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Nachruf Eberhard Graf von Waldburg zu Zeil und Trauchburg (* 30. April 1940, † 3. März 2013)

Geschätzte Freunde, Gäste und Teilnehmer an unserer 64. Tagung der Vereinigung Österreichischer Pflanzenzüchter und Saatgutkaufleute hier in Raumberg-Gumpenstein.

In unsere Freude über diese neuerliche Begegnung der Pflanzenzüchter, Saatgutverantwortlichen und Züchtungswissenschaftler mischt sich die Trauer um den langjährigen Obmann unserer Einrichtung. Graf Eberhard Waldburg-Zeil ist am 3. März dieses Jahres in seiner Heimat Rohrau (Niederösterreich) verstorben.

Widmen wir nun gemeinsam Worte des Gedenkens über diese besondere Persönlichkeit.

Graf Eberhard wurde am 30. April 1940 als jüngstes Kind von sieben Geschwistern im Stammschloss der Waldburg-Zeil im Schloss Zeil bei Leutkirch im schwäbischen Allgäu geboren. Waldburg-Zeil ist der Name einer der zahllosen Linien des ursprünglich welfisch-staufischen Ministerialengeschlechtes derer von Waldburg ab dem Jahr 1595. Wie alle regierenden Linien dieses Hauses in Oberschwaben blieben die Waldburgs dem katholischen Glauben, dem Papst, Kaiser und Reich über Jahrhunderte immer eng verbunden.

Nach harten Jahren in seiner Jugend - im Schloss wurden nach dem 2. Weltkrieg zahllose Flüchtlinge jahrelang untergebracht, einer schweren Lungenkrankheit, gefolgt von einer verspäteten Einschulung und einer harten Internatszeit - zeigte er großes Interesse an Naturwissenschaften, Mathematik und vor allem Geschichte.

Einem umfassenden Studium Generale in Fribourg, wo er sich seine hervorragenden Kenntnisse der französischen Sprache erwarb, und seinen vielfältigen Interessen folgend, begann er Forstwirtschaft in Nürnberg zu studieren.

Nach einer fast halbjährigen Weltreise nach Südamerika, wurde er animiert noch zusätzlich Betriebswirtschaft, ebenfalls in Nürnberg, zu studieren. Diese Kenntnisse über die direkte Beziehung über Landnutzungs-Formen und menschlicher Ernährung hat sein späteres Berufsbild als Landwirt und Betriebsführer nachhaltig geprägt.

Durch die Heirat im Jahre 1966 mit Johanna Gräfin von Harrach zu Rohrau übernahm er die bereits vorhandene Pflanzenzuchtstation am Neuhof und den Landwirtschaftsbetrieb mit ca. 1000 ha Ackerfläche. Durch Pachtung des Zeiselhofes in Deutsch-Jahrndorf, im Besitz der ungarischen Benediktiner in Pannonhalma, traf er damals eine weitsichtige und bereits EU-reife Entscheidung. Mit weiteren Pachtflächen der ehemaligen Herrschaft Potzneusiedl entstand eine geglückte Vereinigung zwischen einer erfolgreichen Zuchtstation und den dafür integrierten großen und bodenmäßig einheitlichen Vermehrungsflächen. Sie waren die ideale Voraussetzung für die Vermehrung von streng kontrolliertem Basissaatgut, das zur Vermehrung von Zertifiziertem Saatgut weitergegeben werden konnte. Diese Synthese war der Beginn einer sehr erfolgreichen Züchtungsperiode unter der Leitung des überaus rührigen „Vollblutlandwirtes“, Getreide- und Maiszüchters DI Josef Adam. So konnte dieser, für die Österreichische Getreide- und Maisproduktion immer bedeutender werdende Betrieb in den Jahren zwischen 1967 und 1987 eine besonders erfolgreiche Sortenzüchtung aufbauen: während dieser Zeit entstanden 26 Sorten von Winter- und Sommergersten, Winter- und Durumweizen, Winterroggen und Maishybriden! Sie alle konnte ohne Auflagen in das österreichische Zuchtbuch eingetragen werden und ermöglichten der inländischen- und auch europäischen Landwirtschaft über drei Jahrzehnte bedeutende Ertrags- und Qualitätssteigerungen.

Persönlich hatte ich zwischen 1975 und 1983 die ehrende Aufgabe die Züchtungsmethoden wissenschaftlich zu begleiten und zu adaptieren. Der Züchtungsbetrieb Neuhof-Rohrau war auch als erster Betrieb nach dem 2. Weltkrieg der Pionier für die Einführung des Hybridmaises in Österreich. Auf der Basis von Inzucht-Material der Universität Wisconsin begann man mit der Produktion von Doppel-Hybriden. Bereits 1960 konnte die Hybridsorte ‘Harrach 470’ der österreichischen Landwirtschaft für den raschen Aufbau einer dringend erforderlichen, eigenständigen Maisproduktionen übergeben werden. In den Folgejahren entstanden weitere adaptierte Hybrid-Sorten, die ab 1969 ‘Neuhof’ Hybriden genannt wurden.

Auch die Einführung des Durumweizen in Österreich ist diesem Betrieb zu verdanken: Die Sorte ‘Adur’ war von Beginn an sehr erfolgreich und der Ausgangspunkt für weitere, hochqualitative Sorten für die expandierende nationale Teigwarenindustrie.

Graf Eberhard Waldburg-Zeil unterstützte maßgeblich diese, oft riskanten züchterischen Entwicklungen, vor allem auch durch seinen selbstlosen Einsatz über 22 Jahre als Obmann der Vereinigung der österreichischen Pflanzenzüchter und Saatgutkaufleute. Dieses Amt übte er mit großer Umsicht, Sachverstand und Toleranz aus. Als langjähriges Mitglied der Zuchtbuchkommission im Landwirtschaftsministerium, die über die wirtschaftlichen und landeskulturellen Werte zur Zulassung von in- und ausländischen Sorten entschied, galt er als ein geschätzter, auch über die Situation im benachbarten Ausland immer hervorragend informierter Fachmann. Auch die Novellierungen des österreichischen Pflanzenzucht- und Saatgutgesetzes und die Anpassungen an die neuen Internationalen Gesetzeswerke (UPOV) tragen seine Handschrift. Seine hohe ethische Verantwortung für die österreichischen Landwirte war auch bei den zweimal jährlich stattfindenden Saatgutpreis-Verhandlungen spürbar, um die Getreide- und Maisanbauern so günstig wie möglich mit Originalsaatgut zu versorgen.

Sein Unternehmertum bewog ihn auch, österreichische Sorten im Ausland zu platzieren. Kooperationen mit Großfirmen, wie Cooperative de Pau in Frankreich und Miln Master in Großbritannien, fanden große Beachtung und Wertschätzung, wie z.B. im Besonderen die Winter- und Sommergerstensorten ‘Rachel’ und ‘Multum’ und die Winterweizen ‘Adam’ und ‘Agron’. Der Staat Österreich dankte ihm 1991 für alle diese besonderen Leistungen mit der Verleihung des Goldenen Verdienstzeichens der Republik.

Eberhard Graf von Waldburg zu Zeil und Trauchburg lebte eine, im christlichen Glauben fest verwurzelte Menschenliebe. Er war seinen Kindern Stephanie, Marie, Johannes und Karl ein liebender Vater und Vorbild.

Seinen Mitarbeitern ein gütiger und umsichtiger Vorgesetzter, seinen Freunden ein hochgebildeter ‘Homo Europaeus’ und wertvoller Begleiter. Seine Person und Lebenshaltung stehen symbolhaft für ein Gebet von Antoine de Saint- Exupéry an den Allmächtigen:

Herr, ich komme zu Dir, denn ich habe in Deinem Namen den Acker bestellt.
Dein ist die Saat. Ich habe diese Kerze gebildet, an Dir ist es, sie anzuzünden.
Ich habe diesen Tempel gebaut, an Dir ist es, sein Schweigen zu bewohnen.

Raumberg-Gumpenstein, 25. November 2013 Em. o. Univ. Prof. DI Dr. Peter RUCKENBAUER

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A brief chronology and current status of plant mutation breeding

Brian P. Forster[1*]

Abstract

Most crops evolve over three phases: (1) gathering from the wild; (2) domestication and agronomy, and (3) improvement through plant breeding. Mutation has been a key factor in both domestication and crop improvement. The first reference to spontaneous mutants can be found in the ancient book of Lulan, China, 300 BC. From 1590 onwards there has been continual documentation of naturally occurring mutants in crop plants in the Western world. 1927 is a significant year as: (1) induced plant mutation was first described (in *Datura stramonium*), (2) Muller provided proof of mutation induction, (3) von Sengbusch developed the first mass screen for a desired mutant trait, and (4) Stadler published the first induced mutants in crop plants. In the 1930s deliberate plant mutation breeding programmes were set up, notably in seed propagated crops in Sweden, USA and Germany. The first mutant crop cultivar was in tobacco cv. 'Vorstenland' released in 1934 in Indonesia (induced by X-rays). 1942 saw the first induced disease resistant mutant (mildew resistance in barley) and in 1944 the term mutation breeding (*Mutationszüchtung*), was coined. The first reported chemical induced mutation was in 1944 and the first report of mutation induction by gamma rays was in 1949. In 1954 the first mutant cultivar in a vegetatively propagated crop 'Faraday' tulip was produced. In 1964 the Joint FAO/IAEA Division was established with a mission to use nuclear technologies to safeguard food security, this included mutation induction and mutation screening for plant breeding. In 1966 the first chemical induced mutant cultivar: barley cv. 'Luther' was released. The Joint FAO/IAEA established a data base of mutant cultivars (http://mvgs.iaea.org) in 1993, this currently lists over 3000 mutant cultivars in over 200 crop species; over 80% of these have been produced by physical mutagenesis, mainly gamma irradiation.

The PBGL of the Joint FAO/IAEA Division provides an irradiation service for Member States (MSs). In the past 50 years the PBGL has received over 1300 requests from 77% MSs with the USA making the most requests (15%) followed by Germany (7%), UK (7%), Pakistan (6%), Kenya (4%), Poland (4%), Nigeria (3%) and the rest 2% or less. In 2013 the PBGL received a record number of plant samples for mutation induction in a wide range of crop species. Resurgence in plant mutagenesis is driven by urgent demands for increased biodiversity in contemporary breeding material, mutant screening is now aided by high-throughput methods in plant (phenotypic) and DNA (genotypic) samples. Increased interest in plant mutation breeding is also being fueled by the current revolution in sequencing and functional genomics which exploit mutations in mapping and gene function studies. Plant mutation breeding is also quick compared to conventional breeding: mutation breeding typically takes a leading, favoured cultivar that is deficient for a trait (e.g. has become susceptible to a disease) and induces a mutation to overcome the deficiency. A recent example is the development of black stem rust (*Ug99*) resistant wheat cultivars in Kenya after just four years from the initial mutation induction.

Keywords

Biodiversity, crop improvement, database, irradiation, mutagenesis

Acknowledgments

The Food and Agriculture Organisation of the United Nations and the International Atomic Energy Agency through the Joint Division Programme of Nuclear Techniques in Food and Agriculture are recognized for data and funding in plant mutation breeding.

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Mutation induction and reverse-genetics for functional genomics and breeding

Bradley J. Till[1*], Ego K. Amos[1,2], Miriam G. Kinyua[2], Souleymane Bado[1], Joanna Jankowicz-Cieslak[1], Owen A. Huynh[1], Isabelle Henry[3], Luca Comai[3] and Pierre Lagoda[1]

Abstract

Induced mutations have remained a powerful means of rapidly generating novel genetic diversity since their first application in the 1920s. Forward-genetic approaches using induced mutations have been applied for mutation breeding and functional genomics. This approach is advantageous because prior knowledge about genes is not required. There are many examples of success. Recently, increased resistance in wheat to black stem rust race *Ug99* has been developed through an IAEA funded multi-country project. Wheat seed were irradiated with varying dosages of gamma irradiation and subjected to phenotypic evaluation for disease resistance. The first two varieties are undergoing seed multiplication for farmer release in Kenya. The mutated gene(s) responsible for the improved disease resistance, however, remain unknown. The task to identify causative mutations in a large polyploid genome remains difficult. We are working to develop exome capture strategies for Illumina based sequencing to identify induced mutations in plant genomes. Pilot work has been conducted with sorghum and cassava. For each species, capture probes covering approximately 20 Mbps of exonic sequences have been designed. Gamma irradiated and EMS mutagenized populations have been developed to optimize mutation discovery using this approach. Such tools will serve several purposes. First, rapid evaluation of induced mutations in early generations will provide a snapshot of the density and spectrum of induced mutations. This will allow a decision to be made regarding the quality of the mutant population and enable calculations to determine the size of a population that needs to be screened to have a reasonable probability to recover desired alleles. Secondly, the ability to sequence all coding sequence of a genome should facilitate the cloning of mutant alleles. Finally, exome sequencing strategies can be used for reverse-genetic approaches.

Since the first description of reverse-genetics using induced mutations (commonly referred to as Targeting Induced Local Lesions in Genomes, or TILLING) in the late 1990s, projects have been developed for more than 25 species. Reverse-genetics is an advantageous approach because it starts with the identification of mutations in genes hypothesized to have a specific function. Hypotheses can be based on homologies to genes in other species with a known role in a particular trait. Since only a handful of plants in a population will carry potentially interesting mutations, the number of plants that need to be characterized phenotypically is dramatically reduced. Our recent work has focused on optimizing mutation induction and dissolution of chimeric sectors using tissue culture approaches.

Our results show that the density and spectrum of induced mutations is similar to that of seed mutagenesis. We have also been developing and adapting low-cost methods for molecular characterization of plants. This includes collection and storage of leaf material at room temperature, extraction of DNA without toxic chemicals, and mutation discovery using self-extracted enzymes. The methods are broadly applicable. For example, we have recently adapted them to validate the production of doubled haploid plants in barley. These efforts to enhance the efficiency of mutation breeding are aimed at assisting developing countries in their progress towards sustainable food security.

Keywords

Barley, sequencing, TILLING, *Ug99*, wheat

Acknowledgments

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Techniques for acceleration of mutation breeding in crop plants

Abdelbagi Mukhtar Ali Ghanim[1] and Brian P. Forster[1*]

Abstract

Plant mutation breeding involves the processes of mutation induction, mutation detection, mutation fixation, mutant line development and release of new mutant cultivars. The length of the process depends largely on the nature of propagation of the crop (for annual crops this normally takes 7 to 12 years), the targeted trait, the ability to recognize and select individuals carrying traits of interest in segregating populations and developing selected lines. Advances in plant propagation, phenotyping, genotyping and supporting technologies provide several opportunities to enhance the efficiency of selection and accelerate the delivery of mutant cultivars. In addition to mutation induction and mutation detection the PBGL of the Joint FAO/IAEA Division has active R&D projects in speeding up plant mutation breeding through rapid generation cycling and doubled haploidy.

Recently, a procedure for rapid generation cycling in wheat and barley was published. This procedure has been adapted for a wider range of wheat genotypes and has been extended to sorghum. Ten wheat and 7 sorghum cultivars from Kenya and Sudan were propagated in different pot sizes, day length and watering regimes.

Embryos were rescued at 10, 15 and 20 days after flowering and germination rates and growth in culture studied. The aim is to provide conditions for immediate germination, sampling and seedling development for transplant. The *in vitro* plantlets produced offer ideal materials for DNA sampling, genotyping and marker assisted selection. Thus only preferred genotypes may be advanced to the next generation. Plants propagated in small pots (8×8×8.5 cm) under continuous lighting flowered in less than 40 days. With embryo rescue, an average of 48 and 60 days were sufficient to complete the generation cycle for the wheat and sorghum cultivars tested, respectively. Sufficient seed (5-20 for small pots and 15-45 for medium sized pots) were produced for the next generation cycle. This enables up to 6-7 generations to be produced in one year which is enough to reach sufficient homozygosity to advance a mutant line for bulking for field evaluation and progression to eventual official testing.

Doubled haploidy is another biotechnology that can speed up mutant line development. It enables the production of homozygous lines from any generation and recessive mutations can be exposed and fixed in one generation. Simplified protocols for anther and microspore culture in wheat, sorghum and barley are being developed. Genotype, growing condition of donor plants, stage of spike collection, pre-treatment of spikes/anthers, media composition and culture condition are among main factors influencing success in anther/microspore culture. Pollen irradiation for doubled haploid production is also being investigated both as a pre-treatment to stimulate microspore development *in vitro* and to stimulate *in vivo* haploid embryo development after pollination via parthenogenesis.

Integration of such biotechnologies together with *in vitro* selection and molecular markers for mutant assisted backcrossing offer potential in speeding up the delivery mutant cultivars and allowing rapid plant breeding responses to new challenges to food production such as changing climate.

Keywords

Barley, breeding cycle, embryo rescue, doubled haploidy, wheat

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High throughput screening for detecting EMS mutations in oilseed rape (*Brassica napus* L.)

Hans-Joachim Harloff[1], Nazgol Emrani[1] and Christian Jung[1*]

Abstract

We have developed two EMS (ethyl methanesulfonate) mutant populations of oilseed rape (*Brassica napus* L.), one from the spring type line 'YN01-429' and the second from the winter type cultivar 'Express 617'. We established a high throughput TILLING (Targeting Induced Local Lesions IN Genomes) protocol to detect mutations in two sinapine synthesis genes with the aim to select low sinapine content rapeseed mutants. Sinapine is an important antinutritive compound and prevents an extended use of the protein-rich extraction meal as animal feed or in human nutrition. We detected 135 missense and 13 non-sense mutations in the two seed-expressed copies of the *BnaX.SGT* gene and 162 missense, 3 nonsense and 7 splice site mutations in the two *BnaX.REF1* gene copies. The mutation frequencies ranged from 1/12 kb to 1/22 kb in the Express 617 population and from 1/27 kb to 1/60 kb in the YN01-429 population, respectively. Due to the presence of multiple paralogs, single non-sense mutations did not result in lower seed sinapine content. Crossing experiments between mutants are on the way to produce double mutants in which both paralogs are mutated.

Keywords

EMS mutagenesis, mutant population, point mutations, TILLING

Introduction

Oilseed rape (*Brassica napus* L.) as the most important oil crop in temperate regions is grown for the production of biodiesel, animal feed and vegetable oil for human consumption. Sinapoylcholine (sinapine) is the major phenolic compound of *B. napus* seeds typically varying from 3 to 12 mg/g (ZUM FELDE et al. 2007). Due to this limited variation within the rapeseed gene pool, genetic modification or mutation induction targeting the sinapine metabolic pathway genes are methods of choice to breed low sinapine rapeseed. The biosynthesis of sinapine in *Brassicaceae* is well known and starts via the phenylalanine/hydroxycinnamate pathway (MILKOWSKI et al. 2004). We focused on two genes encoding key enzymes of the pathway, *SGT* (UDP-glucose:sinapic acid glucosyltransferase) and *REF1* (sinapaldehyde dehydrogenase/coniferaldehyde dehydrogenase) (HARLOFF et al. 2012). Unfortunately, due to the amphidiploid nature of rapeseed, most *Arabidopsis* genes have 2 to 8 paralogs thus complicating any gene knock-down strategy. Because seed sinapine is the target of our project we selected two seed expressed paralogs of either *BnaX.SGT* (MITTASCH et al. 2010) and *BnaX.REF1* (MITTASCH et al. 2013). In previous experiments using an RNAi approach reductions of 76 and 45% in the sinapine content were obtained by downregulating *BnaX.SGT* and *BnaX.REF1*, respectively (HÜSKEN et al. 2005, MITTASCH et al. 2013). As genetically engineered rapeseed is not accepted in the EU, we started a project to identify mutants with a loss of function in the above mentioned genes using TILLING (Targeting Induced Local Lesions IN Genomes) in chemically mutagenized rapeseed populations (*Figure 1*). This technique has been used before in *Arabidopsis*, maize, rice, oat and wheat (CHAWADE et al. 2010, GREENE et al. 2003, SLADE et al. 2005, WEIL and MONDE 2007).

Material and methods

Plant material and EMS mutagenesis

A Canadian yellow-seeded spring type inbred line 'YN01-429' (F_8) kindly provided by Prof. G. Rakow (AAFC Saskatoon, Canada) and a winter type inbred line 'Express 617' (F_{11}) derived from the German cultivar 'Express' were used in this study. Seeds were soaked in tap water for 12 h prior to 12 h EMS treatment. Winter type rapeseed plants with six leaves were vernalized at 4°C for 12 weeks.

DNA extraction and pooling strategy

Leaf samples from M_2 plants were harvested for DNA extraction. Leaves from spring type plants were sampled in 2 ml Eppendorf tubes whereas the winter type leaves were sampled in 96 well plates. Genomic DNA was isolated from freeze-dried material (sample dry weights 20-50 mg spring and 10-20 mg winter type material) in a 96 microtiterplate format using a NucleoSpin® 96 Plant I Kit (Macherey and Nagel, Düren, Germany) and the TECAN Freedom Evo 200 Liquid Handling Robot (4×96 samples/day; TECAN GmbH, Crailsheim, Germany). DNA concentrations were measured in a Genios Microplate Reader (TECAN GmbH, Crailsheim, Germany) using Quant-It-Picogreen dsDNA Reagent (Invitrogen, Karlsruhe, Germany). Average DNA yields were 10.3 ('YN01-429') and 5.8 ('Express 617') µg DNA/sample which is sufficient for screening 50 and 24 amplicons, respectively. As the same kit was used for DNA isolation, differences in DNA yield were due to different amounts of leaf material.

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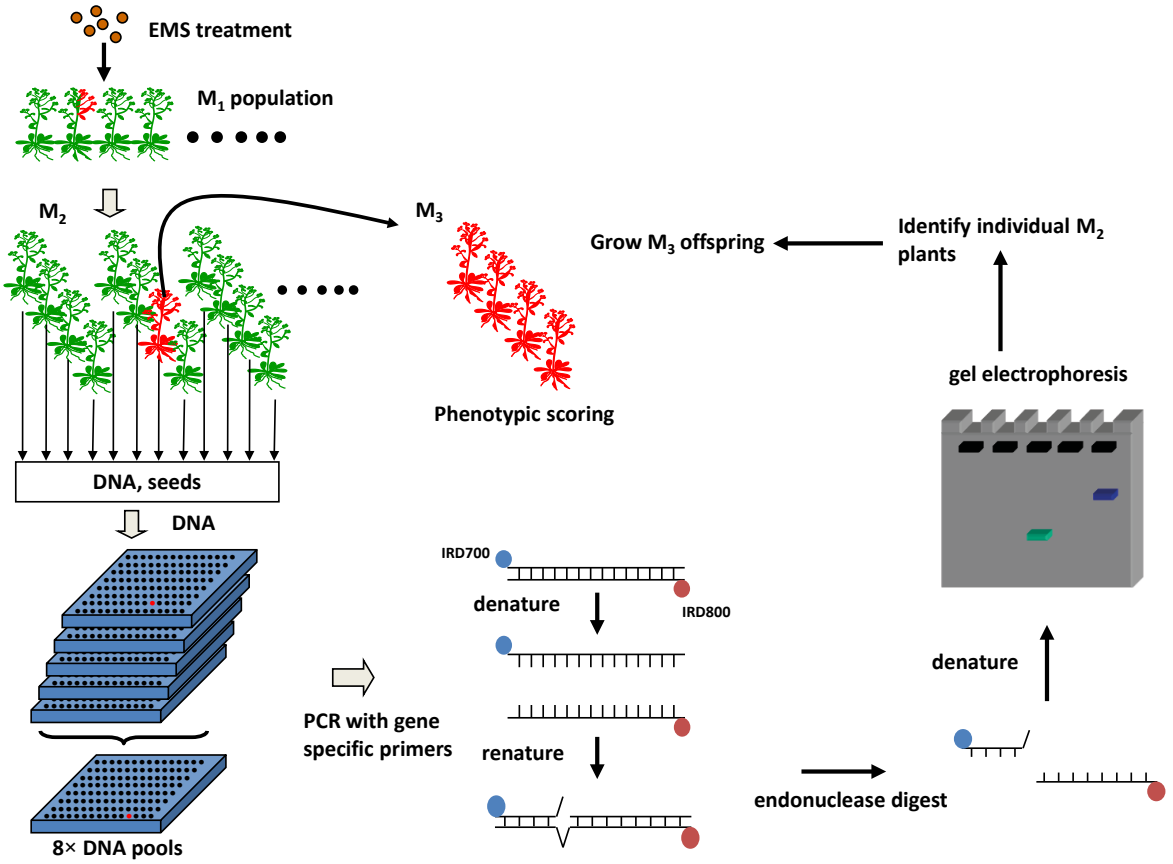

Figure 1: **Mutation screening by genotype: High throughput TILLING**

For normalization, DNA aliquots were diluted to a PCR-ready final concentration of 5 ng/µl and arranged in one-dimensional 4× pools for 'YN01-429' and two-dimensional (2D) 8× pools for 'Express 617'. In the 2D pools 4 microtiter plates with normalized DNA samples were combined in one pool plate with columns 1-6 containing the 8× column pool DNA and columns 7-12 containing the 8× row pool DNA. Due to this arrangement, every single sample is represented twice on a 96 lane LI-COR gel and can be directly identified by assignment of the lanes. As leaf sampling and DNA extraction were performed according to family-number with subsequent column-wise storage in the microtiter plate and 4× pools were built up by combining equivalent positions of 4 plates, 4× pools never contained sibling M_2 samples whereas in the 8× pools the column pools contained samples from 8 families (8×1), whereas the row pools contained samples from only 2 families (4×2). In the case of 1D-4× pools, we sequenced the respective amplicons of all four plants for mutant identification, whereas in the 2D-8× pool the mutant plant could be directly identified and only one amplicon had to be sequenced which greatly facilitated the mutant detection procedure.

Sequence information of the BnaX.SGT and BnaX.REF1 genes

The coding sequence of *BnaX.SGT* (UGT84A9) is 1,494 bp in size organized in one exon, whereas both *BnaX.REF1* genes have 9 exons and 8 introns. Their genomic sequences are 3,977 and 3,973 bp in size with a coding sequence of 1,503 bp. Sequence data and copy numbers are based on BAC library screening, expression studies and Southern hybridization. The GenBank accession numbers for *BnaA.SGT.a* (UGT84A9b), *BnaC.SGT.a* (UGT84A9a), *BnaA.REF.a* and *BnaC.REF.a* are FM872285, FM872284, FN995990, and FN995991, respectively. In addition to BLAST analysis the software CLC Main Workbench (CLC bio, Aarhus, Denmark) was used for *in silico* sequence evaluation.

Primer design and PCR conditions

Locus specific primers for the PCR amplification of the coding regions of these genes were designed with the program FastPCR© (KALENDAR et al. 2009) and tested with unlabeled and 5' labeled primers (IRD labels Dy-681 in the forward and Dy-781 in the reverse primers, Biomers, Ulm, Germany) according to the protocol of TILL et al. (2006). For PCR, we used a DYAD thermal cycler (MJ Research Inc., Waltham, MA, USA).

