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RYE-SUS: a transnational approach to support sustainable grain production in changing environments

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Abstract

Architectural traits such as plant height are important targets to improve the yield potential of crops. The transnational project RYE-SUS makes use of hybrid and genome-based precision breeding to increase target-specific selection efficiency and accelerate breeding processes in rye. Within RYE-SUS, semi-dwarf rye will be developed for the first time ever using the male sterility inducing Pampa cytoplasm and the dwarfing gene *Ddw1*. The new genotypes with an optimized harvest index are expected to increase the yield potential mainly by exploiting heterosis as well as by mobilization of a larger part of the total plant assimilates to the grain, which improves the resource-use efficiency of rye. The genetically reduced gibberellin content of semi-dwarf rye results in new phenotypes to confer lodging tolerance and to strengthen the adaptation of rye to a changing climate. Lodging-resistant genotypes will considerably improve rye production by securing yield quantity and quality, saving cost and energy for grain drying, and reducing environmental impact by forgoing chemical growth regulators. As a further novelty to improve rye health and protection, every single semi-dwarf rye plant will produce pollen in amounts sufficient to minimise the risk of extremely toxic ergot alkaloids in the harvest. A highly diagnostic field phenotyping platform representing target environments for rye production in Europe and

Canada enables to prove the practical potential and to develop and utilze a simulation model of the growth and development of semi-dwarf as well as tall rye under potentially growth-limiting factors such as drought, frost, or nutrient deficiencies.

Introduction

The systematic exploitation of heterosis by hybrid breeding is one of the most sustainable innovations in plant breeding. Heterosis was first described in detail by the American botanist and plant geneticist George Harrison Shull (1908). In rye (*Secale cereale* L.), the systematic exploitation of heterosis by hybrid breeding started around 1970 and the first hybrid rye cultivars were released in 1984 (Geiger & Miedaner 2009). The mating of genetically diverse, pure-bred lines that carry certain desirable traits result in uniform offspring that outperform open-pollinating rye cultivars in grain yield and other agronomic traits (Laidig *et al*. 2017).

In the cross-pollinating rye, hybrid breeding is more flexible than population breeding in creating cultivars with specific traits. In 1983, five population cultivars covered 99.5% of the total rye seed multiplication area in Germany with the most popular variety having a share of 63.5% in total area (Barth *et al*. 2004). In 2018, the German Federal Plant Variety Office describes 32 registered winter rye cultivars for grain use in the descriptive variety list (Bundessortenamt 2018). Hybrids represent 72% of these cultivars. The five largest cultivars include two populations and three hybrids which altogether cover 63% of the seed multiplication area (Bundessortenamt 2018). Hybrid breeding, thus, supplements the opportunities to genetically improve this small grain cereal and triggers the increase of intraspecific biodiversity, *i.e*. the genetic component of biodiversity in rye.

The natural genetic diversity in rye was the fundamental basis to achieve a series of technological advances, which facilitated to establish hybrid breeding. These include self-fertility mutations, which enable the development of inbred lines to capture valuable genetic diversity, nuclear-cytoplasmic gynodioecy to establish a natural, reliable, environmentally friendly and cost-effective production of hybrid seed, effective restorer-of-fertility (*Rf*) genes to enable grain production in CMS-based hybrids, as well as two genetically divergent gene pools, Petkus and Carsten (Hepting 1978), to exploit heterosis. Hybrid breeding has resulted in a substantial breeding progress for yield and quality traits in rye during the last 26 years (Laidig *et al*. 2017). Recently, genome-based prediction of breeding values has been implemented in a hybrid rye breeding program, which is expected to further increase selection gain (Auinger *et al*. 2017).

Despite of these major achievements, major challenges in rye cultivation remain, including, lodging and a much lower harvest index (HI) as compared to wheat. Plant height is a major factor influencing lodging tolerance and counts among the major target traits in rye breeding. Plant height is a quantitative inherited trait with complex genetic architecture in rye (Auinger *et al*. 2017; Miedaner *et al*. 2011, 2012, 2018), rendering the genetic improvement of plant height a challenging task. The dominant dwarfing gene *Ddw1*, that was initially designated *Humilus* (*Hl*) (Kobyljanski 1972) and later renamed by Melz (1989), offers a breeder's option to improve lodging resistance and increase the HI of rye. *Ddw1* has been successfully used in breeding of open pollinating cultivars, which may be characterised by increasing frequencies of tall plants, as genotypes carrying the recessive *ddw1* allele are difficult to detected within the seed multiplication process of a semi-dwarf open-pollinating population (Kobyljanski 1988, McLeod *et al*. 2000, Torop *et al*. 2003, Tenhola-Roininen & Tanhuanpää 2010). However, this mutant has not yet been utilized in hybrid breeding programs (Geiger & Miedaner 2009; Miedaner *et al*. 2011, 2012, 2018). We have applied *de novo* transcriptome sequencing and comparative mapping (Braun *et al*. 2019) to establish and use novel markers for the introgression of *Ddw1* into the Petkus pool. Homozygous semi-dwarf as well as near isogenic tall BC$_3$S$_2$ lines in the male sterility inducing Pampa (P) cytoplasm are now available to develop semi-dwarf P-type hybrids for the first time ever in rye improvement programs (Figure 1).
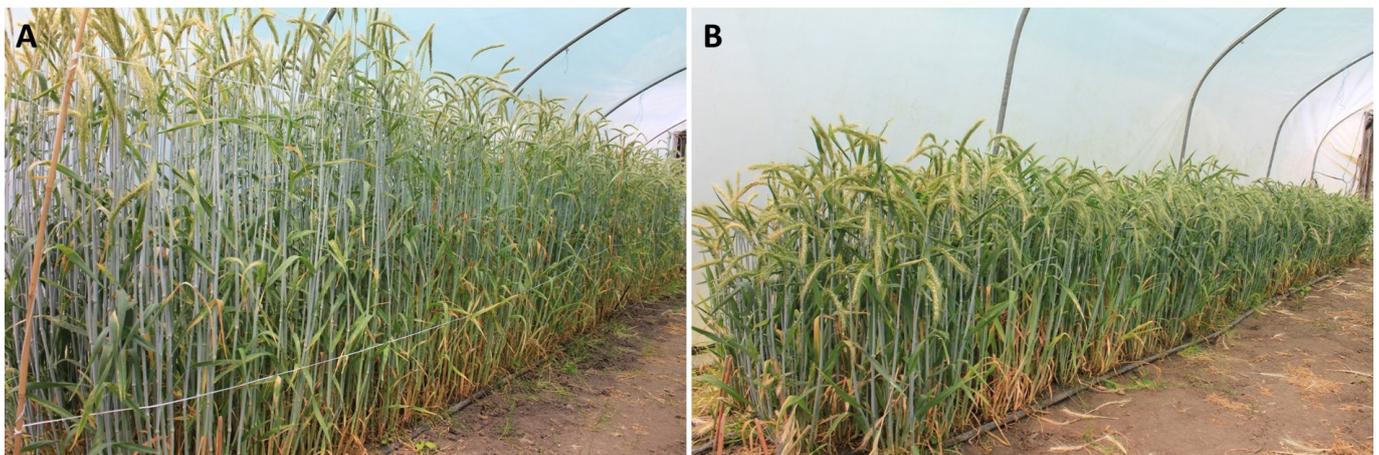

Figure 1: Marker-assisted introgression of *Ddw1* in elite seed parent lines enabled to establish semi-dwarf and near isogenic, tall single cross seed parent lines in the P-cytoplasm.

