiration levels) occurring during the experiment and those that
can be expected during specific periods of the growing season, based on long-term climatic data.
Actual environmental climatic characterization and recording are essential to quantify evapo-
transpiration and crop water requirements, in order to control different water regime treatments
and crop water stress levels. Their comparison with long-term average data is also important to
know to which extent weather data of the year are representative of the climate of the location.
The main atmospheric parameters which must be registered close to the vegetation surface are
air temperature, global solar radiation, air relative humidity (RH), wind speed, air water vapor
pressure deficit (VPD) and precipitation. Acquisition of weather data should be done by means of
an automatic or a standard weather station.

The atmospheric evaporative demand (ETo) is the main factor that drives the water consumption
of the crop and its knowledge is essential to an accurate environment characterization. ETo
can be calculated according to FAO standards (Allen et al., 1998) using the ETo calculator,
missing climatic data from temperature data or from specific climatic conditions. Maximum and
minimum air temperature data are the minimum dataset, but estimations become more precise if
data on air humidity, radiation and wind speed are available.

Some tools have been developed to generate historical information, like the software package
RAINBOW, http://www.iupware.be. (Raes et al., 2006b) that estimates the magnitude of events
nr/climpag/locclim/locclim_en.asp. (FAO, 2005) is a useful tool for choosing suitable experimental
locations (i.e., targeting) and planning experiments. New_LocClim permits an estimate of average
climatic conditions in locations where no observations are available, using climatic data of almost
30,000 meteorological stations worldwide from the FAO and after interpolation, create climatic
maps and graphs of annual cycles of the climate by month and extract numerical data in various formats for further processing.

Soil characterization of potential sites for drought is important as differences in soil depth and water holding capacity can affect the imposition of stress. Soil depth affects rooting volume and consequently nutrient and water availability. Compaction, aluminum toxicity and soil acidity will also reduce root depth. Soil texture is a major determinant of water holding capacity and water release characteristics (Gomide et al., 2011).

As far as the aim is to develop varieties with adaptation to water constraints, it is important to know more about the patterns of water supply and the type of drought faced by the MSE. Water balance models are highly valuable tools to characterize environments based on predicted water availability. Physiologically based crop growth models or mechanistic models like STICS (Brisson et al., 2003), CropSyst (Stöckle et al., 2003) and DSSAT (Jones et al., 2003) have been developed that give a good understanding of the exact influence of environmental characteristics and plant properties on crop development. However, they are sometimes difficult to apply in field situations, due to the relatively large amount of inputs required. Functional or engineering models like BUDGET, AQUASTAT and UPFLOW (Raes et al., 2006b) are more problem-oriented, with more empirically derived functional relationships (Hoogenboom, 2003). BUDGET, http://www.iupware.be. (Raes et al., 2006a) is suitable for assessing crop water stress under rain-fed conditions throughout the season, estimating yield response to water and designing irrigation schedules.

B. Spatial homogeneity

Uniformity represents an essential criterion in the selection of suitable phenotyping sites and any fields with significant heterogeneity must be eliminated as a potential phenotyping site to avoid introducing unwanted experimental error. Without a homogenous phenotyping site, the value of data acquired, regardless of cost and time, is limited (Masuka et al., 2012). Spatial variability affects the detection of treatment differences by inflating the estimated experimental error variance. Moreover, the effects of soil heterogeneity become more apparent under drought (Gomide et al., 2011).

Spatial variability depends on the soil formation process and on complex interactions among natural environmental factors and human activities (Webster, 2000). As variability may be in the range of one meter or less (Solie et al., 2001), the level of resolution of regional soil maps is not sufficient for the objectives of a precise experimental site. In addition, some important agronomic characteristics, such as soil compaction and soil water availability, are not usually displayed in regional soil maps. The past use and management of experimental fields are not always carefully registered and their effects generally not well identified. As a consequence of this, additional information on soil variability should be searched through soil analysis and mapping.

Direct assessment of soil variability within a field site for key soil physical and chemical properties can be made through destructive soil sampling at 30 cm depth intervals (to a depth of 90 or 120 cm soil depth). The location of soil samples could be positioned by GPS to allow the test results to be mapped to the exact location (Campos et al., 2011). Soil samples should be analyzed at a minimum for texture, pH, macro and micro-nutrients. High-throughput techniques are now available for mapping variability within field sites based on penetrometers (Cairns et al., 2011), soil electrical conductivity sensors (Cairns et al., 2012), spectral reflectance (Rossel et al., 2006; Dang et al., 2011) and thermal imagery of plant canopies (Campos et al., 2011).

Knowledge of soil variability can be used to ensure planting within areas of the least spatial variability to further reduce unwanted experimental error (Cairns et al., 2009). This decision, together with the use of adapted trial designs (Federer and Crossa, 2011) is essential to reduce experimental error.
V – Stress monitoring

The ability to manage drought episodes (timing, frequency and intensity) of drought episodes and characterize (soil, plant measurements) is a key factor in mimicking the environmental conditions prevailing in the TPE and ensuring accurate drought phenotyping (Tuberosa, 2012).

1. Stress application and control

   A. Out-of-season testing

   An increasing number of breeding programs are conducting field trials in dry locations or “out-of-season”, i.e., in seasons that are not the cropping season of the crop but are characterized by very low rainfall. Under such conditions the dynamics and intensity of drought episodes can be tightly controlled through the frequency and volume of irrigation treatments. Trials in dry sites also offer the advantage of a lower incidence of noise factors which can bias the evaluation. The option of field testing in dry areas or during dry seasons is however not always available or possible. The dry season should be sufficiently long to cover the whole growth cycle and photoperiod. Furthermore, conditions during the dry season are harsh for plants and generally do not reflect the environmental conditions plants will experience during a natural drought in the main (wet) season, temperatures and vapor pressure deficit (VPD) being generally higher (Jagadish et al., 2011). These differences lead to genotype-by-season interactions and do not allow results obtained from the out-of-season experiments to be easily extrapolated to the growing season conditions.

   B. Water application

   Different traits will confer adaption to different types of drought stress, thus drought experiments should aim to impose a similar water stress (in terms of timing, frequency and intensity) as experienced in the TPE. For example, tolerance to drought stress before anthesis in wheat does not necessarily confer tolerance to drought stress after anthesis (Monneveux et al., 2005). To ensure that drought is imposed at the correct phonological stage, irrigation should be withheld prior to this stage. A crop water balance should be used to determine the last date of irrigation to ensure plants experience drought stress at the target stage.

   As there is generally a substantial variation in phenology across genotypes and drought stress is imposed at the same time across all genotypes within an experiment, genotypes with different phenologies are expected to face different stress duration. The presence of large differences in flowering time among genotypes bias the interpretation of the influence of drought-adaptive traits on yield. To overcome that difficulty, genotypes can be grouped into subsets of similar maturity and planted at different times to ensure phenological synchronization across genotypes at the crucial stage when drought stress is imposed. A preliminary study can be used to determine the phenology of genotypes prior to drought experiments. Another option is to use the information on phenology as a covariate adjustment. Finally, irrigation systems must be carefully chosen to ensure optimum control of the irrigation water. Drip irrigation is recommended to allow plot level control of irrigation.