Heteroduplex analysis, fragment detection and calculation of mutation frequencies

The CEL I enzyme was extracted from celery as described by TILL et al. (2006). Heteroduplex formation of the PCR product and digestion with CEL I were performed according to the same reference. Prior to loading to the gel, 2 µl of the digestion product were mixed with 2 µl formamide loading

dye and denatured for 3 min at 95°C. Aliquots of 0.3 to 0.5µl were applied to a 6.5% polyacrylamide gel (KB^{Plus} Gel Matrix, LI-COR®, Bad Homburg, Germany) and separated on a LI-COR 4300 DNA Analyzer with double laser detection system for IR-labeled primers. The gel was run for 4:15 hours at 1,500 V, 40 mA and 40W. The fragments were analysed with the GelBuddy software (ZERR and HENIKOFF 2005). After the sample assignment of the fragments, mutations were identified by Sanger sequencing of the corresponding PCR products. Sequence analysis was performed using Dye terminator chemistry (Applied Biosystems, Foster City, CA, USA) on a 3730xL DNA Analyzer (Applied Biosystems). Mutation frequencies F [1/kb] were calculated using amplicon sizes corrected by 100 bp for LI-COR gel border effects according to the formula:

$$F\,[1/kb] = 1 / \left(\frac{(amplicon\ size\ [bp] - 100) \times (number\ of\ M_1\ plants)}{(number\ of\ mutations) \times 1{,}000} \right)$$

Determination of sinapic acid metabolites

The M_3 plants were grown in the greenhouse under 16 h light. M_4 seeds were harvested after bag isolation. Sinapine and sinapoylglucose were determined by HPLC as described in MILKOWSKI et al. (2004). If not otherwise indicated, single M_4 seeds were analysed. Sinapic acid ester equivalents (SAE) were determined in single seed extracts after alkaline hydrolysis for 3 h at 50°C in 5 N KOH (WOLFRAM et al. 2010).

Results and discussion

EMS mutagenesis and development of TILLING populations

The purpose of our study was to establish a mutant screening protocol for rapeseed by selecting mutations within two major genes of the sinapine biosynthesis pathway. Two different rapeseed lines were employed in this experiment. First we produced an EMS mutant population with the spring type rapeseed line 'YN01-429'. We used different concentrations of 0.5, 0.8, 1.0 and 1.2% EMS (*Table 1*). Survival rates in the M_1 generation dropped from 80% (0.5% EMS) to 50% (1.2% EMS). Therefore, higher EMS concentrations were avoided and concentrations between 0.5 and 1.2% were chosen for further studies.

According to different EMS treatments, the 'YN01-429' population was subdivided into two subpopulations. The 1st subpopulation consisted of 500 vigorous 'YN01-429' derived M_1 plants derived from 2400 EMS treated seeds (0.5 and 1.0% EMS). They were self-pollinated by bag isolation and M_2 seeds were harvested. Of each M_2 family, 4 plants were grown to avoid loss of mutant alleles due to the chimeric character of the M_1 plants. After bag isolation M_3 seeds were harvested from 1724 M_2 plants grown in the greenhouse. The 2nd subpopulation derived from 0.8 and 1.2% EMS treatments, consisted of 2833 vigorous M_1 plants. Bag isolation resulted in seeds from 2833 M_2 families. Three plants of each M_2 family were grown in the field and M_3 seeds of 3629 plants were harvested without bag isolation. Leaf material was taken and DNA was isolated from all 5361 plants of the 'YN01-429' spring rapeseed M_2 population. DNA samples of all treatments were later jointly investigated by TILLING.

For the production of a winter type rapeseed TILLING population, the 'Express 617' inbred line was treated with 1% EMS, resulting in an M_1 lethality rate of ~40%. A total of 2103 viable M_1 plants were obtained and seeds were harvested from 1902 M_1 plants. A total of 7608 M_2 plants (4 plants/family) were grown in the greenhouse and M_3 seeds were harvested from 6775 M_2 plants. DNA was isolated from 3488 M_2 plants representing 940 M_1 plants. Leaf samples of another 3732 M_2 plants were frieze-dried and stored for further use (*Table 1*).

Primer design and CEL I digest

TILLING in *B. napus* is hampered by the fact that many sequences exist as paralogs and orthologs with high sequence similarity among each other. Therefore, we designed locus specific primers which cover gene regions coding for functional domains of the polypeptide. The amplicon size should not exceed 1500 bp due to resolution and background of the LI-COR gels (TILL et al. 2006). Primers were carefully tested prior to TILLING: first, a so-called PCR crash test was carried out with primer pairs and single primers from paralog and ortholog loci. Suitable primer combinations should not

Table 1: **Features of the rapeseed TILLING platform at the University of Kiel (HARLOFF et al. 2012)**

Population	YN01-429		Express 617
Number of seeds	2400	5000	3120
EMS concentration	0.5%, 1%	0.8%, 1.2%	1%
Number of M_1 plants	2000	3980	2103
Number of M_1 plants with seed set	500	2833	1902
Number of seeds per M_2 family	4	3[a]	4
Number of M_2 plants	2000	3860	7608
Number of selfed M_2 plants	2000	0	7608
Open pollinated M_2 plants	0	3860	0
Leaf samples, DNA extraction	1905	3456	3488[c]
M_1 plants represented	500[b]	1500[b]	940
DNA samples in M_2 population	5361		3488

[a] 8499 seeds were sown in the field, only 3860 M_2 plants survived
[b] estimated values of represented M_1 plants due to combination of different subsets
[c] only half of the leaf material was extracted, leaf material of 3732 additional M_2 plants is available (originating from another 950 M_1 plants)

give any non-specific amplicons visible as additional bands or smear after gel electrophoresis. Second, the obtained PCR products were sequenced to confirm locus specificity and third, the PCR was repeated with IRD labeled primers. *Figure 2* shows the genomic structure of the target genes and the location of the amplicons (HARLOFF et al. 2012). All primers were 20 to 30 nucleotides in size with melting temperatures of 60-65°C to avoid interaction between the two IRD labels during the PCR reaction. It was known that four *BnaX.SGT* loci are present in the *B. napus* genome, but only two of them (*BnaA.SGT.a* and *BnaC.SGT.a*) are expressed in ripening seeds (MITTASCH et al. 2010). Therefore, we designed three locus specific primer combinations for *BnaA.SGT.a* and *BnaC.SGT.a* which gave rise to amplicons in a range between 1270 and 1420 bp covering between 85 and 95% of the coding sequence (HARLOFF et al. 2012).

Two *REF1* homologues (*BnaA.REF1.a* and *BnaC.REF1.a*) had been discovered in the rapeseed genome (MITTASCH et al. 2013). For each locus, primers were designed for two amplicons with sizes between 943 and 1361 bp. Together, they cover 84% of the coding sequence including 7 out of 9 exons.

Apart from primer design an optimized CEL I digest in combination with a refined pooling strategy is critical for successful mutant detection. We performed a number of heteroduplex digestion experiments with varying amounts of CEL I enzyme to determine the optimal signal-to-noise ratio after LI-COR gel electrophoresis (data not shown). An existing SNP within *BnaC.SGT.b* between 'YN01-429' and 'Express 617' served as a positive control as two fragments became visible after CEL I digestion of the mixed amplicons.

Detection and characterization of EMS mutations

We used 4× and 2D-8× pooling strategies to screen the spring type and winter type populations, respectively. Our protocol enabled the detection of TILLING fragments, even in the case of a low signal-to-noise ratio. Fifty to 80% of the polymorphic fragments identified after gel electrophoresis indicated real point mutations as verified by Sanger sequencing. The position of the SNP matched the position of the CEL I cleavage with a precision of 5-20 bp. In the 8× pools no effect of increased effective concentrations of mutant alleles in the 2D row pools containing sibling samples was observed.

TILLING of four sinapine genes resulted in a total of 683 mutations which were later verified by Sanger sequencing (*Table 2*). As in some cases the same mutation was found in more than one plant of the same M_2 family we corrected the number of heritable germ line mutations by counting those mutations only once in each family. This resulted in a total of 570 different transitions by subtracting 113 mutations within one and the same family (HARLOFF et al. 2012). The mutation frequencies varied between the diffe-

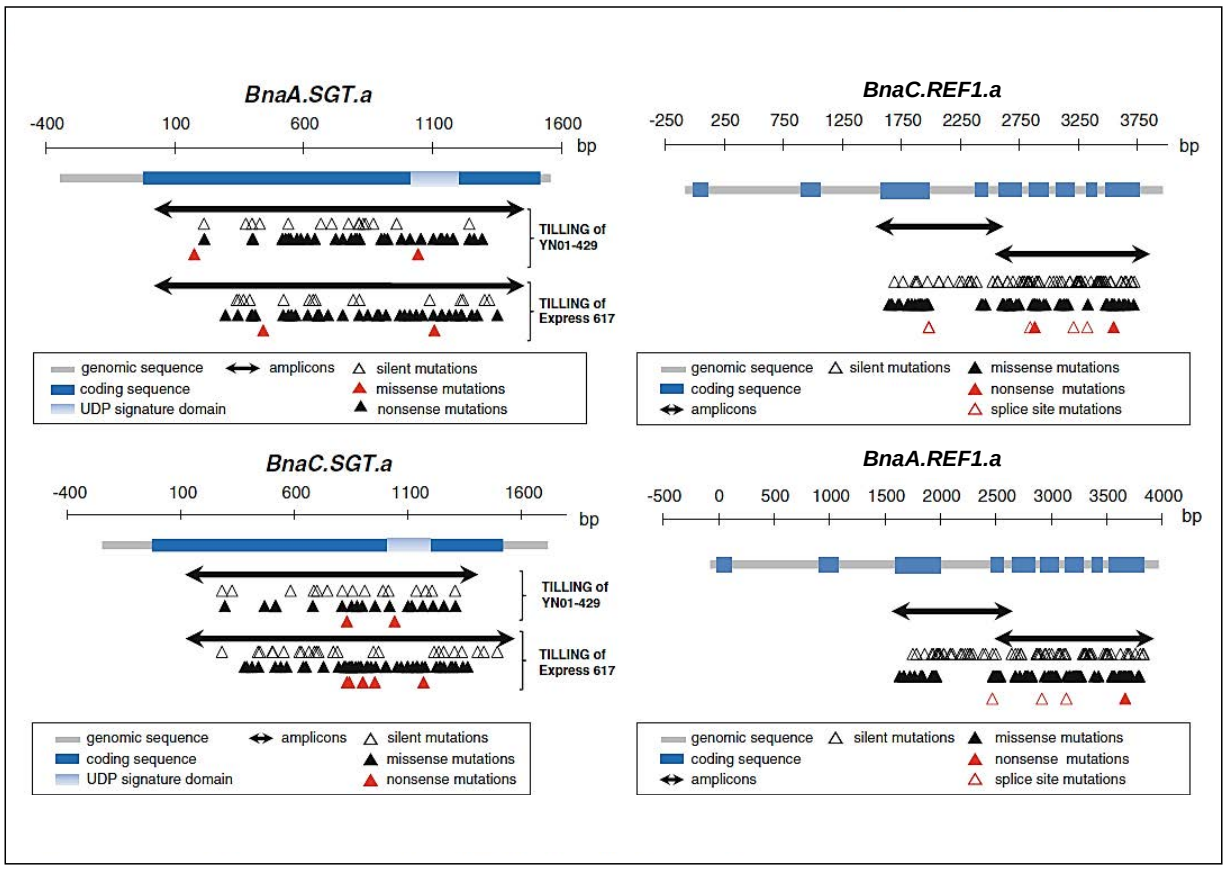

Figure 2: **Gene structure of *BnaX.SGT* and *BnaX.REF1* genes and mutations detected by TILLING (HARLOFF et al. 2012)**

Table 2: **TILLING with sinapine biosynthesis gene sequences. For *BnaX.REF1* values of both amplicons were added. Mutation frequencies were calculated as number of mutations/M_1 plant which was determined by analyzing the M_2 families (HARLOFF et al. 2012)**

Gene	ORF (bp)	% covered by TILLING	LI-COR fragments detected	Mutations verified by sequencing	Mutations verified by M_3 analysis	M_3 phenotypes	Mutation in M_1	Mutations frequency [1/kb][a]	Mutations /1000 M_1/1000 bp
YN01-429									
BnaA.SGT.a	1494	94	95	61	14	2	55	1/27[b]	20
BnaC.SGT.a	1494	85	82	39	13	3	39	1/60[c]	15
Express 617									
BnaA.SGT.a	1494	94	107	60	20	1	56	1/22	42
BnaC.SGT.a	1494	95	127	90	6	1	79	1/16	59
BnaX.REF1.a	1503	84	250	205	4	0	164	1/12	77
BnaX.REF1.b	1503	84	291	228	3	0	177	1/12	83

[a] amplicon correction for LI-COR gel border effects by 100 bp
[b] screening of 1140 M_1
[c] screening of 2000 M_1

rent sequences investigated. We found the lowest mutation frequency within the spring rapeseed population (*BnaC.SGT.a*, 1/60 kb) and the highest frequency within the winter rapeseed population (*BnaX.REF1.a*, 1/12 kb). Average mutation frequencies within the *BnaX.SGT* and *BnaX.REF1* genes were calculated as 1/31 kb and 1/12 kb, respectively. Differences were also found between both populations with lower frequencies in the spring population.

Then we grouped the mutation events into distinct classes to address the question whether EMS mutations are randomly distributed across the different genes. It has been reported that only a limited number of nucleotide triplets can be changed by EMS treatment, with guanine being the predominant target of ethylation (STEPHENSON et al. 2010). Thus, the frequency of mutagenic events should be correlated to the frequency of G on both strands or with the frequency of G/C (G+C) on the coding strand. Accordingly, 99.3% of our mutations were G/C→A/T transitions while only 0.7% (4 among all 570 mutations) were non-G/C→A/T transitions. We classified the mutations in relation to the number of G/C residues (i.e. the maximum number of EMS targets) excluding the 4 non-G/C→A/T transitions. The frequency of mutated G/C residues ranged between 6 % (*BnaX.SGT*) and 20% (*BnaX.REF1*). Moreover, no apparent strand selectivity could be found, with the ratio of G→A to C→T transitions in most cases almost equaling the G/C ratios in the codon strand.

We further calculated the frequency of multiple mutations within one gene (>1 mutation/kb/M_2 plant) with regard to the total number of mutations. It ranged between 3-4% for *BnaC.SGT.a* (disregarding double/triple mutations for *BnaA.SGT.a*) and 2-5% for *BnaX.REF*. We also calculated the average number of mutations per single plant by multiplying mutation frequencies by genome size (2258 Mbp/2C; ARUMUGANATHAN and EARLE 1991) and corrected for an estimated average G/C content in *B. napus* of 35.7% (as a mean of 36.0% in *B. oleracea* (TOWN et al. 2006) and 35.4% in *B. rapa* (TRICK et al. 2009). As a result, the number of mutations/plant in the 'YN01-429' and in the 'Express 617' EMS population were 40000 and 130000, respectively.

Missense and non-sense mutations within sinapine genes

We found a number of putative loss-of-function mutations that cause amino acid changes (missense), stop codons within coding regions (non-sense) or splice site mutations at intron borders. We detected 16 stop codon mutants (2.8%) and 8 splice site mutations (2.3%) with G→A exchanges at the 5' and 3' ends of the introns. Those mutations should result in non-functional enzymes which are expected to have an impact on sinapine content.

We harvested M_3 seeds from all M_2 mutant plants. In a first step, we selected 14 *BnaA.SGT.a* and 13 *BnaC.SGT.a* mutants from the spring rapeseed population and 4 *BnaA.SGT.a* and 5 *BnaC.SGT.a* mutants as well as 4 *BnaX.REF1.a* and 3 *BnaX.REF1.b* mutants from the winter rapeseed population with promising base pair transitions as described above. We aim to select homozygous M_4 offspring for crossing and phenotyping experiments. Homozygous plants were found for all stop codon mutations clearly demonstrating that loss of one gene alone did not seem to have a deleterious or even lethal effect because those plants showed a normal growth habit.

We did first experiments with M_4 seeds to analyze the contents of sinapine, sinapoylglucose and sinapic acid equivalents by HPLC. For these measurements we chose two segregating families (winter types 101612 and 101650), indicating that the parents were heterozygous. No significant reductions could be observed and clearly demonstrate that the knock-down of only one of two seed-expressed genes was not sufficient to produce a measurable effect.

As we expect drastic reductions of sinapine contents after down regulation of both seed-specific *BnaX.SGT* or *BnaX.REF1* genes, crossings of homozygous stop codon and splice site mutants in the *BnaX.SGT* and *BnaX.REF1* genes from the spring and winter rapeseed population have been performed in order to combine two loss-of-function mutations in one plant. First results with double mutants point at drastic reductions of sinapine contents in seeds.

Comparisons between different TILLING platforms

TILLING platforms have been established for a number of plants. The main features of TILLING platforms are the number of M_2 families represented by their DNA samples and the availability of $M_{2:3}$ seeds. Their efficiency relies mainly on (1) the mutation frequency, (2) the number of M_2 plants jointly tested in an experiment (pooling strategy), and (3) the costs for DNA extraction, enzyme reactions and fragment analysis. Here, we will address these questions comparing our results with previously published TILLING protocols. The spring and winter type rapeseed TILLING platforms presented here are open for scientists to screen their sequences in our institute.

The M_1 mutation frequency is a critical parameter for TILLING. It depends on the species and the target tissue, the mutagen, the developmental stage of the mutagenic treatment and the mutagen concentration. Typically, mutation frequencies are measured in the M_2 generation which is derived from selfed M_1 plants. Further generations can be produced by single seed descent with an increased number of families or, to avoid loss of mutations, small sized M_2 families are grown (SUZUKI et al. 2008, RIGOLA et al. 2009, STEPHENSON et al. 2010).

In our winter rapeseed population, we measured an average mutation frequency of 1/15 kb, which is higher as reported for most *Brassicaceae* like *Arabidopsis* (1/170 kb, GREENE et al. 2003), *B. napus* (1/130 kb and 1/42 kb, WANG et al. 2008), *B. oleracea* (1/447 kb, HIMELBLAU et al. 2009) or *B. rapa* (1/30 kb, STEPHENSON et al. 2010). Our mutation frequency is comparable to hexaploid (*Triticum aestivum*, 1/24 kb) or tetraploid (*T. turgidum* subsp. *durum*, 1/40 kb) wheat (SLADE et al. 2005) or oat (1/20 and 1/40 kb, CHAWADE et al. 2010) suggesting that polyploids can tolerate a higher mutation load due to gene redundancy. This is also a reason to use the comparatively high EMS concentration of 1% for mutagenesis resulting in 130000 mutations/plant in our 'Express' population. The number of mutations was substantially lower in the 'YN01-429' population, however, with different EMS concentrations (0.5-1.2% EMS; 40000 mutations/plant). Likewise, much lower mutation frequencies have been reported for EMS treated populations of *B. rapa* 'R-o-18' (2C=2n) (0.3/0.4% EMS; 20000 mutations/plant, STEPHENSON et al. 2010) and *B. napus* 'Ningyou7' (0.6% EMS; 29000 mutations/plant, WANG et al. 2008).

Three *Brassica* TILLING platforms have been published so far. They differ substantially from our TILLING platform with regard to size and screening efficiency. Mutant populations of the diploid species *B. rapa* and *B. oleracea* were screened by a standard 4× or 5× pooling strategy (HIMELBLAU et al. 2009, STEPHENSON et al. 2010). To avoid the selection of locus specific primer combinations, WANG et al. (2008) screened single DNA samples of a *B. napus* M_2 population subtracting natural SNPs for mutant detection. In contrast, we applied a 2D-8× pooling strategy in combination with locus specific primers. This protocol is much more efficient for gel based mutant detection, as it drastically reduces the scoring of false positive fragments due to background and *Taq* polymerase error rate and it enables mutation detection of all orthologous or paralog sequences of a polyploid genome.

The method of choice for accurate measurement of mutation frequencies throughout the whole genome seems to be re-sequencing of EMS mutants and wild type. Here, we were able to show by indirect means, i.e. correlation of mutation events to G/C residues, that there is strong evidence for a random distribution of mutations within genes. We did not find any evidence for individual hotspots for EMS mutations in the *B. napus* genome.

Some recent publications describe alternative techniques for detecting mutations in large populations of tomato (*Solanum lycopersicum*) like Conformation Sensitive Capillary Electrophoresis (CSCE), High Resolution DNA Melting Analysis (HRM) (GADY et al. 2009), and Next Generation Sequencing (NGS) (RIGOLA et al. 2009, TSAI et al. 2011). All these technical alternatives have in common that they avoid the laborious CEL I digestion and LI-COR gel electrophoresis and offer a higher and faster sample throughput. However, with one exception (TSAI et al. 2011) the sequences to be analyzed were much smaller (<400-600 bp) which requires the development of a two to three times higher number of locus specific TILLING amplicons to attain the same gene coverage in a candidate gene. GADY et al. (2009) found an average mutation frequency of 1/737 kb after screening an M_2 population (1% EMS) of *Solanum lycopersicum* by CSCE and HRM. However, they detected a high percentage of false positives which required much proof reading and re-screening activity. In conclusion, they regarded the 'classical' LI-COR method to be more sensitive. This was in line with the NGS technique using the GS FLX 454 (RIGOLA et al. 2009) where a lower mutation rate (1/431 kb) was found as compared to the classical approach (1/322 kb, MINOIA et al. 2010). Another technical improvement has been described recently by TSAI et al. (2011) who used TILLING amplicons <1500 bp for DNA library construction followed by Illumina sequencing based mutation detection. Their results were in line with 'classical' screening methods. However this study was suffering from a very small population size of only 768 plants tested, an underrepresentation of GC rich regions after Illumina sequencing and a loss of rare heterozygous mutants due to statistical noise. The advantage of a faster screening procedure by NGS contrasts to an increased statistical and bioinformatics input to analyze the sequence reads. In addition, our TILLING method is still very cost-effective allowing the screening of 3500 plant DNA samples (1500 bp amplicon) for ~1000 €. Notwithstanding, further technical improvement is needed in the future to facilitate mutant detection in large populations.

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Note: *The online version of the proceedings includes figures in colour. It can be either downloaded from the homepage of the meeting (http://www.raumberg-gumpenstein.at/ - Downloads - Fachveranstaltungen/Tagungen - Saatzüchtertagung - Saatzüchtertagung 2013) or requested from the corresponding author.*

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High throughput analysis of grain quality parameters for hybrid rye breeding

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Abstract

Rye (*Secale cereale* L.) is an invaluable part of crop rotation systems and contributes to increase crop species diversity mainly in European agroecosystems. This small grain cereal is a multi-purpose crop traditionally used in the production of bread or mixed animal feeds and is established as a renewable resource for bioenergy production. Regarding grain quality parameters, these versatile uses of rye require very diverging breeding goals. However, an efficient phenotyping of grain quality parameters, particularly with respect to the content of arabinoxylans as the predominant dietary fibre in the rye grain, is currently the limiting factor in rye breeding.

We have used a near infrared spectroscopy (NIRS) calibration to predict grain quality parameters in experimental rye hybrids. This NIRS calibration was adjusted by a set of 63 selected grain samples from 320 experimental hybrids, which were cultivated in 2011 in two replicates and five environments located in Germany and Poland. After non-destructive NIRS scans of the 3200 samples with two replicates each, the concentration of crude proteins (CP), starch (STC), water-extractable arabinoxylans (WEAX) as well as total arabinoxylans (TAX) was assayed as recently described. Thousand grain weight (TGW) of these samples was included in our analysis as an additional parameter.

The cross-validated NIRS calibration allowed to predict the content of CP, STC, WEAX and TAX in rye grains with unprecedented accuracy. The experimental hybrids revealed significant phenotypic variation for each of the assessed traits. All phenotypic data did not significantly deviate from normal distribution. Coefficients of phenotypic correlation among traits were significant ($P<0.05$) for every trait combination. A strong negative correlation ($r= -0.95$) could be observed between grain protein and starch content. The intensive phenotyping led to high heritability estimates for all traits including water-extractable arabinoxylan content.

We have applied an association mapping approach to unravel the genetic architecture of grain quality parameters in rye. For this purpose, we have tested 1511 DArT markers with an allele frequency >5% in the 320 experimental hybrids for associations with quality traits. The squared correlation of allele frequencies (r^2) representing linkage disequilibrium (LD) were assessed for 483 DArT markers, which we were able to integrate in the recently published transcript map of rye. A critical value of r^2, beyond which LD is likely to be caused by genetic linkage, was estimated at 0.13 for all experimental hybrids. Average LD was observed to decay below the critical level within a map distance of 5-7 cM. We have identified 502 significant ($P<0.05$) marker trait associations of which 159 (32%) have known map positions. QTL for CP (31), STC (26), WEAX (27), TAX (27) and TGW (48) are located on each of the seven rye chromosomes. In total 18 QTL for CP and STC are co-localized, which is consistent with the observed strong negative phenotypic correlation between both parameters. The identified marker/trait associations provide a first step towards a targeted molecular characterization and utilization of genetic resources for precision breeding of hybrid rye varieties with defined grain qualities.

Keywords

Arabinoxylan, DArT marker, NIRS, *Secale cereale*

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High-throughput screening for protein content in blue, yellow and white lupins

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Abstract

Different lupin species (blue lupins, *L. angustifolius*; yellow lupins, *L. luteus*; white lupins, *L. albus)* were analyzed for the protein content in the growing seasons 2010 and 2012. As a reference method for the determination of the protein content, the Kjeldahl method was used. Based on these data a high-throughput screening method for whole lupin seeds was developed using NIRS analyses on a Bruker Multi Purpose Analyzer. The best calibration, cross validation, and prediction of independent samples was observed for whole seeds of blue lupins (RMSECV=0.924, R^2=0.82) followed by yellow (RMSECV=1.05, R^2=0.67) and white lupin seeds (RMSECV=1.08, R^2=0.65).

Keywords

Lupinus albus, *Lupinus angustifolius*, *Lupinus luteus*, NIRS, quality, whole seeds

Introduction

The high protein content in lupin seeds is important for animal feed as well as for human nutrition. Yellow and white lupins have a higher protein content than blue lupins, but still need to be improved with respect to agronomic performance (WEHLING et al. 2012). For all lupin species a high protein content is an important breeding goal. Up to now NIRS methods using ground whole meal (JANSEN et al. 2006, BERK et al. 2008) and whole seeds are described (JANSEN et al. 2006, JANSEN and KUHLMANN 2007). To be able to screen efficiently for the protein content within lupin breeding programmes, NIRS methods facilitating a precise determination of the protein content in different lupin species have to be developed.

Material and methods

Plant material

Seeds of two varieties ('Boruta' and 'Haags Blaue') and six breeding lines of blue lupin grown at the location Bocksee in four replications, sixteen breeding lines grown in Steinach in two replications and eight breeding lines grown in Groß Lüsewitz in two replications were analysed in 2010. Seeds of twelve varieties ('Taper', 'Borena', 'Boresa', 'Borsaja', 'Juno', 'Pootallong', 'Popiel', 'Parys', 'Wasch', 'Bosch', 'Amulett' and 'Piast') and four breeding lines of yellow lupin grown in three locations (Groß Lüsewitz, Steinach and Triesdorf) in three replications were analyzed in 2012. The seeds of blue and yellow lupins were provided by Saatzucht Steinach.

