



# **Wheat drought response strategies with special regards to root diversity**

## **Dissertation**

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submitted by

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## Abstract

Root diversity is considered important for drought resistance. Wheat genotypes of different ploidy levels, origins and breeding intensities were tested under contrasting water supply. Significant root and shoot trait variation was observed, leading to distinct water uptake strategies, *e.g.* dense topsoil rooting, high specific root length and deep rooting. Genotypes with a dominant surface root system benefited most from in-season rainfalls. Root systems of underutilized wheat contrasted with modern cultivars: while genetic resources responded to limiting water condition by allocating more assimilates to roots, advanced cultivars shifted their root morphology towards fine roots. Drought response strategies were analyzed by Passioura's yield-water framework with phenological, morphological, physiological, and root data. Limited water supply resulted in 60% yield loss and substantial reduction of water use (37%), water use efficiency (32.6%) and harvest index (14%). Late flowering underutilized wheats with large root system and vigorous ground cover showed greatest water use. Still there was a link of several water use traits with yield limiting behavior, constraining their potential role for better drought resistance. Lower chlorophyll concentration and stomata conductance of underutilized wheat species also suggested a water saving strategy of transpiration with limited potential growth. Modern cultivars on the contrary had superior water use efficiency via high chlorophyll concentration and stomata conductance. Harvest index was strongly dependent on phenology and yield components: optimized flowering time, reduced tillering and strong grain sink of modern cultivars explained their higher harvest index compared to underutilized genetic resources. The study demonstrated that physiological and root traits within modern cultivars can be used for trait based crop improvement under water limited conditions.

## **Zusammenfassung**

Wurzeldiversität ist eine wichtige Grundlage der Trockenresistenz. Weizengenotypen unterschiedlicher Ploidiestufe, Herkunft und Züchtungsintensität wurden bei unterschiedlicher Wasserversorgung verglichen. Sie zeigten hohe Variabilität in Wurzel- und Sprosseigenschaften, was zu verschiedenen Wasserversorgungsstrategien führte, z.B. dichte Oberbodendurchwurzelung, hohe spezifische Wurzellänge und tiefe Wurzelsysteme. Dicht wurzelnde Genotypen konnten Niederschläge in der Vegetationszeit am effizientesten aufnehmen. Wenig genutzte Weizenarten wie Einkorn unterschieden sich in ihrer Trockenreaktion von modernen Sorten: erstere zeigten höhere Assimilatverlagerung ins Wurzelsystem, während letztere ihre Wurzelmorphologie zu mehr Feinwurzeln verschoben. Die Ertragsreduktion durch Wassermangel wurde mit Passioura's konzeptionellem Modell analysiert. Trockenheit führte im Mittel zu 60% Ertragsverlust, 37% weniger Wasseraufnahme, 32,6% geringerer Wassernutzungseffizienz und 14% niedrigere Ernteindizes. Die spät reifenden wenig genutzten Weizenarten hatten durch ihr intensives Wurzelsystem und rasche Bodenbedeckung die höchste Wasseraufnahme. Die Pflanzeigenschaften für hohe Wasseraufnahme waren jedoch mit Ertrag beschränkenden Eigenschaften verbunden, was das Züchtungspotential dieser Genotypen einschränkt. Geringe Chlorophyllkonzentration und Stomataleitfähigkeit zeigten eine Strategie des Wassersparens. Moderne Sorten dagegen hatten eine überlegene Wassernutzungseffizienz durch hohe Chlorophyllkonzentration und Stomataleitfähigkeit. Der Ernteindex war abhängig von Phänologie und dominierenden Ertragskomponenten: Zuchtsorten hatten aufgrund ihrer optimalen Reifezeit, reduzierter Bestockung und einer hohen Senkenkapazität der Körner einen höheren Ernteindex als genetische Ressourcen. Die Studie zeigte, dass innerhalb der modernen Sorten physiologische und Wurzeleigenschaften vorhanden sind, die für eine gezielte Verbesserung der Trockenresistenz genutzt werden können.



## 1. Introduction

In recent years, climate change and its impacts particularly on crop production (e.g. changes in plant phenology, crop-cycle shortening, and yield instability) have become more evident than ever. Global warming is expected to increase frequency and intensity of extreme climate events (droughts and floods) which result in reduced yields (Padgham 2009). The impacts of climate change on crop production are geographically unevenly distributed. Negative impacts are expected especially for countries near the equator which are already today frequently affected by drought (Cline 2007). Across European regions, the projections indicate an increase in water demand in agriculture along with shifts in rainfall distribution leading to more frequent drought stress (Lavalle *et al.* 2009, Trnka *et al.* 2011). Beside climate change, continued population growth remains among the biggest challenges to world food security. Simulations show that feeding 9.1 billion people in 2050 would require a raise of ~70% for overall food production and 49% for cereals (Bruinsma 2009). This necessitates a yield improvement rate of 1.16-1.31% *per annum* (*p.a.*) to meet the projected demands for cereals (Hall & Richards 2013).

Wheat (*Triticum* L.) is the third most-produced cereal after maize and rice in total world production (FAO 2014). While demand for wheat is predicted to increase by 1.7% *p.a.* by 2050 (Agcaoili & Rosegrant 1995), wheat productivity is increasing globally at a lower rate, *i.e.* 1.1% *p.a.* (Dixon *et al.* 2009). This mismatch represents a serious challenge for future food security (Reynolds *et al.* 2012).

Wheat grain yield is the product of a number of developmental processes occurring throughout crop growth. Thus, it is a complex trait governed by multiple genes and is highly influenced by environmental conditions. Yield improvement in water-limited environments with high variability in seasonal crop water availability is even more complex and depends strongly on the drought regime, *i.e.* drought duration, intensity and time of occurrence (van Ginkel *et al.* 1998, Blum 2011a). This complexity becomes evident when traits contributing to yield loss mitigation in a given environment are not equally useful in other water-limited environments (Richards 2006).

Better understanding of crop yield physiology and identifying stress-adaptive traits are proposed for a more efficient physiological trait-based breeding, particularly when stress adaptation is targeted. This should enhance the rates of yield improvement required to meet

the projected future demand for wheat (Cattivelli *et al.* 2008, Reynolds *et al.* 2009, Richards *et al.* 2010, Slafer *et al.* 2014). Till now, however, only limited incremental gains in yield have been demonstrated using physiological traits in drought prone environments (Richards 2006, Reynolds *et al.* 2009). This is probably due to incomplete understanding of the physiological and genetic basis of drought resistance (Salekdeh *et al.* 2009) as well as insufficient consideration of the drought environments when stress resistance is targeted.

Blum (2009) emphasizes the maximizing plant water use through transpiration for breeding under drought stress because of its general compatibility with high yield. Thus, genotypes showing drought avoidance via maximized water uptake, termed ‘water spenders’ by Levitt (1980), should be focused as valuable sources for better drought resistance. In that respect, enhanced plant root systems are considered as promising trait (de Dorlodot *et al.* 2007, Comas *et al.* 2013). However, due to the lack of fast, non-destructive and cost-effective *in situ* screening methods, root systems have been yet inadequately exploited in crop improvement (Zobel & Waisel 2010, Palta *et al.* 2011, Ehdaie *et al.* 2012).

As root systems of wild wheat species and landraces have been little characterized as a source of potential drought-adaptive traits (Reynolds *et al.* 2005, 2007, Trethowan & Mujeeb-Kazi 2008, Waines & Ehdaie 2007), this study aimed to

- assess the diversity in root properties of a set of wheat genotypes including underutilized wheat species along with durum wheat and bread wheat cultivars and/or breeding lines,
- study the efficiency of root electrical capacitance as an easy, non-destructive *in situ* screening method for root system size,
- analyze the relation between water regime and soil water depletion with root traits,
- dissect the phenological and physiological basis of grain yield,
- assess root and shoot allometries as an alternative indirect method for root screening,
- analyze the grain yield of modern and underutilized wheat germplasm in relation to phenological, physiological and root traits.

Thus, this study is an attempt to provide a new insight into a root-based wheat breeding under rainfed conditions where plant water supply is mostly provided by in-season rainfalls.

## 2. Literature

### 2.1 Wheat

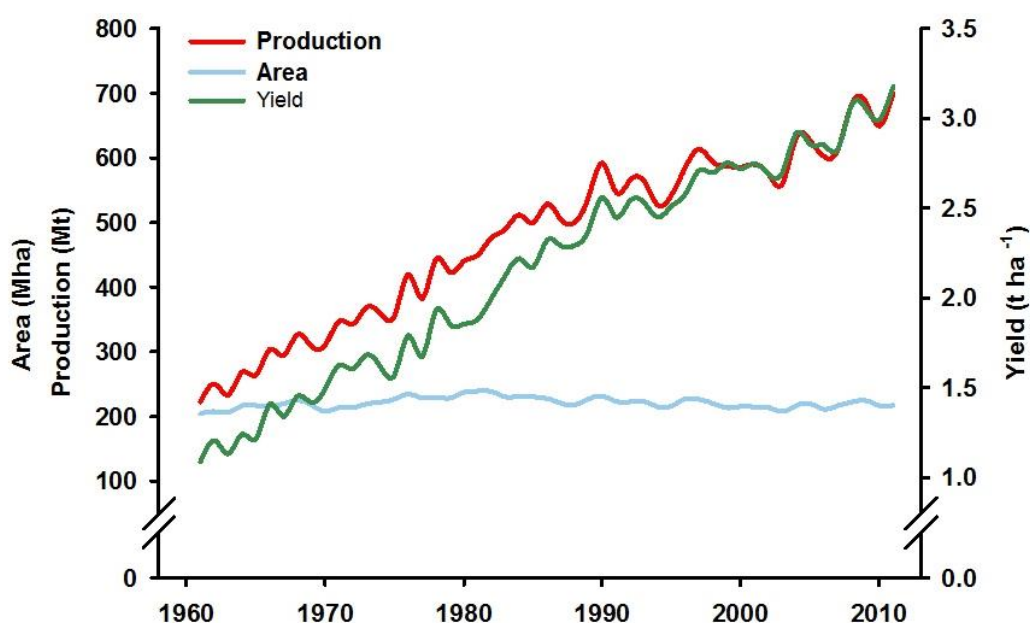
The domestication history of wheat (*Triticum* L.) as food can be traced back to 8000-10000 years ago in the near-eastern Fertile Crescent (Lev-Yadun *et al.* 2000). Common wheat (*T. aestivum* L.) has evolved from a cross between three diploid species within the *Triticeae* tribe and has, therefore, a complex allopolyploid genome. This genomic complexity allowed wheat to spread over varied agro-ecological conditions and cropping systems throughout the world. Today, wheat is the third most-produced cereal after maize and rice in total world production (FAO 2014). Wheat grain, with 60-80% starch and 7-22% storage protein, provides 20% of the world calories consumption and it is an important source of protein in developing countries (Braun *et al.* 2010). The gluten proteins in the seed endosperm confer the unique bread-baking quality of wheat. Therefore, wheat is a staple food for billions of people since ancient times (Gustafson *et al.* 2009). Without doubt, future global food security will highly rely on the productivity of the wheat crop.

### 2.2 Wheat production

World wheat production increased dramatically over the 2<sup>nd</sup> half of the 20<sup>th</sup> century. However, the expansion of wheat acreage has ceased to be the major source of increased wheat production (**Fig. 1**).

Of the 218.5 million hectares (Mha) of wheat sown globally in 2013, India with 29.7 (Mha) land devoted to wheat production had the largest cultivated wheat area, followed closely by China (24.1 Mha), the Russian Federation (23.4 Mha), and the United States (18.3 Mha) (FAO 2014).

In 2013, 713 million tons (Mt) of wheat were produced worldwide, with China (121.7 Mt), India (93.5 Mt), United States of America (58.0 Mt), Russian Federation (52.1 Mt) and France (38.6 Mt) being the major wheat-producing countries. Wheat is the most leading commodity in global trading, *i.e.* 148.3/147.2 MT of total produced wheat in 2011 was exported/imported worldwide. In this year, the most important wheat exporters were USA (32.8 Mt), France (20.3 Mt), Australia (17.7 Mt), Canada (16.3 Mt), and Argentina (8.4 Mt). The five top importers were Egypt (9.8 Mt), Algeria (7.5 Mt), Italy (7.3 Mt), Japan (6.2 Mt) and Brazil (5.7 Mt).

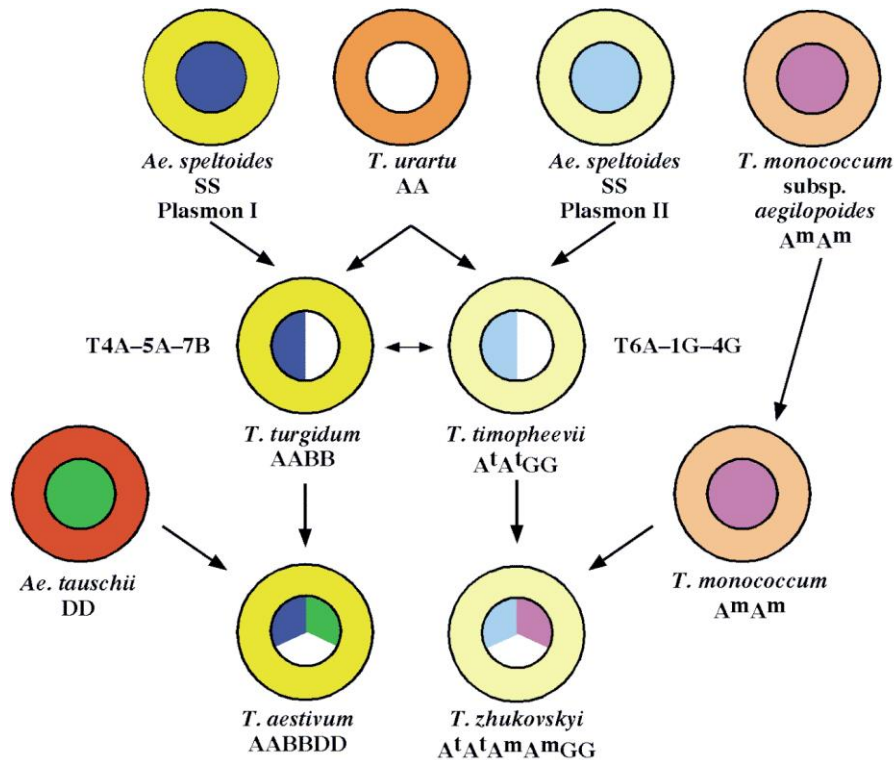


**Fig. 1:** Global wheat area, production and mean grain yield, 1961-2011 (FAO 2014)

### 2.3 Wheat phylogeny

The genus *Triticum* comprises wheat species at three ploidy levels, *i.e.* diploid ( $2n=2x=14$ ), tetraploid ( $2n=4x=28$ ) and hexaploid ( $2n=6x=42$ ). Among diploid wheats, *T. monococcum* L. (einkorn wheat) is still cultivated to a limited extent. The cultivated and wild forms can be found in diploid and tetraploid species. Free-threshing tetraploid durum wheat (*T. durum* Desf.) makes up ~5% of global wheat production (USDA 2009), whereas *e.g.* *T. timopheevii* (Zhuk.) Zhuk. is of little economic importance (Gill & Friebe 2002). Being tolerant to terminal drought, most durum wheat is grown in Mediterranean environments (Monneveux *et al.* 2012). The hulled wheat species *T. dicoccum* Schrank *ex* Schübler (emmer wheat) and *T. monococcum* (einkorn wheat) were founder crops of the Neolithic Revolution (Lev-Yadun *et al.* 2000, Gill & Friebe 2002). ‘Ancient wheat’ often refers to wheat species/subspecies which have never been subjected to any modern plant breeding programs. Recently, neglected and underutilized wheat species such as hulled wheats or Khorasan (Oriental) wheat (*T. turanicum* Jakubz.) have attracted attention of consumers especially interested in healthy and natural foods (Grausgruber *et al.* 2005). Hexaploid bread or common wheat (*T. aestivum* L.) is the most widely cultivated form of wheat representing >90% of total wheat production.

Phylogenetic studies suggest *T. urartu* Tumanian *ex* Gandilyan, *Aegilops tauschii* Cosson and *Ae. speltoides* Tausch as progenitors of the A<sup>u</sup> (in short A), D and B genome, respectively (Petersen *et al.* 2006). In a first step, tetraploid wheat evolved from hybridization between *T. urartu* (2n=2x=14 AA) and *Ae. speltoides* (2n=2x=14 BB). Subsequently, natural hybridization between tetraploid wheat (2n=4x=28 BBAA) and *Ae. tauschii* (2n=2x=14 DD) has resulted in the formation of hexaploid bread wheat (2n=6x=42, BBAADD) (**Fig. 2**).



**Fig. 2:** Current theory of the evolution of polyploidy wheat (Gill & Friebe 2002, KSU 2002)

## 2.4 Wheat growth and development

Wheat is a cool season crop which is widely grown between the latitudes 67° N and 45° S (Gustafson *et al.* 2009) in varying environments from temperate to tropical and from sea level to high altitude under diverse irrigated or rainfed cropping systems (Curtis 2002).

Among the factors controlling growth and development, wheat is most dependent on temperature (Porter & Gawith 1999). The time to any given development stage is measured by the summation of daily mean temperatures between the two thresholds of base and optimum temperature (cardinal temperatures), called thermal time with degree days (°Cd) as unit. Although, cardinal temperatures vary between different phenological stages and for

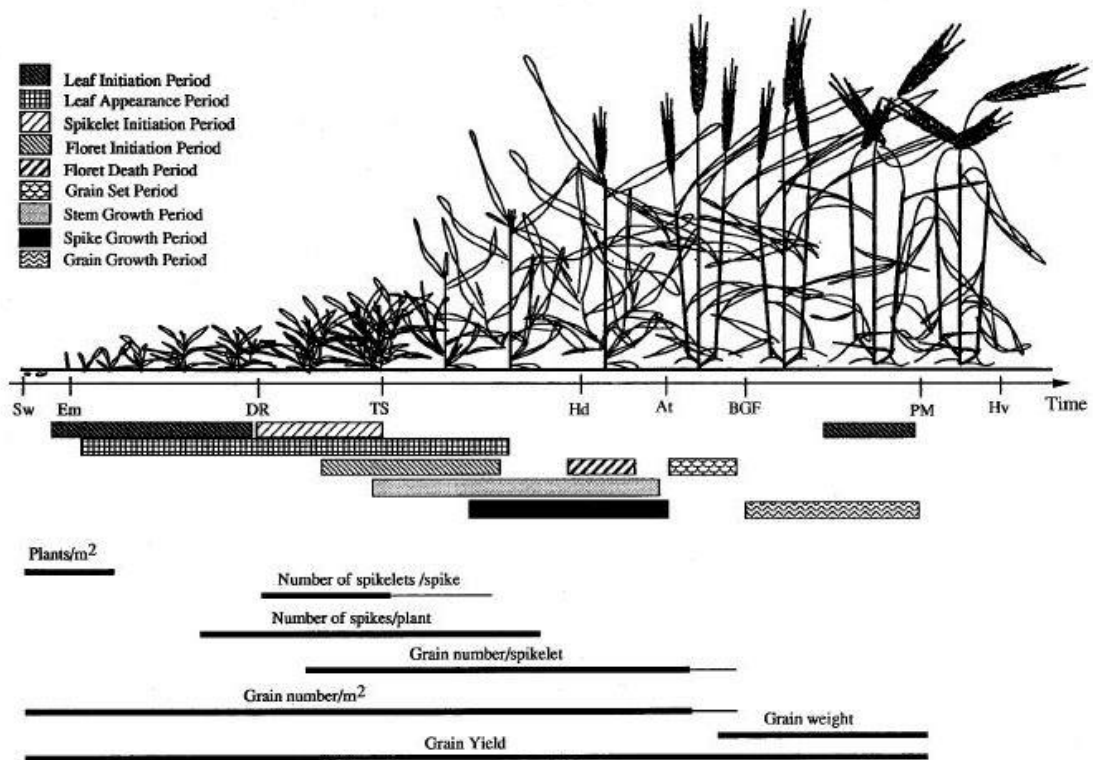
different genotypes, there is a general consensus that the cardinal temperatures for phenological stages rise steadily with plant development. Accordingly, base values increase from  $<0$  to  $>7^{\circ}\text{C}$  during grain filling, while optimum values rise from  $<22$  to  $>25^{\circ}\text{C}$  (Slafer & Rawson 1995, Porter & Gawith 1999, Salazar-Gutierrez *et al.* 2013).

According to their response to vernalization wheat can be grouped into winter or spring types. Winter wheat requires a period of cold temperatures to transit from vegetative to reproductive phase. Wheat is also a photoperiod sensitive crop, therefore, flowers earlier as day-length increases (Evans *et al.* 1975). However, over decades, breeding for photoperiod insensitivity enhanced geographic adaptability of wheat (Langer *et al.* 2014). Vernalization and photoperiod are key factors in adaption of wheat worldwide by hastening or delaying flowering in response to environmental stimuli, to ensure that floral initiation occurs at optimum temperatures (Kamran *et al.* 2014). In wheat, the transition from vegetative to reproductive phase is coincident with ceasing of tillering (Baker & Gallagher 1983) and beginning of stem elongation in which spikes and culms concurrently grow and compete for total assimilates supply (Miralles *et al.* 2000) (**Fig. 3**). On this basis, the higher solar radiation and cool temperatures during spike growth will decrease the competition for carbohydrates by an increase in availability of assimilate for spike growth and lengthening the period of spike growth (Fischer, 1985). This may explain the higher potential yield of wheat in some countries such as in southern New Zealand, Southern Chile, Ireland, England and some regions of China (Hsiao 2012).

## **2.5 Improvement of wheat grain yield**

Wheat yield has remarkably improved over the 2<sup>nd</sup> half of the 20<sup>th</sup> century (Calderini & Slafer 1998) as a result of the fruitful combination of genetic improvement and increased resource availability, particularly water and nitrogen (Sinclair & Rufty 2012). However, in the last decades, the rate of yield improvement has declined (Hall & Richards 2013). The initial genetic gains in wheat yield have been associated with the introduction of semi-dwarf and fertilizer-responsive cultivars during the 1960s and 1970s, the era known as the ‘Green Revolution’. In fact, genetically reduced plant stature has increased harvest index (HI), as a result of reduced competition between the growing stem and spike and, hence, more grains per unit area were realized (Fischer & Stockman 1986). Studies on historic wheat nurseries revealed also an enhanced leaf photosynthetic rate and stomatal conductance in modern varieties (Fischer *et al.* 1998). Improvement in wheat grain yield has largely been driven in the

past by improvements in HI rather than increased biomass. Thus, HI should already approach its theoretical limit (Sayre *et al.* 1997, Shearman *et al.* 2005, Fischer & Edmeades 2010, Foulkes *et al.* 2011, Sadras & Lawson 2011). On this basis, wheat biomass improvement by increasing radiation use efficiency has been suggested as promising opportunity to further increase wheat yield potential beyond its current genetic limits (Parry *et al.* 2011). Reduced competition from non-generative sinks, particularly during stem elongation when the major yield component (*i.e.* grain number) is determined (Fischer 1985), can result in an increase of partitioning to grains (Foulkes *et al.* 2011). In that respect, extending the period from terminal spikelet initiation to heading (stem elongation) has been proposed to further improve yield potential of wheat (Slafer *et al.* 1996).



**Fig 3:** Wheat growth and development stages in relation to periods in which yield components are determined (Sw, sowing; Em, emergence; DR, double ridge appearance; TS, terminal spikelet initiation; Hd, heading; At, anthesis; BGF, beginning of grain filling period; PM, physiological maturity; Hv, harvest (Slafer & Rawson 1994).

