A high-quality genome assembly highlights rye genomic characteristics and agronomically important genes

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Rye is a valuable food and forage crop, an important genetic resource for wheat and triticale improvement and an indispensable material for efficient comparative genomic studies in grasses. Here, we sequenced the genome of Weining rye, an elite Chinese rye variety. The assembled contigs (7.74 Gb) accounted for 98.47% of the estimated genome size (7.86 Gb), with 93.67% of the contigs (7.25 Gb) assigned to seven chromosomes. Repetitive elements constituted 90.31% of the assembled genome. Compared to previously sequenced Triticeae genomes, Daniela, Sumaya and Sumana retrotransposons showed strong expansion in rye. Further analyses of the Weining rye genome assembly shed new light on genome-wide gene duplications and their impact on starch biosynthesis genes, physical organization of complex prolamin loci, gene expression features underlying early heading trait and putative domestication-associated chromosomal regions and loci in rye. This genome sequence promises to accelerate genomic and breeding studies in rye and related cereal crops.

Rye (Secale cereale, 2n = 2x = 14, RR) belongs to the genus Secale in the Triticeae tribe of the grass family Poaceae. Although phylogenetically related to common wheat (Triticum aestivum, Ta) and barley (Hordeum vulgare, Hv), rye has unique characteristics in both agronomic performance and genome properties.

Rye is well known for its strong tolerance to abiotic stresses and high adaptability to barren soils. It is also characterized by potent resistance to many fungal diseases, which often elicit severe economic losses in global Triticeae crops. The disease-resistance genes carried by the rye 1RS chromosome arm, transferred to the wheat genome through wide hybridization, have contributed greatly to the control of powdery mildew and stripe rust diseases in worldwide wheat production. Moreover, rye is essential for developing triticale, a synthetic forage and promising food crop with higher biomass and yield level than rye. Thus, rye is a valuable crop in many countries and a globally important genetic resource for wheat and triticale improvement.

The genome of rye is substantially larger than those of barley and diploid wheat species, and was estimated to be around 7.9 Gb, with transposon elements (TEs) constituting approximately 90% of the genome. However, potential contributions of specific TEs to rye genome expansion remain to be resolved. To date, a high-quality reference genome sequence is still unavailable for rye. By contrast, genome assemblies were published for Ta and its diploid progenitor species Triticum urartu (Tu) and Aegilops tauschii (Aet). The genomes of wild emmer wheat (Triticum turgidum ssp. dicoccoides, WEW), Hv and durum wheat (T. turgidum ssp. durum) were also decoded in recent times.

Weining rye, an early flowering variety cultivated in China, is outstanding because of its broad-spectrum resistance to both powdery mildew and stripe rust. To understand the genetic and molecular basis of rye elite traits and to promote genomic and breeding studies in rye and related crops, we sequenced and analyzed the genome of Weining rye.

Results

Genome assembly and gene annotation. The genome size of Weining rye was estimated to be 7.86 Gb using flow cytometry.
which is consistent with the previous estimate of around 8 Gb for rye genome size\(^\text{20}\). To construct the genome sequence of Weining rye, we integrated the datasets generated by long-range PacBio RS II and short-read Illumina sequencing, as well as those from barley, rye, we integrated the datasets generated by long-range PacBio RS II and short-read Illumina sequencing, as well as those from barley, rye, and comparatively smaller for 1R (0.94097 Gb) and 5R (0.99891 Gb) chromosome size was larger for 2R, 3R, 4R, 6R and 7R (above 1 Gb) seven pseudo-chromosomes (1R–7R), accounting for 93.67% of the genome size of Weining rye, of which 7.25 Gb was anchored on chromosomes 1R–7R, three families (Gypsy, Copia and CACTA) together represented about 56.5% of the assembled Weining genome; CACTA DNA transposons were the dominant TEs and occupied 84.49% of the annotated TE content and 76.29% of the assembled Weining genome; CACTA DNA transposons were the second most abundant TE, constituting 11.68% of the annotated TE content and 10.55% of the assembled Weining genome.

