GAMYB gene in rye – sequence, polymorphisms, map location, allele-specific markers and relationship with selected agronomic traits

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Abstract

Background. A master GA-induced regulatory protein, crucial for development and germination of cereal grain and involved in anther formation is MYB transcription factor GAMYB, activating a vast number of genes including high-molecular-weight glutenin and α -amylase gene families. This paper presents the first attempt to characterize rye gene encoding GAMYB in relation to its sequence, polymorphisms and phenotypic effects.

Results. ScGAMYB gene was identified and mapped on rye chromosome 3R using high-density DArT/DArTseq-based maps developed in two mapping populations. Comparative analysis of the gene sequence revealed its high level of homology to wheat and barley orthologues. Single nucleotide polymorphisms detected among rye inbred lines allowed developing AS-PCR markers for ScGAMYB (ten pairs of primers) which might be used to detect this gene in wide genetic stocks of rye and triticale. Segregation of ScGAMYB alleles showed significant relationship with quantitative traits including plant height, thousand grain weight, α -amylase activity, earliness per se and leaf rolling.

Conclusions. The research showed the strong similarity of rye GAMYB sequence to its orthologues in other Graminae and confirmed the position in the genome consistent with the collinearity rule of cereal genomes. The statistically significant, however moderate association of ScGAMYB with many agronomic features has been pointed out, which proved that this gene is a QTL of pleiotropic character. The effect of ScGAMYB on flowering time was statistically the most significant. Developed sequence-based, allele-specific PCR markers could be useful in research and application purposes.

Background

The expression of structural genes encoding α -amylase and other hydrolases in aleurone layer of germinating grain is enhanced by a transcription factor GAMYB through its direct binding to a highly conserved GA-responsive element (GARE, TAACAA/GA) in the promoter [1]. The constitutive expression of *GAMYB* in aleurone cells in the absence of GA is sufficient to activate α -amylase promoter, however silencing or loss-of-function mutation of *GAMYB* detain α -amylase activity in GA-treated aleurone cells [2,3]. Thus, GAMYB activity is indispensable for elevated expression of α -amylase genes in response to gibberellin signal [4]. GAMYB is also involved in production of storage proteins during grain development [5,6] and in developmental mechanisms of anther formation [4,7].

GAMYB production in aleurone layer is controlled by the quantitative ratio of gibberellins (GA) and abscisic acid (ABA). Genes encoding GAMYB are suppressed by ABA signal transduced by protein kinase PKABA1 [8] and by GAMYB binding protein KGM, representing MAK-kinases [9]. GA induces rapid increase in *HvGAMYB* gene expression in barley aleurone layers through degradation of its repressor SLN1 representing DELLA proteins [4,10,11].

There is only one copy of *GAMYB* gene per cereal genome and it is located in syntenic position on homologous group 3 chromosomes in barley and wheat [12] and on collinear rice chromosome 1 [13].

Sequences of *Hv-* and *Ta-GAMYB* comprise four exons and three introns being differentiated to several haplotypes within wide germplasm collections of both species [12].

Functional polymorphisms in *GAMYB* gene may be an important factor affecting variation of α -amylase activity (AA) and possibly other important traits of cereals. This possibility should be explored since wheat, barley and rye chromosome 3 was shown to contain a number of QTL for AA, preharvest sprouting (PHS) and plant height (PH) [14–19]. Till now neither *ScGAMYB* sequence, polymorphisms and map location nor its relationship with agronomic traits have been characterized in rye.

This paper reports about sequence identification, mapping and association of *ScGAMYB* gene polymorphisms with selected quantitative traits of rye.

Results

Sequence of ScGAMYB

The 769 bp fragment of *ScGAMYB* amplified from DNA of Ot1–3 and 541 parental lines showed 95–97% identity with orthologous genes of wheat and barley deposited in NCBI database (Table 1). The E value between rye and wheat or barley sequences was 0.0.

Comparative analysis of *ScGAMYB* chromatograms representing gene fragment of 769 bp length revealed 4 SNPs differentiating Ot1–3 and 541 lines (C-T, A-G transitions at positions 470 and 476, respectively and G-C transversions at 485 and 495 positions of the *ScGAMYB* fragment sequence). All identified point mutations (ESM1) were used to design primers allowing to generate allele-specific products (Tab. 2). AS-PCR markers uncovered polymorphisms not only between parental 541/Ot1–3 lines but also between S32N/RXL10 lines and within mapping populations. In total, 10 pairs of primers were designed (Table 2). All of them amplified stable and repeatable products specific to the alleles tested.

Geneious software, version 10.2.4. was used to align the fragment of *ScGAMYB* sequence to the whole genome shotgun sequence assembly of rye cultivar Lo7 [20]. This approach allowed to identify homologous gene in the scaffold no. *Sc170168* being a DNA fragment containing entire sequence of *ScGAMYB* located on chromosome 3R in position of 92.15706326 cM. The identity coefficients between the analyzed sequences and that found within the scaffold were 97.46% and 96.56% for Ot1–3 and 541 lines, respectively. Moreover, the sequence alignment of the *ScGAMYB* gene fragments to sequences of rye transcriptome (Górska et al. unpublished) revealed their high level of identity to contig no. *c81081_g3_i1* i.e. 98.23% for line Ot1–3 and 97.33% for line 541.