Seeds of two varieties ('Àmiga' and 'Feodora') and fifteen breeding lines of white lupin grown at three locations (Groß Lüsewitz, Steinach and Triesdorf) in three replications were analyzed in 2012. Seeds of white lupins were provided by Landwirtschaftliche Lehranstalten, Pflanzenbau und Versuchswesen Triesdorf.

Methods

The chemical determination of raw protein content was conducted using the Kjeldahl method (KJELDAHL 1883). For the development of a high-throughput screening method for the determination of the protein content in different lupin species, a NIRS method was used. Seed samples were scanned in duplicate with a Bruker-Fourier-Transform-Spectrometer Multi Purpose Analyzer (MPA, Bruker Optik GmbH, Ettlingen, Germany) equipped with the OPUS software package (Version 6.5). Absorption spectra were recorded between 800 and 2500 nm, using 32 scans per sample. To develop calibration models, the OPUS Quant multivariate calibration software of Bruker Optik incorporating Partial Least Squares (PLS) regressions was used to develop calibration models.

Results and discussion

The mean value and the variation in the protein content of different lupin species is described by JANSEN and BALKO (2012). In the present study yellow lupins had the highest seed protein content followed by white lupins and blue lupins. The variation and mean values of the investigated lupin seeds using the Kjeldahl-method is shown in *Table 1*. Assuming moisture content of the seeds of approximately 10% the values are similar to those already reported. The protein content of the investigated blue, white and yellow lupin seeds varied between 27 and 40% (mean: 33%), 32 and 43% (mean: 39%) and 38 and 48% (mean: 43%), respectively, in whole dry seeds.

Table 1: **Protein content (% in DM) of different lupin species**

Species	*n*	Mean	Variation
L. angustifolius	79	33	27-40
L. albus	147	39	32-43
L. luteus	144	43	38-48

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Table 2: **Results of calibration and validation data of protein prognosis on different whole lupin seeds**

Species	Parameter	Calibration	Validation	Test-Set-Validation
L. angustifolius	R^2	0.93	0.82	0.76
	RPD	3.86	2.36	2.08
	Error	RMSEE 0.585	RMSECV 0.924	RMSEP 1.14
L. luteus	R^2	0.70	0.67	0.63
	RPD	1.83	1.73	1.65
	Error	RMSEE 1.01	RMSECV 1.05	RMSEP 1.13
L. albus	R^2	0.76	0.65	0.57
	RPD	2.04	1.68	1.52
	Error	RMSEE 0.908	RMSECV 1.08	RMSEP 1.15

R^2, coefficient of determination; RMSEE, root mean square error of calibration; RMSECV, root mean square error of cross validation; RMSEP, root mean square error of prediction; RPD, ratio of sample standard deviation to standard error of prediction

An effective NIRS-method for determining the protein content in *L. angustifolius* with high precision was described by JANSEN and KUHLMANN (2007) using single seeds as a matrix (transmission measurements, Infratec® 1255, Fa. Foss). Another method was presented by JANSEN et al. (2006) using whole meal and whole seeds (reflection measurements, NIRS™ 5000, Fa. Foss). Reflection measurements on whole seeds were also applied in this paper. Data concerning quality of the calibration and validation experiments are presented in *Table 2*.

Best results were obtained for the prediction of the protein content in whole seeds of blue lupins, which RMSECV=0.924 and R^2=0.82. The use of NIRS-methods for the determination of protein content is well known in other species. An overview about applications in e.g. wheat quality control (breeding, production, trade, milling) is given by POJIĆ et al. (2012). NIRS methods have been accepted as standard methods by the ISA, AACC, AOAC and ICC. A calibration for predicting protein in single kernel of barley (Validation R^2=0.84) was reported by FOX et al. (2011). MÍKA et al. (2003) described the prediction of the protein content in whole rape seed (Validation R^2=0.83, VDLUFA network).

In experiments to predict the digestible protein of lupin kernel meal GLENNCROSS et al. (2008) determined a RMSECV=2.7% and R^2=0.47. In comparison to these experiments it may be concluded that the NIRS calibration developed in this study can be efficiently used for screening the protein content in lupin breeding programs.

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New resources and approaches for gene cloning in cereals

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important crops, but due to its size (17 Gb) and complexity (allohexaploid and over 80% repetitive elements), its genome represents a challenge for mapping, sequencing, gene cloning and marker assisted breeding. To facilitate wheat genomics, the genome of 'Chinese Spring' has been dissected to particular chromosomes and chromosomal arms by flow cytometric sorting. Flow sorted chromosomes found a large portfolio of applications, including physical mapping using PCR, cytogenetic mapping, protein immunolocalization, chromosome ultrastructure, development of DArT markers, linear DNA amplification suitable for DNA markers development, mapping on DNA arrays, and next generation sequencing. Out of variety of applications, the most important and most demanding application has been the construction of chromosome-specific BAC libraries. In the effort to sequence wheat genome, coordinated by the International Wheat genome Sequencing Consortium (IWGSC, www.wheatgenome.org), this allowed sharing the job between laboratories around the world. Physical maps constructed from the chromosomal specific libraries already facilitate marker development and gene cloning. One example is the cloning of powdery resistance gene *QPm-tut-4A* introgressed to hexaploid wheat from tetraploid wheat *T. militinae* Zhuk & Migush. The gene was mapped to 4AL chromosomal arm. However, recombination suppression in the gene region was observed and marker order in the gene locus could not be resolved by traditional approaches. To overcome this difficulty, a combination of traditional approaches and recent advances in wheat genomics were used. For marker ordering, 4AL-specific radiation hybrid panel and three additional recombination mapping populations were used. To facilitate marker development, 4AL-chromosome specific BAC library was constructed, fingerprinted and ordered to contigs. The assembly of 4AL shotgun sequence was used to construct GenomeZipper (virtual gene order along the chromosome) and all genes from the collinear regions were mapped to our mapping population. Marker development was facilitated using DNA amplified from flow-sorted chromosome arm 4AL of 'Chinese Spring' and the same arm carrying the *T. militinae* translocation. Using these resources, the *QPm-tut-4A* gene was delimited to 0.25 cM region flanked with markers *gpw356* and *Mag974*.

The flanking markers were used to anchor the region with contigs of the 4AL physical map. The anchoring was facilitated by sequencing 3D pools of MTP from whole 4A physical map. Any marker sequence can be anchored in this way to the 4AL physical map. Using sequences of the *QPm-tut-4A* flanking markers, three large contigs spanning the *QPm-tut-4A* gene region and the flanking regions were identified and the sequence of their MTP will be used to identify candidate *QPm-tut-4A* gene/genes. The physical map of 4AL and sequences of the MTP 3D pools are becoming very important genomic resource not only for the *QPm-tut-4A* gene cloning, but also for other agronomically important genes. We have provided complete physical map of gene loci for three additional genes affecting pre-harvest sprouting, gene affecting yield and resistance locus *Yr51*.

Keywords

Chromosome translocation, DArT marker, physical map, *Triticum aestivum*, wheat genome

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Development of marker for resistance genes by using next generation sequencing technologies

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Abstract

By using high trough-put sequence technologies it is possible to sequence whole genomes in a cost efficient way. Therefore in a current project we aim to index the diversity of all major resistance genes (*R* genes) within the genepool of *Solanum* with emphasis on the common potato (*S. tuberosum*). The goals of the project are to explore and catalog nearly all diverse *R* alleles (*R* haplotypes) conferring disease and pest resistance, at the genetic loci of *R* genes belonging to the NBS-type meta-family in *Solanum* (with emphasis on the common potato), to determine in a large-scale approach *R* allele fragments by their association with the resistance phenotype, to explore evolutionary, structural, and diversification aspects of the plant *R* genes and to set up a method for the development of molecular tools (markers; sets of PCR primers) that can be used in research and by plant breeders, for fast tracking of *R* alleles conferring resistance to pathogens and pests in *Solanum*.

For this study 96 potato samples from different breeding programs and gene banks were collected. For the NBS profiling new primer for the NBS domain were developed and tested. For the amplification six different primers tagging the p-loop motif, 3 primers tagging the kinase 2 motif and 4 primers for the GLPL motif were used. The obtained amplification products were sequences using the HiSeq (Illumina) machine at GATC Biotech (Germany). The obtained sequences were analysed using standard bioinformatics tools and aligned to the potato reference sequence (PGSC_DM_v4.03_pseudomolecules) with NextGenMap 0.4.4. The obtained data will be available in a database.

To prove our concept we selected parents from a breeding program where one parent 'Alegria' carries a new PVY virus resistance gene. To localize the resistance gene on the genetic linkage map microsatellite markers were applied to the population. The raw linkage map was calculated using TetraploidMap. The PVY resistance was linked to SSR markers from chromosome 9. Therefore to find a NBS region linked to the resistance the search for SNPs among the two parents were concentrated on chromosome 9. Several markers based on the NGS data were tested and all markers were placed on chromosome 9 in the right order according to the reference genome. Until now no linked marker to the PVY resistance could be found. New primer for different position on chromosome 9 will be further tested.

Keywords

Microsatellite marker, potato, *Solanum tuberosum*

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Genomic selection of wheat - First experiences

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Abstract

Genomic selection (GS) was first successfully applied in animal breeding and has since become the predominate way of breeding cattle. GS identifies individuals that have superior genetic breeding values based on their marker profiles and holds promise to reduce breeding cycles in order to improve gain per year. Only recently GS found its way into plant breeding and just a few studies are available that report on the use of GS in real plant breeding situations. Here we present our first experiences when applying this new paradigm in a breeder's current breeding scheme.

A total of four GS models, which predict breeding values for yield, protein-yield, *Fusarium graminearum* resistance and rust resistance were developed based on a training population of about 700 elite winter wheat lines. The lines were genotyped using the DArT-Seq genotyping platform and the marker information co-analysed together with several thousand phenotypic records that were collected over several years and locations. The phenotypes were adjusted for linear trends per environment using linear mixed models. We treated the phenotypes as random effects in the mixed model equations thus estimating the best linear unbiased prediction per line. To assess the influence of non-additive (e.g. epistatic) effects a series of different statistical models were implemented and their relative performance evaluated. The accuracy of a specific model was estimated as the correlation between the observed phenotypes and the predicted phenotypes. We used 10-fold stratified cross-validation as well as a test set of 167 lines for which initial phenotypes were available.

The accuracy of the model predictions generated by cross-validation agrees well with published literature. For the additive effects model it ranged from 0.46 for protein-yield to 0.66 for yield, which is high: In other studies accuracies above roughly 0.3 led to an increased relative efficiency compared to traditional breeding efforts.

When comparing different statistical models we used a reduced training set of 585 lines and compared models that were commonly used in literature. The models, which differ in computation time as well as their ability to account for gene interactions or epistatic effects.

We found that the simple additive model rr-BLUP is well-suited in terms of computational speed as well as accuracy (0.51). Bayesian models achieve the same accuracy as the rr-BLUP model, whereas support vector machines lag behind. Random forest, a statistical model that can account for non-additive effects, achieved the highest accuracy of 0.57 but at the cost of computation time.

To estimate the relative efficiency of one selection cycle of GS compared to one selection cycle of traditional breeding, we selected 70 lines based on their genetic estimated breeding values and 70 lines that have been selected in traditional breeding programs from the pool of 800 lines. Furthermore, we chose 25 lines randomly to assess the gain from either selection method. The results of this comparison are due for summer 2015.

Keywords

BLUP, DArT sequencing, *Triticum aestivum*, yield

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Optimierung der Entwicklung von Introgressionspopulationen

Optimizing the development of introgression populations

Eva Herzog[1*]

Abstract

Introgression populations are valuable genetic resources which are developed by marker-assisted backcrossing. Their development is labor-intensive, costly and time consuming, as commonly numerous backcrosses and large population sizes are required. Crossing and selection schemes which are suitable for a limited number of individuals and high-throughput marker analyses would therefore greatly improve the efficiency of this process. We employed computer simulations to design selection strategies for the development of maize introgression populations of 100 lines with population sizes of 360 individuals per backcross generation for DH and S_2 crossing schemes. The number of simultaneous backcross programs was reduced by pre-selection for complete donor chromosomes or donor chromosome halves. Selection for complete donor chromosomes in generations BC_1 and BC_2 was the most efficient strategy if no selection was conducted in generation BC_3. With an additional selection step in generation BC_3, selection for donor chromosome halves improved the separation of target segments. Gradually increasing population sizes over backcross generations was advantageous for both DH and S_2 crossing schemes. For DH schemes, this approach reduced the total number of required high-throughput marker analyses. For S_2 crossing schemes, large population sizes in the final backcross generation enabled selection for short target segments and thus reduced the fixation of large donor chromosome segments. The suggested crossing and selection schemes provide guidelines for breeders which help to make the development of introgression populations more efficient.

Keywords

Computer simulation, high-throughput marker analysis, introgression library, marker-assisted backcrossing, *Zea mays*

Einleitung

Introgressionsbibliotheken sind Sets nahe-isogener Linien (NILs), die kurze, durch Marker kontrollierte Segmente des Genoms eines exotischen Donors in einem gemeinsamen genetischen Hintergrund tragen (ESHED und ZAMIR 1994). Sie sind nützliche Ressourcen sowohl für die Identifikation neuer Allele mit vorteilhaften Eigenschaften, als auch für den anschließenden Zuchtprozess, da die neu gefundenen Allele bereits in einem adaptierten Hintergrund vorliegen.

Introgressionsbibliotheken werden für gewöhnlich durch marker-gestützte Rückkreuzung entwickelt, der sich Selbstung oder die Induktion doppelt haploider (DH) Linien anschließt. Die Rückkreuzungsprogramme für ihre Entwicklung sind arbeits- und zeitaufwendig, wenn eine komplette Abdeckung des Donorgenoms durch kurze, gleichmäßig verteilte Chromosomensegmente angestrebt wird, da dann separate Rückkreuzungsprogramme für jedes der Zielsegmente erforderlich sind. Trotz des hohen Aufwands bei der Erstellung wird häufig nur eine unvollständige Abdeckung des Donorgenoms erreicht (SCHMALENBACH et al. 2008, FALKE et al. 2008).

Ein möglicher Ansatz zur Kosteneinsparung ist, die Anzahl der simultan durchgeführten Rückkreuzungsprogramme zu reduzieren, indem während der Rückkreuzung zunächst Individuen vorselektiert werden, die jeweils ein komplettes Donorchromosom tragen (FALKE et al. 2009). Wird dieses Konzept angepasst auf Rückkreuzungsprogramme mit begrenzten Ressourcen, lassen sich Introgressionspopulationen entwickeln, die aber noch einige zusätzliche Donorsegmente im genetischen Hintergrund tragen. Solche Introgressionspopulationen können jedoch mit weniger Individuen und Markeranalysen entwickelt werden. Für diese Introgressionspopulationen sollte als Mindeststandard möglichst komplette Abdeckung des Donorgenoms und gleichmäßig Verteilung der Zielsegmente angestrebt werden.

Die Entwicklung von Linien durch *in vivo* Induktion doppelt haploider (DH) Linien wird routinemäßig in Maiszüchtungsprogrammen angewandt. Dennoch gibt es bislang keine Richtlinien, wie sich diese Technologie bei der Entwicklung von Introgressionsbibliotheken effizient einsetzen lässt, und was im Unterschied zur immer noch kostengünstigeren Selbstung zu beachten ist. Die Ziele unserer Simulationsstudie waren daher die Entwicklung geeigneter Kreuzungs- und Selektionsschemata für die Entwicklung von Introgressionspopulationen mit begrenztem Kontingent an Rückkreuzungsindividuen und Hochdurchsatzchips, und die Erstellung von Richtlinien für das optimale Versuchsdesign für DH- und S_2-Kreuzungsschemata.

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Material und Methoden

Genetisches Modell

Computersimulationen wurden durchgeführt für ein genetisches Maismodell mit 10 Chromosomen von 200 cM Länge. Molekulare Marker für die Selektion waren gleichmäßig mit einer Dichte von einem Marker alle 1 cM im Genom verteilt. Es wurde angenommen, dass die Genotypisierung mit Hochdurchsatzchips durchgeführt wird und alle Marker im Genom in einem Analyseschritt genotypisiert werden können.

Kreuzungsschemata

Es wurden BC_3DH- und BC_3S_2-Kreuzungsschemata untersucht. In den Generationen BC_1-BC_3 erfolgte die Selektion einer festen Anzahl bester Individuen anhand eines Selektionsindexes. Diese Individuen wurden, je nach Generation, rückgekreuzt oder zur Linienentwicklung mittels Selbstung oder *in vivo* DH-Induktion verwendet. Die resultierenden Introgressionspopulationen bestanden jeweils aus 100 NILs.

Selektionsindizes

Die NILs sollten je ein Zielsegment des Donorgenoms von 20 cM Länge tragen. Um den Selektionsindex i für ein Individuum in Bezug auf ein bestimmtes Zielsegment zu bestimmen, wurde mit t_c der Anteil Donorgenom in Prozent auf dem Chromosom, auf dem das Zielsegment sich befindet, bezeichnet. Mit t_h wurde der Anteil Donorgenom in Prozent auf der Chromosomenhälfte, auf der das Zielsegment sich befindet, bezeichnet. Mit t_s wurde der Anteil Donorgenom in Prozent im Zielsegment selbst bezeichnet. Die Werte für den genetischen Hintergrund b_c, b_h, b_s sind komplementär zu t_c, t_h und t_s und bezeichnen den Anteil an Rezipientengenom außerhalb der jeweiligen Selektionsregion. Bei der Selektion auf ganze Chromosomen wurde für jedes Chromosom $c = 1, 2, \ldots, 10$ eine feste Anzahl bester Individuen für den Selektionsindex $i = t_c + b_c$ selektiert. Für Selektion auf Chromosomenhälften $h = 1,2, \ldots, 20$ oder Zielsegmente $s = 1, 2, \ldots, 100$ wurde anhand der Indizes $i = t_h + b_h$ und $i = t_s + b_s$ analog verfahren.

Selektionsstrategien

Selektion auf ganze Chromosomen (C), auf Chromosomenhälften (H) und auf Zielsegmente (S) wurden in den Generationen BC_1-BC_3 kombiniert, um verschiedene Selektionsstrategien zu bilden. Die untersuchten Kombinationen von Kreuzungsschema und Selektionsstrategie sind in der ersten Spalte von *Tabelle 1* aufgelistet. Für alle Selektionsstrategien wurden die besten 100 NILs für den Selektionsindex $i = t_s + b_s$ in Generation DH oder S_2 selektiert.

Simulationsreihen

In der ersten Simulationsreihe wurde Selektion nur in den Generation BC_1 und BC_2 durchgeführt. In der zweiten Simulationsreihe wurde zusätzlich Selektion in Generation BC_3 durchgeführt. Die Gesamtpopulationsgröße pro Generation in der ersten und zweiten Simulationsreihe war konstant und betrug $n_{tot} = 360$ Individuen. In der dritten Simulationsreihe wurden optimierte Kreuzungsschemata mit Selektion in allen drei Rückkreuzungsgenerationen und ansteigenden Populationsgrößen untersucht.

Ergebnisse und Diskussion

Mit fast allen untersuchten Kombinationen aus Kreuzungsschema und Selektionsstrategie wurde eine Abdeckung des Donorgenoms ≥ 99% erzielt (*Tabelle 1*). Diese Werte bezogen sich allerdings nicht nur auf die Zielsegmente, sondern beinhalteten auch zusätzliche unerwünschte Donorsegmente. Die Abdeckung des Donorgenoms war daher nicht ausreichend, um die Qualität einer Introgressionspopulation zu beschreiben. Erhebliche Unterschiede zwischen den Introgressionspopulationen wurden deutlich, wenn der Gesamtdonorgenomanteil, der Donorgenomanteil auf dem Chromosom, welches das Zielsegment trägt, und der durchschnittliche Donorgenomanteil in den Zielsegmenten betrachtet wurden.

Tabelle 1: **Donorgenomabdeckung (Cov), Gesamtdonorgenomanteil (DGP_{tot}), Donorgenomanteil auf den Trägerchromosomen (DGP_{CC}), Donorgenomanteil in den Zielsegmenten (DGP_{TS}) und benötigte Anzahl von Hochdurchsatzchips (HT) für verschiedenen Strategien zur Entwicklung von Introgressionspopulationen bei Mais** (Mittelwerte über 1000 Replikationen der Simulation)

Table 1: **Coverage of the donor genome (Cov), total donor genome proportion (DGP_{tot}), donor genome proportion of the carrier chromosomes (DGP_{CC}), donor genome proportion of the target segments (DGP_{TS}) and required number of high-throughput chips (HT) for different strategies for developing introgression populations in maize** (arithmetic means over 1000 replications of the simulation)

Simulationsreihe	Strategie	Cov	DGP_{tot} (%)	DGP_{CC} (%)	DGP_{TS} (%)	HT
1	BC_3DH-CC	99.2	5.0	35.2	97.8	1080
	BC_3DH-HH	99.8	5.1	33.3	94.2	1080
	BC_3DH-CH	99.6	5.1	35.1	93.7	1080
2	BC_3DH-CCC	99.1	4.8	35.8	98.0	1800
	BC_3DH-HHH	99.9	4.7	33.9	98.9	1800
3	BC_3DH-HHH*	99.9	5.0	33.3	98.4	1440
1	BC_3S_2-CC	99.3	5.7	39.2	97.0	1080
	BC_3S_2-HH	99.9	5.3	33.3	90.3	1080
	BC_3S_2-CH	99.7	5.3	35.0	89.9	1080
2	BC_3S_2-CCC	98.3	5.7	44.6	97.5	1440
	BC_3S_2-HHH	99.7	5.0	38.1	98.6	1440
3	BC_3S_2-HHS*	99.8	4.3	30.4	96.2	1400

Ein hoher Donorgenomanteil auf den Trägerchromosomen deutet auf unerwünschte Donorgenomsegmente hin, die nicht von den Zielsegmenten getrennt werden konnten. Solche großen Donorsegmente in Nachbarschaft zu den Zielsegmenten können die Detektion von QTL beeinträchtigen. Darüber hinaus bergen große Donorgenomsegmente das Risiko, dass unerwünschte Eigenschaften des Donors übertragen werden, und erfordern oft zusätzliche Rückkreuzungsschritte zur Abtrennung der Zielsegmente. Der Donorgenomanteil auf den Trägerchromosomen sollte daher niedrig sein. Innerhalb der Zielsegmente sollte der Donorgenomgehalt hingegen so hoch wie möglich sein, um die gesamte Bandbreite an genetischer Variation im Donor zu erfassen.

Die Selektionsstrategie ist der wichtigste Faktor für die Verteilung und Länge der Donorsegmente in Introgressionspopulationen. Selektionsstrategien, die zunächst Individuen vorselektieren, die ganze Donorchromosomen tragen, reduzieren zwar die Anzahl der Rückkreuzungsprogramme im Vergleich zu Selektionsstrategien, die direkt auf Zielsegmente selektieren. Bei langen Chromosomen von 200 cM Länge oder mehr bergen sie jedoch das Risiko, dass große Teile des Donorgenoms auf dem Zielchromosom bis zur Linienentwicklung konserviert und dann fixiert werden. Zusätzlich zu Selektion auf ganze Chromosomen mit den Selektionsstrategien CC und CCC wurde daher mit den Selektionsstrategien HH und HHH auch Selektion auf Chromosomenhälften untersucht. Damit wurde eine Reduktion des Donorgenomanteils auf den Trägerchromosomen von bis zu 6,5% erreicht, während die Maßzahlen für den genetischen Hintergrund in etwa gleich blieben. Selektion auf Chromosomenhälften in den Rückkreuzungsgenerationen ist daher von Vorteil für Kulturarten mit langen Chromosomen, wie z.B. Mais, Weizen oder Raps, da dadurch das Risiko der Übertragung unerwünschter Eigenschaften vom Donor verringert wird.

Wenn keine Selektion in Generation BC_3 durchgeführt wurde, führte Selektion auf Chromosomenhälften jedoch zu einer beträchtlichen Reduktion des Donorgenomanteils in den Zielsegmenten von 3,6-7,1%, was auf häufigeren Verlust der Zielsegmente hindeutet. Dies ist vermutlich den sehr kleinen Populationsgrößen von $n = 18$ Individuen zuzuschreiben, die mit der Unterteilung der konstanten Gesamtpopulationsgröße von $n_{tot} = 360$ Individuen in 20 Subpopulationen für die Chromosomenhälften einhergeht.

Der gleiche Verlust von Zielsegmenten wurde auch für die kombinierte Selektionsstrategie CH beobachtet, bei der in Generation BC_1 auf ganze Donorchromosomen und in Generation BC_2 auf Chromosomenhälften selektiert wurde. Zusätzlich wurde bei dieser Selektionsstrategie ein ähnlich hoher Wert für den Donorgenomanteil auf dem Trägerchromosom beobachtet wie bei ausschließlicher Selektion auf ganze Chromosomen. Dies ist vermutlich auf die effiziente Selektion auf ganze Chromosomen aus der verhältnismäßig großen BC_1 Population von $n_{tot} = 360$ Individuen zurückzuführen. Die selektierten ganzen Donorchromosomen blieben zum großen Teil bis zur Linienentwicklung erhalten. Die Selektionsstrategie CH vereint daher die Nachteile von Selektion auf ganze Donorchromosomen mit den Nachteilen von Selektion auf Donorchromosomenhälften und ist nicht empfehlenswert für Rückkreuzungsprogramme mit kleinen, konstanten Gesamtpopulationsgrößen. Für kleine Rückkreuzungsprogramme mit Gesamtpopulationsgrößen von $n_{tot} = 360$ Individuen ohne Selektion in Generation BC_3 ist daher Selektion auf ganze Donorchromosomen in den Generationen BC_1 und BC_2 die günstigste Lösung.

Für effektive Reduktion des Donorgenomanteils auf den Trägerchromosomen durch Selektion auf Chromosomenhälften ohne Verlust der Zielsegmente muss die Anzahl der Träger von Zielsegmenten für die Linienentwicklung erhöht werden. Ein möglicher Lösungsansatz für dieses Problem wäre ein zusätzlicher Selektionsschritt in Generation BC_3. Während dieser für die S_2-Kreuzungsschemata ohne Probleme möglich ist, da durch Selbstung viele Linien aus einem Rückkreuzungsindividuum erzeugt werden können, kann mit *in vivo* Induktion von DH Linien oft nur eine Linie aus einem Rückkreuzungsindividuum gewonnen werden. In den Kreuzungsschemata ohne Selektion in Generation BC_3 war ein direkter Vergleich der Ergebnisse zwischen DH- und S_2-Krezungsschemata bei gleichem Einsatz von Individuen und Hochdurchsatzchips möglich. In diesem Fall waren die DH-Kreuzungsschemata den S_2-Kreuzungsschemata überlegen, was vor allem auf die komplette Homozygotie der DH Linien zurückzuführen ist.

Für Selektion in Generation BC_3 wurde in den DH-Kreuzungsschemata eine Verdopplung der Populationsgröße vorgenommen, und nur die bessere Hälfte der Individuen wurde für die DH-Induktion selektiert. In den S_2-Kreuzungsschemata wurde die konstante Populationsgröße beibehalten und nur ein bestes Individuum für den jeweiligen Selektionsindex selektiert. Dieser Unterschied zwischen DH- und S_2-Kreuzungsschemata zeigt sich nicht nur in der Anzahl der benötigten Hochdurchsatzchips, sondern führt auch zu einem Unterschied in der Selektionsintensität. Es ist daher zu erwarten, dass unterschiedliche Selektionsstrategien für diese Formen der Linienentwicklung optimal sind.

