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Drought stress and the response of wheat: nursery and complex stress diagnostic experiments

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Abstract

Breeding for drought tolerance is becoming a more and more important challenge in case of crop plants, notably in wheat in Europe, not only in the Mediterranean area, but in central Europe too. The breeding process includes the characterization of the basic breeding material in regard to performance under well-watered and drought stressed conditions. For our experiments we set up a mobile automatic rain shelter (MARS) system in the breeding nursery and a complex stress diagnostic system (CSDS) in greenhouse tests of the Cereal Research Non-Profit Limited Company, where we could analyze the responses of different wheat materials to drought stress. Wheat plants were grown under ideal water regime in parallel experiments using sprinkler irrigation and shadowing by MARS. In greenhouse the tested wheat materials were grown under optimal (watering to 60% of the 100% soil water capacity) and suboptimal stress (watering to 20%) conditions. The effect of water withholding on plant growing was registered by a digital imaging system in CSDS and traditional way under MARS. After harvesting, plant heights, spike lengths, grain numbers, total grain weights and other agronomical parameters were measured and values of well-watered and stressed plants were compared.

Keywords

Digital image analysis, rain shelter, *Triticum aestivum*, water stress, yield loss

Introduction

Limited water condition, i.e. drought, is one of the most important abiotic stress factors. Depending on the season drought can limit crop production seriously. Plant responses to drought stress are complex mechanisms which include molecular changes and extend to the whole plant metabolism influencing the morphology and phenology of plants (BLUM 1996, CHAVES et al. 2003, CONDON et al. 2004, MOLNÁR et al. 2004). Breeding for drought tolerance is an important challenge in case of crop plants, especially in wheat (*Triticum aestivum* L.). The breeding process includes the characterization of the basic breeding material in regard to performance under well-watered and drought stressed conditions. In recent years many approaches to select wheat genotypes which are resistant to drought were described, e.g. improved water use efficiency (BLUM 2005, CHAERLE et al. 2005, HU et al. 2006), drought resistance indices (MARDEH et al. 2006) or simulation of drought conditions in the greenhouse (GÁSPÁR et al. 2005, HOFFMANN and BURUCS 2005). It is clear that an extensive approach is needed to test a complex trait like drought tolerance. Therefore, in our experiments mobile automatic rain shelter (MARS) and complex stress diagnostic system (CSDS) were set up in the breeding nursery and in the greenhouse, where we could analyze the responses of different wheat germplasm to drought. In this way tolerant genotypes could be tested and selected.

Materials and Methods

Field drought tolerance tests

For testing drought tolerance in the field, the MARS was constructed and installed in Szeged. The MARS covers an area of 720 m². Rain sensors manage the closing mechanism which completely covers the field plots by a convertible plastic tunnel. Drain ditches prevent the side-wetting from the neighboring soil profiles. Drought can be traced by two automatic meteorological stations which continuously measure the rainfall, sun radiation, dew point, soil moisture, soil temperature, air temperature, wind direction and speed. About 100 winter wheat genotypes have been tested on two-row plots in three replications in 2007/2008. Most of the tested genotypes were advanced breeding lines of the Cereal Research Co., and check varieties. All genotypes were planted in two-row plots in 3 replications by a Seedmatic sowing machine (Wintersteiger, Ried, Austria) in October 2007. Plot size was 0.5 m². Effects of drought were evaluated by measuring plant height, acceleration in heading time, depression of the yield components, grain yield and the difference between the canopy temperatures of stressed vs. control plots of the different genotypes. The later method has been considered to be effective in screening wheat genotypes for drought tolerance (WINTER et al. 1988, BLUM 1998, INAGAKI and NACHIT 2008, MATUZ et al. 2008) since tolerant genotypes can maintain photosynthesis (and evaporation) longer (WINTER et al. 1988) and their canopy

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temperature is lower. Canopy temperature was determined by a Crop Trak infrared thermometer (Spectrum Technologies, Plainfield, IL). Out of the MARS a sprinkler irrigated parallel experiment was sown with a similar randomization. The data from the two different experimental conditions were compared in our computing evaluation.