Additionally, bioinformatics analysis of the raw sequences data deposited in Sequence Read Archive (SRA), in GeneBank (NCBI) for DS2, RXL10, M12 and L35 rye inbred lines allowed to disclose the complete mRNA sequence of the *ScGAMYB* geneaccessions SRX2636904–SRX2636920). The alignment of the obtained rye sequences of *GAMYB* gave a total gene length of 3,700 bp for M12 and DS2 lines. Sequences for RXL10 and L35 rye inbred lines were incomplete within exon 1. All sequences contained

the entire coding sequence of *ScGAMYB*. Comparative analysis of these four sequences revealed SNPs in 22 positions (ESM1).

The structure of *ScGAMYB* gene was derived by comparing sequencing data reported in this paper with rye DNA sequences presented by Bauer et al. [20] and those deposited in NCBI database. It has a total length of 3,700bp and contains 4 exons and 3 introns, similarly as coding sequences of orthologous genes in wheat and barley. The particular exons of *ScGAMYB* gene sequence contain 248bp, 387bp, 1009bp and 597bp being interspaced by three introns of 618bp, 829bp and 82bp lengths, with the coding sequence spanning from exon 2 to exon 4 (Fig. 1).

The coding sequence of *ScGAMYB* gene having 1659bp was translated to protein sequence containing 552 amino acids (ESM2), using Geneious software. This analysis revealed that particular SNPs resulted in change of amino acid sequences at positions: 76 (F/N), 87 (A/I), 233 (P/Q), 367 (S/C) and 459 (S/P) (ESM2). In addition, the protein structure prediction conducted by EMBOSS 6.5.7 plug showed that these polymorphisms affected on secondary structure of *ScGAMYB* (Fig. 2). Moreover, two polymorphisms resulted in change of protein sequence were observed in region (44–93 AA) coding functional MYB domain, responsible for DNA-binding. The second region of DNA-binding domain (97–144 AA) was highly conservative.

The similarity relationships between rye inbred lines and related species established based on *GAMYB* sequences are shown on the Figure 3.

Mapping of the *ScGAMYB* on chromosome 3R

Polymorphic AS-PCR markers of *ScGAMYB* segregated within the rye mapping populations in a ratio not significantly deviated from the 1 : 1 and 3 : 1 expectations (population RIL and F₂, respectively), thus allowing for successful mapping. *ScGAMYB* was mapped on proximal part of the long arm of chromosome 3R in tight linkage with the DArT marker *XrPt401390* on the 541×Ot1–3 (RIL-K) map and between DArTseq-silico 3589123 and 3357900 on the S32N/07×RXL10 (BSR-F₂) map (Fig. 4, ESM 3).

Associations of *ScGAMYB* polymorphisms with selected phenotypic traits

ScGAMYB locus showed significant relationship with trait variation for leaf rolling (RL), α-amylase activity in the grain (AMY), flowering date (FD), plant height (PH), spike length (SL), grain number per spike (GNPS), grain weight per spike (GWPS) and thousand grain weight (TGW) (Table 3). While relationship with leaf rolling, amylase activity, grain number per spike and grain weight per spike were detected in one mapping population and in one year of study, the remaining traits showed significant relationship across years and populations (spike length, plant height) or across years of study (thousand-grain weight and flowering date).

Discussion

The sequence of *ScGAMYB* characterized in this paper shows high homology to *TaGAMYB* and *HvGAMYB* in wheat and barley [12]. The gene structure is also similar to wheat and barley orthologues having four exons and three introns where the start codon and functional MYB domain are located on exon 2. *ScGAMYB* map position identified on the proximal part of the long arm of chromosome 3R is syntenic to that found in wheat and in barley [12]. The alignment of the *ScGAMYB* sequence to the whole genome shotgun sequence assembly of rye cultivar Lo7 [20] confirmed this location. The gDNA scaffold no. *Sc170168* containing entire sequence of *ScGAMYB* was also located on chromosome 3R, in position of 92 cM [20].

Out of 22 polymorphisms (SNPs) found within the coding sequence, 5 affected amino acid composition and secondary structure of the ScGAMYB protein. The level of polymorphism detected in rye was thus higher than that reported for wheat and barley within a much wider genetic material [12]. It is not surprising, since rye as an outcrossing species is more heterogeneous than self-pollinated cereals. Finding SNPs which affect secondary structure of ScGAMYB gives opportunity to develop functional markers for this key regulatory protein.