Enhancing photosynthesis efficiency in rye

Photosynthesis is the primary determinant of crop yield. Enhancing photosynthesis, electron transport and photorespiration has, thus, been accepted as a key target for increasing crop productivity (Simkin *et al*. 2019). Hybrids are characterized by a particularley effective light interception machinery beginning with seedling vigour and including homeostasis of photosynthesis over a broad range of environmental conditions as the basis for a strong source (Blum 2013). Transcriptional profiling in rice revealed, that hybrid breeding enables to increase activity of enzymes involved in carbon fixation pathways and net photosynthetic rate (Song *et al*. 2010). The well established heterotic pattern of rye, therefore, offers a proven option to systematically increase and secure basic grain productivity in terms of converting solar irradiation into biomass on finite arable land without increasing water and fertilizer use.

Likewise and as opposed to wheat, the route for improving grain yield by increasing the HI of rye is not approaching its end. The main sites of photosynthesis are leaves, and plants with leaves of optimal shape and size harvest light more efficient, leading to a rapid growth rate and increased yield. Morphological characters of the plant like the size and shape of leaves as well as their spatial arrangement determine a plants architecture. The importance of the plant architecture on crop yield potential became evident during the Green Revolution, when the genetic improvement of plant height in wheat and rice resulted in an unprecedented increase in agricultural production (Hedden 2003). Similar to wheat, the spike has proven to be supplied with assimilates essentially via the flag leaf (F) and F-1 in semi-dwarf *Ddw1* genotypes, while in their tall equivalents the stem is the main photosynthesis organ (Kobyljanski & Babuzhina 2007). A genetically optimized allocation of dry matter to the grain by means of *Ddw1*, together with the systematic utilization of heterosis, offers a strategy to increase rye productivity that is substantially different from current methods in the genetic improvement of rye and wheat.

Translating fundamental research into climate resilient crops

Ddw1 belongs to the group of gibberellin (GA) sensitive dwarfing genes (Börner & Melz 1988). Noteworthy in this context, it is becoming increasingly evident that the GA class of plant hormones is of pivotal relevance in the response of plants to abiotic stress (Colebrook *et al*. 2014). Positive effects on grain yield, lodging tolerance as well as drought tolerance in tef (*Eragrostis tef* (Zucc.) Trotter) and finger millet (*Eleusine coracana* Gaertn) have been reported as a result of chemically induced GA deficiency (Plaza-Wüthrich *et al*. 2016). In rice, induced mutants of the GA deactivation gene GA2-oxidase 6 (GA2ox6) moderately reduced GA concentration and reprogrammed transcriptional networks, leading to reduced plant height, more productive tillers, an expanded root system, higher water use efficiency and photosynthesis rate, and elevated abiotic and biotic stress tolerance (Lo *et al*. 2017). In accordance with these observations the analysis of the Arabidopsis NAC-like GIBBERELLIN SUPPRESSING FACTOR (GSF) showed a novel function in the regulation of gibberellin biosynthesis (Chen *et al*. 2019). The ectopic expression of GSF lacking a transmembrane domain (GSF-TM) caused a dwarf phenotype, which was correlated with the upregulation of GA2ox2/6 and an increased drought tolerance compared to the wild-type plants.
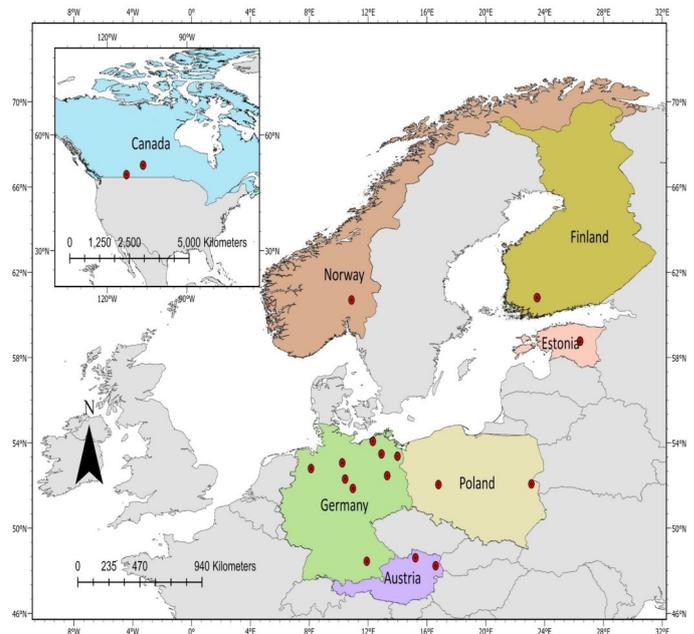

Figure 2: The RYE-SUS phenotyping network.