Greenhouse drought tolerance tests

For greenhouse drought experiments the CSDS was established in the greenhouse of the Cereal Research Co., Szeged, where we could analyze the responses of different wheat material to indoor drought stress conditions. Five pots per genotype were exposed to drought stress conditions and five others were treated as controls. Water capacity of soil was determined and pots were watered twice a week to 20% (stressed) and 60% (well-watered) of the soil water capacity, respectively. Watering was done automatically by a plant watering system including a balance in connection with a computer-mediated peristaltic pump. As pots had a radiofrequency identifier, watering data could be stored automatically by computer. Days to heading were registered individually for each plant. The effect of water withholding on plant growing was tracked weekly by a digital imaging system on the basis of number of plant pixels (Olympus Camedia C-7070 digital camera). Plants were harvested after ripening. Plant heights (measured from ground to the last node) and spike lengths (without awns) were measured. Shoots were dried at 40°C for 4 days to permanent water content. Shoot dry weight, number of grains and total grain yield weight were measured. In this experiment (after previous practical data), the tolerant genotypes were as ‘Tolerant control’ (Tol. 1) and GK 11-05 and two sensitive genotypes were: ‘Sensitive control’ (Sens. cont.) and ‘Chinese sensitive’ (Ch. sens). Wheat seedlings were vernalised at 3°C for 6 weeks. Plants were transferred into pots containing a mixture of 50% Terra peat soil and 50% Maros sandy soil, two in each pot. Equal quantities of chemical fertilizer (Substral Osmocote Plus) were put in each pot at the time of planting. After a week, plants were thinned and one was left in each pot.

Breeding for drought tolerance

Our drought tolerant breeding system is a modified pedigree method, based on manual crossing, head selection from F_2 until uniform head-rows are available. Generally from F_4 information yield trials, later four-replicated yield trials, and at last multi-location performance tests help to select the best advanced lines. From F_5 quality tests and parallel scoring in rust (*Puccinia recondita*, *P. graminis*) and virus nurseries (under provocative conditions) give additional information for successful selection. In the younger, segregating generations (F_3-F_5) visual scoring of morphological and phenological characters is the only effective method to evaluate drought tolerance of a large number of genotypes (10 000-20 000 accessions per year). The most important traits that may be checked visually are: leaf firing, leaf rolling, leaf colour under serious stress, hairiness or glaucosity of leaves, kernel size and healthiness. Fast seedling emergence, rapid phenological development in spring, earliness in heading, anthesis and maturity are also advantageous parameters in regard to drought tolerance (CSEUZ et al. 2009).

Results

MARS experiments

The effect of water withdrawal (under MARS) caused significant differences on plant height and thousand kernel mass and significantly decreased grain yield and changed heading time and canopy temperature. Among the tested genotypes plant height was 89.4 cm in the treated (shaded) and 94.5 cm in the control treatment. Water shortage decreased plant height by 5.1 cm which means a 5.4% depression. Tolerant genotypes’ depression in growth was more moderate than susceptible ones’ (*Table 1*). Water stress also affected heading time. Mean of heading time was 137.7 days after 1st January (17th May) in the control treatment while it happened on 12th May in the stress treatment. Here heading accelerated and on average of the tested genotypes heading time started earlier by 5.8 days, which means a 4.2% shorter time from the beginning of the year. Grain yield of the 85 tested genotypes decreased on average by 36.8% while thousand kernel mass (TKM) decreased by 7.8%. The two-row plots’ average grain yield was 394 g in the irrigated, and 249 g in the stress treatment, which means a 36.8% depression. Only about 22% of grain yield loss could be explained by TKM depression. The rest of the yield depression could be happen due to the lesser number of fertile and productive spikes. The higher number of secondary spikes also decreased the difference of TKM between the two treatments. Midday canopy temperature measurements were executed on two hot days, the 28th and 30th May. Air temperature was 34°C and 30°C during the measurements. Canopy temperature was 22.7°C and 19.1°C on average of the control plots and 27.9°C and 24.2°C in the treated (stressed) treatments. Generally, the results of drought stress trials has no correlation to yield data. The best correlation with MARS data were found with grain yield (r = 0.628-0.836***).

CSDS experiments

In case of days from planting to heading the sensitive ‘J4-11’ and ‘Sens cont.’ genotypes responded with earlier heading to

Table 1: **Effects of water deficiency on the tested genotypes (average of 85 wheat genotypes)**