ScGAMYB is located within a near-centromeric region on chromosome 3R where QTL for α -amylase activity were found in rye [17,19,21]. As expected, analysis performed here indicated significant relationship between *ScGAMYB* allelic segregation and α -amylase activity in grain. Relationship of *ScGAMYB* and α -amylase activity revealed in this paper comprise with its function as transcriptional activator of α -amylase structural genes in cereal grain. This molecular function of GAMYB may be however negatively affected by interactions with a number of transcription factors such as SLN1, Vp1 and PKABA1 [8,10,22]. Also interfering with two members of the WRKY family i.e. ABF1 and ABF2, zinc finger protein HRT, a MAK-like kinase KGM and a DOF transcription factor BPBP [4,9,23,24], may reduce GAMYB's effectiveness in α -amylase induction. In spite of this complex regulatory network *ScGAMYB* seems to be a candidate gene for at least partial control of α -amylase production in rye grain.

Finding of relationship between *ScGAMYB* alleles segregation and variation of other studied traits has not such a straightforward explanation as for α -amylase activity. However knowing that GAMYB is active during grain development in promoting protein synthesis [5], it's role in enhancing thousand-grain weight seems possible. Also connection with flowering date and grain number per spike can be found in a literature since GAMYB's activity in development of anthers and pollen grains have been established. Overexpression of GA-related genes often leads to male sterility and failure to set seed; for example, transgenic barley overexpressing *HvGAMYB* exhibits increased male sterility, which causes a loss of grain production [25]. Important signaling and/or response roles in flowering of GAMYB factors were proved *in Arabidopsis thaliana* [26] and *Lolium temulentum* [27]. Rice GAMYB is involved in almost all instances of GA-regulated gene expression in anthers [7].

Enhancement of plant, spike and leaf growth by GAMYB is also possible at least as a pleiotropic effect of this potent regulatory gene. Our previous study [18] identified QTL for plant height, thousand-grain weight

and awn length within map interval containing *ScGAMYB*, thus confirming results presented here. Further study of *ScGAMYB* polymorphisms using wider collection of genetic stocks should bring more information about functions of this gene in various aspects of plant development.

Conclusions

Plant genomes have undergone significant reshaping during evolution, enabling each species to adapt to its ecological niche. Many changes can be detected within a family, genus or even each species. Sequential analyzes of cereal genomes indicate unusually rapid evolution of intergenic regions, which has consequences for the gene conservation. The priority of cereal genomics should be to develop efficient tools for the isolation of agronomic genes in every important family [28]. Rye belonging to family *Graminae*, genus *Secale*, has great research potential as a species with a much larger basic genome than other crops like rice or even, more related, barley and wheat. It can be used to analyze similarities and confirm orthology, as well as to test hypotheses for other species, especially with respect to gene function. It can also be a source of markers useful in the process of improving wheat and triticale cultivars, enabling breeding progress. The presented research showed the strong similarity of rye *GAMYB* sequence to its orthologues in other *Graminae* (*Triticum*, *Hordeum*, *Brachypodium*, *Avena*) and confirmed the position in the genome (chromosome 3R) consistent with the collinearity rule of cereal genomes. Provided sequence-based, allele-specific PCR markers (ten pairs of primers) can be useful in research and application purposes. The statistically significant, however moderate association of the gene segregation with many agronomic features has been pointed out, which proved that *ScGAMYB* is a QTL of pleiotropic character. An especially important result is the influence of rye *GAMYB* on the date of flowering, stated in two years, but in one population only, which suggest that this function may depend on the allelic form of the gene and/or the genetic background and/or environmental conditions, which should be verified in further studies.

Methods

Plant material and genetic maps

All the plant material used was collected and bred for many years by the employees of the Department of Plant Genetics, Breeding and Biotechnology of the West-Pomeranian University of Technology, Szczecin. All lines of winter rye used were advanced in inbred (at least S₁₀). This material has not been deposited in a publicly available herbarium.

Four rye inbred lines of different pedigree were chosen to the transcriptome sequencing analyses. DS2 is derived from *S. dighoricum* × cv Smolickie; the ancestor of line RXL10 is cv Zeelandzkie; L35 and M12 are recombinant inbred lines (RIL) derived from the two different interline hybrids: DS2 × RXL10 (mapping population RIL-L) and S120N/95 × S76N/95 (mapping population RIL-M), respectively.

For the purpose of genetic mapping several different pairs of parental inbred lines were screened to find out polymorphism at the *ScGAMYB* locus using allele-specific SCAR. Two mapping population were selected: RIL-K and BSR. RIL-K is a population derived from the 541 × Ot1–3 intercross. Origin of line 541 is described as: KaH6 × [(MS69–8–1 × cv Smolickie) F₂MS×KaH] F₁MP. Line Ot1–3 originates from Swedish cv Otello. BSR is the new F₂ mapping population obtained from a cross between inbred lines S32N/07 and RXL10. S32N/07 was developed and provided by Danko Plant Breeding Ltd. (Choryń, Poland) for the research purposes. The parental components varied in term of many morphological traits.