We have identified ScGA2ox12 that is up-regulated in semi-dwarf rye and co-segregating with the GA-sensitive dwarfing gene *Ddw1* (Braun *et al*. 2019). The described progress in our understanding of the importance of GA-homeostasis for drought stress tolerance, thus, implicates that the established semi-dwarf seed parent lines most likely confer both, lodging and drought tolerance in rye, as the *Ddw1* mutant alters the GA content in a favourable manner. Interestingly, a root-derived precursor of bioactive gibberellins has recently been described to mediate thermo-responsive shoot growth in Arabidopsis suggesting that root-to-shoot translocation of GA12 enables a flexible growth response to ambient temperature changes (Camut *et al*. 2019). According to this research it can be assumed, that the root signal cannot trigger stem growth in GA-sensitive semi-dwarf rye, at least to the same extend, as in wild-type plants. As a consequence, dry matter in semi-dwarf rye will be allocated to the grain rather then to the stem, even under elevated temperature. The genetic adaptation of rye to a changing climate by means of GA-sensitive semi-dwarf rye is of particular importance for grain production, as rye is mainly cultivated on light soils with low fertility and water holding capacity. Previous research emphasised the improvement of drought stress tolerance in rye an indispensable task. An average drought induced grain yield reduction of 23.8% has been reported for hybrid rye in non-irrigated compared to irrigated regime under natural drought stress conditions (Hübner *et al*. 2013), while up to 57% grain yield reduction was observed in controlled environments under different drought regimes (Kottmann *et al*. 2016). By conducting transnational field trials with semi-dwarf rye in target environments of rye cultivation (Figure 2), RYE-SUS represents an unique opportunity for the proof of concept, that mutants in the GA biosynthesis pathway provide valuable genetic variants to create lodging tolerant and climate-smart crops. In this context, canopy temperature as an indicator of crop water status (Kottmann *et al*. 2013) might be a useful trait to assess and understand the drought-stress response of semi-dwarf rye.
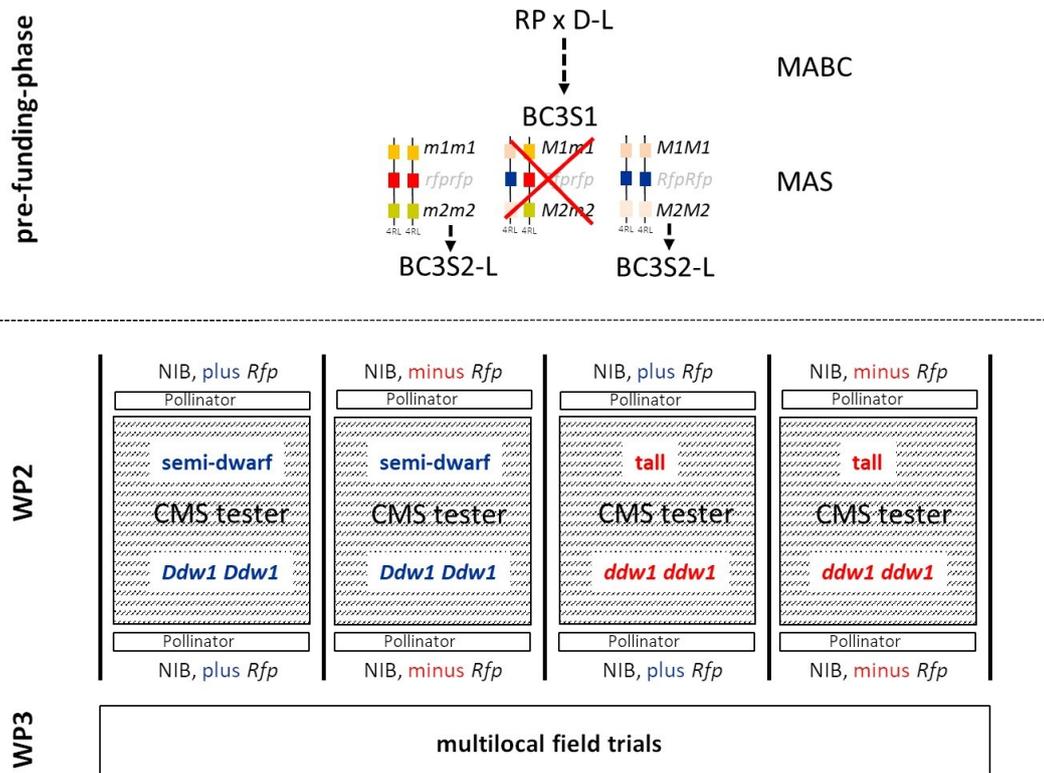
Figure 3: Flow diagram illustrating seed production of semi-dwarf and near-isogenic tall experimental rye hybrids (RP: recurrent parent; D-L: donor line; MABC: marker assisted backcrossing; MAS marker assisted selection; NIB: near isogenic bulk)

A new era in cereal breeding

Ergot is a disease of cereals and grasses caused by fungi in the genus *Claviceps* (Miedaner & Geiger 2015). At anthesis the open, non-fertilized florets of rye enable ergot spores to access the stigma and mimic pollination. CMS-based hybrids with an unsatisfactory restoration level and reduced pollen shedding are notably susceptible to ergot as the fungal spores have no competitors during the infection of the stigmatic tissue (Hackauf *et al*. 2017). The risk of ergot contamination in rye can be particularly high in years with cool and rainy weather during rye flowering which in turn results in high costs for cleaning the harvested seed. *Claviceps purpurea* produces all three major groups of ergot alkaloids: clavine alkaloids, D-lysergic acid and its derivatives, and ergopeptines (Miedaner & Geiger 2015). Because of the toxicity of ergot sclerotia for human and animals, the European Community regulation No. 687/2008 restricts the concentration of ergot bodies to 0.05% in wheat for human consumption. This threshold is commercially used in rye as well. The European directive 2002/32/EC on undesirable substances in animal feed fixes a limit of 0.1% for ergot in feedstuff containing unground cereals. The compliance of thresholds for ergot contamination in the harvest is, thus, critical for a reliable marketing of rye products. As a consequence, ergot infection counts among the economically most important diseases in rye and should be minimized in commercial production. *Rf* genes are of central importance for cereal hybrid breeding, both for achieving maximum seed setting as well as for minimizing ergot infestation. However, hybrids carrying an effective *Rf* gene for the P cytoplasm (*Rfp*) suffer from a significant reduction in grain yield (Miedaner *et al*. 2017). As a consequence of the high yield penalty, a restricted integration of *Rfp* genes from weedy rye in the pollinator gene pool gaining a restorer index of ≈50% is considered as

a feasible practice (Miedaner *et al*. 2017). However, rainy weather at flowering time reduces pollen shedding and pollen movement. As wet pollen agglutinates and distributes over short distances only, a restorer index of ≈50% may result in insufficient quantities of pollen to combat the fungus adequately. In order to comprehensively reduce the risk of ergot infection in hybrid rye, varities should be developed with a restorer index of 100%, *i.e.* male fertility restoration is realized for every single plant.