## **2.6 Wheat root system**

As global crop production is more and more threatened by water and nutrient scarcity, plant root systems have attracted attention as an opportunity for yield improvement (de Dorlodot *et al.* 2007, Lynch 2007, Vadez *et al.* 2007, Comas *et al.* 2013). However, decades of breeding for yield under high input conditions have led to a genetically narrow germplasm with losses of potential adaptive traits for stress-environments. This is particularly evident for the wheat root system as demonstrated by Waines & Ehdaie (2007).

Root systems are still inadequately exploited in crop improvement, as there are limited high-throughput phenotyping methods for roots in the field (Zobel & Waisel 2010, Palta *et al.* 2011, Ehdaie *et al.* 2012, Wasson *et al.* 2012). Moreover root system traits that affect root architecture and root function have a polygenic nature (Lynch 2007, Hall & Richards 2013). Nevertheless, continued efforts resulted in the identification of root traits conferring tolerance to water stress, *e.g.* (i) deep rooting systems with greater radial hydraulic conductivity at depth in rainfed systems where crops rely on deep water for grain filling (Wasson *et al.* 2012), (ii) small root diameter, large specific root length and root length density (Comas *et al.* 2013) and (iii) optimized seminal root angle (Manschadi *et al.* 2006, 2008). There is also limited evidence of yield improvement through the introgression of root-adaptive traits (*e.g.* small xylem diameter of seminal axile root) into elite parental lines under drought prone environments (Richards & Passioura 1989).

## **2.7 Yield improvement in drought-prone environments**

Drought is the most significant environmental stress in agriculture worldwide. Since the 1970s droughts have become more common, especially in the tropics and sub-tropics. By 2100 a temperature rise between 1 and 6°C is predicted (Stott & Kettleborough 2002, Meehl *et al.* 2007). Cereal yields are expected to decline by 5 and 10% for a temperature rise of 2 and 4°C, respectively (Turrall *et al.* 2011); for wheat production in South Australia even a yield loss of up to 30% was projected (Luo *et al.* 2005). Maize yields in Africa and Latin America are predicted to decrease by 10% by 2055 (Jones *et al.* 2003). For most production areas a yield enhancing effect of increased CO<sub>2</sub> concentration is much smaller than expected and insignificant compared to the overriding influence of decreased rainfall (Luo *et al.* 2005, Leakey *et al.* 2009). Improving yield potential and stability (Powell *et al.* 2012) under drought



is, therefore, a major goal of plant breeding alongside integrated soil fertility and cropland management (FAO 2005a,b, Vanlauwe *et al.* 2010).

Further yield improvements under drought conditions are necessary in the future to feed the world. A better understanding of secondary traits for an easy and efficient selection is a must. Sophisticated experimental designs and statistical analysis of field trials in the targeted population of environments can significantly improve heritability and, therefore, response to selection. The complexity of drought resistance can be dissected into single traits (**Fig. 4**). An improvement in any of these traits can contribute to an increased resistance (Blum 2011a, Tardieu 2012). Multidisciplinary research will be necessary to identify drought tolerant genotypes, to understand the biophysical, physiological and biochemical basis of tolerance, to identify the responsible genes and finally to transfer these genes into high-yielding material (Maiti *et al.* 2000, Blum 2011b). The main challenge in the process will be the development of rapid, reliable, cost-effective and non-destructive selection methods which can be applied for high-throughput phenotyping on the field.

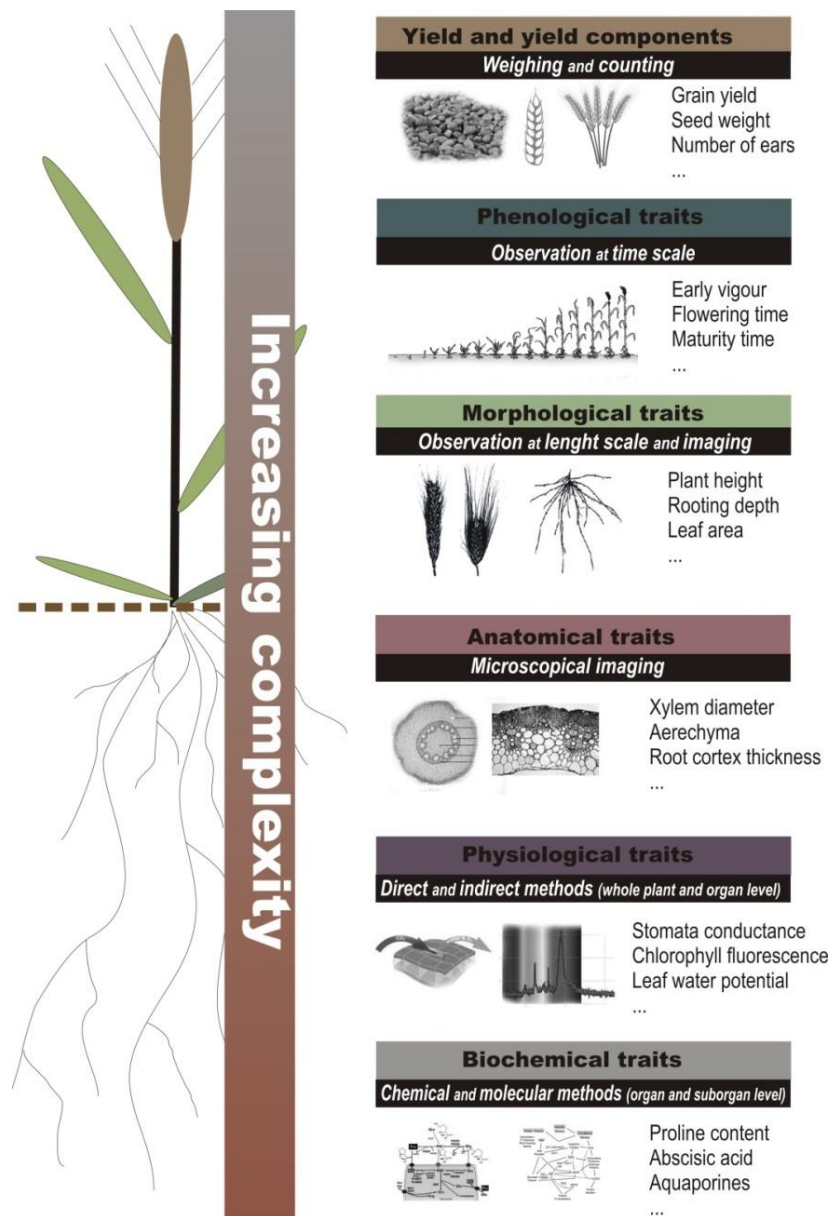
#### ***Drought definition and site characterization***

Stress can be defined as any situation where external constraints limit dry-matter accumulation (Jones & Jones 1989). Drought, in its agricultural sense, is defined as an imbalance of water supply in relation to plant demand, inducing plant adaptive response and eventually growth and yield reduction. By meteorological definition; however, drought is defined when there is a prolonged period reduction of precipitation compared to long term averages (Heim 2002, Blum 2011a).

Understanding crop response to drought and relevant traits conferring better stress resistance requires a precise environmental characterization (Blum 2011a). Several climatic indices were described to characterize drought stress environment (Heim 2002). Ecohydrology is a new approach that integrates meteorological, hydrological and vegetation perspectives on drought at the ecosystem scale (Rodriguez-Iturbe *et al.* 2001) in order to explain *e.g.* vegetation composition and shifts as well as characteristic ecosystem traits (Porporato *et al.* 2001, Preti *et al.* 2010).

In the temperate region of central-eastern Europe, semi-arid to sub-humid continental climates are typically found. These regions are characterized with a higher proportion of in-season rainfall compared to stored soil moisture as a source of crop water supply. Thereby, they

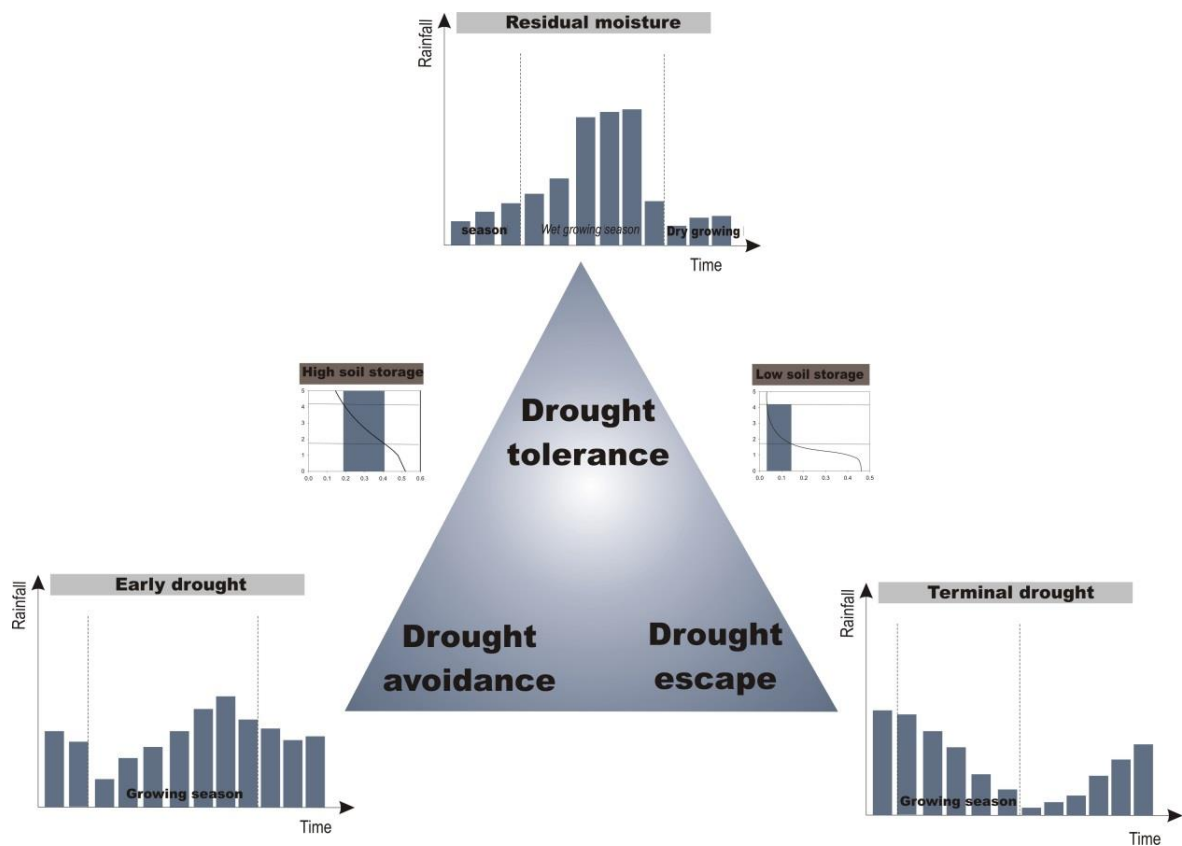
differ essentially from storage driven Mediterranean climates or subtropical sites where crops are grown on residual soil moisture. However, stored soil water can be still essential to buffer temporary dry periods affecting crop yield particularly when their occurrence coincides with sensitive growth stages such as flowering.



**Fig. 4:** The world of drought tolerance: from simple to complex aboveground and belowground traits

### ***Adaptive drought strategies***

From the plant's point of view the most objective drought impact is a reduction in plant water potential (Blum 2011b). Studying adaptation and resistance to stress in natural plant communities, most authors rely on Levitt's framework (Levitt 1980) in which three main responses to water stress are identified: (i) drought escape, (ii) dehydration tolerance and (iii) dehydration avoidance. Yield improvement by breeding for drought resistance mechanisms depends strongly on the drought regime *i.e.* drought duration, severity and time of occurrence (van Ginkel *et al.* 1998, Farooq *et al.* 2009a, Blum 2011b). **Fig. 5** gives an overview of drought regimes and plant adaptive responses of major interest in the distinct environments following van Ginkel *et al.* (1998).



**Fig 5:** Major drought regimes and related plant adaptive mechanisms considered most relevant for superior performance of crops

Drought escape by *e.g.* early maturity (phenological adaptation) might cost vegetation time in early drought environments, while being effective in summer-dry Mediterranean regions. Dehydration avoidance by “water saving” might result in suboptimal use of available water,

while in other situations a “conservative” water use saves water for grain filling and yield formation in environments with decreasing rainfall over the vegetation period (Mori *et al.* 2011). Dehydration avoidance by quick root depth penetration and sustained water uptake during drought periods (“water spenders”) is of major interest for early drought environments and generally considered as relevant breeding target (Blum 2009). Dehydration tolerance, as found in xerophytic plants, is relevant for continuous and high intensity stress conditions that are found in residual moisture environments or soils with low water storage capacity. Productivity of such extreme environments might be too low for agriculture or require irrigation to ensure an acceptable yield level. Overall, different drought regimes may require different selection strategies (Blum 2011a, Tardieu 2012).

## **2.8 Screening methodology for drought resistance**

### ***Test sites, experimental design and analysis***

Progress in yield was demonstrated to be small in areas prone to water stress (*e.g.* Argentina, Australia, Canada, USA) compared to countries with good water conditions (*e.g.* France, Germany, UK) (Araus *et al.* 2002). Due to the multigenic character of yield, its heritability is low and can be once again lower in stressed environments caused by greater genotype by environment interaction (GEI). Therefore, genetic gains are harder to realize under drought stress. Reliable screening methods are necessary to improve heritability of yield under drought stress. Alternative strategies and selection based on secondary traits, which are correlated with yield and have a higher heritability *per se*, have been proposed by various authors (Araus *et al.* 2002, Cattivelli *et al.* 2008, Richards *et al.* 2010, Badu-Apraku *et al.* 2012, Powell *et al.* 2012).

Yield improvements in most arable crops were realized in the last decades by intensive testing of advanced breeding lines or hybrids in multiple sites over multiple years (METs, multi-environment trials) which were expected to represent the target environments. Genetic gain (response to selection) depends on selection pressure (intensity), total variation and heritability (repeatability) of the trait. Heritability in turn depends on the variation caused by genotype, GEI and experimental error; the higher GEI and experimental error, the lower is heritability. Therefore, choice of test sites, experimental design and statistical analysis has to bear in mind the minimization of GEI and experimental error. Targeted management of stress

conditions (Bänziger *et al.* 2004), classification of sites and years into mega-environments (DeLacy *et al.* 1994, Yan & Tinker 2006, Yan & Holland 2010) and/or adjustment for flowering/maturity (Bänziger *et al.* 2004) can improve the accuracy of MET results. Appropriate experimental designs and corresponding statistical analysis can account for natural and extraneous variation within a field trial (Gilmour *et al.* 1997, Barker *et al.* 2005, Stefanova *et al.* 2009, Stringer *et al.* 2012) and reduce the experimental error. Spatial analysis using linear mixed models are perfectly suited to resolve unreplicated trials with checks (Payne 2006). Thereby, (i) field trials can be carried out already in early generations when less amount of seeds is available, (ii) the number of tested genotypes per site can be increased for the same space and workload, and (iii) additional test sites can be included instead of replications. Once the optimal (spatial) model is identified for each trial, this information can be included in the MET analysis using appropriate mixed models with an optimal variance-covariance structure between environments (Smith *et al.* 2001). Finally the breeder should end up with better genotypic estimators and/or predictors. Breeding of many crops rely today on the production of doubled haploid plants which allows an early testing of the total variation present in homozygous lines. Therefore, advanced experimental designs and analyzing methods allowing a multi-environment testing of a huge amount of breeding lines becomes more and more important for an efficient selection and thus genetic gain.

#### ***Aboveground traits: simplicity or complexity?***

Significant advances in genomics in recent years led to breeding programmes based on *e.g.* marker-assisted selection, genomic selection, etc. Gene expression studies revealed a long list of genes involved in drought response suggesting a very complex nature of drought tolerance hardly to handle for a field-based breeder. However, from an agronomic point of view drought resistance appears much simpler. Constitutive or adaptive resistance mechanisms are well known and breeding successes in various crops demonstrate that they work (Blum 2011b). Especially with respect to drought breeding progress is restricted if the selection environments and traits are not appropriate. Different drought regimes may require different selection strategies (Blum 2011a, Tardieu 2012).

#### ***Morphological and anatomical traits***

Morphological traits (*e.g.* plant height, pubescence and glaucousness of leaves or stems, epicuticular wax deposition, cuticle thickness, leaf area and thickness, leaf rolling, stay

green/leaf senescence, size and compactness of palisade tissue, thick collenchyma in stout petiole, partial sterility and size of inflorescence, size and number of xylem vessels, stem solidness, tillering), yield potential and components should be used for selection in early generations since they are rapid, simple, accurate and cost-effective to determine at this stage of a breeding program (Richards *et al.* 1986, Blum 1989, Armstrong *et al.* 1994, Bänziger *et al.* 2000, Duggan *et al.* 2005, Zou *et al.* 2007, Richards *et al.* 2010, Saint Pierre *et al.* 2010, Maiti *et al.* 2012). In further generations other secondary traits can be incorporated in selection.

#### *Phenological traits*

Seedling vigour and coleoptile/mesocotyl length are important traits for crop establishment in dry season cropping systems (residual moisture). Thereby, the seedling is able to emerge from deep planting (where soil moisture is greater) and from soils with high surface temperature (Ellis *et al.* 2004, Reddy *et al.* 2009). Moreover, nutrient and water uptake is increased by early root growth (Liao *et al.* 2006). Vigorous juvenile growth results in early ground cover which can optimize water use efficiency (WUE) and limit water loss due to direct evaporation from the soil surface (Cooper *et al.* 1987). However, excessive canopy development may cause early depletion of soil moisture leaving little soil water for grain filling (Richards *et al.* 2010).

Selection for early flowering and maturity is the most widely used drought escape mechanism where terminal water stress constrains grain filling. In wheat, anthesis and other developmental stages can be controlled by allelic combinations of vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*) genes (Worland & Snape 2001). In maize grain yield under drought stress was significantly improved by shortening the anthesis-to-silking interval (Bänziger *et al.* 2000, Barker *et al.* 2005, Araus *et al.* 2012). For various other crops better performance in low rainfall environments by earlier flowering was reported (Rose *et al.* 1992, Thurling & Kaveeta 1992, Hall 2012).

#### *Physiological traits*

In the early 1980s remote sensing methods became adopted in agriculture. Today precision farming uses various sensors to manage *e.g.* fertilization or irrigation. In breeding for drought tolerance either data from spectral reflectance or infrared (IR) thermometry have been widely used to assess the water and chlorophyll status of crops and/or canopy temperature (Amani *et*

*al.* 1996, Winterhalter *et al.* 2011, Boureima *et al.* 2012, Lopes & Reynolds 2012, Ndjiondjop *et al.* 2012). High correlations between canopy water mass and spectral indices and IR-temperature, respectively, and between grain yield and normalized difference vegetation index (NDVI) was observed in maize (Lu *et al.* 2011, Winterhalter *et al.* 2011) demonstrating their suitability as non-destructive high throughput phenotyping methods.

Stomatal closure is a rapid and effective drought avoidance response which in turn leads to a decrease in net photosynthesis. An increase in WUE through decrease of stomatal conductance and increase of stomatal density was observed in alfalfa (He *et al.* 2012) indicating the ability for regulation of CO<sub>2</sub> intake and water loss which is related to anti-oxidation and osmotic adjustment. Likewise, transgenic tomato showed resistance to soil water deficit by reduction of their stomatal density and stomatal pore size (Mishra *et al.* 2012). In wheat leaf chlorophyll content and stomatal conductance were proposed as proper criteria for identifying drought tolerant genotypes under field conditions (Khamssi & Najaphy 2012). A decrease of stomatal conductance in response to water deficit has been reported also in other studies (Taheri *et al.* 2011, Boureima *et al.* 2012, Fenta *et al.* 2012). Yield potential increase of CIMMYT wheat varieties between 1962 and 1998 was mainly attributed to increased stomatal conductance and canopy temperature depression (Fischer *et al.* 1998). A strong correlation between grain yield and canopy conductance was also observed in rice (Horie 2000). Leaf porometers and IR gas analysers have been mostly used to measure stomatal conductance (Taheri *et al.* 2011, Fenta *et al.* 2012, Khamssi & Najaphy 2012). Although, leaf porometers are easy-to-handle and inexpensive they are not suitable for high throughput phenotyping unless several porometers are used simultaneously. Otherwise, diurnal fluctuation in temperature, humidity, air flow and radiation cause a bias in results (Araus *et al.* 2012). Stomatal conductance is, however, well correlated with canopy temperature which can be measured by remote sensing methods in a high-throughput manner if conditions are appropriate (low cloud cover, low wind speed) (Amani *et al.* 1996, Munns *et al.* 2010, Romano *et al.* 2011).

The effects of drought stress on photosynthetic activity have been investigated in recent years in various crops, *e.g.* wheat (Roostaei *et al.* 2011, Kumar *et al.* 2012), soybean (Fenta *et al.* 2012), chickpea (Rahbarian *et al.* 2011), sesame (Boureima *et al.* 2012), olive (Faraloni *et al.* 2011) and ornamentals (Ow *et al.* 2011). Gas exchange techniques to assess photosynthesis are too time consuming for breeding purposes. Photochemical efficiency of photosystem II

(PSII) can be assessed via the maximum chlorophyll a florescence ratio  $F_v/F_m$  (Khamssi *et al.* 2012). Fluorescence parameters were successfully applied to identify drought-tolerant genotypes in *e.g.* wheat (Roostaei *et al.* 2011), soybean (Fenta *et al.* 2012), chickpea (Rahbarian *et al.* 2011) and sesame (Boureima *et al.* 2012). Hitherto, no method is available to measure photosynthesis and its components directly in a high throughput manner. Leaf chlorophyll concentration as an indirect indicator of photosynthesis activity can be measured easy and fast with a portable SPAD-meter. A high heritability was observed for SPAD values of CIMMYT wheat lines tested globally across environments where heat and drought stress often constrain yield (Lopes & Reynolds 2012).

#### *Biochemical traits*

Osmoregulation (osmotic adjustment, OA) of cells as adaptive mechanism to environmental stress involves an increase in low molecular mass compounds (compatible solutes) leading to the maintenance of turgor (Morgan 1983). OA is a major component of drought resistance and can be estimated by regression of leaf relative water content (RWC) on leaf osmotic potential (Babu *et al.* 1999). OA was demonstrated to be a suitable method for identifying drought tolerant genotypes (Morgan 1983, McCree *et al.* 1984, Blum 1989), however, the method can be applied only to a moderate number of genotypes and not to large breeding populations (Blum 1989, Setter 2012).

Absciscic acid (ABA) accumulates under drought stress. It is believed that the extent of ABA accumulation is a good indicator of adaptation to drought. Furthermore, ABA as a stress-signalling hormone can mediate many stress responses by modification of protein synthesis (Blum 2011b, Peleg & Blumwald 2011, Bano *et al.* 2012, Setter 2012, Wilkinson *et al.* 2012). Under drought stress an increase of ABA results in RWC enhancement (Blum 2011b). In wheat exposed to drought stress during anthesis ABA increased while seed set and consequently grain yield decreased (Weldearegay *et al.* 2012). Therefore, lower ABA concentration in reproductive organs under drought can result in higher grain yield and be an indicator of drought tolerance. An ideal genotype was described having low levels of leaf ABA, good root depth and water transport properties and high stomatal sensitivity to ABA (Setter 2012). ABA can be determined by physical-chemical methods like mass spectrometry or immuno-chemical methods like radioimmunoassay. The low cost and ease of scale-up of the latter could be considered for high-throughput projects. Care has to be taken with respect to timing of tissue sampling for measurements as leaf ABA levels depend on environmental



and tissue development conditions and results can be significantly influenced by GEI (Setter 2012).

