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

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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.

Keywords. Drought – Secondary traits – Target populations of environments – Managed stress environments – *Triticum durum* – Phenotyping.

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

Résumé. *Dans la zone de culture du blé, l'eau est généralement rare. D'après les prévisions, le changement climatique s'accompagnera de l'apparition plus fréquente des sécheresses. Par conséquent, les sélectionneurs du blé dur considèrent actuellement la tolérance à la sécheresse comme un objectif essentiel pour la sélection. Toutefois, le phénotypage représente encore un obstacle majeur dans la sélection pour les caractères de tolérance abiotique. Un phénotypage efficace implique i) la définition précise des populations cibles des environnements, basée sur la performance des variétés connues, ii) le choix et la caractérisation de l'environnement sous conditions de stress, iii) le suivi du stress et iv) la mesure des caractères « secondaires » (associés à la tolérance à la sécheresse). L'amélioration du phénotypage de la sécheresse chez le blé dur devrait tirer parti des nouvelles technologies développées pour affiner la définition des populations cibles des environnements, décrire précisément les environnements sous condition de stress, et suivre efficacement le stress de la sécheresse. Cela permettra d'établir une typologie précise des populations cibles des environnements basée sur des scénarios de sécheresse, de mieux prévoir l'adaptation du matériel génétique testé et enfin, d'augmenter la réponse à la sélection. L'utilisation d'un système d'information géographique (SIG) et l'intégration des méthodes d'évaluation des caractères liés à la tolérance à la sécheresse devraient être encouragées. Le développement de réseaux de recherche entre les différents partenaires et la création de plateformes de phénotypage dans les principales zones de culture du blé dur pourraient stimuler le partage de connaissances et d'expérience et favoriser une évaluation plus rapide du matériel génétique dans des environnements divers, facilitant ainsi la diffusion des ressources phytogénétiques et assurant une plus grande efficacité des efforts de sélection.*

Mots-clés. *Sécheresse – Caractères secondaires – Populations cibles des environnements – Gestion des environnements de stress – Triticum durum – Phénotypage.*

I – Introduction

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 it 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).

ME[a]	Sub ME	Moisture regime	Temperature	Wheat type	Area (%)	Production (Ml t)
ME1		Irrigated	Temperate	Spring	36.1	83
ME2		High Rainfall (>500 mm)	Temperate	Spring	8.5	25
ME3		High Rainfall (>500 mm) Acid Soil	Temperate	Spring	1.9	3
ME4		Low Rainfall (<500 mm)	Temperate/hot	Spring	14.6	20
	ME4A	Winter rain or Mediter.-type drought				
	ME4B	Winter drought or Southern Cone-type rainfall				
	ME4C	Continuous or subcont.-type drought				
ME5		Tropical	Hot	Spring	7.1	12
	ME5A	Low-humidity tropics				
	ME5B	High humidity tropics				
ME6		Semi-arid	Temperate	Spring	6.2	13
	ME6 A	High rainfall				
	ME6 B	Semi-arid				
ME7		Irrigated	Cool	Facult.		
ME8		High Rainfall	Cool	Facult.	10.0	23
ME9		Semi-arid	Cool	Facult.		
ME10		Irrigated	Cold	Winter		
ME11		High Rainfall	Cold	Winter	15.0	30
ME12		Semi-arid	Cold	Winter		

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

Mega-environment	Area (million ha)	Percentage of the total durum wheat cultivated area	Percentage of the total wheat area	Average durum wheat yield (t ha^{-1})
ME1	0.6	7	1.6	4.15
ME2A	2.1	26	29.6	1.99
ME4A	4.0	50	67.8	1.19
ME4B	0.1	1	3.1	2.06
ME4C	0.1	1	1.5	0.97
ME11	0.1	1	2.8	4.80
ME12	1.1	14	19.3	1.45

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), ie, 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 (Loffler *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).