The trials were conducted on experimental fields of the West Pomeranian University of Technology in Szczecin (53.45°N, 14.53°E). The BSR population was analyzed in two vegetation seasons (2016–2017 and 2017–2018). 300 grains of F₂ were sown in 1 m long rows with the distance of 18 cm between rows and 20 cm between plants in a row. Finally, F₂ generation consisted of 255 plants. F₃ generation were the sublines of F₂ - each represented by 8–16 plants. Molecular analyses were conducted on the DNA extracted from the leaves of F₂ plants. Set of 183 individuals and two parental lines were sent to the external institution (Diversity Arrays Technology Pty Ltd., Canberra, Australia) for genotyping by sequencing [29]. Received segregations of DArTseq were used to localize *ScGAMYB* locus on the newly developed genetic map of population BSR. Segregation of *ScGAMYB* was analyzed within 253 F₂ plants.

Phenotype analyses

All vigorous individual plants of the BSR population were analyzed in terms of the following traits: tiller number (TN), plant height (PH), length of main spike (SL), number of spikelets per spike (SNPS), number of grains per spike under isolation (GNPS), weight of grains per spike (GWPS). Thousand grain weight (TGW) was calculated based on GNPS and GWPS. Trait values of F₃ were calculated as means for plants representing each genotype. Spike parameters of F₃ generation were assessed for isolated main spikes of 2–3 randomly chosen plants. Additionally, time of flowering was determined as the number of days, starting from May the 1th to the day when the first anthers was visible on the F₂ plant, and on the first plant of F₃ genotype. BSR plants were differentiated and assessed also in terms of leaf rolling according to the scale described by Myśków et al. [30]. Each line of the 82 RILs-K population was represented by 33 plants grown in randomized block design in three replications in 2010–2014. The amount of 1.5 g of mature kernels from each RIL and each replicate were milled and α-amylase activity in grain was assessed according to the simple diffusion method [31]. Means of plant height, spike length and thousand-grain weight were determined for each RIL using 15–20 mature plants samples from each replicate.

Statistical analysis

Statistical relationships between the segregation of molecular markers and studied traits were analyzed with the nonparametric Kruskal–Wallis' (K-W) test using STATISTICA v. 12.0 software

DNA isolation

Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen) from leaves of two-weeks seedlings, frozen in -80°C or lyophilized in -56°C in Alpha 1–2 LD plus Lyophilizer and stirred into powder in a Retsch MM200 mill. Concentration of DNA was established in EPOCH (Biotek) spectrophotometer. DNA samples were equilibrated to $10\text{ng}/\mu\text{l}$.

Gene amplification, cloning and sequencing

Sequences of primers used for amplification of rye *ScGAMYB* were derived from conserved sequences of *TaGAMYB*, *HvGAMYB*, *OsGAMYB* and *BdGAMYB* available in NCBI databases (accession no JF951917.1, AY008692.1, X98355.1, XM_003564404.3 respectively) using Primer 3 and Oligo 7 computer programs. Phusion HotStart II Polymerase (Finnzymes) was used to specifically amplify the fragments of *GAMYB* gene from rye genome. Amplifications were performed in $20\ \mu\text{l}$ reaction volume containing 5×Phusion HF Buffer, 2 mM dNTPs, 5 pmol/ μl of forward primer and reverse primer, 0.2 U polymerase and 30 ng of DNA template. Amplifications were carried out in BioRad Thermal Cycler according to the thermal profile: initial denaturation in 98°C for 30 sec, 10 cycles of denaturation in 98°C for 10 sec, primers annealing $60^{\circ}\text{C} - 1^{\circ}\text{C}$ per cycle for 15 sec, extension in 72°C for 20 sec and 25 cycles of denaturation in 98°C for 10 sec, primers annealing 50°C for 15 sec, extension in 72°C for 20 sec, and final extension 72°C for 300 sec.

PCR products were separated in a 1.5% agarose gel (Serva-Molecular Biology Grade) in a BioRad apparatus using 1×TBE buffer and current voltage 8V/cm. Bands were visualized by EtBr in UV using G:Box apparatus from Syngene. The monomorphic rye amplicons from lines 541 and Ot1–3 were cut off the gel using sterile extractor from Promega and eluted using MiniElute Gel Extraction Kit (Qiagen). Amplification products were cloned using ligation into pCR2.1-TOPO vectors in the presence of topoisomerase I and transformation of chemically competent *E.coli* cells by heat shock method (Invitrogen). Plasmids were isolated using Plasmid Mini Kit (A&A Biotechnology, BLIRT). Sequencing of PCR products was carried out using Genome Lab DTCS reagents (Beckman Coulter). PCR products were cleaned by magnetic beads (AgencourtCleanSEQ, Beckman Coulter) and sequenced in a Beckman Coulter CEQ 8000 Genetic Analysis System. Sequencing was carried out for both strands, which allowed to obtain a consensus sequence of gene fragments by means of Geneious 10.2.4. computer program [32].