### Analysis of TEs

A total of 6.99 Gb, representing 90.31% of the Weining assembly, was annotated as TEs, which included 2,671,941 elements belonging to 537 families (Table 1 and Supplementary Table 14). This TE content was clearly higher than those previously reported for Ta (84.70%)\(^\text{10}\), Tu (81.42%)\(^\text{11}\), Aet (84.40%)\(^\text{12}\), WEW (82.20%)\(^\text{13}\) or Hv (80.80%)\(^\text{15}\). The LTR-RTs, including Gypsy, Copia and unclassified retrotransposon elements, were the dominant TEs and occupied 84.49% of the annotated TE content and 76.29% of the assembled Weining genome; CACTA DNA transposons were the second most abundant TE, constituting 11.68% of the annotated TE content and 10.55% of the assembled Weining genome.

### Cross-genome comparisons

With Tu, Aet and Hv showed that LTR-RTs, especially Gypsy elements, contributed the most to the genome expansion of Weining rye (Fig. 2a). Weining rye had \(\sim 5.25 \text{~Gb}\) more of LTR-RTs than did barley, and this contributed 85.42% to the 2.95-Gb increase in the genome size of rye versus barley. The top 15 abundant TE families (11 Gypsy, three Copia and one CACTA) together represented about 56.5% of the assembled Weining genome, with the most abundant elements being from Sabrina, a family of non-autonomous Gypsy retrotransposons comprising 10.5% of the Weining assembly (Fig. 2b). Three LTR-RT families (Daniela, Sumaya and Sumana) exhibited substantially elevated abundance in Weining rye relative to those in Tu, Aet and Hv, with Daniela displaying the greatest elevation (Fig. 2b). Daniela accounted for 5.03% of the Weining assembly but less than 0.8% of those of Tu, Aet and Hv; Sumaya made up 3.61%, 2.38%, 0.48% and 0.14% of the genomes of Weining rye, Tu, Aet and Hv, respectively; and Sumana occupied 1.82% of the Weining genome but less than 0.6% of the genomes of Tu (0.58%), Aet (0.52%) and Hv (0.21%) (Fig. 2b).

A distinct bimodal distribution was found for the insertion times of intact LTR-RTs in Weining rye, whereas a unimodal distribution was observed for Tu, Aet and Hv (Fig. 2c). Weining rye had a comparatively high proportion of recent LTR-RT insertions, with the peak of amplification appearing around 0.5 million years ago (Ma), which was the most recent among the four species; the other peak, occurring approximately 1.7 Ma, was older and also seen in barley (Fig. 2c).

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**Table 1 | Statistics of Weining rye genome assembly and annotation**

| Genomic feature          | Value      | Percentage (%) |
|--------------------------|------------|----------------|
| Estimated genome size    | 7.86 Gb    |                |
| Assembled genome size    | 7.74 Gb    |                |
| Assembled genome percentage | 98.47%    |                |
| Sequence assigned to chromosomes | 7.25 Gb    | 93.67          |
| Number of scaffolds      | 18,028     |                |
| N50 scaffold length      | 1.04 Gb    |                |
| N90 scaffold length      | 0.95 Gb    |                |
| Number of contigs        | 63,244     |                |
| N50 contig length        | 480.35 kb  |                |
| N90 contig length        | 3750 kb    |                |
| Longest contig           | 9.02 Mb    |                |
| GC content               | 45.89%     |                |
| Size of retrotransposons | 5,996.0 Mb | 77.46          |
| Size of DNA transposons  | 912.7 Mb   | 11.79          |
| Other repeats            | 81.9 Mb    | 1.06           |
| Size of total repeat sequences | 6,990.6 Mb | 90.31          |
| Number of HC genes       | 45,596     |                |
| Number of HC transcripts | 84,179     |                |
| Mean length of HC genes  | 4,864.34 bp|                |
| Total length of HC genes | 223,740,142 bp |            |

\(^{a}\)The percentages were each calculated based on the assembled genome size (7.74 Gb).
Copia elements in Weining rye at 0.3 Ma, while amplifications of Gypsy retrotransposons dominantly shaped the bimodal distribution pattern of LTR-RT burst dynamics (Fig. 2d).

Therefore, recent large-scale bursts of retrotransposons around 0.3–0.5 Ma, including the Gypsy retroelements Daniela, Sumaya and Sumana, contributed directly to rye genome expansion. Consistent with our analysis, past studies also showed that increases in the abundance of specific retrotransposon families can lead to plant genome expansion over short periods of time. Identification of greatly enlarged TE families in rye may stimulate deeper studies of the dynamic changes of TEs and their consequences on genome expansion and function in Triticeae.

