CORNET EFFICIENT WHEAT - Improving nitrogen efficiency in European winter wheat under drought stress

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Abstract

EFFICIENT WHEAT is a collective research project of the Austrian Agency for Health and Food Safety (AGES), the Technical University of Munich, Chair of Plant Breeding (TUM), and the Bavarian State Research Center for Agriculture (LfL). The major aims of the project are the identification of cultivars with high yield and quality potential carrying valuable alleles for enhanced NUE which become effective under drought stress, establishing of screening techniques for nitrogen associated traits, and the development of molecular markers. A core set of 30 winter wheat genotypes bred in Austria, France, Germany, Hungary and the UK represents the elite breeding material of different European climates. These genotypes are tested over two seasons (2011-2013) in field trials at (climatic) different locations in Germany, Austria and Hungary. In addition to the field trials, greenhouse experiments are conducted to grow the plants under controlled conditions. First results of the project are presented.

Keywords

Drought tolerance, nitrogen use efficiency, *Triticum aestivum*, yield stability

Introduction

The development of cultivars with high nitrogen use efficiency (NUE), high yield stability and high baking quality even under unfavorable weather conditions is one challenge regarding the increasing risk of weather extremes like heat and persistent drought in wide regions of central and eastern Europe. EFFICIENT WHEAT is a collective research project of the Austrian Agency for Health and Food Safety (AGES), the Technical University of Munich, Chair of Plant Breeding (TUM), and the Bavarian State Research Center for Agriculture (LfL) which aims to accelerate wheat breeding by improving the existing breeding material in regard to drought tolerance and NUE without influencing yield and quality. In detail, we focus on the identification of cultivars with high yield and quality potential carrying valuable alleles for enhanced NUE which become effective under drought stress, establishing of screening techniques for nitrogen associated traits, and the development of markers for marker-assisted selection (MAS). Three different work packages are conducted: (1) phenotypic characterization of 30 European elite winter wheat varieties as well as of a DH population derived from a cross of two contrasting varieties; (2) indirect/and direct analysis of baking quality, and (3) genotyping and marker-trait associations. The core set of 30 winter wheat genotypes bred in Austria, France, Germany, Hungary and the UK represents the elite breeding material of different European climates. Over two seasons (2011/2012 and 2012/2013) these genotypes are cultivated in field trials at (climatic) different locations in Germany, Austria and Hungary. In addition to the field trials, greenhouse experiments are conducted to grow the plants under controlled conditions.

Material and methods

During the vegetation period the following phenological data were recorded: heading date, flowering time and date of grain maturity. The percentage of yellow flag leaf area was rated every 2 to 3 weeks by visual scoring in order to estimate the progress in flag leaf senescence (FLS). This assessment was carried out by the same person for each experiment. Thousand kernel weight (TKW) was recorded as well as grain yield. Using the SPAD 502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) readings were repeatedly taken at the lower end of the upper third of flag leaves throughout the experiments. For each entry, the average value from 10 flag leaf measures was recorded. The relative water content (RWC) was determined by sampling 6 flag leaves per entry, weight the fresh weight (W), place them in water for at least 4 hours and weight them again after rehydration and removal of surface moisture (TW). Subsequently the samples were oven dried at 80°C for 24 h and the dry weight (DW) was estimated. Flag leaf relative water content was calculated as:

$$RWC\ (\%) = (W\text{-}DW)/(TW\text{-}DW) \times 100$$

Analysis of variance was performed using Plabstat software (University Hohenheim, Stuttgart, Germany).

Results

Analysis of variance showed significant effects of genotypes and treatments for all investigated traits at<0.001 (*Table 1*) with the exception of plant biomass at harvest. Genotype by environment (treatment) interaction effects were significant for all phenotypic data analyzed so far. Heritability for phenological data calculated across all environments

Table 1: **Variability, variance components and heritability estimates for selected phenotypic data collected in season 2011/2012 (***, *P*≤0.001; **, *P*≤0.01; *, *P*≤0.05)**