We have recently shown, that the yield penalty associated with a defined donor chromosome segment (DCS) on chromosome 4RL, carrying *Rfp2* or *Rfp4* from forage rye, is less pronounced than a DCS carrying the most prominent *Rfp1* gene from a weedy rye (Hackauf *et al*. 2018). Therefore, natural biodiversity of effective *Rf* genes offer valuable means to minimise the costs of restoration in terms of grain yield. BC₃S₁ single plants have been selected carrying either the elite (E) non-restorer allele or the donor (D) restorer allele from weedy (*Rfp1*) and forage rye (*Rfp2* and *Rfp4*) introgressed in two elite inbred lines using previously developed markers (Hackauf *et al*. 2012) to establish near isogenic homozygous BC₃S₂ lines (NIL), that will be used in RYE-SUS as pollinators for crossing with three male sterile semi-dwarf as well as their near isogenic tall single-cross non-restorer genotypes between isolation walls (Figure 3). The genetic makeup of these hybrids enables to calculate linkage drag effects as the difference (ΔE-D) between the means of individual NIL partners, which either carry the E or the D at the 4RL DCS. Similarly, the effect of *Ddw1* will be determined by pairwise comparisons between semi-dwarf and near isogenic tall genotypes. As a consequence, our research conducted in RYE-SUS will clarify, if the fitness costs of *Rfp* genes can be counterbalanced by using beneficial effects of *Ddw1* on grain yield. This proof of concept will open a new era in breeding

hybrid cultivars with high yield potential and minimized risk of ergot infestation. While the technology of hybrid breeding enables to rapidly develop and precisely evaluate prototypes of new rye genotypes, the knowledge gained will be applied within RYE-SUS for the genetic improvement of open-pollinated cultivars with simple and less expensive breeding procedures and short development cycles as well.

Uncover the hidden half of rye

The main driver enabling rye cultivation on light soils with low fertility and low water capacity is its highly developed root system, which facilitates a very efficient uptake of water and nutrients (Starzycki 1976). The power of rye roots becomes apparent by different observations. The entire root system of a single rye plant consisted of 13,815,672 branches, with a total length of 622 km, a surface area of 401 m², and a total root hair length of 11,000 km (Dittmer 1937, Ryser 2006). Winter rye showed the lowest yield reduction compared to winter wheat, winter barley, and winter triticale, when rainfall was fully excluded by means of rain-out shelters from tillering until harvest (Schittenhelm *et al*. 2014). Rye reduced nitrate leaching at the field scale by up to 93%, followed by barley (87%) and wheat (57%), as compared to the estimated seasonal average of 43 kg ha^{-1} over the winter fallow period without cover crop (Yeo *et al*. 2014). The increased nitrogen use efficiency (NUE) of rye is mainly attributed to its higher specific root length (Paponov *et al*. 1999). The high NUE of winter rye also enables grain production with the lowest carbon footprint as compared to wheat, triticale, barley and oats (Wojcik-Gront & Bloch-Michalik 2016). Thus, the cultivation of rye contributes to reduce nitrogen losses and greenhouse gas emissions. Both are serious challenges for (i) a sustainable agriculture that should ensure food security for a growing human population as well as (ii) the need to manage the world's continuously increasing demand for energy and fresh water in view of climate change (Sawamoto *et al*. 2005; Philippot & Hallin 2011).

However, there is currently no information available about possible changes in the rooting pattern of hybrid rye cultivars as a result of breeding activities during the last decades. In wheat, the introduction of GA insensitive dwarfing genes caused a reduction of root mass and root length, even though crop productivity was significantly increased (Waines & Ehdaie 2007, Subira *et al*. 2016, Voss-Fels *et al*. 2017). It is currently under discussion whether the reduction in plant height does also reduce the water uptake capacity of cereal crops. Zhang *et al*. (2009) suggested that recent semi-dwarf wheat cultivars are even more efficient with regard to water use, because the decrease in total root length was mainly at the expense of root length in the top soil, and led to a higher proportion of roots in deeper soil layers. On the other hand, Waines & Ehdaie (2007) assume that the root system sizes of modern, GA insensitive cultivars are sufficient under optimal growth conditions, but insufficient when growth conditions are unfavourable. Root phenotyping, thus, provides an important information for a comprehensive evaluation of GA sensitive, semi-dwarf rye. Root phenotyping can be conducted in controlled environments like tubes and rhizotrones. However, results from controlled environments cannot be easily transferred to field conditions, especially for deep roots. Although direct root sampling in the field is time consuming, it enables an unbiased detection of genetic effects on depth and density of roots (Clarke *et al*. 2017). To uncover the hidden half of rye, root phenotyping within RYE-SUS will be conducted directly in the field within the first three weeks after anthesis to determine total root length as well as root length density, and root dry weight of semi-dwarf as compared to near-isogenic tall rye up to a soil depth of 150 cm.

New perspectives for rye cultivation in northern latitudes

Winter hardiness gains increasing importance for establishing highly productive rye cultivars in marginal high latitude production regions of Europe and Canada, where continental climates and severe winters require high levels of frost tolerance. Recently, new insights on winter-hardiness in rye has been reported (Bahrani *et al*. 2019). The winter survival rate of both cultivars 'Halo' and 'Kustro' was classified with the highest winter survival score (very high), while the cultivars 'Petkuser Kurzstroh' and 'Petkuser' both belong to the opposite class with very low winter survival rate. Likewise, winter survival of the cultivars 'Carokurz' and 'Carstens' was classified as moderate and low, respectively. These results demonstrate, that desired and indispensable alleles for winter hardiness are available in adapted material of the Petkus pool and may exist at low frequency in the Carsten pool as well. The broad genetic diversity available in both heterotic genepools may, therefore be useful to identify valuable alleles and mechanisms of winterhardiness in rye.

Tagging of specific genes or defined genomic regions with gene-specific markers enables an efficient introgression of favorable alleles from donor populations in elite material by efficient marker-assisted introgression programs. Frost tolerance (FT) contributes to winter hardiness and is a complex biological process involving at least two main pathways and many additional processes encompassing a large number of genes (Babben *et al*. 2018). In barley and wheat, two major FT loci, frost resistance 1 (*Fr1*) and frost resistance 2 (*Fr2*), were identified on the long arm of the homeologous group 5 chromosomes (Vagujfalvi *et al*. 2003, Francia *et al*. 2004). The *Fr2* locus on chromosome 5R in rye has been reported to improve the recovery of plants after winter (Li *et al*. 2011, Erath *et al*. 2017). Interestingly, none of the analyzed genes residing at the *Fr2* locus affected the survival after winter. This result indicates, that factors beyond the *Fr2* locus might control the outstanding winter hardiness of rye. However, it needs to be considered that phenotypic data on winter hardiness may be influenced by environmental factors that mask genetic differences in FT (Erath *et al*. 2017, Babben *et al*. 2018). Notably, the timing of floral transition is one of the determining factors for FT tolerance in winter wheat, where the timing of the transition from a vegetative to a generative growth stage has been found to be associated with for how long the plants are able to maintain a high level of FT (Fowler & Limin 2004). Genetic diversity in temperature sensing and/or signaling results in differences of leaf initiation and maturation and/or vernalization rates in wheat (Båga *et al*. 2009). Thus, the final leaf number is an additional, yet not studied parameter in rye, that can contribute to unravel the frost tolerance network. Genes increasing the final leaf number may hence be of importance for the development of elite rye germplasm with improved winter hardiness.