Durch den zusätzlichen Selektionsschritt in Generation BC_3 wurden die Maßzahlen für den genetischen Hintergrund kaum verändert, der Donorgenomanteil in den Zielsegmenten wurde jedoch verbessert. Dies war besonders deutlich für Selektion auf Chromosomenhälften mit Selektionsstrategie HHH, bei der eine Verbesserung von bis zu 8,6% beobachtet wurde. Insgesamt lieferte Selektion auf Chromosomenhälften jetzt die besseren Ergebnisse. Für das beste DH-Kreuzungsschema BC_3DH-HHH fiel die Verbesserung nur sehr gering aus im Vergleich zu Schema BC_3DH-CC ohne Selektion in Generation BC_3. Dafür mussten jedoch 720 zusätzliche Hochdurchsatzchips aufgewendet werden. In den S_2-Kreuzungsschemata ging der zusätzliche Selektionsschritt in Generation BC_3 mit einem stark erhöhten Donorgenomanteil auf den Trägerchromosomen von 38-48% einher. Dies deutet auf Fixierung der Selektionsregionen der letzten Rückkreuzungsgeneration hin. Selektion in Generation BC_3 hat daher das Potential, die Abdeckung der Zielsegmente zu verbessern. Für DH-Kreuzungsschemata ist sie jedoch nur effizient, wenn die Anzahl der benötigten Hochdurchsatzchips reduziert werden kann. Für die S_2-Kreuzungsschemata muss die Fixierung großer Donorchromosomensegmente vermieden werden.

Bei konstanten Gesamtpopulationsgrößen von n_{tot} = 360 Individuen pro Generation ist die Populationsgröße in Generation BC_1, in der noch keine Unterteilung in Subpopulationen vorgenommen wird, relativ groß im Vergleich zum Selektionsgewinn, der durch Selektion einer relativ kleinen Fraktion von 10 oder 20 Individuen erzielt werden kann. Es liegt daher nahe, die Populationsgröße in dieser Generation zu reduzieren und die freiwerdenden Ressourcen fortgeschrittenen Generationen zuzuschlagen. Für die DH-Kreuzungsschemata werden so größere Populationsgrößen in Generation BC_3 möglich, ohne dass insgesamt mehr Ressourcen aufgewendet werden müssen als für die S_2-Kreuzungsschemata. Für die S_2-Kreuzungschemata ermöglichen größere Populationsgrößen die Selektion auf Zielsegmente schon in Generation BC_3.

Das Kreuzungsschema BC_3DH-HHH* mit ansteigenden Populationsgrößen führte zu ähnlichen Ergebnissen wie das bislang beste Schema BC_3DH-HHH, benötigte aber 360 Individuen und Hochdurchsatzchips weniger. Im Vergleich zum billigeren Schema BC_3DH-CC wurde der Donorgenomanteil auf den Trägerchromosomen verringert und der Donorgenomanteil in den Zielsegmenten erhöht. Die Investition in die zusätzlichen Hochdurchsatzchips scheint daher lohnenswert. Das Kreuzungsschema BC_3S_2-HHS* mit ansteigenden Populationsgrößen resultierte in der gewünschten Reduktion des Donorgenomanteils auf den Trägerchromosomen von 38% auf 30% im Vergleich zum bislang besten Schema BC_3S_2-HHH, obwohl 40 Hochdurchsatzchips weniger verwendet wurden. Der Donorgenomanteil in den Zielsegmenten wurde zwar um 2,4% reduziert, dafür wurde allerdings das Verhältnis von Donorgenomanteil auf den Trägerchromosomen zu Donorgenomanteil in den Zielsegmenten erheblich verbessert. Für S_2-Kreuzungsschemata ist Selektion auf Zielsegmente in der letzten Rückkreuzungsgeneration daher zu empfehlen. Im Vergleich zum insgesamt besten aber auch teuersten DH-Kreuzungschema BC_3DH-HHH, erreichte das Kreuzungsschema BC_3S_2-HHS* nur leicht schlechtere Werte, brauchte jedoch 400 Hochdurchsatzanalysen weniger. S_2-Kreuzungsschemata bieten daher ökonomische Alternativen zu DH-Kreuzungsschemata.

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CORNET Efficient Wheat: The influence of *Rht-D1* on agronomic performance and quality traits in common winter wheat

Volker Mohler[1*], Manuela Diethelm[1], Adelheid Castell[2], Theresa Albrecht[1], Regina Friedlhuber[1], Maren Livaja[2] and Lorenz Hartl[1]

Abstract

The gibberellic acid-insensitive dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) are widely distributed in wheat breeding programmes due to grain yield benefits associated with the useful reduction in plant height for these mutant alleles.

The donor of both dwarfing genes in most wheat cultivars is 'Norin 10' that inherited dwarfism from the Japanese landrace 'Daruma'. Pleiotropic effects of 'Norin 10' derived dwarfing genes on grain yield and its components have been reported.

The objectives of the present study were to: (1) analyze the influence of the dwarfing gene *Rht-D1* on agronomic performance and quality traits using a bi-parental winter wheat population, and (2) to compare its effects to that of the gibberellic acid-responsive dwarfing gene *Rht8*.

The population Pamier (*Rht-D1b*, *Rht8a*)/Format (*Rht-D1a*, *Rht8b*) consisting of 114 doubled haploid lines along with the parents were grown at three locations (Roggenstein, Feldkirchen and Hadmersleben) in Germany during the 2011/2012 and 2012/2013 cropping seasons, for a total of six environments.

The field trials included the genotypes with two replications, plot sizes of locations ranged between 5.7 m² and 10 m². The population was genotyped using 928 single nucleotide polymorphism (Illumina® Wheat 90k SNP array) and 80 simple sequence repeat markers including markers functional for *Rht-D1* and linked to *Rht8* (*Xgwm261*).

Quantitative trait locus (QTL) analysis revealed significant ($P<0.001$) QTL for plant height (2012 and 2013), ear emergence time (2012), 1000-kernel weight (2012 and 2013), grain yield (2012 and 2013), harvest index, grain protein content, grain protein yield, sedimentation volume and falling number that were strongly linked to *Rht-D1*. At this stage of analysis, data for both growing seasons were only available for plant height, ear emergence time, 1000-kernel weight and grain yield. Whereas the *Rht-D1b* mutant allele was found to increase grain yield at location Hadmersleben in 2012 and 2013 by 4.0 dt·ha^{-1} and 8.4 dt·ha^{-1}, no positive effect of the dwarfing allele on grain yield was observed at south German field sites Roggenstein and Feldkirchen in both years. When compared to the mean of tall sister lines (those carrying the *Rht-D1a* wild-type allele), *Rht-D1b* lines reduced plant height by 21.6% (2012) and 19.4% (2013). In comparison, height reduction associated with *Rht8b* (vs. lines carrying *Rht8a*) averaged 5.4% in 2012 and 5.1% in 2013. An influence of the *Rht-D1b* allele on reduction in 1000-kernel weight was observed and estimated at 4.5% (2012) and 7.3% (2013) of the *Rht-D1a* wild-type allele, whereas the influence of *Rht8* on this character was negligible in both years. In 2012, *Rht-D1b* reduced grain protein content, grain protein yield and sedimentation value by 4.1%, 5.0% and 13.2%, respectively, and improved harvest index and falling number by 4.9% and 11.6%, respectively. In contrast, *Rht8b* showed a slight increase in harvest index (3.0%) without compromising quality traits in bread wheat.

Keywords

Dwarfing genes, QTL, quality traits, SNP, *Triticum aestivum*

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Moving Fields - Using high-throughput phenotyping to select winter barley for the production of biogas

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Abstract

Winter barley (*Hordeum vulgare*) lends itself well for crop rotation, as it can be harvested earlier than most other winter crops. As such, it holds potential to contribute to the production of biogas from whole crop silage. To select candidates promising for further breeding, we compared the biomass production of 48 winter barley varieties. Creating 8 replicated 'fields' per genotype, we planted these varieties in 384 boxes (0.1 m^2×20 cm deep), at a density of 300 plants per m^2. After vernalization, we photographically captured biomass production throughout development using the 'Moving Fields' high-throughput phenotyping installation (LemnaTec, Aachen, Germany). Built into a greenhouse, this installation consists of a conveyor belt system (Bosch Rexroth TS 2 plus; capacity: 390 carriers), 3 measuring stations and 4 photo cabins (LemnaTec Scanalyzer 3d), which together enable fields to be automatically moved, watered, weighted and photographed. Using RGB cameras (2456×2058 px) in visual light range (300-700 nm) and RGB cameras (1390×1038 px) capturing fluorescence, we documented plant growth on a weekly basis. A preliminary comparison using images made by the RGB cameras in the visual light range demonstrated that growth of the 48 genotypes could be traced non-invasively, pointing at genotypic differences in biomass production over time. Analysis of dry weight biomass at harvest confirmed six-rowed genotypes to be generally heavier than two-rowed genotypes. Despite both considerable variation within genotypes and considerable overlap between genotypes, weight differences of various genotypes were statistically significant. Variety 'Titus', for instance, harvested significantly more biomass than all other genotypes except 'Highlight', whereas 'Anisette' developed significantly less biomass than all but three genotypes.

Keywords
Biomass, *Hordeum vulgare*, image analysis, renewable energy

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Impact of climate change on cereal growth and its potential yield

Jan Křen[1*], Lubomír Neudert[1], Petr Hlavinka[1], Petr Martinek[2] and Vladimír Smutný[1]

Abstract

Climate and weather are important factors which influence plant growth and development as well as yield quality and quantity. However, it is very difficult to assess the impact of climate on yield development in field crops, because a series of related positive and negative impacts have to be quantified and these are mutually interactive. An increase of CO_2 can stimulate the intensity of photosynthesis and thereby increase biological yield and economic profit. This 'fertilization effect' of CO_2, however, is dependent on the growth stage of a plant and other factors. With regard to yield formation, the indirect effects of increased temperature, in combination with precipitations and global radiation, are of crucial importance. The increased appearance of risk factors has a negative impact on the utilization of yield potential and production quality of crops. For the prediction of the impact of climate change on the yield of winter wheat and spring barley under Czech conditions the model CERES-wheat and CERES-barley were used. The results showed negative impacts of climate change on yield of cereals in the South Moravian plains, which are in contrast to the potential yield increase in the colder locations of the Czech-Moravian Highland which enjoy fertile, well-watered soils. As a consequence of the presumed impacts which might be obtained, adaptation possibilities available in breeding and crop management increasing utilization of cereal yield potential are discussed based on the genotype×environment×management interaction.

Keywords

Adaptation, cereals, genotype by environment by management interactions, yield potential

Introduction

In recent years, a number of research projects and scientific papers have been focused on climate change and its impacts on the global and regional levels (THOMAS et al. 2004, PARRY et al. 2007). In crop production, climate and weather are considered as important factors which influence plant growth and development as well as yield quantity and quality. However, it is very difficult to assess the impact of climate on yield development in field crops, because a series of related positive and negative impacts have to be quantified and these are mutually interactive. The negative impacts of climate change on crop production can be reduced by using adaptive measures that ensure the utilization of the potential yield of field crops under changing conditions (REYNOLDS 2010).

The main impacts of climate change on crop production are listed in *Table 1*. The negative (indirect) effects usually prevail over the positive (direct) ones. That has adverse impacts on the utilization of yield potential due to increased risk of: (i) availability of water (enlargement of dry areas), (ii) water and wind erosion, (iii) lodging of cereal stands, (iv) leaching of nutrients, especially NO_3^- ions, (v) ocurrence of pests and diseases, (vi) influence of terms of crop management measures. This has been reflected in increased variability of grain yields in recent years (*Figure 1*). This contribution is focused on the analysis of climate change effects on cereal grain yield formation and on the possibility of mitigating its negative impact through breeding and crop management.

Material and methods

To assess whether in terms of grain yield positive fertilization effect of CO_2 concentration prevail over the negative impact of increased temperatures and changes in other

Table 1: **The main impacts of climate change on weather, soil and plants**

Changes in	Positive	Negative
Weather	Increase in temperature in colder areas with higher level of moisture.	The increase in temperature combined with precipitation and global radiation cause increase in variability of meteorological elements associated with the occurrence of extreme events (reduction of snow cover, the occurrence of spring frosts, significant periods of drought, heat waves, torrential rains at the expense of drizzle, hails, etc.)
Soil	Increased anaerobic conditions in the topsoil change the dynamics of organic matter transformation in the soil.	Intensive salinisation and alkalinizing processes, crusting, compaction of the soil.
Plants	The increase in CO_2 concentration has a positive effect on the efficiency of photo synthesis and efficient use of water and nutrients (so called CO_2 fertilization effect).	Higher temperature affects evapotranspiration and phenological development of the crop (sum of effective temperatures), acceleration of development and shortening the growing season reduce yield. The increase in CO_2 concentration can increase the proportion of carbohydrate components of biomass.

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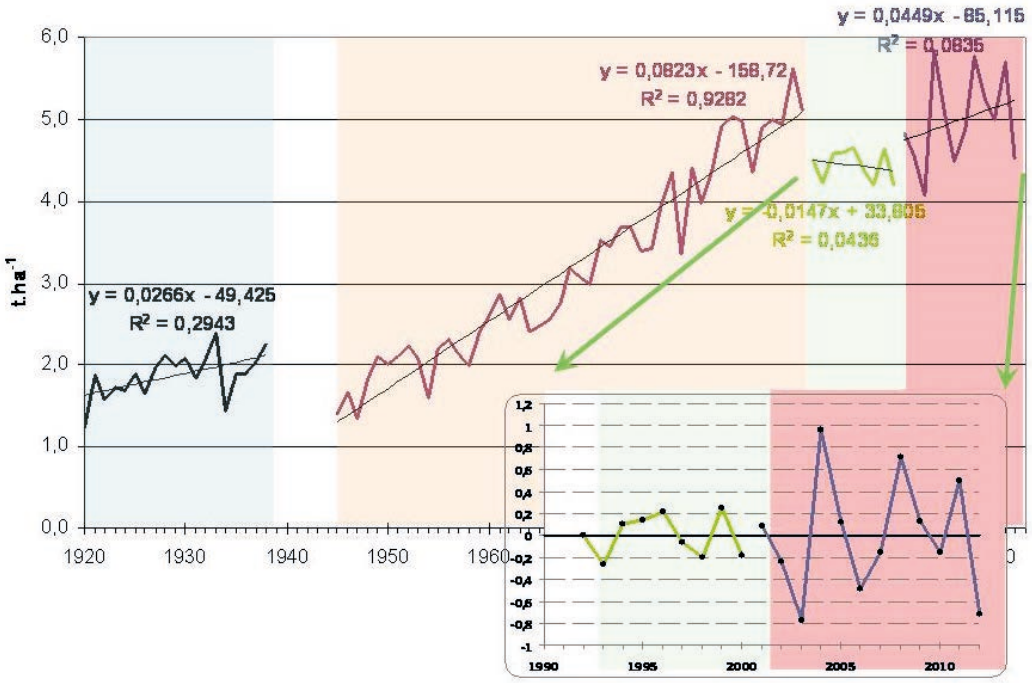

Figure 1: **Development of wheat yield (t.ha^{-1}) in the Czech Republic**

meteorological elements the simulations of yield formation using growth models CERES-Wheat and CERES-Barley was performed. These models were parameterized for the current climate using the results of variety small plot field trials performed by the Central Institute for Supervising and Testing in Agriculture. Meteorological data was subsequently replaced by the outputs of the GCMs (Global Climate Models), which represent the expected climate. Evaluation methodology was described in detail in TRNKA et al. (2004a,b). Basic scenario parameters used for the evaluation of the employed circulation models are listed in *Table 2*.

Results and discussion

Simulation of climate change impact

Figure 2 presents the simulation results corresponding to the variation of emission SRES-A2 scenario and three GCMs (ECHAM - b, HadCM - c and VCAR - d) for winter wheat and spring barley. The maps show the differences between the yield levels in the period 1961-2000 (map a) and 2050 (maps b, c, d).

The performed analyses show: (i) deficit in the water balance in major agricultural regions with negative consequences in arid areas and some positive effects in wet areas in particular years, (ii) asymmetric impact of climate change in different regions, negative effect on yield in dry areas and positive one in areas with suboptimal temperatures, sufficient rainfall and fertile soil, (iii) significant link between the occurrence of drought and variability of yields at the local and national level.

Even if the simulation results obtained were in recent years partly confirmed by the differences of grain yields in agricultural practice (*Figure 3*), it should be emphasized that the model is a tool to recognize the context but provides a simplified picture of reality. Therefore the knowledge of yield formation processes, yield potential utilization and adaptation mechanisms is very important.

Fundamentals about yield formation

Yield potential is defined as the yield of a cultivar when grown in environments to which it is adapted, with non-limiting nutrients and water and with pests, diseases, weeds, lodging and other stresses effectively controlled (EVANS and FISCHER 1999). As such, it is distinguished from potential yield, which is defined as the maximum yield which could be reached by the crop in given environments, as determined, for example, by simulation models with plausible physiological and agronomic assumption.

REYNOLDS et al. (2011) proposed a conceptual platform for the synergistic combination of traits as a model for increasing the yield potential (*Table 3*). Of the listed traits, the

Table 2: **Basic parameters used for the evaluation of models CERES-Wheat and CERES-Barley and for yield simulation**

Characteristic	Source of information
Varieties	winter wheat 'Hana'; spring barley'Akcent'
Locations	7 test sites, representing Czech soil-climatic conditions
Soil database	pedological soil survey (394 soil pits)
Climate database	1961-2000; 125 Czech meteorological stations
Arable land database	Corine Land Cover (CLC2000) 100 m, Vers. 8/2005 (EEA, 2005)
Emission scenario	SRES A2 (with a more rapid increase in greenhouse gases)
Global circulation models	ECHAM, HadCM and NCAR
Grid vector network	500×500 m
CO_2 conc. initial period	350 ppm
Simulation	Potential yield (excl. negative impact of diseases and pests)
Software	ArcGIS using polygon layer of soil types

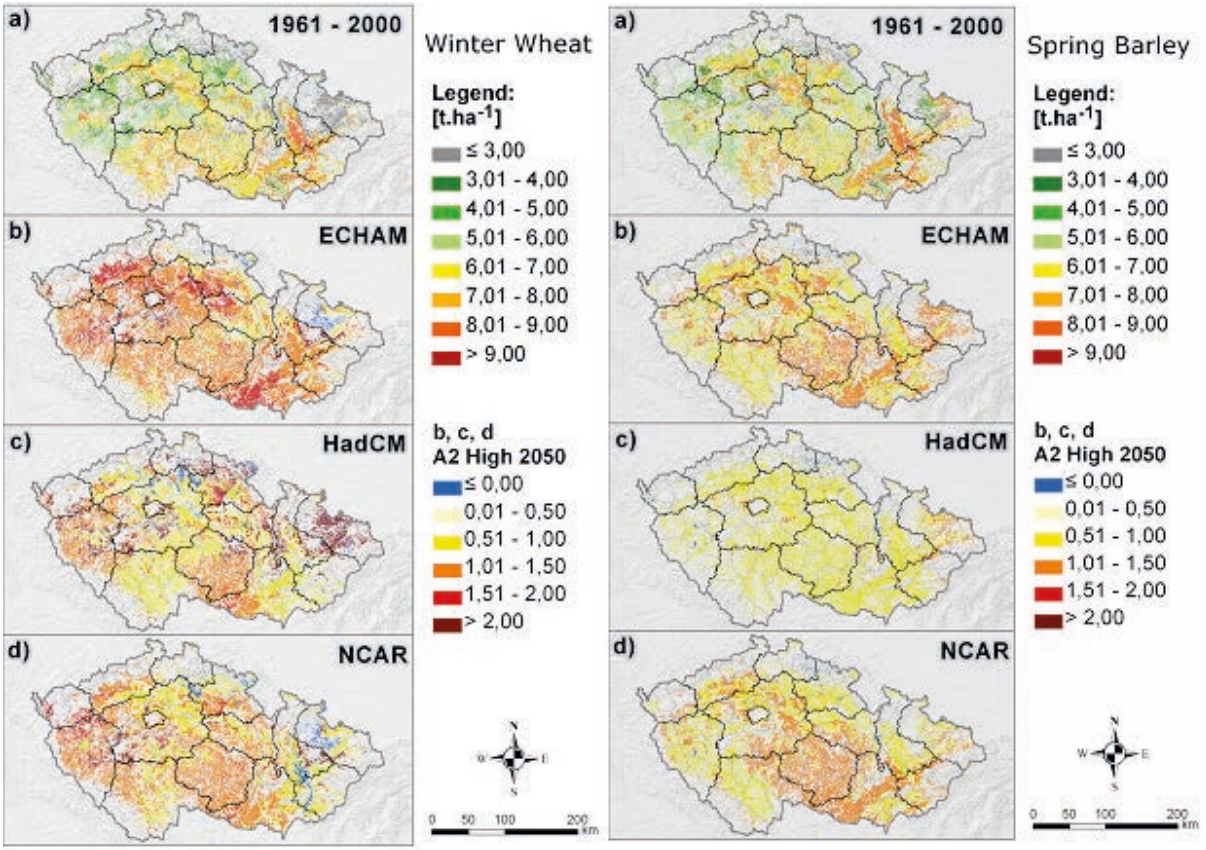

Figure 2: **Potential yield (t.ha^{-1}) of winter wheat (left) and spring barley (right) in the Czech Republic for the climatic conditions in 1961-2000 (a) and the difference between this yield level and the simulated yields for 2050 according to the emission SRES-A2 variant and three global circulation models of climate change (b: ECHAM; c: HadCM; d: VCAR).** The median of 99 simulation results for grid on arable land is depicted (ŽALUD 2009).

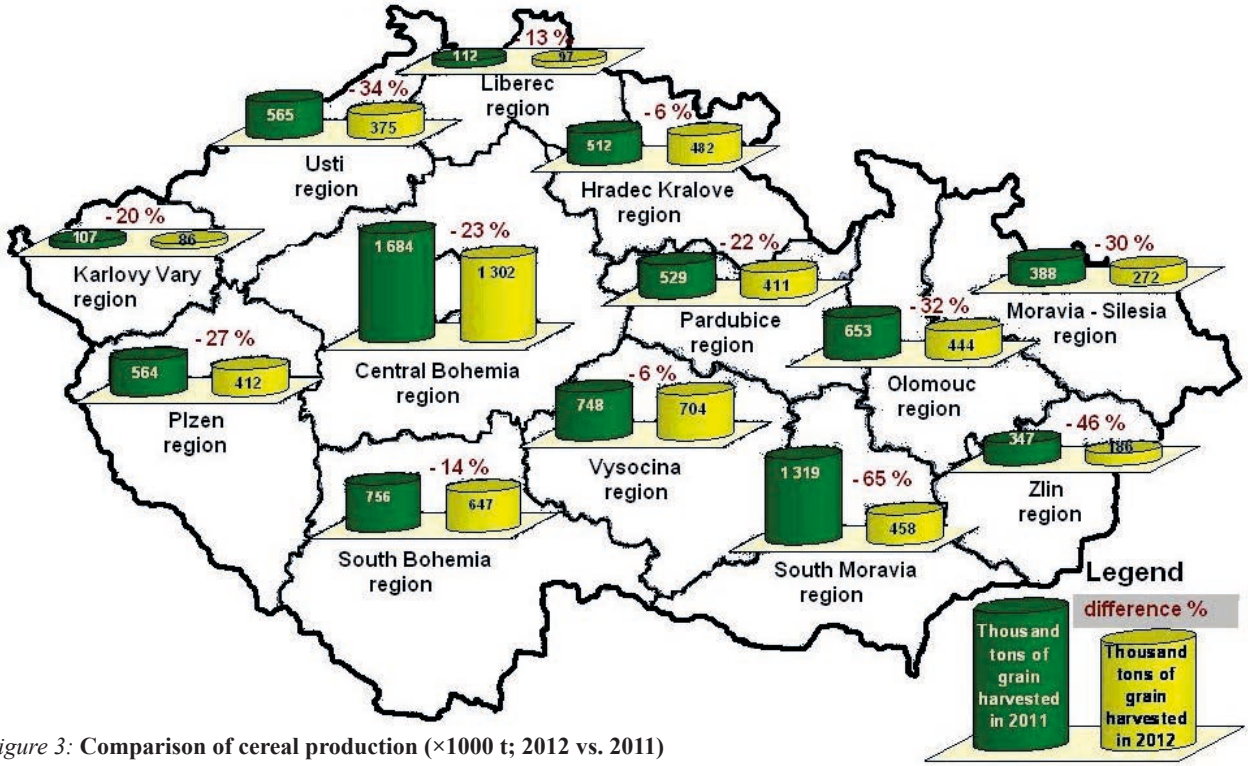

Figure 3: **Comparison of cereal production (×1000 t; 2012 vs. 2011) in individual regions of the Czech Republic**

Table 3: **Model of yield potential traits - a conceptual platform for combination of synergistic traits (REYNOLDS et al. 2011)**

Sink	Source
Pre grain filling	
Increase partitioning to developing spike	Light interception
Reduce floret abortion	Radiation use efficiency (RUE)
Optimize phenological pattern	Rubisco
Lodging resistance	C4 type traits
Grain filling	
Partitioning to grain (Rht)	Canopy photosynthesis
Spike capacity	Cellular (e.g. heat tolerance)
Abort weak tillers	Light distribution
Adequate roots for resource capture	N distribution/partitioning
	Spike photosynthesis

following are important for adaptation to climate change: (a) in the source: light interception, canopy photosynthesis, heat tolerance, (b) in the sink: abort weak tillers, floret abortion, roots for resource capture, phenological pattern and lodging.

The use of vegetation and production factors and biological potential of varieties is conditioned by activities of both breeders and growers of field crops. The aim of breeders should be to create varieties with high and stable yields and required grain quality. The aim of growers is to utilize such biological potential. Stability of grain yield and quality is achieved by the breeder through homeostasis of a variety and by the grower through ensuring stability of the environment, i.e. the modification of farming practices, and optimization of crop management. The role of breeding and crop management in utilization of the potential yield can be explained based on the interaction of G×E×M (*Figure 4*), where *G* is the variety and its biological potential, *E* the environmental components not influenced or partly influenced by the grower (E_S, soil and climatic conditions of the site; E_W, weather; E_E, prices of inputs and outputs), and *M* the environmental components influenced by the grower (crop management as a set of measures used during the growing season and modified according to the requirements of variety, site conditions, course of weather, intensity of farming, way of production use, prices of inputs and outputs, etc.).