	HEAD[1]	GMAT	YLD	TKW	FLS	SPAD	RWC	BIOM
Minimum	134.4	188.8	47.2	38.0	0.0	8.95	58.2	0.78
Maximum	159.0	207.3	117.1	65.7	66.7	61.30	89.2	2.76
Variance components								
Genotype (G)	20.08***	9.83***	12.89***	29.45***	16.18***	8.63***	4.09***	1.55*
Environment (E)	205.28***	23.85***	11.73***	86.19***	4.15**	44.40***	18.52***	3.20*
G×E	4.68***	6.62***	3.95***	3.48***	1.62***	6.33***	1.46**	1.31*
LSD5%	1.63	2.24	6.50	1.89	6.80	5.36	2.81	0.28
Heritability	0.97	0.90	0.92	0.97	0.94	0.88	0.76	0.36

[1] HEAD, heading date; GMAT, date of grain maturity; YLD, grain yield; TKW, 1000 kernel weight; FLS, flag leaf senescence; SPAD, relative chlorophyll content estimated on flag leaves; RWC, relative water content; BIOM, biomass yield at harvest

was between 0.90 and 0.97. Heritability estimates for yield, TKW, FLS and chlorophyll content across all experiments were 0.92, 0.97, 0.94 and 0.88, respectively.

As basis for the aimed genetic association mapping, a combination of morpho-physiological trait evaluation and nitrogen analyses of wheat straw and grain are performed together with laboratory investigations of N-related genes. A limited number of candidate genes associated with N efficiency have already been identified using expression analysis in the model species maize (GALLAIS and HIREL 2004) and rice (TABUCHI et al. 2005). Also in wheat, partial sequences of genes controlling nitrogen metabolism have been identified (BOISSON et al. 2005). Using expressed sequence tag (EST)-based wheat microarrays (LU et al. 2005) showed that gene expression is significantly influenced by the amount and form of N-fertilizer. Many of the genes showing differential expression are known to participate in N metabolism and storage protein synthesis. Putative candidate genes were identified encoding enzymes involved in N assimilation and recycling (LEA and AZEVEDO 2007). These genes were present in chromosomal regions for which co-localizations between agronomic and physiological traits related to N metabolism were identified (HIREL et al. 2001, OBARA et al. 2001). Further it has been highlighted that glutamine synthetase (GS) activity is correlated to the amount of N remobilisation in kernels and grain protein content using quantitative genetic approaches (BERNARD et al. 2008, HABASH et al. 2007, KICHEY et al. 2007). Several co-localizations between QTL related to yield, physiological traits related to NUE and enzyme activities involved in the control of N assimilation and recycling were detected for nitrate reductase (NR) and glutamate dehydrogenase (GDH) in maize (HIREL et al. 2001), and proteases in barley (YANG et al. 2004). Based on these findings, candidate genes implicated

in NUE and identified from the literature will be screened for allelic variation within the 30 winter wheat genotypes (*Table 2*). After identification of single nucleotide polymorphism (SNP) within these gene sequences, comparative mapping will be performed using available populations.

Whole-gene Sanger-sequencing of glutamine synthetase using chromosome 2A specific primer pairs revealed two alleles of GS within our variety set. The two haplotypes correspond to GQ169687.1 and GQ169685.1 (NCBI) which were identified for 'Chinese Spring' and 'Xiaoyan54' (here one SNP was detected), respectively (*Table 3*). In total, 15 SNPs and one Indel of 239 bp length were detected between the START- and STOP codons. Hybrid wheat 'Hybred' wears both glutamine synthetase alleles. Unfortunately, varieties 'Format' and 'Pamier', from which our DH population derives, have the same GS allele, at least on chromosome 2A.

Table 3: **Alleles of GS on chromosome 2A identified in the tested varieties**

GS_2Aa (GQ169687)	GS_2Aa (GQ169685)	
Bitop	Capo	GK Szala
Brilliant	Element	Hybred
GK Hunyad	Energo	Hyland
GK Körös	Eurofit	JB Asano
Hybred	Eurojet	Komarom
Premio	Exklusiv	Midas
	Format	Pamier
	GK Bekes	Pegassos
	GK Bereny	Robigus
	GK Feny	SZD9296
	GK Kalasz	Tacitus
	GK Petur	Tiger
	GK Raba	

Table 2: **Screening for allelic variation in candidate genes**

Candidate gene	Species	References
NR (nitrate reductase)	Maize	HIREL et al. (2001)
NiR (nitrite reductase)	Maize	GALLAIS and HIREL (2004)
GS (glutamine synthetase)	Wheat	HABASH et al. (2007)
GDH (glutamate dehydrogenase)	Maize	GALLAIS and HIREL (2004)
ASN (asparagine synthetase)	Maize, Arabidopsis	CHEVALIER (1996), LAM (2003)
NTR (nitrate transporter)	Wheat	ZHAO (2004), YIN (2007)
TIP (tonoplast intrinsic protein)	Wheat	BERTL and KALDENHOFF (2007)