Treatments	Plant height (cm)	Heading time (d)	TKM (g)	Grain yield (g)	Canopy temp. (28/05) (°C)	Canopy temp. (30/05) (°C)
Control	94.5	137.7	44.7	394	22.7	19.1
Stress	89.4	131.9	41.2	249	27.9	24.2
Difference	5.1	5.8	3.5	145	-5.2	-5.1
LSD5%	3.3	3.4	3.9	23.5	4.8	5.1
Control %	94.6	95.8	92.2	63.2	122.9	126.7

stress. Earlier heading is a general response of sensitive wheat plants to drought. In case of 'GK 11-05' and 'Tol. cont.' no significant differences in time of heading in the two different water treatments were observed. Growing curves of the two varieties ('GK 11-05' and 'Tol. cont.') were constructed from the data of complex stress diagnostic system and the curves were studied. GK 11-05 has not reduced growing in response to stress until the end of the growing period, while sensitive J4-11 stopped growing after heading and used its so far collected reserves to produce grain. Since wheat varieties of different origin differ in morphological and agronomical parameters it is better to compare the parameters in relative values (percentages) instead of absolute values. Agrobotanical (plant height, spike length, dry weight) and yield parameters (grain number, grain weight) of the well-watered plants were set to 100%. Concerning yield parameters of the tolerant varieties there was less depression in response to stress. The most significant differences between treatments could be observed in the number of seeds. However, in case of plant height and spike length all varieties suffered only a slight depression. There were differences in shoot dry weight, too: the 'Tolerant cont.' and GK 11-05 varieties suffered 45% and 55% depression, respectively, while the 'Sensitive cont.' and J4-11 varieties suffered 60% and 67% depression, respectively. Therefore, the polygons representing the tolerant and the sensitive genotypes differ in their areas. In CSDS the plants grown under drought conditions were significantly different in their morphological aspects and in their yield parameters from the well-watered plants. Drought had serious effects on plant growing (green and dry weights): stems were thinner and spikes were smaller than those of their ideally watered parallels. Yield depression was remarkable in case of all varieties, but depressions were more significant in sensitive genotypes.

Discussion

In our nursery and greenhouse stress diagnostic system we could analyze the responses of different wheat genotypes by modeling drought stress. Water withholding had serious effects in case of all wheat genotypes on morphological and yield parameters. Sensitive genotypes responded with earlier heading and, therefore, shortened life cycle to stress (HOFFMANN and BURUCS 2005). Varieties referred as tolerant had no significant differences in time of heading. Hence, registering time of heading proved to be a useful tool to characterize genotypes. Tracking the growing rate of plants can serve as a useful tool in testing varieties for drought tolerance. Digital imaging is a modern and non-invasive method in evaluating green weight of plants on the basis of pixel number without cutting and measuring them (KACIRA and LING 2001). Furthermore, with this method the growing of plants can be followed week by week and a growing curve can be drawn for each plant and (a cumulated growing curve) for each genotype. Hence, the size of control and stressed plants can be compared at any period of growing. There were no significant differences between the genotypes tested in the depression in plant height and stem length, but shoot dry weight was more reduced in sensitive genotypes than in tolerant ones. Therefore, it can be assumed that shoot dry weight measured after harvesting is also a relevant parameter in characterizing wheat genotypes for drought tolerance.

Yield parameters are the most important agronomical traits in selecting drought tolerant genotypes. Depression in grain number and total grain yield was significantly smaller in tolerant genotypes. We would like to note that in the selection of a drought tolerant genotype with high yield, one has to consider not only yield stability but high yield at good producing conditions, too (ARAUS et al. 2002). In respect to this, our results can be completed with this factor by using different stress indices (MARDEH et al. 2006). However, so far there are no reports on the use of stress indices in greenhouse experiments. Greenhouse experiments mean somehow artificial conditions to field crops. Therefore, results gained in greenhouse experiments are further evaluated by comparing them to results of field nursery tests. Besides characterizing wheat genotypes in regard to drought tolerance our stress diagnostic system can also be useful in testing other plant species, e.g. rice or barley, for different kinds of abiotic stresses, e.g. heat or frost, or for biotic stresses too. Also mapping populations can be effectively screened by our diagnostic system. The system is currently under development. We are going to broaden the range of measured parameters by installing infrared thermal imaging and fluorescent imaging systems. These modern non-invasive methods could complete our diagnostic system by giving a better physiological characterization of plants (CHAERLE and VAN DER STRAETEN 2001). The mentioned greenhouse selection method was integrated in our conventional winter wheat breeding programme. All these tests can only be additional methods in the breeding procedure besides visual scoring of morphological and phenological traits. Important information can be obtained also from multi-location yield trials, especially at drier locations and/or in drier years. Significant improvement in drought tolerance of future wheat varieties can only be achieved by the combination of all these data. The advance in drought tolerance can be found among our latest registered wheat varieties and numerous new advanced winter wheat breeding lines (candidates for registration) with a higher level of adaptability to dry environments.

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