The recently published rye reference genome sequence (Rabanus-Wallace *et al*. 2019) facilitates many downstream applications including efficient development of gene specific primers. An in-

depth characterization of candidate genes beyond the CBF regulatory hub (Thomashow 2010) residing at the *Fr2* locus offers a novel opportunity to get insights into the genetic architecture of winter hardiness in rye. Genes involved in the anthocyanin biosynthesis pathway count among our targets, as for the most cold-hardy an overall higher abundance and diversity of anthocyanins has been noted as compared to the less hardy genotypes (Bahrani *et al.* 2019). It needs to considered that the regulation of anthocyanin biosynthesis is less well-described in rye than in Arabidopsis (Shi & Xie 2014), maize (Petroni *et al.* 2014), or rice (Zheng *et al.* 2019). However, genetic diversity in the anthocyanin pigmentation of rye coleoptiles served to identify the six recessive genes *vi1–vi6* (Voylkov *et al.* 2015). A novel 20k custom wheat/rye/triticale SNP array, that contains 5k SNPs selected from the rye 600k SNP array (Bauer *et al.* 2017), and bulked segregant analysis (Michelmore *et al.* 1991) enabled us to map these genes on chromosomes 2R, 3R, 4R and 7R (Figure 4). A phenotyping strategy based on a $F_{2:3}$ design and testcross performance in multi-environmental field trials (Hackauf *et al.* 2017) will be used to unravel the relevance of these genes to control winter hardiness in rye.

Figure 4: Chromosomal localization of genes controlling anthocyanin pigmentation of rye coleoptiles.

The way forward: predicting the future of rye

Process-based crop models are essential tools in current agronomic research. They enable simulations of crop growth, development and yield under diverse soil, climate and management conditions (Jones *et al*. 2003). Crop models consider the soil-plant-atmosphere-system and the involved carbon-, water- and nitrogen fluxes. They make it possible to complement and upscale empirical research, and may hence contribute to complement the necessity of expensive field trials. In addition, they enable testing and evaluation of scenarios for future altered climatic, technological or cropping systems related conditions (Munz *et al*. 2014). Crop models may be used to generate missing data in large-scale agronomic and experimental data sets, such as crop phenological information (Dreccer *et al*. 2018) or potential yield levels (van Rees *et al*. 2014). In recent years the application of crop models for climate change impact assessment and development of climate change adaptation strategies (Qin *et al*. 2018) has gained increasing importance. In this context, crop models may also contribute to the development of crop ideotypes for current and future production conditions (Gérardeaux *et al*. 2018), ideotypes that feature necessary phenotypic and genotypic traits in order to optimize yield under given pedo-climatic conditions. Another useful application of crop models is to test and evaluate the introduction of a new crop or crop cultivar to a specific environment in virtual experiments (George & Kaffka 2017). Up to now mainly phenotypic data have been used for model parameterization (Gouache *et al*. 2017). Those data may, as planned in RYE-SUS, be complemented by genetic information on QTL governing growth and development, which could improve the prediction of a genotype's performance in different environments even further (Hwang *et al*. 2017).

A specific challenge regarding models for winter cereals is the simulation of winter survival and hence the remaining field plant cover in spring. The FROSTOL model (Bergjord *et al*. 2008) was developed to simulate course of frost tolerance from sowing on towards spring. The implementation of a spring field plant coverage factor resulted in improved predictions of wheat growth and yield (Bergjord O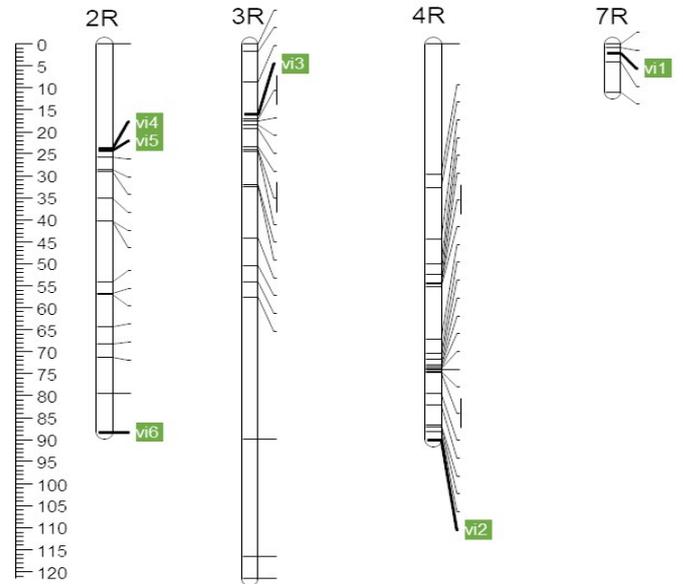lsen *et al*. 2018). By quantifying relationships between climatic factors and winter survival, the FROSTOL model makes it possible to perform risk calculations for winter survival of winter cereals under different climatic conditions and to evaluate consequences of anticipated future climatic changes for overwintering. Increased weather variability with repeated cycles of freezing and thawing is expected to be one of the consequences of climate change. RYE-SUS will advance current models (Mirschel *et al*. 2005, Balkovič *et al*. 2013) to reproduce the whole phenology process between sowing and maturity for rye by (i) parametrizing the genotype of semi-dwarf and standard rye hybrids and (ii) integrating their ability to tolerate dehardening and rehardening conditions during winter and hence their robustness in face of climate change. As such, RYE-SUS will integrate studies of different rye genotypes ability to tolerate dehardening and rehardening conditions during winter and hence their robustness in face of climate change.

Conclusions

On a global scale, rye contributes to crop species diversity mainly from the North German Plain to the Ural Mountains and between the 50th and 60th latitude. Farmers in the Rye Belt are globally in competition with other regions of cereal production. Research in RYE-SUS will promote the international competitiveness of rye growing farmers by developing high-performing and environmentally adapted cultivars. We have recently shown, that the rich genetic diversity of the cross-pollinating rye, together with emerging genomic tools, offer a so far underutilized genetic system to enhance our understanding of complex agronomic traits including grain yield in small grain cereals (Hackauf *et al*. 2017).

The genomics-assisted research in RYE-SUS balances current economic models for plant breeding, which have resulted in major investment and subsequent advances for a limited number of major crops, with little, if any, benefit for minor or orphan crops (Abberton *et al*. 2016). RYE-SUS is going to counterbalance this development by tapping the full yield potential of 'the overlooked

cereal' (Buttriss 2006) rye. The breeding research of RYE-SUS is directed to a sustainable intensification in marginal production environments. RYE-SUS advances the Green Revolution with its achievement in terms of agricultural productivity improvement, as we will utilize heterosis in rye to develop genotypes, which gain an improved yield potential and counterbalance drought stress effects through a genetic mechanism. The output of RYE-SUS will increase the competitiveness of rye in agricultural production systems and will ensure access to the health potential of rye bread and other rye-based foods (Jonsson *et al*. 2018).