   C. Rainout shelters

   Static or moveable rainout shelters represent another alternative of investigating the adaptive response of crops to a desired level of drought stress, avoiding the bias of unpredictable rainfall patterns. Major inconvenient to the use of rainout shelters are (in addition to the high construction and operating costs), the usually rather limited area protected by a shelter which, in turn, limits the number and size of experimental plots that can be tested.
**D. Controlled environments**

As the environment where selection and testing work are done is often variable in terms of rainfall, breeders are searching for more reliable phenotyping protocols that can accelerate progress. This can be made by controlling the environment and phenotyping in greenhouses or growth chambers, with increasingly sophisticated systems (eg, high-throughput screening based on robotized systems and advanced image analysis software). Greenhouse research increases the speed at which large numbers of plants can be phenotyped in a reproducible and precise manner. It also allows control of other environmental influences on phenotype expression that could confound data interpretation. Carefully controlled environments (such as pots, soil-filled pipes and hydroponics) are generally favored by molecular-oriented researchers because unwanted environmental variation can be minimized. However, by choosing to work in highly controlled environment, breeders should be aware that controlled conditions tend to be very different to those prevailing in the target population of environments (TPE) and may limit the application of results in germplasm development. In particular, irrigation in pots creates a situation that is very different from that occurring under field conditions (Passiourea, 2005). Significant differences in transpiration response were noted by Wahbi and Sinclair (2005) between plants grown in a potting mixture and in field conditions, plants in pots being exposed to stress earlier in the drying cycle and with a more rapid depletion of moisture. An additional factor to be also considered is the more uniform pore distribution existing in potting mixtures, compared to natural soils, which can lead to hypoxia (Passiourea, 2005). Finally, the temperature of the substrate used to fill pots or containers used in greenhouse experiments can be different from field soil temperature (Passiourea, 2005).

**2. Stress characterization**

Drought covers different ranges of intensity and timing. These differences cause differential responses of the genotypes under consideration. Therefore, the intensity timing and timing of drought in the phenotyping experiment should be very well controlled and in areas where drought severity fluctuates widely, phenotyping should preferably be carried out under well-watered conditions and at different levels of drought stress (e.g., intermediate and severe). A sound interpretation of the results of an experiment conducted under conditions of water shortage requires an accurate characterization and monitoring of the water status of both soil and plant. In a review of molecular papers focusing on the effects of drought on gene expression or transgenes under drought stress, Jones (2007) highlighted that over half of the published papers had no measure of plant or soil water status. Measuring soil and plant water status also permits to optimize irrigation scheduling and crop management and allows the repetition of the experiment under comparable conditions. Soil or plant water status can be monitored by measuring the amount of water or its energy status (Kirkham, 2004).

At the plant level, emphasis has traditionally been devoted to water potential (Blum, 2009). The relative water content of the leaf also provides important information on the water status of the plant (Riga and Vartanian, 1999), offering the advantage of collecting a high number of samples in a short time. Both leaf water potential and relative water content provide an integrated measurement of the interaction among the factors involved in maintaining the flow of water through the plant. As components of leaf water relations change during the day as irradiance and temperatures vary, the change is small for about two hours at and after solar noon. Therefore, this is an appropriate time window for investigating leaf water relations in a large number of genotypes.

Different methods are available to measure the amount of water stored in the soil. The gravimetric method (i.e., weighing samples of soil columns before and after oven drying) provides an accurate but cumbersome measurement of soil moisture. Furthermore, the gravimetric method is destructive and requires dedicated plots distributed across the other experimental plots. Tools such as the neutron probe extensively used to estimate soil water status since the 1970’s (Hignett and Evett, 2008) and the capacity probe (Nagy et al., 2008) allow quicker and less labor-intensive
measurement. Several dielectric based soil water monitoring techniques have been developed, like the time-domain reflectometry (TDR), and the (single and multi-sensor) capacitance probe (CP) systems (Fares and Polyakov, 2006). These techniques greatly simplify the real-time determination of water content on a fine spatial and temporal scale. TDR techniques are of the most widely used thanks to their high precision, non-ionising radiation and low influence of soil salinity, bulk density and texture (Noborio, 2001). However, they generally not permit detailed measurement along the soil profile (Manieri et al., 2007). Because of their relatively low cost and ease of operation, CP systems have met widespread acceptance as a means of closely monitoring soil moisture by collecting high-resolution soil-water content data in the rhizosphere. More recently, two dimensional geo-electrical tomography has been used for monitoring soil-water redistribution due to water uptake (Werban et al., 2008). This technique permits to image and monitor diurnal soil-water redistribution. An additional option is provided by the use of a polymer-based tensiometer (POT) designed to measure matric potentials down to −1.6 MPa, thus allowing a better resolution of levels of local water stress and quantification of root water uptake in dry soils (van der Ploeg et al., 2008). The choice of methodology used for monitoring soil water content will depend on many factors including the cost, intensity of drought, field variability, and accuracy and precision required.

3. Reducing noise factors

Experimental conditions on the MSE should ensure target stress to be imposed without interference from additional stresses, and with minimal environmental heterogeneity to reduce experimental error. The crop facing water deficit or heat stress simultaneously experiences a number of additional stress factors (e.g., micronutrient deficiency, soil compaction, salinity, nematodes, fungal pathogens) that exacerbate the effects of studies stresses. Typical case scenarios are those involving factors that cause mechanical damage to roots (e.g., nematodes, root-worms), impair root growth (e.g., soil acidity, boron toxicity, salinity) and reduce water availability to the crop (e.g., presence of weeds) and source capacity (e.g., foliar diseases, insect damage to the canopy). When one or more of these constraints affects the experimental plots, genetic variability among the tested germplasm for resistance to these stress agents inevitably biases an accurate evaluation of the effects of the drought or heat tolerance. Important and more subtle interactions may also occur when the effects of water deficit are evaluated in the presence of other abiotic stress factors (eg, high temperatures) that enhance leaf senescence and the role of specific adaptive mechanisms, such as the relocation of stem water soluble carbohydrate. This is typically the case for durum wheat experiencing combining drought and heat stress during grain filling in Mediterranean environments.

Efforts should be made to remove all other constraints except drought, or to implement additional trials where only this constraint is applied, in order to evaluate its specific impact (eg, trial under full irrigation in heat prone areas to isolate the specific effect of high temperatures). Soil surveys may allow the identification of selection sites or fields that avoid confounding factors. In some cases, these surveys allow identifying sites where the selection pressure for these stress factors permit the selection of genotypes targeted for regions where these stresses interact with drought. They could also identify the within-site distribution of e.g., nematodes (Nicol and Ortiz-Monasterio, 2004) or zinc deficiency (Ekiz et al., 1998). These ‘noise’ factors can be partially overcome through adequate replication within and across environments.

Another solution to this problem, at least for traits other than grain yield and its components, which are best evaluated under field testing, is to collect phenotypic data from plants grown in controlled facilities (greenhouse, growth chamber, etc). This allows for an accurate control of the main environmental parameters (temperature, air humidity, light, etc...) but, as already mentioned, makes more difficult to mimic the real conditions of the target environment. Other major inconvenient is the limited volume of genetic material that can be evaluated and the high operating costs.
4. Accurate statistical designs and interpretations

It is recognized that an important part of the efficiency of modern breeding is due to the accurate phenotyping of large numbers of plots, made possible by more sophisticated and high-throughput experimental machinery (e.g., plot combines able to measure yield directly in the field), as well as the automation of tedious manual operations. The labeling of a large number of plots and samples, data collection and storage are now facilitated by the use of electronics (e.g., bar-coding) and dedicated software (e.g., spreadsheets, databases, etc). The effectiveness of field experiments and the management and interpretation of phenotypic data can be enhanced through the utilization of the most appropriate experimental designs (Federer and Crossa, 2011), to allow for better control of within-replicate variability and reduce or remove spatial trends.

VI – Traits measurement

1. General Requirements

After having used yield under drought as an exclusive breeding objective, most breeders have progressively replaced this empirical approach by a more analytical one, the so-called “indirect selection” (Jackson et al., 1996) based on the selection for “secondary traits” or plant characteristics other than grain yield that provide additional information about how the plant performs under a given environment (Laitte et al., 2003). For a secondary trait to be useful in breeding programs, it has to comply with several requirements (Edmeades et al., 1997). A secondary trait should ideally be: (i) genetically associated with grain yield under drought; (ii) genetically variable; (iii) highly heritable; (iv) easy, inexpensive and fast to observe or measure; (v) non-destructive; (vi) stable over the measurement period; and (vii) not associated with yield loss under unstressed conditions. The heritability of indirect traits itself varies according to the genetic make-up of the materials under investigation, the conditions under which the materials are investigated and the accuracy and precision of the phenotypic data. The identification of secondary traits requires analyzing their association with yield on genetic pool with wide genetic basis, a condition not always met (Annichiarico et al., 2005). The accuracy of secondary traits measurement is closely related to precision or repeatability, the degree to which further measurements show the same or similar results. For a number of traits measured with mechanical or electronic devices, accuracy and precision in measurements require calibration of the instrument prior to data collection. Finally, secondary traits can improve the selection response for stress conditions only if they avoid any confounding effects of stress timing on yield (e.g., drought and flowering dates). The set of genotypes to be evaluated may be composed accordingly, grouping the genotypes by similar earliness or using irrigation methods (e.g., drip irrigation) allowing precise water supply at the plot level.