Investigation of rye genome evolution and chromosome synteny. We computed 2,517 single-copy orthologous genes by comparing the genomes of Weining rye, Tu, Aet, Ta (subgenomes TaA, TaB and TaD), Hv, Oryza sativa ssp. japonica (Os), Brachypodium distachyon (Bd), Zea mays, Sorghum bicolor and Setaria italica. Phylogenetic and molecular dating investigations with these genes revealed that the divergence between rye and diploid wheats took place after the separation of barley from wheat, with the divergence times for the two events being approximately 9.6 and 15 Ma, respectively (Fig. 3a). These values were older than those based on chloroplast sequences but close to the higher end of the estimates based on Acc homoeolooci.

All grass species experienced three ancient whole-genome duplications, which resulted in 12 ancestral grass karyotype (AGK) chromosomes that are largely preserved in modern-day rice. We therefore used the rice genome as an ancestral reference to investigate the chromosomal evolution of Weining rye. A total of 23 large syntenic blocks enfolding 10,949 orthologous gene pairs between Weining rye and rice were identified (Supplementary Table 15), which enabled us to deduce the arrangements of ancestral chromosome segments in 1R–7R (Fig. 3b). In essence, 3R was derived...
from a single ancient chromosome, AGK1 or Os1, although a segment of this chromosome was translocated to 6RL; 1R and 2R were each formed by two ancestral chromosomes, with 1R involving a nested insertion of AGK10 or Os10 into AGK5 or Os5 and 2R forming by nested insertion of AGK7 or Os7 into AGK4 or Os4; 4R, 5R, 6R and 7R were each derived from at least three ancestral chromosomes via complex translocations (Fig. 3b and Supplementary Table 15). In the comparison of the Weining assembly with the three subgenomes of common wheat, 22,648, 22,212 and 22,830 orthologous gene pairs, representing 51.98%, 50.98% and 52.40% of the HC genes of TaA, TaB and TaD, respectively, were identified (Supplementary Table 16). These orthologous genes facilitated the identification of syntenic blocks between rye and common wheat chromosomes (Fig. 3c). Chromosomes 1R, 2R and 3R were entirely collinear with group 1, 2 and 3 chromosomes of wheat, respectively. In 4R, three regions showing collinearity with parts of 4(A, B, D), 7(A, B, D) or 6(A, B, D) were found. Chromosome 5R was entirely collinear with 5A and partly collinear with 5B and 5D due to the fusion of translocated 4B or 4D segments at the distal ends of 5BL or 5DL. In 6R, three regions displaying collinearity with parts of 6(A, B, D), 3(A, B, D) or 7(A, B, D), were observed. Chromosome 7R was partly collinear with 7A, 7B and 7D; the non-collinear regions in 7A, 7B and 7D were caused by two translocations (from 4A and 2A) to 7A and three translocations (from 5B or 5D, 4B or 4D and 2A or 2B) to 7B or 7D (Fig. 3c). Together, the above data will encourage the use of rye in comparative genomic research of grasses and in future wide hybridization studies between rye and common wheat.

Analysis of gene duplications and their impact on starch biosynthesis genes. The chromosomally located HC genes of Weining rye were analyzed with MCScanX software\(^3\), which yielded 4,217 singletons, 23,753 dispersed duplicated genes, 6,659 proximally duplicated genes, 7,077 tandemly duplicated genes and 1,866 segmentally duplicated genes (Fig. 4a and Supplementary Table 17). Notably, the numbers of tandemly duplicated genes and proximally duplicated genes in Weining rye were both higher than those found for Tu, Aet, Hv, Bd and Os (Supplementary Table 17).

The increased TE content of Weining rye (Fig. 2a) led us to investigate transposed duplicated genes (TrDGs), which are induced by TE activities and constitute a major part of the dispersed duplicated genes. We identified 10,357 TrDGs in Weining rye using Hv as a reference, which was substantially larger than the number of TrDGs in Tu (7,145) or Aet (7,351) (Fig. 4b). The TrDGs unique to Weining...
rye (5,926) were also more numerous than those specifically found for Tu (3,513) or Aet (3,327) (Fig. 4b).