ScGAMYB sequence analysis

Fragments of *ScGAMYB* sequence representing Ot1–3 and 541 inbred lines were compared to their homologues deposited in NCBI database (megaBLAST) and aligned to identify SNP mutations. Additionally, rye sequences were mapped to RNA-seq libraries created for 4 rye inbred lines (DS2, RXL10,

L35, M12). Each line was represented by 2–3 biological repetitions (Górska et al. unpublished). Finally, the contig covering the entire coding sequence of *ScGAMYB* was revealed using Geneious software version 10.2.4. The same software was used to map the fragment of *ScGAMYB* to the whole genome shotgun sequence assembly of rye cultivar Lo7 [20]. This approach allowed verification of the gene structure and its chromosomal location.

Polymerase chain reaction for detecting *ScGAMYB* polymorphisms

Identification of single nucleotide polymorphisms (SNPs) between *ScGAMYB* clones from Ot1–3 and 541 parental lines allowed designing primers for allele-specific PCR (AS-PCR) using Oligo7 software. Three types of primers were designed to detect individual SNPs. The first was complementary specifically to the allele sequence from line Ot1–3 expressing low α -amylase activity in grain. The second primer was specific to the sequence of allele from line 541 having grain with high α -amylase activity, and the third primer was complementary to the sequences of both alleles. Differentiating nucleotides in this assay were located in the last pentamer at the 3' terminal base of the primers. The highly specific polymerase without 3'→5' exonuclease activity—SNPase Hot Start (Bioron) was used to produce amplicons. The allele specific products were amplified in PCR mix in total amount 12.5 μ l containing 5 $\times$ SNPase buffer, 2 mM dNTPs, 2.5 mM MgCl₂, 5 pmol/ μ l of primer 1 and primer 2.5 U polymerase and 15 ng of DNA template. Amplifications were carried out in BioRad Thermal Cycler. The cycling parameters were 94°C for 2 min of pre-denaturation, followed by 35 cycles of 94°C for 30 s, 60°C for 15 s, 72°C for 30 s, and final extension at 72°C for 5 min.

Genetic mapping

ScGAMYB was genetically mapped on the 541 $\times$ Ot1–3 (RIL-K) and S32N/07 $\times$ RXL10 (BSR-F₂) high-density DArT based maps developed by Milczarski et al. [33] and Myśków et al. (unpublished). The genetic map construction of the population BSR-F₂ was conducted using Multipoint 3.2 software [34]. The group of DARTseq markers segregating in the combination “b, d” (the same as *GAMYB* segregation) were used to construct the genetic map. The “order” command was used for marker groups formed at a maximum threshold level of recombination frequencies at 0.005. For detection and removing problematic markers that caused neighborhood instabilities the “control of monotony” command was used. Finally, the ordering was repeated. For reducing the inflation of genetic distances on a high-density genetic map, the average length of the consensus map [33] was used for scaling of obtained linkage groups, as previously described [35,36]. Genetic map of population RIL-S [37] consisted of both DArT and DArTseq markers was used to compare position of *GAMYB* gene on two analyzed maps using the MapChart software [38].

List Of Abbreviations

AMY: α -amylase activity; AS: allele-specific; DArT: diversity array technology; FD: flowering date; GNPS: grain number *per* spike; GWPS: grain weight per spike; PH: plant height; QTL: quantitative trait locus; RIL: recombinant inbred line; RL: leaf rolling; SL: spike length; SNP: single nucleotide polymorphism; TGW: thousand grain weight

Declarations

Ethics approval and consent to participate:

This study has not directly involved humans and animals. The plant material belonged to the West-Pomeranian University of Technology, Szczecin; the mapping population was bred by the authors. No special permissions and/or licences were necessary.

Consent for publication:

Not applicable

Availability of data and material:

The mapping data are submitted as the supplementary material ESM 3 and are available in the article by Milczarski [33,37]. The raw transcripts sequences data for rye inbred lines are deposited in Sequence Read Archive (SRA), in GeneBank (NCBI) (accessions SRX2636904–SRX2636920).

Competing interests:

The authors declare that they have no competing interests.

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Authors' contribution:

Conceived and designed the experiments: AB, MG. Performed the experiments, analyzed the data: AB, MG, PMi, BM. Wrote the paper: PMa. Participated in writing: AB, MG, BM. Provided funds: BM. All authors read and approved the final version of the manuscript.

