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Masterarbeit

Field-screening of durum wheat (*Triticum durum* Desf.) for drought tolerance

eingereicht von

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TABLE OF CONTENTS

1. Introduction

Within the last few years climate change and its consequences on environment and especially agriculture (crop production) like global warming, extreme weather events and changes in rainfall patterns attain more and more attention mainly in regard to food security. Increased aridity and higher temperatures are following implications and especially in southern regions nature is racked with heat and drought. Worldwide adaptation and mitigation strategies of plants are developed to counter these adversities. Within Europe regions of southern Europe and the Mediterranean basin are especially vulnerable to heat and drought, but also for the Atlantic zones or the Continental North and South an increased risk of drought is predicted (Iglesias et al. 2007).

A perfect object of investigation for agricultural products is durum wheat (*Triticum durum* Desf.), a tetraploid crop which is resilient and well adapted to drought climates (Kutschera et al. 2009) and also well suited for breeding research because of the high diversity of tetraploid wheat species. Breeding for grain yield under water stress conditions can be realised by direct selection for high yield or by indirect selection for specific morphophysiological traits which can be associated with drought tolerance (Ali Dib et al. 1992). The importance of the durum root system for drought stress tolerance was established by Benlaribi et al. (1990). Ali Dib and Monneveux (1992) demonstrated that root characteristics can vary in relation to the type of drought. Physiological traits associated with drought adaptation and their use in practical breeding programmes were reviewed by Araus et al. (2008).

In the present study a durum nursery including international, European and Austrian germplasm was studied for a wide range of agronomic and morpho-physiological traits (phenological traits, physiological measurements and yield components) with the aim to roughly characterise the plant material in regard to drought tolerance. Thereby, genotypes with specific traits should have been selected for further studies on root system characteristics.

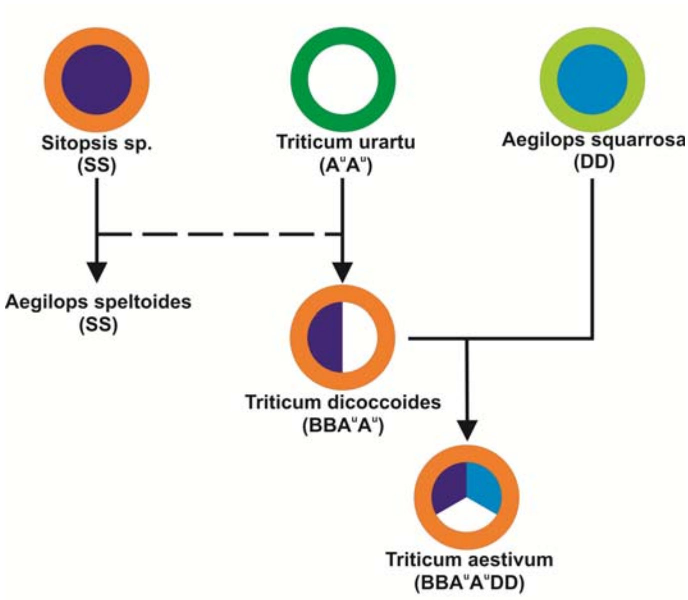
2. Literature review

2.1 Durum

Taxonomy, evolution and origin of durum wheat

Figure 1: Spikes and grains of *Triticum durum* Desf. (Virtueller Sortengarten Getreide, ETH Zürich 2008)

Durum wheat (Figure 1) belongs to the family of *Poaceae* (grasses), the genus *Triticum* (wheat) and the species *Triticum durum* Desf. (Desfontaines 1798). Within the genus *Triticum* to which all wheats belong three ploidy levels exist; each of it featuring an additional genom. In cytogenetic view there are diploid (2n = 2x = 14 chromosomes) wheat like *T. monococcum* (AA), tetraploid (2n = 4x = 28) wheat like *T. dicoccoides* (BBAA) and hexaploid (2n = 6x = 42) wheat like the common wheat *T. aestivum* (BBAADD) (Bozzini 1988) as well as naked and hulled forms of wheat. The ploidy levels are also designated as einkorn, emmer and spelt series (Figure 2). Durum is a free-threshing ("naked") grain and belongs to the emmer series. As a tetraploid species it has a BA genome and has wild einkorn (*T. urartu*) as paternal ancestor (Breiman & Graur 1995). At the tetraploid level the highest diversity within the genus *Triticum* can be observed making tetraploid wheat species interesting for wheat breeding, e.g. the creation of synthetic hexaploid wheat.



free-threshing	hulled
Einkorn series	
T.sinskajae	*T. monococcum*
Emmer series	
T.durum	*T. dicoccoides*
T. turgidum	*T. dicoccum*
T. turanicum	*T. isaphanicum*
T. polonicum	*T. karamyschevii*
T. aethioticum	
T. carthlicum	
T. jakubzineri	
Spelt series	
T. eastivum	*T. spelta*
T. compactum	*T. macha*
T. spaerococcum	*T. vavilovii*

Figure 2: Phylogeny of wheat (modified after Wheat Genetic Resources Centre, Kansas State University) and classification of cultivated wheat species

Wild tetraploid wheat *T. dicoccoides* (wild emmer) has its origin in the Fertile Crescent, an area which runs along the Israelian coast to southeast Turkey, including Syria, Iraq and western Iran (MacKey 2005). In this region agriculture emerged about 12000 to 15000 years ago (Lev-Yadun et al. 2000). Because of its grain size and high nutritional value tetraploid wheat was preferred over diploid forms and started to be domesticated. Durum itself is a more advanced type of wheat compared to wild emmer concerning ear morphology (no brittle rachis, free threshability), grain size, grain yield, etc. It is usually a spring crop which does not require vernalization, however, in Mediterranean regions cultivation is often carried out in autumn and winter hardy material is available in eastern European regions (Lafferty 2011).

Distribution and production

Durum wheat is traditionally cultivated in regions with limited rainfall and therefore is a characteristically drought tolerant crop. The main production areas are the Mediterranean Basin, North America, Morocco and Russia (Table 1). The worldwide acreage of durum cultivation is about 13.5 Mio ha. The highest yields per ha are globally achieved by Mexico and France, followed by Italy, Syria and India (Table 2).

Table 1: Acreage of *Triticum durum* (in 1000 ha) in main production areas (USDA 2009)

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010
Canada	2036	2246	2459	2141	2297	1518	1926	2416	2230
Italy	1664	1733	1690	1870	1426	1190	1340	1380	1160
Turkey	900	1100	1100	1100	1300	1300	1300	1300	1300
Algeria	1112	1351	1318	1369	1100	1300	1300	1300	1300
Russia	1000	2100	1000	1000	1000	1000	1000	1000	1000
Marocco	977	882	1093	1111	1059	1069	843	928	1000
World	13017	14708	14356	14370	14370	13711	12297	12812	13396

Table 2: Mean grain yield ($t \cdot ha^{-1}$) of *Triticum durum* in main production areas (USDA 2009)

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010
Canada	1.47	1.73	1.74	2.24	2.58	2.20	1.91	2.28	2.42
Italy	2.10	2.54	2.21	3.05	2.66	2.86	2.50	3.04	3.02
US	2.01	1.99	2.27	2.56	2.50	1.98	2.29	2.19	3.02
Spain	1.99	2.24	2.48	3.10	1.18	2.52	2.49	1.85	2.02
Syria	2.72	2.76	2.61	2.53	2.77	2.77	2.77	2.77	2.77
India	2.67	2.80	2.29	2.67	2.67	2.67	2.67	2.67	2.67
France	4.37	4.82	4.05	5.12	4.83	4.66	4.30	4.91	4.91
Mexico			3.48	5.22	5.22	5.22	5.22	5.22	5.22
World	1.79	1.73	1.97	2.32	2.14	2.16	2.04	2.16	2.38

In Austria durum breeding and cultivation started after World War II. In the early 1950s first steps were made to breed durum wheat for adaptation to Austrian climate north of the Alps. Because of its annual precipitation of about 500 mm the area east of Vienna seemed to be well suited for spring durum cultivation. The first Austrian variety, i.e. Extradur, was released in 1959. The first Austrian varieties were based on American and Algerian genotypes and exhibited high quality but low levels of grain yield. Therefore, since 1963 it has been necessary to secure Austrain durum production contract based cultivation and premium payments (Hänsel & Seibert 1989). With the introgression of the Italian semi-dwarf mutant CpB132 (Castelporziano) the yield level could be increased significantly and the production became profitably. In the last ten years Austrain durum acreage varied between 12000 and 16000 ha and the average yield was around 40 dt·ha^{-1} (Figure 3).

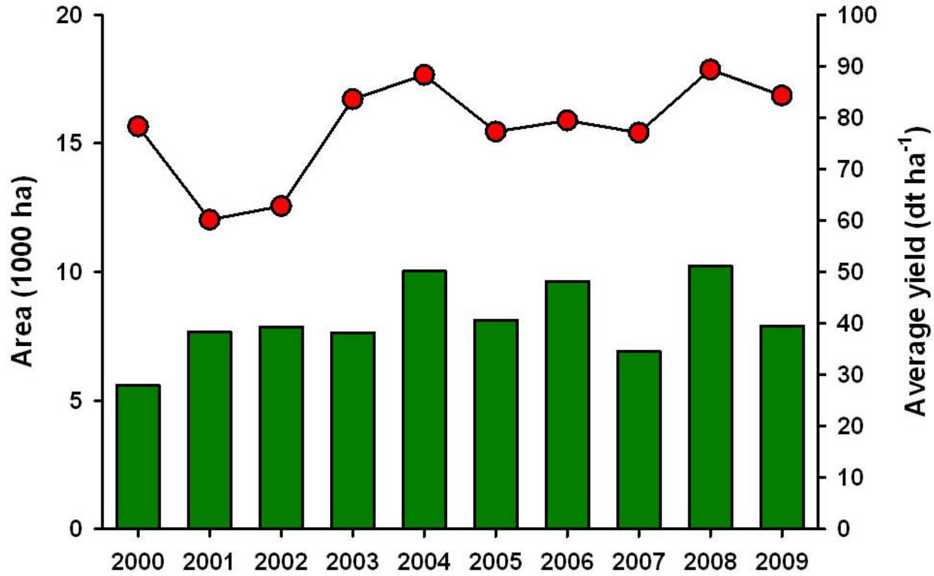


Figure 3: Cultivation area (1000 ha, red circles) and average yield (dt ha^{-1}, green columns) in Austria from 2000 to 2009 (BMLFUW 2011, 2009, 2007, 2005, 2004, 2001)

Recent data revealed a slight increase of grain yield with a parallel decrease of production area (2009: 16865 ha, 39 dt·ha^{-1}; 2010: 17497 ha, 45 dt·ha^{-1}; 2011: 15310 ha, estimated 46 dt·ha^{-1}) (AMA 2011). About 900 ha of the Austrian durum production are used for seed multiplicaton. The annual demand of the milling industry is around 55000 to 65000 t (AGES 2011).

Grain characteristics and products

Although durum wheat is cultivated on a limited acreage the special qualities of its grain and its end products, e.g. good cooking properties, led to worldwide importance of durum. Its naturally large sized kernels are vitreous and of an amber-yellow colour, caused by carotinoids (Quaglia 1988; AGES 2011). They contain a high amount of protein as well as gluten and starch and they are said to be the hardest of all wheat kernels. The quality of the grain itself and its ingredients are important because they influence the milling characteristics and, therefore, the quality of end products. Concerning pasta quality the grain should have good gluten strength, high kernel weight, plump kernel size, uniform kernel shape and size. The protein content of each kernel should be higher than 13%. Vitreousness of grain is important since it is associated with high protein content and related to the fracturing ability of the endosperm during milling (Dexter et al. 1990). This characteristic arises from the compact aggregation of starch and protein during a hot and dry grain ripening (AGES 2011). Semolina used for end use products should have a small and equal particle size for a good hydrating quality. Most durum products belong to the Mediterranean kitchen. They can be divided in paste products, like noodles or couscous, and non paste products, like bread or bulgur (Dick & Matsuo 1988) or semi finished products like semolina. It depends on the region if mainly pasta or other durum products are consumed and produced.

Figure 4: Durum products: pasta and Altamura bread (B. Boscolo & Associazone Amici de il mangione, respectively)

In European and American countries nearly all durum is used for pasta production while in the Middle East mostly local bread, bulgur or couscous is produced (Varughese 1975). Couscous presents nowadays the main diet in most of the countries in North Africa. Nearly 10% of all durum wheat in the Near East is used to produce couscous. Nevertheless the oldest known and probably the most popular form of using wheat for food is pasta. Already in the 9th century BC the Etruscans in Italy should have prepared a kind of lasagne (Agnesi 1996).

2.2 Drought stress

Mandre (2002) defined stress as '... *reaction of a biological system to extreme environmental factors that, depending on their intensity and duration, may cause significant changes in the system'*. Stress restricts the growth and life of each plant. It can be distinguished between biotic stress, e.g. infestation with pests, pathogens or weeds (Peterson & Higley 2001) and abiotic stress, e.g. lack of nutrients, too high or too low temperatures, deficit of water or light, soil salinity (Jenks & Hasegrawa 2005). Abiotic stress can lead to yield losses >50% (Bray et al. 2000).

Drought stress is caused by arid conditions, implicating hot temperatures and inadequate water supply. The amount of water to create stress situations is different and strongly depends on the temperature which has an influence on reaction (time) of the cellular interior of plants (Masle et al. 1989) and soil characteristics. It can be differentiated into early, midterm and terminal drought stress depending during which development stage of the plant the stress appears. Terminal drought occurs when plants are exposed to water deficiency during later stages of reproductive growth. The greatest loss occurs if drought appears during flowering (Frahm et al. 2004). Therefore, time of stress occurrence is important because it can implicate different serious damages. The effect on the plant and it's reactions depend also on duration and density of stress (Brar et al. 1990).

According to Levitt (1980) avoidance and tolerance of dehydration are ways of the drought tolerant behaviour of plants. To prevent serious damages and for efficient drought avoidance the plant has different strategies. One strategy is to save water, following the principle of 'minimal loss', as well as to survive through a 'maximum uptake' (Levitt 1980) for which a good root system is necessary (Bodner et al. 2010). Unfortunately yield orientated breeding targets are directed against the underground part (Waines & Ehdaie 2007) even if in case of drought tolerance it is a needful part to look at.

Durum itself is a drought tolerant plant (Kutschera et al. 2009) but has a low intraspecific genetic variability for drought tolerance (Kara et al. 2000, Valkoun 2001). Therefore, genetic ressources of wild relatives and landraces like *T. turgidum* ssp. *dicoccoides* and *T. urartu* as potential drought tolerant donors (Kara et al. 2000; Valkoun 2001) could contribute interesting and important genetics.

2.3 Roots

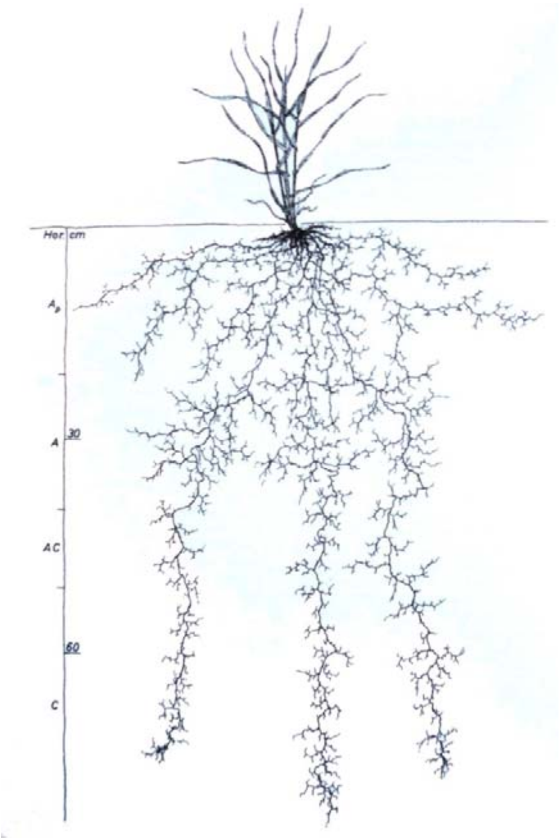


Figure 5: Plant and root system of durum wheat (Kutschera et al. 2009)

The wheat root system consists of nodal as well as seminal roots which have a cylindrical shape and an average root radiation of around 130 cm (Klepper et al. 1984, Conert 1998). In parts of Lower Austria the roots reach down to 83-100 cm below ground surface (Kutschera et al. 2009).

Although the main focus of economical use is devoted to above ground crop parts the section which can be found below ground is at least as important. The roots have several general functions, e.g. incorporation, storage and transfer of water and its solved nutrients, incorporation and storage of assimilates, ground anchorage, etc.. To increase nutrient ability in the rhizosphere roots have the possibility to excrete specific substances (Kutschera et al. 2009). To measure the root size different ways of examination are known. The measurement of the electrical capacitance is an easy and non destructive field method. Thereby, the electrical capacitance values can be correlated to the root size (Chloupek 1977). A destructive, more complex and time consuming method but resulting in more root parameters is to scan root samples. Thereby, root length, root diameter, root surface, root volume and root dry matter can be determined. Root samples are taken in different soil depths, cleaned through washing and sieving in the laboratory (removal of soil and other organic material) and afterwards the roots are stained, scanned and analyzed with respective computer software (Bouma et al. 2000).

3. Material and Methods

3.1 Plant material

In total 82 genotypes of spring durum wheat from various geographical origin were tested. The majority of the nursery, i.e. 63 genotypes, were from the 40th ISDN (International Spring Durum Nursery) of CIMMYT[1] in Mexico. Furthermore, 11 varieties and/or breeding lines from Austria and 4 from other countries were included. Additionally, 4 tetraploid genetic resources were included, i.e. 2 old durum varieties, 1 *T. durum*×*T. dicoccum* line and *T. turanicum* variety QK-77 (KAMUT® brand wheat).