We next investigated gene duplications in rye starch biosynthesis-related genes (SBRGs). Of the Weining rye SBRGs identified (Fig. 4c and Supplementary Table 1), nine had one or two duplicated copies on the same chromosome or a different chromosome. Transposed duplication occurred for five (ScSSIV, ScDPEI, ScSuSy1, ScSuSy2 and ScUGPaseI) SBRGs, while tandem, proximal and dispersed duplications occurred for one (ScPHO2), two (ScAGP-L2-p and ScSBE1) and one (SSIIIa) SBRGs, respectively (Fig. 4c). The duplicates of the same SBRGs often showed differences in expression (Fig. 4d and Supplementary Data 2). For example, the parental copy of ScSuSy2 (ScWN2R01G169900) was strongly expressed in developing grains at 10 and 20 d after anthesis but with fairly low expression in stem and root tissues; one of its transposed duplicates (ScWN4R01G484200), however, had little expression in developing grains but was rather highly expressed in stem and root tissues.

Thus, it appears that rye genome expansion is accompanied by larger numbers of gene duplications. The increased TE bursts in rye may have led to an elevated number of TrDGs. As illustrated by analyzing SBRGs, the various types of gene duplications can enrich the diversities of rye genes functioning in important biological processes. Elucidation of the whole set of rye SBRGs will facilitate their use in improving yield potential and nutritional quality traits. The new changes in rye SBRGs may provide new enzyme activities for manipulating plant starch biosynthesis and properties.
Dissection of rye seed storage protein gene loci. Similar to wheat and barley, rye accumulates abundant prolamin-type seed storage proteins (SSPs) in endosperm tissues. Although four chromosomal loci (Sec-1 to Sec-4) specifying rye SSPs were identified, their structures remain to be fully elucidated. We therefore dissected rye SSP loci and genes using the Weining genome assembly.

The secalin genes we identified are listed in Supplementary Table 19. As shown in Fig. 5a, the size of Sec-1 was ~12 Mb, and it contained two separate clusters of genes encoding γ- or ω-secalins, with a total of seven active gene members. Sec-2 was ~591 kb and carried two active genes coding for one γ- and one ω-secalin. Sec-3 was ~38 kb and harbored two active genes specifying one γ- and one ω-type of high-molecular-weight (HMW)-secalin (HMW-1Rx and HMW-1Ry), respectively. Sec-2 was about 33 kb and comprised three active genes encoding 75k γ-secalins. In agreement with these results, SDS–polyacrylamide gel electrophoresis (PAGE) analysis indicated that mature Weining rye grains accumulated two HMW-secalins, three 75k γ-secalins and high amounts of ω-secalin and 40k γ-secalins (Extended Data Fig. 5).

Fig. 4 | Analysis of rye gene duplications and their impact on the diversity of SBRGs. a, Higher numbers of proximally duplicated genes (PDGs) and tandemly duplicated genes (TDGs) in rye as compared with those in Tu, Aet, Hv, Bd and rice. b, Venn diagram showing the numbers of specific and shared transposed duplicated genes between Weining rye, Tu and Aet, computed using barley as a reference. c, A diagram illustrating the main enzymatic reactions catalyzed by the protein products of different SBRGs (definitions for abbreviations can be found in Supplementary Table 18). PPi, inorganic pyrophosphate; p, phosphate. d, Expression patterns of SBRGs in the root, stem, leaf, spike and developing grain tissues of Weining rye. Root, stem, leaf and spike samples were collected at the heading stage. The grain samples were collected at 10, 20, 30 and 40 d after anthesis (DAA), respectively. TPM, transcripts per million. In c,d, the nine genes shown in red exhibit different types of duplications relative to their counterparts in the A, B and D subgenomes of common wheat. Note that the SBRG duplicates often showed expression differences in different organs (d). DDG, dispersed duplicated gene.
specifying rye HMW-secalins was collinear with the barley locus carrying the D-hordein gene and the wheat homoeologous loci harboring HMW glutenin subunit genes (Fig. 5b). The 75k γ-secalins specified by Sec-2 were phylogenetically related to wheat γ-gliadins and barley γ-hordeins (Extended Data Fig. 6), but the wheat and barley chromosomal segments collinear to Sec-2 contained no gliadin or other annotated SSP genes (Fig. 5b). Lastly, we did not find α-gliadin genes in the Weining genome assembly, which is compatible with the suggestion that α-gliadin genes evolved only recently in wheat and closely related species after the divergence of wheat from rye 35. These SSP analysis results clarify the structure and composition of secalin loci, which will assist future efforts to refine the processing and nutritional qualities of rye, triticale and wheat.