Under drought stress an increase of ABA is followed by increased activities of antioxidant enzymes, *e.g.* superoxide dismutase (SOD) and peroxidase (POD) in order to scavenge harmful reactive oxygen species (ROS) (Mittler & Zilinskas 1994, Wang *et al.* 2009). In transgenic rice SOD was demonstrated to improve drought tolerance (Wang *et al.* 2005). A wide range of other constituents was described to be influenced by drought stress, *e.g.* amino acids (proline, tryptophan, etc.), compatible solutes, sugars and antioxidants (Liu *et al.* 2011, Bowne *et al.* 2012, Setter 2012). Analysis of these constituents is often cumbersome or if suitable for high-throughput screening associated with high costs. Metabolomic methods will for sure be applied in large-scale projects in the future and unravel the importance of these constituents for drought tolerance breeding programs.

#### *Stable isotopes*

A promising approach to identify plant varieties with enhanced WUE in drought-prone environments is carbon isotope discrimination (CID) and oxygen isotope composition. The method relies fully on the natural variability of the target crops (Condon *et al.* 2002, 2006) and is less time consuming. CID has been proposed as an indirect selection criterion for wheat grain yield under drought (Farquhar & Richards 1984, Merah *et al.* 2001a, Monneveux *et al.* 2005, Adu-Gyamfi *et al.* 2012a) and was successfully employed in breeding Australian wheat varieties with higher WUE (Condon *et al.* 2004). Naturally two stable isotopes of carbon occur, *i.e.*  $^{12}\text{C}$  and  $^{13}\text{C}$ . The discrimination against the heavier  $^{13}\text{C}$  isotope in favor of the lighter  $^{12}\text{C}$  during photosynthesis forms the basis of CID ( $\Delta^{13}\text{C}$ ,  $\Delta$ ) technique (O'Leary 1981). Since CID is related to WUE it can be used as surrogate marker for the identification of drought tolerant varieties. For  $\text{C}_3$  plants  $\Delta$  is related to  $\text{CO}_2$  diffusion in air, discrimination against  $^{13}\text{CO}_2$  by RuBisCo (ribulose-1,5-bisphosphate carboxylase oxygenase) and the ratio of intercellular to ambient partial pressure of  $\text{CO}_2$ . Similarly for  $\text{C}_4$  plants the fraction of the dissolution of  $\text{CO}_2$  to  $\text{HCO}_3$  and fixation by PEP (phosphoenolpyruvate) and the fraction of  $\text{CO}_2$  fixed by PEP carboxylase have to be considered (Farquhar *et al.* 1989). Positive correlations between CID and transpiration efficiency and/or productivity have been reported in various crops (Farquhar *et al.* 1982, Condon *et al.* 1987, Ehleringer 1990, Acevedo 1993, Ismail *et al.* 1994, Wright *et al.* 1994, Nageswara Rao *et al.* 1995, Merah *et al.* 2001b, Condon *et al.* 2002, Araus *et al.* 2004). CID has practical advantages over measuring stomatal

conductance by instantaneous measurements. Plant samples (*e.g.* leaves) can be easily collected for analyses and the method enables the analysis of a large number of samples.

Results from the International Atomic Energy Agency (IAEA) Coordinated Research Project (CRP) carried out in Algeria, Australia, Bangladesh, China, India, Morocco, Pakistan, Philippines, Syrian Arab Republic and Yemen, have shown that many variables are known to affect CID including phenotype, type and age of plant organ, environmental conditions (especially water regime) and edaphic factors (*e.g.* soil salinity), while very little is known in regard to other soil-related factors, *e.g.* plant nutrition. Thus, it is important to specify as precisely as possible measurement parameters. Results from CRP countries showed that considerable genetic variation in CID exists within large populations of rice and wheat. Observed extreme values were  $\Delta = 4.7\text{‰}$  for rice,  $4.8\text{‰}$  for common wheat and  $5.7\text{‰}$  for durum wheat (sampled across phenotypes, plant organs and abiotic stress factors), whereas for a given plant organ within a small population  $\Delta$  is usually relatively small ( $\leq 2.6\text{‰}$ ) (IAEA 2012).

Earliness has to be considered if CID is used in breeding. Earliness is a widely exploited drought escape mechanism in environments affected by terminal water stress. There, early genotypes can fix much of their carbon in a period relatively free of drought. Negative correlations between  $\Delta$  and total biomass production under field conditions are the result and were observed in various crops (Hubick *et al.* 1986, Craufurd *et al.* 1991, Ehdaie *et al.* 1991, Condon *et al.* 1993, Menéndez & Hall 1997) which is contradictory to the studies mentioned above. Hence, when applying CID in breeding programs two types of environments have to be considered: (i) post-anthesis drought stress where the selection for high grain CID would give grain yield advantage (Condon *et al.* 2002), and (ii) pre-anthesis drought stress as under rainfed conditions relying on residual soil moisture. Based on earlier Australian studies the hypothesis is that selection for low leaf CID would give a grain yield advantage (Condon *et al.* 1993). However, this hypothesis was refuted when tested in Pakistan, Yemen, India and China. When grain CID was measured, positive correlations were observed in environments with high air temperature during grain filling (IAEA 2012). A more comprehensive classification of environments in crop specific growing regions is needed to determine the applicability of the technique in a wider range of target regions.

CID is usually determined on early leaves (BBCH 14, 4 leaves unfolded) and grain samples which are collected before water stress and at maturity, respectively.  $\delta^{13}\text{C}$  is measured with an

isotope ratio mass spectrometer. The primary reference standard for  $\delta^{13}\text{C}$  measurements is fossil carbonate (Pee Dee Belemnite), limestone formation in South Carolina, USA. Traits such as number of tillers, plant height, heading/flowering date, maturity, length of inflorescence, number of grains per inflorescence, 1000 grain weight, biomass and grain yields are also recorded. Rainfall, air temperature, relative humidity and solar radiation need to be recorded. Grain and biomass WUE are determined by dividing the respective yield by the quantity of water consumed during the growth period.

Relationships between CID and crop yields can vary (positive or negative) depending on time and severity of stress during crop development, plant organ which is studied as well as the crop itself. Therefore, a more robust technique is required that is less sensitive to such variations. Plant  $^{18}\text{O}/^{16}\text{O}$  ratios have been shown to vary with transpiration rate, which is closely related to carbohydrate assimilation during photosynthesis, and could, therefore, be used as an indicator of drought tolerance (Condon & Hall 1997). In the absence of rainfall, crops that are better able to access subsoil water may have more open stomata. Thus, genotypic differences in stomatal conductance and canopy temperature may reflect differences in access to water. Stable isotopes laid down in plant dry matter could provide a time integrated measure of differences in stomatal conductance. Variation in oxygen isotope composition may be more strongly related to stomatal conductance than CID and, therefore, provide stronger relationships with water extraction. Genotypes that are better able to extract subsoil water during grain filling might be expected to have higher values of  $\Delta^{13}\text{C}$  and/or lower values of  $\delta^{18}\text{O}$  in the grain (Condon & Hall 1997).

There may be another influence on the C and O isotopic composition of grain. Grain filling in wheat relies to varying degrees on carbohydrates assimilated by photosynthesis during grain filling and on carbohydrates acquired earlier in the season and stored mainly in the stems for later re-translocation to the grain. The C and O isotopic signatures of carbohydrates acquired by the crop during grain filling should reflect the prevailing conditions of evaporative demand and soil water availability during that period, whereas the C and O isotopic signatures of stored assimilates re-translocated to the grain are likely to be different, *i.e.* representative of conditions when those assimilates were first acquired (Barbour *et al.* 2000). In Mediterranean environments, conditions are usually more favorable before grain filling. Given this scenario, genotypes that are more reliant on re-translocation of assimilates for grain filling might be expected to have higher values of  $\Delta^{13}\text{C}$  and/or lower values of  $\delta^{18}\text{O}$ . Preliminary results of

field-grown wheat have shown that variations in  $^{18}\text{O}$  in grains could be a more robust indicator of drought tolerance compared to the currently used CID (Adu-Gyamfi *et al.* 2012b). More research is required to compare the two isotopic techniques in evaluating crop plants for their drought tolerance. From plant breeding, molecular-marker development and gene-discovery perspectives, it would be very useful if the contribution to yield attributable to either better access to subsoil water or re-translocation of assimilates could be separated. This may be possible by measuring C and O isotope composition of grain and other plant parts harvested before and during grain filling. Although working well cheaper alternatives to CID are needed for screening whole breeding programs. There is an exciting opportunity to exploit other surrogates such as correlation between grain ash and grain CID.

#### *High-throughput phenotyping*

High-throughput phenotyping platforms (HTPP) developed for plants under controlled conditions are used to provide reliable estimates of phenotypic traits. HTPP are usually equipped with sensors for thermal infrared, visible and near infrared, and fluorescence imaging (Berger *et al.* 2010). Plants are grown under controlled conditions in pots and transported via conveyer belt automatically and regularly to the fixed recording cabinet (Berger *et al.* 2010) or the recording device is movable to fixed places of the plants, *e.g.* if the crop is too tall for transport (van der Heijden *et al.* 2012). Phenotyping experiments under controlled conditions require careful planning (*e.g.* pot size, growth medium, water and nutrient supply, light quantity etc.) to ensure within-laboratory replicability (Poorter *et al.* 2012a ,b).

High-throughput phenotyping on the field uses various sensor technology (Maes *et al.* 2012, Montes *et al.* 2011, Römer *et al.* 2012). The challenge is the optimal platform (Comar *et al.* 2012, White *et al.* 2012) to ensure records of high quality. Wherever the spectral data are gained, greenhouse or field, sophisticated biometrical methods are necessary to calibrate and validate the data and build robust prediction models (Cabrera-Bosquet *et al.* 2012, Römer *et al.* 2012).

#### ***Belowground traits: the roots mystery***

A fundamental part of the plant to provide superior dehydration avoidance is the root system. Systematic breeding efforts for root system properties have been realized in rice (Price *et al.* 2002, Kato *et al.* 2006, Farooq *et al.* 2009b) and chickpea (Kashiwagi *et al.* 2005, Gaur *et al.*

2008). However, in most breeding programs roots are absent as target trait mainly due to measurement difficulties. Root methods can be distinguished between *in situ* (field) methods, rhizotron methods in soil or soil-like medium, and observation methods in non-soil medium (gel, filter paper). The latter are mostly restricted to the measurement of the primary root characteristics of small plants (seedlings) (Smit *et al.* 2000, Luster & Finlay 2006, Neumann *et al.* 2009).

### *Root morphology*

Field sampling of rooting depth and distribution ensures natural root expansion and avoids artifacts due to restrictions of growth volume. The core-break method using an automatic sampler is effective in sampling a high number of genotypes (Wasson *et al.* 2012). However, washing roots from soil cores and subsequent image analysis for a detailed picture of root morphology is time consuming (Himmelbauer *et al.* 2004, Benjamin & Nielsen 2005). Indirect field screening methods for root morphology are pulling resistance (Ekanayake *et al.* 1985), induced restriction of the root zone by a mesh (McKenzie *et al.* 2009) or herbicide layer (Trebuil *et al.* 1996).

Soil columns or rhizotrons are advantageous compared to field sampling in regard to (i) better control of water regime, (ii) easier sampling, and (iii) potential inclusion of architectural observations. However, also handling of soil columns/rhizotrons of sufficient size for unrestricted root growth is laborious due to their weight. Rhizotrons were applied successfully in the screening of chickpea and rice (Price *et al.* 2002, Kashiwagi *et al.* 2005). Root zone restriction using *e.g.* a wax layer of different density was applied to evaluate rooting depth and penetration strength (Löfkvist *et al.* 2005, Whalley *et al.* 2013).

Specific root length or specific surface area has been used as indicator of assimilate partitioning in the root system, particularly fine root formation (Ryser 2006). Fine rooting provides an intense root-soil contact to exploit water resources under dry conditions. Fine laterals also show a distinct growth response upon soil drying compared to primary axes (Comas *et al.* 2010). A new semi-hydroponic system for phenotyping root traits including specific root length was described recently (Chen *et al.* 2011). An indirect measure related to both, total root surface area as well as finer functional root axes, is root capacitance (Chloupek *et al.* 2010). However, despite its biophysical basis (Dalton 1995), multiple influences on the measurement signal others than root system size limit its clear biological interpretation. Other described indirect indicators like plant height, harvest index, stomata

conductance, leaf temperature and leaf water potential (Chloupek *et al.* 2006, Richards 2008, Zhang *et al.* 2009, Tuberosa 2012) require validation with direct measurement to ensure proper interpretation.

Root architecture parameters are fundamental to understand plant response and adaptation to drought. There is a lack of direct field methods to measure root architecture *in situ* beyond excavation. A quick field quantification of root branching traits of maize based on visual scoring was described as “shovelomics” (Trachsel *et al.* 2011). Ground penetrating radar has been used to detect coarse roots, but resolution is still a limitation for application in annual crops (Guo *et al.* 2013). To obtain root architecture for larger samples most studies use laboratory methods, mostly based on seedling roots in non-soil growth media (Sanguineti *et al.* 2007, Manschadi *et al.* 2008, Hund *et al.* 2009). Thereby, information on branching strategies of mature root systems is lacking and results could be biased by the artificial growth medium (Wojciechowski 2009). For characterizing mature root system architecture in soil, rhizobox systems are commonly used (Smit *et al.* 2000, Price *et al.* 2002, Neumann *et al.* 2009). Root phenotyping in such semi-natural systems could be done by spectral image analysis (Nakaji *et al.* 2008, Pierret 2008) instead of time consuming root washing. Depending on the wavelength spectrum, several information on the root-soil system is obtained, *e.g.* root architecture, root age (Kusumo *et al.* 2011) and soil water content (Mouazen *et al.* 2006). Spectral analysis is currently implemented as root screening method for large scale phenotyping platforms (Rascher *et al.* 2011).

#### *Root anatomy*

Root anatomy is a sensitive indicator for plant response to drought (Shao *et al.* 2008). Roots protect against anoxia from waterlogging by formation of aerenchyma in the root cortex (Changdee *et al.* 2008). Under drought roots frequently show the formation of suberinization to avoid water outflow (Henry *et al.* 2012). Anatomical traits such as cortex thickness, root endodermal silicification, and number and diameter of xylem vessels could be targeted in drought resistance breeding (Lux *et al.* 2002, Watt *et al.* 2008, Maiti *et al.* 2012). In wheat, genotypes with a narrower xylem diameter were identified in landraces (Richards & Passioura 1981a).

### *Root physiology*

Root physiology is difficult to use in breeding due to a lack of proper methods. Direct measurement of water uptake requires complex measurement of hydraulic conductance between soil, root and stem (Steudle 2000). Active regulation of water uptake is mediated by aquaporins which could contribute to drought tolerance (Sade *et al.* 2009). Indirectly root functioning can be assessed by water depletion profiles measured by soil moisture sensors or non-invasive methods. This is particularly important for sites where deep water resources are essential for better crop performance (Khan *et al.* 2010). Soil water monitoring in large populations however is restricted by time required for sensor installation as well as costs. Non-invasive screening methods are promising (Srayeddin & Doussan 2009), but resolution can limit their application. Aboveground methods indicating dehydration avoidance by enhanced uptake are proper indicators of distinct root functioning and can provide information for further in depth direct measurements.

### 3. Material and Methods

#### 3.1 Plant material

Root characteristics and above-ground traits of wheat genotypes from different ploidy levels, origins and domestication/breeding pressure were examined in a two-year field experiment (**Table 1**). In 2011 seven durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husnot), two Khorasan wheat (*T. turgidum* subsp. *uranicum* (Jakubz.) Á. Löve & D. Löve), two einkorn wheat (*T. monococcum* L. subsp. *monococcum*), and one Zanduri wheat (*T. timopheevii* (Zhuk.) Zhuk. subsp. *timopheevii*) were tested. In 2012, six contrasting genotypes from the previous year were examined along with two common wheat (*T. aestivum* L. subsp. *aestivum*) varieties and one Persian wheat (*T. turgidum* subsp. *carthlicum* (Nevski in Kom.) Á. Löve & D. Löve).

**Table 1:** Characteristics and origin of the wheat germplasm used in the experiment

Year/Genotype	Origin <sup>1</sup>	Donor/Breeder	Ploidy/Genome	Species
2011				
SZD3146	AT	Saatzucht Donau, AT	4×, BA <sup>u</sup>	durum
Clovis	FR	GIE Eurodur, FR	4×, BA <sup>u</sup>	durum
7060 <sup>2</sup> ; 7063; 7094	MX	CIMMYT, MX	4×, BA <sup>u</sup>	durum
TRI5254	?	IPK Gatersleben, DE	4×, BA <sup>u</sup>	Khorasan
2011-2012				
QK-77 (Kamut <sup>®</sup> )	US	AGES, Vienna, AT	4×, BA <sup>u</sup>	Khorasan
Floradur	AT	Saatzucht Donau, AT	4×, BA <sup>u</sup>	durum
Matt	US	Arizona Plant Breeders, US	4×, BA <sup>u</sup>	durum
PI428154; PI428165	TR	NSGC, Aberdeen, US	2×, A <sup>m</sup>	einkorn
W9	GE	GSAU, Tbilisi, GE	4×, GA <sup>m</sup>	Zanduri
2012				
W13	GE	GSAU, Tbilisi, GE	4×, BA <sup>u</sup>	Persian
Tabasi	IR	IFA Tulln, AT	6×, BA <sup>u</sup> D	common
Taifun	DE	KWS Lochow GmbH, DE	6×, BA <sup>u</sup> D	common

<sup>1</sup> AT, Austria; DE, Germany; FR, France; GE, Georgia; IR, Iran; MX, Mexico; TR, Turkey; US, United States

<sup>2</sup> Entry codes of the 40<sup>th</sup> IDS (International durum wheat screening nursery)



### 3.2 Experimental conditions

Field experiments were carried out under rainfed conditions at an experimental field located in Raasdorf (48°14'N, 16°35'E, 156 m) in the Pannonian plains of Austria. The experimental site is characterized by continental climate (Köppen-Geiger Cfb) (Rubel & Kottek 2010) with a calcic chernozem soil type (FAO 2007). Long term annual precipitation and mean temperature are 538 mm and 10.6°C, respectively. Daily weather data were obtained from a weather station located at the trial site. Physical and chemical soil properties of the experimental site are given in **Table 2**.

**Table 2:** Physical and chemical soil properties of the experimental site Raasdorf

Horizon	Depth (cm)	Sand (kg·kg <sup>-1</sup> )	Silt (kg·kg <sup>-1</sup> )	Clay (kg·kg <sup>-1</sup> )	Texture (USDA)	C <sub>org</sub> (kg·kg <sup>-1</sup> )	Field capacity (cm <sup>3</sup> ·cm <sup>-3</sup> )	Wilting point (cm <sup>3</sup> ·cm <sup>-3</sup> )
A	0-40	0.19	0.56	0.24	silt loam	0.025	0.29	0.16
AC	40-55	0.23	0.54	0.23	silt loam	0.015	0.33	0.16
C	>55	0.22	0.62	0.16	silt loam	0.008	0.24	0.09

Hydrological condition of the experimental site were characterized using the simulation model HYDRUS 1D (Šimůnek *et al.* 2013), to assess the soil water availability during the growing season in two experimental years in relation to the long term average site hydrology. Field experiments were machine sown after post-winter soil drying and following a shallow seedbed preparation using a rotary harrow on 8<sup>th</sup> March 2011 and 20<sup>th</sup> March 2012 with a seeding rate of 400 seeds per m<sup>2</sup>. The experiments were conducted in a four replicate randomized complete block design with a plot size of 7.5 m<sup>2</sup>. Each plot consisted of 10 rows spaced 12.5 cm apart. Nitrogen fertilizer was applied at the mid to late boot stage at a rate of 50 kg N ha<sup>-1</sup>. Pesticides were applied if necessary. Detailed information on crop management is provided in **Table 3**.

**Table 3:** Soil and crop management of the field experiments at experimental site Raasdorf

2011	Crop/Treatment <sup>1</sup>
Precrop	Winter oilseed rape
	12 July 2010: disc harrow, 8 cm
	26 July 2010: cultivator, 20 cm
Soil cultivation	11 August 2010: plough, 18 cm
(date/machine/working depth)	12 August 2010: harrow
	10 November 2010: spike-tooth harrow, 10 cm
	7 March 2011: rotary harrow, 8 cm
Sowing	8 March 2011
Fertilization:	3 May 2011: 50 kg N/ha
Pesticides <sup>1</sup> :	17 March 2011: Aniten duo <sup>®</sup> 2 L/ha
	18 May 2011: Prosaro <sup>®</sup> 1 L/ha + Fastac <sup>®</sup> 0.2 L/ha
Harvesting	11 July 2011
2012	
Precrop	Winter wheat
	28 July 2011: disc harrow, 8 cm
	4 August 2011: cultivator, 20 cm
Soil cultivation	18 August 2011: plough, 18 cm
(date/machine/working depth)	22 August 2011: harrow
	29 November 2011: spike-tooth harrow, 10 cm
	18 March 2012: rotary harrow, 8 cm
Sowing	20 March 2012
Fertilization:	24 May 2012: 50 kg N/ha
Pesticides <sup>1</sup> :	25 May 2012: Aniten duo <sup>®</sup> 2 L/ha
	29 May 2012: Prosaro <sup>®</sup> 1 L/ha + Fastac <sup>®</sup> 0.2 L/ha
Harvesting	18 July 2012

<sup>1</sup> Aniten duo<sup>®</sup>: carfentrazone-ethyl + mecoprop-P plus 2,4-D; Prosaro<sup>®</sup>: prothioconazole + tebuconazole + n,n-dimethyldecanamide); Fastac<sup>®</sup>: alpha-cypermethrin

### 3.3 Phenological and physiological traits

#### *Developmental growth and time to flowering*

Crop phenology determines that to what extent water demand during different crop growth stages is synchronized with seasonal water availability. Phenology was assessed using the BBCH scale (Lancashire *et al.* 1991). Flowering is scored when ~50% of spikes contained dehiscent anthers (*i.e.* BBCH 65). Time to any given developmental stage was expressed in cumulative thermal time (CTT), measured in degree-days (°C d) as described by Salazar-Gutierrez *et al.* (2013) and assumed a constant base temperature ( $T_b$ ) of 0°C as no information

was available with respect to the specific  $T_b$  of different investigated genotypes (Slafer & Rawson 1994, Salazar-Gutierrez *et al.* 2013).

#### *Early vigor*

To assess the early leaf area development or early vigor, ground cover was measured by digital imaging, using a Canon EOS20D (Canon Inc., Tokyo) digital camera at 1.5 m height above the canopy, at early emergence and when canopy almost closed. Digital images were analyzed individually by SigmaScan Pro vers. 5.0 software (Systat Software Inc., Chicago) to identify green leaves and calculate the percentage of green ground cover as described by Richardson *et al.* (2001). Ground cover rate was defined based on the differences in ground covers between two measurements, i.e. early emergence and canopy closure, divided by thermal times cumulated during the corresponding period.

#### *Leaf chlorophyll content*

Leaf chlorophyll content, as a proxy of leaf photosynthesis (Nageswara Rao *et al.* 2001), was measured using a nondestructive method, chlorophyll meter SPAD-502Plus (Konica Minolta Holdings, Inc., Tokyo) in SPAD unit (**Fig. 6**). The principal is based on leaf chlorophyll (650 nm) and non-chlorophyll, *e.g.* cell walls (940 nm) light absorbance (Lopes & Reynolds 2012). Chlorophyll content was determined twice over the growing season, once around heading (BBCH 50) as an estimation of photosynthetic capacity and again at end of flowering and coincident with onset of seed development (BBCH 70) in order to assess the stay-green of investigated genotypes. Ten plants were randomly selected in each plot and SPAD values of the last fully developed leaf on main stem were recorded at five points along the proximal-distal axis of the leaf.