Examining morpho-physiological traits in landraces from different origins can eventually help in the identification of traits of adaptation to specific environments and understanding of adaptation patterns. Ali Dib et al. (1992) compared the two durum landraces Haurani (from Middle-East) and Oued-Zenati (from Algeria) and found that the latter was characterized by later heading, taller stature, more developed root systems, larger and decumbent leaves, lower number of fertile tillers, longer awn, and heavier kernels, compared to the Middle-East landrace. They suggested that some of these characteristics could confer specific adaptation to stress conditions prevailing in the two regions, i.e., longer cold spells and intermittent drought in Algeria and severe terminal drought stress in the Middle-East. Moragues et al. (2006) reported that durum wheat landraces from South Mediterranean regions had larger plot stand at jointing, produced more biomass at anthesis (distributed mostly in the main stem) and were more efficient in the allocation of biomass to reproductive organs because their higher mean harvest index (HI). They suggested that these traits could have major importance in harsh Mediterranean environments.
Most of the traits currently mentioned in the literature associated with drought adaptation in durum wheat are shown in Table 4. Secondary traits can be classified according to their relationship to drought escape, pre-anthesis growth, access to water, water-use efficiency and photoprotection. In addition to these traits that may improve yield under drought, any other characteristic of socio-economic importance may obviously be considered. A good example is, for durum wheat, the case of straw production in cereal-livestock Mediterranean farming system which can be used to feed animals (Isaac and Hrimat, 1999). Traits that confer this characteristic like stem height (Annicchiarico and Pecetti, 2003) or tillering and should be consequently considered in the breeding process.

2. Traits related to drought escape

In low rainfall areas, earliness is considered as fundamental adaptive trait (Blum, 1988). In Mediterranean conditions characterized by drought developing increasingly throughout the late reproductive and grain-filling phases (ME4A mega-environment), earliness allows grain filling to take place under conditions of lower drought and high temperature stress (Loss and Siddique, 1994). Breeding for earliness of flowering is relatively simple, as major genes responsible for insensitivity to photoperiod and vernalization which allows anticipating heading are well known and relatively easily manipulated (Slafer, 1996). However, in most Mediterranean regions where cereal breeding has been carried out for decades, selection for earliness has already taken place (Siddique et al., 1989) and there may be only marginal scope for further raising yield due to selecting for even earlier flowering crops (Slafer et al., 2005). Under optimal conditions, as grain yield is often positively correlated with crop duration, selection for shorter duration may impose a substantial yield penalty (Evans, 1993). In high altitude or continental areas, a compromise is requested between the need of escaping late frosts prior to anthesis on one hand and terminal drought and heat stress on the other (Annichiarico and Pecetti, 1998; Hafsi et al., 2006).

3. Traits related to pre-anthesis growth

A. Controlled environments

Under drought prone environments, rapid ground cover through vigorous crop establishment is a highly desirable trait as it improves radiation interception by the crop at the early stages of growth (Ludlow and Muchow, 1990) and helps to shade the soil and suppress weeds that compete for water (Richards, 1987). In Mediterranean types of drought environment (ME4A) where 40% of available water may be lost by evaporation (Loss and Siddique, 1994), it also increases water use efficiency by reducing evaporation (Turner and Nicolas, 1987). Early vigor and associated larger root mass may also help to maintain a better water balance under early water stress (ME4B) if water is available deeper in the soil profile (Mian and Nafziger, 1994). Significant association has been found between biomass at the second leaf stage and final yield in durum wheat by Royo et al. (2000) and Aparicio et al. (2002). Ground cover can be estimated visually, recorded quantitatively by measuring plant dry weight, or assessed by digital image analysis (Regan et al., 1992).

Large seed and embryo size favors early vigor. In durum wheat, seed size has been showed to be strongly associated with seedling development and seedling biomass by Aparicio et al. (2002). Similar associations were reported by Amin and Brinis (2013). Akinci et al. (2008) also reported an association of seed size and emergence rate. Rapid ground cover was found to be associated to thinner and wider leaves in bread wheat (Richards, 1996) but not in durum wheat (Araus et al., 2002). In addition, a negative association between large leaves and frost tolerance has been reported in durum wheat (Pecetti et al., 1993) suggesting that this trait could be a disadvantage in continental or high altitude areas.
Another seedling trait useful to improve crop establishment under drought conditions is coleoptile length. Genotypes with a long coleoptile allow sowings at greater soil depth. This trait is particularly useful when the crop grows exclusively on stored soil moisture (ME4C), to avoid extremely hot soil surface temperatures and rapid soil drying. The association between the presence of dwarfing gene \textit{Rht1} and coleoptile length, stronger in durum wheat than in bread wheat because of dosage effect, makes the selection for long coleoptile quite difficult in durum wheat. A significant genetic variation was however observed for this trait in durum wheat by Alaei \textit{et al.} (2010).

\textbf{B. Tillering survival and recovery}

An intermediate level of potential tillering is favorable in drought prone areas (Loss and Siddique, 1994). In durum wheat, a positive association has been reported in Morocco under early-season drought between high tiller survival rate and yield (El Haid \textit{et al.}, 1998). Garcia del Moral \textit{et al.} (2003) also reported that the number of spikes per square meter predominantly influenced grain production in the warmer environments of Spain.

\textbf{C. Total biomass}

\textit{T} Final grain yield is determined in durum wheat by total biomass production and the proportion of biomass allocated to grains (Van den Boogaard \textit{et al.}, 1996). As a consequence biomass should be considered in breeding programs targeting drought prone environments. Significant correlation has been reported in durum wheat between grain yield and biomass at maturity (Waddington \textit{et al.}, 1987) and anthesis (Villegas \textit{et al.}, 2001; Royo \textit{et al.}, 2005). Under Mediterranean climate (ME4A), the magnitude of the correlation is expected to increase with drought intensity association between biomass and grain yield, since canopy photosynthesis is inhibited by post-anthesis drought and final yield depends increasingly on the re-mobilization (Blum, 1998).

Measurement of total biomass is cumbersome and destructive. Samplings reduce the final area available for determining final grain yield on small plots (Whan \textit{et al.}, 1991). The measurement of the spectra reflected by crop canopies has been largely proposed as a quick, cheap, reliable and noninvasive method for estimating plant above-ground biomass production in cereals (Aparicio \textit{et al.}, 2002; Elliot and Regan, 1993; Smith \textit{et al.}, 1993). Biomass can be estimated by measuring the spectra reflected by crop canopies in the visible (VIS, \(\lambda=400-700\) nm) and near-infrared (NIR, \(\lambda=700-1300\) nm) regions of the electromagnetic spectrum (the crop’s ability to intercept radiation and photosynthesize (Ma \textit{et al.}, 1996). Estimation is now feasible using spectro-radiometers to measure the spectra of light reflected by the canopy (Royo \textit{et al.}, 2003). Spectral reflectance information from leaves or canopies is used to build vegetation indices which are simple operations (e.g., ratios and differences) between spectral reflectance data at given wavelengths. The normalized difference vegetation index (NDVI) and simple ratio (SR) have been reported as the best traits to assess biomass (Table 5), and stages 65 and 75 of the Zadoks scale the most accurate period for measurements (Aparicio \textit{et al.}, 2002; Cabrera-Bosquet \textit{et al.}, 2011). Vegetation indices have been used to estimate biomass (Aparicio \textit{et al.}, 2002) and yield (Aparicio \textit{et al.}, 2000) of durum wheat, but phenotypic correlation coefficients found are usually weak and largely dependent on the range of variation of the tested material (Royo \textit{et al.}, 2003).