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

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 (ET_o) is the main factor that drives the water consumption of the crop and its knowledge is essential to an accurate environment characterization. ET_o can be calculated according to FAO standards (Allen *et al.*, 1998) using the ET_o calculator, http://www.fao.org/nr/water/eto.html. (FAO, 2009). Procedures are incorporated to estimate 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 by the mean of frequency analysis. Together with RAINBOW, New_LocClim, http://www.fao.org/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

T 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 (Passioura, 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 (Passioura, 2005). Finally, the temperature of the substrate used to fill pots or containers used in greenhouse experiments can be different from field soil temperature (Passioura, 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 (eg, 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 (Lafitte *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 (eg, 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 (eg, 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 *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 *et al.* (2010).

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 Hafid *et al.*, 1998). Garcia del Moral *et al.* (2003) also reported that the number of spikes per square meter predominantly influenced grain production in the warmer environments of Spain.

C. Total biomass

T Final grain yield is determined in durum wheat by total biomass production and the proportion of biomass allocated to grains (Van den Boogaard *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 *et al.*, 1987) and anthesis (Villegas *et al.*, 2001; Royo *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 *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 *et al.*, 2002; Elliot and Regan, 1993; Smith *et al.,* 1993). Biomass can be estimated by measuring the spectra reflected by crop canopies in the visible (VIS, λ=400-700 nm) and near-infrared (NIR, λ =700-1300 nm) regions of the electromagnetic spectrum (the crop's ability to intercept radiation and photosynthesize (Ma *et al.*, 1996). Estimation is now feasible using spectro-radiometers to measure the spectra of light reflected by the canopy (Royo *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 *et al.*, 2002; Cabrera-Bosquet *et al.*, 2011). Vegetation indices have been used to estimate biomass (Aparicio *et al.*, 2002) and yield (Aparicio *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 *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 *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 (Lopes and Reynolds, 2010). Optimal root characteristics can vary in relation to the type of drought (Ali Dib *et al.*, 1992). Deep rooting appears more important when the crop depends on residual soil moisture (Mian *et al.,* 1994) whereas higher root density at intermediate soil depths (0.15–0.60 m) is more important in Mediterranean environments (Gregory *et al.,* 2009). In durum wheat, Motzo *et al.* (1993) reported an association between high root mass and tolerance to severe drought. However, extensive root systems also have higher respiration costs for plants. Root depth (Simane *et al.*, 1993) and root length density (El Hafid *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 e for high throughput phenotyping. Table 6 provides a list of the main techniques that are available actually, with their mains 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 a.l*, 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 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 evapo-transpiration 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. Photosyntetic 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 nonphotochemical 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. ΦPSII 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. Antioxydants

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, Zaefyzadeh *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 Δ^{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 (R680), 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.

Secondary trait	Associated characteristics	Measurement method	References	Heritability	Ease of use	Target environment
Traits related to drought escape						
Earliness	Drought escape	scoring	Annichiarico and Pecetti (1998), Hafsi *et al.* (2006)	high	+++	ME4A, ME4C
Traits related to pre-anthesis growth						
Early ground cover	Decrease of evaporation, increase of radiation use	scoring, digital image analysis	Regan *et al.* (1992), Annicchiarico and Pecetti (1993)	moderate	+++	ME4A, early
Large seed size	Emergence, early ground cover	measurement	Aparicio *et al.* (2002a), Amin and Brinis (2013)	high	+++	ME4A
Long coleoptiles	Emergence from deep sowing	measurement	Giriyappanavar *et al.* (2010)	moderate	+++	ME4C
Number of sp kes (fertile tillering)	Tiller Survival and recovery	scoring	El Hafid *et al.* (1998) Annichiarico *et al.* (2002)	low	++	ME4A (early-season drought)
Pre-anthesis biomass		Measurement NDVI	Villegas *et al.* (2001), Royo *et al.* (2005)	low	++	ME4A
Traits related to remobilization and sink strength						
Stem water soluble carbohydrates	Storage of carbon products	biochemical analysis	Kameli and Lösel (1996)	moderate	+	ME4A
Peduncle length	Storage of carbon products	measurement	Kaya *et al.* (2002), Bogale *et al.* (2011)	moderate	+++	ME4A
Spike fertility	Sink strength		Gebeyehou *et al.* (1982)	moderate	+++	-
Grain filling duration	Grain filling, thousand kernel weight	measurement	Simane *et al.* (1993), Annicchiarico and Pecetti (1998)	low to moderate	+++	Drought around flowering
Traits relating to water status						
Root mass	Water uptake	see Table 5	Motzo *et al.* (1993)	low	+	Severe drought
Root depth	Water uptake	see Table 5	Simane *et al.* (1993)	low	+	ME4C
Root length density	Water uptake	see Table 5	El Hafid *et al.* (1998)	low	+	ME4A
Stomata conductance	Transpiration and CO_2 assimilation	gas exchange, porometry	Monneveux *et al.* (2006)	moderate	++	ME4A
[18]Oxygen	Transpiration	mass spectrometry	Cabrera-Bosquet *et al.* (2011)	high	++	ME4A
Canopy temperature depression	Stomata conductance	infra-red thermometry	Royo *et al.* (2002)	moderate	+++	Hot and dry environments
Leaf rolling	Loss of turgor	score	Bogale *et al.* (2011)	high	+++	-