#### *Stomatal conductance*

Stomatal conductance, concurrently underling the transpiration and photosynthetic rates, was measured using AP4 porometer (**Fig. 6**; Delta-T Devices Ltd., Cambridge, UK) at the same date as SPAD measurement (*i.e.* BBCH 50). Despite being non-destructive, the method is laborious and slow. As stomata are highly sensitive to small environmental changes, care has to be taken in regard to the adequate number of measurements and also homogeneity of other environmental conditions (Clarke & Clarke 1996, Rebetzke *et al.* 2001). Stomatal conductance of five sunlit, fully expanded leaves from each plot was measured on cloudless, low-wind condition between 9:30 and 12:00 a.m.



**Fig. 6:** Leaf chlorophyll (SPAD) meter, leaf porometer and linear ceptometer (from left to right)

#### *Leaf area index*

In 2012 leaf area index (LAI), an indicator of leaf photosynthetic and evaporative area, was measured around end of flowering (BBCH 68±5.7; 83 d after flowering), using a linear ceptometer AccuPAR LP-80 (Decagon Devices Inc., Pullman, WA, USA) (**Fig. 6**). The sensor was positioned perpendicular to the row direction, below the canopy close to ground level, at the center of each plot. An external PAR sensor connected to the AccuPAR provided simultaneously above and below canopy PAR readings.

### **3.4 Root traits**

#### *Morphological root characterization and root system distribution*

In the second week of June, *i.e.* BBCH 68 and BBCH 61 of early and late maturity genotypes, respectively, when wheat roots reach their maximum depth (Gregory 2006), roots were sampled using the soil core method (Böhm 1979). Samples were taken by one augering at the center of each plot at three soil depths (10-20, 30-40 and 50-60 cm) using a hand-driven auger (cylinder diameter: 7 cm). Root samples were washed, sieved (1.6 and 0.6 mm mesh size), scanned and analyzed by WinRHIZO software (Regent Instruments Inc., Québec City, Canada) as described by Himmelbauer et al. (2004) (**Fig. 7**). Measured root parameters included root length, root diameter, root surface and root volume. After washing, the roots were dried at 60°C and weighted after 48 h in order to determine root dry matter. Root length density (RLD, cm cm<sup>-3</sup>), root tissue mass density (TMD, mg cm<sup>-3</sup>) and specific root length (SRL, m g<sup>-1</sup>) were calculated as follows:

$$RLD = \frac{\text{root length}}{\text{soil sample volume}}$$

$$TMD = \frac{\text{root dry mass}}{\text{root volume}}$$

$$SRL = \frac{\text{root length}}{\text{root dry mass}}$$

Moreover, RLD was calculated for different root diameter classes: very fine roots ( $\leq 0.4$  mm), fine roots (0.4 to 1.5 mm) and thick roots (1.5 to 10 mm).

To quantify the distribution of root over the soil profile, rooting distribution,  $\beta$ , was calculated based on an asymptotic equation ( $Y_r = 1 - \beta^d$ ) according to Jackson *et al.* (1996). High values of  $\beta$  correspond to a greater proportion of roots with depth and vice versa. To calculate root to shoot ratio (R:S), root biomass over 60 cm soil depth was divided by shoot biomass.

#### *Root functionality and water use*

Water use ( $WU_{ET}$ ) was calculated by a simplified water balance from measured soil water depletion ( $\Delta$ ) and cumulative rainfall. Soil water depletion ( $\Delta$ ) was defined as the difference in soil water content ( $\theta$ ) between seeding and harvest time. Soil water depletion ( $\Delta$ ) was calculated as a functional indicator of root water extraction capacity over the season and soil profile. Soil water content ( $\theta$ ) was measured weekly every 10 cm down to 90 cm soil depth by a capacitance probe (Diviner 2000®, Sentek Pty Ltd., Stepney, Australia). Claimed accuracy is  $\pm 0.5\%$ . PVC access tubes were installed after seeding in the center of each plot as described by Silva *et al.* (2007) (**Fig. 8**). At the flat experimental field site, surface runoff can be neglected. Deep drainage cannot be quantified from soil water content measurements. From lysimeter studies it is, however, known that due to low amount of rainfall, high soil water holding capacity and high profile depth at the site, the amount of seepage water is very small (Nolz *et al.* 2014). The term  $WU_{ET}$  was taken to indicate that water use includes both productive plant transpiration as well as soil evaporation. These two water balance components cannot be measured separately in a water balance approach.



**Fig. 7:** Preparing roots for image analyzing: (i) washing, sieving and separation of roots; (ii) staining with Giemsa solution (azur-eosin-methylenblue); (iii) scanning and image analysis; (iv) individual stained root samples



**Fig. 8:** Installation of PVC access tubes for capacitance probe and measurement of root electrical capacitance

#### *Root electrical capacitance*

Root electrical capacitance was measured by using an Escort ELC-133A LCR-meter (Instruments Techno Test Inc., Laval, Canada) at a frequency of 1 kHz at flowering. The two-terminal LCR-meter is connected to a soil and a plant electrode (**Fig. 8**). The soil electrode is a stainless steel rod (20 cm length, 3 mm diameter) which is inserted 15 cm into the soil, 10 cm away from the plant base where the plant electrode was clamped 2 cm above soil surface. The measurements were carried out on eight different points in the two center rows of the plots.

#### *Root anatomical characterization*

To evaluate sectional area of xylem vessels and seminal axes at seedling stage, an experiment with four replications was conducted under growth chamber conditions following Hund *et al.* (2009). Two uniformly medium-size seeds were soaked for a few hours in deionized water and directly planted in a position of 10 cm apart each other and 4 cm from top edge of a moistened blotting paper (21×29.5 cm). The blotting papers were covered by a PE foil like a pouch and the pouches were attached to a rod and hung into plastic containers. The lowest 2 cm were submerged in nutrient solution. The containers were placed in the growth chamber at 22°C and 60% relative humidity, and 14 h photoperiod with photosynthetic active radiation (PAR) of  $480 \mu\text{mol s}^{-1} \text{m}^{-2}$ . The seminal axes of two weeks old seedlings were counted. Roots



were transversely cross-sectioned in 30 µm thick segments by a vibrating blade microtome in 5 cm distance from base of main axes. To stain the specimens, phloroglucinol-HCl reagent was prepared by mixing 4 volumes of 2% (w/v) phloroglucinol in 70% ethanol with 1 volume of concentrated HCl. After embedding root slices for several minutes in phloroglucinol, lignin will appear red-violet. The area of main xylem vessels were measured using a confocal laser scanning microscope.

### 3.5 Yield and yield components

After full ripening (BBCH 92), plants were hand harvested from an area consisting of two, 1 m long center rows in each plot (0.25 m<sup>2</sup>). Total aboveground biomass, seed yield (oven dried at 60°C for 48 h), number of seeds per ear and number of fertile tillers were measured and expressed per unit area. Thousand grain weight was determined by weighing 400 seeds and the means were converted to one-thousand seed weight.

Sensitivity of genotypes to water limitation was characterized by relative stress response of traits between the two experimental years which differed strongly in seasonal water supply.

Relative stress response (RSR) of yield and its components was calculated as:

$$RSR = \frac{Trait_{wet} - Trait_{dry}}{Trait_{wet}}$$

where  $Trait_{wet}$  is the trait value under high water availability and  $Trait_{dry}$  is the value under low water availability conditions.

*Water use efficiency for biomass (WUEb)*

Water use efficiency equals  $B/WU$ , where  $B$  is biomass, and  $WU$  is water use.

### 3.6 Statistical analysis

Using linear mixed model, a combined analysis of variance across years was performed with the core set of genotypes (six genotypes presented in both experimental years) in order to test main (year, genotype, soil depth) and interaction effects. The genotype and soil depth effect and their interaction were considered as fixed effects, whereas block(year), year and all interaction terms with year were considered as random. The best linear mixed models were selected according to the corrected Akaike information criterion (AICC).



Analyses of single years were performed with the respective set of genotypes. Genotypes, soil depth and their interaction were treated as fixed effects, while the blocks (replications) were treated as random effects. All mixed model analyses of variances were carried out using procedure MIXED of SAS 9.2 software (SAS Institute, Inc., Cary, NC). In case of a significant fixed interaction effect (*i.e.* RLD and TMD in 2011) this term was partitioned into non-crossover (change in scale) and crossover (change in ranks) interaction (Bowman 1972) according to Muir *et al.* (1992) using the GenStat (GenStat 16 Ed., VSNi, Hemel Hempstead, UK) program devised by Emebiri *et al.* (2005).

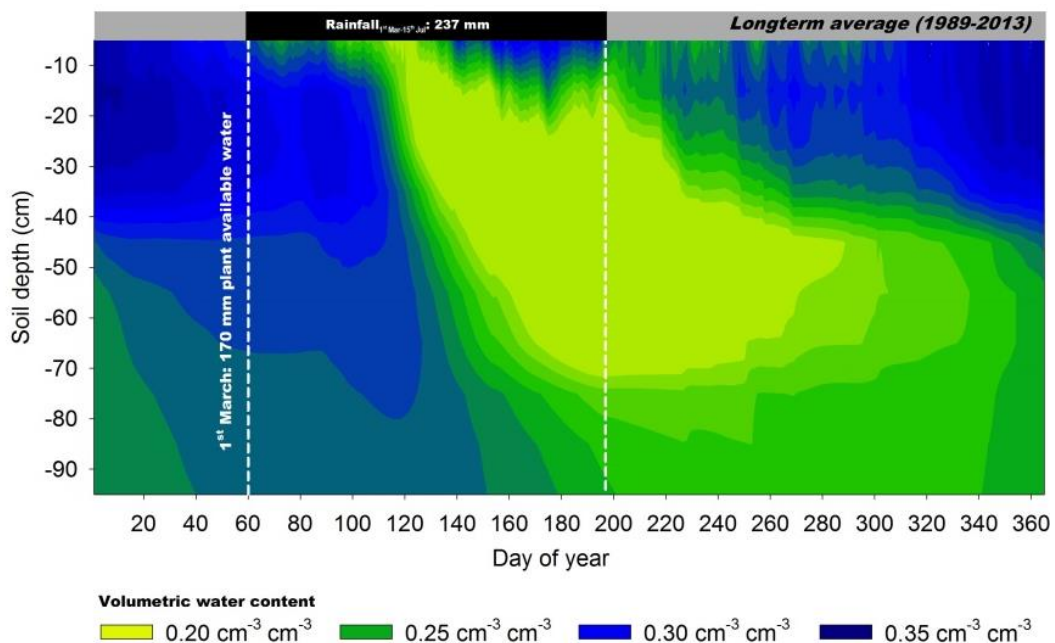
In order to study the relations of investigated traits, regression analysis was applied via the REG procedure of SAS. Following Bodner *et al.* (2013) we used cluster analysis (PROC CLUSTER) as a multivariate approach to determine similar groups of genotypes based on root and shoot traits.

## 4. Results

### 4.1 Rainfall pattern and soil water availability

Hydrological conditions at the experimental site are displayed in **Fig. 9**. Longtime rainfall during the vegetation period of spring cereals is 237 mm, while simulated plant available water (PAW) in the soil from stored winter moisture at time of sowing is 170 mm, *i.e.* 42% of total seasonal crop water supply (**Fig. 9**). Monthly in-season rainfall increases towards summer, resulting in a favorable balance between climatic demand and supply. Therefore, the site can be described as predominantly supply driven.

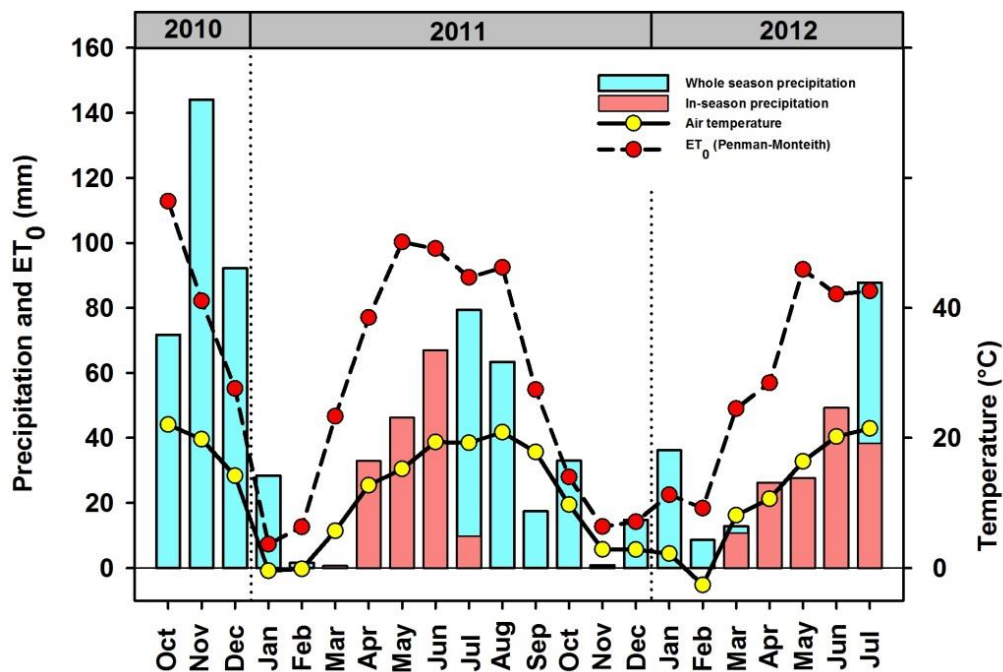
The two experimental years showed distinct hydrological conditions. Although annual mean temperature and precipitation were similar (2011: 10.5°C, 395 mm; 2012: 10.9°C, 402 mm), in-season rainfall distribution and stored soil moisture at sowing differed strongly. During May and June, *i.e.* time of stem elongation, heading, anthesis and early grain filling, rainfall was significantly lower in 2012 (77 mm) than in 2011 (113 mm) (**Fig. 10**). Differences in previous autumn precipitations (2010: 308 mm; 2011: 49 mm) resulted in substantially lower PAW in 2012 (38 mm on 4<sup>th</sup> April, *i.e.* two weeks after sowing) compared to 2011 (114 mm on 30<sup>th</sup> March, *i.e.* three weeks after sowing) (**Fig. 11**).



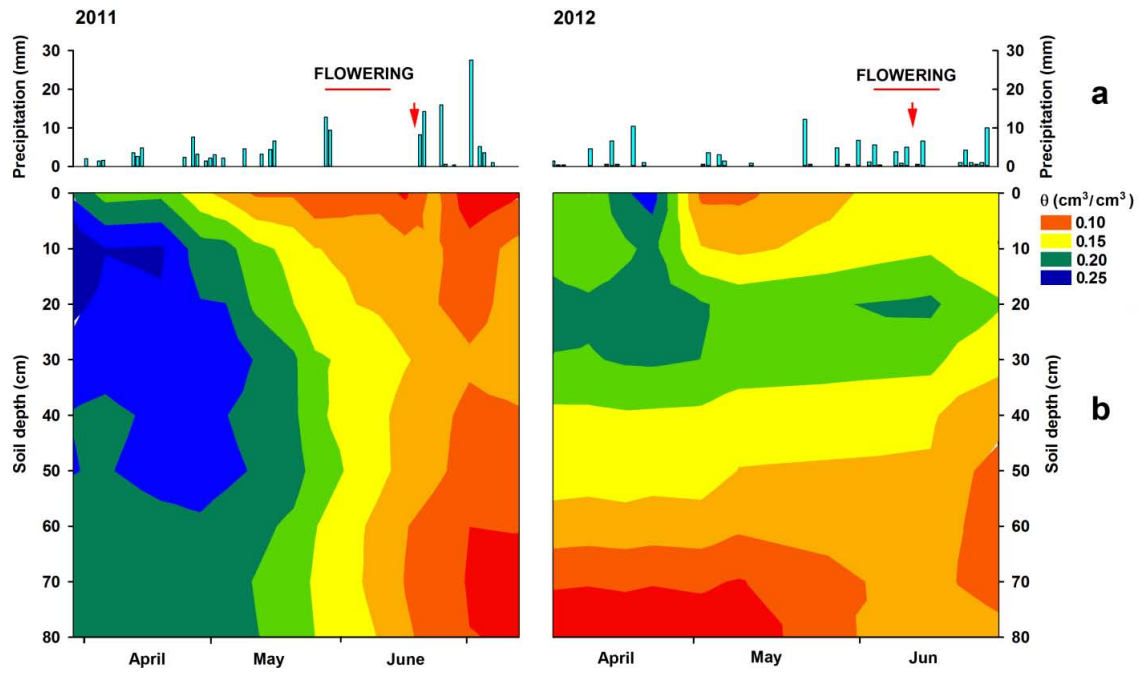
**Fig. 9:** Simulated spatio-temporal dynamics of soil profile water content using the simulation model HYDRUS 1D for long term average conditions (1989 to 2013). Dashed lines specify the range of the growing season for spring wheat.

Based on the simulated site hydrology, we determined the number of stress days using a threshold of  $\leq 50\%$  PAW and calculated the probability of occurrence of the two experimental years compared to the last 25 year average (1988-2013) (**Fig. 12**). Seasonal water availability revealed that in 2011 hydrological conditions in May were among the wetter half of years, while June water availability was similar to 65% of years. Contrary, 2012 was a particularly dry year with a low probability of occurrence in 25 years. Due to low water storage over winter and reduced rainfall in spring, prolonged dry periods with water contents below 50% PAW were observed in May and June. The probability of occurrence of dry conditions of similar intensity as in 2012 is 8% for May and 32% for June, respectively.

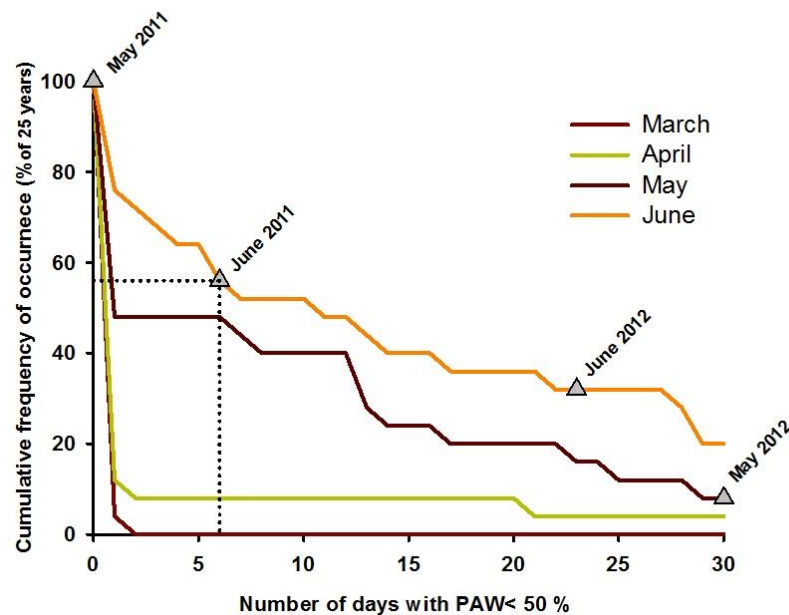
Thus, site hydrology revealed that only limited water stress occurred in June 2011, whereas 2012 was a particularly dry year with high stress incidence. Consequently, changes in crop performance between the two years can be interpreted in terms of drought response.



**Fig. 10:** Temperature, precipitation and potential evaporation (ET<sub>0</sub>) from October 2010 until July 2012 at the experimental site Raasdorf



**Fig. 11:** Daily rainfall (a) and spatio-temporal dynamics of soil profile water content averaged over the core set plots (b) at the experimental site during the two growing seasons. Arrows and horizontal lines indicate dates of root sampling and duration of flowering, respectively



**Fig. 12:** Cumulative frequency of occurrence of days with less than 50% plant available water (PAW) over 1 m soil profile depth during growing season. Calculation based on annual vs. longtime soil water content simulation. Triangles indicate number of days observed in 2011 and 2012 with <50% PAW. Dotted line exemplifies interpretation for June 2011: 6 stress days with <50% PAW means that 56% of years have  $\geq 6$  stress days, while 44% of years have <6 stress days, that means June 2011 is within the wetter half of years for this site.

## 4.2 Phenological and physiological traits

For all phenological and physiological traits a significant ( $P<0.05$ ) difference between genotypes was observed. Combined ANOVA of the core set showed significant variation for year and genotype×year interaction (**Table 4**) for almost all traits.

**Table 4:** Selected linear mixed models of the analyses of variance of the core set genotypes across the two experimental years. Significant ( $P\leq 0.05$ ) fixed effects are printed in bold capital (B: block; G: genotype; Y: year)

Trait	Fixed effects	Random effects
Time to full flowering (thermal time)	<b>G</b>	B(Y) + Y + G×Y
Time to full flowering (calendar time)	<b>G</b>	Y + G×Y
Early vigor	<i>g</i>	Y + G×Y
Leaf chlorophyll content (SPAD)	<b>G</b>	B(Y) + G×Y
Stomatal conductance	<i>g</i>	Y + G×Y
Water use	<b>G</b>	B(Y) + Y
Water use efficiency for biomass	<b>G</b>	Y
Seed yield	<b>G</b>	Y + G×Y
Shoot biomass	<b>G</b>	Y
Harvest index	<b>G</b>	Y + G×Y
Number of fertile tillers	<i>g</i>	B(Y) + G×Y
Seed number per ear	<b>G</b>	Y + G×Y
Thousand kernel weight	<b>G</b>	Y + G×Y
Plant height	<i>g</i>	B(Y) + G×Y

### *Developmental growth and time to flowering*

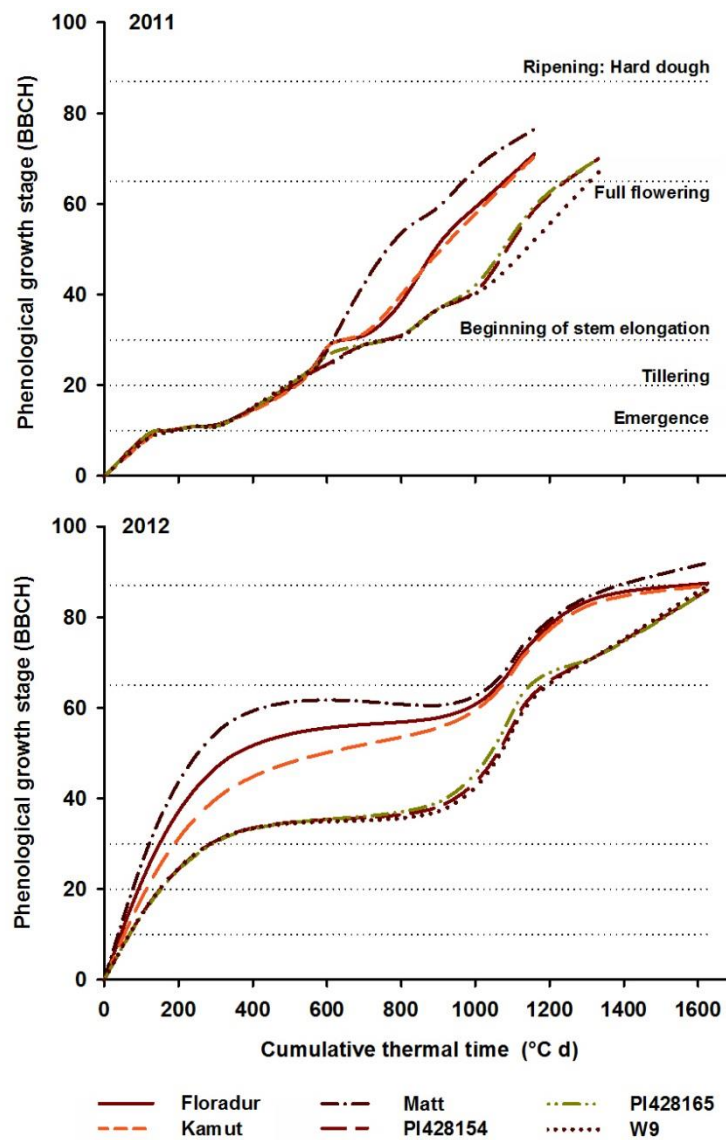
Based on time to flowering, investigated genotypes can be classified into three groups (**Fig. 13, Table 5**): (i) early flowering durum cv. ‘Matt’, (ii) intermediate flowering group incl. ‘Floradur’ and other tetraploid and hexaploid wheat genotypes, and (iii) very late flowering underutilized wheat species *T. monococcum* and *T. timopheevi*.