Table 3: Origin and names (or ID codes) of the 82 genotypes used in the experiment

Origin	Name / ID
40th ISDN, CIMMYT, Mexico	7005, 7015-7027, 7030-7050, 7052-7054, 7056, 7057, 7060, 7063-7065, 7069, 7075, 7094, 7097, 7105, 7109
Saatzucht Donau, Probstdorf, Austria	Durobonus, Duroflavus, Floradur, Malvadur, Rosadur, Topdur, SZD3146, SZD3180, SZD4774, SZD4854, SZD5643, SZD5658D
Syngenta Seeds / C.C. Benoist, Orgerus, France	Babylone, D07643
GIE Eurodur, Maisse, France	Clovis
Arizona Plant Breeders, Casa Grande, AZ, USA	Matt
LSA Hohenheim, Stuttgart, Germany	P103, P104, P107
Kamut International Ltd., Big Sandy, MT, USA	QK-77 (KAMUT®)
Agriculture and Agri-Food Canada, Winnipeg, Manitoba	8772

[1] Centro Internacional de Mejoramiento de Maíz yTrigo (International Maize and Wheat Improvement Center); found in 1943; non-profit organization to improve productivity of maize and wheat on global behalf increasing food security and decreasing poverty (www.cimmyt.org/en/about-us/who-we-are)

3.2 Experimental conditions

The field experiment was laid out as a row-column (30×10) design with six replications (Figure 6). Plot size was 1.25×1.40 m (1.75 m²). The number of replicates per genotype was variable. Three check varieties, i.e. Durobonus, Floradur and Rosadur, were replicated 15 to 33 times throughout the experimental design in order to account for natural and extraneous spatial variation. The field trial was sown on 22nd March 2010 in Pysdorf, Raasdorf (16°35' E, 48°14' N) in the Pannonian plains, north east of Vienna.

The mean annual temperature at the experimental site is 9.8°C, the longterm annual precipitation is 550 mm. The type of soil is a chernozem of chalky fine sediments above quaternary gravel. Soil texture is silty loam with a moderate water retention capacity of about 150 mm·m^{-1} (Bodner et al. 2010). During the past few years the amount of annual rainfall has been nearly constant but its distribution has changed; a drought period in early spring was regularly observed (Figure 7). Such a period of early drought is especially critical for spring sown crops resulting in poorly established crops and furthermore significant yield losses.

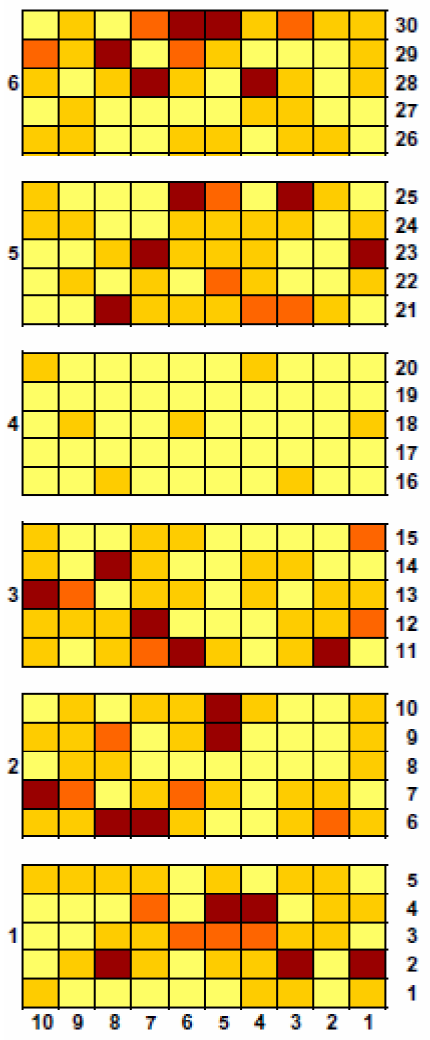


Figure 6: Field plan of the experiment: varieties of the same origin are indicated by the same colour (yellow: CIMMYT 40th ISDN; dark yellow: Austrian varieties and breeding lines; orange: French and US varieties and breeding lines; dark red: genetic resources)

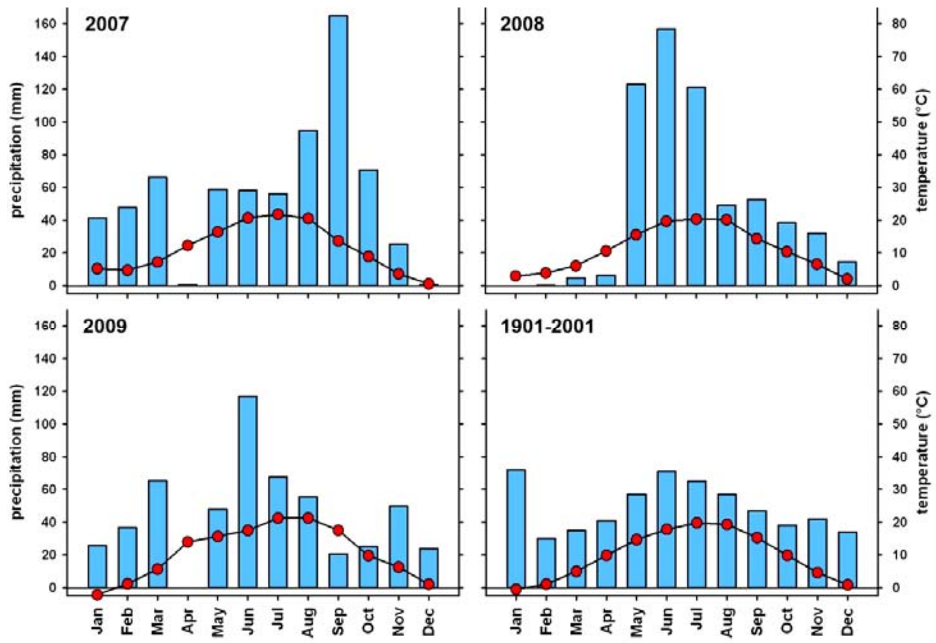


Figure 7: Climate diagram for the experimental site Raasdorf (16°35' E, 48°14' N) for the years 2007 to 2009, and the longterm trend (1901-2001) of the experimental station Groß-Enzersdorf (16°33' E, 48°12' N)

3.3 Phenological traits

Ground cover

After plant emergence the ground cover was measured by digital image analysis (Figure 8). Therefore, digital images of each single plot were taken on four dates (19^{th} and 30^{th} April, 7^{th} May, 30^{th} June) with a Canon EOS20D (Canon Inc., Tokyo, Japan) digital camera from about 1.5 m height. The digital images were downloaded in JPEG-format to a personal computer and analyzed individually by SigmaScan Pro vers. 5.0 software (Systat Software Inc., Chicago, IL). Thresholds for hue and saturation were manually chosen to selectively identify green leaves and distinguish them from the colour of the ground. The total number of selected green pixels were counted and then divided by the total number of pixels of the image to give the percentage of green ground cover in reference to the ground (Richardson et al. 2001). The data of the first dates can be used as an index for early growth vigour, while the data for the last date (early summer) gives information for the stay green effect of the leaves.

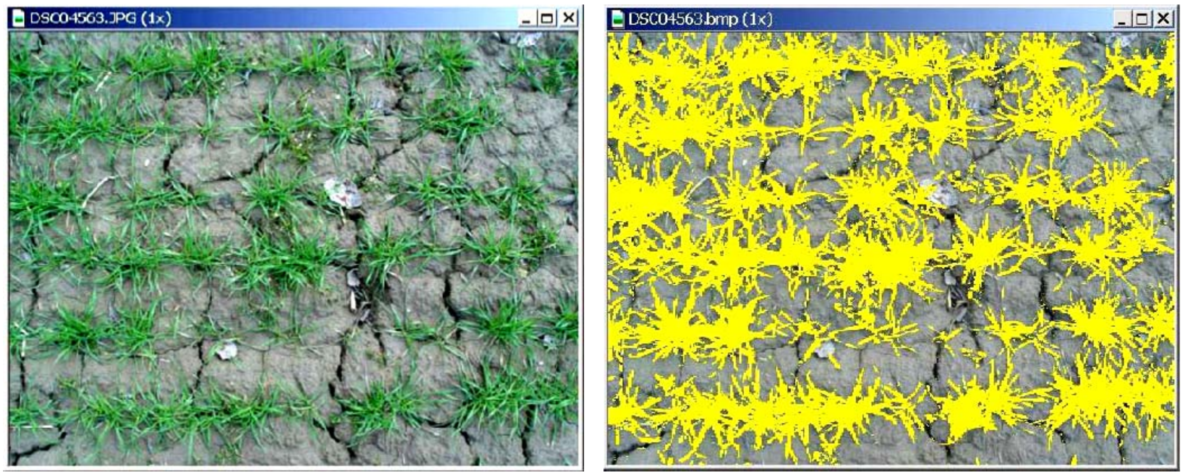


Figure 8: Estimation of crop ground cover by digital image analysis: uploaded digital image of a plot (left); green pixels overlayed with yellow colour (right) (J. Vollmann)

Heading date

The date of middle of heading (BBCH55), i.e. half of the inflorescences of the plot emerged (Witzenberger et al. 1989, Lancashire et al. 1991), was recorded for each variety and plot. For statistical data analysis the heading dates were converted into “Days after 31st March” values.

3.4 Physiological traits

Electrical capacitance

Root surface area was characterized indirectly by electrical capacitance measurements (Chloupek 1977) on three dates, i.e. 20th and 29th May, and 15th June, using an Escort elc-133 lcr-meter (Instruments Techno Test Inc., Laval, Canada) with a 1 kHz measuring frequency. In each plot four plants (sub-samples) were measured on each date. According to Dalton (1995) it is a non destructive and an in-situ measurement for assessing plant root development. It is based on the principle of the polarisation of living membranes or living cells of the root system (Kupecsek & Molnárová 2009) and functions as a capacitor or rather a sum of many capacitors while the capacity is directly proportional to the root length. Following that principle the plant acts as first plate and the soil as second plate of the capacitor while an equivalent parallel resistance-capacitance circuit is formed by the interface between soil-water and the plant root surface (Dalton

1995). At the contact surface of those two substances a thin double-layer develops and creates an electric field (Chloupek et al. 2010).The electrical capacitance is measured between an electrical contact made at the plant shoot and an electrode needle which is inserted in the ground above the roots (Figure 9). The electrical contact with the plant is made by clipping the electrode onto the base of the plant shoot 2 cm above the ground without any contact to the soil (Dalton 1995). The electrical capacitance depends on the active root surface area and the root length (Kupecsek & Molnárová 2009). To be able to compare results and to establish a relationship between the size of living tissue and its electrical capacitance the environmental circumstances like soil water content and/or development stages of the measured plants have to be equal. The development stage is important since values for electrical capacitance change with the amount of living and dead cells. After flowering the root system becomes more and more lignified and, therefore, electrical capacitance decreases (Chloupek et al. 2010). If the plants have a wet surface, for example after rainfall, the measurement of electrical capacitance is not possible.

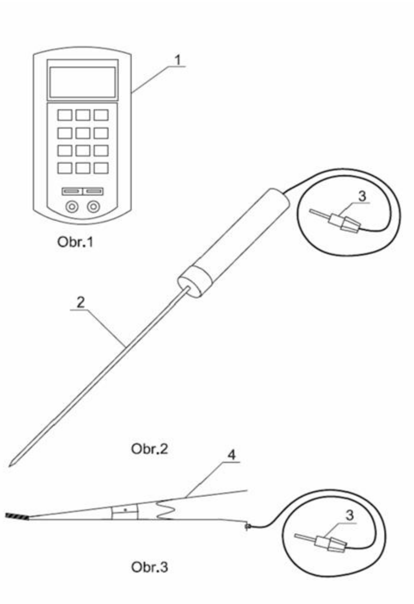


Figure 9: Measurement of electric capacitance in the field with the Escort elc-133 lcr-meter (left) and schematic presentation (right) of the measurement device: lcr-meter (Obr. 1), electrode needle (Obr. 2) and electrode clip (Obr. 3) (G. Bodner & V. Dostál, respectively)

Chlorophyll concentration

The chlorophyll concentration is a good indicator for plant health of leaf plants in case that leaf thickness is not higher than 1.2 mm. Chlorophyll concentration was measured as a leaf chlorophyll index (SPAD units) on 16 June using a SPAD-502Plus meter (Konica Minolta Holdings, Inc., Tokyo; Figure 10). The method is non-destructive and quick to implement on the field. Each measurement was carried out on the flag leaf. Three plants per genotype were investigated. The method is based on the difference of optical density at two wavelengths. The SPAD (Soil Plant Analysis Development) results are correlated to the status of nutrition of the plant and leaf photosynthesis (Botha et al. 2010), and especially reflects the N status of the plant (Follett et al. 1992, Vidal et al. 1999, Vouillot et al.1998). From the SPAD values the chlorophyll A and chlorphyll B content ($mg \cdot cm^{-2}$) can be derived using a calibration curve (Botha et al. 2010).

Figure 10: SPAD-502Plus meter

Stomatal conductance

Stomatal conductance (mmol H_2O m^{-2} s^{-1}) is a physiological parameter often used for evaluating drought tolerance (Aminian et al. 2010). It was measured on 24 June with the SC1 steady-state leaf porometer (Decagon Devices, Inc., Pullman; Figure 11). Three samples per plot were investigated. The principle is based on the passage of water vapour through stomata. For measurements the flag leaf is clamped into the

Figure 11: Leaf porometer

sensor head of the porometer and the difference of water vapour inside and outside the sensor head is measured. At the same time air temperature is also recorded which is necessary for the instrument to calculate the water vapour inside the leaf. The method is non-destructive but in regard to its complex calibration very time consuming. According to Bahar et al. (2009) the method has a great relevance to drought stress. Hyeon-Hye et al. (2004) report that stomata can adjust conductance to realign the uptake of CO_2 and the transpiration under different environments.

Leaf area index

The size of the assimilation area was measured as the leaf area index (LAI) through hemispherical photography with a LAI-2000 plant canopy analyzer (Li-Cor Environmental, Lincoln; Figure 12) on 24 June. The LAI was determined for each single plot through radiation measurements based on a fisheye optical sensor. To obtain reliable results light conditions should be constant throughout the measurements. The method is non-destructive. Measurements are made above and below the canopy. From the difference between the entrance radiation and the radiation passing through the canopy, the assimilating area is calculated automatically by the analyzer.

Figure 12: LAI-2000 plant canopy analyzer (Eurosep Instruments)

3.5 Yield traits

Harvest of the field trial was on 20 July. Plant height and lodging scores were recorded before harvest. From each plot the whole plants of the two center rows (Figure 13) representing 0.35 m² were cut 1 cm above ground for the determination of total dry matter yield and number of fertile tillers per unit area. After threshing (Figures 13 & 14) grain yield per unit area was calculated, as well as harvest index (ratio of grain yield and total dry matter yield). Additionally 1000 grain mass was determined using a Contador seed counter (Figure 14). Furthermore, hectolitre weight ($kg \cdot hL^{-1}$), kernel plumpness (percentage of seeds remaining on 2.8×25 and 2.5×25 mm slotted sieves, respectively) and protein content by near-infrared spectroscopy were determined.

Figure 13: Harvest of the field trial: combine harvest of plots (left) and whole plant harvest from the two center rows of each plot (right)

Figure 14: Stored samples for the determination of total dry matter yield (top left), mechanical seed counting for the determination of 1000 grain mass (bottom left), and threshing machine for the determination of grain yield of the manually harvested two center rows (right)

3.6 Statistical analysis

Accounting for the randomization scheme the linear mixed models were fitted with the fixed genotype and block effects. Linear trends along rows and/or columns, random row and/or column effects and spatial covariance were confined (nested) within blocks using GenStat 13th Ed. (VSN International Ltd, Hemel Hempstead, UK). Models with the smallest deviance and/or Akaike Information Criterion (AIC) were preferred as the optimized model of spatial analysis to calculate adjusted genotypic means (Gilmour et al. 1997, Payne 2006, Piepho & Williams 2010). Subsequently, the mean values of all varieties were sorted and transferred into relative values. This transformation followed the concept of 'site highest performance' according to Jensen (1976). Thereby the results of the genotypes were ranked according to their performance and the highest value was set 100. The relative values of eight traits, i.e. ground cover (measured on 19 April and 30

June), SPAD values (chlorophyll concentration), number of spikes (fertile tillers), dry matter yield, grain yield, harvest index and 1000 grain mass, were selected to create star plots: Finally, the area within the star was determined and used as multivariate index to rank the tested germplasm following the principle that the higher the area the better the performance of the genotype in regard to the eight traits. Correlation analysis was carried out to determine the relationships among traits.

4. Results

4.1 Spatial models

Optimized spatial models for the diverse characters are demonstrated in Table 4. Genotypic effects were significant for almost all traits with the exception of two dates of electrical capacitance measurements (EC2005: P=0.835; EC1506: P=0.294).

Table 4: Optimized spatial models and significance tests for fixed genotype and block effects.

	Linear mixed model			*Pr>F*	
Trait [1]	Fixed effects: Gen + block +	Random effects:	Error variance model	Gen	Block
GC1904	lin(row)+ lin(col)		AR1×AR1	<.001	<.001
GC3004	lin(row)		AR1×AR1	<.001	<.001
GC0705	lin(row)		AR1×AR1	<.001	0.009
GC3006	lin(col)	row	ID×AR1	<.001	0.011
EC2005			ID×AR1	0.835	0.670
EC2905		row	ID×AR1	0.028	0.029
EC1506			ID×AR1	0.294	0.014
HEAD			ID×AR1	<.001	<.001
SPAD			ID×AR1	<.001	0.004
SC		row	ID×AR1	0.004	0.003
LAI	lin(col)	row	ID×ID	<.001	<.001
PH	lin(col)	row	ID×ID	<.001	<.001
DMYLD		row	ID×AR1	<.001	<.001
SPK		row	AR1×AR1	<.001	<.001
GYLD		row	AR1×AR1	<.001	<.001
HI			ID×ID	<.001	<.001
TGM		row	AR1×ID	<.001	<.001
HLW			AR1×AR1	<.001	0.020
KS28			AR1×AR1	<.001	<.001
PROT		row	ID×AR1	<.001	0.009

[1] GC, ground cover measured on 19 and 30 April, 7 May and 30 June, respectively (%); EC, electrical capacitance measured on 20 and 29 May, and 15 June, respectively (nF); HEAD, heading date (days after 31 May); SPAD, chlorophyll concentration (SPAD values); SC, stomatal conductance (mmol·m^{-2}·s^{-1}); LAI, leaf area index; PH, plant height (cm); DMYLD, dry matter yield (g·m^{-2}); SPK, number of spikes·m^{-2}; GYLD, grain yield (g·m^{-2}); HI, harvest index; TGM, thousand grain mass (g); HLW, hectolitre weight (kg·hL^{-1}); KS28, kernel plumpness >2.8 mm (%); PROT, protein content (%)

4.2 Correlation analysis

Crop ground cover at early (GC1904) and late (GC3006) growth stages showed positive and significant correlations to LAI (r =0.43-0.53, P<0.01) and several yield related traits. Correlation coefficients were highest for the relationships to grain yield and dry matter yield (r =0.56-0.71, P<0.0001), followed by the number of fertile tillers per unit area (r =0.54-0.63, P<0.01), while the relationship to TGM and kernel plumpness (r =0.41-0.48, P<0.01) was worth mentioning only for GC1904. Within yield related traits grain yield was highly correlated to the dry matter yield (r =0.87, P<0.0001), whereas correlation was lower to the number of fertile tillers per unit area (r =0.69, P<0.0001), and especially low to TGM (r =0.35, P=0.001). Physiological traits didn't show any remarkable correlations with the exception of the already above mentioned relationship between crop ground cover and LAI, heading date and EC1506 (r =0.55, P=0.0002), as well as LAI and TGM (r =0.45, P=0.003). The most pronounced relationships to grain yield are demonstrated in Figure 15.