Optimization of the system (harmonization of all components based on their importance and functions) should be directed to: (i) maximum biological potential of yield and

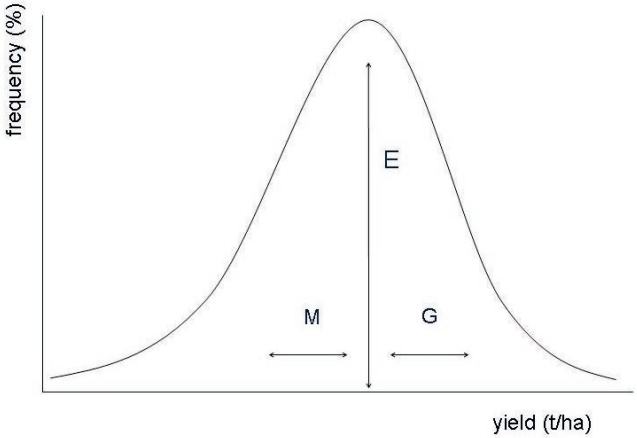

Figure 5: **The role of individual components of the interaction G×E×M**

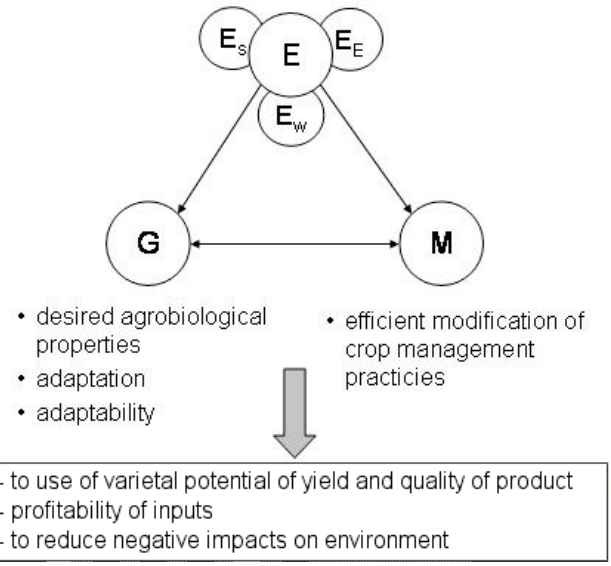

Figure 4: **Genotype by environment by management interaction (G×E×M)**

production quality utilization, (ii) maximum profitability of inputs, (iii) reduction of the negative impacts of farming on the environment. In practice it means the choice of the proper crop variety and decision about consecutive crop management measures that should be performed in the right place, at the right time, and at the right intensity.

The roles of individual components of the system in yield creation are shown in *Figure 5*. The graph shows that the utilized level of the variety yield potential (*G*) is limited by uncontrollable environmental components (*E*). Crop management practices (*M*) should eliminate the negative effects of environmental components by the realization of potential yield, the level of which is in ideal conditions identical with yield potential.

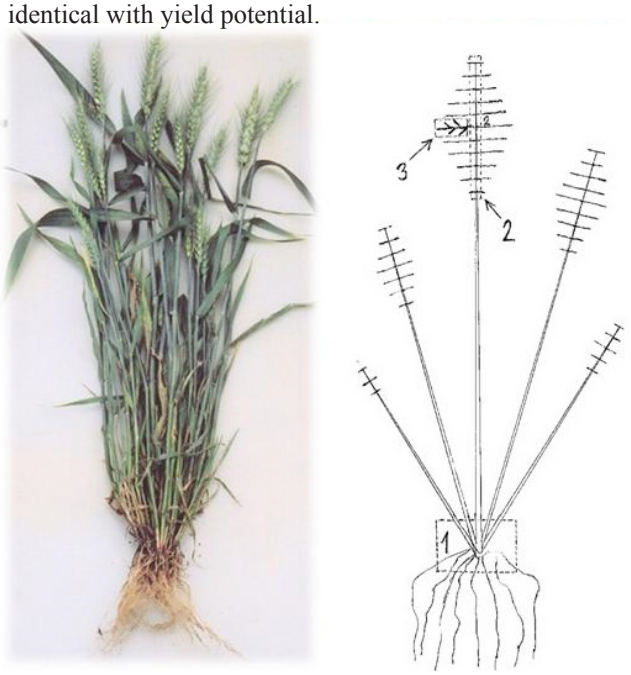

Figure 6: **The modular structure of cereal plant (wheat) and its hierarchical organization**

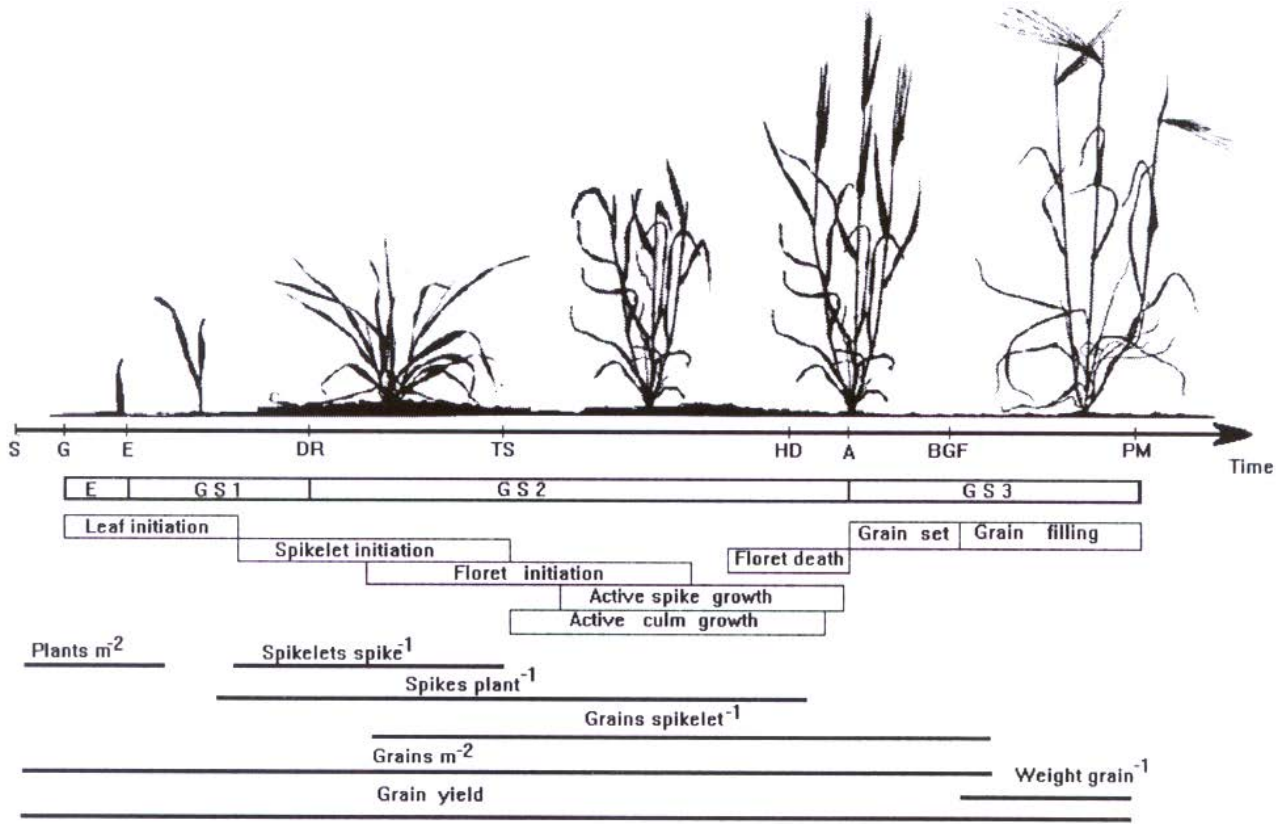

Figure 7: **The course of cereal plant growth (ACEVEDO et al. 2002)**

Climate change increases the role of environment (*E*) in this system, i.e. increases the importance of uncontrollable environmental factors and thus the risk of negative effects (damage of crop stands, lower effectiveness of cropping measures). The knowledge of adaptation to the negative impacts of climate change is therefore of great importance.

Adaptability and formation of yield elements

Grasses in general, and thus the cereals own high level of adaptability due to their modular structure (ARBER 1941, WHITE 1979, PORTER 1983a,b). Classic wheat morphotype (*Figure 6*) responds to changes of environment (ability of resources) using few (three) levels of branching (hierarchical structures - tillers in tillering node, spikelets in the spike, grains in the spikelets) and grain filling. These metameric organs (*Figure 7*) allow plants to respond to environmental changes during their growth and development (ACEVEDO et al. 2002). Hierarchical organisation of plants (NICOLIS et al. 1977) ensures their reproduction even in unfavourable conditions (MIRALLES and SLAFER 2007), when increase of apical dominance and inter-and intra-plant competition and decrease of crop yield occur. On the other hand, high yield in favourable conditions is associated with the decrease of apical dominance and competition in the stand. Thus, adaptability relies on the formation, growth, and reduction of metameric organs used by plants to respond to environmental changes (MASLE-MEYNARD and SEBILLOTTE 1981a,b, REYNOLDS et al. 2005). Cereal plants can thus be investigated as developing modular systems and their growth can be described analogously to the processes of population type, and source×sink theory can be used to explain the regulation processes at plant level (KREN et al. 1992, KŘEN 2012).

Adaptation possibilities

Basic possibilities of adaptation to the negative impacts of climate change and their mitigation during the grain yield formation are shown in *Table 4*.

Breeding

Breeding for higher grain yield resulted in changes of plant proportions, stand architecture (stand density increases) and extended duration of the canopy assimilation (AUSTIN et al. 1980). A significant increase in yield potential has been associated with the achievement of a number of physiological and morphological changes in plants. The consequence was a significant increase in grain weight in spike and shortening of straw (increased harvest index value). These improved the resistance to lodging and increased the number of ears per unit area. Since the mid-1990s, the upward trend in yields and production has begun to slow mainly due to environmental and energy limits. The trend in decreasing yield stability and a gradual slowing down or stopping shortening of straw in new varieties has also been observed. Further shortening of the stem is genetically feasible, but currently it is constrained by ecological limits (FOULKES et al. 2011).

It is also becoming apparent that a higher yield can be achieved more easily when formed by a higher proportion

Table 4: **The main issues to be addressed in climate change by utilization of cereal yield potential**

Issue	Mitigation strategy	
	Breeding	Crop management
Drought	Earliness Optimize phenological pattern Large root system	Choice of crop Suitable previous crops Minimum soil tillage Early sowing Nitrogen application
Frosts without snow	Frost resistance	Time of sowing Growth regulators Balanced crop nutrition
Increased frequency of extreme weather	Adaptability Lodging resistance Resistance to diseases and pests	Application of fertilizers, growth regulators and pesticides Timely modification of cropping measures

of the polysaccharide (starch) components of grains and a lower proportion of the protein component. This is probably related to the amount of metabolic energy that the plant needs for the synthesis of a unit amount of protein and starch, and this difference may enhance the increase in the concentration of CO_2 in the atmosphere in the future (NÁTR 2000).

Cereal varieties should have the following properties that allow adaptation and mitigation of the negative impacts of climate change:

- lower transpiration coefficient, more powerful and deeper root system or faster growth and earlier ripening,
- resistance to frost and low temperatures in winter and during regeneration periods,
- resistance to drought in the tillering-earing stage,
- lower level of inter- and intra-plant competition,
- resistance to lodging (stem length is probably ecologically limited),
- resistance to selected biotic harmful agents,
- adaptability to climatic extremes (probably negatively correlated with the increase of harvest index and potential yield).

Cereal growers should take the above properties into consideration during decisions making concerning varieties and varieties assortment. It should be noted that varieties performing all these requirements are hard to find, and if they do, unfortunately they usually provide low but stable yields (universal organism usually shows average performance).

Crop management

Decision making about cropping measures is a crucial farming activity. Farmers spend most of the working time with implementation of crop management practices. The basic problem, which has to be solved, is the balance of production factors in space and time, i.e. making them available to the needs of developing crops while controlling crop structure according to their levels. In doing so, growers face a number of agronomic, economic and administrative problems:

- implementation of crop measures in right agrotechnical terms, according to the needs of stands depending on the weather,
- achieving good prices of inputs and output,
- compliance with all accounting and administrative requirements.

From this point of view the crop management practices feature economically implemented agronomic knowledge in mostly uncontrolled environments. Impacts of climate change affect terms and methods of crop management practices and result in their efficiency and in the level of yield potential utilization.

From the view of the above considerations about the grain yield formation and of the increasing importance of *E* in the G×E×M system (as a result of climate change), it would be useful to create varietal crop management practices. This is complicated by difficult orientation of growers in numerous registered varieties and in their various properties. On the other hand, it is worth highlighting the stagnation of the rising trend in the potential yield of new varieties in small plot field experiments and in practice (AHLEMEYER and FRIEDT 2012). Therefore, the concept based on the optimization of all three components of G×E×M interaction, in particular of the links between G×E (choice adaptable varieties) and E×M (cropping measures modified according to the weather course), seems to be promising.

Crop management practices should be implemented as a coherent set of optimized cropping measures for specific site conditions and for a certain way of using the produced grain. It is important to:

- determine the economically efficient intensity of growing dependent on the changing prices of seeds, agrochemicals, agricultural machinery and production;
- be flexible in diagnosing the state of crop stand and subsequent modification of cropping measures according to weather changes (adaptability).

It should be noted that with the increasing importance of uncontrollable environmental components (*E*) as a result of climate change high intensification is economically and ecologically risky. This situation can be solved by the establishing and improving the early warning systems (e.g. agrometeorological monitoring).

Conclusions

We can assume that breeding and growing of field crops always include the endeavour to eliminate the impact of climatic and weather conditions on soil and growth processes. The utilization of yield potential of cereal cultivars under the conditions of changing climate requires both the grown cultivars and the growers' activities to be more adaptable.

Successful implementation of this approach in practice requires broad knowledge and experience including the need to solve often opposed biological or agronomic and economic requirements.

Acknowledgement

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The effect of drought on the composition of selected substances in barley grains

Marta Bradáčová[1*], Helena Pluháčková[1], Jaroslava Ehrenbergerová[1], Ludmila Holková[1] and Eva Truhlářová[1]

Abstract

Drought-induced stress affects a number of processes in plants. The protective mechanisms in plants are mainly based on reducing water losses, however, during drought stress also other enzymatic systems are activated, e.g. as a protection against oxidative stress. Vitamin E isomers (α-tocopherols and α-tocotrienols) are synthesized in plants and inhibit lipid peroxidation. The biosynthetic pathway of vitamin E isomers is very complicated. One of the important enzymes is 4-hydroxyphenylpyruvate dioxygenase (HPPD) due to its strategic location in the pathway. The content of tocotrienols is associated with HGGT enzyme. This study was aimed to assess the expression of the *Hppd* and *Hggt* genes in three barley genotypes in relation to the amount of vitamin E isomers in grains and the intensity of drought stress as an activation factor. Plants were cultivated under three different regimes (optimal conditions, early and late drought stress). The regulation of vitamin E synthesis and the composition of tocols were influenced both by temperature and relative air humidity, and the genotype.

Keywords

Gene expression, *Hordeum vulgare*, RT-PCR, tocopherols, tocotrienols

Introduction

A number of processes in plants which result in changes of metabolic activity is affected by drought induced stress. Plant reaction is highly influenced by the duration and intensity of exposure to stress. The protective mechanisms in plants are mainly based on reducing water losses, however, during drought stress also other enzymatic systems are activated, e.g. as a protection against oxidative stress.

To prevent oxidative damage of cellular components there is a complex network of antioxidants. Vitamin E isomers (α-tocopherols and α-tocotrienols) are synthesized in plants and inhibit lipid peroxidation. The most important source of tocopherols is plant oil and tocotrienols are abundant in seeds of monocotyledonous plants such as wheat and barley. The biosynthetic pathway of vitamin E isomers is very complicated. The content of tocotrienols in barley grains is associated with the activity of HGGT enzyme (CAHOON et al. 2003). Another important enzyme is 4-hydroxyphenylpyruvate dioxygenase (HPPD) due to its strategic location in the pathway (DÖRMANN 2007). The expression of the homologic gene in mango fruit (MiHPPD) is related to ripening and is rapidly induced by ethylene. The increase in MiHPPD transcript accumulation was followed by an increase in tocopherol levels during ripening. The ripening-related increase in MiHPPD expression was also seen in response to abscisic acid and to a lesser extent to indole-3-acetic acid (SINGH et al. 2011).

This study was aimed to assess the expression of the *Hppd* and *Hggt* genes in barley in relation to the amount of vitamin E isomers in grains and intensity of drought stress as an activation factor. Intensity of stress depends not only on the quantity of water available in the environment, but also on the stage of plant development and genotype. Therefore, plants were cultivated under three different water regimes in the years 2009 to 2011.

Material and methods

Three genotypes of spring barley (*Hordeum vulgare* L.) with different levels of tocols in the grain were cultivated in pots under controlled watering in the years 2009 to 2011; 'Krona' represented a malting barley, 'Wanubet' a hull-less, waxy starch barley and breeding line KM1057 a hull-less, high lysine barley developed from a cross with 'Hiproly'. Early-drought stress was induced at the end of tillering, when the water content in soil was reduced from 75% of full water capacity to 35% and kept at this level until maturity. Late-drought was applied in the same way from the stage of ear emergence to ripening. An optimal variant was continuously watered at 75% water capacity. Ear samples were taken five times (I: before pollination; II: 4 days post anthesis (dpa); III: 8 dpa; IV: 12 dpa; V: 15 dpa) for the evaluation of *Hppd* and *Hggt* genes' expression. The concentration of tocol isomers was measured in fully matured grains. The relative expression of *Hppd* and *Hggt* genes was assessed using RT-PCR (KOSAŘ et al. 2010). The content of tocopherols and tocotrienols was analysed using the HPLC with fluorescence detection (EHRENBERGEROVÁ et al. 2006). The data were analysed by two-way analysis of variance and Fisher´s LSD multiple comparison test (Statistica 8.0, StatSoft, Inc.).

Results and discussion

The previous studies showed that the content of isomers of vitamin E in barley grains was significantly influenced

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Table 1: **Content of tocols in barley grains** (Mean values with different letters within columns are statistically significant at $P \leq 0,05$; α-T, α-tocopherols; α-T3, α-tocotrienols)

Year	Water regime	cultivar/line	Content of tocols (mg·kg^{-1} dry matter)		
			Sum of tocols	α-T	α-T3
2009	early drought	Wanubet	29.03 ^{g}	4.53 $^{e-i}$	15.51 ^{lm}
		Krona	28.95 ^{g}	6.70 ^{jk}	13.14 ^{jk}
		KM1057	38.26 ^{ij}	13.70 ^{m}	11.86 $^{h-j}$
	late drought	Wanubet	29.42 ^{g}	5.61 ^{ij}	14.50 ^{kl}
		Krona	21.36 $^{d-f}$	4.99 $^{f-j}$	9.64 ^{fg}
		KM1057	32.99 ^{gh}	11.36 ^{l}	10.23 $^{f-h}$
	optimal conditions	Wanubet	39.45 ^{j}	5.84 ^{ij}	20.98 ^{o}
		Krona	31.46 ^{gh}	8.12 ^{k}	11.73 $^{h-j}$
		KM1057	37.77 ^{ij}	11.09 ^{l}	12.43 ^{ij}
2010	early drought	Wanubet	21.17 $^{d-f}$	3.55 $^{c-h}$	10.23 $^{f-h}$
		Krona	20.09 $^{c-f}$	5.35 $^{h-j}$	7.52 $^{c-e}$
		KM1057	12.57 ^{a}	2.54 $^{a-d}$	4.02 ^{a}
	late drought	Wanubet	22.61 ^{ef}	3.54 $^{c-g}$	8.70 ^{ef}
		Krona	20.10 $^{c-f}$	5.09 $^{g-j}$	6.17 ^{bc}
		KM1057	15.98 $^{a-c}$	3.71 $^{d-h}$	4.73 ^{ab}
	optimal conditions	Wanubet	24.06 ^{f}	5.14 $^{g-j}$	10.70 $^{g-i}$
		Krona	21.03 $^{d-f}$	6.16 ^{ij}	7.39 $^{c-e}$
		KM1057	17.84 $^{b-d}$	4.51 $^{e-i}$	5.68 $^{a-c}$
2011	early drought	Wanubet	12.40 ^{a}	1.06 ^{a}	6.08 ^{bc}
		Krona	14.72 ^{ab}	1.87 $^{a-c}$	6.51 ^{bc}
		KM1057	19.30 $^{c-e}$	3.26 $^{b-f}$	8.45 $^{d-f}$
	late drought	Wanubet	29.13 ^{g}	3.22 $^{b-f}$	17.22 ^{mn}
		Krona	12.64 ^{a}	1.58 ^{ab}	5.79 $^{a-c}$
		KM1057	13.82 ^{ab}	1.80 $^{a-c}$	6.57 $^{b-d}$
	optimal conditions	Wanubet	31.73 ^{gh}	3.10 $^{b-e}$	18.17 ^{n}
		Krona	35.08 ^{hi}	5.33 $^{h-j}$	18.39 ^{n}
		KM1057	16.41 $^{a-c}$	2.97 $^{b-e}$	6.52 $^{b-d}$

not only by the genotype, but also by growing conditions (EHRENBERGEROVÁ et al. 2006). From *Table 1* it is obvious that the water regime has a significant effect on the content and composition of vitamin E isomers (tocopherols and tocotrienols). We focused on α-T and α-T3 due to low concentrations of other isomers. 'Wanubet' had the lowest content of tocols and α-tocopherols under drought conditions. The content of α-tocotrienols was lowest in the late drought variant and highest in optimal conditions. The content of α-T3 in 2009 was the highest of all years, regimes and genotypes. 'Krona' had the lowest content of tocols and α-T and α-T3 in late drought conditions and highest in optimal conditions. The content of vitamin E isomers of KM1057 was variable with respect to years and water regimes. The sunny and warm weather in 2009 and 2011 affected the content of tocols and isomers, which was highest in early drought conditions and lowest in late drought conditions. In 2010 (cold weather with high air humidity) the highest content was realized in optimal condition. The ratio α-T3/α-T depended on water regime, year and genotype (*Table 2*). The highest α-T3/α-T value in grains was observed for hull-less 'Wanubet'. Genotypic differences of the T3/T ratio were also shown by ZIELINSKI et al. (2007), but contrary to our results these authors observed relatively low T3/T ratios in whole grains. It is known that tocopherols and tocotrienols are distributed differently within the kernel: tocopherols have their highest concentration in the outer layers and decrease gradually to zero in the inner endosperm, while tocotrienols have their lowest concentrations in the seed coat layer (WINTER and DAVIS 2006). This justifies results of TSOCHATZIS et al. (2012), where the tocopherol contents were higher in conventionally grown barley samples that had high ash contents, whereas organic cultivation resulted in an increase of tocotrienols and consequently in increased T3/T ratios.

The gene expressions of the two enzymes HPPD and HGGT connected with tocols biosynthetic pathway were evaluated during experiment. The results showed significant correlations between genes' expression and T3 and T contents in grains 12 dpa (*Hppd*) and 15 dpa (*Hggt*). The close correlation was found in the drought stressed variant. Hence, the influence of drought on the biosynthetic pathway was demonstrated (*Table 3*). The close relationship between Hggt and *Hppd* genes' expression and the content of vitamin E iso-

Table 2: **Ratio of the mean content of α-tocotrienols (α-T3) and α-tocopherols (α-T) in barley grains under different water regimes**

Year	Water regime	Genotype		
		Wanubet	Krona	KM1057
2009	early drought	3.42	1.96	0.87
	late drought	2.58	1.93	0.90
	optimal conditions	3.59	1.44	1.12
2010	early drought	2.88	1.41	1.58
	late drought	2.46	1.21	1.27
	optimal conditions	2.08	1.20	1.26
2011	early drought	5.74	3.48	2.59
	late drought	5.35	3.66	3.65
	optimal conditions	5.86	3.45	2.20

Table 3: **Correlation between the content of α-T3 and α-T and the expression of *Hggt* gene 15 days post anthesis and *Hppd* gene 12 days post anthesis** (ns, not significant; *, **, significant at $P<0.05$ and 0.01, respectively)

		NRE	
Water regime	Tocols	*HGGT (V)*	*HPPD (IV)*
early drought	α-T3	0.44	0.52
	α-T	ns	0.87**
late drought	α-T3	0.78*	0.55
	α-T	ns	0.47
optimal conditions	α-T3	ns	ns
	α-T	ns	ns
all regimes	α-T3	0.53**	ns
	α-T	ns	0.63**

mers is determined by the position and function of vitamin E biosynthetic pathway enzymes. It remains still unclear why this relationship was not observed in optimal conditions. The plants growing under optimal water conditions could have been influenced by another type of abiotic stress, e.g. high temperature. This type of stress was not observed in the drought-stressed variants due to a masking effect of the drought stress *per se*. MUNNÉ-BOSCH (2005) described the effect of various types of abiotic stress on genes connected to the regulation of the tocols biosynthetic pathway.

The regulation of vitamin E isomers synthesis and the composition of tocols isomers were influenced both by growing conditions, i.e. water availability, temperature, relative air humidity, and the genotype.

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Genotypische Unterschiede in der Verlagerung von Rohprotein bei Winterweizen

Genotypic differences in the translocation of protein in winter wheat

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Abstract

Thirty cultivars selected by Austrian, German and Hungarian breeders were tested in 12 Austrian trials (2012-2013). Genotypic differences were observed for crude protein content and yield of green matter at flowering, grains and straw. Austrian high quality wheats showed a low protein content in the aboveground biomass at flowering. Especially high quality wheat 'Energo' exhibited outstanding low values with respect to protein yield at flowering. High protein content was detected in the grain of high quality cultivars. The protein yield was medium to high. German high quality cultivars showed high biomassprotein content at flowering, but only a medium grain protein content. The Hungarian cultivars were in between. 'Ubicus' of the medium quality group showed the highest protein yield at flowering as well as in the grains. In the straw Austrian wheats had the lowest protein content independent of the quality group. The German cultivars showed the highest protein values.

Keywords

Green matter, protein content, straw, *Triticum aestivum*

Einleitung

Von Oktober 2012 bis Dezember 2013 führten die Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), die Bayerische Landesanstalt für Landwirtschaft (LfL) und die Technische Universität München (TUM) gemeinsam mit österreichischen und deutschen Züchtern das CORNET-Projekt *Efficient Wheat* durch. Unter anderem wurde dabei die N-Effizienz von Winterweizen unter Trockenstressbedingungen untersucht. Neben dem Korngut wurden auch die Biomasse zur Blüte und das Stroh auf den Proteingehalt analysiert. Im folgenden Beitrag werden die Ergebnisse der österreichischen Versuche dargestellt.

Material und Methoden

In den Jahren 2011/12 und 2012/13 wurden Winterweizenversuche an den Standorten Niederweiden (NWe, Bezirk Gänserndorf), Tattendorf (Tat, Bez. Baden) und St. Andrä (StA, Bez. Neusiedl) sowie nur in der Saison 2012/13 in Wien (WSP) durchgeführt. In NWe, Tat und WSP wurden die Sorten mit zwei Wasserversorgungsvarianten getestet. An den Standorten NWe und Tat erfolgte dies neben der Variante „natürlicher Niederschlag" (I) mit zwei bis drei Beregnungsgaben von zusätzlich je 25-30 $l \cdot m^{-2}$ (II). Am Standort WSP (*Abbildung 1*) wurden neben der Variante I die Niederschläge durch ein Rollglashaus von April bis Juni großteils abgehalten und somit künstlich Trockenstress erzeugt (II).