Easy-to-handle spectro-radiometers such as the GreenSeeker are now available which gives the basic spectro-radiometric index of green biomass, NDVI. As the GreenSeeker includes its own radiation source, it may be used independently of atmospheric conditions. Spectro-radiometric measurements are been quite intensively used to evaluate biomass in durum wheat. Alternative techniques such as the use of an affordable conventional digital camera may provide information about the portion of the soil occupied by green biomass, the percentage of yellow leaves, or even yield components such as the number of spikes per unit land area (Casadesús \textit{et al.}, 2007).
4. Traits related to remobilization and sink strength

A. Carbohydrates reserves

When drought stress occurs after anthesis, as it is frequently the case in Mediterranean drought environments (ME4A), photosynthesis is limited and yield depends greatly on the remobilization to the grain of pre-anthesis assimilates accumulated in leaves and stems (Álvaro et al., 2008). Post-anthesis maximum water soluble carbohydrates (WSC) content has been consequently proposed as a selection criterion to stabilize grain yield under stressful environments (Edhaie et al., 2006). In durum wheat an accumulation of WSC has been noted under water stress in vegetative tissues by Kameli and Lösel (1996).

Traits that may also contribute to remobilization during grain filling include long and thick stem internodes and peduncle, and solid stems. In studies where crosses were made between bread wheat lines contrasting in the solid-stem trait, the solid-stem progeny contained more soluble carbohydrate per unit of stem length (Ford et al., 1979). In durum wheat, Kaya et al. (2002) and Bogale et al. (2011) reported a positive association between peduncle length and yield under drought.

The capacity of a genotype to support grain filling from mobilized stem and leaf reserves can be also assessed through application of chemical desiccants as potassium iodide which inhibit stem and leaves photosynthesis (Blum, 1988). Although chemical selection seems to have successfully used to screen for remobilization of pre-anthesis reserves (Blum et al., 1991), the method is not currently used in breeding programs.

B. Spike fertility

Annicchiarico and Pecetti (1993), Simane et al. (1993), Kiliç and Yağbasanlar (2010) found that spike fertility was the component most highly correlated with yield in drought prone environments.

C. Grain filling duration

A significant positive association between grain filling rate and grain yield has been found in durum wheat (Gebeyehou et al., 1982). It is generally accepted that grain filling duration is largely affected by environmental conditions, as its heritability is medium to low (Egli, 1998).

5. Traits related to water status: Root characteristics

Root systems determine the potential volume of soil that can be explored for water and nutrients. Variation in root characteristics includes differences among wheat genotypes in the ability to establish a deep root system quickly (Siddique et al., 1990), in root length density (Mian et al., 1994), in root distribution (Ford et al., 2006), in post-anthesis root growth (Ford et al., 2006) and in the numbers of seminal roots (Robertson et al., 1979) and total roots (Box and Johnson, 1987). Manschadi et al. (2006) found a relation between the angular orientation of wheat seminal roots, root and water uptake. reported an Associations have been postulated between drought tolerance and root length density in deeper soil layers (Manske and Vlek, 2002) and rooting depth (Simane et al., 1993) and root length density (El Haid et al., 1998) appears as better candidate traits for drought tolerance in durum wheat in Mediterranean conditions.
In the practice, root patterns have been poorly studied because root trait evaluation under field conditions is tedious and impractical for large populations. Nakhforoosh et al. (2012) reported some encouraging results concerning the use of electrical capacitance to screen for root length and root surface. In order to reduce the variability observed in field studies, root screening can also be made under controlled environments using rhizotrons, pots, hydroponics, or gel-filled containers. Some attempts have also been made to follow root growth in controlled and field conditions using Nuclear Magnetic Resonance but this technique is not yet available for high throughput phenotyping. Table 6 provides a list of the main techniques that are available actually, with their main advantages and limitations.

6. Traits related to drought escape

A. Stomata conductance

Traits that are indicative of the water status of a plant, especially when measured during periods of peak stress, are useful indicators of the plant's capacity to match evaporative demand by exploring and extracting soil water. Significant correlation between stomata conductance and yield has been reported in durum wheat by Monneveux et al. (2006). Viscous-flow porometers have been developed that allow a quick assessment of stomata conductance (Richards et al., 2001). It is however difficult to accurately assess stomata conductance in a large number of plants while properly accounting for the fluctuation in the main environmental factors that affect stomata conductance during the day (wind, solar radiation, humidity, etc.).

A more integrative way of monitoring stomata conductance is based on the measurement of the natural oxygen isotope composition ($\delta^{18}O$) in leaf and grain materials (Barbour et al., 2000). Measuring $\delta^{18}O$ in plant material allows for the collection of a large number of samples, and requires very little labor in the field. Significant association was found between leaf $\delta^{18}O$, stomata conductance and grain yield in bread wheat (Barbour et al., 2000) and durum wheat (Cabrera-Bosquet et al., 2011).

B. Abscisic acid

An increase in ABA concentration is a universal response observed in plants subjected to drought (Quarrie, 1991). ABA is a fundamental component of the mechanisms allowing the plant to match water demand with water supply and optimize growth and survival in response to environmental fluctuations. ABA has been shown to affect many of the traits that influence the water balance of the plant through both dehydration avoidance and dehydration tolerance (Thompson et al., 2007). It also appears to pre-adapt plants to stress by reducing rates of cell division, reducing organ size, and increasing the rate of development. The analysis of the effects of ABA accumulation on other drought-related traits and yield showed some contradictory results (Tuberosa, 2012), thus limiting potential applications in breeding.

C. Canopy temperature depression

Among the traits relating to access to water, by far the easiest to measure in the field is canopy temperature depression (CTD) or difference in temperature between the canopy surface and the surrounding air, a quick and non-destructive method. Because a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of how much transpiration cools the leaves under a demanding environmental load. Higher transpiration means colder leaves and higher stomata conductance, both aspects favoring net photosynthesis and crop duration. A relatively lower canopy temperature in drought-stressed crops also indicates a relatively greater capacity for taking up soil moisture or for maintaining a better plant water status. The addition of CTD as a selection criterion in wheat nursery improved
considerably the identification of the highest yielding materials (van Ginkel and Ogbonnaya, 2007).

CTD is useful mainly in hot and dry environments typical of countries with a Mediterranean climate. Although canopy temperature may seem very easy to measure, in practice there are methodological problems, particularly when there is variation in the air temperature with wind or cloudiness (Araus et al., 2002; Royo et al., 2002). Screening by canopy temperature measurements under drought stress can be done only after full ground cover has been attained and before inflorescence emerges, at high vapour-pressure deficits and without the presence of wind or clouds (Royo et al., 2005).

In durum wheat, association was found between CTD and yield under stress by Royo et al. (2002) in Spain and further by Bahar et al. (2008) in Turkey, Guendouz et al. (2012) in Algeria, Karimizadeh and Mohammadi (2011), Moayedi et al. (2011) and Shefazadeh et al. (2012) in Iran.

D. Plant water status

Ability to maintain leaf hydration under drought stress is related to root growth, low residual transpiration and osmotic adjustment. Leaf rolling protects the leaf against excess of solar radiation which cannot be dissipated by transpiration, but is also an indicator of turgor loss (Nachit et al., 1992). Positive association was found between leaf rolling and yield in durum wheat in Ethiopia (Bogale et al., 2011). Low residual transpiration, the sum of cuticular transpiration and residual stomata transpiration (due to an incomplete closure of stomata) is expected to limit water loss under harsh drought conditions (Rawson and Clarke, 1988). Genotypes with low RT tend to have higher yield under drought conditions (Clarke and Romagosa, 1991). Lower residual transpiration was found by Febrero et al. (1991) in durum wheat landraces from the Middle-East, compared to landraces from North-Africa and improved cultivars.