4.3 Multivariate index

Relative values of eight traits were used to create a star plot. The area within the star was used as multivariate index. Examples for the star area of selected genotypes are presented in Figure 16. The mean star area of the nursery was 14.92×10^3. Relative values used for the creation of star plots are demonstrated in Table 5, star areas are demonstrated in Table 6.

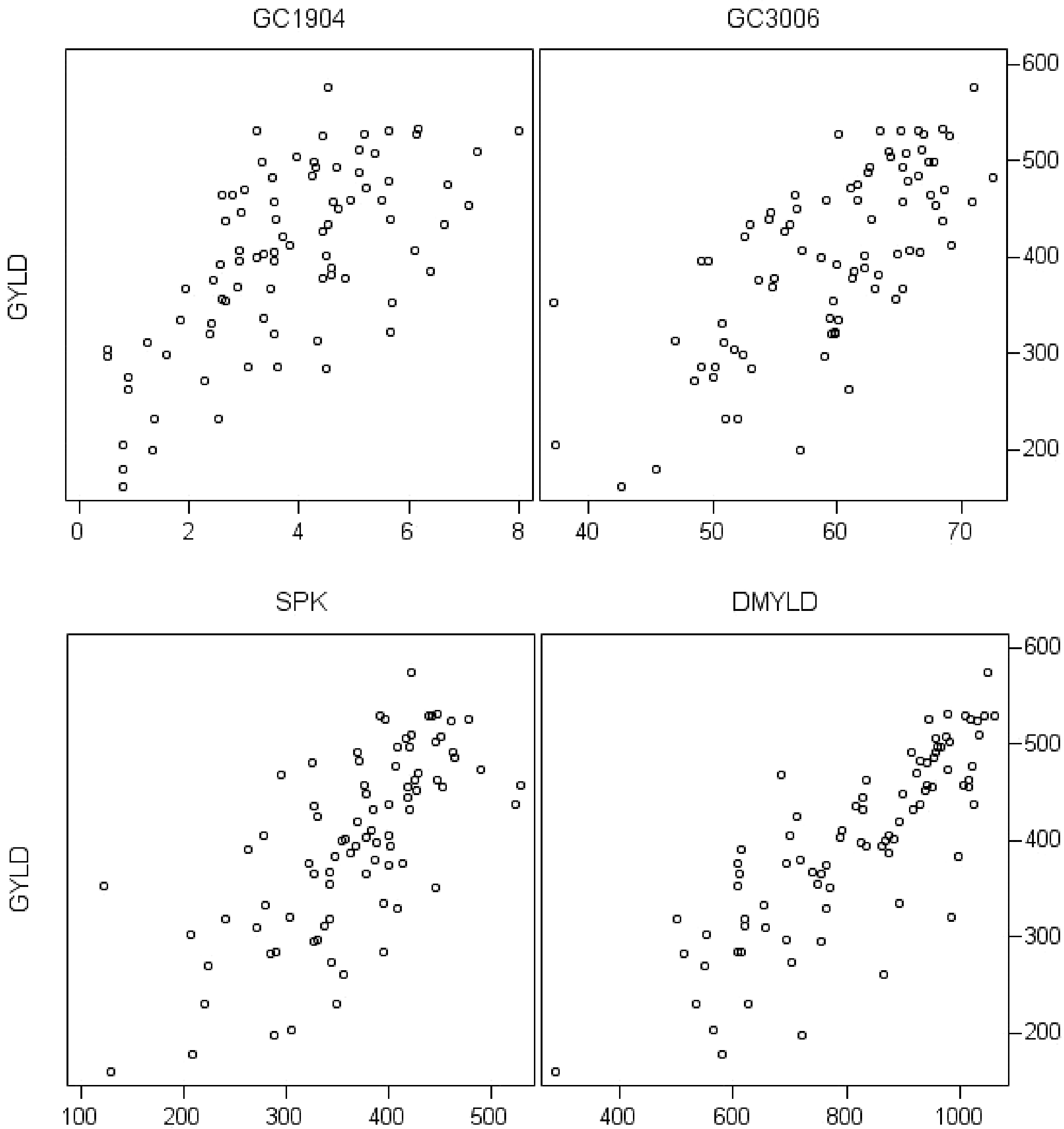


Figure 15: Relationship between grain yield (GYLD) and crop ground cover at early (GC1904) and late (GC3006) growth stage, number of fertile tillers per unit area (SPK) and dry matter yield (DMYLD)

Table 5: Relative values for eight selected traits used to create star plots (abbreviations of traits see Table 4)

Genotype	GC1904	GC3006	SPAD	SPK	TMSQM	YLDSQM	HI	TGM
7005	61.77	81.50	83.03	100.00	94.69	79.71	98.06	70.63
7006	55.33	84.32	81.74	60.88	57.32	65.52	92.23	71.58
7007	38.25	67.67	80.27	54.87	57.37	49.47	90.29	66.82
7008	19.94	72.26	86.80	62.57	65.50	51.79	92.23	58.50
7009	31.80	70.33	82.05	65.99	59.02	39.98	86.40	71.19
7010	56.21	73.15	88.65	53.79	48.49	49.16	92.23	67.52
7011	15.27	70.22	95.56	51.41	61.84	53.82	96.11	65.88
7012	6.37	71.28	77.63	39.22	52.20	52.61	88.34	68.76
7013	45.19	69.27	81.07	74.58	57.85	49.53	65.03	63.44
7014	36.62	75.41	83.47	79.24	78.12	77.46	100.00	74.98
7015	49.35	88.72	79.62	84.31	92.54	87.50	96.93	67.91
7016	67.27	90.28	88.37	78.93	90.34	88.13	90.29	65.17
7017	58.42	89.94	79.55	87.61	90.21	85.62	86.40	72.32
7018	54.27	64.83	77.15	63.73	58.42	54.27	86.40	60.01
7019	30.00	69.94	68.43	77.27	72.09	57.50	53.38	58.23
7020	11.21	69.03	87.54	65.21	66.37	47.74	84.46	60.63
7021	24.09	86.83	77.36	71.54	71.21	63.74	88.34	54.36
7022	64.96	82.91	82.13	90.30	96.09	91.64	96.11	64.55
7023	32.23	82.66	75.06	49.69	57.94	67.95	90.29	65.60
7024	30.45	74.01	76.01	75.57	71.97	65.06	94.17	63.95
7025	6.45	81.29	82.41	61.79	71.33	51.53	98.06	59.75
7026	11.11	84.01	78.61	67.45	81.54	45.45	49.49	74.62
7027	22.91	82.83	85.36	52.85	61.67	57.96	90.29	78.60
7030	37.75	94.53	79.38	55.75	64.51	81.57	94.17	69.51
7031	58.83	78.21	86.10	71.48	84.66	77.91	88.77	74.83
7032	44.36	91.82	83.75	71.61	74.21	70.32	84.89	68.59
7033	57.26	87.33	76.35	73.16	67.64	66.01	78.63	68.06
7034	44.48	67.60	88.39	76.11	78.65	68.61	84.89	64.53
7035	44.42	97.70	77.70	85.47	89.51	79.40	88.34	67.26
7036	70.87	75.12	79.28	99.15	87.70	76.02	90.29	57.90
7037	33.39	94.32	67.47	62.01	76.97	75.89	90.29	69.67
7038	36.36	68.41	82.25	69.67	81.23	68.75	94.99	62.89
7039	63.74	86.07	85.45	87.69	89.77	84.67	92.23	66.11
7040	34.96	92.98	76.53	80.51	95.78	80.66	87.61	66.77
7041	35.97	75.46	78.87	64.71	69.69	63.86	92.23	63.41
7042	53.89	86.39	78.13	69.92	86.14	85.42	91.88	73.26
7043	36.51	78.86	87.98	52.59	66.05	70.55	96.11	65.92
7044	55.28	76.81	87.13	62.54	67.11	74.12	96.11	66.58
7045	44.33	82.43	77.15	64.72	58.61	55.41	84.46	66.25
7046	65.25	84.20	80.65	81.08	86.89	81.78	85.66	70.01
7047	9.92	51.45	78.54	57.60	53.25	35.55	94.17	59.80
7048	32.31	89.17	85.02	64.88	70.71	61.69	82.52	71.30

Table 5: Continued

Genotype	GC1904	GC3006	SPAD	SPK	TMSQM	YLDSQM	HI	TGM
7049	29.61	82.12	89.38	45.58	47.27	55.38	78.63	68.06
7050	60.63	75.72	87.81	78.04	65.45	65.42	94.17	72.26
7052	43.43	89.90	79.89	61.85	57.71	63.50	90.29	64.42
7053	16.89	71.74	88.56	41.80	50.42	40.24	90.29	61.80
7054	32.26	78.06	82.33	84.72	78.61	80.46	96.11	64.95
7056	28.57	66.79	80.90	42.32	51.76	47.06	100.00	79.05
7057	9.74	58.78	74.16	24.42	27.10	27.87	82.52	64.10
7060	9.80	62.66	84.68	39.45	54.69	31.07	84.46	60.26
7063	70.31	87.39	83.81	83.13	100.00	92.03	88.77	78.24
7064	52.91	91.75	78.24	70.13	87.55	84.07	86.83	77.34
7065	40.50	89.73	85.86	74.13	98.17	92.24	96.93	75.03
7069	41.40	93.48	87.05	79.49	90.47	86.36	88.00	69.40
7075	43.79	100.00	80.91	61.45	88.81	83.56	89.16	75.40
7094	56.47	97.79	89.42	79.82	98.79	100.00	90.33	72.09
7097	47.99	95.33	74.82	72.58	74.61	71.51	87.22	71.25
7105	57.45	85.74	88.41	68.48	82.39	67.43	87.61	66.54
7109	44.76	86.42	81.95	75.69	96.42	76.30	89.16	63.51
7111	56.34	85.76	79.30	67.08	81.98	69.59	86.44	64.44
7125	40.41	80.89	82.70	73.24	77.71	69.14	90.33	79.01
7139	56.69	73.11	100.00	72.74	78.20	75.20	97.71	63.82
8772	16.62	78.68	67.92	54.61	68.10	34.46	61.19	54.28
BABYLONE	57.86	90.03	81.47	79.10	95.73	79.35	82.56	86.20
CLOVIS	100.00	91.78	86.73	83.70	94.96	92.06	89.94	81.42
D07643	55.49	95.10	82.31	87.17	97.19	91.05	83.33	64.29
DUROBONUS	42.11	81.89	80.12	74.65	84.21	58.31	71.81	72.31
DUROFLAVUS	76.49	90.81	85.15	75.76	82.42	70.38	77.51	70.83
FLORADUR	53.23	92.92	90.72	77.15	91.16	86.52	85.30	72.21
KAMUT	70.87	82.40	84.53	57.41	92.70	55.88	61.97	100.00
MALVADUR	68.91	85.01	93.88	71.10	88.81	79.75	81.31	80.17
MATT	71.22	51.28	92.24	84.16	72.70	61.10	89.94	65.77
P103	46.30	72.52	82.45	69.77	84.12	73.13	90.71	73.31
P104	70.46	90.58	77.75	77.04	96.28	83.07	86.44	69.55
P107	79.87	84.68	82.53	65.86	94.07	66.85	67.02	75.69
ROSADUR	63.55	92.14	91.02	79.92	97.33	88.68	86.05	75.88
SZD3146	76.90	92.32	92.74	74.86	88.92	91.66	86.83	73.82
SZD3180	41.93	89.48	95.69	67.52	83.15	69.89	81.78	74.90
SZD4774	90.37	88.38	82.64	85.09	91.80	88.48	89.34	73.73
SZD4854	83.03	77.55	92.37	79.45	86.54	75.28	83.33	73.38
SZD5643	77.09	94.48	78.66	84.67	92.28	92.58	87.61	73.87
SZD5658D	84.00	84.95	85.63	92.57	92.07	82.37	81.00	74.05
TOPDUR	88.53	93.68	88.61	80.85	88.33	78.77	78.13	76.99

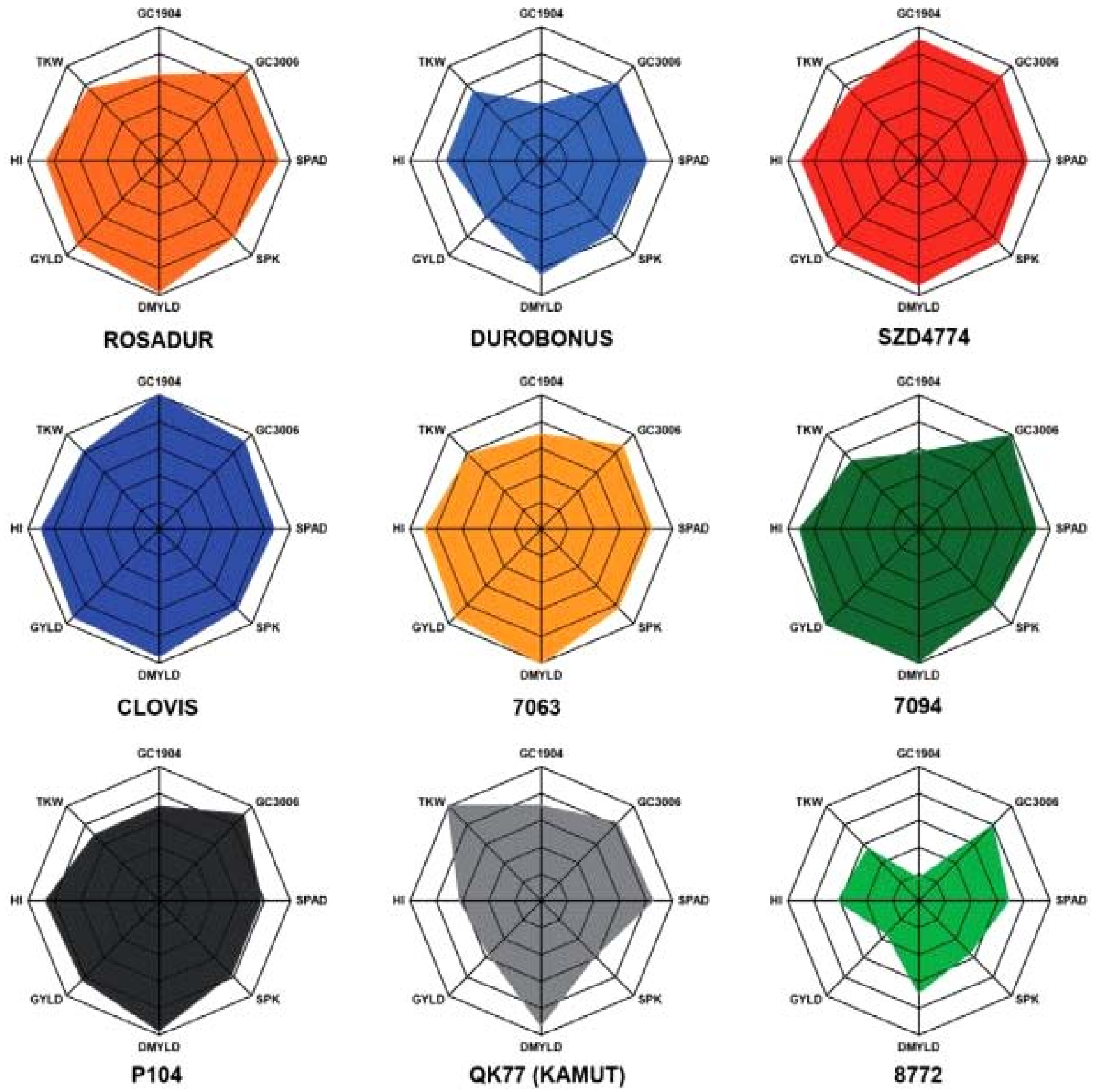


Figure 16: Star plot area of nine selected genotypes (origin of genotypes see Table 3; abbreviations of traits see Table 4)

Table 6: Star plot areas (×10³) of the investigated genotypes and their originating genpool

Genotype	Genepool	Area	Genotype	Genepool	Area
7005	CIMMYT	19.76	7049	CIMMYT	10.26
7006	CIMMYT	14.20	7050	CIMMYT	15.84
7007	CIMMYT	10.97	7052	CIMMYT	13.26
7008	CIMMYT	10.96	7053	CIMMYT	8.57
7009	CIMMYT	11.02	7054	CIMMYT	15.69
7010	CIMMYT	12.18	7056	CIMMYT	10.19
7011	CIMMYT	10.66	7057	CIMMYT	5.05
7012	CIMMYT	8.42	7060	CIMMYT	7.10
7013	CIMMYT	11.11	7063	CIMMYT	20.65
7014	CIMMYT	16.12	7064	CIMMYT	17.39
7015	CIMMYT	18.44	7065	CIMMYT	18.70
7016	CIMMYT	19.12	7069	CIMMYT	17.61
7017	CIMMYT	18.61	7075	CIMMYT	16.91
7018	CIMMYT	11.84	7094	CIMMYT	20.62
7019	CIMMYT	10.18	7097	CIMMYT	15.55
7020	CIMMYT	10.05	7105	CIMMYT	15.94
7021	CIMMYT	12.44	7109	CIMMYT	16.45
7022	CIMMYT	19.70	7111	CIMMYT	15.32
7023	CIMMYT	11.71	7125	CIMMYT	15.40
7024	CIMMYT	13.34	7139	CIMMYT	16.65
7025	CIMMYT	10.95	DUROBONUS	AT	13.84
7026	CIMMYT	9.79	DUROFLAVUS	AT	17.45
7027	CIMMYT	11.89	FLORADUR	AT	18.45
7030	CIMMYT	14.43	MALVADUR	AT	18.53
7031	CIMMYT	16.95	ROSADUR	AT	20.01
7032	CIMMYT	15.12	SZD3146	AT	20.32
7033	CIMMYT	14.52	SZD3180	AT	15.69
7034	CIMMYT	14.35	SZD4774	AT	21.01
7035	CIMMYT	17.33	SZD4854	AT	18.68
7036	CIMMYT	17.74	SZD5643	AT	20.48
7037	CIMMYT	14.20	SZD5658D	AT	20.16
7038	CIMMYT	13.90	TOPDUR	AT	20.06
7039	CIMMYT	18.93	BABYLONE	EU	18.75
7040	CIMMYT	16.54	CLOVIS	EU	22.95
7041	CIMMYT	12.90	D07643	EU	18.82
7042	CIMMYT	17.21	MATT	EU	15.16
7043	CIMMYT	13.23	8772	PGR	7.76
7044	CIMMYT	15.03	KAMUT	PGR	16.05
7045	CIMMYT	12.44	P103	PGR	15.40
7046	CIMMYT	17.81	P104	PGR	18.69
7047	CIMMYT	8.09	P107	PGR	16.74
7048	CIMMYT	13.30			

Table 7 represents the absolute performance values for selected traits of those genotypes which showed a star area above the mean index.

