Lehr- und Forschungszentrum für Landwirtschaft
Raumberg-Gumpenstein

61. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2010, 147 – 154
ISBN: 978-3-902559-53-1

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 m^2, 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

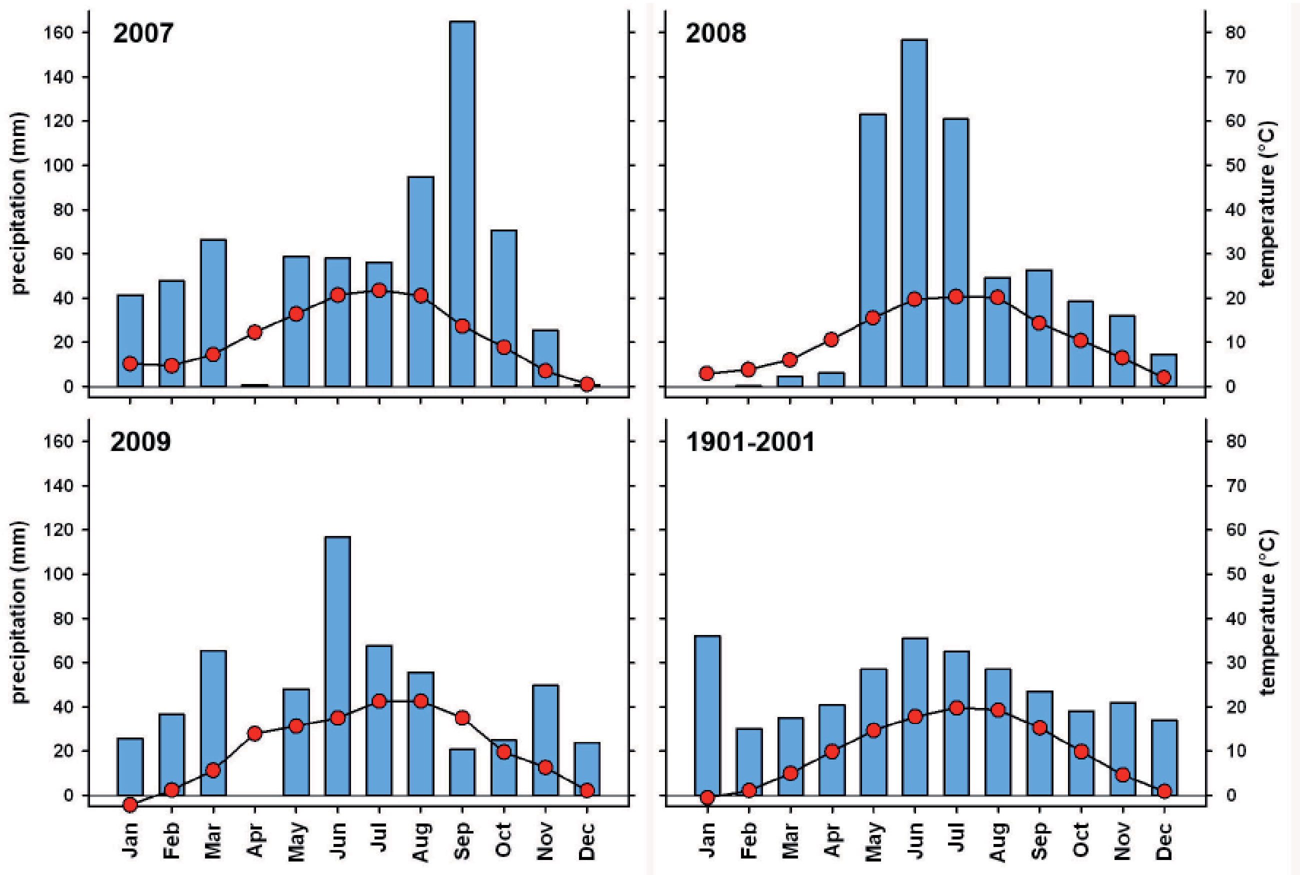

Figure 1: **Climate diagram for the experimental site Raasdorf for the years 2007 to 2009, and the longterm trend illustrating the recurrent period of not sufficient precipitation (bars) in early spring in recent years**