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References

1. Gubler F, Jacobsen J V. Gibberellin-responsive elements in the promoter of a barley high-pl alpha-amylase gene. *Plant Cell* [Internet]. 1992 Nov;4(11):1435–41. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/1477556>
2. Zentella R, Yamauchi D, Ho TD. Molecular dissection of the gibberellin/abscisic acid signaling pathways by transiently expressed RNA interference in barley aleurone cells. *Plant Cell* [Internet]. 2002 Sep;14(9):2289–301. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12215521>
3. Kaneko M, Inukai Y, Ueguchi-Tanaka M, Itoh H, Izawa T, Kobayashi Y, et al. Loss-of-function mutations of the rice GAMYB gene impair alpha-amylase expression in aleurone and flower development. *Plant Cell* [Internet]. 2003/12/19. 2004 Jan;16(1):33–44. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/14688295>
4. Woodger FJ, Millar A, Murray F, Jacobsen J V, Gubler F. The Role of GAMYB Transcription Factors in GA-Regulated Gene Expression. *J Plant Growth Regul* [Internet]. 2003;22(2):176–84. Available from: <https://doi.org/10.1007/s00344-003-0025-8>
5. Diaz I, Vicente-Carbajosa J, Abraham Z, Martínez M, Isabel-La Moneda I, Carbonero P. The GAMYB protein from barley interacts with the DOF transcription factor BPBF and activates endosperm-specific genes during seed development. *Plant J* [Internet]. 2002 Feb 1;29(4):453–64. Available from: <https://doi.org/10.1046/j.0960-7412.2001.01230.x>
6. Guo W, Yang H, Liu Y, Gao Y, Ni Z, Peng H, et al. The wheat transcription factor TaGAMYb recruits histone acetyltransferase and activates the expression of a high-molecular-weight glutenin subunit gene. *Plant J* [Internet]. 2015 Oct 1;84(2):347–59. Available from: <https://doi.org/10.1111/tpj.13003>
7. Aya K, Ueguchi-Tanaka M, Kondo M, Hamada K, Yano K, Nishimura M, et al. Gibberellin Modulates Anther Development in Rice via the Transcriptional Regulation of GAMYB. *Plant Cell* [Internet]. 2009 May 1;21(5):1453 LP–1472. Available from: <http://www.plantcell.org/content/21/5/1453.abstract>
8. Gómez-Cadenas A, Zentella R, Walker-Simmons MK, Ho T-HD. Gibberellin/Abscisic Acid Antagonism in Barley Aleurone Cells: Site of Action of the Protein Kinase PKABA1 in Relation to Gibberellin Signaling Molecules. *Plant Cell* [Internet]. 2001 Mar 1;13(3):667 LP–679. Available from: <http://www.plantcell.org/content/13/3/667.abstract>
9. Woodger FJ, Gubler F, Pogson BJ, Jacobsen J V. A Mak-like kinase is a repressor of GAMYB in barley aleurone. *Plant J* [Internet]. 2003 Feb 1;33(4):707–17. Available from: <https://doi.org/10.1046/j.1365-313X.2003.01663.x>
10. Gubler F, Chandler PM, White RG, Llewellyn DJ, Jacobsen J V. Gibberellin signaling in barley aleurone cells. Control of SLN1 and GAMYB expression. *Plant Physiol* [Internet]. 2002 May;129(1):191–200. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12011350>

11. Tuan PA, Kumar R, Rehal PK, Toora PK, Ayele BT. Molecular Mechanisms Underlying Absciscic Acid/Gibberellin Balance in the Control of Seed Dormancy and Germination in Cereals. *Front Plant Sci* [Internet]. 2018 May 23;9:668. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29875780>
12. Haseneyer G, Ravel C, Dardevet M, Balfourier F, Sourdille P, Charmet G, et al. High level of conservation between genes coding for the GAMYB transcription factor in barley (*Hordeum vulgare* L.) and bread wheat (*Triticum aestivum* L.) collections. *Theor Appl Genet* [Internet]. 2008/05/17. 2008 Aug;117(3):321–31. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/18488187>
13. Kaneko M, Ashikari M, Matsuoka M. Mapping and expression of rice GAMYB. *Rice Genet Newsl.* 2002;19:60.
14. Ullrich SE, Clancy JA, del Blanco IA, Lee H, Jitkov VA, Han F, et al. Genetic analysis of preharvest sprouting in a six-row barley cross. *Mol Breed* [Internet]. 2008;21(2):249–59. Available from: <https://doi.org/10.1007/s11032-007-9125-7>
15. Mohan A, Kulwal P, Singh R, Kumar V, Mir RR, Kumar J, et al. Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. *Euphytica* [Internet]. 2009;168(3):319–29. Available from: <https://doi.org/10.1007/s10681-009-9935-2>
16. Masojć P. A4 - Lebiecka, K. A4 - Milczarski, P. A4 - Wiśniewska, M. A4 - Łań, A. A4 - Owsianicki, R. PA-M. Three classes of loci controlling preharvest sprouting in rye (*Secale cereale* L.) discerned by means of bidirectional selective genotyping (BSG). *Euphytica*. 2009;v. 170(1–2):123–129–2009 v.170 nos.1–2.
17. Masojć P, Wiśniewska M, Łań A, Milczarski P, Berdzik M, Pędziwiatr D, et al. Genomic architecture of alpha-amylase activity in mature rye grain relative to that of preharvest sprouting. *J Appl Genet* [Internet]. 2011;52(2):153–60. Available from: <https://doi.org/10.1007/s13353-010-0025-x>
18. Masojć P, Milczarski P, Kruszona P. Comparative analysis of genetic architectures for nine developmental traits of rye. *J Appl Genet* [Internet]. 2017/05/09. 2017 Aug;58(3):297–305. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28488059>
19. Myśków B, Stojalowski S, Łań A, Bolibok-Bragoszewska H, Rakoczy-Trojanowska M, Kilian A. Detection of the quantitative trait loci for α -amylase activity on a high-density genetic map of rye and comparison of their localization to loci controlling preharvest sprouting and earliness. *Mol Breed*. 2012;30(1).
20. Bauer E, Schmutzer T, Barilar I, Mascher M, Gundlach H, Martis MM, et al. Towards a whole-genome sequence for rye (*Secale cereale* L.). *Plant J*. 2017;89(5).
21. Milczarski P, Masojć P, Krajewski P, Stochmal A, Kowalczyk M, Angelov M, et al. QTL mapping for benzoxazinoid content, preharvest sprouting, α -amylase activity, and leaf rust resistance in rye (*Secale cereale* L.). *PLoS One* [Internet]. 2017 Dec 21;12(12):e0189912–e0189912. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29267335>
22. Abraham Z, Iglesias-Fernández R, Martínez M, Rubio-Somoza I, Díaz I, Carbonero P, et al. A Developmental Switch of Gene Expression in the Barley Seed Mediated by HvVP1 (Viviparous–1)