In Figure 17 the multivariate index (star area) is plotted against heading date. It is clearly visible that the major part of the genotypes didn't reach a star area above 18×10^3 which was the level of check variety Floradur. Heading of genotypes with an index $>18 \times 10^3$ occurred between 6 and 9 June indicating an optimal window of heading date. In the group of best performing genotypes most of the Austrian germplasm was included. Some genotypes like French variety Clovis or CIMMYT line 7063 showed excellent performance and an earlier heading date.

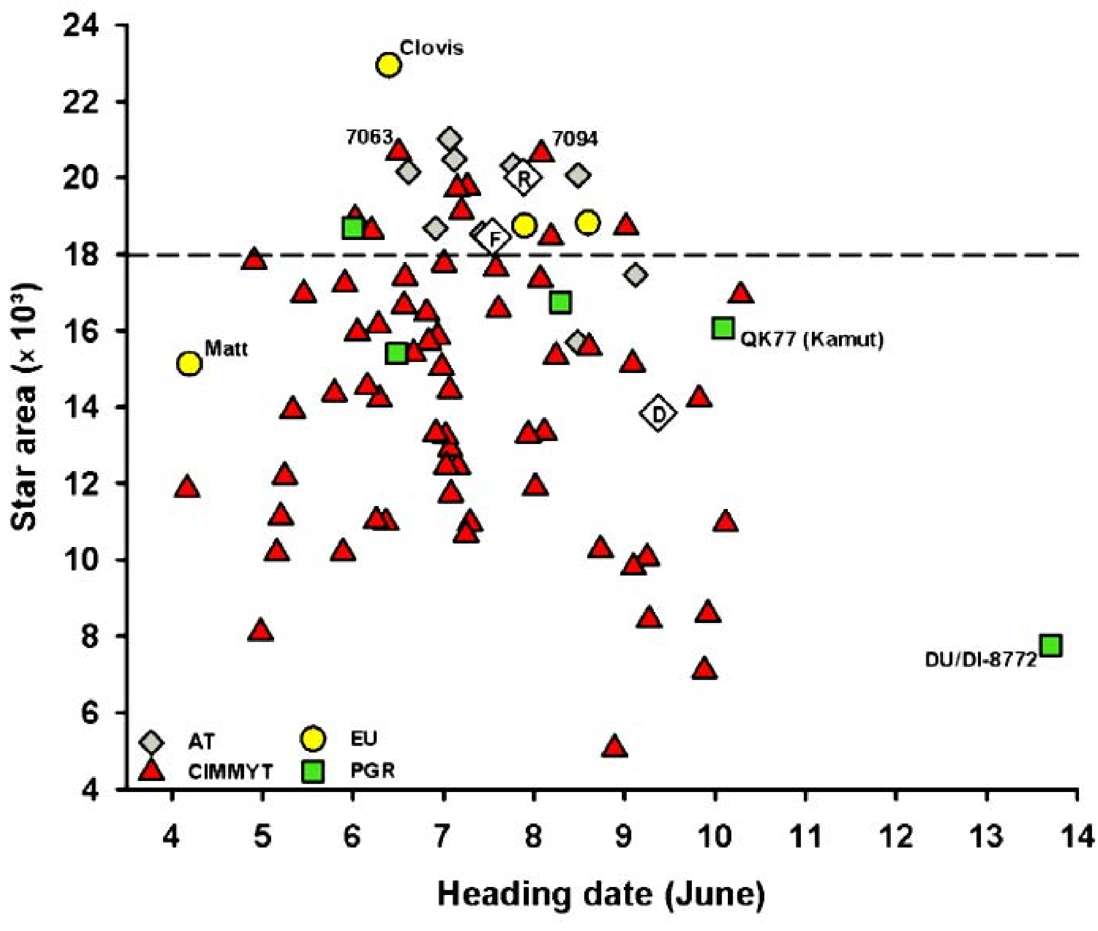


Figure 17: Relationship between heading date and star area. Genotypes of different genepools are indicated by different symbols; check varieties Durobonus. Floradur and Rosadur are indicated by white diamonds including the initial letters of the variety names

Table 7: Means of selected traits for above average performing genotypes in regard to the multivariate index (star area) (Abbreviations of traits see Table 4; Minimum, maximum and mean values refer to the complete nursery)

Genotype	GC1904	HEAD	SPAD	GC3006	SPK	DMYLD	GYLD	HI	TKW	HLW	PROT
7005	4.94	7.3	49.3	59.1	529	1004	458.2	0.50	41.4	78.1	13.9
7015	3.95	8.2	47.2	64.4	446	981	502.9	0.50	39.8	80.1	13.0
7016	5.38	7.2	52.4	65.5	417	958	506.6	0.46	38.2	78.8	15.8
7017	4.67	6.2	47.2	65.3	463	957	592.2	0.44	42.4	77.9	15.1
7022	5.19	7.2	48.7	60.2	478	1019	526.8	0.49	37.8	77.6	13.5
7036	5.67	7.0	47.0	54.5	524	930	437.0	0.46	33.9	77.3	13.9
7039	5.10	6.0	50.7	62.5	464	952	486.7	0.47	38.8	76.6	14.1
7046	5.22	4.9	47.9	61.1	429	921	470.0	0.44	41.0	79.9	14.6
7063	5.62	6.5	49.7	63.4	440	1060	529.0	0.46	45.9	78.3	15.7
7065	3.24	9.0	50.9	65.1	392	1041	530.2	0.50	44.0	78.2	13.7
7069	3.31	7.6	51.7	67.8	420	959	496.4	0.45	40.7	76.7	14.6
7094	4.51	8.1	53.1	71.0	422	1048	574.8	0.47	42.3	78.9	13.9
BABYLONE	4.63	7.9	48.3	65.3	418	1015	456.1	0.43	50.5	75.2	16.4
CLOVIS	8.00	6.4	51.5	66.6	443	1007	529.2	0.46	47.7	78.5	15.0
D07643	4.44	8.6	48.8	69.0	461	1031	523.4	0.43	37.7	76.1	15.5
DUROFLAVUS	6.12	9.1	50.5	65.9	401	874	404.6	0.40	41.5	75.0	16.1
FLORADUR	4.26	7.5	53.8	67.4	408	967	497.3	0.44	42.3	79.1	14.6
MALVADUR	5.51	7.4	55.7	61.7	376	942	458.4	0.42	47.0	76.9	14.5
ROSADUR	5.08	7.9	54.0	66.9	423	1032	509.7	0.44	44.5	79.2	15.2
TOPDUR	7.08	8.5	52.6	68.0	428	937	452.8	0.40	45.1	76.8	16.0
SZD4774	7.23	7.1	49.0	64.1	450	973	508.6	0.46	43.2	77.1	14.6
SZD4854	6.64	6.9	54.8	56.3	420	918	432.7	0.43	43.0	77.2	15.2
SZD5643	6.16	7.1	46.7	68.6	448	978	532.1	0.45	43.3	77.1	13.3
SZD5648D	6.72	6.6	50.8	61.6	490	976	473.5	0.42	43.4	77.0	15.4
P104	5.63	6.0	46.1	65.7	407	1021	477.5	0.45	40.8	78.7	15.0
Minimum[2]	0.51	4.2	40.0	37.2	122	287	160.2	0.25	31.8	72.8	12.5
Maximum	8.00	13.7	59.3	72.6	529	1060	574.8	0.51	58.6	80.1	17.5
Mean	3.79	7.4	49.2	59.4	368	817	399.0	0.45	40.6	77.2	14.8

5. Discussion

5.1 Plant material and experimental conditions

The tested germplasm showed a broad variation concerning almost all investigated traits. About half of the CIMMYT lines performed inferior than the lowest performing Austrian check variety Durobonus, whereas the four old durum varieties with tall plant height, i.e. Kamut, P103, P104 and P107, performed above average (Figure 17). Due to the relatively high amount of rainfall until mid June the expected drought period which was repeatedly observed during the past few years did not show up. Therefore, the plants were not exposed to an early drought stress. Only the late maturing genotypes were finally exposed to terminal drought stress at their late grain filling period (Figure 18).

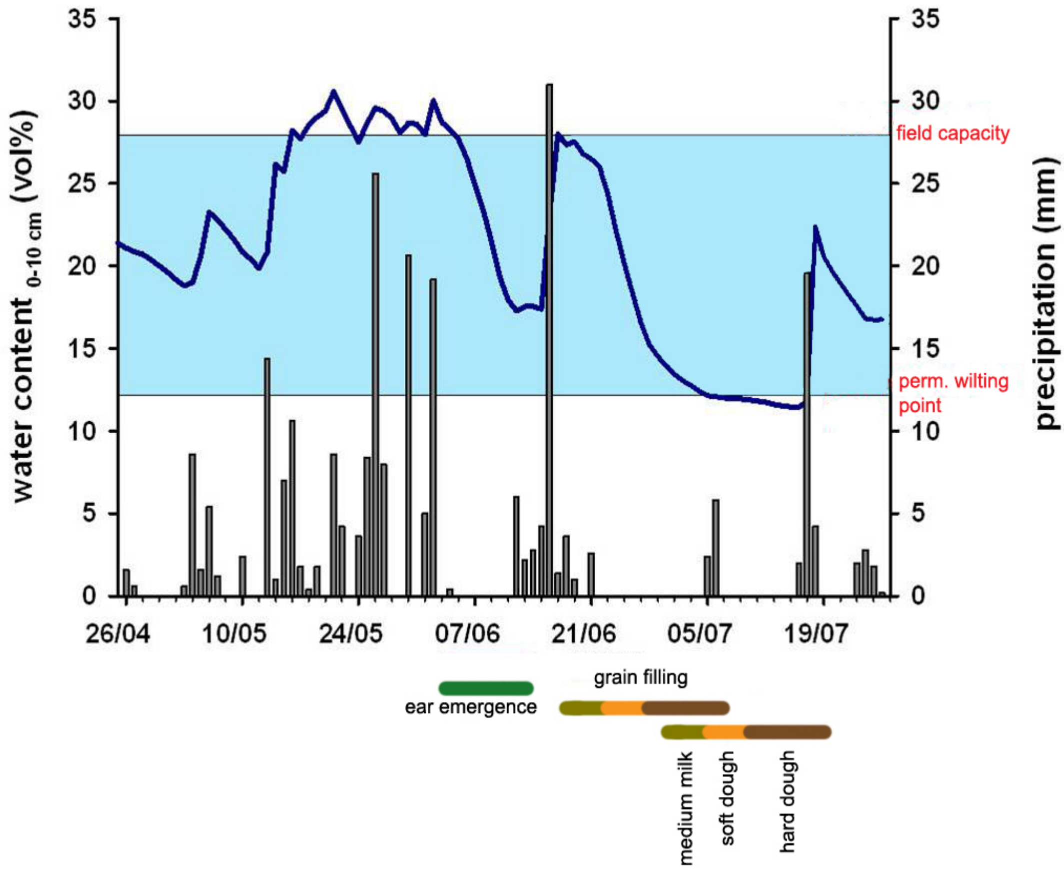


Figure 18: Water dynamics (precipitation and water content in the upper 10 cm soil layer) of the durum trial in Raasdorf 2010. Time span of ear emergence within the nursery is indicated, as well as the duration of the grain filling (medium milk; soft dough; hard dough) for early and late maturing genotypes

The set of varieties most probably did not differentiate appropriately for drought resistance because of sufficient rainfall. The obtained low grain yields of genotypes resulted most likely from other causes, e.g. susceptibility to fungal diseases. Nevertheless, most of the traits determined are constitutive traits which are expressed independently of the degree of stress (Blum 1996). It has also to be considered that the breeding strategy of CIMMYT is focused on distributing semi-dwarf wheat material combined with disease resistance that would perform well in relatively wet or irrigated environments while not collapsing under dry conditions (Reynolds & Borlaug 2006). Another promising strategy to identify valuable germplasm could be an intensive use of eco-geographic parameters of collection sites of durum genetic resources. Valuable resources might be durum genotypes originating from areas with severe drought stress (annual precipitation between 180 and 300 mm; excluding collection sites with known irrigation). This approach is followed by the Focused Identification of Germplasm Strategy (Mackay & Street 2004, Street et al. 2008, Endresen 2010). Some durum improvement programs are using wild relatives for the introgression of valuable traits, however, in this case intensive backcrossing is necessary (Valkoun 2001).

5.2 Phenological and physiological traits

Digital image analysis used for the determination of early ground cover and the late stay green effect showed a significant correlation to grain yield (Figure 15). The positive influence of that trait on grain yield and, therefore, yield potential has also been mentioned by Fischer (1980) and Turner & Nicolas (1987). The methodology of using conventional digital cameras and subsequently analyse the pictures by appropriate software is an affordable and easy-to-use tool to generate phenotypic data. The methodology seems to be suitable for selection in wheat breeding programs for drought resistance, especially if optimal processing of the colour information is applied (Casadesús et al. 2007). Early vigour and rapid ground covering have been proposed as important traits in regard to an economic water use and early drought tolerance (Rebetzke & Richards 1999, Royo et al. 2000, López-Castañeda et al. 1996). Early heading/flowering plays a major role in escaping terminal drought stress in rainfed environments. Early vigour is genetically fixed and positively related to a larger kernel size (López-Castañeda et al. 1996). Considering this criteria for the selection of genotypes in breeding programs addicted to drought tolerance could be a worth possibility. Stomatal conductance, electrical capacitance, chlorophyll concentration and leaf area index clearly showed their limitation. The methods require dry and/or clear weather conditions for the measurements. However, due to continuous rainfall until mid June the time frame for measurements at several critical phenological stages was restricted. Moreover, physiological traits are prone to variation within a trial and between environments, therefore, having only intermediate heritability (Clarke & Clarke 1996, Richards et al. 2001, Martínez & Guiamet 2004). Estimation and consideration of appropriate covariates, e.g. exact phenological stage, climate variables etc., can significantly improve results from such measurements (Clarke & Clarke 1996). The fact that physiological traits can work as indicators for drought stress was hitherto demonstrated in several studies (Fischer et al. 1998, Rebetzke et al. 2000, Ommen et al. 1999, Chloupek et al. 2010).

5.3 Yield related traits

In this study grain yield was highly correlated to biomass yield and number of fertile tillers per area unit (Figure 15). Recent studies of Fischer & Edmeades (2010) and Reynolds et al. (2010) confirmed that yield progress on a global level is still associated closely with an increased number of grains per area. Thus, increasing grain weight and grain size might be a way worth to be followed to improve grain yields, especially in case of early water stress which affects mainly spikelet and floret initiation and, therefore, limits grain number per area unit, whereas grain weight is affected by terminal drought. Grain weight in durum wheat can be improved by using e.g. genetic resources of *T. polonicum* or *T. turanicum* which are known for their characteristic high thousand kernel weight (Sissons & Hare 2002, Grausgruber et al. 2005). Identifying yield limiting traits and indirect traits and applying them effectively in a breeding program are major challenges because of the different types of drought and seasonal variation in the severity of drought (Richards et al. 2001). A high correlation to grain yield independent of environmental influence and a rapid, easy and cheap determination of such traits are prerequisites for a successful integration in breeding programs.

6. Conclusions

The results show significant genotypic effects for almost all investigated traits with the exception of two electrical capacitance measurements. Significant positive correlations with grain yield were observed for early and/or late ground cover, dry matter yield and number of fertile tillers per area unit, respectively.

Using a multivariate index most genotype values performed inferior than check variety Floradur. Varieties performing better than the check included varieties of Austrian and European origin. All these varieties showed a similar heading date which was optimal in this experimental year; varieties with earlier flowering were yield limited while later maturing genotypes suffered from drought at grain filling.

Exploring the broad variation in regard to the investigated traits it is worth to mention that the old durum genotype P103 and the Khorasan wheat QK-77 (Kamut) showed above average performance for some traits, whereas about half of the breeding lines from the CIMMYT nursery were inferior than the lowest performing Austrian variety Durobonus.

Sufficient rainfall in spring didn’t allow a testing for early drought stress, only terminal drought stress was present during grain filling of late maturing genotypes. Therefore, yield was not that much influenced by drought but more by other causes like lodging and fungal diseases. Nevertheless, most of the determined traits are constitutive traits which are expressed independently of the degree of stress.

Early ground cover and late ground cover (stay green effect) were highly correlated to grain yield and their digital analysis is easy and affordable. Early vigor and fast ground covering are related to the plant’s economic water use and tolerance to drought. Early heading and flowering is another plant’s strategy to escape drought stress. It is genetically fixed, easy to determine and related to a larger kernel size. Another good indicator for drought tolerance is the number of fertile tillers per unit area, which is highly correlated to grain yield. In regard to yield parameters early drought affects mainly spikelet and floret initiation and grain number per area whereas late drought influences grain weight.

Concerning stomatal conductance, electrical capacitance, chlorophyll concentration and leaf area index it must be noted that these traits represent well known stress indictors but show difficulties in their use. Their measurements require dry conditions, a certain time window of determination, i.e. certain critical developmental stages, and more time. Hence, this time consuming methods can not be used in practical breeding programs at early generations where a large amount of breeding lines must be screened.