In the core set, flowering hastened in 2012 (2011: 91.9 d; 1173.5°Cd; 2012: 81.7 d, 1104.8°Cd). This was more evident with respect to early growth stages, *i.e.* from emergence to stem elongation. Late flowering wheat relatives inevitably showed shorter grain filling periods than durum wheat over two years.

**Table 5:** Genotypic mean values of phenological and physiological traits in 2011 and 2012

Year/Genotype	ANTH1 (°Cd)	ANTH2 (d)	GCR (% °Cd <sup>-1</sup> )	SPAD	SC (mmol m <sup>-2</sup> s <sup>-1</sup> )	LAI
2011						
7060	1045.3	85.5	0.148	48.2	420.8	—
7063	1065.0	86.5	0.159	50.4	547.8	—
7094	1096.6	88.0	0.165	50.0	418.2	—
Clovis	1035.6	85.0	0.154	52.2	522.9	—
Floradur	1096.6	88.0	0.154	51.0	457.5	—
Matt	972.3	81.5	0.158	48.0	569.3	—
SZD3146	1106.5	88.5	0.152	53.4	544.7	—
Kamut	1106.5	88.5	0.151	51.5	625.1	—
TRI5254	1116.5	89.0	0.156	49.2	603.7	—
W9	1331.4	100.0	0.156	41.2	287.0	—
PI428154	1269.4	97.0	0.152	38.7	362.4	—
PI428165	1264.6	96.8	0.164	44.9	440.9	—
s.e.d. <sup>2</sup>	7.9	0.4	0.004	1.8	60.1	
d.f.	36	36	36	36	36	
2012						
Tabasi	1007.5	76.0	0.177	45.0	219.5	2.6
Taifun	1014.7	76.5	0.167	47.8	190.2	2.2
Floradur	1018.4	76.8	0.165	54.0	184.5	2.2
Matt	983.5	74.8	0.156	48.9	275.2	2.2
Kamut	1037.4	78.0	0.163	48.6	185.2	2.5
W13	1056.3	79.0	0.168	44.2	233.4	2.6
W9	1219.4	88.0	0.180	38.2	86.6	3.2
PI428154	1208.3	87.5	0.182	34.8	128.0	3.0
PI428165	1161.7	85.0	0.180	37.0	140.5	2.7
s.e.d.	13.2	0.7	0.005	1.9	29.3	0.19
d.f.	27	27	27	27	24	24

<sup>1</sup> ANTH1, anthesis (BBCH 65) based on cumulative thermal time (CTT); ANTH2, anthesis based on calendar time (days after flowering); GCR, ground cover rate (between emergence and almost closed canopy); SPAD, leaf chlorophyll content (SPAD values); SC, stomatal conductance; LAI, leaf area index; <sup>2</sup> s.e.d., standard error of differences; d.f., degrees of freedom



**Fig. 13:** Genotypic variation in phenological growth development of core set genotypes in 2011 and 2012. Cumulative thermal time (CTT) was calculated from day of sowing onwards.

#### *Early vigor*

Early vigor, as determined by ground cover rate, was significantly higher for the core set in 2012 than 2011 ( $0.171$  vs  $0.156\%$   $^{\circ}\text{Cd}^{-1}$ , respectively) with a significant genotype by year interaction (**Table 4**). Despite an initial lag phase, which was especially evident for *T. timopheevi* W9, underutilized wheat accessions closed their canopy more swiftly than durum and Khorasan wheat, particularly in 2012.

#### *Leaf chlorophyll content*

Chlorophyll concentration, as an indicator for photosynthetic capacity and measured by SPAD, showed a significant decrease for the core set in 2012 which was more evident for underutilized wheat species. In 2011, durum wheats SZD3146 and ‘Clovis’ were the genotypes with highest chlorophyll content followed by ‘QK-77’ and ‘Floradur’, while in 2012 ‘Floradur’ was the superior genotype. Accessions of einkorn and Zanduri wheat constantly had the lowest SPAD values in both years (**Table 5**).

#### *Stomatal conductance*

Stomatal conductance declined substantially for the core set in response to water scarcity in 2012 (*i.e.* from 457.0 to 166.6 mmol m<sup>-2</sup> s<sup>-1</sup>). In 2011 Khorasan wheat along with durum cv. ‘Matt’ showed highest stomatal conductance while einkorn and Zanduri wheat were characterized by the lowest stomatal conductance. In 2012, ‘Matt’ and *T. carthlicum* W13 had highest stomatal conductance whereas, like in 2011, the underutilized species *T. monococcum* and *T. timopheevii* showed the lowest stomatal conductance (**Table 5**).

#### *Leaf area index*

Leaf area index (LAI), the ratio of the leaf area to ground area, in 2012 year showed significant variation between genotypes with highest value for wild wheat genotypes and lowest values for early maturing cv. ‘Matt’ and common wheat cv. ‘Taifun’ (**Table 5**).

### **4.3 Root diversity and root functional characterization**

#### *Root morphology and root system shape*

Combined ANOVA of the core set for root traits obtained from the soil core samples revealed similar results, *i.e.* significant genotypic effects were observed for all parameters except root weight, and year×soil depth and genotype×year×soil depth were included as random effects in the best mixed model except for root diameter where only year×soil depth was included (**Table 6**). A summary of the genetic variation within wheat species, soil depths and years for the root traits obtained from the soil core samples is presented in **Table 7**. Root diameter and derived root traits are presented in detail in the following.



**Table 6:** Selected linear mixed ANOVA models of root traits of the core set across the two years. Significant ( $P \leq 0.05$ ) fixed effects are printed in bold capital (B: block; D: soil depth; G: genotype; Y: year)

Trait	Fixed effects	Random effects
Root length	<b>G</b> + d + g×d	Y×D + G×Y×D
Root surface	<b>G</b> + d + g×d	Y×D + G×Y×D
Root volume	<b>G</b> + d + g×d	Y×D + G×Y×D
Root weight	g + d + g×d	Y×D + G×Y×D
Root diameter	<b>G</b> + d + g×d	Y×D
Root length density	<b>G</b> + d + g×d	Y×D + G×Y×D
Tissue mass density	g + d + g×d	Y×D + G×Y×D
Specific root length	<b>G</b> + <b>D</b> + <b>G×D</b>	Y + G×Y
Rooting distribution	g	Y
Root system size	<b>G</b>	Y + G×Y
Root to shoot ratio	g	G×Y
Soil water depletion	<b>G</b> + d + g×d	B(Y) + Y + Y×D + G×Y

**Table 7:** Genetic variation in root traits subject to different soil depths and wheat species

Year	2011		
Soil depth (cm)	10-20	30-40	50-60
Root length (cm)			
<i>T. monococcum</i>	4247-5536	1640-1675	2528-2854
<i>T. turanicum</i>	1853-2746	769-983	1726-2446
<i>T. durum</i>	2025-2612	786-1191	1239-2312
<i>T. timopheevi</i>	3538	949	1818
Root surface (cm <sup>2</sup> )			
<i>T. monococcum</i>	462.9-606.0	212.7-218.7	286.7-298.3
<i>T. turanicum</i>	238.5-362.1	112.6-153.5	209.5-317.8
<i>T. durum</i>	225.0-371.5	100.1-186.6	150.6-305.1
<i>T. timopheevi</i>	466.9	135.9	205.8
Root volume (cm <sup>3</sup> )			
<i>T. monococcum</i>	4.06-5.35	2.22-2.29	2.49-2.60
<i>T. turanicum</i>	2.47-3.89	1.32-1.91	2.05-3.30
<i>T. durum</i>	2.65-4.76	1.03-2.35	1.48-3.25
<i>T. timopheevi</i>	5.02	1.58	1.86
Root weight (mg)			
<i>T. monococcum</i>	240.2-357.6	106.4-107.8	111.3-115.4
<i>T. turanicum</i>	300.9-409.9	74.3-104.0	97.9-152.1
<i>T. durum</i>	315.7-692.6	55.8-116.0	68.9-139.8
<i>T. timopheevi</i>	383.8	71.6	86.4

**Table 7:** continued

Year	2012		
Soil depth (cm)	10-20	30-40	50-60
Root length (cm)			
<i>T. monococcum</i>	1377-1409	2264-2312	1668-1872
<i>T. turanicum</i>	983	1658	2181
<i>T. durum</i>	913-1005	1432-1755	1462-1856
<i>T. timopheevi</i>	1203	1568	2259
<i>T. carthlicum</i>	715	1558	1689
<i>T. aestivum</i>	869-1005	1652-1743	1444-2371
Root surface (cm <sup>2</sup> )			
<i>T. monococcum</i>	198.6-210.3	324.2-328.7	218.9-247.4
<i>T. turanicum</i>	169.5	243.0	276.5
<i>T. durum</i>	147.8-163.6	209.4-264.9	193.0-244.4
<i>T. timopheevi</i>	193.5	226.2	304.9
<i>T. carthlicum</i>	112.5	212.1	203.0
<i>T. aestivum</i>	141.0-151.9	234.0-255.1	200.7-317.0
Root volume (cm <sup>3</sup> )			
<i>T. monococcum</i>	2.28-2.52	3.63-3.82	2.29-2.62
<i>T. turanicum</i>	2.34	2.85	2.13
<i>T. durum</i>	1.92-2.13	2.44-3.19	2.03-2.58
<i>T. timopheevi</i>	2.50	2.61	3.28
<i>T. carthlicum</i>	1.41	2.30	1.95
<i>T. aestivum</i>	1.82-1.83	2.65-2.98	2.24-3.38
Root weight (mg)			
<i>T. monococcum</i>	82.3-90.0	118.3-130.5	80.5-86.8
<i>T. turanicum</i>	92.3	103.3	102.0
<i>T. durum</i>	77.5-80.9	89.5-117.9	63.5-94.1
<i>T. timopheevi</i>	86.7	85.1	97.3
<i>T. carthlicum</i>	50.9	72.7	61.5
<i>T. aestivum</i>	65.1-73.9	97.7-98.9	72.9-115.7

### Root diameter

For root diameter (RD) significant genotype and soil depth effects were observed in both years, while the genotype×soil depth interaction was not significant (**Table 8**). Mean root diameter was somewhat higher in 2012. The distribution across soil profile, however, was different between the years: in 2012, RD decreased with increasing soil depth, whereas in 2011 the highest value was observed for the second layer (30-40 cm) (**Fig. 14**).

With respect to genotypes it was observed that in both years ‘Floradur’ and the einkorn wheat accessions had the thickest and thinnest roots, respectively. Also the Persian wheat accession tested in 2012 showed very thin roots.

### Root length density

For root length density (RLD) genotype and soil depth effects were significant in both years, whereas their interaction was significant only in 2011 (**Table 8**). Partitioning of the interaction sum of squares following the method of Muir *et al.* (1992) revealed that >80% of the interaction was due to a change in scale (variances) and not to a change in rank order (cross-over interaction) of the genotypes. The two years were different with respect to the distribution of RLD (**Fig. 14**). In 2011 highest RLD was found in the topsoil layer (7.4 cm cm<sup>-3</sup>), whereas in 2012 highest RLD was observed for the subsoil layers (4.6-4.9 cm cm<sup>-3</sup>). Furthermore, a trade-off between RLD and RD can be recognized in soil profile (**Fig. 14**).

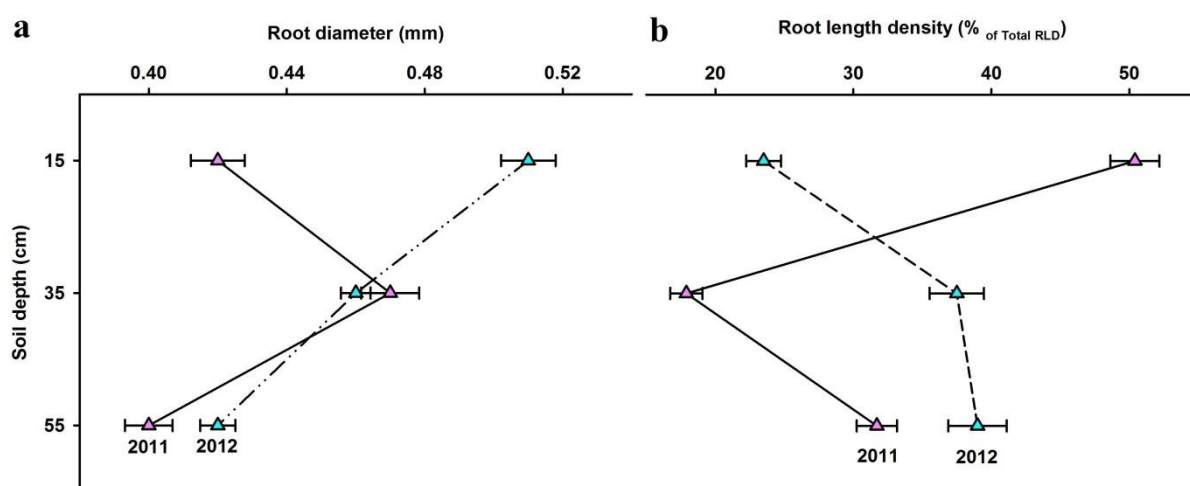
**Table 8:** Analysis of variance and mean values of fixed effects for root traits

Year	RD <sup>1</sup> (mm)	RLD (cm·cm <sup>-3</sup> )	TMD (mg·cm <sup>-3</sup> )	SRL (m.g <sup>-1</sup> )	Δ (mm)
2011					
Genotype (G)	<.001	<.001	<.001	<.001	<.001
Soil depth (D)	<.001	<.001	<.001	<.001	<.001
G×D	0.860	<.001	<.001	0.060	0.599
Soil depth					
10-20 cm	0.42	7.40	110.2	80.7	12.1
30-40 cm	0.47	2.90	49.5	124.3	9.2
50-60 cm	0.40	5.30	46.6	184.6	10.4
s.e.d.	0.010	0.288	2.719	6.695	1.931
Genotype					
7060	0.44	4.40	69.8	126.8	9.9
7063	0.44	5.10	65.3	122.2	7.3
7094	0.45	4.20	80.2	112.2	11.6
Clovis	0.45	4.90	77.5	102.9	8.4
Floradur	0.45	5.00	84.2	105.7	10.1
Matt	0.41	3.50	73.5	127.8	7.8
SZD3146	0.45	4.70	71.5	111.2	9.1
Kamut	0.42	3.80	76.0	115.3	10.2
TRI5254	0.45	5.40	67.8	109.7	12.0
W9	0.42	5.50	55.3	149.2	14.2
PI428154	0.37	8.70	53.3	191.8	12.4
PI428165	0.38	7.30	50.7	183.3	13.9
s.e.d.	0.020	0.575	5.438	13.39	2.25
d.f.	108	108	108	108	105

**Table 8:** continued

Year	RD <sup>1</sup> (mm)	RLD (cm·cm <sup>-3</sup> )	TMD (mg·cm <sup>-3</sup> )	SRL (m.g <sup>-1</sup> )	Δ (mm)
2012					
Genotype (G)	0.038	0.027	0.004	<.001	<.001
Soil depth (D)	<.001	<.001	<.001	<.001	0.680
G×D	0.145	0.350	0.722	0.364	0.689
Depth					
10-20 cm	0.51	2.70	37.5	135.0	4.1
30-40 cm	0.46	4.60	34.6	177.1	4.0
50-60 cm	0.42	4.90	33.2	226.3	4.3
s.e.d.	0.008	0.295	0.998	6.908	1.551
Genotype					
Tabasi	0.46	4.43	35.8	176.2	4.1
Taifun	0.47	3.43	35.4	165.9	3.3
Floradur	0.49	3.92	38.9	151.1	3.6
Matt	0.47	3.38	34.7	176.3	2.5
Kamut	0.48	4.18	37.6	161.2	4.2
W13	0.44	3.43	33.1	209.3	4.8
W9	0.47	4.36	32.4	187.8	4.9
PI428154	0.45	4.84	33.0	202.7	4.2
PI428165	0.45	4.60	34.9	184.8	5.7
s.e.d.	0.014	0.512	1.728	11.97	1.632
d.f.	81	81	81	81	78

<sup>1</sup> RD, root diameter; RLD, root length density; SRL, specific root length; TMD, tissue mass density; Δ, soil water depletion



**Fig. 14:** Distribution of root diameter (a) and root length density (b) throughout the soil profile for the core set varieties in the two experimental years (mean ± standard errors)

Highest RLD values were observed in both years for einkorn wheat, alongside the Iranian bread wheat ‘Tabasi’ in 2012, whereas the early maturing durum ‘Matt’ showed the lowest values in both years. Generally, a significantly higher variation in RLD was observed in 2011 (3.5-8.7 cm cm<sup>-3</sup>) compared to 2012 (3.4-4.8 cm cm<sup>-3</sup>). Correlation and regression analysis revealed that the observed variation in RLD is mainly explained by the RLD of very fine roots ( $\leq 0.4$  mm). A significant positive correlation was observed between LAI (measured only in 2012) and RLD ( $r = 0.72$ ,  $P < 0.05$ ).

#### *Rooting distribution*

Rooting distribution expressed by numerical index  $\beta$  showed no significant genotypic but a significant year effect for the core set varieties (**Table 6**). While all genotypes of the core set reacted to lower rainfall by rooting into deeper soil layers, *T. timopheevi* W9 responded more significantly to lower rainfall in 2012 compared to the other genotypes by developing 45% of total RLD in 50-60 cm soil depth compared to only 29% in 2011.

The analyses of rooting distribution revealed no significant variation among genotypes in individual years (**Table 9**). Nevertheless, Khorasan wheat, common wheat ‘Tabasi’, *T. carthlicum* W13 and adapted durum wheat ‘Floradur’ with high values of  $\beta$  represented genotypes with deep rooting system. On the contrary, einkorn accessions and early maturing durum ‘Matt’ appeared to have a dominant surface rooting system.

#### *Root tissue mass densities and specific root length*

Significant main effects for root tissue mass density (TMD) and specific root length (SRL) were observed in both years while their interaction was significant only in case of TMD in 2011 (**Table 8**). Likewise RLD, the interaction was mainly (>70%) due to a change in scale and not in rank. TMD values were higher in 2011, whereas higher SRL was observed in 2012. In response to increasing soil depth TMD decreased while SRL increased. These responses were consistent in both years; however, for TMD the difference between topsoil and subsoil layers was more expressed in 2011.

Einkorn wheat accessions showed stable and high values of SRL in both years, whereas tetraploid varieties of the core set, *i.e.* ‘Floradur’, ‘Matt’ and ‘QK-77’, showed a significant increase (+45-49 m g<sup>-1</sup>) in the drier year 2012.

A similar grouping was observed for TMD in 2011, *i.e.* ‘Floradur’ showed the highest values followed by other durum wheats, while the accessions of *T. monococcum* and *T. timopheevi*

exhibited the lowest TMD. Due to a considerable lower variation this grouping was not so obvious in 2012. However, the underutilized wheat species still show the lowest values.

**Table 9:** Analysis of variance and mean values of fixed effects for different root and shoot traits

Year	$\beta^1$	R:S	RC (nF)	$\Delta_{60-90}$ (mm)
2011				
Genotype (G)	0.053	0.122	<.001	0.552
7060	0.960	0.30	0.216	35.7
7063	0.965	0.29	0.281	27.2
7094	0.957	0.40	0.402	33.2
Clovis	0.964	0.44	0.253	29.0
Floradur	0.960	0.42	0.282	28.9
Matt	0.956	0.30	0.303	22.9
SZD3146	0.956	0.38	0.296	28.6
Kamut	0.963	0.29	0.285	35.3
TRI5254	0.964	0.41	0.323	34.4
W9	0.953	0.29	0.578	38.8
PI428154	0.952	0.33	0.521	39.7
PI428165	0.957	0.26	0.645	37.3
s.e.d.	0.005	0.068	0.084	9.57
d.f.	36	36	33	33
2012				
Genotype (G)	0.082	0.029	<.001	0.007
Tabasi	0.973	0.44	1.008	8.7
Taifun	0.969	0.32	0.771	-0.5
Floradur	0.971	0.31	0.782	-4.1
Matt	0.969	0.31	0.577	-7.4
Kamut	0.972	0.32	0.788	-0.8
W13	0.972	0.26	0.633	-5.4
W9	0.971	0.40	1.158	-3.7
PI428154	0.967	0.50	1.007	-5.6
PI428165	0.967	0.47	0.905	0.7
s.e.d.	0.002	0.073	0.106	4.35
d.f.	27	27	27	24

<sup>1</sup>  $\beta$ , rooting distribution index; R:S, root to shoot ratio; RC, root capacitance;  $\Delta_{60-90}$  Soil water depletion at 60-90 cm soil depth

#### *Root to shoot ratio*

Assimilate allocation to roots, as expressed by root to shoot ratio (R:S), showed no significant genotypic but a significant genotype by year interaction effect for the core set varieties (**Table**

7). This interaction is obvious by the opposite response across years for the adapted durum variety Floradur and the einkorn wheat accessions (**Fig. 15**).

With respect to the single year analyses no significant genotypic differences were observed in 2011 with R:S ranging from 0.26 to 0.44 (**Table 8**). A higher variation (0.26 to 0.50) and significant genotypic effects were observed in 2012. Besides einkorn wheat a high R:S was observed also for the Iranian landrace variety ‘Tabasi’ while *T. carthlicum* showed the lowest R:S.

#### 4.4 Seminal root anatomy

##### *Seminal root number*

Seminal root number differed significantly between ‘QK-77’ with 6 seminal roots and the two einkorn accessions PI428154 and PI428165 with 3.6 and 3.7 seminal roots, respectively. The rest of the material showed similar seminal root numbers about 5 (**Table 10**).