Examination of transcription factor and disease-resistance genes. We predicted transcription factor (TF) genes in Weining rye encoding HMW-secalins/D-hordein or other annotated SSP genes (Fig. 5b). Lastly, we did not find α-gliadin genes in the Weining genome assembly, which is compatible with the suggestion that α-gliadin genes evolved only recently in wheat and closely related species after the divergence of wheat from rye 35. These SSP analysis results clarify the structure and composition of secalin loci, which will assist future efforts to refine the processing and nutritional qualities of rye, triticale and wheat.

Investigation of gene expression features associated with early heading trait. In this work, we observed that Weining rye was heading 10–12 d earlier than Jingzhou rye under long-day conditions (Fig. 6a), which correlated with a more rapid development of the shoot apical meristem of Weining rye (Fig. 6b). Because of the key role of the flowering locus T (FT) gene in flowering-time control in higher plants 39, we examined FT expression in the two lines.

Two FT genes with relatively high expression levels under long-day conditions, ScFT1 (ScWN4R01G446100) and ScFT2 (ScWN3R01G192500), were annotated in the Weining genome assembly. The expression levels of ScFT1 and ScFT2 were significantly higher in Weining plants than those in Jingzhou plants at 7 and 10 d after sowing (DAS) (Fig. 6c, Extended Data Fig. 8 and 9).
Supplementary Data 4). Consistently, rye FT (ScFT) proteins accumulated to relatively high levels in Weining plants but were barely detectable in Jingzhou rye at 7 and 10 DAS (Fig. 6d). Surprisingly, the size of ScFT proteins detected in rye (~29 kDa) was larger than the calculated molecular mass of ScFT1 or ScFT2 (both around 19 kDa) (Fig. 6d and Extended Data Fig. 9a), indicating potential post-translational modification of ScFT proteins. Analysis using Phos-tag SDS–PAGE, which is highly efficient at detecting phosphoproteins, showed that ScFT was phosphorylated in Weining rye (Extended Data Fig. 9b).

Two amino acid residues in ScFT2 (S76 and T132), strictly conserved among the main FT proteins in grass species and Arabidopsis...
Fig. 7 | Analysis of chromosomal regions and loci potentially related to rye domestication. a, Putative selection sweeps revealed using three different methods, with dashed lines indicating thresholds for significant selection sweeps (top 5% outliers). ScBC1, ScBtr, ScGW2, ScMOC1, ScID1 and ScWx were found in the putative sweeps. b, Specific and shared selection sweeps (SLS) uncovered by different methods. c, Selection sweeps associated with ScBC1, ScBtr, ScMOC1 or ScID1 uncovered by joint DRI and XP-CLR analysis. d, Microsynteny analysis of collinear ID1 loci in rye and related Triticeae genomes. Red and gray lines connect orthologous ID1 or flanking genes. e, Different expression profiles of ScID1.1 and ScID1.2 genes in Weining and Jingzhou rye plants at 3 DAS, revealed by RT-qPCR. Each value is the mean (± s.d.) of five samples taken from n = 5 plants, and the results shown were reproducible in n = 3 separate assays. f, Boxplots for days to heading, based on the genotype of the ScID1 locus in the Weining x Jingzhou F2 population (n = 66 for WN/WN, n = 161 for WN/JZ and n = 64 for JZ/JZ). Box edges represent 0.25 and 0.75 quantiles, respectively, with the median values shown by bold lines. The lower and upper whiskers correspond to the minima values, at most the first quartile – 1.5 x interquartile range (IQR) or the maxima values no further than the third quartile + 1.5 x IQR. All data are shown by gray dots, with statistical analysis performed using two-tailed t-tests.
thaliana, were predicted to be phosphorylated (Supplementary Fig. 4). We therefore mutated the two residues and created a series of desphosphomimic (S76A, T132A and S76A+T132A) and phosphomimic sites (S76D, T132D and S76D+T132D) for ScFT2. When ectopically expressed in tobacco using a potato virus X-based viral vector, ScFT2 and the desphosphomimic double mutant ScFT2S76A+T132A consistently enhanced tobacco growth relative to free GFP (control) and other ScFT2 mutants (Fig. 6c). Compared to GFP, ectopic expression of ScFT2 and the three desphosphomimic mutants (ScFT2S76A, ScFT2T132A and ScFT2S76A+T132A) promoted the percentage of flowering plants, which was especially evident for ScFT2S76A+T132A but such promotion was not observed when expressing the three phosphomimetic mutants (ScFT2S76D, ScFT2T132D or ScFT2S76D+T132D) (Fig. 6f). Immunoblotting assays showed that ScFT2, ScFT2S76A, ScFT2T132A and ScFT2S76A+T132A accumulated to similarly high levels in the tobacco plants, but the amounts of ScFT2S76D, ScFT2T132D and ScFT2S76D+T132D were very low (Fig. 6g). Hence, alteration of the conserved S76 and T132 residues affected the ability of ScFT2 to control plant flowering, which was associated with altered ScFT2 protein stability. To our knowledge, previous studies have not documented FT phosphorylation and its impact on flowering-time control. Our finding provides a new avenue to more comprehensively explore the molecular and biochemical mechanisms underlying the control of plant flowering by FT proteins.