7. Abstract

Due to the climate change plants are forced to develop adaption and mitigation strategies to survive in more hot and dry environments. The negative effects of changing environmental conditions affect significantly crop production. Breeding for drought tolerance is of increased interest also for European cereal breeders. In the Pannonian hills and plains growing region of eastern Austria spring sown cereals like durum wheat can be gravely impaired by recurring water stress. The selection for grain yield can be accomplished either directly under drought conditions or by indirect selection for morpho-physiological traits which are associated with drought tolerance. In the present study spring durum germplasm of diverse origin, has been investigated in a field trial for a wide range of agronomic and morpho-physiological criteria (phenological traits, physiological measurements and yield components) with the aim to roughly characterize the plant material in regard to drought tolerance. The results showed significant genotypic effects for almost all investigated parameters. Positive correlations to grain yield were observed for early ground cover, stay green effect, number of fertile tillers and biomass yield. Selected parameters were used to create a multivariate index which was graphically displayed as star plot. Plotting the index against heading date showed that the best performing genotypes were found within a period of three days. Especially Austrian and European genotypes were present in this group. Due to sufficient rainfall throughout the vegetation period water stress was observed only for the terminal growth stages of late maturing genotypes. Hence, evaluation of some physiological traits was hampered and did not lead to differentiating results.

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Fig. 4: *Bunte Pasta 6*, Bernd Boscolo (http://www.suite101.de/view_image.cfm/532792)

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Fig. 5: In: *Wurzelatlas der Kulturpflanzen gemäßigter Gebiete mit Arten des Feldgemüsebaus*, Kutschera et al. (2009).

Fig. 6: *Field plan of the experiment*, Heinrich Grausgruber, Dept. Crop Sci., Univ. Natural Resour. Life Sci., Vienna.

Fig. 7: *Climate diagram for the experimental site Raasdorf*, Experimental Station Groß-Enzersdorf, Univ. Natural Resour. Life Sci., Vienna. (Data available online: http://www.dnw.boku.ac.at/5751.html)

Fig. 8: *Estimation of crop ground cover by digital image analysis*, Johann Vollmann, Dept. Crop Sci., Univ. Natural Resour. Life Sci., Vienna.

Fig. 9: *Electric capacitance measurement*, Gernot Bodner, Dept. Crop Sci., Univ. Natural Resour. Life Sci., Vienna.

Schema of electric capacitance measuring device, Vítězslav Dostál, Dept. Crop Sci., Breed. & Plant Med., Mendel Univ., Brno.

Fig. 10: *SPAD-502Plus meter*, Doris Schuhwerk, Dept. Crop Sci., Univ. Natural Resour. Life Sci., Vienna.

Fig. 11: *SC1 steady-state leaf porometer*, D. Schuhwerk

Fig. 12: *LAI-2000 plant canopy analyzer*, Eurosep Instruments, Cergy Pontoise. (http://www.hellotrade.com/eurosep-instruments/plant-canopy-analyser.html)

Fig. 13: D. Schuhwerk.

Fig. 14: D. Schuhwerk

Fig. 15: D. Schuhwerk

Fig. 16: D. Schuhwerk

Fig. 17: D. Schuhwerk

Fig. 18: D. Schuhwerk

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Tab. 2: see Tab. 1

Tab. 3: D. Schuhwerk

Tab. 4: D. Schuhwerk

Tab. 5: D. Schuhwerk

Tab. 6: D. Schuhwerk

Tab. 7: D. Schuhwerk

9. Appendix

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Publications

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Field-screening of durum wheat (*Triticum durum* Desf.) for drought tolerance

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Abstract

Due to global warming and its negative effects on crop production, e.g. heat and drought, breeding for drought resistance is of increased interest also for European cereal breeders. In Eastern Austria, the Pannonian hills and plains growing region, especially spring sown cereals like durum wheat can be seriously damaged by recurring water stress. Selection for grain yield can be carried out either directly under drought conditions or by indirect selection for morphophysiological traits associated with drought tolerance. In the present study spring durum germplasm of diverse origin has been investigated in a field trial for a wide range of agronomic and morphophysiological criteria (phenological traits, physiological measurements and yield components) with the aim to roughly characterize the plant material in regard to drought tolerance. The results showed significant genotype effects for every parameter except for electrical capacitance measurements. Positive correlations were observed between grain yield and early ground cover, late stay green area, number of fertile tillers and biomass yield. Selected parameters were used to create a multivariate index based on star plots. Plotting the index against heading date revealed that the best performing genotypes were found within a period of three days difference and that genotypes from all genpools were present in this group. Due to unexpected rainfall throughout the period water stress was observed only for the terminal growth stages of late maturing genotypes. Hence, evaluation of some physiological traits was hampered and did not lead to differentiating results.

Keywords

Adaptation, global warming, heat, root system, water stress

Introduction

Worldwide adaptation and mitigation strategies are developed to counter the consequences of climate change, i.e. melting ice and rising sea levels, global warming, extreme weather events and changes in the rainfall patterns. The impact of global warming on crop production can already be seen by increased aridity and warmer temperatures in some regions. In Europe regions of southern Europe and the Mediterranean basin are especially vulnerable to heat and drought. But also for other European regions like the Atlantic zones or the Continental North and South an increased risk of drought is predicted (IGLESIAS et al. 2007).

Durum wheat (*Triticum durum* Desf..) is traditionally cultivated in regions with limited rainfall. The main production areas are the Mediterranean Basin and North America. Other countries with a production worth to mention are India, Russia, Mexico and Australia (BOZZINI 1988). In Austria durum breeding and cultivation started after World War II. The first varieties were based on American and Algerian genotypes and exhibited high quality but low yield levels. Therefore, it was necessary to secure the durum production by contract based cultivation and premium payments. With the introgression of the Italian semi-dwarf mutant CpB132 (Castelporziano) the yield level could be increased significantly (HÄNSEL and SEIBERT 1989).

Breeding for grain yield under water stress conditions can be realised by both direct selection for yield and by indirect selection for specific morphophysiological traits which are associated with drought tolerance (ALI DIB et al. 1992). The importance of the durum root system for drought stress tolerance was established by BENLARIBI et al. (1990), however, root characteristics can vary in relation to the type of drought (ALI DIB and MONNEVEUX 1992). Recently, ARAUS et al. (2008) have published an excellent review on physiological traits associated with drought adaptation and the use of secondary traits in practical breeding.

In the present study a durum nursery including international, European and Austrian germplasm was studied for a wide range of agronomic and morphophysiological traits with the aim to roughly characterise the plant material in regard to drought tolerance in order to select genotypes with specific traits for further studies on root system characteristics.

Material and methods

Plant material

In total 82 genotypes of spring durum wheat were tested. The majority of the nursery, i.e. 63 genotypes, were varieties and/or breeding lines from the CIMMYT 40th ISDN. Furthermore, genotypes of Austrian and other European origin, and a few tetraploid genetic resources (i.e. two old durum varieties, one *T. durum* x *T. dicoccum* line, and T. turanicum QK-77) were included in the trial.

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Experimental conditions

The field experiment was laid out as a row-column (30x10) design with six blocks. Number of replications per genotype was variable. Three check varieties (i.e. Durobonus, Floradur, Rosadur) were replicated 15 to 33 times throughout the experimental lay-out in order to optimally account for natural and extraneous spatial variation. The field trial was sown on 22nd March 2010 in Raasdorf (16°35'E, 48°14'N) in the Pannonian plains growing region. Plot size was 1.25x1.4 m. Mean annual temperature at the experimental site is 9.8°C, the precipitation is around 550 mm. During the last years a drought period in early spring was regularly observed (*Figure 1*).

Phenological traits

After plant emergence digital images of the plots were obtained four times (19th and 30th April, 7th May, 30th June) with a Canon EOS20D (Canon Inc., Tokyo) digital camera from about 1.5 m height. Digital images were downloaded in the JPEG format to a personal computer and analyzed individually by SigmaScan Pro vers. 5.0 software (Systat Software Inc., Chicago). Thresholds for the hue and saturation range were chosen to selectively identify green leaves. The total number of selected green pixels were counted and then divided by the total number of pixels of the image to give the percentage of green ground cover (RICHARDSON et al. 2001). Data for the first dates in spring indicate early growth vigour, while data for the summer date give an indication for the stay green effect of leaves. Moreover, heading date (days after 31st May) was recorded for each plot if 50% of the spikes were visible.

Physiological traits

Root surface area was characterised indirectly by electrical capacitance measurements (CHLOUPEK 1977) on 20th and 29th May, and 15th June using an Escort elc-133 lcr-meter (Instruments Techno Test Inc., Laval, Canada). Chlorophyll-concentration was measured on 16th June using a SPAD-502Plus meter (Konica Minolta Holdings, Inc., Tokyo). The SPAD results are correlated to the nutrition status of the plant and the leaf photosynthesis (BOTHA et al. 2010). Stomatal conductance is a parameter to describe the stomata opening and is measured with the SC1 Steady-State Leaf Porometer (Decagon Devices, Inc., Pullman). Size of the assimilation area (leaf area index, LAI) was measured through hemispherical photography with a LAI-2000 Plant Canopy Analyzer (Li-Cor Environmental, Lincoln) (QARIANI et al. 2000, INOUE et al. 2004) on 24th June.

Yield traits

Whole plants of the two centre rows of each plot, i.e. 0.35 m², were cutted about 1 cm above ground for the determination of number of fertile spikes, total dry matter yield and grain yield per unit area, and 1000 kernel weight. Harvest

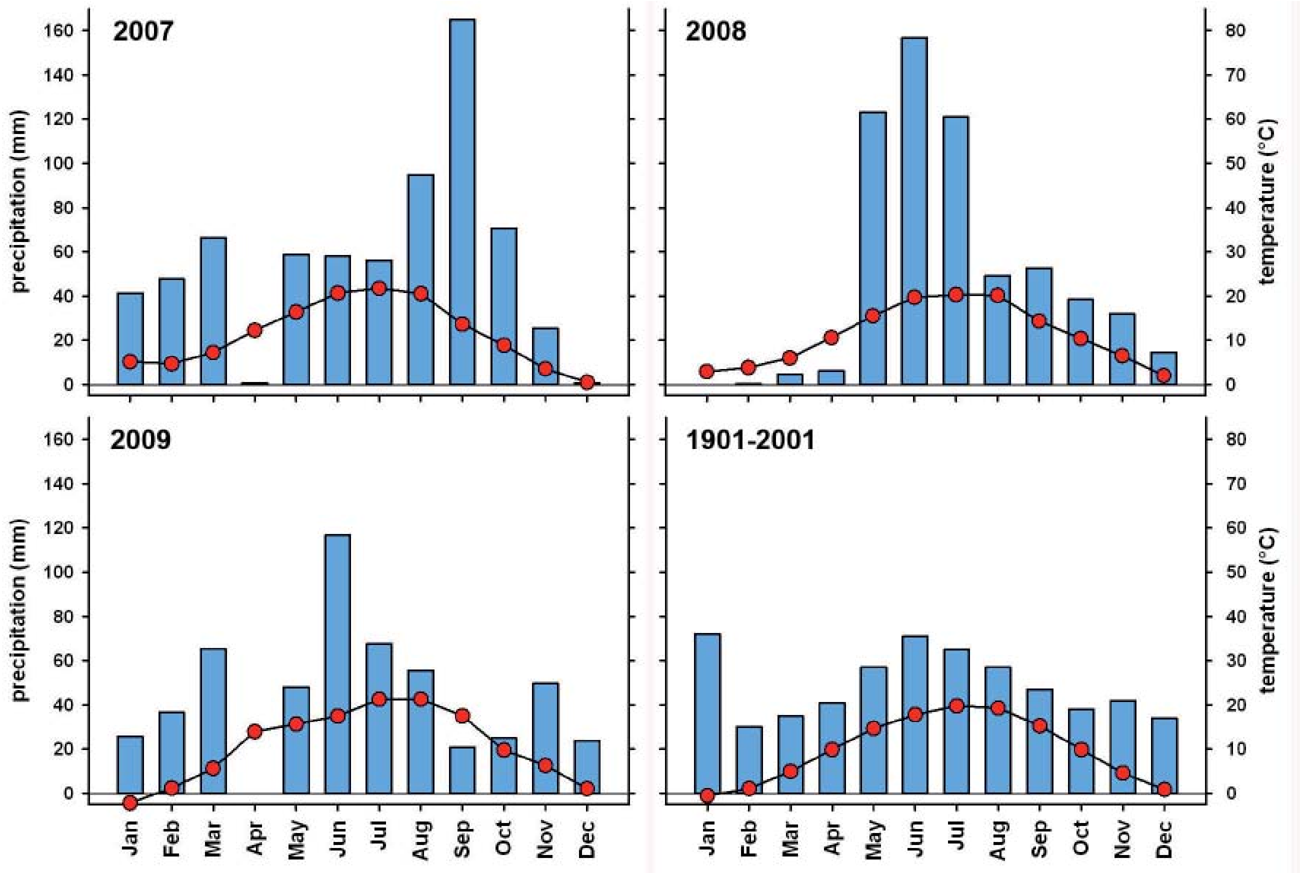

Figure 1: **Climate diagram for the experimental site Raasdorf for the years 2007 to 2009, and the longterm trend illustrating the recurrent period of not sufficient precipitation (bars) in early spring in recent years**

Table 1: **Optimized spatial models and significance tests for fixed genotype and block effects**

	Linear mixed model			*Pr>F*	
Trait[1]	Fixed effects: gen + block	Random effects	Error variance model	gen	block
GC1904	+ lin(row) + lin(col)		AR1xAR1	<.001	<.001
GC3004	+ lin(row)		AR1xAR1	<.001	<.001
GC0705	+ lin(row)		AR1xAR1	<.001	0.009
GC3006	+ lin(col)	+ row	IDxAR1	<.001	0.011
EC2005			IDxAR1	0.835	0.670
EC2905		+ row	IDxAR1	0.028	0.029
EC1506			IDxAR1	0.294	0.014
HEAD			IDxAR1	<.001	<.001
SPAD			IDxAR1	<.001	0.004
SC		+ row	IDxAR1	0.004	0.003
LAI	+ lin(col)	+ row	IDxID	<.001	<.001
PH	+ lin(col)	+ row	IDxID	<.001	<.001
DMYLD		+ row	IDxAR1	<.001	<.001
SPK		+ row	AR1xAR1	<.001	<.001
GYLD		+ row	AR1xAR1	<.001	<.001
HI			IDxID	<.001	<.001
TKW		+ row	AR1xID	<.001	0.020
HLW			AR1xAR1	<.001	<.001
KS28			AR1xAR1	<.001	<.001
PROT		+ row	IDxAR1	<.001	0.009

[1] GC, ground cover measured on 19th and 30th April, 7th May, and 30th June, respectively (%); EC, electrical capacitance measured on 20th and 29th May, and 15th June, respectively (nF); HEAD, heading date (days after 31st May); SPAD, chlorophyll concentration (SPAD values); SC, stomatal conductance ($mmol.m^{-2}.s^{-1}$); LAI, leaf area index; PH, plant height (cm); DMYLD, dry matter yield ($g.m^{-2}$); SPK, number of spikes.m^{-2}; GYLD, grain yield ($g.m^{-2}$); HI, harvest index; TKW, thousand kernel weight (g); HLW, hectolitre weight ($kg.hl^{-1}$); KS28, kernel plumpness >2.8 mm (%); PROT, protein content (%)

index was calculated as the grain yield/total dry matter yield ratio. The residual plots were combine harvested and samples were further used for the determination of hectolitre weight, kernel plumpness (2.8x25 and 2.5x25 mm slotted sieves, respectively) and protein content. Total plot grain yield was calculated by adding the data of the manual and combine harvested plot parts. Plant height and lodging scores were recorded before harvest.

Statistical analysis

Accounting for the randomization scheme, we fitted linear mixed models with fixed genotype and block effects, linear trends along rows and/or columns, random row and/or column effects and spatial covariance confined (nested) within blocks using GenStat 13th Ed. (VSN International Ltd, Hemel Hempstead, UK). Among models those with the smallest deviance and/or Akaike Information Criterion (AIC) were preferred as the optimized model of spatial analysis to calculate adjusted genotypic means (GILMOUR et al. 1997, PAYNE 2006, PIEPHO and WILLIAMS 2010). Subsequently the mean values were sorted and transferred into relative values setting the highest performance 100 (JENSEN 1976). The relative values of eight traits (i.e. crop cover 19th April, crop cover 30th June, SPAD values, number of spikes per square meter, dry matter yield per square meter, grain yield per

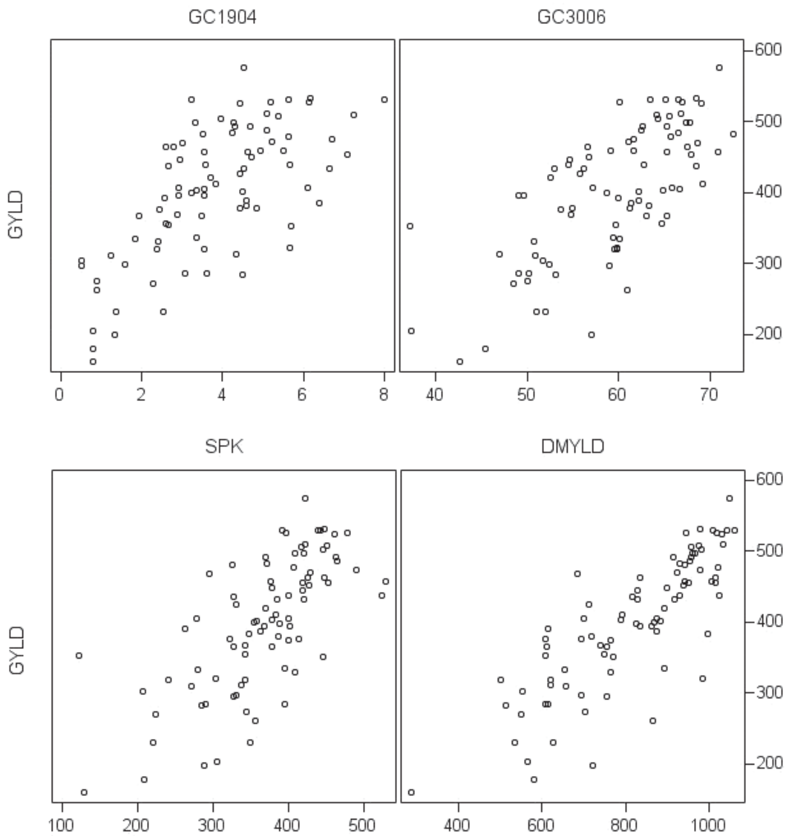

Figure 2: **Relationship between grain yield (GYLD) and crop ground cover at early (GC1904) and late (GC3006) growth stage, number of fertile tillers per unit area (SPK) and dry matter yield (DMYLD)**

square meter, harvest index, and 1000 kernel weight) were selected to create star plots. Finally, the area within the star was determined and used as multivariate index to rank the tested germplasm. Correlation analysis was carried out to determine the relationships among traits.