30 Winterweizensorten deutscher (DE), österreichischer (AT) und ungarischer (HU) Züchter (= Herkunft) wurden getestet. Sie zeigten eine unterschiedliche Backqualität (= Qualität) und wurden nach AGES (2013) bzw. OBERFORSTER et al. (1994) in die drei Gruppen Qualitätsweizen (QW), Mahlweizen (MW) und Futterweizen (FW) gegliedert.

Der Anbau erfolgte zwischen 3. und 25. Oktober nach Winterraps, Sommergerste, Sonnenblume, Kartoffel, Körnermais bzw. Erbse in einer Gitteranlage mit drei Wiederholungen. Zur Blüte wurde ein Teil der Parzellen beerntet; die Erntefläche betrug 0,1 m² im Rollglashaus bzw. 1,9 bis 2,0 m² in den Großparzellenversuchen. Beim Rest der Parzelle wurde zur Totreife die Korn- und Strohernte durchgeführt (1,0 m² im Rollglashaus bzw. 8,1 bis 13,6 m² in den Feldversuchen). Die Versuche wurden mit 57-128 kg $N \cdot ha^{-1}$ gedüngt, aufgeteilt auf zwei bis drei Gaben.

Der Rohproteingehalt wurde nach ICC-Standard Nr. 167 von der Biomasse bei der Blüte und dem Stroh (N×6,25) sowie vom Korn (N×5,7) untersucht (= Material). Der Proteinertrag resultiert aus Biomasse- bzw. Kornertrag (TS) und Proteingehalt.

Mit zwei linearen gemischten Modellen wurde der Einfluss des Materials auf den Proteingehalt untersucht. Als fixe Effekte wurden die Genotypen (Modell A) bzw. die Qualitätsgruppe, die Herkunft sowie deren Interaktion (Modell B) berücksichtigt. Als zufälliger Effekt fand eine Variable ID in beiden Modellen Berücksichtigung die aus den drei Messungen pro Sorte (Grünpflanze, Stroh und Korn) generiert wurden. Dadurch konnte auf Abhängigkeiten eingegangen werden, die sich aus den Messwiederholungen

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Abbildung 1: **Versuch am Standort Wien 2013** (links: trockengestresst infolge Abhaltung des Niederschlages durch ein Rollglashaus, rechts: natürlicher Niederschlag)

Figure 1: **Trial in Vienna 2013** (wheat under drought stress caused by a rain out shelter (left) and natural precipitation (right), respectively)

ergaben. Im Modell B wurde zusätzlich zur ID auch der Standort als zufälliger Effekt berücksichtigt, um ortsspezifische Schwankungen herauszurechnen. Die Variation dieser zufälligen Effekte wurde bei der Berechnung der Prädiktionsintervalle einbezogen. In beiden Modellen wurde das Hauptaugenmerk auf die Signifikanz der fixen Effekte gelegt. Die Identifizierung von signifikanten Einflussfaktoren erfolgte über eine schrittweise forward-selection mittels eines χ^2-Tests. Dabei wurden, ausgehend vom Intercept-only Modell (in dem keine erklärenden Variablen berücksichtigt werden), schrittweise erklärende Variablen hinzugefügt und mit dem vorherigen Modell verglichen. Ist die Änderung im Erklärungsgrad des Modells signifikant zum Niveau α=0,05 bei Hinzunahme der jeweils erklärenden Variable, so wird diese ins Modell aufgenommen. Nach der Varianzanalyse wurden Post-Hoc-Tests (Tukey's HSD Test) durchgeführt. Die Modellierung erfolgte mit der Statistiksoftware R (R DEVELOPMENT CORE TEAM 2012).

Ergebnisse und Diskussion

Rohproteingehalt

Die Analyse der Ergebnisse zeigte große genotypische Unterschiede (*Abbildung 2*). So wiesen die Qualitätsweizen 'Bitop', 'Energo', 'Element' und 'Capo' bei der Blüte die geringsten Proteingehalte auf. Sie unterschieden sich signifikant (α=0,05) von 'Format' und 'Robigus'. DIEKMANN

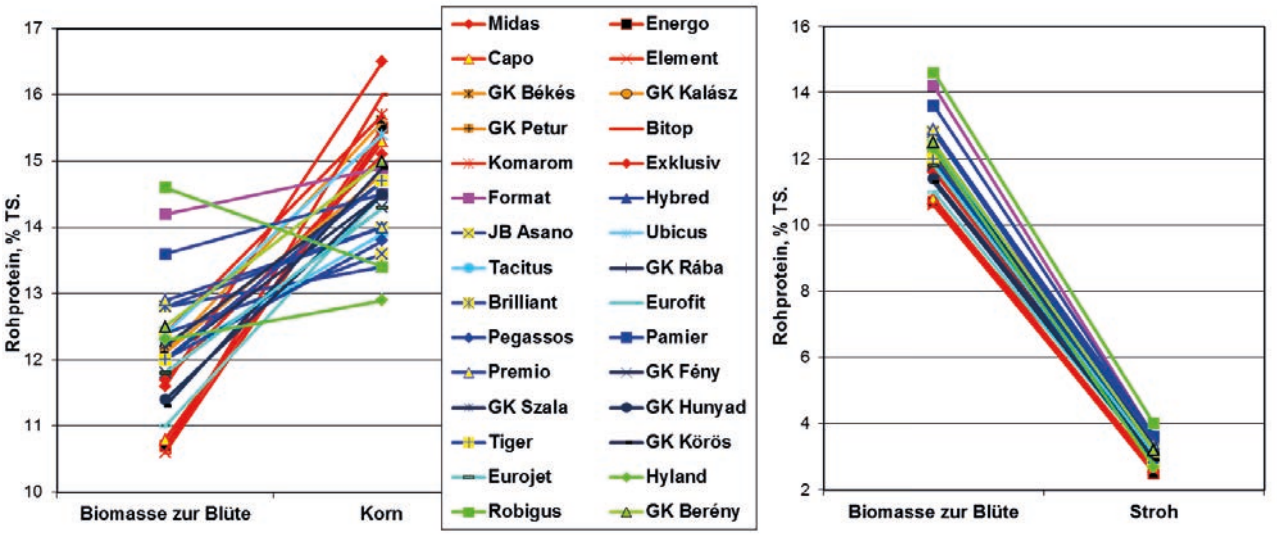

Abbildung 2: **Verlagerung des Proteingehaltes von der Biomasse zur Blüte (BBCH 65) ins Korn (links) und Stroh (rechts) bei 30 Winterweizensorten** (Mittel aus 12 Versuchen)

Figure 2: **Translocation of protein content from the biomass at flowering (BBCH 65) into grains (left) and straw (right) of 30 winter wheat cultivars** (mean of 12 trials)

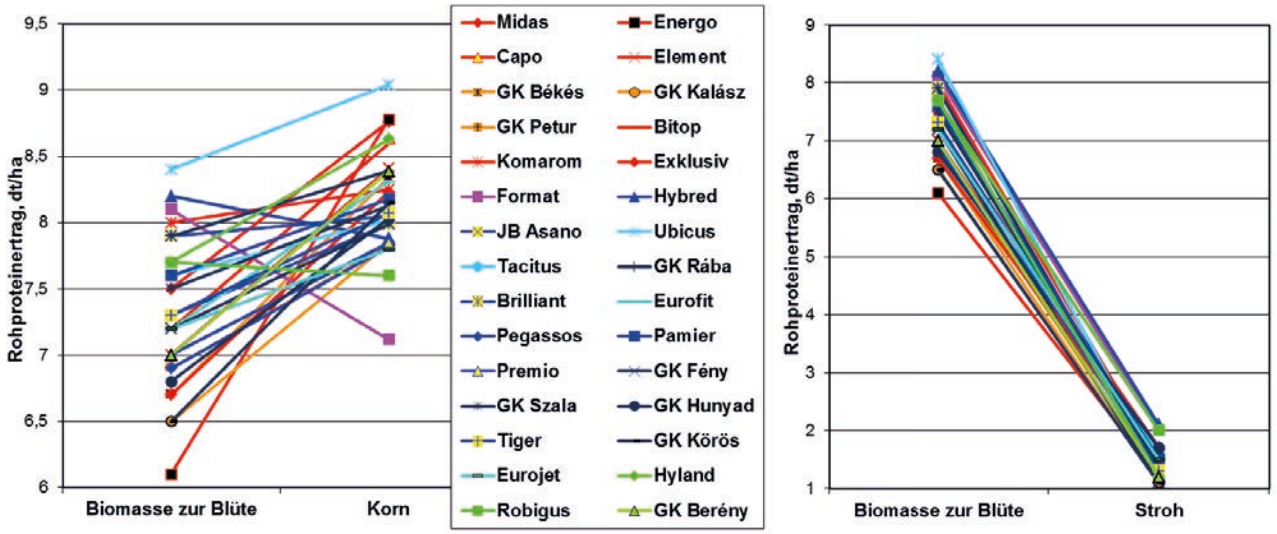

Abbildung 3: **Verlagerung des Proteinertrages von der Biomasse zur Blüte (BBCH 65) in Korn (links) und Stroh (rechts) bei 30 Winterweizensorten** (Mittel aus 12 Versuchen)
Figure 3: **Translocation of the protein yield from the biomass at flowering (BBCH 65) into grains (left) and straw (right) of 30 winter wheat cultivars** (mean of 12 trials)

(2002) fand ebenfalls große N-Konzentrationsunterschiede in der oberirdischen Biomasse von zwei Winterweizensorten. Er führte dies auf differenziertes Aneignungsvermögen zurück. Im Korn fanden sich hingegen bei diesen Sorten gemeinsam mit 'Exklusiv', 'Komarom' und 'GK Békés' die höchsten Werte. 'Exklusiv' unterschied sich darin signifikant von 'Hybred' und 'Hyland'. Der Futterweizen 'Robigus' verhielt sich abweichend zu allen anderen Sorten. Der vergleichsweise hohe Proteingehalt bei der Blüte fand sich nur teilweise im Korn wieder. Im Stroh konnte im Vergleich zur Biomasse bei der Blüte nur mehr wenig Protein festgestellt werden. Dabei verhielten sich die Sorten alle relativ ähnlich. Die Variation zwischen den Sorten wurde zur Reife hin geringer, sodass signifikante Sortenunterschiede nicht beobachtet wurden.

Rohproteinertrag

Im Proteinertrag zeigte sich teilweise ein anderes Bild (*Abbildung 3*). Die Qualitätsweizen 'Energo', 'GK Kalász', 'GK Békés' und 'Exklusiv' wiesen bei der Blüte die geringsten Proteinerträge auf. Diese Sorten waren jedoch großteils im Korn gemeinsam mit 'Midas', 'Bitop', 'Capo', 'Element' und 'Komarom' am N-effizientesten. 'Ubicus' erzielte als Mahlweizen sowohl in der Biomasse zur Blüte als auch im Korn den höchsten Proteinertrag. Die hochqualitative Sorte 'Format' hatte bei der Blüte einen sehr hohen Proteinertrag, im Korn war sie jedoch die Sorte mit der geringsten N-Effizienz. Dies bedeutet, dass 'Format' bei der Blüte mehr Protein aufgenommen hat, als er eigentlich benötigt. COX et al. (1986) fanden in Weizenlinien ebenfalls einen Überschuss an Protein bei der Blüte. GREGORY et al. (1981) begründeten variierende N-Verlagerung mit unterschiedlichem Photosyntheseverhalten.

Auch der Futterweizen 'Robigus' steigerte den Proteinertrag von der Blüte zum Korn nicht. Mit dem Stroh wurden etwa 1 bis 2 dt·ha^{-1} Protein abgeführt. Der Proteinertrag veränderte sich von Blüte zur Strohernte in einem ähnlichen Verhältnis wie der Proteingehalt. Die Sortenunterschiede waren statistisch nicht absicherbar. Laut DIEKMANN (2002) basieren genotypische Unterschiede im Korn-N-Ertrag auf einer differenzierten N-Aufnahme und Mobilisation aus der vegetativen Biomasse. Die Variation in der N-Einlagerung war bei ihm im Korn ebenfalls viel höher als im Stroh.

Einfluss von Genotyp und Material

Material, Sorte sowie die Interaktion von Material und Sorte konnten in Modell A als signifikante Einflussfaktoren auf den Proteingehalt identifiziert werden. Für die Referenzsorte 'Bitop' ist der erwartete Proteingehalt in der Biomasse zur Blüte 10,6%. Der erwartete Gehalt im Korn ist um 5,4% höher und im Stroh um 7,2% geringer als in der Biomasse zur Blüte. Der im Modell erwartete Proteingehalt verhält sich ähnlich wie im Mittelwertsvergleich: So hat 'Robigus' den höchsten erwarteten Wert in der Biomasse zur Blüte, gefolgt von 'Format', 'Pamier' und 'Premio'. Die geringsten Werte weist das Modell bei 'Bitop', 'Element', 'Energo' und 'Capo' aus. Im Korn finden sich dagegen die niedrigsten Werte bei 'Hyland', 'Hybred', 'Robigus' und 'JB Asano' (12,9-13,6%). Die höchsten Werte sind bei 'Exklusiv', 'Bitop', 'Energo', 'GK Békés', 'Komarom', 'Capo' und 'Element' zu erwarten (15,4-16,5%). Im Stroh ist der geringste Proteingehalt für die Sorte 'Energo' (2,5%), gefolgt von 'Element', 'Hyland' und 'Capo', zu erwarten. Der höchste erwartete Proteingehalt ergibt sich für Sorte 'Robigus' (4,0%) gefolgt von 'Pamier', 'Hybred' und 'Format'. Die erwarteten Proteingehalte von Sorte und Material sind in *Tabelle 1* dargestellt.

Aus den Varianzkomponenten der zufälligen Effekte kann geschlossen werden, welcher Teil der Gesamtvarianz auf den jeweiligen Effekt bzw. die Restvarianz entfällt. Unter Gesamtvarianz ist jene Varianz zu verstehen, die nicht durch die berücksichtigten fixen Effekte erklärt werden kann. Der hier berücksichtigte zufällige Effekt ID erklärt 52,9% dieser Gesamtvarianz.

Tabelle 1: **Ergebnisse der Modellierung des Proteingehaltes in Abhängigkeit von Material und Genotyp sowie deren Interaktion (Modell A)**
Table 1: **Results of the modelling of the protein content depending on the material and the genotype as well as their interaction (model A)**

			Erwarteter Proteingehalt (% TS)		
Sorte	Herkunft	Qualität	Biomasse BBCH65	Korn	Stroh
Bitop (Referenz)	AT	QW	10,56	16,00	3,32
Brilliant	DE	MW	12,81	14,10	3,19
Capo	AT	QW	10,76	15,46	2,74
Element	AT	QW	10,64	15,42	2,60
Energo	AT	QW	10,68	15,64	2,52
Eurofit	AT	MW	11,04	14,55	2,89
Eurojet	AT	MW	11,81	14,45	3,08
Exklusiv	AT	QW	11,61	16,52	3,13
Format	DE	QW	14,18	15,01	3,56
GK Békés	HU	QW	12,13	15,62	3,15
GK Berény	HU	FW	12,46	15,04	3,25
GK Fény	HU	MW	11,84	14,29	3,00
GK Hunyad	HU	MW	11,43	14,59	3,13
GK Kalász	HU	QW	12,19	14,69	3,36
GK Körös	HU	MW	11,30	14,87	2,91
GK Petur	HU	QW	12,05	14,81	3,11
GK Rába	HU	MW	12,03	14,40	3,13
GK Szala	HU	MW	12,16	14,72	3,31
Hybred	DE	MW	12,81	13,38	3,58
Hyland	DE	FW	12,33	12,93	2,73
JB Asano	DE	MW	12,38	13,62	3,03
Komarom	AT	QW	12,39	15,61	3,36
Midas	AT	QW	11,66	15,19	3,08
Pamier	DE	MW	13,64	14,53	3,65
Pegassos	DE	MW	12,00	13,83	3,25
Premio	DE	MW	12,94	13,94	3,42
Robigus	DE	FW	14,59	13,40	4,01
Tacitus	AT	MW	12,03	13,98	2,98
Tiger	DE	MW	12,03	14,83	2,78
Ubicus	AT	MW	12,43	15,40	3,09

Zufälliger Effekt	Varianzkomponente	Anteil an Gesamtvarianz
ID	1,639	52,90%
Rest	1,458	47,10%

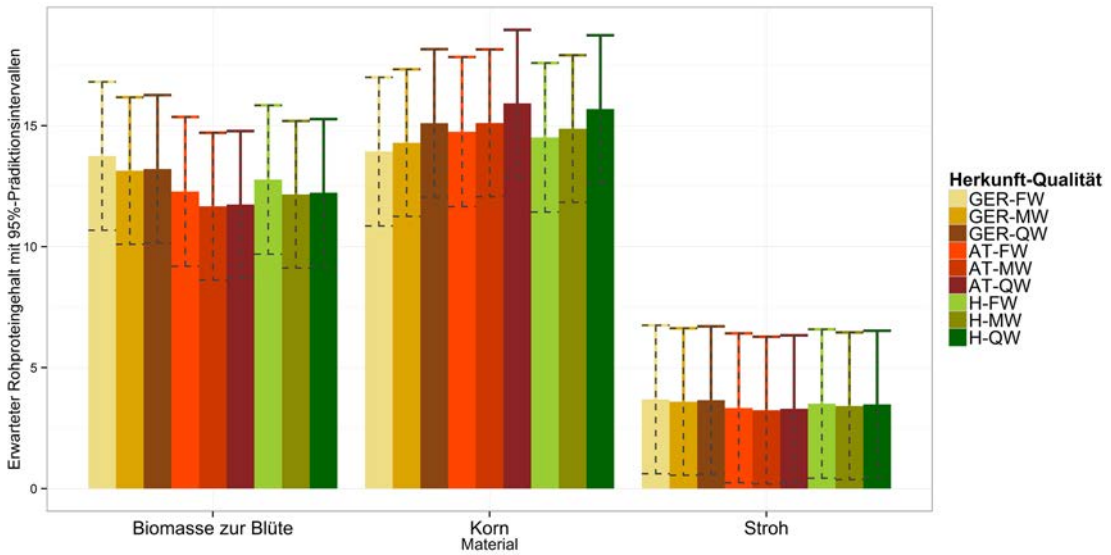

Abbildung 4: **Erwarteter Proteingehalt basierend auf den Modellergebnissen (Modell B) nach Qualität, Herkunft und Material.** Für die Berechnungen wurde angenommen, dass die zufälligen Effekte den Wert Null annehmen.

Figure 4: **Expected protein content based on modelling results (model B) of quality and origin of the genotypes and material.** For the calculations it was assumed, that the random effects accept the value null.

Einfluss von Qualität und Herkunft

Der erwartete Proteingehalt in der Biomasse zur Blüte beträgt 13,8% für Genotypen der Qualitätsgruppe Futterweizen und Herkunft Deutschland. Für Sorten der Qualitätsgruppe Mahlweizen verringert sich der erwartete Proteingehalt in der Biomasse zur Blüte um 0,6%, für Qualitätsweizen um 0,5%. Im Korn erhöht sich der erwartete Wert bei Qualitätsweizen um 1,7% und bei Mahlweizen um 1,0%. Österreichische Weizen weisen durchschnittlich um 2,3% und ungarische um 1,6% mehr Protein im Korn auf als deutsche.

Im Stroh findet sich bei ungarischen Sorten um 0,8% und bei österreichischen um 1,1% mehr Protein als bei jenen mit deutscher Herkunft. Die Qualitätsweizen und Mahlweizen liegen im Stroh nur wenig (jeweils 0,5%) über den Futterweizen.

Alle betrachteten erklärenden Variablen konnten als signifikante Einflussfaktoren auf den Rohproteingehalt identifiziert werden. Die erwarteten Proteinwerte für die einzelnen Gruppen von Genotypen sind in *Abbildung 4* dargestellt. Die 95%-Prädiktionsintervalle geben die Genauigkeit des geschätzten Proteingehaltes an. Hinsichtlich der Random Effects ist zu sehen, dass 36,3% der Gesamtvarianz durch ortsspezifische Schwankungen erklärt werden können.

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Dehydrin genes expression and transpiration intensity of drought stressed maize (*Zea mays* L.)

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Abstract

Drought is the most significant environmental stress worldwide and improving yield under drought is a major goal of plant breeding. The significance of drought increases with the time of its effect during the vegetation period and with its occurrence in the critical phases of the plants' development. For maize (*Zea mays* L.), critical periods are flowering and early maturity. The objective of the work was to determine the intensity of stress response of maize plants using physical (sap flow) and molecular methods to quantify stress reaction with respect to the plant's growth phase and drought intensity.

The experiment was performed on plants of drought-resistant maize line 2087 (breeding material provided by CEZEA Čejč, Czech Republic). A container experiment was established in natural conditions with limited irrigation. Four variants were maintained under different soil moisture conditions from phase BBCH 40: Variant A: control (90% available water holding capacity, AWHC); variant B: mild stress (50% AWHC); variant C: moderate stress (25% AWHC); and variant D: high stress (15% AWHC).Transpiration was monitored by continuous measuring of xylem sap flow. The EMS 62 measuring system (EMS Brno, CZ) uses the 'stem heat balance' method. Plant biomass for assessing the expression of the selected genes *DHN1* and *DHN2* was sampled on four terms (BBCH 63, 67, 75, 83-85). Total RNA was isolated from 100 mg leaf discs taken from the second youngest leaf. qPCR was performed with gene-specific primers for *DHN1* and *DHN2*. Gene expression was evaluated as normalised relative gene expression. The results were values of gene expression relative to the value of internal calibrator, i.e. value of expression in the first taking of the control variant.

Intensity of drought confirmatively influenced plant transpiration only in the two most stressed variants. A significant dependence between the average diurnal values of sap flow and volumetric soil moisture appeared only in the moderate-stressed variant C (r=0.528**, $P\leq$0.01) and in high-stressed variant D (r=0.395**, $P\leq$0.01). Significant differences were found out in transpiration ($P\leq$0.05). At the beginning of the measured period (flowering), transpiration in variants C and D was by 60% lower than transpiration of the control. At the end of the growing period, (BBCH 83-85), the transpiration flow was almost comparable in all experimental variants.

Expression of both evaluated genes *DHN1* and *DHN2* was detected from the beginning of the experiment (BBCH 63) also in plants grown under the optimum moisture conditions. The level of *DHN2* expression was 1000× higher at the beginning of the assessment than the level of *DHN1* expression. Increasing expression of both genes was observed up to the second sampling. At this time high increase in *DHN1* and *DHN2* expression was observed mainly in the high-stressed variant (*DHN1* $10^5\times$ and *DHN2* $10^3\times$) compared to the control.

Keywords

Drought tolerance, gene expression, qPCR, stem heat balance

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Analysis of genotype by environment interaction in an international winter wheat ring test and consequences for direct and indirect selection strategies for organic agriculture

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Abstract

Within the framework of the EU-COST action 860 SUSVAR (Sustainable low-input cereal production: required varietal characteristics and crop diversity), a ring test with 14 winter wheat genotypes from 5 different countries (AT, CH, DE, FR, RO) was performed in 36 field trials between 2006-2008 in Romania, Switzerland, France and Austria. Based on the observation of about 43 phenotypic traits, the ring test aimed at comparing the performance of wheat genotypes under low and high input cropping practices in many different European environments. Overall, 13 trials were sown under organic conditions (ORG), and out of the conventional trials, 6 were sown without N supply (NI, 'no input'), 8 under 'low input' (LI) conditions using maximum 100 kg N·ha^{-1}, and 9 trials were sown under 'high input' (HI) conditions. All trials were conducted without fungicide or growth regulator application. GGE-Biplots were used for the analysis of genotype stability and differential behavior under ORG, NI, LI and HI. Results were more variable between countries and individual trials than between systems. To represent all countries in each set, environments were re-grouped combining ORG plus NI in N0 and LI plus HI in N, i.e. groups without and with synthetic nitrogen.

Variance components and heritabilities were calculated for N0 and N plus additional groupings of environments (ORG, NI, LI, HI; years and countries). Heritability was 0.85, 0.83, 0.44, and 0.61 for HI, LI, NI, and ORG, respectively. Subsequently, relative selection efficiencies (RE) were calculated in order to compare direct and indirect selection for ORG, N0 and N and for countries and years. The question 'Can 3 years of trialling be replaced by trialling in one year' was answered by comparing sets of 12 trials from 2006 and 2007 with a set comprising 12 trials for 2006-2008 (4 in each year). Using 2006 as test environment gave a poor RE with respect to all 3 years' results, while selection in the year 2007 alone was sufficiently efficient.

Many traits, e.g. plant growth habit or leaf inclination as well as soil coverage were scored at different developmental stages. Data for 13 traits are represented in all 4 intensity groups, 31 traits are represented in both the N0 and N group of trials. Many traits were found to be highly correlated across the four systems HI, NI, LI and ORG.

For specific traits relevant mainly in organic agriculture (e.g. soil coverage) this work gave evidence that direct selection in N0 or ORG can be advantageous due to better differentiation. There seem to be two classes of traits: Those where available nitrogen increases differentiation (e.g. grain yield, plant height) and those where it blurs differentiation (e.g. soil coverage, number of tillers). Therefore, it may be promising to work with both types of environments (N and N0) when varieties are bred being adapted to organic and low input conditions. Conversely, if traits are highly correlated first among each other and second among systems, then it does not matter where selection is performed. Highly correlated traits can replace each other in practical breeding in order to save costs for selection.

The SUSVAR ring test experiment gave evidence that, in order to select suitable lines for organic and low input agriculture, higher selection efficiency at lower cost can be achieved by combining information from organic, conventional low input and high input trials. This enables a commercially more sustainable breeding program for organic and low input agriculture.

Keywords

Genotype by environment interaction, organic breeding, stability, *Triticum aestivum*

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Yield stability of hybrids versus lines in wheat, barley, and triticale

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Abstract

Hybrid breeding in the self-pollinating crops wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and triticale (×*Triticosecale* Wittmack) has the potential to lead to higher yield and enhanced yield stability. Yield stability can be assessed by the genotype-specific genotype by environment interaction (GEI) variance, which is termed stability variance. Small stability variance indicated high dynamic yield stability. A genotype that possesses high dynamic yield stability has changes in yield performance across environments, which correspond to the average changes in yield performance of all genotypes across environments.

Precise estimation of stability variance of individual genotypes requires intensive testing of genotypes. A recent study investigating barley registration trials suggested a minimum of 40 test environments. For group-specific estimates the required number of test environments is expected to be lower due to the larger sample of GEI effects.

We investigated three published experiments of the three crops. The wheat experiment comprised 1606 single-cross hybrids and 143 inbred lines, the barley experiment 45 single-cross hybrids, 15 three-way hybrids, and 36 inbred lines, and the triticale experiment 80 single-cross hybrids and 50 inbred lines. Each experiment was conducted at always five European locations. Single-cross hybrids, three-way hybrids and inbred lines were considered as separate genotypic groups.

The stability variance of each genotypic group was calculated for the three different crops. We found in all three crops a significant ($P<0.05$) smaller stability variance of hybrids compared to inbred lines, indicating higher dynamic yield stability of hybrids. In the barley experiment, stability variance of three-way hybrids was smaller than for the single-cross hybrids, but the difference was not significant. Our results agreed well with previous studies measuring dynamic yield stability with the stability variance. But several studies investigating dynamic yield stability with the deviation variance of the regression approach reported no advantage of hybrids in yield stability.