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

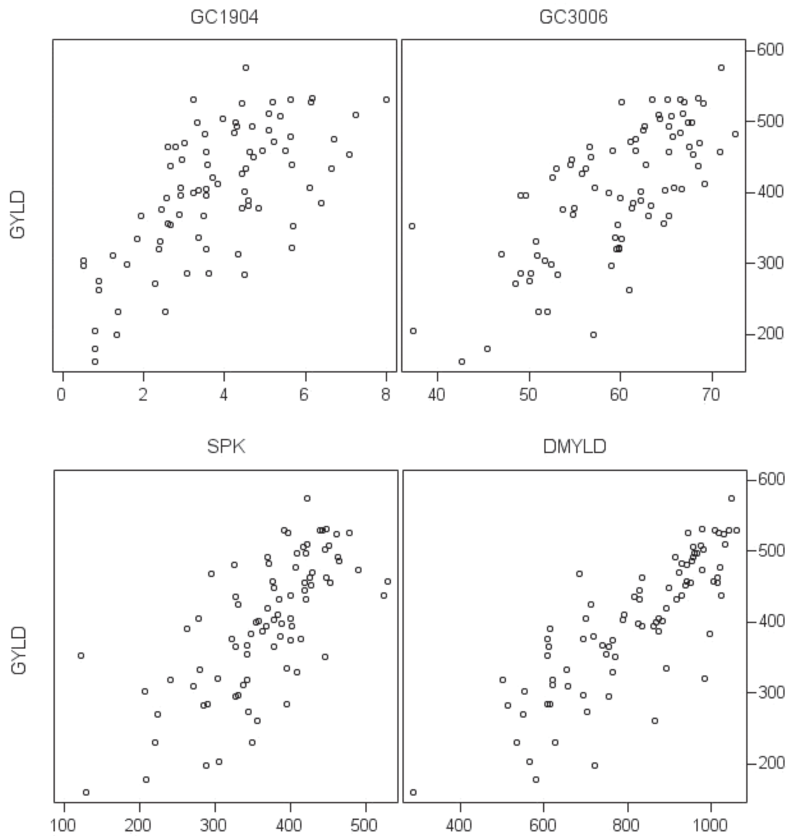

Figure 2: **Relationship between grain yield (GYLD) and crop ground cover at early (GC1904) and late (GC3006) growth stage, number of fertile tillers per unit area (SPK) and dry matter yield (DMYLD)**