##### *Xylem vessels*

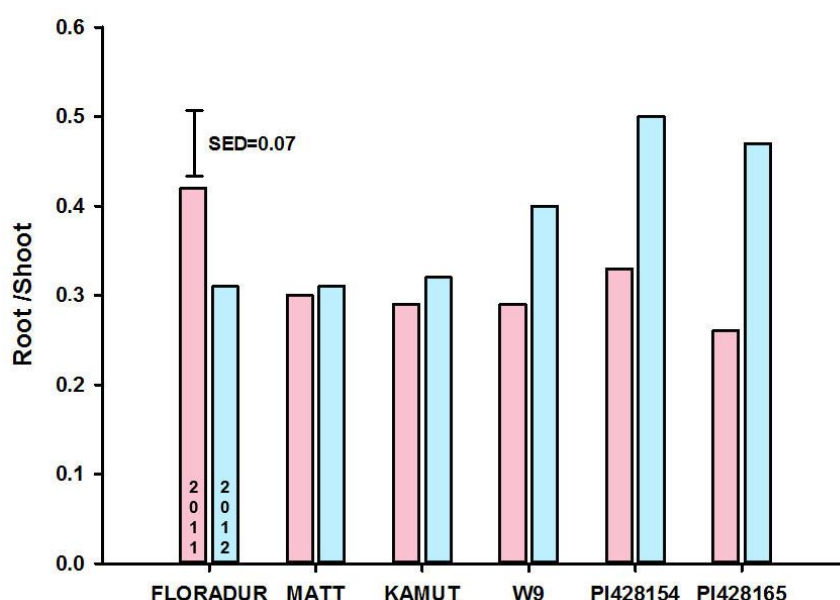
Significant variation for cross-sectional area of main xylem vessels based on cross sections of seminal roots was observed in the 2011 nursery, grown under controlled growth chamber conditions. *T. turanicum* accession TRI5254 showed the largest xylem vessel cross-sectional area followed by durum genotypes ‘Clovis’ and 7094, whereas ‘Matt’, SZD3146 and 7063 showed the lowest main xylem cross-sectional area (**Table 10**). Genotypic variation was also observed for the presence of multiple main xylem vessels (**Fig. 16**).

A significant and positive correlation ( $r = 0.61$ ,  $P < 0.05$ ) was observed between seminal root number and depth rooting defined as RLD at 50-60 cm soil depth divided by total RLD.

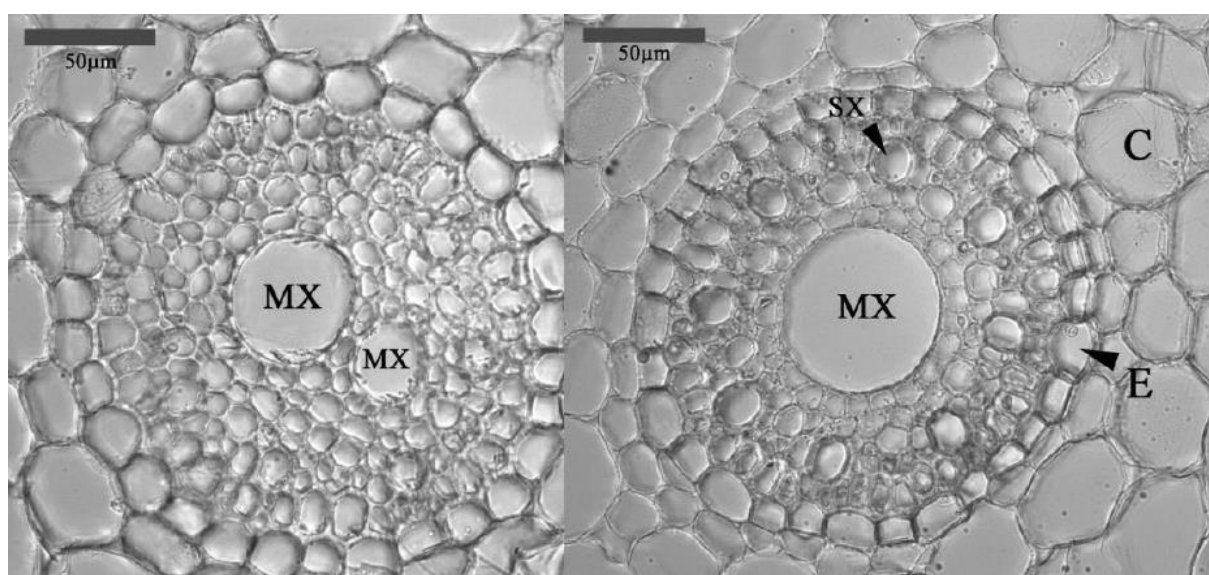
#### 4.5 Root functionality

The spatio-temporal dynamics of water content in soil profile is shown for both experimental years in **Fig. 11**. In 2011, the initial water content at sowing shows a rather homogeneously wetted profile. A progressive water depletion was observed as soil depth increased. Between mid May and mid June, soil water depletion was distributed evenly over depth, with the exception of a higher depletion in the upper layer due to soil surface evaporation. From mid June onwards a higher depletion took place in the upper and lower parts of the profile compared to the middle layer. In 2012 a low soil water content was available already at the

beginning of the vegetation period, with a strongly depleted deep profile. Higher variability of water dynamics during growing season was limited to the upper 15 cm soil depth. Deeper soil layers showed only small changes in water content with stronger depletion of deeper layers only during the last half of June.



**Fig. 15:** Root to shoot ratio of core set genotypes in 2011 and 2012 (s.e.d., standard error of difference)



**Fig. 16:** Root cross-sections of 'Floradur' (*T. durum*; left) and PI428165 (*T. monococcum*; right) of a seminal root axis; MX, main xylem vessel; SX, subsidiary xylem vessel; C, cortex; E, endode



**Table 10:** Analysis of variance and mean values of root anatomical traits of two week old seedlings grown under controlled conditions

Year	XVA <sup>1</sup> (mm <sup>2</sup> ×10 <sup>-2</sup> )	SRN (n)
Genotype (G)	<.001	<.001
Genotype		
7060	0.27	5.13
7063	0.20	5.00
7094	0.41	5.13
Clovis	0.41	4.88
Floradur	0.27	4.75
Matt	0.20	4.43
SZD3146	0.16	5.13
Kamut	0.33	6.00
TRI5254	0.51	5.00
W9	0.32	5.13
PI428154	0.33	3.75
PI428165	0.38	3.63
s.e.d.	0.048	0.366
d.f.	19	79

<sup>1</sup> XVA, main xylem vessel area; SRN, seminal root number

**Fig. 17** shows the dynamics of soil water depletion as the most appropriate descriptor of root functionality for the soil depths 0-60 cm, where the roots were sampled, and 60-90 cm soil depth. Soil water depletion was averaged over the plots of the core set. The strongest differentiation between varieties was observed at the late growth stages (post-flowering), which is most probably related to (i) the highest evaporative demand at this stage and (ii) genotypic differences with respect to maturity.

Soil water depletion ( $\Delta$ ) revealed highest root functionality at topsoil (10-20 cm) in 2011. Highest water uptake was measured for *T. monococcum* and *T. timopheevi* germplasm, followed by Khorasan wheat (TRI5254). ‘Clovis’, ‘Matt’ and 7063 showed the lowest values of water depletion (**Table 8**). Substantial but not significant genotypic differences for soil water depletion were also observed in deeper soil layers (60-90 cm) with *T. monococcum* and *T. timopheevi* extracting the highest amounts of water (**Table 9**). In 2012 *T. monococcum*, *T. timopheevi* and *T. carthlicum* achieved highest soil water depletion, while no significant differences were observed between soil depths (**Table 8**). In subsoil layers (60-90 cm) no depletion of soil water was observed with the exception of the Iranian wheat variety ‘Tabasi’ (8.7 mm; **Table 9**).

Linear regression analysis revealed 5.9 and 13.2 mm water depletion per unit of root length density in 2011 and 2012, respectively, indicating the importance of the rooting density in terms of water depletion especially in dry years.

#### 4.6 Root electrical capacitance

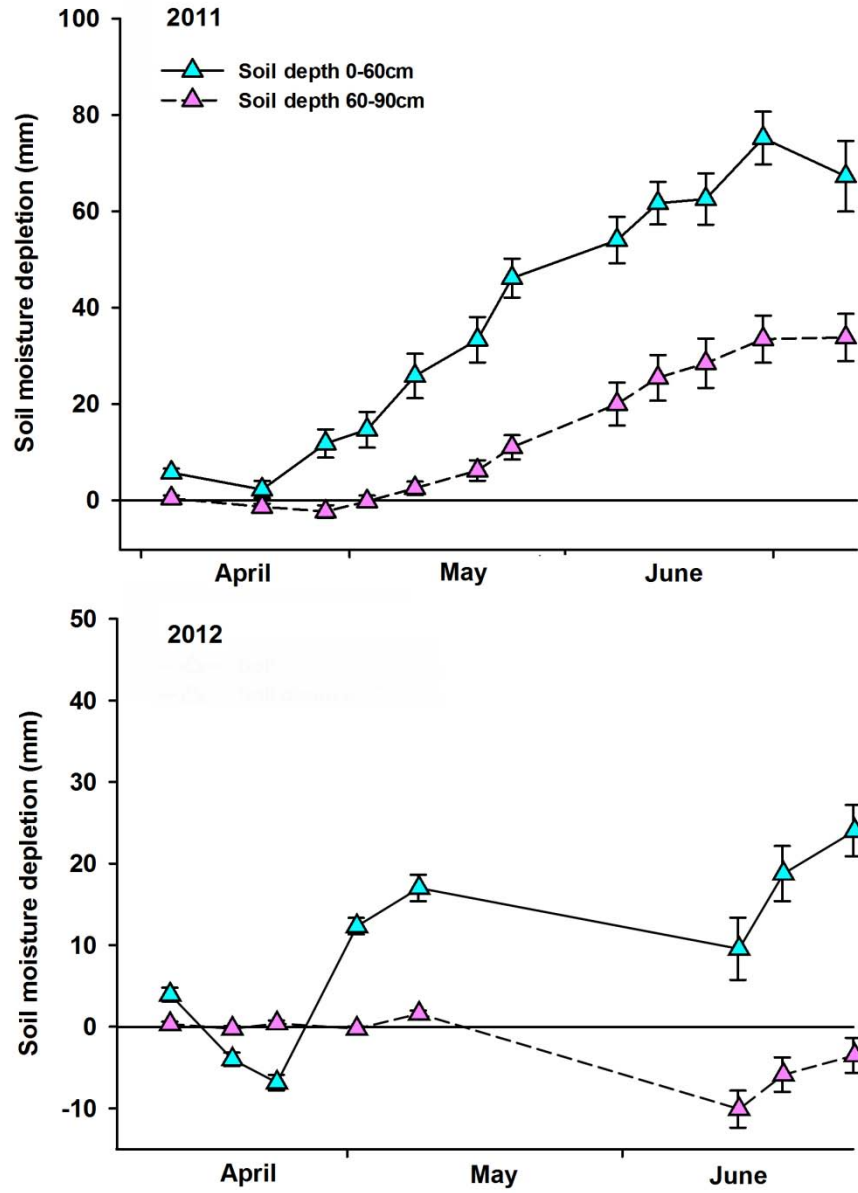
Root electrical capacitance (RC) was strongly influenced by the year and was significantly higher in 2012 compared to 2011 (**Table 9**). In both years RC varied significantly between genotypes. In 2011 RC of *T. monococcum* and *T. timopheevi* significantly differed from the other genotypes. Also in 2012 these two species showed the highest RC values besides bread wheat variety ‘Tabasi’.

Correlation between RC and RLD (0-60 cm) at the time of flowering was significant in both years (2011:  $r=0.7$ ,  $P<0.05$ ; 2012:  $r=0.82$ ,  $P<0.01$ ). Detailed analyses of the correlations showed the highest correlations between RC and RLD in topsoil (0-20 cm) in both years (2011:  $r=0.79$ ,  $P<0.01$ ; 2012:  $r=0.64$ ,  $P=0.058$ ), and RLD in subsoil (50-60 cm) ( $r=0.69$ ,  $P<0.05$ ) in 2012. Furthermore, a significant correlation was observed between RC and soil water depletion in 2011 ( $r=0.86$ ,  $P<0.001$ ), whereas this relationship was not significant in the dry year 2012 ( $r=0.6$ ,  $P=0.09$ ).

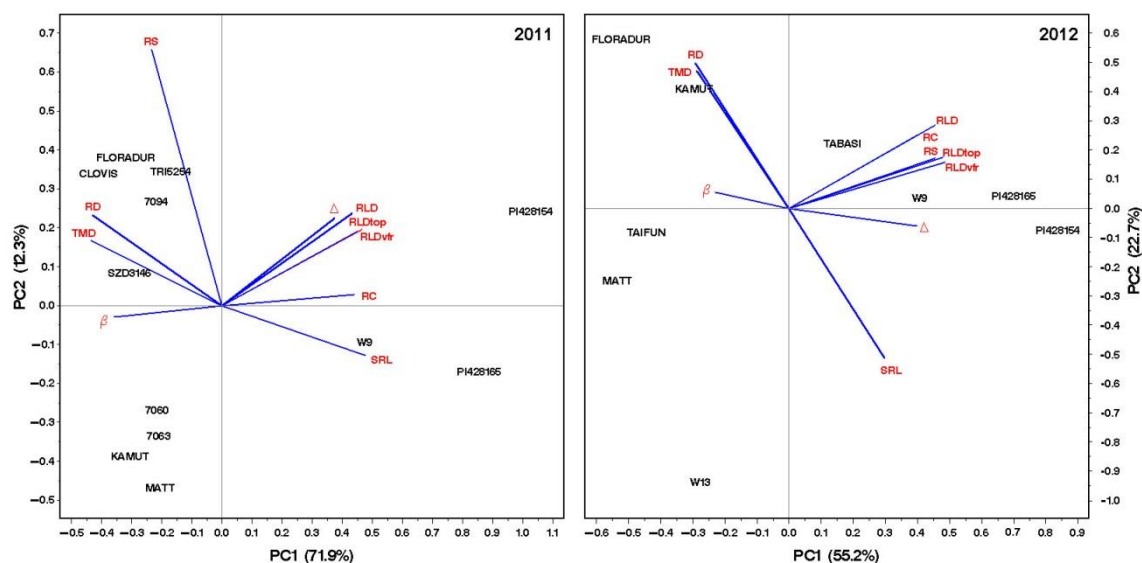
#### 4.7 Root system classification

Principal components analysis of root traits revealed characteristic groups of genotypes and traits (**Fig. 18**). In both years the *T. monococcum* and *T. timopheevi* germplasm formed a distinctive group. The *T. durum* and *T. turanicum* genotypes form another group which is, however, more heterogenous and consists of two subgroups. The *T. carthlicum* accession W13 makes an isolated group in 2012, whereas the two bread wheat varieties ‘Taifun’ and ‘Tabasi’ are associated with different groups.

With respect to root traits a uniform pattern across years was observed for most parameters: the diverse RLD parameters together with root capacitance and soil water depletion ( $\Delta$ ) formed one group, RD, TMD and rooting distribution  $\beta$  formed another group, whereas SRL makes an isolated group which is not or negatively correlated to the two groups mentioned before. R:S showed a year specific reaction (**Fig. 18**).



**Fig. 17:** Dynamic of soil water depletion ( $\Delta$ ) averaged over the core set plots during the growing seasons 2011 and 2012 in soil depths 0-60 cm and 60-90 cm ( $\Delta = \theta_{t0} - \theta_{ti}$ ;  $\theta_{t0}$  = soil water content on 30<sup>th</sup> March and 4<sup>th</sup> April in 2011 and 2012 respectively;  $\theta_{ti}$  = soil water content at any time)



**Fig. 18:** Biplots of root traits (*RD*, root diameter; *RLD*, root length density; *RLD<sub>top</sub>*, root length density at topsoil (10-20 cm); *RLD<sub>vfr</sub>*, root length density of very fine roots (*RD*<0.4 mm);  $\beta$ , rooting distribution; *RS*, root to shoot ratio; *TMD*, tissue mass density; *SRL*, specific root length; *RC*, root capacitance;  $\Delta$ , soil water depletion) and the tested genotypes in 2011 and 2012, respectively

#### 4.8 Yield and yield components

Significant ( $P<0.001$ ) genotypic variation was observed for grain yield and all other yield components. Combined ANOVA of the core set revealed also significant variation for year and genotype $\times$ year interaction (**Table 4**).

Grain yield varied from 209.2 (TRI5254) to 541.3 g m<sup>-2</sup> (7060) and 37.9 (PI428154) to 237.7 g m<sup>-2</sup> (‘Floradur’) in 2011 and 2012, respectively (**Table 11**). Mean drought-induced grain yield loss in 2012 was 60.6% for the core set. Adapted durum cv. ‘Floradur’ showed the highest grain yield among core set genotypes followed by early flowering cv. ‘Matt’ and Khorasan wheat ‘QK-77’ (Kamut®), whereas *T. monococcum* and *T. timopheevi* accessions were lowest yielding. Yield reduction in 2012 was lowest for Khorasan (20.5%), intermediate for ‘Matt’ and ‘Floradur’ (51.2 and 54.7%, respectively) and highest for the einkorn and Zanduri wheat (80.1-85.2%).

Total above ground biomass ranged from 615.1 (TRI5254) to 1171.2 g m<sup>-2</sup> (7060) and from 504.4 (‘Floradur’) to 326.7 (PI428154) g m<sup>-2</sup> in 2011 and 2012 respectively (**Table 11**). A mean biomass loss of 57.7% was observed for the core set in respond to drought in the second year, with more evident loss for the einkorn and Zanduri wheat. Durum cv. ‘Floradur’ and ‘Matt’ produced the highest and lowest-biomass over two years, respectively.

**Table 11:** Genotypic mean values of grain yield and yield components in 2011 and 2012

Year/Genotype	YLD <sup>1</sup> (g m <sup>-2</sup> )	BM (g m <sup>-2</sup> )	HI	TIL <sub>f</sub> (n m <sup>-2</sup> )	SPE (n)	TGW (g)	PH (cm)	WU <sub>ET</sub> (mm)	WUE <sub>b</sub> (g m <sup>-2</sup> mm <sup>-1</sup> )
2011									
7060	541.3	1171.2	0.46	354.3	36.5	41.8	66.3	247.8	4.78
7063	472.5	1087.5	0.43	324.0	29.2	50.8	73.8	223.2	4.92
7094	404.1	939.4	0.43	310.1	29.8	44.8	68.8	258.0	3.76
Clovis	413.9	941.4	0.43	296.7	25.9	54.0	73.8	234.7	4.17
Floradur	525.0	1165.4	0.45	383.3	28.1	48.7	72.5	242.9	4.86
Matt	365.9	810.6	0.45	286.7	26.3	48.4	60.0	224.3	3.67
SZD3146	395.9	908.6	0.44	302.1	25.6	51.9	72.5	238.2	3.84
Kamut	254.2	856.3	0.29	227.6	17.1	67.2	105.0	248.6	3.51
TRI5254	209.2	615.1	0.34	248.6	16.8	52.5	107.5	260.1	2.39
W9	256.4	1001.9	0.26	530.8	17.5	27.8	70.0	277.8	3.63
PI428154	240.5	909.6	0.26	759.6	12.7	25.2	67.5	272.0	3.39
PI428165	247.5	926.0	0.27	994.8	11.4	21.8	71.3	275.7	3.41
s.e.d. <sup>2</sup>	55.8	111.7	0.016	56.2	2.8	2.5	2.2	20.7	0.65
d.f.	33	33	36	33	33	33	33	33	33
2012									
Tabasi	141.3	348.9	0.40	276.9	13.2	38.2	70.3	173.3	2.03
Taifun	126.4	387.6	0.33	325.0	11.2	35.3	61.0	161.4	2.48
Floradur	237.7	504.4	0.47	362.5	18.1	36.1	63.3	156.3	3.33
Matt	178.9	402.4	0.44	295.2	14.5	42.5	50.0	145.9	2.73
Kamut	202.1	481.7	0.42	261.6	15.7	49.4	79.8	163.5	2.97
W13	152.0	373.2	0.41	345.2	17.5	25.2	78.8	162.6	2.33
W9	37.9	368.2	0.11	355.8	4.2	25.7	74.8	167.2	2.21
PI428154	37.9	326.7	0.12	447.1	4.2	20.7	73.3	161.7	2.03
PI428165	49.3	328.8	0.15	527.9	4.7	20.1	69.0	177.0	1.87
s.e.d.	21.2	49.4	0.022	37.8	1.5	1.4	3.8	12.6	0.40
d.f.	27	27	27	27	27	24	24	24	24

<sup>1</sup> YLD, grain yield; BM, shoot biomass; HI, harvest index; TIL<sub>f</sub>, number of fertile tillers; SPE, seeds per ear; TGW, thousand grain weight; PH, Plant height; WU<sub>ET</sub>, water use; WUE<sub>b</sub>, Water use efficiency for biomass

<sup>2</sup> s.e.d., standard error of differences; d.f., degrees of freedom

In 2011 the highest harvest index (HI) values were observed for durum wheat (mean 0.42), followed by Khorasan wheat (0.32) and the underutilized wheat species *T. monococcum* and *T. timopheevii* (0.26). In 2012 HI of the latter underutilized wheat species decreased significantly (0.13) in response to drought, while ‘Floradur’ and ‘Matt’ almost retained their HI. Interestingly, Khorasan wheat ‘QK-77’ showed even an increase in HI.

Einkorn and Zanduri wheat showed a significantly higher number of fertile tillers, whereas Khorasan wheat had the lowest tillering capacity. Number of fertile tillers correlated significantly with RLD in topsoil (0-20 cm) in both years (2011:  $r=0.86$ ,  $P<0.001$ ; 2012:  $r=0.70$ ,  $P<0.05$ ).

Number of seeds per ear varied from 11.4 (PI428165) to 36.5 (7060) and 4.2 (PI428154) to 18.1 ('Floradur') in 2011 and 2012, respectively. Elite durum germplasm had a significantly higher number of seeds per ear compared to underutilized wheats, showing that seed number is a key component for high yielding cultivars.

*T. turanicum* and *T. monococcum* showed the largest and smallest seed weight, respectively. Particularly for Khorasan wheat, seed weight was the component ensuring a relatively high yield.

With respect to plant height *T. turanicum* was significantly taller than the other wheat species. **Fig. 19** shows the sensitivity of the core set genotypes for yield components in response to low water availability. It is evident that seed number per ear and tillering were highly sensitive (46 and 29% average reduction, respectively) providing plants with high plasticity in response to water availability. Contrary, seed weight was relatively insensitive to changing conditions (18.6% average reduction).

Within the core set, underutilized wheat species were most drought sensitive with respect to seeds per ear (68.5%) and tillering (41.8%), while they had a relatively stable seed weight (11%). Durum varieties had high sensitivity for seeds per ear (40%) followed by seed weight (19.1%). Khorasan wheat responded to drought stress mainly with seed weight loss (26.5%) along with plant height reduction.

#### **4.9 Water use and water use efficiency**

Water use, achieved from total in-season rainfall plus soil water depletion ( $\Delta$ ) in depth 0-90 cm, varied significantly between genotypes in each year and ranged from 223.2 (7063) to 277.8 mm (W9) and from 145.9 ('Matt') to 177 mm (PI428165) in 2011 and 2012, respectively (**Table 11**).

Water shortage in 2012 resulted in a 37% reduction in average  $WU_{ET}$  among core set genotypes (i.e. from 256.9 in 2011 to 161.9 mm in 2012). Einkorn wheat PI428165 (226.3

mm) and durum cv. ‘Matt’ (185.1 mm) showed the highest and lowest  $WU_{ET}$  over the two years.

Water use efficiency ( $WUE_b$ ) showed significant differences among the germplasm in both years. ‘Floradur’ along with durum lines 7060 and 7063 had highest  $WUE_b$  in 2011. ‘Floradur’ remained superior in  $WUE_b$  also in 2012. Genotypes with lowest  $WUE_b$  were Khorasan wheat TRI5254 in 2011 and einkorn wheat along with Iranian wheat ‘Tabasi’ in 2012. Average  $WUE_b$  of the core set dropped from 3.7 to 2.5 g m<sup>-2</sup> mm<sup>-1</sup>. ‘QK-77’ was the most stable genotype of the core set in sustaining  $WUE_b$  (15.4%) while ‘Floradur’ (31.5%) and ‘Matt’ (25.6%) had an intermediate response. Underutilized wheat species were most susceptible to drought stress (41.4%).