- and HvGAMYB Interactions. *Plant Physiol* [Internet]. 2016 Apr 1;170(4):2146 LP–2158. Available from: <http://www.plantphysiol.org/content/170/4/2146.abstract>
23. Xie Z, Zhang Z-L, Zou X, Yang G, Komatsu S, Shen QJ. Interactions of two abscisic-acid induced WRKY genes in repressing gibberellin signaling in aleurone cells. *Plant J* [Internet]. 2006 Apr 1;46(2):231–42. Available from: <https://doi.org/10.1111/j.1365-313X.2006.02694.x>
 24. Lovegrove A, Hooley R. Gibberellin and abscisic acid signaling in aleurone. *Trends Plant Sci*. 2000;5(5):102–10.
 25. Murray F, Kalla R, Jacobsen J, Gubler F. A role for HvGAMYB in anther development. *Plant J* [Internet]. 2003 Feb 1;33(3):481–91. Available from: <https://doi.org/10.1046/j.1365-313X.2003.01641.x>
 26. Gocal GF, Sheldon CC, Gubler F, Moritz T, Bagnall DJ, MacMillan CP, et al. GAMYB-like genes, flowering, and gibberellin signaling in Arabidopsis. *Plant Physiol* [Internet]. 2001 Dec;127(4):1682–93. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/11743113>
 27. Gocal GF, Poole AT, Gubler F, Watts RJ, Blundell C, King RW. Long-day up-regulation of a GAMYB gene during *Lolium temulentum* inflorescence formation. *Plant Physiol* [Internet]. 1999 Apr;119(4):1271–8. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/10198085>
 28. Delseny M. Re-evaluating the relevance of ancestral shared synteny as a tool for crop improvement. *Curr Opin Plant Biol* [Internet]. 2004;7(2):126–31. Available from: <http://www.sciencedirect.com/science/article/pii/S1369526604000081>
 29. Li H, Vikram P, Singh RP, Kilian A, Carling J, Song J, et al. A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. *BMC Genomics* [Internet]. 2015;16(1):216. Available from: <https://doi.org/10.1186/s12864-015-1424-5>
 30. Myśków B, Góralska M, Lenarczyk N, Czyczyło-Mysza I, Stojakowski S. Putative candidate genes responsible for leaf rolling in rye (*Secale cereale* L.). *BMC Genet* [Internet]. 2018;19(1):57. Available from: <https://doi.org/10.1186/s12863-018-0665-0>
 31. Masojć P, Larsson-Raźnikiewicz M. Genetic variation of alpha-amylase levels among rye kernels, tested by gel diffusion technique. *Swedish J Agric Res*. 1991;21:141–5.
 32. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* [Internet]. 2012/04/27. 2012 Jun 15;28(12):1647–9. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22543367>
 33. Milczarski P, Bolibok-Bragoszewska H, Myśków B, Stojakowski S, Heller-Uszyńska K, Góralska M, et al. A high density consensus map of Rye (*Secale cereale* L.) based on dart markers. *PLoS One*. 2011;6(12).
 34. Ronin Y, Mester D, Minkov D, Belotserkovski R, Jackson BN, Schnable PS, et al. Two-phase analysis in consensus genetic mapping. *G3 (Bethesda)* [Internet]. 2012/05/01. 2012 May;2(5):537–49. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22670224>
 35. Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, et al. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and

- cultivars. Proc Natl Acad Sci [Internet]. 2013 May 14;110(20):8057 LP–8062. Available from: <http://www.pnas.org/content/110/20/8057.abstract>
36. Maccaferri M, Ricci A, Salvi S, Milner SG, Noli E, Martelli PL, et al. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnol J [Internet]. 2015 Jun 1;13(5):648–63. Available from: <https://doi.org/10.1111/pbi.12288>
37. Milczarski P, Hanek M, Tyrka M, Stojalowski S. The application of GBS markers for extending the dense genetic map of rye (*Secale cereale* L.) and the localization of the Rfc1 gene restoring male fertility in plants with the C source of sterility-inducing cytoplasm. J Appl Genet [Internet]. 2016/04/16. 2016 Nov;57(4):439–51. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27085345>
38. Voorrips RE. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J Hered [Internet]. 2002 Jan 1;93(1):77–8. Available from: <https://doi.org/10.1093/jhered/93.1.77>