In the regression approach, for each genotype a linear regression of the genotypic yields in the individual environments on corresponding environmental indices is performed. The environmental indices should describe the yield level of the environments. The variance of the deviations of observed yield from the expected yields based on the regression line can be calculated and used as dynamic stability measure in analogy to the stability variance. In experimental studies comparing different yield stability measures, the deviation variance was closely related with the stability variance. Therefore, we were surprised about the contrasting results in literature and reviewed the studies using the regression approach thoroughly. In at least two of the four studies, the environmental indices were calculated based on yield performance of inbred lines only. We expected that this definition favours a smaller deviation variance of inbred lines, in case hybrids and lines react different across environments.

We analyzed the triticale experiment with the linear regression approach and found that inbred lines showed a lower deviation variance than hybrids when the mean performance of inbred lines was used as environmental index. But when the mean performance of all genotypes or the mean performance of hybrids was used as environmental index, hybrids showed a smaller deviation variance. We concluded that also the studies investigating yield stability with the regression approach would have found a higher dynamic yield stability of hybrids, when they would have used the mean yield of all genotypes as environmental index.

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Correlation between line *per se* and testcross performance for rye: Multi-cross QTL analysis results

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Abstract

The genotypic correlation between line *per se* and testcross performance is an important quantitative-genetic parameter to design hybrid breeding programs. The two main goals were to study (1) the association between line *per se* and testcross performance in two segregating winter rye populations (A, B) with each of 220 progenies tested in six environments and (2) the advantage of QTL mapping in single analysis and across populations (multiple cross) for plant height, thousand-kernel weight, test weight, total pentosan, and starch. The segregating progenies were genotyped with single nucleotide polymorphism (SNP), simple sequence repeat (SSR), and DArT markers (2176 for population A and 1072 for population B, respectively) to generate the consensus map.

High heritabilities were found for all traits for line *per se* and testcross performance (H^2=0.7-0.9). The only exception was pentosan content with moderate heritabilities throughout (H^2=0.6). Genotypic correlations between line *per se* and testcross performance were high for most comparisons (r_g=0.7-0.8) except for pentosan content (r_g=0.4-0.7). Selection of these traits for line *per se* performance in early generations will save field plots in further testing testcross performance.

By single QTL analysis we detected 19 QTL for line *per se* and 43 QTL for testcross performance across all traits in population A, and for line *per se* and testcross performance each of 16 QTL in population B, respectively.

In the multi cross QTL analysis we detected 49 QTL for line *per se* and 44 QTL for testcross performance. The highest explained phenotypic variance across all QTL was found for starch for line *per se* (51.6%), and for plant height for testcross performance (54.2%). For plant height, test weight, total pentosan, and starch contents we detected five, three, two, and four QTL overlapping for line *per se* and testcross performance, respectively.

In contrast, the other traits did not show any overlapping QTL. This can be explained by epistasis and/or masking dominance effects of the tester. For plant height and most quality traits, the higher heritability for line *per se* than for testcross performance and the large congruency of QTL results clearly suggests selection on line *per se* performance should be successful and increase efficiency of hybrid breeding.

Keywords

DArT marker, heritability, hybrid breeding, *Secale cereale*

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Phenotypic and genetic analysis of biomass and grain yield in hybrid rye

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Abstract

Winter rye (*Secale cereale* L.) is becoming increasingly important as substrate for biogas production in Central Europe and contributes to diversify crop rotation systems in agricultural bioenergy production. Dry matter yield has evolved as a novel breeding goal comparably important than traditional grain yield. In this study, we aimed at the identification of indirect phenotypic and molecular selection paramaters for dry matter yield to increase the genetic gain of this parameter.

The study was conducted with 258 elite testcross progenies for dry matter yield harvested at late milk stage and grain yield harvested at full ripening at three to four locations in Germany in 2011 and 2012. Further, we collected data for heading time, plant height in three stages and number of spikes per square meter. We observed a wide range of dry matter yield (10-24 t·ha^{-1}) and grain yield (6-15 t·ha^{-1}) among testcross progenies. Genetic variances were significantly (P<0.01) different from zero for all traits. High entry-mean heritabilities (H^2=0.76-0.94) were found for three plant height measurements and moderate heritabilities (H^2=0.52 and 0.49, respectively) for grain and dry matter yield. We observed a moderate correlation between plant height at EC 51-55/ EC 73 and dry matter yield (r=0.64, r=0.52, respectively, P<0.01) and a low correlation between grain yield and dry matter yield (r=0.33, P<0.01). Expected gain from direct selection for dry matter yield was 0.15 t·ha^{-1} per year. Indirect selection for dry matter yield using plant height at EC 51-55 was more effective (0.19 t·ha^{-1} per year). Indirect selection of dry matter yield by grain yield was less effective (0.08 t·ha^{-1} per year).

For the construction of the genetic map, we used a total of 911 SSR- and DArT-markers covering 964 cM of the rye genome. In total, we observed 23 QTL based on adjusted entry means using software PLABMQTL of the assessed agronomic traits. Most QTL were found for heading time (7) followed by plant height at EC 73 (6). Single QTL explained between 46% (plant height at EC 51-55) and 5% (third plant height at EC 73) of the genotypic variance. We have identified co-localized QTL for plant height and dry matter yield on chromosomes 2R and 5R, which supports the strong phenotypic correlation between both traits.

Indirect selection for dry matter yield using plant height at EC 73 should be successful to improve dry matter yield in rye. However, additionally improved lodging resistance should be selected. QTL with high effects on plant height were found that allow to improve selection gain by marker-assisted selection. The observed broad genetic variation for biomass yield in elite experimental hybrids gives good prospects for breeding improved hybrid rye as a renewable source of biogas production.

Keywords

Biogas, dry matter yield, indirect selection, plant height, *Secale cereale*

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Ermittlung von Resistenzquellen gegen Ährenfusariose für Weizenzüchtung und -anbau

Sources of resistance to Fusarium head blight for wheat breeding and growing

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Abstract

The response of 35 spring wheat varieties and breeding lines of four *Triticum* species to spray inoculation with *Fusarium culmorum* was evaluated in field experiments over three years. Besides symptom scores the content of deoxynivalenol (DON), the percentage of *Fusarium* damaged kernels, the reduction of thousand grain weight and the reduction of grain weight per spike due to infection were recorded. The resistance to *Fusarium* head blight (FHB) determined on the basis of the five recorded traits was variable in the investigated material and very high only in the non-adapted check variety 'Sumai 3'. The common wheat landrace Červená perla, four *T. dicoccum* genotypes (May Emmer, Weisser Sommer, Tábor, Rudico), *T. spelta* (Ruzyně), and bread wheat variety 'Vánek' can be considered moderately resistant to FHB. DON accumulation was significantly higher in modern common wheat varieties than in old landraces and other *Triticum* species. Similar experiments with 47 winter wheat varieties showed lowest DON contents in varieties 'Dagmar', 'Turandot', 'Balada' and 'Sakura'. Mid- and better-parent heterosis for DON content and FHB symptoms was studied in F_1 progenies of crosses between current varieties. Heterosis for resistance was common, therefore, selection of transgressive segregates from heterotic combinations should be feasible. The obtained results are encouraging with respect to the development of FHB resistant hybrid wheat varieties.

Keywords
Common wheat, DON content, *Fusarium culmorum*, heterosis, hulled wheat

Einleitung

Die Ährenfusariosen gehören weltweit zu den wichtigsten Getreidekrankheiten bei Weizen. Ihre Bedeutung ist weiter im Steigen begriffen. Eine wesentliche Rolle spielen dabei die Veränderungen in der landwirtschaftlichen Produktion wie eine Zunahme der Minimalbodenbearbeitung oder der Übergang zu einer intensiveren Produktion von Getreide und Mais in engen Fruchtfolgen. Der Befall führt zu Ertragsverlusten und hat auch die Bildung von Mykotoxinen im Erntegut und in der Folge eine Gefährdung von Lebensmitteln zur Folge. Pilze der Gattung *Fusarium* bilden eine ganze Reihe von Mykotoxinen. Am bekanntesten und am meisten untersucht ist Desoxynivalenol (DON).

Wirksame Schutzmaßnahmen gegen Ährenfusariosen basieren auf einer Kombination von Maßnahmen, die den Anbau von Sorten mit einer höheren Resistenz und den Einsatz wirksamer Fungizide zum Zeitpunkt der Blüte umfassen. Auch die Einhaltung einer weiten Fruchtfolge ist von Bedeutung, d.h. keine ausschließliche Konzentration auf Getreide und Mais. Die Resistenzzüchtung gegen Ährenfusariose bleibt weiterhin eine wichtige Herausforderung für den Weizenzüchter. Auch wenn eine mittlere bis hohe Anfälligkeit im kommerziell genutzten Weizensortiment dominiert so gibt es dennoch Unterschiede in der Resistenz, die beim Management der Bestände genutzt werden können.

Die Resistenz gegen Ährenfusariose beim Weizen ist ein quantitatives Merkmal, das polygen vererbt wird und von der Umwelt beeinflusst wird. QTL für die Resistenz befinden sich auf allen Chromosomen des Weizen (BUERSTMAYR et al. 2009). Ziel dieses Beitrags ist die Evaluierung eines breites Sortiments einschließlich von F_1-Hybriden hinsichtlich deren Resistenz gegen Ährenfusariose.

Material und Methoden

Inokulation und Mykotoxinbestimmung

Die Versuche mit 3 Wiederholungen wurden mit *Fusarium culmorum* künstlichen infiziert (MESTERHÁZY 1997). Dabei wurden während der Blüte 10 zufällig ausgewählte Ähren infiziert und über 24 Stunden mit Plastiktüten umhüllt. Zur Unterstützung der Infektion erfolgte eine Mikrobewässerung. Der Befall der Ähre wurde zu 3 Zeitpunkten an Hand einer Skala von 1 bis 9 entsprechend dem Prozentsatz des Befalls der Ährchen in der Ähre bewertet (1=kein Befall). Nach der Ernte wurden der Prozentsatz der von Fusariose befallenen Körner und die Reduktion der Ertragsmerkmale im Vergleich zur nicht infizierten Kontrollprobe bestimmt. Die Feststellung des DON-Gehalts erfolgte mittels ELISA unter Verwendung des RIDASCREEN® FAST DON Kits (R-Biopharm GmbH, Darmstadt, Deutschland).

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Sommerweizen

Von 2010 bis 2012 wurden durch künstliche Infektion 6 in der Tschechischen Republik registrierte Sorten Sommerweizen, sowie 23 genetische Ressourcen geprüft. Letztere Gruppe beinhaltete Dinkel (*Triticum spelta*), Einkorn (*T. monococcum*), Emmer (*T. dicoccum*) und eine alte Landsorte Weichweizen (*T. aestivum*). Als Kontrolle wurde die resistente Weichweizensorte 'Sumai 3' verwendet.

Winterweizen

Von 2010 bis 2012 wurden 30 in der Tschechischen Republik registrierte Winterweizensorten geprüft, davon wurden 12 Sorten in der Tschechischen Republik gezüchtet während 15 auf Basis einer Eintragung in den gemeinsamen Sortenkatalog der EU angebaut werden. Als Kontrollen wurden die ausländischen Sorten 'Arina' und 'Petrus' in die Versuche aufgenommen für die in zahlreichen Untersuchungen eine mittlere Resistenz gegen Ährenfusariose nachgewiesen wurde.

F_1-Hybride

Im Jahr 2013 wurden 28 F_1-Hybride von gegenwärtig kommerziell genutzten Sorten gemeinsam mit den Elternsorten in zwei Umgebungen (Feld, kaltes Treibhaus) getestet. Die Kreuzungen umfassten 8 Sorten, die sich im Grad der Resistenz unterscheiden: 'Bakfis', 'Petrus', 'Sakura', 'Federer' (mäßig bis mittel resistent), 'Bohemia', 'Elly' (mäßig anfällig), 'Cubus' und 'Biscay' (anfällig).

Ergebnisse und Diskussion

Sommerweizen

Die Ergebnisse der Resistenzprüfungen sind in *Tabelle 1* angeführt. Das höchste Resistenzniveau wies die Kon-

Tabelle 1: **Zusammenfassung der Resistenzdaten von Sommerweizen nach künstlicher Infektion mit *F. culmorum*: DON (mg·kg^{-1}), Ährensymptome (ÄS, 1-9), Fusarium befallene Körner (FDK, %), Reduktion des TKG (RTKG, %), Reduktion des Korngewichts an der Ähre (RKGÄ, %)**

Table 1: **FHB resistance of spring wheat: DON content (mg·kg^{-1}), visual FHB symptom scores (ÄS, 1-9; 1=no symptoms), Fusarium damaged kernels (FDK, %), reduction of thousand grain weight (RTKG, %) and reduction of grain weight per spike (RKGÄ, %)**

Sorte	Taxonomie	Zulassungsjahr/Genbankcode	DON	ÄS	FDK	RTKG	RKGÄ
Sumai 3	*T. aestivum*		5,2	1,9	14,2	11,1	17,6
Červená perla	*T. aestivum*	01C0100124	8,1	3,0	15,3	12,5	25,8
May-Emmer	*T. dicoccum*	01C0203990	8,2	2,5	16,5	7,3	34,1
Weisser Sommer	*T. dicoccum*	01C0203993	11,1	2,6	18,4	12,0	28,6
T. dicoccum (Tábor)	*T. dicoccum*	01C0204318	7,3	3,4	18,4	10,4	27,8
T. spelta (Ruzyně)	*T. spelta*	01C0201257	6,5	2,5	19,3	14,9	34,5
Vánek	*T. aestivum*	2004	14,5	3,1	24,6	14,9	25,7
Rudico	*T. dicoccum*	01C0200948	7,1	3,5	22,7	16,1	32,2
T. monococcum (Georgia)	*T. monococcum*	01C0204038	23,8	2,5	18,3	13,3	37,5
Schwedisches Einkorn	*T. monococcum*	01C0204053	13,5	3,4	35,0	15,4	28,8
T. spelta (Tábor 2)	*T. spelta*	01C0204323	9,1	3,6	22,5	18,6	33,9
Kaštická přesívka 203	*T. aestivum*	01C0200031	14,0	3,3	26,8	16,5	35,6
T. monococcum No.8910	*T. monococcum*	01C0204542	14,5	3,4	26,3	22,5	41,9
T. spelta (Tábor 1)	*T. spelta*	01C0204322	11,4	3,6	23,5	22,7	44,1
Rosamova česká červená přesívka	*T. aestivum*	01C0200051	13,8	4,3	27,2	20,9	34,6
Trappe	*T. aestivum*	2007	22,7	3,4	28,4	25,7	34,3
T. dicoccum (Tapioszele)	*T. dicoccum*	01C0201280	16,5	3,3	29,0	24,1	45,0
T. spelta (Kew)	*T. spelta*	01C0200984	19,6	3,4	34,6	24,6	42,3
Postoloprtská přesívka 6	*T. aestivum*	01C0200043	22,9	3,6	35,4	25,0	41,9
T. dicoccon (Palestine)	*T. dicoccum*	01C0201261	16,6	3,9	40,5	28,4	39,2
KWS Scirocco	*T. aestivum*	2011	22,7	3,7	34,8	25,1	49,3
Izzy	*T. aestivum*	2011	29,0	4,4	40,8	22,9	37,7
Dafne	*T. aestivum*	2011	31,0	4,0	38,5	30,9	41,3
T. spelta (VIR St.Petersburg)	*T. spelta*	01C0204865	21,2	4,2	35,8	34,7	47,5
KWS Chamsin	*T. aestivum*	2012	34,4	4,2	38,7	28,6	46,5
T. monococcum (Albania)	*T. monococcum*	01C0204044	27,2	3,6	50,1	30,4	51,0
Špalda bílá jarní	*T. spelta*	01C0200982	25,7	4,2	38,3	37,4	53,0
T. spelta No 8930	*T. spelta*	01C0204506	22,9	4,7	39,2	33,5	50,7
Seance	*T. aestivum*	2008	41,4	4,5	47,6	30,4	43,6
Astrid	*T. aestivum*	2012	38,9	4,6	42,4	32,2	46,8
Septima	*T. aestivum*	2008	40,9	4,9	50,3	37,2	46,9
Tercie	*T. aestivum*	2008	45,8	4,6	54,9	38,4	49,7
SW Kadrilj	*T. aestivum*	2006	51,3	4,6	44,0	40,1	54,1
T. dicoccon (Dagestan ASSR)	*T. dicoccum*	01C0204016	31,0	5,1	53,3	44,6	60,0
T. dicoccon (Brno)	*T. dicoccum*	01C0204022	37,9	5,6	61,1	41,2	65,6
2010			19,4	4,0	29,6	24,4	40,6
2011			22,3	3,8	39,7	27,2	45,4
2012			24,4	3,6	32,3	23,5	36,9
Mittelwert			22,4	3,8	33,9	25,1	41,5

trollsorte ‘Sumai 3’ auf, es folgten Červená perla (Rote Perle, *T. aestivum*), May Emmer, Weißer Sommer, Tábor (alle *T. dicoccum*), *T. spelta* Ruzyně, ‘Vánek’ (*T. aestivum)* und Rudico (*T. dicoccum*) (CHRPOVÁ et al. 2013). Ein relativ niedriger und stabiler DON-Gehalt wurde bei *T. spelta* Ruzyně, sowie bei Rudico und *T. dicoccum* Tábor festgestellt. Eine niedrige, jedoch variable DON-Akkumulation wiesen Červená perla, May Emmer und *T. spelta* Tábor 2 auf. Insgesamt kann festgestellt werden, dass ein statistisch höherer mittlerer DON-Gehalt (33,9 mg·kg^{-1}) bei den gegenwärtigen Hochzuchtsorten im Vergleich zu Spelzweizen (Dinkel, Emmer, Einkorn) oder alten Weichweizen Landsorten (17,3 mg·kg^{-1}) beobachtet wurde. Die Verringerung des Korngewichts war bei ‘Sumai 3’ (17,6%)

Tabelle 2: **Zusammenfassung der Resistenzdaten von Winterweizen nach künstlicher Infektion mit *F. culmorum*: DON (mg·kg^{-1}), Ährensymptome (ÄS, 1-9), Fusarium befallene Körner (FDK, %), Reduktion des TKG (RTKG, %), Reduktion des Korngewichts an der Ähre (RKGÄ, %)**

Table 2: **FHB resistance of winter wheat: DON content (mg·kg^{-1}), visual FHB symptom scores (ÄS, 1-9; 1=no symptoms), Fusarium damaged kernels (FDK, %), reduction of thousand grain weight (RTKG, %) and reduction of grain weight per spike (RKGÄ, %)**

Sorte	Zulassung	Herkunft	Zulassung in CZ	DON	ÄS	FDK	RTKG	RKGÄ
Cimrmanova raná	CZ	CZ	2012	31,4	3,3	37,9	23,9	43,4
Turandot	CZ	CZ	2012	30,8	3,6	44,2	29,9	52,5
Dagmar	CZ	CZ	2012	29,7	4,0	41,6	36,1	50,5
Sakura	CZ	CZ	2007	31,2	2,8	58,8	29,1	45,9
Alana	CZ	CZ	1997	46,9	3,8	50,0	34,0	50,7
Petrus		DE		39,8	3,3	52,6	37,2	50,7
Simila	CZ	CZ	2006	39,8	3,6	51,4	37,7	50,9
Elly	CZ	CZ	2010	38,4	4,1	51,3	33,5	56,1
Levis	EU	CH		41,1	4,1	52,8	27,8	51,4
Bohemia	CZ	CZ	2007	43,9	3,4	45,6	40,0	52,9
Solitär	EU	DE		53,3	3,7	52,8	35,2	53,2
Evina	CZ	EU	2012	39,1	3,9	49,0	39,0	58,4
Bazilika	EU	SK		41,9	4,1	55,3	36,4	53,0
Baletka	CZ	CZ	2008	43,8	4,0	43,5	40,1	55,5
Arina				44,4	4,0	52,3	37,2	58,0
Balada	EU	CZ		31,0	4,7	56,1	26,4	58,5
Aladin	CZ	DE	2010	38,1	4,6	55,7	37,4	57,1
Brilliant	CZ	DE	2009	48,0	3,9	50,7	42,2	57,0
Bardotka	EU	SK		46,2	4,7	54,8	34,4	58,7
Matylda	CZ	CZ	2011	52,4	4,7	49,5	39,0	61,4
Mulan	CZ	DE	2007	60,7	3,8	58,7	39,3	61,4
JB Asano	CZ	DE	2012	52,2	4,9	60,9	36,7	53,4
Princeps	CZ	DE	2012	57,1	4,4	50,7	43,3	59,2
Penalta	EU	CZ		70,6	4,3	64,3	37,3	60,4
Tiguan	CZ	FR	2012	58,9	4,1	64,3	37,8	64,3
Akteur	CZ	DE	2004	69,4	4,8	55,7	41,7	63,2
Potenzial	CZ	DE	2012	55,2	4,3	55,8	48,6	67,3
Privileg	EU	DE		77,4	4,4	57,8	40,7	66,4
Sultan	CZ	CZ	2008	82,8	4,3	56,7	44,8	63,6
Beduin	CZ	FR	2011	92,7	4,5	59,4	38,1	67,9
Henrik	CZ	DE	2010	65,7	5,4	57,1	42,1	62,0
Brentano	CZ	DE	2010	83,0	4,5	59,5	40,8	67,7
Carroll	CZ	NL	2011	89,5	4,2	63,5	42,0	64,8
Contra	EU	DE		76,0	5,2	69,5	40,1	63,6
Pannónia NS	EU	RS		72,7	5,2	76,5	38,7	63,9
SW Topper	EU	DE		66,0	5,1	67,1	41,0	69,3
KWS Ozon	CZ	DE	2012	82,2	5,0	56,2	48,0	68,2
Hewitt	CZ	NL	2012	93,9	4,5	65,9	46,6	67,9
Cubus	CZ	DE	2004	96,1	4,7	63,2	45,8	68,7
IS Karpatia	EU	SK		94,4	4,6	62,2	46,1	73,7
Samurai	EU	DE		126,9	5,4	75,9	38,3	70,6
Venistar	EU	SK		77,0	6,4	72,5	44,8	75,0
Sogood	EU	FR		89,6	6,0	74,8	42,7	71,9
Biscay	CZ	DE	2005	100,3	5,8	60,4	51,2	79,9
Seladon	CZ	CZ	2009	107,8	5,3	70,6	52,7	70,9
Buzogány	EU	HU		109,3	5,6	76,0	49,0	73,8
Altigo	CZ	FR	2011	128,9	5,8	80,0	61,0	80,6
2010				62,4	4,7	69,9	38,5	65,0
2011				73,7	5,0	55,8	47,6	68,8
2012				56,9	3,8	47,9	33,4	50,6
Mittelwert				64,8	4,5	58,3	39,9	61,6

signifikant geringer als bei den derzeitig kommerziell genutzten Sorten (43,3%), Spelzweizen (42,6%) oder alten Landsorten (33,8%). Die größte Variabilität bei der Reduzierung des Korngewichts wurde bei Genotypen der Arten *T. monococcum* und *T. dicoccum* festgestellt. Relativ niedrige Werte zeigten auch die beiden Hochzuchtsorten 'Vánek' und 'Trappe'. Die festgestellten Unterschiede zwischen modernen und älteren Sorten stehen in Einklang mit anderen Untersuchungen. Die dichte Umschließung des Korns durch dickere Spelzen kann eine effektive Barriere für das Pilzmyzel und somit einen passiven Resistenzfaktor darstellen, wodurch die etwas geringere Anfälligkeit von Spelzweizen erklärt werden könnte (BUERSTMAYR et al. 2003, SUCHOWILSKA et al. 2010). Ein weiterer Mechanismus einer passiven Resistenz kann eine lockere Ähre mit festen Spelzen und einer zarten Ährenspindel sein (BUERSTMAYR et al. 2003). Von praktischem Wert ist die Resistenz gegen FHB bei *T. dicoccum* Rudico, May Emmer, Weißer Sommer und Tábor, die auch einen hohen Eiweißgehalt (≥16%) aufweisen (CHRPOVÁ et al. 2013). Eine besonders interessante Resistenzquelle ist die Sorte 'Vánek', die auch eine ausgezeichnete Backqualität besitzt.

Winterweizen

Die Ergebnisse der Winterweizenprüfungen sind in *Tabelle 2* angeführt. Das höchste Resistenzniveau wurde bei den Sorten 'Cimrmanova raná', 'Turandot', 'Dagmar', 'Sakura', 'Alana' und der Kontrollsorte 'Petrus' festgestellt. Der niedrigste DON-Gehalt konnte bei 'Dagmar', 'Turandot', 'Balada' und 'Sakura' beobachtet werden. Die höchste DON-Akkumulation (>100 mg·kg^{-1}) wurden bei 'Biscay', 'Seladon', 'Buzogány', 'Samurai' und 'Altigo' beobachtet. Diese Sorten waren auch in den anderen Merkmalen als anfällig bewertet. Die in der Tschechischen Republik gezüchteten Sorten weisen ein statistisch signifikant höheres Resistenzniveau auf als die registrierten, ausländischen Sorten oder die auf Grundlage des Gemeinsamen Sortenkatalogs angebauten Sorten. Aus den Ergebnissen wird deutlich, dass es im bestehenden Winterweizensortiment für die Landwirte sehr wohl die Möglichkeit gibt Sorten mit einem höheren Resistenzniveau gegen Ährenfusariose zu wählen. Vor allem in Risikogebieten sollen v.a. bei Vorfrucht Mais und bei Minimalbodenbearbeitung resistentere Sorten zum Einsatz kommen.