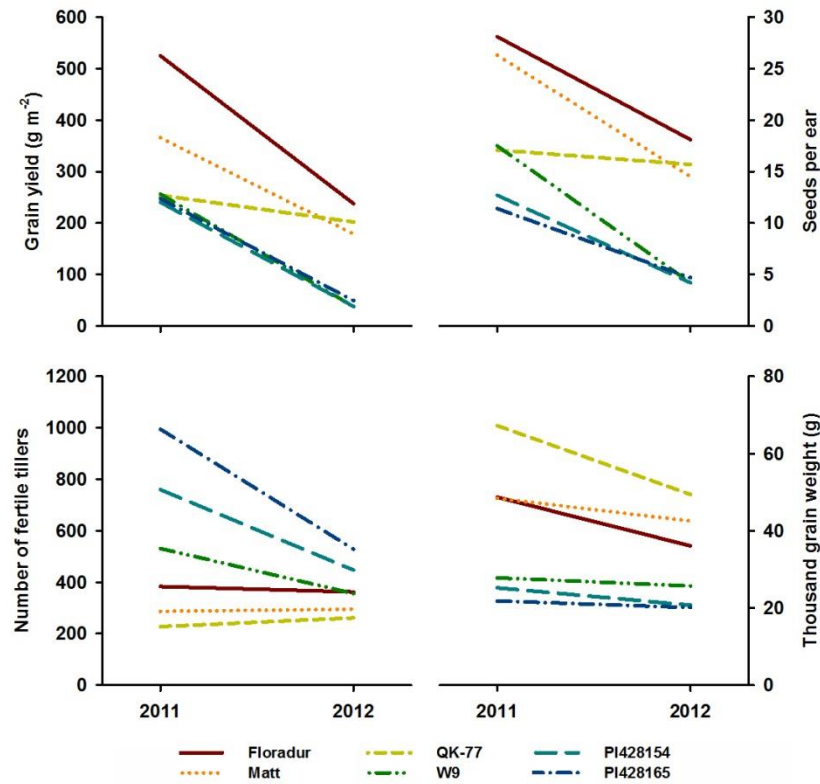
#### **4.10 Components of Passioura’s yield-water framework and related traits**

**Fig. 20** shows the components of the yield-water framework according to Passioura (1977) and relations to traits that we hypothesized to constitute the crops’ phenological, morphological and physiological drivers of  $WU$ ,  $WUE$  and  $HI$ . Beside direct relations of traits with Passioura’s components, we also provide some secondary intra-trait relations suggesting hierarchical dependences among traits.

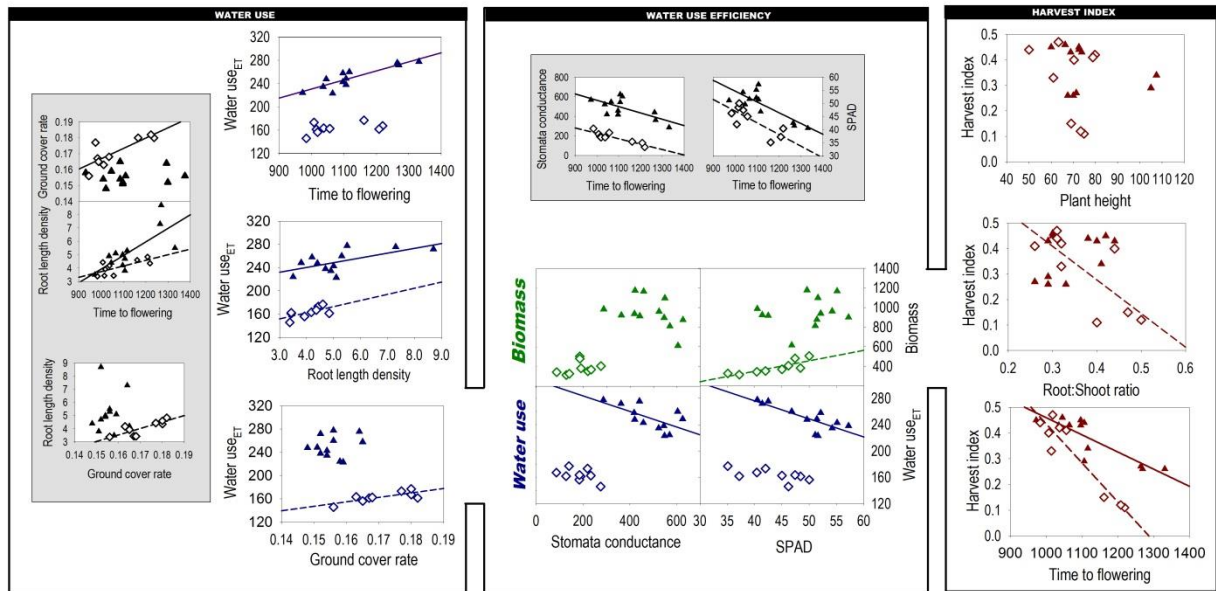
The relation of flowering time to  $WU_{ET}$  was significant in 2011 while in 2012 there was less variation in  $WU_{ET}$ . In both years a strong relationship between root length density and  $WU_{ET}$  was observed. Under water limited condition of the second year, a significant relation was observed between early vigor and  $WU_{ET}$  (**Fig. 20**).

#### **4.11 Trait based grouping of genotypes**

Association between genotypes (and years) based on (i) yield components, (ii) Passioura components ( $WU_{ET}$ ,  $WUE_b$ ,  $HI$ ), (iii) phenological, morphological and physiological traits related to Passioura’s components, and (iv) all traits was revealed by cluster analysis (**Fig. 21**). Including all genotypes reveals the strength of group linkage driven by genotypic similarity (constitutive) and environmental influence (adaptive), respectively. Using different clustering variables shows which group of traits mainly expresses constitutive or adaptive linkage between genotypes.



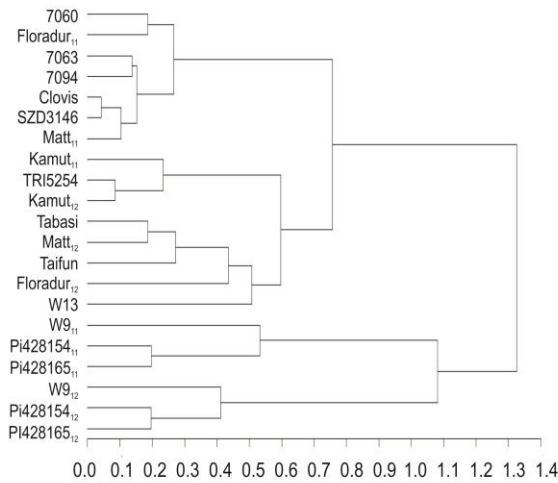
**Fig. 19:** Response of grain yield and yield components of core set genotypes in a wet (2011) and dry (2012) year indicating distinct phenotypic plasticity ('Floradur', 'Matt': *T. durum*; 'QK-77': *T. turanicum*; W9, *T. timopheevii*; PI428154, PI428165: *T. monococcum*)



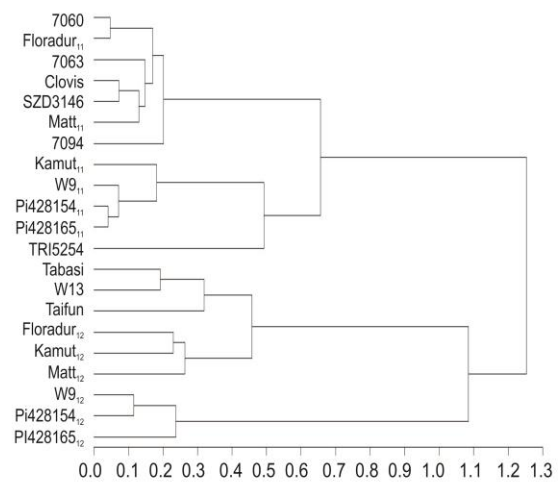
**Fig. 20:** Phenological, morphological, physiological and root traits underlying the components of Passioura's yield-water framework. Figures on grey background indicate secondary inter-trait associations. (Closed triangles: 2011; open diamonds: 2012; regression lines indicate significant relations)



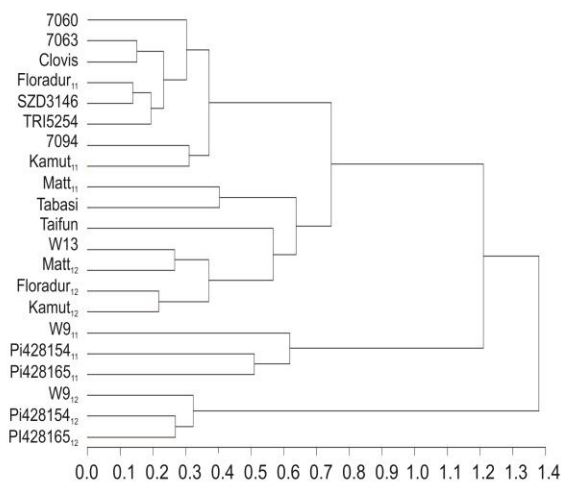
### (i) Yield components



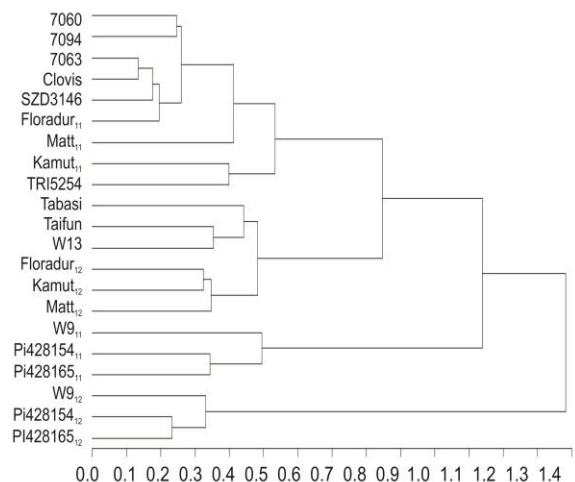
### (ii) Passioura components



### (iii) Shoot-Root traits



### (iv) All traits



**Fig. 21:** Hierarchical clustering of wheat genotypes based on (i) yield components, (ii) components of Passioura's yield-water framework, (iii) phenological, morphological, physiological and root traits related to components of Passioura's yield-water framework, and (iv) all traits.

The distinction between underutilized einkorn and Zanduri wheat and the other genotypes appeared at the highest hierarchical with the exception of (ii) Passioura components. In this case the first grouping was according to years, which is explained by the strong water dependence of these traits. At a lower hierarchical level four clusters can be distinguished,

subdividing the whole sample according to years and/or wheat species. For clustering based on yield components a differentiation at a lower level is even obvious between durum and Khorasan wheat. With respect to Passioura components only four main clusters can be distinguished. Interestingly, Khorasan wheat 'QK-77' changes the group between years: in 2011 (high water availability) 'QK-77' is group together with the other underutilized wheat species *T. monococcum* and *T. timopheevii*, while in 2012 (low water availability) it joins the group of modern durum cultivars. The most meaningful grouping at high and low distances is provided when considering all traits. Here, in 2011 *T. durum* and *T. turanicum* are grouped in different clusters. Among the 2012 clusters, hexaploid wheats are next to each other, while einkorn and Zanduri wheat form distinct groups.

## 5. Discussion

### 5.1 Drought environment characterization

Understanding crop response to drought and relevant traits conferring better stress resistance requires a precise environmental characterization (Blum 2011a). Simulation models have been shown to be an appropriate tool for a proper description of the target environment for crop management and breeding activities (Chauhan *et al.* 2013). Continental climates as found in central-eastern Europe are distinguished by a higher proportion of in-season rainfall compared to stored soil moisture as source of crop water supply. Thereby, they differ essentially from storage driven Mediterranean winter rainfall climates or subtropical sites where dry season crops grow on residual soil moisture. Still, stored water can be essential to buffer temporary dry periods affecting crop yield particularly when their occurrence coincides with sensitive growth stages.

The substantial change of crop performance due to low stored soil moisture together with low precipitation around flowering in our experiment clearly revealed that average climate variables (*e.g.* annual or seasonal rainfall sum) are insufficient to provide an appropriate picture on crop water stress.

### 5.2 Phenological and physiological characterization

#### *Phenology*

Flowering is the most sensitive stage to water shortage (Farooq *et al.* 2012). Synchronizing plant water demand with seasonal water availability during pre- and post-anthesis growth is a substantial breeding target in rainfed drought prone environments (Passioura 2006, Blum 2009, Passioura 2012).

Progress has been achieved by breeding for earliness allowing crops to escape terminal drought stress and access enough soil water during flowering and grain filling (Salekdeh *et al.* 2009). However, vigorous growth and developing enough biomass prior to flowering is also critical for yield potential. In the present in-season rainfall environment yield limitation due to earliness was clearly demonstrated by low grain yields of early maturing cv. ‘Matt’ compared to other advanced varieties and/or breeding lines (**Tables 5 & 11**). Grain yield of early

maturing genotypes is largely limited by the potential number of grains per unit area which is determined between stem elongation and post-anthesis (Slafer *et al.* 2014).

A significant relation was observed between time to flowering and  $WU_{ET}$  in 2011 (**Fig. 20**) when wetter soil profile alongside with in-season rainfalls provided appropriate conditions for longer root water uptake of late flowering genotypes. However, low water availability in May and June in 2012 obviously restricted the prolonged water extraction by late flowering varieties and hence reduced variation in  $WU_{ET}$ . Lower water use, limiting yield potential of very early cultivars, can be attributed to a reduced rooting intensity (**Fig. 20**). Mitchell *et al.* (1996) reported a negative relationship between days to flowering and grain yield and greater water extraction of late maturing varieties. Siddique *et al.* (1990) reported similar results comparing old and modern wheat varieties where late-maturing old varieties showed more water use in the pre- than post-anthesis period.

Beside the constitutive differences among genotypes, there was also phenological plasticity in response to water availability. Accelerated development in 2012 was more evident for early stages, i.e. from emergence until stem elongation (**Fig. 13**). Transition from vegetative into reproductive phase was obviously stimulated by water stress. This is a well-known plasticity in wheat, *e.g.* McMaster & Wilhelm (2003) reported that cumulative thermal time (CTT) to stem elongation was highly variable while CTT of subsequent growth stages was very stable.

Accelerated growth development limited time for developing tillers in 2012. Water stress, therefore, resulted in a reduced number of tillers in this year (**Table 11**). In regard to water use, tillering is relevant due to the secondary nodal root system developing from tillers (Zobel & Waisel 2010). Thus, a shortened period between emergence and stem elongation can limit the development of nodal roots, resulting in lower water use. Strong association between  $WU_{ET}$  and root length density, observed in both years, indicates that roots are key determinant for the  $WU_{ET}$  component in Passioura's framework (**Fig. 20**).

#### *Early vigor*

An interesting trait promoting water use under conditions of limited availability was early vigor. Rapid ground cover can protect stored soil moisture by shading the soil to reduce evaporation losses (López-Castañeda & Richards 1994), increase total photosynthesis by extending the duration of light capture (Parry *et al.* 2011) and enhance weed competitiveness of the crop (Bertholdsson 2005). Botwright *et al.* (2002) emphasized that the benefit of early

vigor for higher yields is environment-specific. Rebetzke *et al.* (2014) suggested that early vigor might serve as a proxy for the improvement of root vigor under high strength and no-till soils. Our results showed a significant association of early vigor with water use only in the dry year 2012, suggesting secondary associations of this trait with phenology and root length density (**Fig. 20**). With respect to inter-trait relations, however, results should be treated with caution if the data are concentrated at the two ends of the regression line. The associations might be a consequence of constitutive differences between underutilized wheat species and modern varieties rather than expressing causal inter-trait relations.

#### *Stomatal conductance and photosynthetic capacity*

Crop growth depends on acquiring CO<sub>2</sub> through open stomata, which in turn results in water loss through transpiration. However, suitability of stomatal conductance as selection criterion has been demonstrated under both drought stress and well watered conditions (Rebetzke *et al.* 2013). The significant genetic variation observed for stomatal conductance over the two study years is consistent with previous studies (Fischer *et al.* 1998, Araus *et al.* 2002, Rebetzke *et al.* 2003). Early maturing durum ‘Matt’ was in both years among the genotypes with highest stomatal conductance, suggesting an association between earliness and/or crop growth rate with stomatal conductance. Araus *et al.* (2002) pointed to higher stomatal opening as a consequence of crop earliness and lower leaf area index (LAI). Also in our study stomatal conductance was significantly and negatively correlated with LAI in the dry year 2012 ( $r = -0.75$ ,  $P < 0.05$ ; **Table 5**). Contrary, late maturing *T. monococcum* and *T. timopheevii* had the lowest values of stomatal conductance. An influence of ploidy level on stomata characteristics with diploid species, having the smallest stomata, was demonstrated by Khazaei *et al.* (2010). Low stomata conductance of einkorn and Zanduri wheat suggested a conservative gas exchange strategy. Their comparatively high water use is, therefore, explained rather by prolonged duration of transpiration than a high rate of water extraction due to conductive stomata.

Recent evidence indicated that historic gains in wheat grain yield are not exclusively a consequence of changed dry matter partitioning, but also of increased total shoot biomass (Fischer & Edmeades 2010, Sadras & Lawson 2011). Therefore, Parry *et al.* (2011) suggested that enhanced photosynthetic capacity might lead to further yield improvement. In the present study photosynthetic capacity, was estimated by leaf chlorophyll measurements at heading, which revealed an inherently low photosynthetic capacity of underutilized wheat species.

Stomatal conductance and photosynthetic capacity, both traits underlying intrinsic WUE (Condon *et al.* 2002), seem to be strongly related to constitutive differences resulting from different breeding intensities. Similar to other studies, we found a significant association between stomata conductance and photosynthetic capacity (2011:  $r=0.73$ ,  $P<0.01$ ; 2012:  $r=0.65$ ,  $P=0.058$ ). This indicates a tight functional link between stomata opening ensuring high CO<sub>2</sub> inflow and photosynthetic capacity providing efficient fixation of available carbon in modern high yielding varieties. It also confirms the challenge of improving intrinsic WUE by lower stomata conductance without compromising crop productivity (Blum 2005, Lawson *et al.* 2012). Fischer *et al.* (1998) demonstrated the association of leaf photosynthetic rate and stomatal conductance with yield progress in CIMMYT wheat genotypes. Also Reynolds *et al.* (1994) reported a significant association between photosynthetic rate and stomatal conductance with grain yield. Combining stomata conductance and leaf chlorophyll content measurements could allow the identification of germplasm combining improved WUE and productivity under both well watered or water limited conditions (Rebetzke *et al.* 2013).

### **5.3 Root diversity and root functional characterization**

#### **Root morphology and root system shape**

##### *Root diameter*

The importance of RD was already indicated by Böhm (1979). Among the investigated root traits RD showed the lowest variation over years. The non-significant genotype×year interaction indicates the stability of this trait and, thus, its suitability for selection. The slight increase of RD in the drier year 2012 might be a response to the greater mechanical resistance of drying soils (Kirkegaard *et al.* 1992) which requires thicker roots with improved penetration ability (Materechera *et al.* 1991, Clark *et al.* 2008).

Our results revealed a dominance of fine roots in the tested accessions of underutilized einkorn wheat. The advantage of a dense fine root system during water and nutrient deficiency has been frequently reported (Fitter 1994). Zobel & Waisel (2010) demonstrated that within the ‘tertiary’ fine root system different diameter classes were not only functionally different, but also under distinct genetic control. Still further investigations are essential to better interpret the functional implications of differences in RD, *e.g.* higher penetration ability (Clark *et al.* 2008).

### *Root length density*

In the present study RLD showed a high variation (3.4 to 8.7 cm cm<sup>-3</sup>). A similar broad variation in wheat (2 to 10 cm·cm<sup>-3</sup>) has been reported by Manschadi *et al.* (2013) depending on the stage of plant development, soil depth and environmental factors. From our results it seems that underutilized wheat species such as *T. monococcum* and *T. timopheevi* have a higher potential in rooting density. The tested genotypes of these species exploited well the high water availability in 2011 by producing the highest root length density in all soil depths. But they were also the superior genotypes with respect to RLD at deep soil layers under more limited moisture condition of 2012. We suppose that the higher tillering capacity of these species results in a more intense shoot-borne root system arising from lower stem nodes (Klepper *et al.* 1984, Zobel & Waisel 2010). This is particularly evident for the topsoil RLD under the high moisture condition of 2011. A positive correlation between RLD in topsoil and/or root number with tiller number in cereals was also reported by Hockett (1986) and Manske *et al.* (2000).

### *Rooting distribution*

The root system was reversely distributed through the soil profile in the two experimental years due to the different rainfall availability (**Fig. 14**). The Iranian bread wheat ‘Tabasi’ was not only similarly competitive as *T. monococcum* with respect to total RLD, but it appears that ‘Tabasi’ also successfully exploits deeper soil layers. This suggests the potential of this landrace as genetic resource for improving the deep rooting potential of other varieties. The (intermediate) drought tolerance of ‘Tabasi’ was also confirmed by other studies using different traits (Hasheminasab *et al.* 2012). Deep rooting and high RLD at depth has been known as a common strategy of plants to avoid dehydration (Hurd 1968, Price *et al.* 1997, Ehdaie *et al.* 2012) and thereby positively influence grain yield especially under terminal drought condition (Passioura 1983, Ludlow & Muchow 1990, Gaur *et al.* 2008). More roots at depth as a response to lower rainfall in 2012 was also observed for the *T. timopheevi* accession W9. This response can be regarded as adaptive phenotypic plasticity (Nicotra & Davidson 2010) improving the performance over a range of soil moisture conditions.

### *Root tissue mass density and specific root length*

Genotypes with low tissue mass density (TMD) invest fewer assimilates to build up one unit of volume of roots. This enables plants to develop a more extensive root system, resulting in

high acquisition capacities for below-ground resources (Wahl & Ryser 2000). In 2012 TMD showed a significant decrease in response to less water availability compared to 2011 (**Table 8**), indicating differences in assimilate allocation within root system. Moreover, TMD decreased with soil depth which is likely to also reflect differences in water and nutrient availability (Stetson & Sullivan 1998).

The tested underutilized wheat species (*T. monococcum*, *T. timopheevi*, *T. carthlicum*) were among the genotypes exhibiting low TMD values and, therefore, invested low assimilation costs for the construction of one unit of volume of roots. The efficiency of the root system can be expressed in terms of water gain (soil water depletion) per unit root mass. On this basis, a modern durum cultivar such as ‘Floradur’ significantly differed from e.g. the einkorn wheat accessions PI428154 and PI428165, *i.e.* 193 mm g<sup>-1</sup> vs. 331 and 447 mm g<sup>-1</sup>, respectively.

It is believed that specific root length (SRL) represents the economic response of the root system to environmental changes (Ostonen *et al.* 2007). Higher SRL refer to a higher proportion of fine roots length (Ryser 2006). Genotypes with a dominant fine root system produce a relatively larger root system and achieve a high SRL (Løes & Gahoonia 2004), and hence, exploit a higher volume of soil per unit of root biomass. Durum cultivars ‘Floradur’ and ‘Matt’, and also ‘QK-77’ (Kamut®) wheat showed a considerable increase of SRL in response to less rainfall in 2012, whereas the underutilized wheat species, particularly the einkorn wheat accessions, appeared to be more stable with respect to this root trait (**Table 8**). We hypothesize that *T. durum* cultivars rely more on alteration of assimilate allocation within their root system.