We further investigated the expression of the photoperiod (Ppd) gene, which positively regulates FT expression under long-day conditions43-44. One gene expressing Ppd, ScPpd1 (ScWN201G043000), was found in the transcriptome analysis of Weining and Jingzhou plants (Extended Data Fig. 8). This gene was expressed very early in Weining rye, with the peak of expression detected at 2 DAS; in contrast, ScPpd1 expression occurred relatively late in Jingzhou plants and peaked at 4 DAS (Fig. 6h). In line with the involvement of the product of ScPpd1 in regulating heading date, we detected a major quantitative trait locus (QTL) (Hd2R) with an logarithm of the odds (LOD) score of 8.19, explaining 12.16% of the heading date variation, in the chromosomal region harboring ScPpd1 using the F2 population of Weining×Jingzhou plants (Fig. 6i and Supplementary Data 1). The same analysis also identified another two heading date QTL, Hd5R and Hd6R, located on chromosomes 5R and 6R, respectively (Fig. 6i). Hd2R, Hd5R and Hd6R together explained 33.63% of phenotypic variance, with the alleles from Weining rye exhibiting earliness additive effects (Supplementary Data 1). The identification of Hd2R, Hd5R and Hd6R is in line with the discovery of heading date QTL on chromosomes 2R, 5R and 6R in previous studies43-44.

Mining of chromosomal regions and loci potentially involved in rye domestication. Analysis of domestication genes can accelerate rye domestication. In line with our findings, a recent study discovered the selection of flowering-time genes during soybean domestication, and it was suggested that domestication selection of flowering-time genes may allow proper adjustment of crop maturity and thus better adaptation to the growth environment51. However, considering that the ScID1-containing 6R region identified by all three methods is quite large (~12 Mb, Supplementary Data 5), further work is needed to verify whether the ScID1 locus might indeed function in heading date control and be selected during rye domestication.

Discussion

Through the complementary sets of analyses described above, we generated new insights into the genomic characteristics of rye and its genes involved in agronomic trait control, identifying potentially useful chromosome regions and loci for further studies of the genetic basis of rye domestication. Therefore, the Weining genome assembly is of high value for deciphering rye genome biology, deepening comparative cereal genomic research and accelerating the genetic improvement of rye and related cereal crops.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41588-021-00808-z.

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References

1. Martis, M. M. et al. Reticulate evolution of the rye genome. Plant Cell 25, 3685–3698 (2013).
2. Bauer, E. et al. Towards a whole-genome sequence for rye (Secale cereale L.). Plant J. 89, 853–869 (2017).
3. Bartlomiej, S., Justyna, R.-K. & Ewa, N. Bioactive compounds in cereal grains—occurrence, structure, technological significance and nutritional benefits—a review. Food Sci. Technol. Int. 18, 559–568 (2012).
4. Crespo-Herrera, L. A., Garkava-Gustavsson, L. & Åhman, I. A systematic review of rye (Secale cereale L.) as a source of resistance to pathogens and pests in wheat (Triticum aestivum L.). Hereditas 154, 14 (2017).
5. Schreiber, M., Himmelbach, A., Börner, A. & Mascher, M. Genetic diversity and relationship between domesticated rye and its wild relatives as revealed through genotyping-by-sequencing. Evol. Appl. 12, 66–77 (2019).
6. Singh, R. P. et al. Disease impact on wheat yield potential and prospects of genetic control. *Annu. Rev. Phytopathol.* 54, 303–322 (2016).