Residual transpiration	Cuticular and residual stomata transpiration	weighting	Febrero *et al.* (1991)	high	+++	Severe drought
Osmotic adjustment	Minimization water loss	measurement of water status parameters under controlled conditions	Rekika *et al.* (1998)	moderate	+	Moderate drought
Relative water content	Maintenance of cell function	Weighting reflectance (WI)	El Hafid *et al.* (1998)	moderate	+	-
[13]Carbon	Stomata conductance	mass spectrometry	Araus *et al.* (1998), Merah *et al.* (2001)	high	++	Mainly for ME4A
Ash content	[13]Carbon, transpiration	Combustion, near-Infrared spectrometry (NIRS)	Araus *et al.* (1998), Merah *et al.* (1999), Ferrio *et al.* (2001)	high	++	-
Traits relating to water-use efficiency						
Root xylem diameter	reduction in root conductance	measurement	Richards and Passioura 1989)	high	+	ME4C (Australia)
Spike photosynthesis	Contr bution to photosynthesis	gas-exchange measurements, Δ of water soluble fraction, fluorescence (?)	Araus *et al.* (1993)	moderate	+	ME4A
Awn length	Contr bution to photosynthesis	measurement	Villegas *et al.* (2006)	moderate	+++	ME4A
Senescence	drought avoidance, partitioning	SPAD	Hafsi *et al.* (2003), Hafsi *et al.* (2006), Guendouz and Maamari (2011)	moderate	++	-
Traits relating to photo-protection						
Glaucousness	radiation load to the leaf surface, water loss	scoring	Qariani *et al.* (2000), Merah *et al.* (2000)	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).

Measured trait and corresponding indices	Calculation	Reference
Photosynthetic size of canopy		
Simple ratio	$SR = R_{NIR}/R_{red}$	
Normalized difference vegetation index	$NDVI = (R_{NIR} - R_{red})/(R_{NIR} + R_{red})$	
Modified NDVI	$NDVI = (R_{701} - R_{520})/(R_{701} + R_{520})$	Carter (1998)
Soil adjusted vegetation index	$SAVI = [(R_{NIR} - R_{red})/(R_{NIR} + R_{red} + L)] (1 + L)*$	Huete (1988)
Transformed soil adjusted vegetation index	$TSAVI = a(R_{NIR} - R_{red} - b)/[R_{red} + a(R_{NIR} - b) + 0.08 (1 + a^2)]**$	Baret and Guyot (1991)
Perpendicular vegetation index	$PVI = [(R_{redsoil} - R_{red\ vegetation})^2 + (R_{NIR\ vegetation} - R_{NIRsoil})^2]^{1/2}$	Richardson and Wiegand (1977)
Water status		
Water index	$WI = R_{900}/R_{970}$	Pañuelas *et al.* (1993)
Normalized water index - 1	$NWI-1 = (R_{970} - R_{900}) / (R_{970} + R_{900})$	Babar *et al.*, 2006b
Normalized water index - 2	$NWI-2 = (R_{970} - R_{850}) / (R_{970} + R_{850})$	Babar *et al.*, 2006b
Normalized water index - 3	$NWI-3 = (R_{970} - R_{920}) / (R_{970} + R_{920})$	Prasad *et al.*, 2007
Normalized water index - 4	$NWI-4 = (R_{970} - R_{880}) / (R_{970} + R_{880})$	Prasad *et al.*, 2007
Chlorophyll		
Simple chlorophyll index	R_{675}	Jacquemoud and Baret (1990)
Simple chlorophyll index	R_{550}	Jacquemoud and Baret (1990)
Ratio of reflectance	R_{750}/R_{550}	Lichtenthaler *et al.* (1996)
Ratio of reflectance	R_{750}/R_{700}	Lichtenthaler *et al.* (1996)
Green normalized difference vegetation index	$NDVI_{green} = [R_{NIR} - R_{540}/R_{570})]/ [R_{NIR} + R_{540}/R_{570})]$	Gitelson and Merzlyak (1997)
Wavelength of the red edge	λ_{re}	Filella *et al.* (1995)
Maximum amplitude in the first derivative of the reflectance spectra	dR_{re}	Filella *et al.* (1995)
Sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra	$\Sigma dR_{680-780}$	Filella *et al.* (1995)
Normalized difference red edge	$NDRE = (R790 - R720) / (R790 + R720)$	Barnes *et al.* (2000)
Modified spectral ratio (chlorophyll concentration)	$MSR = (R750 - R445) / (R705 - R445)$	Sims and Gamon (2003)
Chlorophyll degradation		
Normalized phaeophytinization index	$NPQI = (R_{415} - R_{435})/(R_{415} + R_{435})$	Pañuelas *et al.* (1995c)
Chlorophyll a		
Ratio analysis of reflectance spectra (Chla)	$RARSa = R_{675}/R_{700}$	Chapelle *et al.* (1992)