Tables

Table 1. Similarity of *ScGAMYB* sequence with sequences of related cereal species

Species	Max Score	Query cover	% Identity	Accession no.
<i>Hordeum vulgare</i>	1277	99%	96.74	AY008692.1
<i>Triticum aestivum</i>	1279	100%	96.63	JF951917.1
<i>Triticum monococum</i>	1254	100%	96.10	AB214883.1
<i>Avena sativa</i>	878	99%	87.50	AJ133638.1
<i>Brachypodium distachyon</i>	852	100%	86.68	XM_003564404.3

Table 2. Characteristics of allele specific PCR for *ScGAMYB* identification in rye

Forward primer	Reverse primer	T _a	Product size	Allele present in line:
catcagggcgacgcagtgctc	ccgtcagtgaaatcggagtg	59.0	274	Ot1-3, 8/03LM
catcagggcgacgcagtgctc	ccgtcagtgaaatcggagtc	59.0	274	541, DS2, S32N/07
catcagggcgacgcagtgctc	aatcggagtstgcgtcgccc	59.0	264	Ot1-3
catcagggcgacgcagtgctc	aatcggagtctgcgtcgccg	59.0	264	541, DS2
ggagagctgaaaaacatcagg	ccgtcagtgaaatcggagtg	59.0	288	Ot1-3, RXL10, 8/03LM
ggagagctgaaaaacatcagg	ccgtcagtgaaatcggagtc	59.0	288	541, DS2, S32N/07
ggagagctgaaaaacatcagg	aatcggagtstgcgtcgccc	59.0	278	Ot-3, RXL10
ggagagctgaaaaacatcagg	aatcggagtctgcgtcgccg	59.0	278	541, DS2
gctgttcctcggcgacgcac	cggtcgatcagttctcaaagact	59.0	341	Ot1-3, RXL10, 8/03LM
gctgttcctcggcgacgcag	cggtcgatcagttctcaaagact	59.0	341	541, DS2, S32N/07

Table 3. Relationship between allele polymorphism in the *ScGAMYB* locus and variance of morphological traits established using Kruskal-Wallis test in BSR and RIL-K populations

Trait/ unit	Population	Year	GAMYB genotype	N	Mean trait value	Mean rank	H	P	Significance
RL	BSR-F ₂	2017	b	58	1.7	86.2	10.01	0.002	***
[1-3 scale]			d	156	2.0	115.4			
AMY	RIL-K	2014	a	42	8.1	46.7	5.06	0.024	*
[U]			b	39	1.8	34.9			
FD	BSR-F ₂	2017	b	64	33.7	146.7	11.35	0.001	****
[days]			d	179	33.0	113.2			
FD	BSR-F ₃	2018	b	63	27.5	146.6	8.70	0.003	***
[days]			d	184	26.9	116.3			
PH	BSR-F ₂	2017	b	66	114.1	144.3	5.33	0.021	*
[cm]			d	186	106.9	120.2			
PH	RIL-K	2012	a	43	96.8	46.7	4.29	0.038	*
[cm]			b	39	90.7	35.8			
SL	BSR-F ₂	2017	b	64	8.6	144.9	6.58	0.010	**
[cm]			d	185	7.9	118.1			
SL	BSR-F ₃	2018	b	64	8.1	139.0	3.83	0.050	*
[cm]			d	183	7.8	118.7			
SL	RIL-K	2010	a	43	8.9	46.5	4.04	0.044	*
[cm]			b	39	8.4	35.9			
GNPS	BSR-F ₂	2017	b	64	38.5	145.3	6.86	0.009	**
[number]			d	185	34.2	118.0			
GWPS	BSR-F ₂	2017	b	64	1.4	143.7	5.78	0.016	*
[g]			d	185	1.2	118.5			
TGW	RIL-K	2010	a	43	21.2	47.2	5.15	0.023	*
[g]			b	39	19.4	35.2			
TGW	RIL-K	2011	a	43	20.9	47.6	5.99	0.014	**
[g]			b	39	19.2	34.7			
TGW	RIL-K	2012	a	43	20.7	47.6	5.94	0.015	*
[g]			b	39	18.9	34.8			

Figures

Figure 1

The structure of ScGAMYB gene in rye.

Figure 2

The changes in secondary structure of ScGAMYB resulting from SNPs.

Figure 3

Relationships between rye inbred lines and related species established based on GAMYB sequences using UPGMA method.

Figure 4

Location of the *ScGAMYB* gene on the chromosome 3R of populations BSR-F2 and RIL-K [33]. To integrate these two maps the map of RIL-S population [37] was used.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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