F_1-Hybride

Bei den geprüften F_1-Hybriden wurde hinsichtlich der FHB Symptome und des DON-Gehaltes eine im Vergleich zum Elterndurchschnitt höhere Resistenz festgestellt. Wie aus *Tabelle 3* ersichtlich ist, erreichte die *mid-parent* Heterosis bei allen Hybriden negative Werte. Zu ähnlichen Ergebnissen kamen auch BUERSTMAYR et al. (1999) bei der Bewertung der Intensität des Ährenfusariose-Befalls an Nachkommen europäischer Weizensorten in der F_1. Der

Tabelle 3: **Heterosis in der F_1 in Bezug auf das Elternmittel (MPH) bzw. auf den resistenteren Elter (BPH) für die Merkmale DON Gehalt (mg·kg^{-1}) und Ährenbefalls (ÄS, %).**

Table 3: **Mid-parent (MPH) and better-parent (BPH) heterosis of F_1 with respect to DON content (mg·kg^{-1}) and Fusarium infected spikelets (ÄS, %).**

Saatelter	Pollenelter	DON	MPH	BPH	ÄS	MPH	BPH
Bakfis	Cubus	16,69	-55,6	-5,7	10,83	-28,6	-17,0
Bakfis	Elly	17,25	-37,7	-5,1	14,56	-25,2	-13,3
Federer	Sakura	21,94	-28,6	-27,4	17,22	-4,8	-4,2
Bakfis	Bohemia	24,09	-21,8	1,7	14,83	-15,7	-13,0
Bakfis	Biscay	25,03	-79,4	2,7	20,33	-23,4	-7,5
Bakfis	Sakura	26,78	-10,3	4,4	13,78	-10,9	-7,7
Bakfis	Petrus	27,89	-20,4	5,5	9,61	-17,4	-16,5
Sakura	Petrus	28,03	-34,9	-23,8	13,06	-10,7	-8,4
Bakfis	Federer	35,24	-0,6	12,9	16,39	-8,9	-6,3
Bohemia	Petrus	39,91	-31,9	-29,6	12,22	-17,4	-13,9
Petrus	Elly	40,40	-40,4	-33,7	14,94	-23,9	-11,2
Sakura	Bohemia	41,92	-18,7	-9,9	15,83	-11,5	-5,6
Federer	Bohemia	43,48	-15,9	-5,8	21,33	-6,6	-1,3
Federer	Elly	43,76	-24,6	-5,6	28,83	-8,3	6,2
Bohemia	Elly	46,71	-31,8	-22,8	26,33	-16,1	-6,9
Federer	Petrus	49,61	-12,1	0,3	14,39	-10,0	-8,3
Sakura	Cubus	51,67	-35,4	-0,1	30,61	-5,6	9,2
Petrus	Biscay	58,32	-72,0	-15,8	21,33	-21,6	-4,8
Federer	Cubus	63,94	-21,8	14,6	25,56	-11,3	2,9
Sakura	Biscay	70,42	-48,7	18,6	35,00	-5,6	13,6
Sakura	Elly	73,25	3,6	21,4	20,33	-16,2	-1,1
Elly	Cubus	74,81	-30,0	-12,6	26,50	-24,8	-24,5
Bohemia	Cubus	75,68	-20,2	6,2	32,56	-9,6	-0,7
Federer	Biscay	80,88	-37,0	31,6	37,11	-4,1	14,4
Petrus	Cubus	82,75	-15,4	8,6	29,89	-8,7	3,8
Bohemia	Biscay	120,83	-7,1	51,4	43,53	-2,9	10,3
Elly	Biscay	128,26	-8,7	40,8	38,89	-16,8	-12,8
Cubus	Biscay	128,98	-25,4	6,7	43,80	-11,6	-7,2
Mittelwert		54,95	-28,0		23,20	-13,5	-4,7

Heterosiseffekt wird offensichtlich durch die Anwesenheit verschiedener Gene bei den Elternsorten hervorgerufen.

Sorten, die in langfristigen Versuchen ein vergleichbares Resistenzniveau erreichten, unterschieden sich jedoch (insbesondere beim DON-Gehalt) in der allgemeinen Kombinationsfähigkeit (GCA). Diese war am höchsten und sehr bedeutend bei 'Bakfis' (-29,6**) und unbedeutend bei 'Sakura' (-9,4) und 'Petrus' (-7,6*). Die spezifische Kombinationseignung (SCA) war am höchsten bei den Hybriden von 'Bakfis' mit den anfälligen Sorten 'Cubus' und 'Biscay'. Auf Grundlage der *better-parent* Heterosis wurde beim DON-Gehalt eine stärkere Ausgeglichenheit bei Hybriden mit der Sorte 'Bakfis' festgestellt, demgegenüber jedoch eine deutlich spezifische Reaktion nach der Kreuzung mit den Sorten 'Sakura', 'Petrus' und 'Federer' (*Tabelle 3*). Es zeigt sich, dass die Wahl einer geeigneten Elternsorte (mit einer hohen GCA) den Selektionserfolg eines FHB resistenten Hybriden bedeutend erhöhen kann. Eine hohe SCA ist für die Auswahl der Hybriden, die für eine Verbesserung des gesamten FHB Merkmalkomplexes in Frage kommen, entscheidend. Die Ergebnisse ermöglichen sowohl eine Elternauswahl für Kreuzungen bei denen Transgressionen in der aufspaltenden Nachkommenschaft genutzt werden können, als auch direkt die Möglichkeit der Nutzung der Heterosis bei der Schaffung von Hybridweizen.

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Association between anther-retention and Fusarium head blight susceptibility in wheat

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Abstract

Plant morphological and developmental traits play an important role in Fusarium resistance, such as plant height and the extent of anther-retention during flowering. We evaluated three series of wheat germplasm for Fusarium head blight (FHB) resistance in replicated and artificially spray-inoculated field trials during 2-3 seasons. FHB severity was evaluated using visual scorings at several dates after inoculation which were used to calculate the area under disease progress curve (AUDPC) as an integrated measure for FHB severity. The same lines were evaluated for the extent of anther retention. We counted in each plot 20 florets per line 4-6 days after main flowering for florets with at least one anther still trapped inside the floret and expressed this as % anther retention. Population I consisted of 192 winter wheat breeding lines and cultivars, mainly from France and Austria. Population II was a bi-parental mapping population of 171 F_6 recombinant inbred lines from the cross Capo×Arina. Population III was a doubled haploid population with 203 DH lines from the cross Hermann×Skalmeje.

All three populations showed quantitative variation for FHB severity and for percentage of anther retention. Both traits were highly heritable, with broad sense heritability coefficients H^2=0.77 and 0.86 for % anther-retention and H^2=0.85 and 0.82 for AUDPC in populations III and II, respectively. In all three populations % anther retention was significantly correlated with AUDPC (r=0.63-0.65).

In the Capo×Arina population a linkage map with SSR and DArT markers is available which allowed mapping of QTL for both traits. Among four medium effect QTL for FHB resistance mapping to chromosomes 6B (r^2=10.4), 4A (r^2=10.9), 2A (r^2=9.6) and 5A (r^2=8), two co-mapped with large effect QTL for anther retention on 6B (r^2=22.4), and 4A (r^2=18.8). In both cases the 'Arina' allele contributed to increased FHB resistance and to reduced anther retention.

The Hermann×Skalmeje population segregates at two major dwarfing genes: *Rht-B1* and *Rht-D1*. Both semi-dwarf alleles (*Rht-B1b* and *Rht-D1b*) had very similar effects on reducing height by 12-14 cm compared to the tall lines, but different associations with FHB severity and anther retention. Relative to lines with both tall alleles (*Rht-B1a*/*Rht-D1a*) on average lines with *Rht-B1b*/*Rht-D1a* showed moderately increased in FHB severity (+22%) and anther retention (+12%), while lines possessing *Rht-B1a*/*Rht-D1b* showed strongly increased FHB severity (+61%) and anther retention (+60%). Double dwarfs (*Rht-B1b*/*Rht-D1b*) were on average very short (-31 cm), and were highly FHB susceptible (+128%) and had a high degree of anther retention (+99%), relative to tall lines with *Rht-B1a*/*Rht-D1a*.

In summary, FHB severity is strongly associated with the trait anther-retention. We speculate that anthers are an easy to conquer nutritious tissue for Fusarium that stimulate fungal development and thus give Fusarium an advantage for penetrating the floret. We assume that anther extrusion is a passive resistance factor which is primarily relevant for type 1 resistance of wheat. Selection of lines with high degree of anther extrusion should result in a correlated selection response towards increased FHB resistance.

Keywords

Dwarfing genes, flowering, heritability, resistance mechanism, *Triticum aestivum*

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Fusarium head blight of wheat: Involvement of salicylic acid and jasmonic acid in disease resistance response

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Abstract

Fusarium head blight (FHB) is a devastating disease affecting maize and small grain cereals mainly caused by the hemi-biotrophic fungus *Fusarium graminearum*. Infection of wheat results in severe grain yield losses and trichothecene contamination (e.g. deoxynivalenol). This is not only toxic for the plant itself, but more importantly also for humans and livestock. Controlling FHB outbreaks and the disease is difficult and achieved by cultural techniques and the use of chemicals. Therefore, breeding of highly resistant cultivars is the most effective way of coping with the disease. FHB resistance in hexaploid wheat is quantitative. More than 200 QTL have been detected in various studies. Only a few are stable in different environments and diverse genetic backgrounds and therefore useful for breeding. An accepted resistance mechanism is the prevention of fungal spread within the spike (type II resistance). However, the underlying molecular mechanisms are still poorly understood. Further research at the molecular level of the *Triticum aestivum* - *F. graminearum* interaction will deepen our understanding and forward FHB resistance breeding.

In our study, four bread wheat genotypes differing in FHB resistance level were evaluated: highly-resistant 'Sumai 3', moderately-resistant 'Dream', moderately-susceptible 'Lynx' and highly-susceptible 'Florence Aurore'. Single-floret inoculation was used to infect wheat heads at early anthesis (1000 *F. graminearum* macroconidia per floret, or distilled water). Spike samples were collected at 12 time points after inoculation, i.e. 0, 0.33, 1, 1.33, 2, 3, 4, 5, 7, 14, 21 days after inoculation and at maturity. Furthermore, spike samples were dissected in four subsamples, which were analyzed independently: inoculated spikelets, rachis, uninoculated spikelets and upper stem. Fresh weights were recorded. Quantification of salicylic acid, jasmonic acid, fungal biomass and DON are in progress. Also, expression analysis of marker genes associated with salicylic- and jasmonic acid-dependent signaling pathways will be performed.

Comparison of *F. graminearum* and mock-inoculated subsample weights showed the importance of the rachis tissue in highly-resistant 'Sumai 3'. Here, the weight was not affected by *F. graminearum* attack, whereas in highly-susceptible 'Florence Aurore' the rachis was completely blighted 14 days after inoculation. Gene expression analysis confirmed importance of the rachis tissue. The gene expression of *Non-Expressor Of Pathogenesis-Related Gene 1* (*NPR1*) was examined. *NPR1* is a master regulator controlling immune responses in the systemic acquired resistance (SAR) pathway. Three days after inoculation the *NPR1* gene was 5-fold up-regulated compared to the water control in 'Sumai 3', and seven days after inoculation still 3.5-fold. However, this gene was not up-regulated in 'Florence Aurore'. Indicating an absence of, or late response to *F. graminearum* attack. Fungal biomass quantification using qPCR resulted in a clear differentiation between FHB resistance levels of the two cultivars. In the rachis of 'Sumai 3', 2.5-fold less *F. graminearum* biomass was detected than in 'Florence Aurore' after 21 days. Furthermore, in the 'Sumai 3' subsample from uninoculated spikelets 0.21 ng fungus per mg plant fresh weight were found at 21 days after inoculation, i.e. 4-fold less than in 'Florence Aurore'. Our results show that analyzing the dissected spike provides a more detailed insight into systemic signaling and defense response of wheat attacked by *F. graminearum*.

Keywords

Disease resistance, *Fusarium graminearum*, gene expression, NPR1, *Triticum aestivum*

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Occurrence of maize redness disease in Hungary

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Abstract

Maize redness (MR) is a severe disease of corn associated with stolbur phytoplasma (16SrXII-A) which is transmitted to the host plant by the cixiid planthopper *Reptalus panzeri* (Low). The disease is characterized by midrib, leaf and stem reddening, followed by reddening and desiccation of the whole plant and abnormal ear development with poor seed set. During the MR epidemic, disease symptoms can be present in up to 90% of the plants, and yield losses can be over 50%. Previously reported presence of *R. panzeri* in Hungarian vineyards and their natural infection with stolbur phytoplasma, in addition to economic importance of maize production, increased the need for a survey of MR presence in the territory of Hungary.

During August and September of 2010 selected maize fields in several production areas of Hungary were surveyed for the occurrence of reddening symptoms on maize. This year *R. panzeri* and *Hyalesthes obsoletus* cixiids were also collected from a single maize field in the vicinity of village Monorierdő. A total of nine sampling sites were included in the survey. In the autumn of 2013 MR symptomatic maize plants were sampled from the post-control plots of the National Food Chain Safety Office in Central Hungary, Monorierdő. Phytoplasma identification was performed using a nested PCR on the 16S rRNA gene with primer pairs P1/P7 and R16F2n/R16R2. In parallel, all samples were tested with TaqMan real-time PCR amplifying phytoplasma nonribosomal *map* gene of the 16SrXII-A subgroup, applying plant endogenous control (EC) with slight modification of probe labelling and PCR conditions.

Only in three out of 25 symptomatic maize plants, collected from nine sampling sites in 2010, was the stolbur phytoplasma (16SrXII-A) identified by PCR/RFLP based analysis of the 16S rRNA gene. All three stolbur-infected corn samples originated from the same locality, Monorierdő, Central Hungary where the potential planthopper vectors were identified. As for analysed insects, two out of six *R. panzeri* and three out of eight *H. obsoletus* specimens proved to be positive for the presence of stolbur phytoplasma. The results were confirmed by real-time PCR. None of the other maize samples, collected at other localities were positive for any phytoplasma. In one out of three MR symptomatic maize samples originated from post-control plots of Monorierdő in 2013, the stolbur phytoplasma was also detected and significant differences were registered in the susceptibility of maize genotypes tested.

Based on these results it is concluded that stolbur phytoplasma associated with maize redness disease and the identified vector of the disease, *R. panzeri* are present in corn in Hungary. The role of *H. obsoletus* in MR epidemiology in Hungary is yet to be studied since this cixiid was not reported to play a significant role in MR epidemiology in Serbia.

Keywords

Cixiids, real-time PCR, stolbur phytoplasma, *Zea mays*

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Use of wheat gene resources with different grain colour in breeding

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Abstract

The interest in wheat genetic resources with different grain colour has recently increased. Differences in grain colour are caused by the presence of polyphenols, tannins, anthocyanins and carotenoids. Resources of uncommon grain colour have blue aleurone (*Ba* genes), purple pericarp (*Pp* genes) and yellow endosperm (*Psy* genes), which are determined by the presence of anthocyanins and carotenoids, respectively. These substances have antioxidant activity and are useful for the production of functional foods with positive effects on consumers' health. In 2011, the winter wheat variety Skorpion with blue aleurone, which had been bred in the Czech Republic, was registered in Austria. The used donor of blue aleurone comes from the heritage of Erich von Tschermak-Seysenegg (1871-1962). The breeding program at Agrotest Fyto, Ltd., Kroměříž, is focused on the development of winter wheat genotypes with dark grain combining different genes for grain colour and simultaneously agronomic important traits (yield, resistance to stress) and good baking quality. In different kernel tissues there are different amounts of coloured substances that affect their content in bran and flour and, thus, their content in bakery and biscuit products.

Keywords

Antioxidants, blue aleurone, purple pericarp, *Triticum aestivum*, yellow endosperm

Introduction

Various types of plants synthesize a number of substances from the group of flavonoids that cause the characteristic colour of tissues in response to the respective environments. The occurrence of these substances is associated with adaptive role to stressful environmental factors (KHLESTKINA 2013, ZEVEN 1991). Also, the grain colour of cereals may be different. For the vast majority of current wheat varieties the grain colour is red, less often it is white. However, there are genetic resources of wheat with a grain colour significantly differing from current varieties due to the presence of coloured pigments (e. g. carotenoids, flavonoids, anthocyanins, some phenolic compounds). Like many types of vegetables and fruits, these substances are characterized by antioxidant properties and have an irreplaceable role in a healthy diet for people. Generally, antioxidants are considered essential for humans to prevent inflammation, diabetes, cancer, oxidative stress and ocular diseases (LAMY et al. 2006). Antioxidants in cereals could be used for the production of functional foods and positively affect consumers' health. The use of existing wheat genetic resources with different genes for coloured substances and understanding the pathway of their biosynthesis could be employed for the breeding of varieties accumulating a higher number of relevant genes, which would allow to increase the content of health-promoting substances. The successful application of varieties with coloured grains into practice will depend on the level of yield and agronomic properties comparable to commercially used varieties. The current donors of genes for blue and purple grain, and yellow flour have usually lower yield compared to standard varieties. Therefore, wheat breeding programmes should focus on eliminating this deficiency.

It will be also necessary to know the extent of natural degradation of dyes during thermal processing of the wheat grain when during Maillard reaction chemical changes occur. These new compounds can have a differerent influence on health compared to the original compounds. Production and processing technology will have to be adapted in order to conserve and use best these natural substances. An alternative technology could be extrusion or expansion (puffing) where the exposure of the raw material to high temperatures is reduced.

Wheat grain colours and their genes

Red and white grain

The red colour of grain occurs in most common European wheat varieties. It is controlled by one to three dominant alleles *R-A1* (on chromosome 3AL), *R-B1* (3BL) and *R-D1* (3DL). Contrary, white grain colour is determined by the recessive alleles, i.e. *r-A1*, *r-B1* and *r-D1*. The pigment is composed of catechin and tannin derivatives generated in the process of biosynthesis of flavonoids (HIMI and NODA 2005). The red colour of the grain is associated with a higher content of bitter phenolic components, lower activity

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of hydrolytic enzymes, and better resistance to sprouting. Phenolic acids are low molecular weight compounds with antioxidant activities and can be oxidised in the grain by polyphenol oxidase to darker colour compounds (tannins and lignin). They inhibit lipoxygenase and are uncompetitive inhibitors with protective effects against diseases (LACHMAN et al. 2003). White grains have low amounts of polyphenol oxidase, the absence of bitter substances makes the product naturally sweeter, which can be important in confectionery. Wheats with white grains are more susceptible to sprouting, the milling allows higher yield of flour and, thus, the flour can contain more fibre, minerals and proteins.

Purple pericarp

The purple grain colour is caused by genes for purple pericarp *Pp*, which were transferred to common wheat from tetraploid wheat *Triticum turgidum* L. subsp. *abyssinicum* Vavilov, coming from the Abyssinian region in Ethiopia. It is characterized by the presence of anthocyanins in the surface layer (pericarp) of the grain. According to ABDEL-AAL and HUCL (2003) and KNIEVEL et al. (2009) cyanidin 3-glucoside, cyanidin 3-rutinoside, and succinyl glucoside are most represented in purple grains. The mean content of total anthocyanins was 104 mg·kg^{-1} in wholemeal flour and 251 mg·kg^{-1} in the bran (ABDEL-AAL and HUCL 1999). So far several genes for purple pericarp were identified. Using monosomic analysis two genes, i.e. *Pp1* and *Pp2*, on chromosomes 7B and 7A, respectively, were detected in variety 'Purple Feed', whereas variety 'Purple' contained the genes *Pp1* and *Pp3* (ARBUZOVA and MAYSTRENKO 2000). Later it was found that *Pp3* is composed of two alleles that have been named *Pp3a* and *Pp3b* (DOBROVOLSKAYA et al. 2006). Both are located in the centromeric region of chromosome 2A. For genes *Pp1* and *Pp3* complementary effect was described. Feeding hens with purple wheat resulted in increased weight of meat (+6.2%) and a higher number of eggs (+3.4%), whereas no effect was found on the colour of yolk (RÜCKSCHLOSS et al. 2010).

Blue aleurone

Blue grain colour is determined by genes for blue aleurone *Ba*. QUALSET et al. (2005) reported that blue aleurone in spring wheat 'UC66049' is controlled by the codominantly acting gene *Ba1*. This gene has been transferred to wheat by an entire chromosome arm from *Thinopyrum ponticum* Podp. which was incorporated into chromosome 4B (4BS-4el$_2$). Dr. Emil Šebesta from USDA-ARS at Oklahoma State University developed during 1958-1988 the blue grained lines 'SB-1', 'SB-2', and 'SB-3' ('Sebesta Blue') with chromosomal segments from *Th. ponticum* (MORRISON et al. 2004). This material seems to have the similar origin as 'UC66049'. Gene *Ba2* has been transferred to wheat from *T. monococcum* ssp. *aegilopoides* (syn. *T. boeoticum*) as disomic substitution of 4A (4A^{m}L) (DUBCOVSKY et al. 1996, SINGH et al. 2007). It is assumed *Ba2* is present in 'Thatcher Blue', originating from the John Innes Centre (Watanabe, personal commun.). Deviations in the inheritance demonstrate that *Ba1* and *Ba2* are distinct genes (METTIN et al. 1991). According to the 'Catalogue of Genetic Symbols for Wheat', there is also weaker gene expression 'half-blue' in addition to the above two genes, which occurred in a sample of *T. boeoticum*. Another possible gene for blue aleurone could be on chromosome 4D, which has been substituted by chromosome pair of *Agropyron elongatum* in the variety 'Xiao Yian' from China (ZELLER et al. 1991). Genbank accession TRI2401 (*T. aestivum* var. *tschermakianum* Mansf.; IPK Gatersleben, Germany) with the name 'Tschermaks Blaukörniger Sommerweizen' is reported to have a different origin and gene (ZEVEN 1991). The results based on the FISH and GISH analysis show that the transferred chromosomal segments of *Th. ponticum* in blue grained wheats have different length, position and we can divide the material according to the length, position and number of transferred segments into six different groups (BUREŠOVÁ et al. 2013). This demonstrates a great genetic diversity among individual blue grained gene sources. It is also necessary to consider that the donors *Agropyron elongatum* and *Th. ponticum* are some of the many synonyms of the highly heterogeneous decaploid group with chromosomal constitution 2n = 10x = 70 (StStEeEbEx).

Blue grain differs from purple grain by the composition and presence of individual anthocyanins (ABDEL-AAL and HUCL 2003), which can be seen clearly on cross sections of kernels. For blue aleurone wheat the dominant anthocyanins were delphinidin 3-glucoside and delphinidin 3-rutinoside, whereas cyanidin 3-glucoside and cyanidin 3-rutinoside were, unlike in purple grains, present only in smaller quantities (KNIEVEL et al. 2009). Wholemeal flour contained 157 mg·kg^{-1} and bran 458 mg·kg^{-1} anthocyanins (ABDEL-AAL and HUCL 1999). Generally, the content of total anthocyanins seems to be higher in blue grained wheat compared to purple wheat (SYED JAAFAR et al. 2013).

In the Czech Republic, Miroslav Škorpík from the Crop Research Institute Prague-Ruzyně was interested in blue grained wheat for a long time. After World War II, he received donor material coming from the heritage of Erich von Tschermak-Seysenegg (one of the rediscoverers of Mendel's laws in 1900) (ŠKORPÍK et al. 1983). We assume it was a material similar to 'Tschermaks Blaukörniger Sommerweizen', which is preserved in the genebank of IPK Gatersleben. The initial material was gradually significantly improved by crossing with conventional wheat varieties. The activities led to the development of winter wheat variety 'Skorpion', which was registered in Austria in 2011. In 2012 it was enrolled in the European Catalogue of Varieties. 'Skorpion' has blue grain, but in comparison with common winter wheat varieties it has lower grain yield, medium winter hardiness and lower resistance to Fusarium head blight (FHB) (MARTINEK et al. 2013).

Yellow endosperm

The yellow endosperm colour is determined by two loci *Psy1* and *Psy2*, located on homoeologous chromosome groups 7 and 5 (POZNIAK et al. 2007). They affect the biosynthetic pathway of carotenoids, in particular phytoen synthase enzyme. The most investigated loci are *Psy1-A1* (7AL), *Psy1-B1* (7BL), *Psy1-D1* (7DL), *Psy2-A1* (5A) and *Psy2-B1* (5B) (HOWITT et al. 2009, Catalogue of Genetic Symbols

for Wheat). The content of the yellow pigment is currently the most studied in *T. durum* (ZHANG and DUBCOVSKY 2008, HE et al. 2008) due to a need of yellow products in the pasta production industry. In the Czech Republic, winter wheat 'Citrus' and spring wheat 'Luteus' from Germany containing the carotenoids lutein and zeaxanthin were registered in 2011. The yellow substances favourably affect the colour of egg yolk in feeding trials with poultry.

Breeding for high anthocyanin content

The aim is to develop breeding lines of wheat with a high content of anthocyanins, good baking quality and satisfactory yield levels. In the available wheat genetic resources with uncommon grain colour the genetic similarity was evaluated using SSR markers (MUSILOVA et al. 2013). This information is used together with the assessment of the length of chromosomal segments in blue grained wheats (BUREŠOVÁ et al. 2013) to select parental combinations for crossing. At Agrotest Fyto, Ltd., we have now advanced lines with dark purple or blue grain colour which were selected on visual assessment. In some cases a combination of both colours, which is characterized by a dark purple with a violet shade, was observed. The problem is still the significant yield penalty, especially in the lines with blue grains. We assume that the low yield is conditioned by the negative influence of genes linked to the gene for blue aleurone on the chromosome segment from the wild species. It would be desirable to disrupt these linkages by evoking recombination. Most contemporary breeding lines with blue grain are characterized by a low resistance to FHB and snow mould, and frequent drying of grain. Lines with purple pericarp often exhibit small grains, whereas their resistance to FHB is usually good. Back-crosses are used for the transmission of non-traditional colour into the genetic background of common wheat varieties. It will be important to understand the biosynthetic pathways of anthocyanins in grain and regulatory mechanisms of their expression in different tissues of the grain. It would be useful to find such genetic mechanisms that enable gene expression also in the grain endosperm through unlocking regulatory genes or responsible transcription factors. Currently, the first steps have been made to clarify the biosynthetic pathway of enzyme chalcone synthase and the corresponding gene in wheat has been sequenced (TROJAN et al. 2013). New candidate sequence for other genes of the biosynthetic pathway of anthocyanins, e. g. dihydroflavonol 4-reductase, chalcon isomerase, including the first data on their expression during caryopsis maturation have been described.

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Analytical identification of wheat subspecies - A contribution to consumer protection

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Abstract

In the last years the consumer was faced with an increasing number of baking products made from neglected wheat subspecies such as spelt wheat (*Triticum aestivum* L. subsp. *spelta* (L.) Thell., *T. aestivum* L. subsp. *macha* (Dekapr. & Menabde) MacKey) and their crossbreeds.

In the interest of producers and consumers reliable analytical methods for the distinction of these subspecies from common wheat (*T. aestivum* L.) are necessary. The consumer has to be protected against fraudulent use of common wheat in spelt wheat products and the producer against dishonest competitors. Under the aspects of emerging non coeliac wheat incompatibilities in some groups of the population the purity of raw material gets also a public health dimension.

The task of this study was the development of a method for analytical distinction of wheat subspecies based on HPLC-spectra of the albumin/globulin, gliadin and glutenin fractions resulting from a modified Osborn-fractionation. The peak areas of the separated proteins were quantified and evaluated by principal component analysis. Additionally the influence of the gliadin/glutenin-ratio on the distinguishability was investigated.

From five locations of the Austrian VCU trials of 2011 three *T. aestivum*, four *T. spelta* varieties and two crossbreds were analysed (45 samples). To increase the number of wheat samples 29 wheat samples from two locations of the VCU trials of 2012 were included in the study. Moreover, 47 samples of *T. spelta*, *T. macha* and *T. macha*×*T. spelta* from single locations in Austria, Germany and Italy, were provided by the breeding programme of the Department of Crop Sciences of the University of Natural Resources and Life Sciences, Vienna.

The best results with respect to the differentiation were obtained by the application of a standardised evaluation of the principal component analysis. In this mode the absolute magnitude of the peaks is neglected and only their relative variability is important for the alignment of the principal components. In this way the influence of small peaks is enhanced and their importance for the distinction of subspecies may be recognized. All three subspecies could be separated properly by this method, however, the detection of crossbreds (*T. spelta*×*T. aestivum*) was not possible.

Keywords

Food security, HPLC, principal component analysis, spelt wheat, *Triticum*

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