Ratio analysis of reflectance spectra (Chla)	RARSa* = R_{680}/R_{800}	Blackburn (1998)
Pigment specific simple ratio (Chla)	PSSRa = R_{800}/R_{675}	Blackburn (1998)
Chlorophyll b		
Ratio analysis of reflectance spectra (Chlb)	RARSb = $R_{675}/(R_{650} \times R_{700})$	Chapelle *et al.* (1992)
Pigment specific simple ratio (Chla)	PSSRb = R_{800}/R_{650}	Blackburn (1998)
Carotenoid		
Ratio analysis of reflectance spectra (car)	RARSc = R_{760}/R_{500}	Chapelle *et al.* (1992)
Carotenoid to chlorophyll ratio		
Pigment simple ratio (PSR)	PSR = R_{430}/R_{680}	Pañuelas *et al.* (1993)
Normalized difference pigment index (NDPI)	NDPI = $(R_{680} - R_{430})/(R_{680} + R_{430})$	Pañuelas *et al.* (1993)
Structural independent pigment index (SIPI)	SIPI = $(R_{800} - R_{435})/(R_{800} + R_{435})$	Pañuelas *et al.* (1995a)
Zeaxanthin		
Photochemical reflectance index (PRI)	PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$	Pañuelas *et al.* (1995b)

*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 $R_{NIRsoil} = a\ R_{redsoil} + b$

Table 6. Main techniques available for assessing root characteristics (adapted from Herrera *et al.* (2012).

Method	Short description	Reference	Accu-racy	Time-effecti-veness	Cost effecti-veness	Through-put
Trench walls	The soil next to a plant is dug in such a way that the root systems become visible	-	++	---	+++	---
Mesh bags	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	-	-	--	++	--
Monoliths	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.	McCully (1999)	+++	---	+++	---
Soil core	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	Kumar *et al.* (1993); Yamaguchi (2002); Pierret *et al.* (2005),	++	---	+++	---
Two-dimensional (2D) rhizotrons	the plant is grown in a flat container with side walls made of a transparent material such as glass	-	+	-	-	+
Mini-rhizotrons	small-diameter transparent tubes inserted into the soil for the observation of root	Smit *et al.* (2000a)	-	-	-	+
Optical scanners	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	Dannoura *et al.* (2008)	+	+	--	+
Electrical capacitance	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	Chloupek *et al.* (2006) Amato *et al.* (2009)	--	+++	+	++
Ground-penetrating radars	Used to study the root biomass of trees, to be validated for cereals	Hruska *et al.* (1999)	?	++	--	++
Computed tomography methods	allow to image root growing and water uptake in the soil non-invasively	Tracy *et al.* (2010)	+++	++	---	+

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