Osmotic adjustment (OA) is the process by which plants accumulate solutes in their cells to minimize water loss and maintain cell function under drought conditions. OA has been identified as a mechanism to maintain grain yield under stressed conditions by allowing root growth and maintaining water and nutrient capture (Morgan and Condon, 1986), thereby mitigating some of the most detrimental effects of plant water deficit. A number of experiments have shown that wheat lines selected for high OA in response to the lowering of leaf water potential have higher grain yields in field experiments. However, OA is difficult to measure in large samples under field conditions. Moreover, filed conditions generate confounding effects related to genotypic differences in soil water exploration by roots. In durum wheat genetic variation in OA has been established under controlled conditions (Rekika et al., 1998).

A positive relationship was noted by El Hafid et al. (1998) between relative water content (RWC) and grain yield in durum wheat. As RWC measurement is cumbersome, plant water status can be assessed directly by reflectance (Table 5), using the water index, WI = R900/R970 (Peñuelas et al., 1993). WI has been used to detect variation in relative water content, leaf water potential and canopy temperature depression, but only when plant water stress is well developed. The ratio of WI to NDVI has also been proposed for estimating relative water content (Peñuelas et al., 1997).

E. Carbon isotope discrimination

Carbon isotope discrimination (\(\Delta^{13}\)C) measures the ratio of stable carbon isotopes (\(^{13}\)C/\(^{12}\)C) in the plant dry matter compared to the ratio in the atmosphere (Condon et al., 1990). Because of differences in leaf anatomy and mechanisms of carbon fixation between species with C\(_3\) and C\(_4\) photosynthetic pathway, studies on \(\Delta^{13}\)C have wider implications for C\(_3\) crops (Monneveux et al., 2007). \(\Delta^{13}\)C is generally negatively associated with water use efficiency over the period of dry mass accumulation (Condon et al., 2004) and positively associated to stomata conductance (Condon et al., 2002). In wheat, the relationship between \(\Delta^{13}\)C and grain yield depends on the
environmental conditions, the phenology of the crop and the plant organ (e.g., leaf or grain) from which the samples are collected (Merah et al., 2002). In durum wheat cultivated in Mediterranean environments, $\Delta^{13}C$ (particularly when measured in mature grains) is positively correlated with grain yield (Araus et al., 1998; Hafsi et al., 2001; Merah et al., 2001; Monneveux et al., 2005). One of the reasons for this positive relationship is that a genotype exhibiting higher $\Delta^{13}C$ has higher stomata conductance. The higher correlation generally observed under Mediterranean conditions with harvest index and grain yield, compared to those with biomass, suggest that higher $\Delta^{13}C$ values also indicate higher efficiency of carbon partitioning to the kernel (Merah et al., 2001). High genetic variation and heritability was reported $\Delta^{13}C$ (Merah et al., 2001). For all these characteristics, $\Delta^{13}C$ is an attractive breeding target for improving WUE and yield, while the high cost required for measuring each sample makes it an interesting candidate for marker assisted selection.

F. Ash content

Carbon isotope discrimination ($\Delta^{13}C$), despite being a very promising trait, is probably less widely accepted because of the cost of its determination. Several surrogate approaches have been proposed that are cheaper, faster and easier. The option most studied has been to use the mineral or ash content of leaves (Araus et al., 1998; Merah et al., 1999) or grains (Monneveux et al., 2005; Misra et al., 2006). A significant negative association was found in durum wheat between ash content and grain yield by Bogale and Tesfaye (2011) in Ethiopia. A promising option relies on the estimation of ash content through the near-infrared spectroscopy (NIRS) technique (Ferrio et al., 2001) which has the additional advantage to be non-destructive.

7. Traits related to water use efficiency

Measurement of carbon isotope discrimination of grain or other tissues can be used to estimate the water-use efficiency (WUE) of the crop, since their signals are based on the integration of plant water status over a period of time (Condon et al., 1993). However, these data must be interpreted with care. While in Australia, under conditions where wheat is grown on stored soil moisture, better performance of wheat cultivars indicated an advantage for high WUE genotypes (Rebetzke et al., 2002), under Mediterranean drought conditions high yield is associated with lower WUE, reflected by high $\Delta^{13}C$ values (Monneveux et al., 2005).

A. Spikes photosynthesis

Spikes photosynthesis contributes up to 40 percent of total carbon fixation under moisture stress (Evans et al., 1972) and to 10-70 percent of final grain weight (Duffus et al., 1985). Spikes have higher WUE than leaves due to the fact that they can refix respiratory carbon (Bort et al., 1996). Moreover, they are able to maintain a better water status than leaves, through a higher OA and a more xeromorphic structure (Tambussi et al., 2005). While gas exchange measurement of spikes is time consuming and difficult to standardize (Araus et al., 1993), chlorophyll fluorescence should be considered as a more rapid means of screening for spike photosynthetic capacity under stress.

B. Awn length

In durum wheat, awns contribute substantially to spike photosynthesis and longer awns are a possible selection criterion (Villegas et al., 2006).

C. Harvest index

Genes that increase partitioning of assimilates to the sink, resulting in a higher harvest index (HI), would be expected to improve yield under drought. They however often affect root development and access to soil water. As a consequence, a compromise should be found, depending on
environmental conditions (input level, occurrence of constraints) and particularly on drought stress intensity.

**D. Senescence**

Changes in leaf color can reflect a variation in partitioning of assimilates to the sink. Stress accelerates the senescence of leaves. Delayed senescence of leaves has been proposed as a secondary trait for performance under drought by Rharrabti et al. (2001). However, the relationship between delayed senescence and yield has been found by other authors to be unstable and highly dependent on drought intensity (Hafsi et al., 2006; Guendouz and Maamari, 2011). According to Blum (1998), the stay-green trait may indicate the presence of drought avoidance mechanisms and contribute to yield per se if there is no water left in the soil profile by the end of the cycle to support leaf gas exchange, but may be detrimental if it indicates lack of ability to remobilize stem reserves. To check for delayed senescence of leaves, particularly flag leaves, portable chlorophyll meters such as the Minolta SPAD are extensively used, due to their speed and ease of use. Image analysis techniques are more precise but less time-effective (Hafsi et al., 2000).

8. Traits relating to photo-protection

Decreased stomata conductance in response to drought leads to warmer leaf temperatures and insufficient \( \text{CO}_2 \) to dissipate incident radiation, both of which increase the accumulation of harmful oxygen radicals and photo-inhibitory damage. Photo-inhibition can be mitigated by some leaf adaptive traits such as glaucousness, pubescence, rolling, thickness or posture (Richards, 1996). These traits decrease the radiation load to the leaf surface. Benefits include a lower evapotranspiration rate and reduced risk of irreversible photo-inhibition. However, they may also be associated with reduced radiation use efficiency, which would reduce yield under more favorable conditions. In durum wheat, glaucousness (waxy covering over the plant cuticle) was found to reduce water loss after stomata closure (Qariani et al., 2000) and provide a yield advantage under drought stress (Merah et al., 2000).

A. Photosynthetic pigments

In theory, chlorophyll content is a desirable characteristic as it indicates a low degree of photo-inhibition. However, in hot and high light intensity environments, a pale-green color, related to low chlorophyll content, could limit the energy load from strong sunlight, as suggested in barley (Tardy et al., 1998) and the wild wheat *Aegilops geniculata* (Zaharieva et al., 2001). No clear relationship with yield under drought was found in durum wheat (Royo et al., 2000). Additionally to handheld devices for measurements of chlorophyll indices (e.g., SPAD meter), parameters of canopy reflectance via remote sensing approaches have been intensively investigated. Several reflectance methods have been proposed to estimate the concentration of chlorophyll and other pigments (Table 5). Chlorophyll concentration can be assessed by direct measurement at 675 nm \( (R_{675}) \) and 550 nm \( (R_{550}) \). \( R_{675} \) is very sensible to changes in chlorophyll concentration at relatively high concentrations. \( R_{550} \) can be used at low chlorophyll concentrations, but is less sensible (Lichtenthaler et al., 1996).