Acknowledgments

References

BERNARD S, MØLLER A, DIONISIO G, KICHEY T, JAHN T, DU-BOIS F, BAUDO M, LOPES M, TERCÉ-LAFORGUE T, FOYER C, PARRY M, FORDE B, ARAUS J, HIREL B, SCHJOERRING J, HABASH D, 2008: Gene expression, cellular localisation and function of glutamine synthetase isozymes in wheat (*Triticum aestivum* L.). Plant Mol Biol 67, 89-105.

BERTL A, KALDENHOFF R, 2007: Function of a separate NH3-pore in aquaporin TIP2;2 from wheat. FEBS Lett 581, 5413-5417.

BOISSON M, MONDON K, TORNEY V, NICOT N, LAINE AL, BAHR-MAN N, GOUY A, DANIEL-VEDELE F, HIREL B, SOURDILLE P, DARDEVET M, RAVEL C, LE GOUIS J, 2005: Partial sequences of nitrogen metabolism genes in hexaploid wheat. Theor Appl Genet 110, 932-940.

CHEVALIER C, BOURGEOIS E, JUST D & RAYMOND P, 1996: Metabolic regulation of asparagine synthetase gene expression in maize (*Zea mays* L.) root tips. Plant J 9, 1-11.

GALLAIS A, HIREL B ,2004: An approach to the genetics of nitrogen use efficiency in maize. J Exp Bot 55, 295-306.

HABASH D, BERNARD S, SCHONDELMAIER J, WEYEN J, QUAR-RIE S, 2007: The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. Theor Appl Genet 114, 403-419.

HIREL B, BERTIN P, QUILLERE I, BOURDONCLE W, ATTAGNANT C, DELLAY C, GOUY A, CADIOU S, RETAILLIAU C, FALQUE M, GALLAIS A, 2001: Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiol 125, 1258-1270.

KICHEY T, HIREL B, HEUMEZ E, DUBOIS F, LE GOUIS J, 2007: In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crop Res 102, 22-32.

LAM H-M, WONG P, CHAN H-K, YAM K-M, CHEN L, CHOW C-M, CORUZZI G M ,2003: Overexpression of the ASN1 gene enhances nitrogen status in seeds of Arabidopsis. Plant Physiol 132, 926-935.

LEA P, AZEVEDO R, 2007: Nitrogen use efficiency. 2. Amino acid metabolism. Ann Appl Biol 151, 269-275.

LU C, HAWKESFORD MJ, BARRACLOUGH PB, POULTON PR, WIL-SON ID, BARKER GL, EDWARDS KJ, 2005: Markedly different gene expression in wheat grown with organic or inorganic fertilizer. Proc Roy Soc B Biol Sci 272, 1901-1908.

OBARA M, KAJIURA M, FUKUTA Y, YANO M, HAYASHI M, YAMA-YA T, SATO T, 2001: Mapping of QTLs associated with cytosolic glutamine synthetase and NADH-glutamate synthase in rice (*Oryza sativa L.*). J Exp Bot 52, 1209-1217.

TABUCHI M, SUGIYAMA K, ISHIYAMA K, INOUE E, SATO T, TA-KAHASHI H, YAMAYA T, 2005: Severe reduction in growth rate and grain filling of rice mutants lacking OsGS1;1, a cytosolic glutamine synthetase1;1. Plant J 42, 641-651.

YANG L, MICKELSON S, SEE D, BLAKE TK, FISCHER AM, 2004: Genetic analysis of the function of major leaf proteases in barley (*Hordeum vulgare* L.) nitrogen remobilization. J Exp Bot 55, 2607-2616.

YIN L P, LI P, WEN B, TAYLOR D, BERRY J O ,2007:. Characterization and expression of a high-affinity nitrate system transporter gene (TaNRT2.1) from wheat roots, and its evolutionary relationship to other NTR2 genes. Plant Science 172, 621-631.

ZHAO X Q, LI Y J, LIU J Z, LI B, LIU Q Y, TONG Y P, LI J Y, LI Z S, 2004: Isolation and expression analysis of a high-affinity nitrate transporter TaNRT2.3 from roots of wheat. Acta Bot Sin 46, 347-354.