Results

Spatial models

Optimized models for the diverse characters are demonstrated in *Table 1*. Genotypic effects were significant for almost all traits with the exception of two dates of electrical capacitance measurements.

Correlation analysis

Crop ground cover at early and late growth stages showed positive and significant correlations to LAI (*r* =0.43-0.53, *p*<0.01) and several yield related traits: correlation coefficients were highest for the relationships to grain yield and dry matter yield (*r* =0.56-0.71, *p*<0.0001), followed by the number of fertile tillers per unit area (*r* =0.54-0.63, *p*<0.01), while the relationship to thousand kernel weight and kernel plumpness (*r* =0.41-0.48, *p*<0.01) was worth mentioning only for crop ground cover at early growth stages. Within yield related traits grain yield was highly correlated to dry matter yield (*r* =0.87, *p*<0.0001), whereas correlation was lower to the number of fertile tillers per unit area (*r* =0.69, *p*<0.0001) and especially low to thousand kernel weight

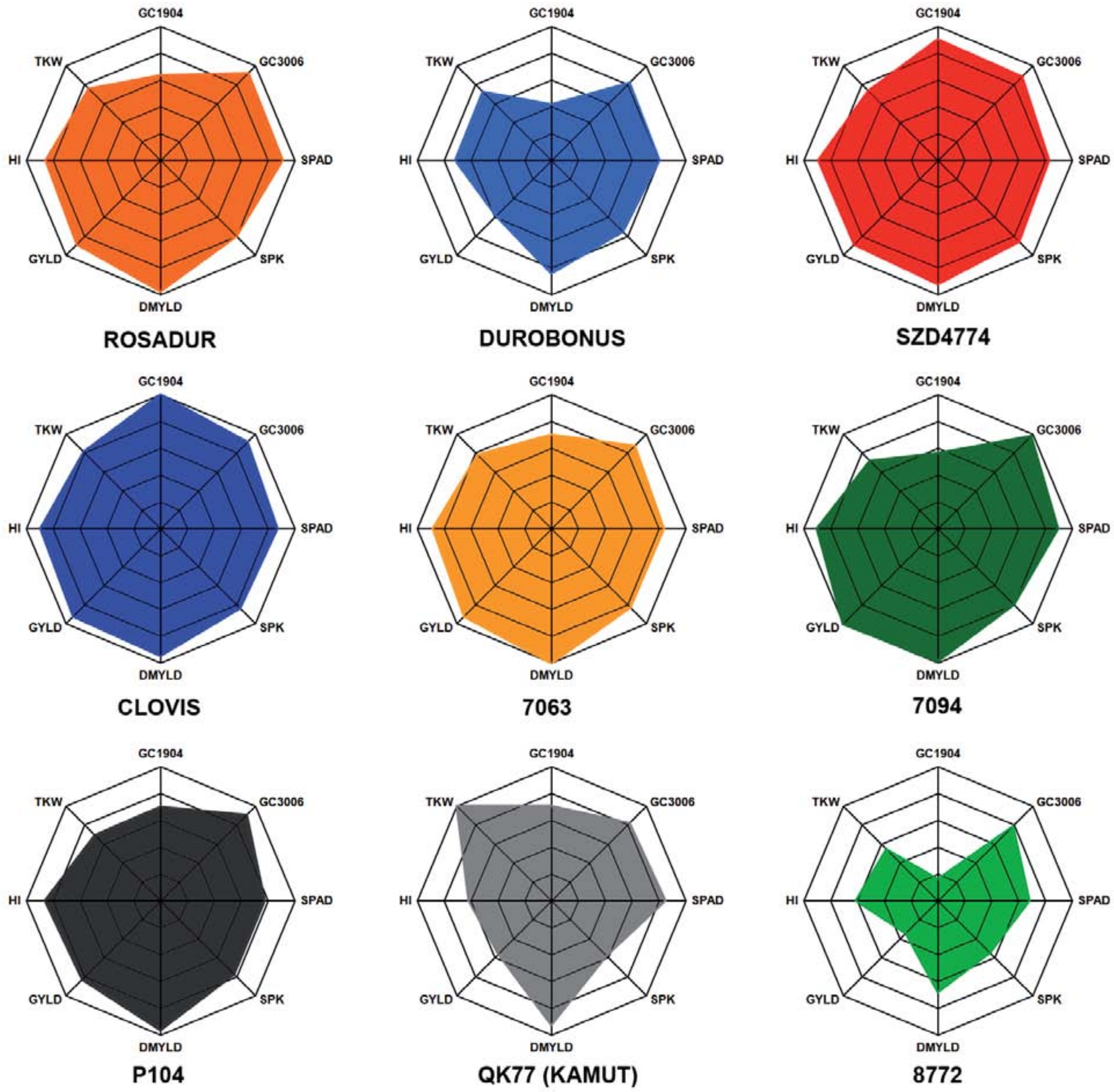

Figure 3: **Star plot area of nine selected genotypes: Rosadur, Durobonus, SZD4774 (AT), Clovis (FR), 7063, 7094 (CIMMYT), P104 (PGR), QK77 (*T. turanicum*), 8772 (*T. durum* x *T. dicoccum*). Abbreviations of traits see *Table 1***

Table 2: **Means of selected traits for genotypes which performed above average in regard to the star area (multivariate index)**

Genotype[1]	GC1904[2]	HEAD	SPAD	GC3006	SPK	DMYLD	GYLD	HI	TKW	HLW	PROT
7005	4.94	7.3	49.3	59.1	529	1004	458.2	0.50	41.4	78.1	13.9
7015	3.95	8.2	47.2	64.4	446	981	502.9	0.50	39.8	80.1	13.0
7016	5.38	7.2	52.4	65.5	417	958	506.6	0.46	38.2	78.8	15.8
7017	4.67	6.2	47.2	65.3	463	957	492.2	0.44	42.4	77.9	15.1
7022	5.19	7.2	48.7	60.2	478	1019	526.8	0.49	37.8	77.6	13.5
7036	5.67	7.0	47.0	54.5	524	930	437.0	0.46	33.9	77.3	13.9
7039	5.10	6.0	50.7	62.5	464	952	486.7	0.47	38.8	76.6	14.1
7046	5.22	4.9	47.9	61.1	429	921	470.0	0.44	41.0	79.9	14.6
7063	5.62	6.5	49.7	63.4	440	1060	529.0	0.46	45.9	78.3	15.7
7065	3.24	9.0	50.9	65.1	392	1041	530.2	0.50	44.0	78.2	13.7
7069	3.31	7.6	51.7	67.8	420	959	496.4	0.45	40.7	76.7	14.6
7094	4.51	8.1	53.1	71.0	422	1048	574.8	0.47	42.3	78.9	13.9
Babylone	4.63	7.9	48.3	65.3	418	1015	456.1	0.43	50.5	75.2	16.4
Clovis	8.00	6.4	51.5	66.6	443	1007	529.2	0.46	47.7	78.5	15.0
D07643	4.44	8.6	48.8	69.0	461	1031	523.4	0.43	37.7	76.1	15.5
Duroflavus	6.12	9.1	50.5	65.9	401	874	404.6	0.40	41.5	75.0	16.1
Floradur	4.26	7.5	53.8	67.4	408	967	497.3	0.44	42.3	79.1	14.6
Malvadur	5.51	7.4	55.7	61.7	376	942	458.4	0.42	47.0	76.9	14.5
Rosadur	5.08	7.9	54.0	66.9	423	1032	509.7	0.44	44.5	79.2	15.2
Topdur	7.08	8.5	52.6	68.0	428	937	452.8	0.40	45.1	76.8	16.0
SZD4774	7.23	7.1	49.0	64.1	450	973	508.6	0.46	43.2	77.1	14.6
SZD4854	6.64	6.9	54.8	56.3	420	918	432.7	0.43	43.0	77.2	15.2
SZD5643	6.16	7.1	46.7	68.6	448	978	532.1	0.45	43.3	77.1	13.3
SZD5658D	6.72	6.6	50.8	61.6	490	976	473.5	0.42	43.4	77.0	15.4
P104	5.63	6.0	46.1	65.7	407	1021	477.5	0.45	40.8	78.7	15.0
Minimum[3]	0.51	4.2	40.0	37.2	122	287	160.2	0.25	31.8	72.8	12.5
Maximum	8.00	13.7	59.3	72.6	529	1060	574.8	0.51	58.6	80.1	17.5
Mean	3.79	7.4	49.2	59.4	368	817	399.0	0.45	40.6	77.2	14.8

[1] Origin of genotypes: 7005-7094: CIMMYT 40th ISDN; Babylone, Clovis, D07643: France (FR); Duroflavus, Floradur, Malvadur, Rosadur, Topdur, SZD4774, SZD4854, SZD5643, SZD5658D: Austria (AT); P104, Plant genetic resource

[2] Abbreviations and units of traits see *Table 1*

[3] Minimum, maximum and mean values refer to the complete nursery

(r=0.35, p=0.001). Physiological traits showed no remarkable correlations with the exception of the already above mentioned relationships between crop ground cover and LAI, heading date and electrical capacitance EC1506 (r =0.55, p=0.0002), and LAI and thousand kernel weight (r =0.45, p=0.003). The most pronounced relationships to grain yield are demonstrated in *Figure 2*.

Multivariate index

Relative values of eight traits were used to create a star plot. The area within the star was used as multivariate index. Examples for the star area of selected genotypes are presented in *Figure 3*. The mean star area of the nursery was 14.92x10³. *Table 2* represents absolute performance values for selected traits of those genotypes which performed above the mean index.

In *Figure 4* the multivariate index is plotted against the heading date. It is obvious that the majority of genotypes reached a star area below 18x10³, the

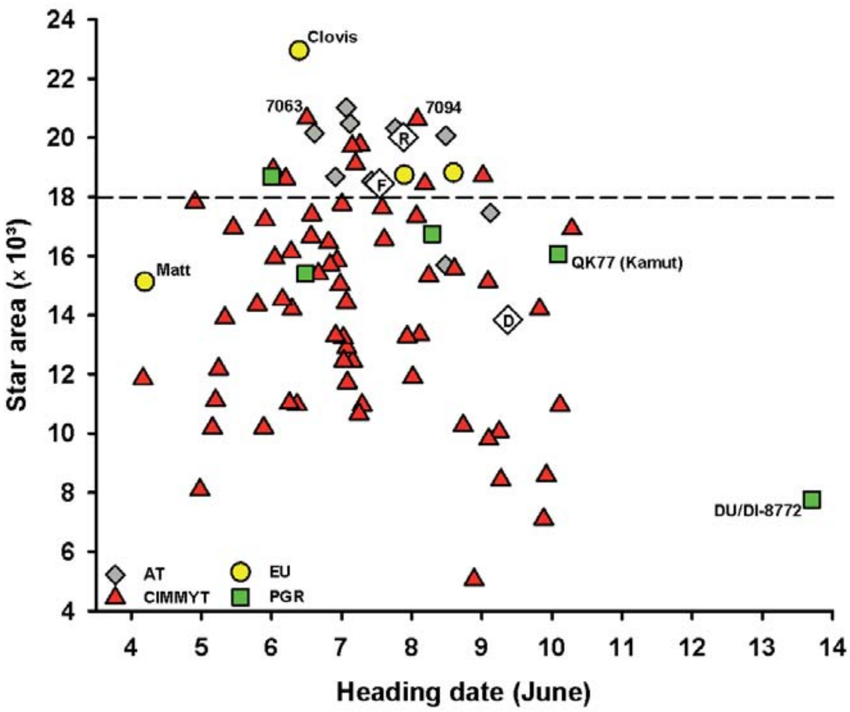

Figure 4: **Relationship between heading date and star plot area. Genotypes of different genpools are indicated by different symbols; check varieties Durobonus, Floradur and Rosadur are indicated by white diamonds including the initial letter of the variety name**

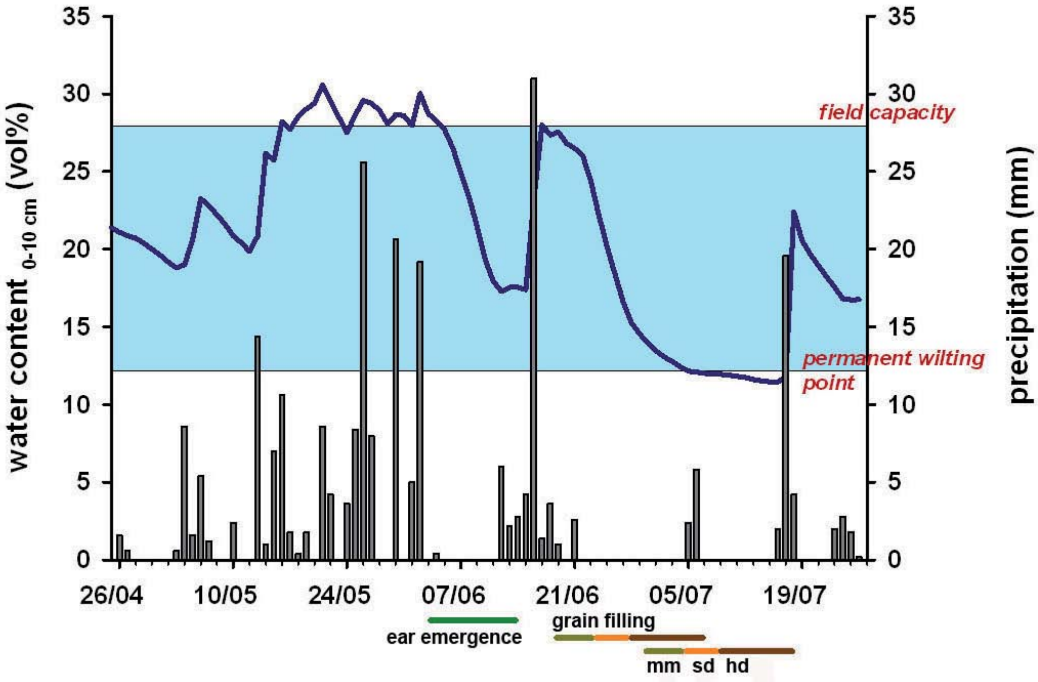

Figure 5: **Water dynamics (precipitation and water content in the upper 10 cm soil layer) of the durum trial in Raasdorf 2010. Time span of ear emergence within the nursery is indicated as well as the duration of the grain filling (mm, medium milk; sd, soft dough; hd, hard dough) for early and late maturing genotypes**

approximate level of Floradur, which was the most popular durum variety in Austria in recent years. Heading of genotypes with an index above 18x10³, occurred between 6th and 9th June 2010 indicating an optimal window of heading date. In the group of best performing genotypes most of the Austrian germplasm was included, but also other genotypes like the French variety Clovis or the CIMMYT line 7063 showed excellent performance with at the same time somewhat earlier heading date.

Discussion

Plant material and experimental conditions

The tested germplasm showed a broad variation concerning almost all traits. About half of the CIMMYT lines performed inferior than the lowest performing Austrian check Durobonus, whereas the three old durum varieties with tall plant height performed above average (see unlabelled squares in *Figure 4*). Due to the relatively high amount of rainfall until mid June water stress appeared only at the late grain filling period of the late maturing genotypes (*Figure 5*). Hence, the nursery most probably did not differentiate appropriately for drought resistance and low grain yields of genotypes resulted from other causes such as fungal diseases since no fungicides were applied. Nevertheless most of the traits determined are constitutive traits which are expressed independently of the degree of stress (BLUM 1996). It has also to be considered that CIMMYT's breeding strategy focused on distributing semi-dwarf wheat material with disease resistance that would perform well in relatively wet (irrigated) environments while not collapsing under dry conditions (REYNOLDS and BORLAUG 2006). Another promising strategy could be an intensive use of the eco-geographic parameters of collection sites of genetic resources to identify valuable germplasm, e.g. search within durum genetic resources originating from areas with severe drought stress (annual precipitation between 180 and 300 mm; excluding collection sites with known irrigation). This approach is followed by the Focused Identification of Germplasm Strategy (MACKAY and STREET 2004, STREET et al. 2008, ENDRESEN 2010). Some other durum improvement programs are using wild relatives for the introgression of valuable traits, however, in this case intensive backcrossing is necessary (VALKOUN 2001).

Phenological and physiological traits

Digital image analysis for early ground cover and late stay green effect showed a significant correlation to grain yield. The methodology of using conventional digital cameras and subsequently analyse the pictures by appropriate software is an affordable and easy-to-use tool to generate phenotypic data. The methodology seems to be suitable for selection in wheat breeding programs for drought resistance, especially if optimal processing of the color information is applied (CASADESÚS et al. (2007). Early vigour and rapid ground cover have been proposed as important traits in regard to an economic water use and early drought tolerance (REBETZKE and RICHARDS 1999, ROYO et al. 2000), whereas early heading/flowering plays a major role in escape of terminal drought stress in rainfed environments.

The measured physiological traits (stomatal conductance, electric capacitance, chlorophyll concentration, leaf area index) clearly showed their limitation. The methods require dry and/or clear weather conditions for the measurements.

However, due to continuous rainfall until mid June the time frame for measurements of physiological traits at several critical phenological stages was restricted. Physiological traits are prone to variation within a trial and between environments, therefore, having only intermediate heritability (CLARKE and CLARKE 1996, RICHARDS et al. 2001, MARTÍNEZ and GUIAMET 2004). Estimation and consideration of appropriate covariates, e.g. exact phenological stage, climate variables etc., can significantly improve results from such measurements (CLARKE and CLARKE 1996). The fact that physiological traits can work as indicators for drought stress was hitherto demonstrated in several studies (e.g. FISCHER et al. 1998, REBETZKE et al. 2000, OMMEN et al. 1999, CHLOUPEK et al. 2010).

Yield related traits

In the present study grain yield was highly correlated to biomass yield and number of fertile tillers per area unit. Recent studies of FISCHER and EDMEADES (2010) and REYNOLDS et al. (2010) confirmed that yield progress on a global level is still associated closely with an increased number of grains per area. Thus, increasing grain weight and grain size might be a way worth to be followed to improve grain yields, especially in case of early water stress which affects mainly spikelet and floret initiation and, therefore, limits grain number per area unit, whereas grain weight is affected by terminal drought. Grain weight in durum wheat can be improved by using e.g. genetic resources of *T. polonicum* or *T. turanicum* which are known for their characteristic high thousand kernel weight (SISSONS and HARE 2002, GRAUSGRUBER et al. 2005).

Identifying yield limiting traits and indirect traits and applying them effectively in a breeding program are major challenges because of the different types of drought and seasonal variation in the severity of drought (RICHARDS et al. 2001). A high correlation to grain yield independent of environmental influence and a rapid, easy and cheap determination of such traits are prerequisites for a successful integration in breeding programs.