The carotenoid to chlorophyll ratio can be used to estimate the intensity of stress faced by the plant (Young and Britton, 1990). It can be estimated using the pigment simple ratio (PSR) or the normalized pigment index (NDPI). As these indices are affected by variation in leaf surface and structure, Pañuelas et al. (1995a) developed a new index, structural independent pigment index (SIPI).

Violaxanthin, a xanthophyll carotenoid present in the photosynthetic apparatus of plants, is rapidly and reversibly de-epoxidized into zeaxanthin via the intermediate antheraxanthin under high-light stress (Horton et al., 2005). This chemical transformation of violaxanthin, called the xanthophyll cycle, is required for the conversion of PSII from a state of efficient light harvesting to a state of
high thermal energy dissipation, which is usually measured as a non-photochemical quenching (NPQ) of chlorophyll (Chl) fluorescence. NPQ protects PSII from photoinhibition, at least under short-term light stress (Niyogi et al., 1998). Zeaxanthin synthesis in high light was also found to prevent photo-oxidative stress and lipid peroxidation (Havaux et al., 2000). In a number of cases, accumulation of zeaxanthin was shown to increase tolerance to photo-oxidative stress (Havaux et al., 2004). In durum wheat, an increase in zeaxanthin was noted under drought stress in the cultivar Adamello by Loggini et al. (1999). A reflectance based measurement of zeaxanthin has been proposed by Pañuelas et al. (1995b) using the photochemical index (PI). Relationship between the non-photochemical quenching and the photochemical Index across different stress intensities has been reported by Tambussi et al. (2000).

B. Chlorophyll fluorescence

Chlorophyll fluorescence can be used to estimate the activity of thermal energy dissipation in photosystem II and has been proposed to screen durum wheat accessions for drought tolerance (Flagella et al., 1995; Flagella et al., 1998; Royo et al. 2000). Under Mediterranean conditions, $F_o$, $F_m$ and $F_v$ have been used successfully to detect differences across genotypes and showed high heritability (Araus et al., 1998). $F_v/F_m$ is only sensitive to very severe stress conditions and has a poor heritability. $\Phi$PSII and $F_v'/F_m'$, as they are sensible to light intensity variation, are difficult to measure in field conditions. Fluorescence imaging should become a promising tool if portable systems are available as this technique accounts for spatial variation within the leaf and plot.

C. Antioxidants

The effects of photo-inhibition can be alleviated by antioxidants such as superoxide dismutase (SOD) and ascorbate peroxidise, which have been shown to increase in quantity in response to drought stress (Mittler and Zilinskas, 1994). Thermal dissipation through the xanthophyll cycle is another protective mechanism that can dissipate as much as 75 percent of absorbed light energy (Niyogi, 1999). In durum wheat, Zaeefyzadeh et al. (2009) found higher SOD in drought tolerant landraces from Iran and Azerbaijan than in susceptible ones.

9. Application of secondary traits in breeding

The use of any trait and its further application in breeding should be first considered in relation to the type of stress (intensity, timing) faced by the crop in the TPE. As mentioned by Tardieu et al. (2011), most traits presumably associated with drought tolerance have a dual effect, positive in some conditions and limited or negative in others. A strong association reported between a given trait and yield in a specific environment may be weaker or disappear in others. A typical case in durum wheat is the association between grain yield and grain $\Delta^{13}C$, constantly positive under the typical post-anthesis drought of Mediterranean countries (ME4A), but highly dependent on the intensity of drought and particularly on the quantity of water stored in the soil in ME4B and ME4C (Monneveux et al., 2005). Some other examples of traits effects changing according to the environment have been mentioned in this paper (earliness, chlorophyll concentration). Others have been reported by Tardieu et al. (2011).

While many traits have been studied for their use in breeding for drought resistance, there is a general consensus among breeders that only a few of them can be recommended for practical use in breeding programs at this time. The use of some traits in breeding is sometimes prevented by their low heritability but more often because of their lack of accuracy and precision. Some traits are difficult to assess on a large number of plants and their measurement is consequently affected by the fluctuation of environmental factors. In many cases, “instantaneous” measurements also face a problem of sampling (e.g., to which extent a measurement done on one leaf of few plants is representative of a plot and to which extent the time of measurement (hour of the day) affects the results of the measure. The development of techniques that are more time- and space-integrative
(spectrometry, thermal imaging) should solve most of these difficulties and the development of new equipment will facilitate measurements. Other traits cannot yet be recommended as part of an ongoing breeding program, because they are too expensive. However, some such as $\Delta^{13}$C can be used for the selection of parents (Misra et al., 2006; Xu et al., 2007).

Vegetation indices have been defined to estimate different plant characteristics such as photosynthetic active biomass, pigment content and water status (Table 5). An extensive study conducted by Royo et al. (2002) on a collection of genotypes showed that Reflectance at 550 nm ($R_{550}$), water index (WI), photochemical reflectance index (PRI), structural independent pigment index (SIPI), normalized difference vegetation index (NDVI) and simple ratio (SR) explained jointly a 95.7% of yield variability when all the experiments were analyzed together, 92% being explained by $R_{550}$. When regression analyses were carried out separately for each experiment, spectral reflectance indices explained from 17.3% to 65.2% of total variation in yield, and the indices that best explained differences in yield were experiment-dependent. The same authors especially recommended the use of reflectance at 680 nm ($R_{680}$), WI and SR as suitable estimators of durum wheat grain yield under Mediterranean conditions, when determined at milk-grain stage. Thermal imaging and color imaging techniques are expected to greatly facilitate large scale evaluations in the next future (Cabrera-Bosquet et al., 2012).

Conventional cameras have been proposed as a selection tool for cereal breeding by Casadesus et al. (2007) and Mullan and Reynolds 2010). In breeding programs, photographic sampling can be cost-efficient because a large number of samples can be obtained with minimum effort. Calculations from those images can also be cost-effective since they are based on rather simple methods that can be automated for application to a large number of images.

VII – Traits measurement

Drought is expected to increasingly affect durum wheat in most regions where it is cultivated, with potential consequences on food security. Genomics approaches to improve drought tolerance will bring new opportunities over the next few years, but their impact in farmer’s fields will mainly depend on the actual progress in our understanding of the physiology and genetic basis of drought-adaptive traits. The effective implementation in breeding programs of accurate and cost-effective phenotyping methods will be consequently essential to ensure research impact.

Efforts should focus on a more precise definition of TPEs, a better control of the stress monitoring in the MSEs and a more accurate assessment of drought tolerance related traits. Geographic information system tools, new equipment for the measurement of soil and plant water content, and more integrative drought tolerance related traits assessment methods can contribute largely in these efforts. But the success will also depend on a closer cooperation among partners. Collaborative efforts could include development of free-access long-term climatic data bases, multi-local and multi-institutional trials including common sets of cultivars, establishment of a well-documented database of durum wheat MSEs, registration of field data in common databases, web-sharing of experiences and organization of training courses. The development of networks among different partners and establishment of shared phenotyping platforms will allow quicker evaluation of germplasm in diversified environments, broader dissemination of germplasm products and larger impact of breeding efforts.
Table 4. Main secondary traits that can be used to improve drought tolerance in durum wheat, associated characteristics, measurement methods, references, ease of use and main target environment of application.