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

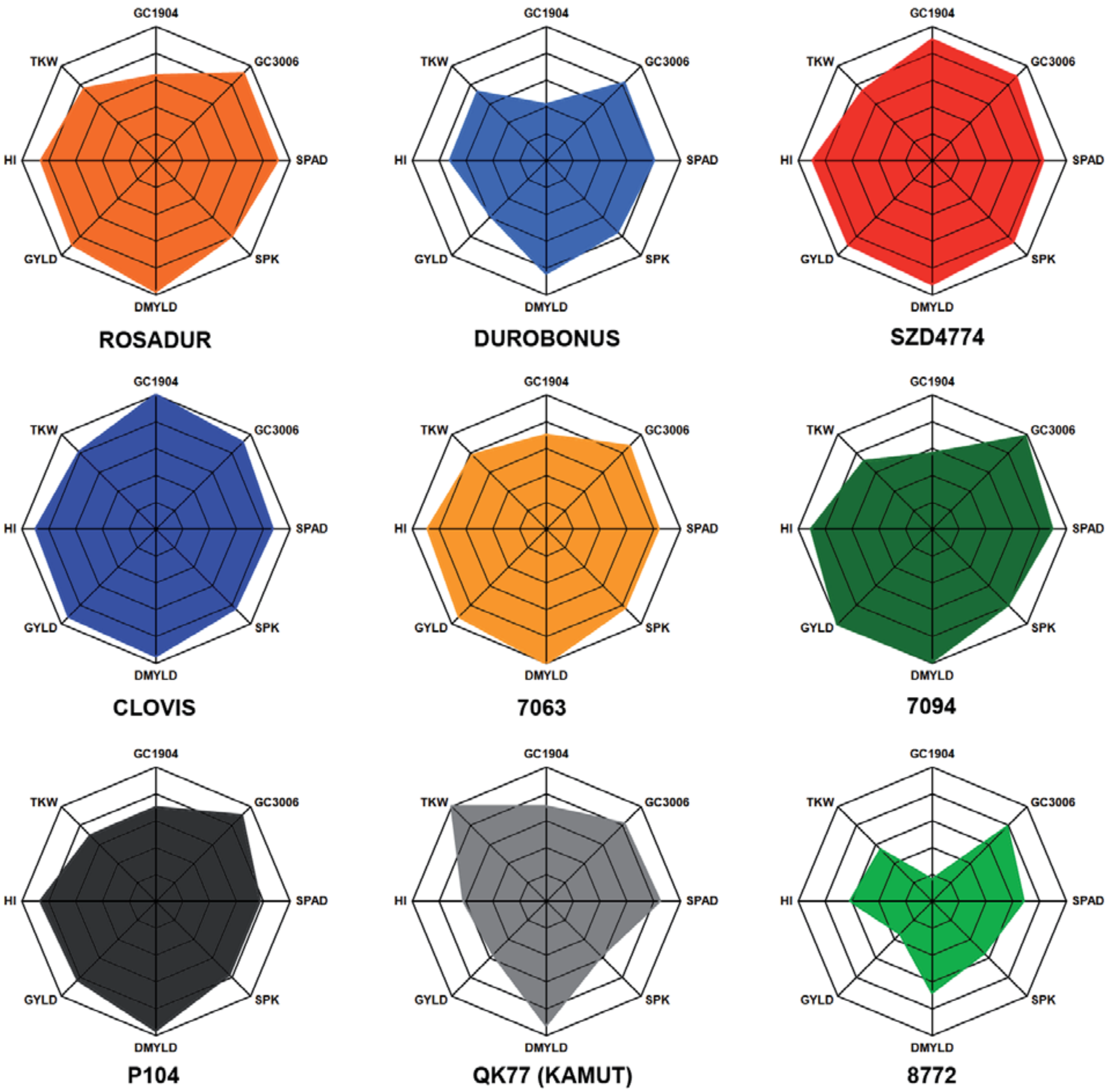

Figure 3: **Star plot area of nine selected genotypes: Rosadur, Durobonus, SZD4774 (AT), Clovis (FR), 7063, 7094 (CIMMYT), P104 (PGR), QK77 (*T. turanicum*), 8772 (*T. durum* x *T. dicoccum*). Abbreviations of traits see *Table 1***

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 40[th] 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