The observed trade-off between RLD and RD (**Fig. 14**) was reflected in an increase of SRL and also decrease of TMD down to the soil profile in both years (**Table 8**). The distribution of soil water content in respective soil depths (10-20, 30-40, 50-60 cm – see Fig. 4-3) underlies the observed distribution for SRL and TMD.

#### *Root to shoot ratio*

Root to shoot ratio (R:S) indicates assimilate partitioning between above-ground and below-ground organs. It has been shown that, for a given water supply, there is an optimal R:S resulting in a maximum above-ground biomass (Passioura 1983, Ehdaie *et al.* 2012). This makes R:S a relevant breeding trait. Our two years results revealed a species-dependent response of genotypes in assimilate allocation to different water availability. Unlike advanced



cultivars, genetic resources of *T. monococcum* and *T. timopheevi* appear to be more dependent on alteration of assimilate allocation between root and shoot. Such high root plasticity is considered as an important feature in environments with uncertainty in water supply (El Hafid *et al.* 1998, Grossman & Rice 2012). An increase of R:S has been reported in plant's response to moderate water deficit (Blum *et al.* 1983, Reynolds *et al.* 2007). However this is not always an observed response (Poorter & Nagel 2000) and root systems frequently respond to moderate water shortage by changing assimilate partitioning within the root system. Such responses were already discussed regarding to the tissue mass density and specific root length.

### **Seminal root anatomy**

In a drying soil, root system is a major component of resistance to water transport in the soil-plant-atmosphere continuum (Blum 2011a). The number of seminal axes and the diameter of their main xylem vessels determine the extent of axial resistance to the longitudinal flow of water (Richards & Passioura 1981a,b). In this study the number of seminal roots varied from 3.6 for einkorn wheat to 6 for Khorasan wheat 'QK-77'. A similar range of variation has been reported by Gregory *et al.* (1978) and Manschadi *et al.* (2008). The significant and positive correlation between seminal root number and RLD at 50-60 cm soil depth suggests the functional importance of seminal roots for deeper soil exploration. These results are consistent with Watt *et al.* (2008) who argued that seminal roots are primarily responsible for root growth to deeper soil layers.

Xylem vessel area has a major influence on hydraulic conductance of mature roots; therefore, it could play a critical role under condition of late season water shortage. A smaller xylem diameter implies a conservative water use during vegetative growth and sufficient stored soil water remaining for the generative period (Richards & Passioura 1989).

Taken main xylem vessels cross sectional area and number of seminal axes together would give an estimation of the longitudinal axes resistance against water flow in the xylem. The highest total area of main xylem vessel and, therefore, the lowest resistance against water flow, observed for the two *T. turanicum* genotypes as well as durum genotypes 'Clovis' and 7094. Similarly, the highest resistance could be expected for SZD3146, 'Matt' and 7063.

Oyanagi (1994) reported that genotypes adapted to drier environments show a deeper root system resulting from a smaller seminal root angle, whereas genotypes originating from environments with frequent rainfall display more horizontal seminal root growth and shallow

root systems. A conservative uptake strategy resulting from high axial resistance might not be appropriate for supply-driven ecohydrologies of drought-prone areas in temperate climates of Central Europe. Here an extensive root system in fertile and frequently refilled upper soil layers with high xylem conductance to transfer water and nutrients from the root to the sinks sites of the shoot is more appropriate for yield optimization. It is shown that there is an interplay between hydraulic properties of root and soil water regime which determines the pattern of the soil water depletion. In this regard, simulating models provide new opportunities to access to promising root traits in order to the maximizing of soil water availability to the plant (Draye *et al.* 2010).

### **Root functionality**

In a water balance framework, soil water depletion is an essential component for total plant water supply, beyond the incoming rainfall during the growth period. The higher the contribution of seasonal rainfall to total plant water supply (supply-driven ecosystems), the more important is a larger root system and vice versa (Palta & Gregory 1997, Palta *et al.* 2011). High late autumn rainfall in 2010 (**Fig. 10**) replenished the soil profile so that the profile was homogeneously wetted at sowing (**Fig. 11**), despite low precipitation in February and March 2011. The average temperature in 2011 was above the long-term average, while rainfall was slightly below the average. This resulted in a strong evaporative demand which depleted the initially wet profile comparatively quick. The lower water depletion of the middle soil layer (25-45 cm) from mid June onwards is in agreement with the root distribution observed in this year (**Fig. 14**). This can also be due to the infiltration of high rainfall down to 35 cm in the second half of June. While the upper profile dried out quickly due to evaporation and transpiration, the middle layer maintained higher water content upon refilling (**Fig. 11**). On the contrary the initial soil water condition at sowing in 2012 was the result of very dry weather in autumn 2011 (**Figs. 10 & 11**). This renders the crop water supply strongly dependent on in-season rainfall. While the uppermost layer (<15 cm) is regularly depleted by evaporation and transpiration, the lower layers (>35 cm) were not refilled by in-season rainfall due to the limited infiltration down to 30 cm. This caused a wetted layer between 15 and 35 cm soil depth.

In a drying soil, water flow to the root surface is limited by increasing soil resistance (Schröder *et al.* 2008, Draye *et al.* 2010, Blum 2011a) and the availability of water is mainly determined by the volume of soil explored. Therefore, higher rooting density lowers the

impact of soil resistance due to higher soil-root contact area (Draye *et al.* 2010, Blum 2011a). A higher linear regression coefficient between soil water depletion and RLD in 2012 reveals the importance of RLD under limited water condition for efficient water uptake. In agreement with our results El Hafid *et al.* (1998) reported an association between total water use and RLD in a Mediterranean climate where grain yield of durum wheat is limited by early season drought.

Our results revealed that genotypes exploit different strategies to maximize water uptake. The consistent superiority of einkorn wheat in regard to soil water depletion could be probably explained by their high topsoil RLD, resulting from high tillering and presumably abundant nodal roots. *T. timopheevi* on the other hand showed a high RLD and a tendency to deep rooting in response to water deficit, while the higher water uptake of Khorasan wheat TRI5254 can be explained by the highest longitudinal conductivity of xylem vessels. *T. carthlicum* W13 tested in 2012 had a high deep rooting along with very low TMD, causing an economic water uptake. Contrary, ‘Matt’ as an early maturing durum variety with the lowest values for RLD and xylem area had the lowest water extraction from the soil over 2 years.

This study revealed the key role of high topsoil RLD for soil water uptake, even under the limited moisture condition of 2012 when genotypes had a higher proportion of roots in deeper soil layers (**Fig. 14b**). At the experimental site the distribution of rainfall and the high soil water storage capacity result in highest plant water availability in the topsoil. Thus, water uptake by roots is dominant from the upper soil layers and genotypes with a dominant surface root system benefit from the ecohydrological conditions at the site. Furthermore, in 2012 soil water distribution at seeding date showed a gradual decrease of water availability with depth (**Fig. 11**). Thus, roots in deeper soil layers could not profit from stored soil moisture.

The Iranian bread wheat ‘Tabasi’ with a high rooting tendency to depth appeared to be an efficient cultivar in extracting soil water at depth. This result is consistent with those reported by Manschadi *et al.* (2006) who observed greater root length and water extraction at depth for drought-tolerant wheat under Mediterranean conditions. Deep rooting and higher RLD in deeper soil layers is, therefore, highly recommended for storage-driven environments where the contribution of stored soil water plays a more important role in plant water supply. In such environments yield is limited by water shortage during flowering and grain filling (Passioura

1983). Selecting such genotypes, however, would require root sampling in deeper soil layers which is time consuming and cost intensive.

### **Root electrical capacitance**

In a breeding program targeting at the improvement of the root system, the access to a rapid field screening method is essential. Our results showed that root electrical capacitance (RC) is strongly influenced by the year. This is explainable as differences in soil moisture are known to be the main factor for variable RC (Chloupek 1977, Kendall *et al.* 1982, Dalton 1995, McBride *et al.* 2008, Dietrich *et al.* 2013). The higher root system size (RSS) values in 2012 can be explained by a shorter time interval between the measurement and preceding rainfall and, thus, higher soil moisture. Hence, a comparison of genotypes by this method is only possible for a single date with homogeneous moisture conditions (Chloupek *et al.* 2010).

In the present study an inter- and intra-specific differentiation by RC was evident: *T. monococcum* and *T. timopheevi* showed the high RC values in both years, Iranian bread wheat landrace ‘Tabasi’ outperformed German variety ‘Taifun’. Inter- and intra-specific effects on the relationship between capacitance and root mass have been demonstrated also in previous studies (Chloupek 1972, McBride *et al.* 2008, Aulen & Shipley 2012). Based on our results RC can be adopted as field screening method at least under supply-driven environments with regular in-season rainfall where high topsoil rooting density is important for dehydration avoidance by maximization of water uptake. Although measured topsoil RLD might not be easily extrapolated to the plant material between the plant electrode and the soil surface which corresponds to the model for capacitance developed by Dietrich *et al.* (2012, 2013), the significant correlation between RC and topsoil RLD in both years reveal the importance of top root tissues in root electrical capacitance measurements. On the other hand, the significant correlation between RC and subsoil RLD in 2012 could be better explained by the model of Dalton (1995) as less water availability resulted in a higher root mass in deeper soil layers.

The methodology was hitherto successfully deployed to select for greater root mass of alfalfa (Chloupek *et al.* 1999), study the effect of dwarfing genes on root system size of barley (Chloupek *et al.* 2006), select barley for drought tolerance (Chloupek *et al.* 2010), and study the diversity of wheat varieties in regard to water use efficiency (Středa *et al.* 2012). Although different models and improvements (Dalton 1995, Dietrich *et al.* 2012, 2013, Ellis *et al.* 2013)

are now existing, the drawbacks of the method are still the lack of exact knowledge concerning the complex electrical circuit of the system. Thus, a straightforward interpretation which component of the measured soil-plant resistance-capacitance system is dominant in a given measurement situation is not possible.

The significant association of RC and soil water depletion revealed that the method is also informative with respect to root functioning. Dalton (1995) observed changes in root biomass and RC of tomato seedlings during their growth and proposed RC for discerning root activity or functionality. The capacitance method, therefore, seems to be capable to obtain information on the size of active roots in moist soil layers where most water extraction is taken place.

### **Root and shoot allometries, tiller number and leaf area index**

Root and shoot allometries have been proposed as an indirect way to screen for root system size (Richards 2008) assumed the root and shoot relationship is well understood at the genetic and phenotypic levels (Richards *et al.* 2010).

Underutilized wheat species such as *T. monococcum*, *T. timopheevi* and *T. carthlicum* seem to produce more shoot-borne roots in topsoil, especially in well-watered soil, due to their higher tillering capacity as revealed by significant association between the number of fertile tillers and RLD in topsoil. This finding is in agreement with reports by Hockett (1986) and Manske *et al.* (2000) indicating a positive correlation between RLD in topsoil and/or root number with tiller number. A higher number of tillers is known as distinctive characteristic of competitive cereal plants offering them an advantage in capturing nutrients and water. However, in terms of yield potential a restricted number of tillers per plant is recommended when water is limited (Richards *et al.* 2007).

Our results revealed a significant and positive correlation between LAI and RLD in the dry year 2012. Ratios of root length or root surface area to leaf surface area are supposed to be more functional descriptive than mass-based ratios (Comas *et al.* 2013).

Shifts in root and shoot allometries are, however, an adaptive plant response to withstand sub-optimal conditions. For instance, this study demonstrated that underutilized wheat species with a survival strategy are more reliant on increase of R:S when facing water stress (**Fig. 15**), while durum genotypes retain the R:S more constant and respond to water deficit by a shift in root morphology reflected in their higher SRL.

## Root system classification

Although some single root parameters may have a dominant role for a given target function, the assessment of the total root system diversity should be taken into account. Univariate analysis of variance can only reveal differences in a single trait. Our results showed that not for all traits the same genotypes differed among each other and there were changes in their ranking. Therefore, Bodner *et al.* (2013) proposed using multivariate approaches for the classification of root systems. We followed this method to determine the diversity in root system within the tested genotypes. Thereby, similar rooting patterns related to phylogenetic relations, ploidy levels, regional origin or other factors should be identified. In fact, the biplots revealed a consistent rooting type for *T. monococcum* and *T. timopheevi* with fine and topsoil concentrated root axes (**Fig. 18**). Adapted durum variety ‘Floradur’ was characterized by thick roots with high TMD, whereas early maturing ‘Matt’ showed a less dense and topsoil concentrated root system. The two common wheat varieties ‘Tabasi’ and ‘Taifun’, originating from contrasting environments (*i.e.* Iran and Germany, respectively) were placed diagonally opposite. Therefore, the results suggest that both regional origin and genetic background were responsible for the observed root system diversity.

From the biplots it is revealed that the topsoil concentrated rooting type of underutilized wheat species resulted in higher functionality of this group in terms of soil water depletion. Length and position of vectors for RLD parameters, root capacitance and soil water depletion were consistent over both experimental years. Root capacitance seems to be efficient for interspecific differentiation (*e.g.* einkorn vs. durum wheat) but not for intraspecific differentiation (*e.g.* varieties within species).

## 5.4 Plasticity of yield components

Grain yield of cereal crops is based on the number of fertile tillers, number of seeds per ear and thousand grain weight. These components allow a plastic plant response to resource availability at different development stages. It has been shown that the number of ears per unit area is the main component responding to environmental variability (*e.g.* water supply). Additionally, other buffering sources (*e.g.* stem carbohydrate reserves) can increase the plasticity of seeds per ear and seed weight (Slafer *et al.* 2014).

In the present study the reduction of yield in 2012 was associated with different genotypic responses of single yield components. For example, ‘QK-77’ (Kamut®) stabilized its grain

yield at the cost of shoot biomass via a significant decrease in plant height, resulting in an increased HI, suggesting a potential for partitioning of biomass to seeds as an important stress adaptive trait frequently found for cereals (Blum 1998, Shearman *et al.* 2005, Dreccer *et al.* 2009). Reduction of competition from alternative sinks (stem and infertile tillers) is hypothesized as an opportunity to increase the partitioning to spikes and further increase HI beyond its current limit (Foulkes *et al.* 2011). Although ‘QK-77’ can be considered a water stress tolerant genotype, it does not show high yield potential under favorable water condition. Contrary, *T. monococcum* and *T. timopheevi* significantly reduced their number of fertile tillers, their main yield component, in response to suboptimum water availability (**Fig. 19**). Number of seeds per ear and seed weight, which are both related to grain sink strength (Miralles & Slafer 2007, Acreche & Slafer 2009), are basically very low in these species, resulting in significantly lower HI despite reasonable biomass production. Restricting tillering capacity is considered beneficial where water limitation requires a more conservative uptake strategy over the growing season to provide the crop with enough water during grain filling (Richards *et al.* 2010). The main yield component of durum varieties was number of seeds per ear followed by seed weight, whereas number of fertile tillers showed no plasticity. Slafer *et al.* (2014) recommended a balanced dependence of grain yield on single components to ensure both high yield potential and sufficient plasticity in response to water limitation.

### **5.5 Modern and ancient wheat varieties in Passioura’s yield-water framework**

Clustering genotypes based on Passioura’s components revealed a clear distinction between tetraploid *T. turgidum* and underutilized *T. monococcum* and *T. timopheevi*. On the other hand, Khorasan wheat, a *turgidum* subspecies genetically similar to durum wheat but with lower breeding intensity, was more variable between and within clusters (**Fig. 21**). Also cluster analysis revealed that constitutive differences between underutilized wheat species and modern varieties can be identified beyond strong adaptive plasticity of genotypes, with the exception of water dependent traits (*i.e.* water uptake, water use efficiency).

The strength of underutilized wheat species in the supply-driven hydrology of continental Europe was water use, while they were clearly inferior in all other components. Efficient root water extraction of the underutilized wheat species were linked to high assimilate translocation to roots, high tillering capacity and long vegetative growth. While the high tiller number of underutilized wheat species was evidently a yield limiting factor as revealed by its

negative correlation to harvest index. Therefore, optimization of tiller, related to nodal rooting, for high water uptake is constraint within tight limits. However, other root system traits might provide alternatives to improve water use under stress conditions. For instance, this study revealed that underutilized wheat species increased root-to-shoot ratio to compensate for limited soil water availability, while the modern durum cultivars as well as Khorasan wheat ‘QK-77’ increased specific root length (**Table 8 & Fig. 15**). Under dry conditions, the latter adaptive response is more desired than alteration of assimilate allocation between roots and shoots, as it does not impose competition for assimilates. This is obvious with less reduction in water use of durum cultivars and ‘QK-77’ (34.9%) compared to einkorn and Zanduri wheat (38.7 %).

For modern high yielding cultivars we provided evidence that physiological traits (*e.g.* stomata conductance combined with leaf chlorophyll concentration) are relevant to understand their superior performance in both well watered and stress conditions. These physiological traits seem to be linked to crop growth rate and, thereby, phenology. Within modern germplasm several unexploited physiological and morphological adaptive traits were observed that should be further explored, such as above-mentioned root plasticity as a means to ensure sufficient water uptake under stress conditions. The high yield stability of *T. turgidum* subsp. *turanicum* provided evidence that, despite limited yield potential, also underutilized genetic material can be a source of interesting adaptive processes for future trait based breeding with respect to drought tolerance.

Genetic variation in harvest index within our germplasm was largely determined by distinct differences in yield components and phenology (**Fig. 20**). Unlike modern cultivars, underutilized wheat species were more dependent on alteration of assimilate allocation between roots and shoot in response to drought (**Fig. 15**). The observed association between harvest index and root to shoot in the second year most probably results from an intrinsic low harvest index of the underutilized wheat species resulting from their high allocation to roots under limited water availability.



## 6. Conclusions

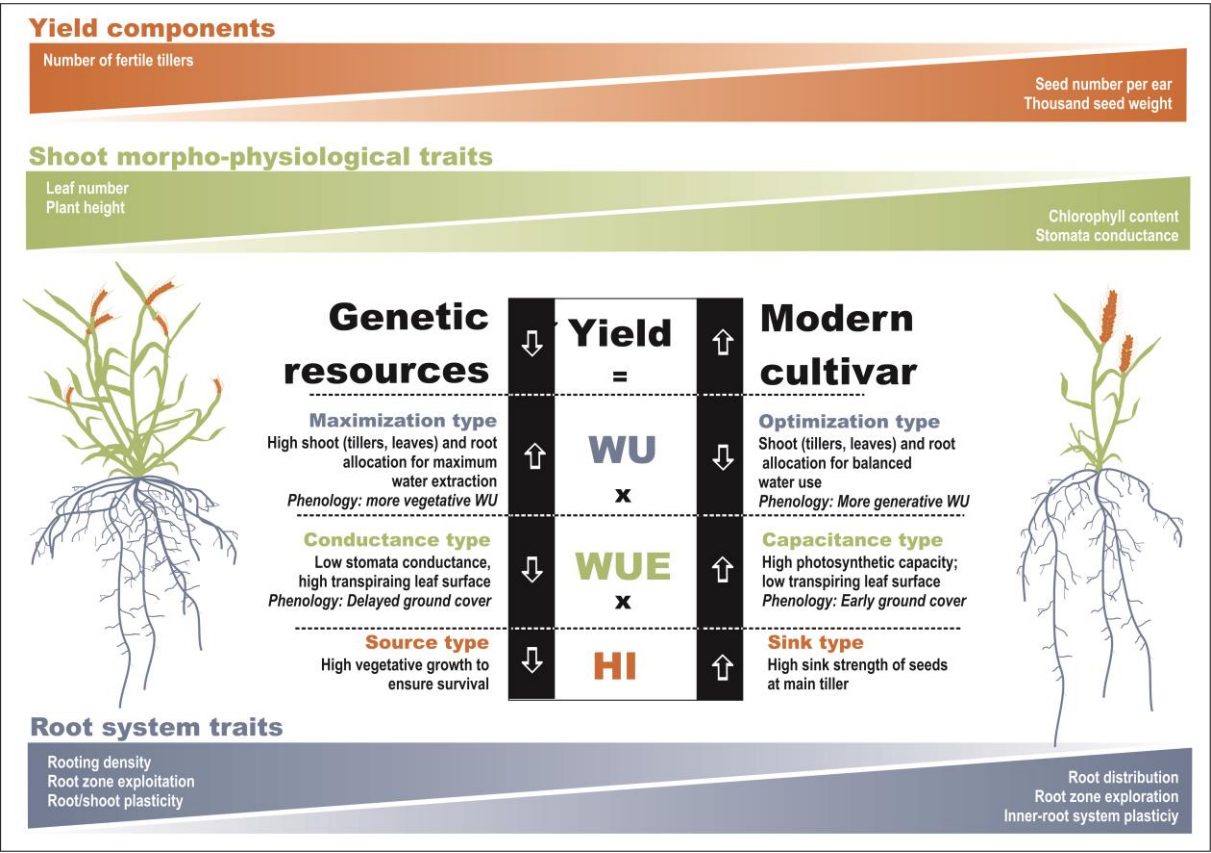
In order to develop a root-based wheat breeding under supply-driven environments where plant water supply is mostly provided by in-season rainfalls, root diversity and functionality of a set of wheat genotypes were examined in relation to their phenological and physiological characteristics.

This study revealed the significant genotypic variation for most root and shoot traits. The investigated genotypes exploited different strategies to maximize soil water depletion, *e.g.* high topsoil root length density, high specific root length and deep rooting. Our results suggest that under intermittent in-season rainfalls of supply-driven environments, dehydration avoidance via water uptake maximization can be achieved through high topsoil rooting density. In this regard, root capacitance can be a useful tool for in situ screening.

Following Passioura's yield-water framework, we could dissect the yield formation and drought response strategies of the investigated germplasm (**Fig. 22**). Accordingly, underutilized wheat species can be considered as maximization types in terms of water use. Their phenology and morphology allows an intensive water extraction as a result of prolonged and excessive vegetative growth. This seems to be a safety strategy based on a high number of tillers. Although the vegetative apparatus may suffer a high reduction of tillers in case of later water limitation, still the crop will avoid complete failure. Contrary, the optimized plant characteristics of modern genotypes provide them with an effective water use through a well-balanced water demand between vegetative and reproductive growth. This strategy is most appropriate to sustainably supply less but still highly demanding generative sinks. In case of high water stress, this strategy may be risky and result in total crop failure if available water for their main yield components is insufficient.

In terms of WUE, underutilized wheat species can be defined as conductance types and modern varieties as capacitance types. The high conductance, however, does not refer to the stomata scale as applied by Udayakumar *et al.* (1998) but to the whole plant scale. The intense vegetative apparatus with high leaf area results in a high transpiring surface. This goes along with a low stomatal conductance and low photosynthetic capacity, both limiting assimilation potential. On the contrary, in modern varieties high stomatal conductance is linked to high photosynthetic capacity which ensures an efficient supply of assimilates. Water

losses are controlled by an optimized total leaf area, ensuring sufficient light interception while avoiding unnecessarily high transpiring surface.



**Fig. 22:** Distinctive behavior of wheat genetic resources and/or underutilized wheat species vs. modern varieties within Passioura’s yield-water framework

Differences in harvest index between old and modern varieties are well documented. We characterized the distinctive pattern as source types for underutilized wheat with an extensive vegetative apparatus and as sink type for modern varieties where available resources are efficiently allocated to a strong generative sink.

Passioura’s yield-water framework provides an appropriate conceptual model to guide trait based analysis of breeding material. Our results suggest that crop improvement in water limited environments will likely profit more from making use of unexploited secondary traits in modern varieties than relying on wide crosses. Khorasan wheat, however, demonstrated that landraces or landrace selections of wheat subspecies of the same ploidy level may reveal promising drought stress response strategies that are currently not present in modern varieties.

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