<table>
<thead>
<tr>
<th>Secondary trait</th>
<th>Associated characteristics</th>
<th>Measurement method</th>
<th>References</th>
<th>Heritability</th>
<th>Ease of use</th>
<th>Target environment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Traits related to drought escape</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earliness</td>
<td>Drought escape</td>
<td>scoring</td>
<td>Annichiarico and Pecetti (1998), Hafsi et al. (2006)</td>
<td>high</td>
<td>+++</td>
<td>ME4A, ME4C</td>
</tr>
<tr>
<td><strong>Traits related to pre-anthesis growth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early ground cover</td>
<td>Decrease of evaporation, increase of radiation use</td>
<td>scoring, digital image analysis</td>
<td>Regan et al. (1992), Annichiarico and Pecetti (1993)</td>
<td>moderate</td>
<td>+++</td>
<td>ME4A, early</td>
</tr>
<tr>
<td>Large seed size</td>
<td>Emergence, early ground cover</td>
<td>measurement</td>
<td>Aparicio et al. (2002a), Amin and Brinis (2013)</td>
<td>high</td>
<td>+++</td>
<td>ME4A</td>
</tr>
<tr>
<td>Long coleoptiles</td>
<td>Emergence from deep sowing</td>
<td>measurement</td>
<td>Giriyanpanavar et al. (2010)</td>
<td>moderate</td>
<td>+++</td>
<td>ME4C</td>
</tr>
<tr>
<td>Number of sp kes (fertile tillering)</td>
<td>Tiller Survival and recovery</td>
<td>scoring</td>
<td>EI Hafid et al. (1998) Annichiarico et al. (2002)</td>
<td>low</td>
<td>++</td>
<td>ME4A (early-season drought)</td>
</tr>
<tr>
<td>Pre-anthesis biomass</td>
<td></td>
<td>Measurement NDVI</td>
<td>Villegas et al. (2001), Royo et al. (2005)</td>
<td>low</td>
<td>++</td>
<td>ME4A</td>
</tr>
<tr>
<td><strong>Traits related to remobilization and sink strength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem water soluble carbohydrates</td>
<td>Storage of carbon products</td>
<td>biochemical analysis</td>
<td>Kameli and Lösel (1996)</td>
<td>moderate</td>
<td>+</td>
<td>ME4A</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>Storage of carbon products</td>
<td>measurement</td>
<td>Kaya et al. (2002), Bogale et al. (2011)</td>
<td>moderate</td>
<td>+++</td>
<td>ME4A</td>
</tr>
<tr>
<td>Spike fertility</td>
<td>Sink strength</td>
<td></td>
<td>Gebeyehou et al. (1982)</td>
<td>moderate</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Grain filling duration</td>
<td>Grain filling, thousand kernel weight</td>
<td>measurement</td>
<td>Simane et al. (1993), Annichiarico and Pecetti (1998)</td>
<td>low to moderate</td>
<td>+++</td>
<td>Drought around flowering</td>
</tr>
<tr>
<td><strong>Traits relating to water status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root mass</td>
<td>Water uptake</td>
<td>see Table 5</td>
<td>Motzo et al. (1993)</td>
<td>low</td>
<td>+</td>
<td>Severe drought</td>
</tr>
<tr>
<td>Root depth</td>
<td>Water uptake</td>
<td>see Table 5</td>
<td>Simane et al. (1993)</td>
<td>low</td>
<td>+</td>
<td>ME4C</td>
</tr>
<tr>
<td>Root length density</td>
<td>Water uptake</td>
<td>see Table 5</td>
<td>EI Hafid et al. (1998)</td>
<td>low</td>
<td>+</td>
<td>ME4A</td>
</tr>
<tr>
<td>Stomata conductance</td>
<td>Transpiration and CO₂ assimilation</td>
<td>gas exchange, porometry, mass spectrometry</td>
<td>Monneveux et al. (2006)</td>
<td>moderate</td>
<td>++</td>
<td>ME4A</td>
</tr>
<tr>
<td>¹⁸Oxygen</td>
<td>Transpiration</td>
<td>mass spectrometry</td>
<td>Cabrera-Bosquet et al. (2011)</td>
<td>high</td>
<td>++</td>
<td>ME4A</td>
</tr>
<tr>
<td>Canopy temperature depression</td>
<td>Stomata conductance</td>
<td>infra-red thermometry</td>
<td>Royo et al. (2002)</td>
<td>moderate</td>
<td>+++</td>
<td>Hot and dry environments</td>
</tr>
<tr>
<td>Leaf rolling</td>
<td>Loss of turgor</td>
<td>score</td>
<td>Bogale et al. (2011)</td>
<td>high</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Measurement/Technique</td>
<td>Source</td>
<td>Suitability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
<td>--------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual transpiration</td>
<td>Cuticular and residual stomata transpiration</td>
<td>weighting</td>
<td>Febrero <em>et al.</em> (1991)</td>
<td>high</td>
<td>+++</td>
<td>Severe drought</td>
</tr>
<tr>
<td>Osmotic adjustment</td>
<td>Minimization water loss</td>
<td>measurement of water status parameters under controlled conditions</td>
<td>Rekika <em>et al.</em> (1998)</td>
<td>moderate</td>
<td>+</td>
<td>Moderate drought</td>
</tr>
<tr>
<td>Relative water content</td>
<td>Maintenance of cell function</td>
<td>Weighting reflectance (WI)</td>
<td>El Hafid <em>et al.</em> (1998)</td>
<td>moderate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>$^{13}$Carbon</td>
<td>Stomata conductance</td>
<td>mass spectrometry</td>
<td>Araus <em>et al.</em> (1998), Merah <em>et al.</em> (2001)</td>
<td>high</td>
<td>++</td>
<td>Mainly for ME4A</td>
</tr>
<tr>
<td>Ash content</td>
<td>$^{13}$Carbon, transpiration</td>
<td>Combustion, near-Infrared spectrometry (NIRS)</td>
<td>Araus <em>et al.</em> (1998), Merah <em>et al.</em> (1999), Ferrio <em>et al.</em> (2001)</td>
<td>high</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

**Traits relating to water-use efficiency**

- **Root xylem diameter**
  - reduction in root conductance
  - measurement: Richards and Passioua (1989)
  - high | + | ME4C (Australia) |
- **Spike photosynthesis**
  - contribution to photosynthesis
  - gas-exchange measurements, $\Delta$ of water soluble fraction, fluorescence (?)
  - Araus *et al.* (1993)
  - moderate | + | ME4A |
- **Awn length**
  - contribution to photosynthesis
  - measurement: Villegas *et al.* (2006)
  - moderate | +++ | ME4A |
- **Senescence**
  - drought avoidance, partitioning
  - moderate | ++ | - |

**Traits relating to photo-protection**

- **Glaucescence**
  - radiation load to the leaf surface, water loss
  - high | +++ | Severe drought |
- **Chlorophyll fluorescence**
  - activity of thermal energy dissipation in photosystem II
  - fluorimetry: Araus *et al.* (1998)
  - high | ++ | Severe drought |
- **Carotenoid content**
- **Antioxidants (S.O.D., ascorbate peroxidise)**
  - biochemical analysis: Zaefyzadeh *et al.* (2009)
  - moderate | ++ | - |
Table 5. Spectral vegetation indices (adapted from Araus et al., 2001 and Mullan, 2012).