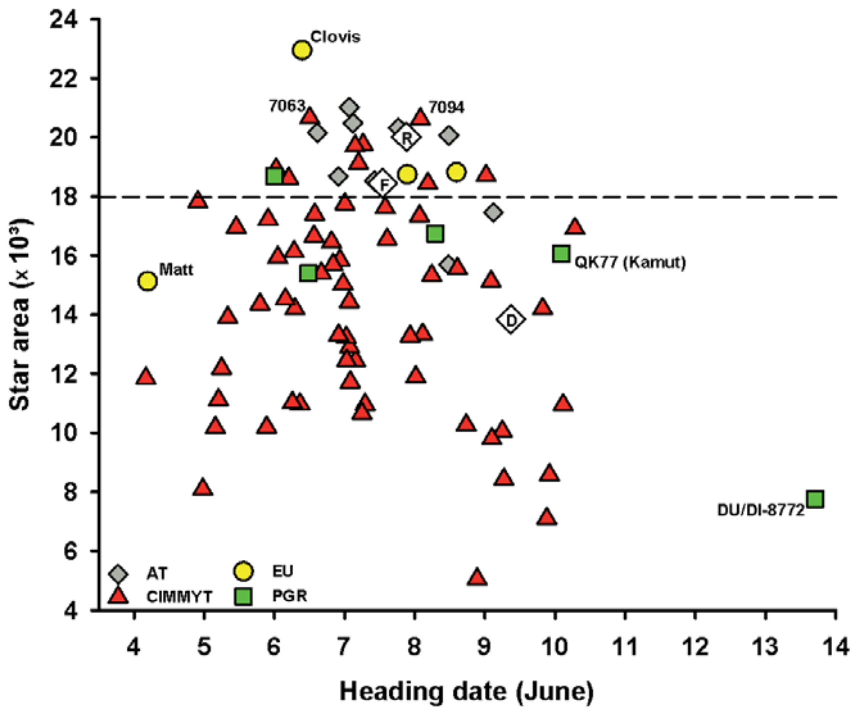

Figure 4: **Relationship between heading date and star plot area. Genotypes of different genpools are indicated by different symbols; check varieties Durobonus, Floradur and Rosadur are indicated by white diamonds including the initial letter of the variety name**

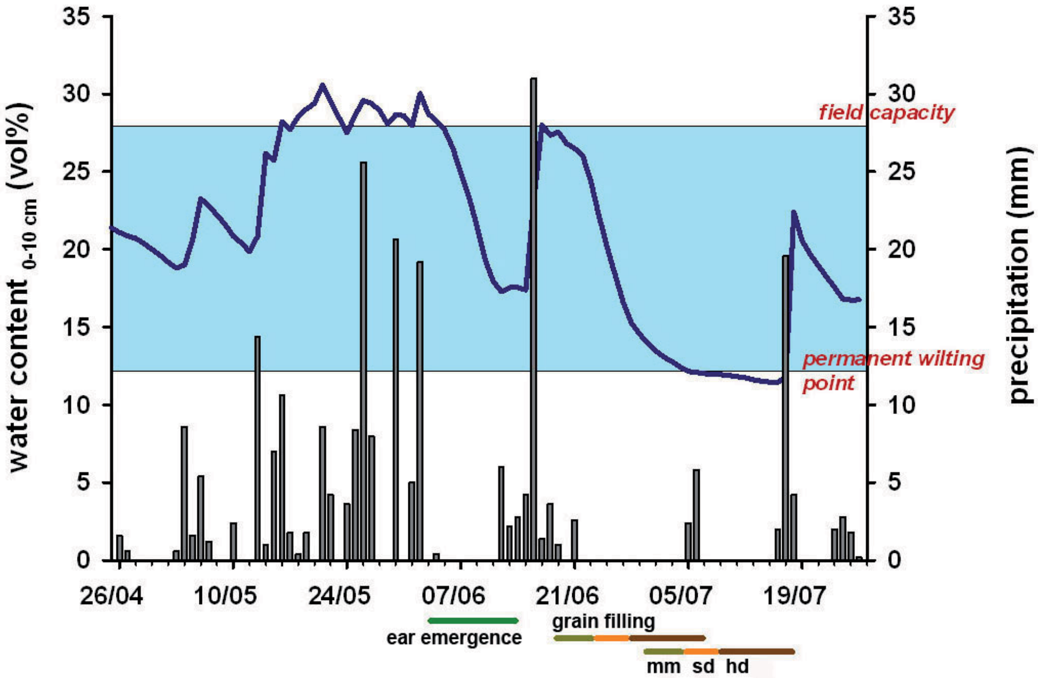

Figure 5: **Water dynamics (precipitation and water content in the upper 10 cm soil layer) of the durum trial in Raasdorf 2010. Time span of ear emergence within the nursery is indicated as well as the duration of the grain filling (mm, medium milk; sd, soft dough; hd, hard dough) for early and late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

Acknowledgements

Seeds were kindly provided by T. Payne, CIMMYT, Mexico and J. Lafferty, Saatzucht Donau, Probstdorf.

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