The demand-less rye enabled post-Roman societies in Europe to survive, but also shaped them in a way that pioneered unique technological developments in world history (Mitterauer 2009). In view on current international efforts to develop solutions combatting the global climate change, rye again offers viable options to help alleviate hunger of a steadily increasing population.

References

Abberton M, Batley J, Bentley A, Bryant J, Cai H, *et al*. (2016) Global agricultural intensification during climate change: a role for genomics. Plant Biotechnol J 14: 1095-1098. DOI: 10.1111/pbi.12467

Auinger HJ, Schönleben M, Lehermeier C, Schmidt M, Korzun V, *et al*. (2016) Model training across multiple breeding cycles significantly improves genomic prediction accuracy in rye (*Secale cereale* L.). Theor Appl Genet 129: 2043-2053 DOI: 10.1007/s00122-016-2756-5

Babben S, Schliephake E, Janitza P, Berner T, Keilwagen J, *et al*. (2018) Association genetics studies on frost tolerance in wheat (*Triticum aestivum* L.) reveal new highly conserved amino acid substitutions in CBF-A3, CBF-A15, VRN3 and PPD1 genes. BMC Genomics 19: 409. DOI: 10.1186/s12864-018-4795-6

Båga M, Fowler DB, Chibbar RN (2009) Identification of genomic regions determining the phenological development leading to floral transition in wheat (*Triticum aestivum* L.). J Exp Bot 60:3575-3585. DOI: 10.1093/jxb/erp199

Bahrani H, Thoms K, Båga M, Larsen J, Graf R, *et al*. (2019) Preferential accumulation of glycosylated cyanidins in winter-hardy rye (*Secale cereale* L.) genotypes during cold acclimation. Env Exp Bot 164: 203-212. DOI: 10.1016/j.envexpbot.2019.05.006

Balkovič J, van der Velde M, Schmid E, Skalský R, Khabarova N, *et al*. (2013) Pan-European crop modelling with EPIC: Implementation, up-scaling and regional crop yield validation. Agric Syst 120: 61-75. DOI: 10.1016/j.agsy.2013.05.008

Barth R, Bilz M, Brauner R, Clausen J, Dross M, *et al*. (2004) Agrobiodiversität entwickeln! Handlungsstrategien für eine nachhaltige Tier- und Pflanzenzucht. Institut für ökologische Wirtschaftsforschung, Berlin [available at https://www.ioew.de/publikation/agrobiodiversitaet_entwickeln].

Bauer E, Schmutzer T, Barilar I, Mascher M, Gundlach H, *et al*. (2017) Towards a whole-genome sequence for rye (*Secale cereale* L.). Plant J 89: 853-869. DOI: 10.1111/tpj.13436

Bergjord AK, Bonesmo H, Skjelvåg AO (2008) Modelling the course of frost tolerance in winter wheat I: Model development. Eur J Agron 28: 321-330. DOI: 10.1016/j.eja.2007.10.002

Bergjord Olsen AK, Persson T, de Wit A, Nkurunziza L, Sindhøj E, Eckersten H (2018) Estimating winter survival of winter wheat by simulations of plant frost tolerance. J Agron Crop Sci 204: 62-73. DOI: 10.1111/jac.12238

Blum A (2013) Heterosis, stress, and the environment: a possible road map towards the general improvement of crop yield. J Exp Bot 64: 4829-4837 DOI: 10.1093/jxb/ert289

Börner A, Melz G (1988) Response of rye genotypes differing in plant height to exogenous gibberellic acid application. Arch Züchtungsforsch 18: 71-74.

Braun EM, Tsvetkova N, Rotter B, Siekmann D, Schwefel K, *et al*. (2019) Gene expression profiling and fine mapping identifies a gibberellin 2-oxidase gene co-segregating with the dominant dwarfing gene *Ddw1* in rye (*Secale cereale* L.). Front Plant Sci 10: 857. DOI: 10.3389/fpls.2019.00857

Bundessortenamt (2018) Beschreibende Sortenliste Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben, Zwischenfrüchte.

Buttriss JL (2006) Rye: The overlooked cereal. Nutr Bull 31: 3-5. DOI: 10.1111/j.1467-3010.2006.00539.x

Camut L, Regnault T, Sirlin-Josserand M, Sakvarelidze-Achard L, Carrera E, *et al*. (2019) Root-derived GA12 contributes to temperature-induced shoot growth in Arabidopsis. Nat Plants 5: 1216-1221. DOI: 10.1038/s41477-019-0568-8

Chen HI, Li PF, Yang CH (2019) NAC-Like Gene GIBBERELLIN SUPPRESSING FACTOR regulates the gibberellin metabolic pathway in response to cold and drought stresses in Arabidopsis. Sci Rep 9: 19226. DOI: 10.1038/s41598-019-55429-8

Clarke CK, Gregory PJ, Lukac M, Burridge AJ, Allen AM, *et al*. (2017) Quantifying rooting at depth in a wheat doubled haploid population with introgression from wild emmer. Ann Bot 120: 457-470. DOI: 10.1093/aob/mcx068

Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. J Exp Biol 217: 67-75. DOI: 10.1242/jeb.089938

Dittmer HJ (1937) A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). Am J Bot 24: 417-420. DOI: 10.2307/2436424

Dreccer MF, Fainges J, Whish J, Ogbonnaya FC, Sadras FO (2018) Comparison of sensitive stages of wheat, barley, canola, chickpea and field pea to temperature and water stress across Australia. Agric For Met 248: 275-294. DOI: 10.1016/j.agrformet.2017.10.006

Erath W, Bauer E, Fowler DB, Gordillo A, Korzun V, *et al*. (2017) Exploring new alleles for frost tolerance in winter rye. Theor Appl Genet 130: 2151-2164. DOI: 10.1007/s00122-017-2948-7

Fowler DB, Limin AE (2004) Interactions among factors regulating phenological development and acclimation rate determine low temperature tolerance in wheat. Ann Bot 94: 717-724. DOI: 10.1093/aob/mch196

Francia E, Rizza F, Cattivelli L, Stanca AM, Galiba G, *et al*. (2004) Two loci on chromosome 5H determine low temperature tolerance in a 'Nure' (winter) × 'Tremois' (spring) barley map. Theor Appl Genet 108: 670-680. DOI: 10.1007/s00122-003-1468-9