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Wurzelmethoden für die Pflanzenzüchtung

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Zusammenfassung

Wurzeleigenschaften wurden in der Pflanzenzüchtung bisher kaum berücksichtigt. Die Züchtung auf Ertragspotential führte sogar zu einer tendenziellen Verringerung der Durchwurzelungsintensität. Für die Verbesserung der Toleranz gegenüber abiotischem Stress und für eine ressourceneffiziente Produktion ist die Wurzel jedoch ein zentrales Pflanzenorgan. Die Aufnahme von Wurzelparametern als Zuchtziel erfordert eine klare Definition des Zielmerkmals in Abhängigkeit des hydrologischen Regimes. Dies kann über Wurzel-Boden-Simulationsmodelle erreicht werden. Bisher existiert keine geeignete Methode zur Phänotypisierung von Wurzelsystemen unter Feldbedingungen, die ausreichende Genauigkeit mit hohem Probendurchsatz verbindet. Ausgehend von einer Übersicht über Labor- und Feldmethoden wird eine Kombination aus Modellierung, Screening-Methoden und Detailbeschreibung von vorselektierten Kandidaten vorgeschlagen, um Wurzeleigenschaften in der Züchtung zu berücksichtigen.

Schlagwörter: Wurzeleigenschaften, Stresstoleranz, Züchtung, Messmethoden

Summary

Root traits are rarely considered as breeding target. Breeding for yield potential in the context of intensive agricultural management has even resulted in reduced rooting vigor of modern cultivars. However, the root is an essential plant organ to improve crop tolerance against abiotic stress as well as for a resource efficient cropping system. Consideration of root properties in breeding requires first an accurate definition of the target trait in relation to the hydrological site conditions which can be assisted by the use of root-soil simulation models. There is still no appropriate method for phenotyping root systems under field conditions that combine sufficient accuracy with the high throughput required in breeding experiments. Following a general overview of field and laboratory methods of root measurement, we propose a scheme integrating modeling, quick screening and detailed description of pre-selected candidates to include root traits in breeding programs.

Keywords: root traits, stress tolerance, breeding, measurement methods

Einleitung

Die züchterische Verbesserung von Kulturpflanzen hat in Verbindung mit Veränderungen des landwirtschaftlichen Managements zu einem stetigen Ertragszuwachs im 20. Jahrhundert geführt. Seit den 1990er Jahren wurde jedoch ein Auseinanderfallen der Ertragssteigerungen und des Wachstums der Weltbevölkerung beobachtet. Die Erschließung neuen Ackerlandes ist begrenzt – Inkulturnahme marginaler Standorte würde nur wenig zu einem höheren Produktionsumfang beitragen, während gleichzeitig die Ausweitung der Anbaufläche auf Kosten schützenswerter natürlicher Ökosysteme geht. Auch die Ertragssteigerung durch Intensivierung der Produktion ist problematisch, da bereits heute intensive Produktionssysteme die Umwelt belasten, während die Verfügbarkeit der Produktionsfaktoren (Wasser, Phosphor) knapper wird und damit auch mit einer tendenziellen Steigerung der Produktionskosten einhergeht.

Demnach ist auf produktionstechnischer Seite vor allem die Steigerung der Ressourcen-Nutzungseffizienz ein wesentliches Ziel. Die Pflanzenzüchtung war über Jahrzehnte auf die Verbesserung des Ertragspotentials unter Bedingungen optimaler Wasser- und Nährstoffversorgung konzentriert. Die künftigen Rahmenbedingungen erfordern verstärkt eine Konzentration auf Ertragsbildung und -stabilität in low-input Systemen. Im Zusammenhang mit der Verknappung von Wasserressourcen sprach LYNCH (2007) von der Notwendigkeit einer „blauen Revolution“, in der die verbesserte Wassernutzung in der Pflanzenproduktion im Zentrum steht. In diesem Zusammenhang wies er auf die Rolle des Wurzelsystems hin. WAINES und EHDAIE (2007) untersuchten züchterische Veränderungen des Wurzelsystems im 20. Jahrhundert bei Weizen. Sie konnten zeigen, dass das Zuchtziel Ertragspotential unter high-input Bedingungen indirekt zu einer Verringerung der Wurzelsystemgröße führte. Mit wenigen Ausnahmen (Reis, Kichererbse) findet sich bis heute keine systematische Züchtung auf Wurzeleigenschaften.

Die Bedeutung der Wurzel als Zuchtziel ergibt sich aus ihrer wesentlichen Rolle in der Stresstoleranz. LEVITT (1980) unterschied drei Mechanismen pflanzlicher Stresstoleranz: das zeitliche Ausweichen vor der Stressperiode, die physiologische Resistenz gegen Dehydratation der Gewebe, und die Vermeidung von Stress durch reduzierte Wasserabgabe

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oder verbesserte Aufnahme. Züchterisch wurde eine Anpassung der Kulturpflanzen an das Auftreten von Stress durch frühreife Sorten in Gebieten mit Sommertrockenheit erreicht. Auch Mechanismen der Stressvermeidung über physiologische Reaktionen am Blatt wurden bearbeitet, etwa die intrinsische Wassernutzungseffizienz über die Methode der Kohlenstoff-Isotop-Diskriminierung (CONDON et al. 2002). BLUM (2005) wies jedoch darauf hin, dass viele Mechanismen der Stresstoleranz zulasten der Ertragsbildung gehen. Statt der Wassernutzungseffizienz, die häufig durch einen hohen stomatären Widerstand erreicht wird (UDAYAKUMAR et al. 1998), solle daher eher die effiziente Wassernutzung im Mittelpunkt stehen (BLUM 2009). Dies wiederum rückt die Wurzel in den Mittelpunkt des Interesses.

Eine der wesentlichen Herausforderungen dabei ist jedoch die Methodik. Eine Vielzahl möglicher Parameter sowie die in der Züchtung übliche hohe Zahl an Genotypen stehen der Schwierigkeit der Beobachtung gegenüber. Im Folgenden wird eine Übersicht über Zielgrößen im Wurzelbereich gegeben sowie Methoden in Hinblick auf ihre züchterische Anwendbarkeit diskutiert. Daraus soll ein Schema für die Bearbeitung von Wurzelparametern im Kontext der Züchtung vorgeschlagen werden. Schwerpunkt für die vorliegende Arbeit bildet der Kontext Trockenstress.

Material und Methoden

Neben einem Literaturüberblick über züchtungsrelevante Wurzelparameter und Methoden, werden ausgewählte Ansätze vorgestellt, die in Versuchen zum Vergleich von Wurzelparametern verschiedener Getreidesorten erhoben wurden. In Feldversuchen wurden dabei bildanalytische Messungen von Wurzeln aus Bodenzylindern mittels WinRhizo durchgeführt, sowie Messungen der Wurzelkapazität nach CHLOUPEK (1977). Als Labormethoden werden Messsysteme und Auswertungsmethoden für Keimpflanzen sowie Rhizoboxsysteme für die Untersuchung ganzer Wurzelsysteme vorgestellt.

Ergebnisse und Diskussion

Wurzelparameter

Tabelle 1 gibt einen Überblick über Wurzelparameter, die von züchterischer Relevanz im Zusammenhang mit verbesserter Trockentoleranz sein können.

CALDERINI et al. (1999) erwähnen, dass die züchterische Ertragssteigerung weniger von einer höheren Gesamtbiomassebildung kommt, denn von einer erhöhten Assimilatverlagerung ins Korn (Harvest Index). Demnach kann bei gleichem Assimilationspotential, die Ausbildung eines intensiven Wurzelsystems auch auf eine Assimilatkonkurrenz hinweisen, die bei wenig bearbeiteten Genotypen verstärkt jene Organe fördert, die das Überleben und Fortbestehen unter Mangelbedingungen sichern (SIDDIQUE und TENNANT 1990). *Tabelle 1* zeigt jedoch, dass zahlreiche Wurzelparameter keine negative Relation zum Ertrag erwarten lassen und sich daher als Zuchtziel für verbesserte Stresstoleranz ohne negative Ertragswirkung anbieten.

Züchterisch ergeben sich daraus mehrere Herausforderungen. Zum ersten gilt es, die genetische Variabilität eines Zielparameters festzustellen. Insofern die Wurzel bisher nur wenig in der Züchtung berücksichtigt wurde, kann erwartet werden, dass sowohl im vorhandenen Sortenspektrum als auch in genetischen Ressourcen ausreichend Variabilität vorliegt (BLUM 2010). Zum zweiten stellt sich angesichts der Vielfalt von Wurzelparametern die Frage, welcher in einer bestimmten Situation als Zuchtziel zu definieren ist, da die Effektivität eines Wurzelparameters für die Pflanzenwasserversorgung von der hydrologischen Standortsituation abhängt. Ergebnisse umfassender Wurzeluntersuchungen

Tabelle 1: **Wurzelparameter, Trockentoleranz und Ertrag***

Parameter	Bedeutung	Referenz	Negative Ertragsbeziehung
Wurzeltiefe	Erhöhung des Bodenvolumens zur Wasseraufnahme	Kage und Ehlers (1996)	Möglich
Wurzellängen-/-oberflächendichte	Wasseraufnahme aus trockenen Bodenschichten	Vamerali et al. (2003)	Möglich
Einschränkung der Seitenwurzelbildung	Vermehrte Assimilatallokation zu tiefen Wurzeln	Xiong et al. (2006)	Nein
Spezifische Wurzellänge/-oberfäche	Höhere Aufnahmefläche pro Einheit Wurzelbiomasse	Ryser (2006)	Nein
Zahl samenbürtiger Wurzeln	Assimilatinvestition in tiefgehende Wurzeln	Grando und Ceccarelli (1995)	Nein
Wurzelleitfähigkeit (radial und axial)	Zeitlicher Verlauf des Wasserentzugs	Richards und Passioura (1989)	Nein
Aquaporine	Regelung der radialen Leitfähigkeit	Javot und Maurel (2002)	Nein
Wurzelplastizität	Morphologische Stress-Anpassung	Bell und Sultan (1999)	Nein
Tiefenverteilung	Räumliche Aufteilung des Wasserentzugs	Bodner et al. (2008)	Nein
Eindringstärke	Durchwachsen von Verdichtungen	Zheng et al. (2000)	Möglich
Wurzelbiomasse	Assimilatallokation zum Wurzelsink	Siddique et al. (1990)	Ja
Wurzelkapazität	Durchwurzelungsintensität und -funktionalität	Chloupek (1977)	Möglich
Stresskompensation	Aufnahmekapazität einzelner tiefer Wurzeln	Šimůnek und Hopmans (2008)	Nein
Membranstabilität	Physiologische Stabilität unter Trockenheit	Huang und Fry (1998)	Nein
Mykorrhizierungsgrad	Erhöhung der Aufnahmefläche	Allen (2007)	Möglich
Trockenheitssignalisierung	Steuerung oberirdische Reaktionen (Stomata)	Hsiao et al. (2000)	Möglich

*Die Literaturliste kann über den Autor bezogen werden.

und begleitender ökohydrologischer Modellierungsstudien zeigten, dass die natürliche Vegetation ihr Wurzelsystem auf die vorliegenden hydrologischen Bedingungen optimiert (z.B. VAN WIJK 2011, JACKSON et al. 2000). Die Nutzung ökohydrologischer Modellansätze und Optimierungshypothesen könnte zu einer standortangepassten Definition geeigneter Zielmerkmale im Wurzelsystem beitragen. Darauf folgt die wesentliche Herausforderung der Selektion aus einer potentiell hohen Vielfalt bei gleichzeitig starker Interaktion der Merkmalsausprägung mit den Umweltbedingungen (Tropismen). Die Plastizität des Wurzelsystems bedeutet hohe Genotyp-Umweltinteraktionen und damit eine besondere Bedeutung der Selektionsbedingungen. Gerade die hohe Variabilität von Niederschlägen als Merkmal eines Trockengebiets lässt die Anpassungsfähigkeit des Wurzelsystems an die aktuelle Wasserversorgung eher als gewünschte Eigenschaft erscheinen denn eine konstitutiv hohe Assimilatallokation in die Wurzel.

Feldmessmethoden

Klassische Feldmethoden der quantitativen Wurzelanalyse beruhen auf der Entnahme von Bodenproben und deren nachfolgender Bearbeitung (Auswaschung, Bildanalyse) im Labor (z.B. HIMMELBAUER et al. 2004). *Abbildung 1* zeigt ein typisches Ergebnis dieser Messmethode. Die arbeitsintensive Probenentnahme und -nachbearbeitung erfordert ein an die Kulturpflanze sowie den Standort angepasstes Beprobungsschema, um bei hoher natürlicher Boden- und Wurzelsystemheterogenität eine statistisch absicherbare Unterscheidbarkeit zu erlangen (BENGOUGHT et al. 2000).

Eine weitere Einschränkung dieser destruktiven Methode ist die Störung des Wurzelraumes und Wasserhaushaltes durch die Beprobung, was besonders auf züchterischen Kleinparzellen eine wichtige Einschränkung ist. Damit wird die Untersuchung von Wachstum und Entwicklung des Wurzelsystems weitestgehend verunmöglicht. Für dynamische Betrachtungen im Freiland bieten Minirhizotrone einen Ansatz, bei jedoch eingeschränktem Beobachtungsfeld,

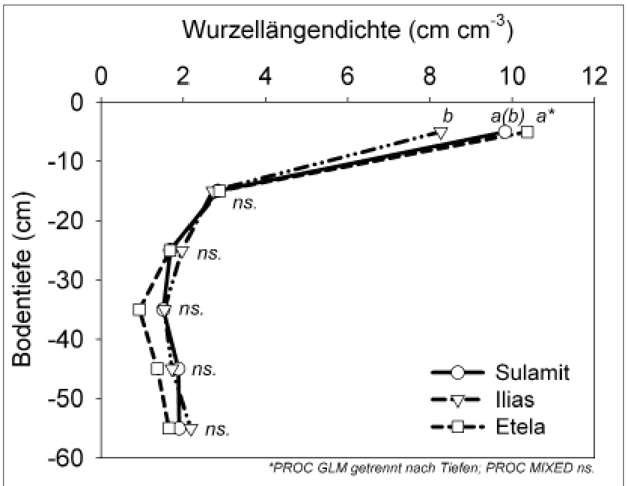

Abbildung 1: **Tiefenverteilung der Wurzellängendichte von drei Winterweizen-Sorten mittels Bodenzylinderentnahme und anschließender Bildanalyse**

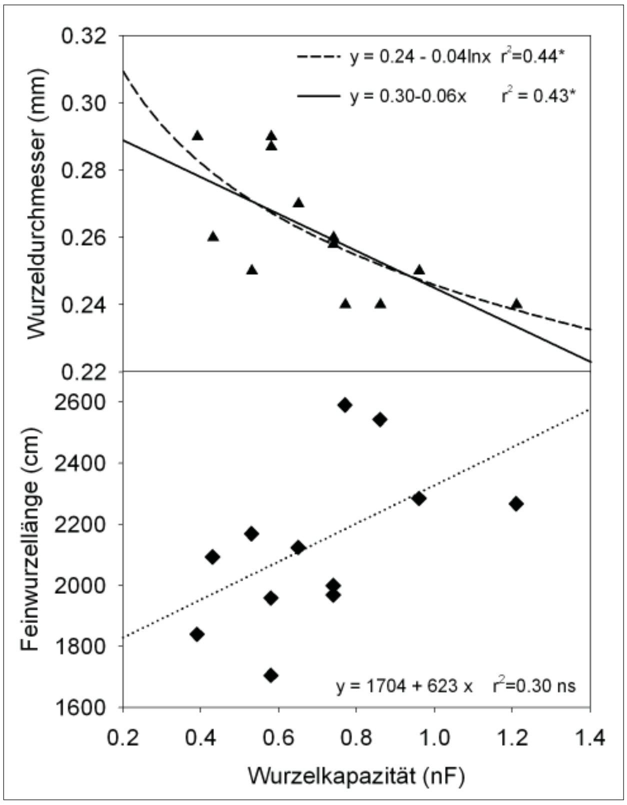

Abbildung 2: **Zusammenhang von Wurzelkapazität, Wurzeldurchmesser und Feinwurzellänge**

erhöhten Kosten für das Messsystem und Anfälligkeit auf Störeinflüsse durch die Zugangsrohre (z.B. JOSLIN und WOLFE 1999).

Zu den nicht-destruktiven Feldmethoden zählt die Messung der Wurzelkapazität (CHLOUPEK 1977), ein Ansatz, der spezifisch aus einem züchterischen Kontext entwickelt wurde. Sie beruht auf einer Messung der Kapazität des elektrischen Feldes zwischen einer Boden- und Pflanzenelektrode, das mit der Größe der, als zylindrischer Kondensator verstandenen, Wurzeloberfläche sowie auch mit dem Wurzeldurchmesser zusammenhängt (DALTON, 1995). Dieser Zusammenhang wird jedoch von zahlreichen anderen Faktoren beeinflusst und teilweise überlagert, die so die Korrelation des Messsignals mit direkt gemessenen Wurzelparametern erschweren (*Abbildung 2*).

Dennoch ist die Wurzelmessung auf Grundlage der elektrischen Eigenschaften im Boden-Wurzel-System ein Ansatz, an dem intensiv gearbeitet wird, um die physikalische Interpretation des Messsignals zu verbessern und mit bildgebenden Verfahren zu koppeln (z.B. AMATO et al. 2009, CAO et al. 2010). Für züchterische Ansätze im Feldversuch scheint die Weiterentwicklung dieses Ansatzes vielversprechend. Für prozessorientierte Wurzelforschung dürfte jedoch, auch bei wesentlicher Verbesserung der Signalauswertung, die erreichbare Auflösung beschränkend bleiben.

In Verbindung mit elektrischen Methoden kommt auch der Bewertung von Wassergehalts – bzw. Wasserpotentialprofilen mithilfe inverser Modellierung eine Bedeutung zu. Da sowohl elektrische Wurzel- als auch kapazitive Wassergehaltsmessung von den dielektrischen Eigenschaften des

Abbildung 3: **Scan einer Rhizobox mit dem Wurzelbild von Sonnenblume**

Systems ausgehen, scheint eine Verbindung dieser Ansätze über eine entsprechende Messsignalinterpretation möglich. Besonders die effektive Funktionalität der Wurzel kann über Wasserentzugsprofile bewertet werden und es könnten Ansätze aus der Geophysik (z.B. Bodenradar) Verwendung finden (ZENONE et al. 2008).