<table>
<thead>
<tr>
<th>Measured trait and corresponding indices</th>
<th>Calculation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Photosynthetic size of canopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple ratio</td>
<td>SR = ( \frac{R_{\text{NIR}}}{R_{\text{red}}} )</td>
<td></td>
</tr>
<tr>
<td>Normalized difference vegetation index</td>
<td>NDVI = ( \frac{(R_{\text{NIR}} - R_{\text{red}})}{(R_{\text{NIR}} + R_{\text{red}})} )</td>
<td></td>
</tr>
<tr>
<td>Modified NDVI</td>
<td>NDVI = ( \frac{R_{701} - R_{520}}{(R_{701} + R_{520})} ) Carter (1998)</td>
<td></td>
</tr>
<tr>
<td>Soil adjusted vegetation index</td>
<td>SAVI = ( \frac{(R_{\text{NIR}} - R_{\text{red}})}{(R_{\text{red}} + L)} (1 + L)^* ) Huete (1988)</td>
<td></td>
</tr>
<tr>
<td>Transformed soil adjusted vegetation index</td>
<td>TSVI = ( a(R_{\text{NIR}} - R_{\text{red}} - b) / (R_{\text{red}} + 0.08) ) Baret and Guyot (1991)</td>
<td></td>
</tr>
<tr>
<td>Perpendicular vegetation index</td>
<td>PVI = ( \left[ \frac{(R_{\text{red}} - R_{\text{red vegetation}})^2 + (R_{\text{NIR vegetation}} - R_{\text{NIR soil}})^2}{(1 + a)^2} \right]^{1/2} ) Richardson and Wiegand (1977)</td>
<td></td>
</tr>
<tr>
<td><strong>Water status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water index</td>
<td>WI = ( \frac{R_{900}}{R_{970}} ) Pañuelas et al. (1993)</td>
<td></td>
</tr>
<tr>
<td>Normalized water index - 1</td>
<td>NWI-1 = ( \frac{(R_{970} - R_{900})}{(R_{675} + R_{900})} ) Babar et al., 2006b</td>
<td></td>
</tr>
<tr>
<td>Normalized water index - 2</td>
<td>NWI-2 = ( \frac{(R_{970} - R_{850})}{(R_{970} + R_{850})} ) Babar et al., 2006b</td>
<td></td>
</tr>
<tr>
<td>Normalized water index - 3</td>
<td>NWI-3 = ( \frac{(R_{970} - R_{920})}{(R_{970} + R_{920})} ) Prasad et al., 2007</td>
<td></td>
</tr>
<tr>
<td>Normalized water index - 4</td>
<td>NWI-4 = ( \frac{(R_{970} - R_{880})}{(R_{970} + R_{880})} ) Prasad et al., 2007</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple chlorophyll index</td>
<td>( R_{575} ) Jacquesmoud and Baret (1990)</td>
<td></td>
</tr>
<tr>
<td>Simple chlorophyll index</td>
<td>( R_{550} ) Jacquesmoud and Baret (1990)</td>
<td></td>
</tr>
<tr>
<td>Ratio of reflectance</td>
<td>( R_{750}/R_{550} ) Lichtenenthaler et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Ratio of reflectance</td>
<td>( R_{750}/R_{700} ) Lichtenenthaler et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Green normalized difference vegetation index</td>
<td>NDVI_{green} = ( \left[ \frac{(R_{\text{NIR}} - R_{540}/R_{570})}{(R_{\text{NIR}} + R_{540}/R_{570})} \right] ) Gitelson and Merzlyak (1997)</td>
<td></td>
</tr>
<tr>
<td>Wavelength of the red edge</td>
<td>( \lambda_{\text{pe}} ) Filella et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>Maximum amplitude in the first derivative of the reflectance spectra</td>
<td>( dR_{\text{re}} ) Filella et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>Sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra</td>
<td>( \Sigma dR_{680-780} ) Filella et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>Normalized difference red edge</td>
<td>NDRE = ( \frac{(R_{790} - R_{720})}{(R_{790} + R_{720})} ) Barnes et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Modified spectral ratio (chlorophyll concentration)</td>
<td>MSR = ( \frac{(R_{750} - R_{445})}{(R_{705} - R_{445})} ) Sims and Gamon (2003)</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll degradation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized phaeophytinization index</td>
<td>NPQI = ( \frac{(R_{445} - R_{430})}{(R_{415} + R_{430})} ) Pañuelas et al. (1995c)</td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll a</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio analysis of reflectance spectra (Chla)</td>
<td>RARSa = ( \frac{R_{675}}{R_{700}} ) Chapelle et al. (1992)</td>
<td></td>
</tr>
<tr>
<td>Pigment</td>
<td>Ratio Analysis of Reflectance Spectra</td>
<td>Simple Ratio</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>Chlorophyll a</strong></td>
<td>( R_{\text{ARS}a}^* = \frac{R_{680}}{R_{660}} )</td>
<td>( P_{\text{SSRa}} = \frac{R_{800}}{R_{675}} )</td>
</tr>
<tr>
<td><strong>Pigment Specific Simple Ratio (Chla)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chlorophyll b</strong></td>
<td>( R_{\text{ARS}b} = \frac{R_{675}}{(R_{650} \times R_{700})} )</td>
<td>( P_{\text{SSRb}} = \frac{R_{800}}{R_{650}} )</td>
</tr>
<tr>
<td><strong>Pigment Specific Simple Ratio (Chla)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carotenoid</strong></td>
<td>( R_{\text{ARS}c} = \frac{R_{760}}{R_{500}} )</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio Analysis of Reflectance Spectra (car)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Carotenoid to Chlorophyll Ratio</strong></td>
<td>( \text{PSR} = \frac{R_{430}}{R_{670}} )</td>
<td></td>
</tr>
<tr>
<td><strong>Pigment Simple Ratio (PSR)</strong></td>
<td>( \text{NDPI} = \frac{(R_{680} - R_{430})}{(R_{680} + R_{430})} )</td>
<td></td>
</tr>
<tr>
<td><strong>Normalized Difference Pigment Index (NDPI)</strong></td>
<td>( \text{SIPI} = \frac{(R_{800} - R_{435})}{(R_{800} + R_{435})} )</td>
<td></td>
</tr>
<tr>
<td><strong>Structural Independent Pigment Index (SIPI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zeaxanthin</strong></td>
<td>( \text{PRI} = \frac{(R_{431} - R_{570})}{(R_{431} + R_{570})} )</td>
<td></td>
</tr>
<tr>
<td><strong>Photochemical Reflectance Index (PRI)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*L = 1 for low soil coverage, L = 0.25 for high soil coverage

\**a is the slope and b the intercept of the linear equation \( R_{\text{NIRsoil}} = a R_{\text{redsoil}} + b \)
### Table 6. Main techniques available for assessing root characteristics (adapted from Herrera et al. (2012)).

<table>
<thead>
<tr>
<th>Method</th>
<th>Short description</th>
<th>Reference</th>
<th>Accuracy</th>
<th>Time-effectiveness</th>
<th>Cost-effectiveness</th>
<th>Through-put</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trench walls</td>
<td>The soil next to a plant is dug in such a way that the root systems become visible</td>
<td>-</td>
<td>++</td>
<td>---</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>Mesh bags</td>
<td>The dynamics of root growth and root turnover can be studied by placing bags containing root-free soil in the field and removing them at regular intervals</td>
<td>-</td>
<td>-</td>
<td>---</td>
<td>++</td>
<td>---</td>
</tr>
<tr>
<td>Monoliths</td>
<td>A cubic section of soil that contains roots (monolith) dug out from the soil or obtained from a container in which the plant has been grown is washed to remove soil and separate roots.</td>
<td>McCully (1999)</td>
<td>+++</td>
<td>---</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>Soil core</td>
<td>A soil core, small compared to the rooting volume is taken from the rhizosphere. The amount of roots can be estimated by breaking the soil core horizontally and counting the roots exposed on both faces of the breakage or by washing the samples and recovering the roots.</td>
<td>Kumar et al. (1993); Yamaguchi (2002); Pierret et al. (2005),</td>
<td>++</td>
<td>---</td>
<td>+++</td>
<td>---</td>
</tr>
<tr>
<td>Two-dimensional (2D) rhizotrons</td>
<td></td>
<td>Smit et al. (2000a)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mini-rhizotrons</td>
<td>the plant is grown in a flat container with side walls made of a transparent material such as glass small-diameter transparent tubes inserted into the soil for the observation of root</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Optical scanners</td>
<td>used to process samples obtained by soil coring or by burying them in the soil to study roots in a similar way as with 2D rhizotrons</td>
<td>Dannoura et al. (2008)</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>Electrical capacitance</td>
<td>based on measuring the electrical capacitance of an equivalent parallel resistance-capacitance circuit formed by the interface between soil water and the plant root surface</td>
<td>Chloupek et al. (2006)</td>
<td>--</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ground-penetrating radars</td>
<td>Used to study the root biomass of trees, to be validated for cereals</td>
<td>Amato et al. (2009)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Computed tomography methods</td>
<td>allow to image root growing and water uptake in the soil non-invasively</td>
<td>Hruska et al. (1999)</td>
<td>?</td>
<td>++</td>
<td>--</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tracy et al. (2010)</td>
<td>+++</td>
<td>++</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>
References


Isaac J., Hrimat N.S., 1999. Agronomic and economic characteristics of improved wheat cultivars under rainfed conditions in the Southern West Bank. Rachis, 18, pp. 4-11.


Richards R.A., Passioura J.B., 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. Australian J. Agricultural Research, 40, pp. 943-950.