Geiger HH, Miedaner T (2009) Rye breeding. In: Carena MJ (Ed), Handbook of plant breeding, Vol. 3: Cereals, pp 151-187. Springer, New York. DOI: 10.1007/978-0-387-72297-9

George N, Kaffka S (2017) Canola as a new crop for california: A simulation study. Agron J 109: 496-509. DOI: 10.2134/agronj2016.04.0247

Gérardeaux E, Loison R, Palaï O, Sultan B (2018) Adaptation strategies to climate change using cotton (*Gossypium hirsutum* L.) ideotypes in rainfed tropical cropping systems in Sub-Saharan Africa. A modeling approach. Field Crop Res 226: 38-47. DOI: 10.1016/j.fcr.2018.07.007

Gouache D, Bogard M, Pegard M, Thepot S, Garcia C, *et al*. (2017) Bridging the gap between ideotype and genotype: Challenges and prospects for modelling as exemplified by the case of adapting wheat (*Triticum aestivum* L.) phenology to climate change in France. Field Crop Res 202: 108-121. DOI: 10.1016/j.fcr.2015.12.012

Hackauf B, Korzun V, Wortmann H, Wilde P, Wehling P (2012) Development of conserved ortholog set markers linked to the restorer gene *Rfp1* in rye. Mol Breed 30: 1507-1518. DOI: 10.1007/s11032-012-9736-5

Hackauf B, Bauer E, Korzun V, Miedaner T (2017) Fine mapping of the restorer gene Rfp3 from an Iranian primitive rye (*Secale cereale* L.). Theor Appl Genet 130: 1179-1189. DOI: 10.1007/s00122-017-2879-3

Hackauf B, Haffke S, Fromme FJ, Roux SR, Kusterer B, *et al*. (2017) QTL mapping and comparative genome analysis of agronomic traits including grain yield in winter rye. Theor Appl Genet 130: 1801-1817. DOI: 10.1007/s00122-017-2926-0

Hackauf B, Siekmann D, Fromme FJ (2018) Hybrid breeding in cereals: lessons from rye. Proc 68th Plant Breeders Conf, 20-22 Nov 2017, Raumberg-Gumpenstein, Austria, pp 17-18.

Hedden P (2003) The genes of the Green Revolution. Trend Genet 19: 5-9. DOI: 10.1016/s0168-9525(02)00009-4

Hepting L (1978) Analyse eines 7×7-Sortendiallels zur Ermittlung geeigneten Ausgangsmaterials fur die Hybridzuchtung bei Roggen. Z Pflanzenzüchtg 80: 188-197.

Hübner M, Wilde P, Schmiedchen B, Dopierala P, Gowda M, *et al*. (2013) Hybrid rye performance under natural drought stress in Europe. Theor Appl Genet 126: 475-482. DOI: 10.1007/s00122-012-1994-4

Hwang C, Correll MJ, Gezan SA, Zhang L, Bhakta MS, *et al*. (2017) Next generation crop models: A modular approach to model early vegetative and reproductive development of the common bean (*Phaseolus vulgaris* L). Agric Syst 155: 225-239. DOI: 10.1016/j.agsy.2016.10.010

Jones JW, Hoogenboom G, Porter CH, Boote KJ, Batchelor WD, *et al*. (2003) The DSSAT cropping system model. Eur J Agron 18: 235-265. DOI: 10.1016/S1161-0301(02)00107-7

Jonsson K, Andersson R, Bach Knudsen KE, Hallmans G, Hanhineva K, *et al*. (2018) Rye and health - Where do we stand and where do we go? Trend Food Sci Technol 79: 78-87. DOI: 10.1016/j.tifs.2018.06.018

Kobyljanski VD (1972) On the genetics of the dominant factor of short-strawed rye. Genetika 8: 12-17.

Kobyljanski VD, Babuzhina DI (2007) Photosynthesis of different plant organs in short stem rye. Vortr Pflanzenzüchtg 71: 62-65.

Kottmann L, Schittenhelm S, Wittich KP, Wilde P (2013) Suitability of canopy temperature depression in a temperate climate with drought-stressed winter rye, determined with three infrared measurement devices. J Agron Crop Sci 199: 385-394. DOI: 10.1111/jac.12035

Kottmann L, Wilde P, Schittenhelm S (2016) How do timing, duration, and intensity of drought stress affect the agronomic performance of winter rye? Eur J Agron 75: 25-32. DOI: 10.1016/j.eja.2015.12.010

Laidig F, Piepho HP, Rentel D, Drobek T, Meyer U, Huesken A (2017) Breeding progress, variation, and correlation of grain and quality traits in winter rye hybrid and population varieties and national on-farm progress in Germany over 26 years. Theor Appl Genet 130: 981-998. DOI: 10.1007/s00122-017-2865-9

Li Y, Böck A, Haseneyer G, Korzun V, Wilde P, *et al*. (2011) Association analysis of frost tolerance in rye using candidate genes and phenotypic data from controlled, semi-controlled, and field phenotyping platforms. BMC Plant Biol 11: 146. DOI: 10.1186/1471-2229-11-146

Lo SF, Ho TD, Liu YL, Jiang MJ, Hsieh KT, *et al*. (2017) Ectopic expression of specific GA2 oxidase mutants promotes yield and stress tolerance in rice. Plant Biotechnol J 15: 850-864. DOI: 10.1111/pbi.12681

McLeod G, Gan YT, Payne JF (2000) AC Remington winter rye. Can J Plant Sci 80: 605-607. DOI: 10.4141/P99-158

Melz G (1989) Beiträge zur Genetik des Roggens (*Secale cereale* L.). PhD thesis, Berlin.

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci 88: 9828-9832. DOI: 10.1073/pnas.88.21.9828

Miedaner T, Müller BU, Piepho HP, Falke KC (2011) Genetic architecture of plant height in winter rye introgression libraries. Plant Breed 130: 209-216. DOI: 10.1111/j.1439-0523.2010.01823.x

Miedaner T, Hübner M, Korzun V, Schmiedchen B, Bauer E, *et al*. (2012) Genetic architecture of complex agronomic traits examined in two testcross populations of rye (*Secale cereale* L.). BMC Genomics 13: 706. DOI: 10.1186/1471-2164-13-706

Miedaner T, Haffke S, Siekmann D, Fromme FJ, Roux SR, Hackauf B (2018) Dynamic quantitative trait loci (QTL) for plant height predict biomass yield in hybrid rye (*Secale cereale* L.). Biomass Bioenergy 115: 10-18. DOI: 10.1016/j.biombioe.2018.04.001

Miedaner T, Geiger HH (2015) Biology, genetics, and management of ergot (*Claviceps* spp.) in rye, sorghum, and pearl millet. Toxins 7: 659-678. DOI: 10.3390/toxins7030659

Miedaner T, Herter CP, Goßlau H, Wilde P, Hackauf B (2017) Correlated effects of exotic pollen-fertility restorer genes on agronomic and quality traits of hybrid rye. Plant Breed 136: 224-229. DOI: 10.1111/pbr.12456

Mirschel W, Wenkel K-O, Schultz A, Pommerening J, Verch G (2005) Dynamic phenological model for winter rye and winter barley. Eur J Agron 23: 123-135. DOI: 10.1016/j.eja.2004.10.002

Mitterauer M (2009) Warum Europa? Mittelalterliche Grundlagen eines Sonderwegs. Verlag C.H. Beck, München.