Labormethoden

Es liegen zahlreiche Labormethoden zur Quantifizierung von stressrelevanten Wurzelparametern vor. Häufig werden Keimwurzeleigenschaften bestimmt unter der Annahme, von der Ausprägung des primären Wurzelsystems auf das voll entwickelte Wurzelbild schließen zu können. Keimwurzelausbildung sowie Wachstum und Architektur samenbürtiger Primärwurzeln von Jungpflanzen wurden für züchterische Fragestellungen verwendet, da sie einen hohen Probendurchsatz erlauben und die Merkmale (Zahl der samenbürtigen Wurzeln, primärer Verzweigungswinkel) eine hohe Heritabilität aufweisen (z.B. SANGUINETI et al. 2007). Verschiedene Substrate können verwendet werden, wie etwa Gel, Filterpapier oder andere poröse Materialien. Diese erlauben eine einfache und rasche Quantifizierung wichtiger primärer Wurzelparameter.

Die Untersuchung ausgewachsener Wurzelsysteme in Laborversuchen erfolgt häufig über transparente Boxen (*Abbildung 3*).

Diese erlauben die Messung der Wurzelarchitektur in einem zweidimensionalen System mit natürlichem Boden oder Sand als Substrat. Verschiedene Bauarten wurden publiziert, wobei das durchwurzelbare Bodenvolumen (Natürlichkeit des Systems) gegen die Sichtbarkeit an der Oberfläche (Vollständigkeit der Beobachtung) abgewogen werden muss (KUCHENBUCH und INGRAM 2002). Die Vermessung kann durch Übertragung der Wurzelbilder auf eine transparente Folie, über Scannen oder Fotografieren der Oberfläche mit anschließender Bildanalyse bis hin zur Nutzung unterschiedlicher Lichtspektren gehen (z.B. NAKAJI et al. 2008). Ein Überblick über vorhandene Software zur Quantifizierung der Wurzelparameter an transparenten Oberflächen findet sich bei LE BOT et al. (2010).

Moderne Methoden zur dreidimensionalen Abbildung von Wurzelsystemen (z.B. NMR-Imaging, Computertomographie) sind für die Untersuchung von Prozessen auf der Einzelwurzelskala sowie von Boden-Wurzelinteraktionen bedeutend, für züchterische Zwecke jedoch derzeit noch weniger zielführend, da die Detailinformation auf Kosten des möglichen Probendurchsatzes, der Größe der Versuchsgefäße als auch der Kosten geht.

Eine hauptsächliche Problematik von Labormethoden ist die Frage der Übertragbarkeit in die Feldsituation. Wenngleich manche Autoren einen Zusammenhang zwischen verbesserter Trockentoleranz im Feldversuch und Wurzeleigenschaften im Laborversuch fanden (z.B. INAGAKI et al. 2010), konnten WOJCIECHOWSKI et al. (2009) zeigen, dass das verwendete Substrat nicht nur die Ausprägung der Wurzel beeinflusste, sondern auch zu unterschiedlichen Schlussfolgerungen im Vergleich von Genotypen führen kann.

Schlussfolgerungen

Bisher liegt keine befriedigende Methode zur Phänotypisierung von Wurzeleigenschaften im Kontext pflanzenzüchterischer Versuche vor. Insbesondere für die Feldanwendung ist ausschließlich die Verwendung indirekter Methoden möglich, wenn ein züchterisches Screening großer Populationen erforderlich ist. Damit ist eine Kombination von Ansätzen notwendig, ausgehend von Modellierung zur Bestimmung des Zielmerkmals in einer gegebenen hydrologischen Situation, über Laborscreening bzw. indirekte Feldscreening-Methoden mit hohem Durchsatz bis zur Selektion weniger Kandidaten, deren Wurzelsystem detailliert mit direkten Methoden auf Morphologie und Architektur beschrieben wird (*Abbildung 4*). Somit ist immer noch die Schlussfolgerung von BLUM (1988) gültig, dass Wurzelforschung in der Züchtung auf Stresstoleranz vor allem einen erklärenden Beitrag leistet, der zum Verständnis der Mechanismen beiträgt, die bekanntermaßen stresstoleranten Sorten zugrundeliegen. Dieser Einschränkung steht jedoch die potentielle Bedeutung der Wurzel für eine mit dem Ertragspotential kompatible Verbesserung der Ressourcennutzungseffizienz gegenüber. Eine Orientierung auf die Entwicklung von Messmethoden für die Pflanzenzüchtung ist daher eine zentrale Aufgabe der Wurzelforschung.

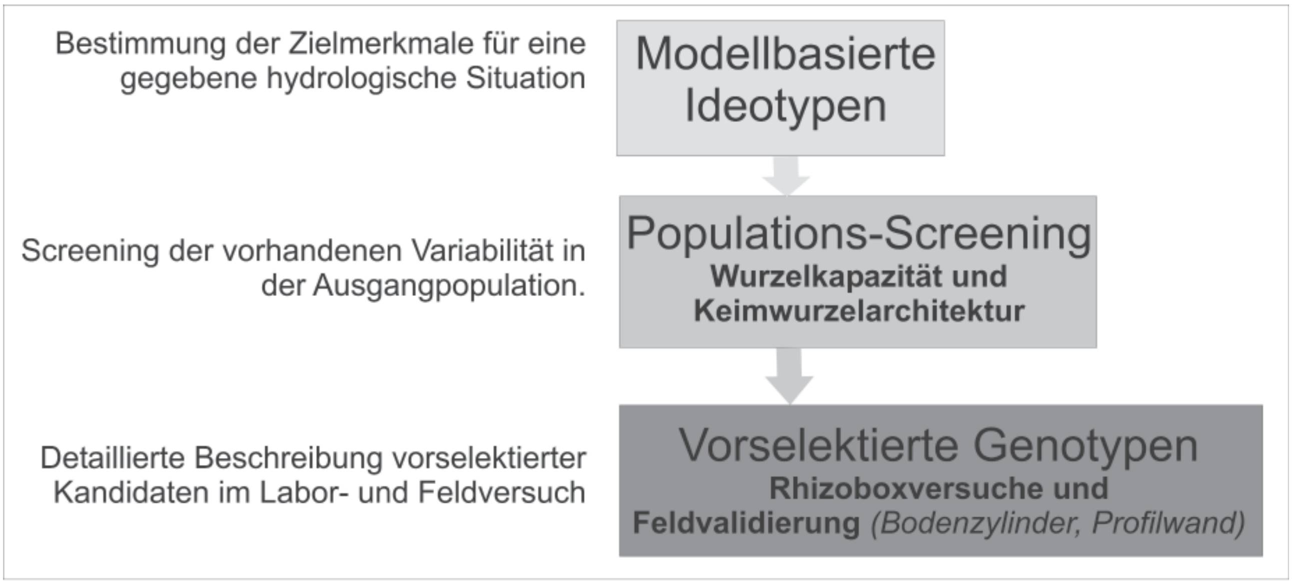

Abbildung 4: **Schema für die Integration von Wurzelmerkmalen in ein Züchtungsprogramm**

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Root characteristics of durum wheat and wheat relatives

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Abstract

Access to a rich genetic diversity, and easy and feasible screening methods are main prerequisites for an efficient breeding program. Under drought condition, roots could play an outstanding role to improve yield by effective absorption of water from soil. Since roots are not easily accessible, root characteristics are hardly exploited in crop breeding so far. In the present study the diversity of root properties of 7 durum wheat genotypes and 5 relatives was determined in 3 soil depths, i.e. 10-20, 30-40 and 50-60 cm, of a field trial. As an easy and non-destructive field screening method in regard to root system size, electrical capacitance was assessed for its efficiency to predict 'true' root characteristics. The results revealed significant differences between genotypes and soil depths. A significant and positive correlation between root capacitance and/or root length and root surface indicates the capability of this method for the screening of genetic material under field conditions.

Keywords

Einkorn wheat, Khorasan wheat, root image analysis, *Triticum durum*, *T. timopheevi*

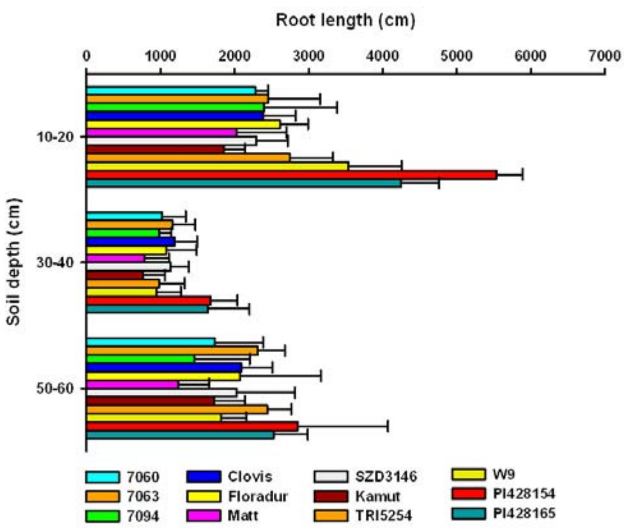

Figure 1: **Root length of different wheat material in three soil depths** (mean values + standard deviations)

Introduction

Improving abiotic stress resistance is a major challenge for plant breeding. Especially drought is among the most important environmental constraints to plant growth. LEVITT (1980) identified three main responses to water stress in natural plant communities: (i) drought escape, (ii) dehydration tolerance and (iii) dehydration avoidance. Dehydration avoidance may be achieved by reduced losses ('water savers') and improved supply ('water spenders'). Although the plant root system is essential to ensure an efficient water uptake (BLUM 2009), it is still hardly exploited in plant breeding (PALTA et al. 2011). A targeted integration of the root system into plant breeding requires knowledge on the existing diversity in root traits. The objective of the current study was to assess root system properties in different durum wheat genotypes and selected accessions of relatives. The main distinguishing features of root system diversity within this nursery are presented.

Material and methods

Plant material

Seven durum wheat (*Triticum durum*) genotypes, i.e. 7060 (CDSS02Y01022T-0TOPB-0Y-0M-21Y-0Y), 7063 (CDSS02Y01082T-0TOPB-0Y-0M-11Y-0Y), 7094 (CDSS02B00667S-0Y-0M-10Y-4M-04Y-0B), Clovis, Floradur, Matt and SZD3146, two Khorasan wheat (*T. turanicum*), i.e. QK-77 (Kamut®) and TRI5254, two einkorn wheat (*T. monococcum*), i.e. PI428154 and PI428165, and one Zanduri wheat (*T. timopheevi*), i.e. W9, were tested in a field experiment with 4 replications. The field trial was sown on 8 March 2011 in Raasdorf (16°35'E, 48°14'N) in the Pannonian plains growing region of Eastern Austria. Plot size was 1.75 m² (1.4×1.25 m).

Trait measurements

Electrical capacitance was measured by an Escort elc-133 lcr-meter (CHLOUPEK 1977) at the physiological stages stem elongation, inflorescence emergence and development of fruit. Thereby, an estimation of root system size was obtained. On 16 June (milk dough stage) the roots were sampled from 3 soil depths (10-20, 30-40 and 50-60 cm) by means of a single root auger from the center of each plot. Samples were stored at -20°C until final processing. After

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storage the samples were defrosted at room temperature. Then the roots were washed and sieved (pore size 0.63 mm). To prepare the roots for scanning they were coloured by an azur eosin methylene-blue solution (1:20 v/v). After staining the roots were scanned by a flat bed scanner and analyzed by winRhizo software (Regent Instruments Inc., Québec City). Measured root parameters included root length, root diameter, root surface and root volume. To measure root dry matter the roots were dried at 60°C and weighted after 24 h.

Results and discussion

This study revealed significant differences between genotypes and different soil depths for all investigated root characteristics. Root length varied from 4049.6 cm (durum cv. Matt) to 10065.3 cm (einkorn PI428154) with an average of 6004.3 cm. All genotypes showed their maximum and minimum root length in the upper and intermediate soil layer, respectively (*Figure 1*). The einkorn wheat accessions showed a significantly higher root length in all three soil depths compared to the other genotypes.

Concerning root surface area an average value of 774.0 cm² was estimated; with a minimum of 502.6 cm² for durum cv. Matt and a maximum of 1123.0 cm² for einkorn accession PI4281154. Root diameter varied between 0.368 mm (PI4281154) and 0.455 mm (Clovis). *Figure 2* shows a part of the observed root diameter diversity. In regard to root volume, minimum and maximum values were obtained for Matt (5.15 cm³) and PI4281154 (10.12 cm³), respectively. Root dry matter varied significantly between 440.4 mg (Matt) and 912.6 mg (Floradur) with an average of 623.3 mg.

As it is shown in *Figure 3* a significant correlation between electrical capacitance and root length was obtained ($P \leq 0.0001$). Moreover, similar correlations were observed between electrical capacitance and root surface and root diameter, respectively ($P \leq 0.0001$). Since electrical capacitance is an easy and non-destructive measurement it could be suggested as a feasible method for screening of genetic material for root system size under field conditions. In cereals the methodology was successfully deployed to study root system size of oats (CHLOUPEK 1972, 1977), the effect of semi-dwarf genes on root system size of barley (CHLOUPEK et al. 2006), to select barley for drought tolerance (CHLOUPEK et al. 2010), and recently to study the diversity in water use efficiency of wheat varieties (STREDA et al. 2012). Drawbacks of the method is the lack of knowledge concerning the electrical circuit of the system. Values are only comparable for plants of the same species grown in the same substrate, at the same soil moisture (a moist medium around the plant root system is necessary) and measured at the same time. The measured aerial parts must be dry (no precipitation immediately before or during measurements), dry leaves at the basal stems have to be removed, the electrode must be consitantly placed (CHLOUPEK et al. 2006), and development stages have to be considered in the analysis.

To summarize, this study confirmed a considerable variation concerning the investigated root properties and also the capability of electrical root capacitance measurements as

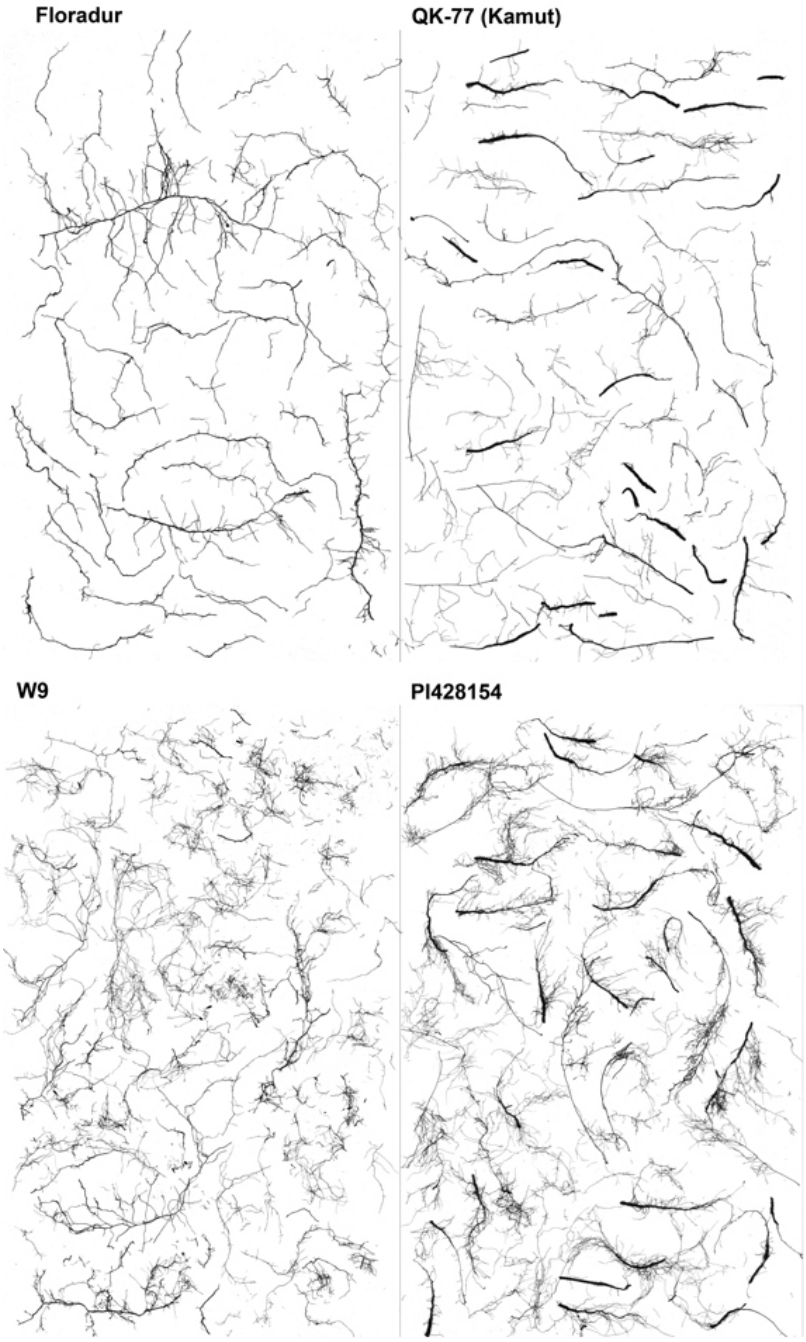

Figure 2: **Scanned roots from 10-20 cm soil depth for Floradur (*T. durum*), Kamut (*T. turanicum*), W9 (*T. timopheevii*) and PI428154 (*T. monococcum*)**

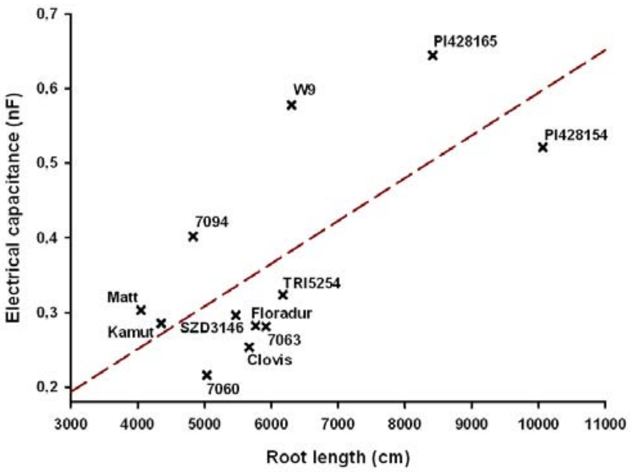

Figure 3: **Relationship between root length and electrical capacitance (measured at milk dough stage on 17 June) for different wheat material**

an easy and feasible method for the screening of genetic material under field conditions. Further experiments with non-destructive methods for studying root architecture are necessary to better understand the differences between the studied genotypes.

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