Munz S, Feike T, Chen Q, Claupein W, Graeff-Hönninger S (2014) Understanding interactions between cropping pattern, maize cultivar and the local environment in strip-intercropping systems. Agric For Met 195-196: 152-164. DOI: 10.1016/j.agrformet.2014.05.009

Paponov IA, Lebedinskai S, Koshkin EI (1999) Growth analysis of solution culture-grown winter rye, wheat and triticale at different relative rates of nitrogen supply. Ann Bot 84: 467-473. DOI: 10.1006/anbo.1999.0935

Petroni K, Pilu R, Tonelli C (2014) Anthocyanins in corn: a wealth of genes for human health. Planta 240: 901-911. DOI: 10.1007/s00425-014-2131-1

Philippot L, Hallin S (2011) Towards food, feed and energy crops mitigating climate change. Trend Plant Sci 16: 476-480. DOI: 10.1016/j.tplants.2011.05.007

Plaza-Wüthrich S, Blösch R, Rindisbacher A, Cannarozzi G, Tadele Z (2016) Gibberellin deficiency confers both lodging and drought tolerance in small cereals. Front Plant Sci 7: 643. DOI: 10.3389/fpls.2016.00643

Qin X, Wang H, He Y, Li Y, Li Z, *et al*. (2018) Simulated adaptation strategies for spring wheat to climate change in a northern high latitude environment by DAYCENT model. Eur J Agron 95: 45-56. DOI: 10.1016/j.eja.2017.12.005

Rabanus-Wallace MT, Hackauf B, Mascher M, Lux T, Wicker T, *et al*. (2019) Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. bioRxiv, preprint DOI: 10.1101/2019.12.11.869693

Ryser P (2006) The mysterious root length. Plant Soil 286: 1-6. DOI: 10.1007/s11104-006-9096-1

Sawamoto T, Nakajima Y, Kasuya M, Tsuruta H, Yagi K (2005) Evaluation of emission factors for indirect N2O emission due to nitrogen leaching in agro-ecosystems. Geophys Res Lett 32: L03403. DOI: 10.1029/2004GL021625

Schittenhelm S, Kraft M, Wittich KP (2015) Performance of winter cereals on field-stored soil moisture only. Eur J Agron 52: 247-258. DOI: 10.1016/j.eja.2013.08.010

Simkin AJ, López-Calcagno PE, Raines CA (2019). Feeding the world: improving photosynthetic efficiency for sustainable crop production. J Exp Bot 70: 1119-1140. DOI: 10.1093/jxb/ery445

Shi MZ, Xie DY (2014) Biosynthesis and metabolic engineering of anthocyanins in *Arabidopsis thaliana*. Recent Patent Biotechnol 8: 47-60. DOI: 10.2174/1872208307666131218123538

Shull GH (1908) The composition of a field of maize. Am Breed Ass Rep 4: 296-301. DOI: 10.1093/jhered/os-4.1.296

Song GS, Zhai HL, Peng YG, Zhang L, Wei G, *et al*. (2010) Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol Plant 3: 1012-1025. DOI: 10.1093/mp/ssq046

Starzycki S (1976) Diseases, pests and physiology of rye. In: Bushuk W (ed), Rye: Production, chemistry and technology, pp 27-61. Am Ass Cereal Chem, St. Paul, MN.

Subira J, Ammar K, Alvaro F, Garcia del Moral LF, Dreisigacker S, Royo C (2016) Changes in durum wheat root and aerial biomass caused by the introduction of the *Rht-B1b* dwarfing allele and their effects on yield formation. Plant Soil 403: 291-304. DOI: 10.1007/s11104-015-2781-1

Thomashow MF (2010) Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. Plant Physiol 154: 571-577. DOI: 10.1104/pp.110.161794

Torop AA, Dedyaev VG, Tschaykin VV, Dokuchaev VV (2003) The results of rye breeding in the central-Chernosem region of Russia. Plant Breed Seed Sci 47: 69-75.

Vagujfalvi A, Galiba G, Cattivelli L, Dubcovsky J (2003) The cold-regulated transcriptional activator *Cbf3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A. Mol Gen Genomics 269: 60-67. DOI: 10.1007/s00438-003-0806-6

Van Rees H, McClelland T, Hochman Z, Carberry P, Hunt J, *et al*. (2014) Leading farmers in South East Australia have closed the exploitable wheat yield gap: Prospects for further improvement. Field Crop Res 164: 1-11. DOI: 10.1016/j.fcr.2014.04.018

Voss-Fels KP, Qian L, Parra-Londono S, Uptmoor R, Frisch M, *et al*. (2017) Linkage drag constrains the roots of modern wheat. Plant Cell Env 40: 717-725. DOI: 10.1111/pce.12888

Voylokov AV, Lykholay AN, Smirnov VG (2015) Genetic Control of Anthocyanin Coloration in Rye. Russ J Genet 5: 262-267. DOI: 10.1134/S207905971503020X

Waines JG, Ehdaie B (2007) Domestication and crop physiology: Roots of Green-Revolution wheat. Ann Bot 100: 991-998. DOI: 10.1093/aob/mcm180

Wojcik-Gront E, Bloch-Michalik M (2016) Assessment of greenhouse gas emission from life cycle of basic cereals production in Poland. Zemdirbyste-Agriculture 103: 259-266. DOI: 10.13080/z-a.2016.103.033

Yeo IY, Lee S, Sadeghi AM, Beeson PC, Hively WD, *et al*. (2014) Assessing winter cover crop nutrient uptake efficiency using a water quality simulation model. Hydrol Earth Syst Sci 18: 5239-5253. DOI: 10.5194/hess-18-5239-2014

Zhang X, Chen S, Sun H, Wang Y, Shao L (2009) Root size, distribution and soil water depletion as affected by cultivars and environmental factors. Field Crop Res 114: 75-83. DOI: 10.1016/j.fcr.2009.07.006

Zheng J, Wu H, Zhu H, Huang C, Liu C, *et al*. (2019) Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves. New Phytol 223: 705-721. DOI: 10.1111/nph.15807