7. Zhu, F. Triticale: nutritional composition and food uses. *Food Chem.* 241, 468–479 (2018).

8. Flavell, R. B., Bennett, M. D., Smith, J. B. & Smith, D. B. Genome size and the proportion of repeated nucleotide sequence DNA in plants. *Biochem. Genet.* 12, 257–269 (1974).

9. Bartoš, J. et al. A first survey of the rye (*Secale cereale*) genome composition through BAC end sequencing of the short arm of chromosome 1R. *BMC Plant Biol.* 8, 95 (2008).

10. International Wheat Genome Sequencing Consortium (IWGSC). et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361, eaar7191 (2018).

11. Ling, H. Q. et al. Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. *Nature* 557, 424–428 (2018).

12. Luo, M. C. et al. Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. *Nature* 551, 498–502 (2017).

13. Zhao, G. et al. The *Aegilops tauschii* genome reveals multiple impacts of transposons. *Nat. Plants* 3, 946–955 (2017).

14. Aveni, R. et al. Wild emmer wheat genome architecture and diversity elucidate wheat evolution and domestication. *Science* 357, 93–97 (2017).

15. Mascher, M. et al. A chromosome conformation capture ordered sequence of the barley genome. *Nature* 544, 427–433 (2017).

16. Maccarferri, M. et al. Durum wheat genome highlights past domestication signatures and future improvement targets. *Nat. Genet.* 51, 885–895 (2019).

17. Yang, M. Y., Ren, T. H., Yan, R. J., Li, Z. & Ren, Z. I. Diversity resistance to *Puccinia striiformis* f. sp tritici in rye chromosome arm 1RS expressed in wheat. *Genet. Mol. Res.***13**, 8783–8793 (2014).

18. Ren, T. et al. Molecular cytogenetic characterization of novel wheat-rye T1RS.1BL translocation lines with high resistance to diseases and great agronomic traits. *Front. Plant Sci.* 8, 799 (2017).

19. Rabanus-Wallace, M. T. et al. Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential. *Nat. Genet.* https://doi.org/10.1038/s41588-021-00807-0 (2021).

20. Doležel, J., Čížková, J., Šimková, H. & Bartoš, J. One major challenge of the barley genome. *Nat. Genet.* 46, 527–532 (2014).

21. Doležel, J., Čížková, J., Simková, H. & Bartoš, J. One major challenge of sequencing large plant genomes is to know how big they really are. *Int. J. Mol. Sci.* 19, 3554 (2018).

22. Ou, S., Chen, J. & Jiang, N. Assessing genome assembly quality using the LTR Assembly Index (LAI). *Nucleic Acids Res.* 46, e128 (2018).

23. Wu, Z. et al. De novo genome assembly of *Oryza granulata* reveals rapid genome expansion and adaptive evolution. *Common. Biol. Inf.* 1, 84 (2018).

24. Piegue, B. et al. Doubling genome size without polyploidization: dynamics of retrotransposition-driven genomic expansions in *Oryza australiensis*, a wild relative of rice. *Genome Res.* 16, 1262–1269 (2006).

25. Lee, J. et al. Rapid amplification of four retrotransposon families promoted speciation and genome size expansion in the genus *Panax* Gen. *Sci. Rep.* 7, 9045 (2017).

26. Middleton, C. P. et al. Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticaceae tribe. *PLOS ONE***9**, e85761 (2014).

27. Chalupská, D. et al. Acc homoeologs and the evolution of wheat genomes. *Proc. Natl Acad. Sci. USA***105**, 9691–9696 (2008).

28. Salje, J. et al. Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *Plant Cell***20**, 11–24 (2008).

29. Jiao, Y., Li, J., Tang, H. & Paterson, A. H. Integrated syntenic and collinearity analyses reveal an ancient genome duplication in a 1.75-Mb genomic region harboring the α-gladin gene family in *Aegilops tauschii*, the source of wheat D genome. *Plant J.* 92, 571–583 (2017).

30. Zheng, Y. et al. tAK: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. *Mol. Plant***9**, 1667–1670 (2016).

31. Xie, Z., Nolan, T. M., Jiang, H. & Yin, Y. AP2/ERF transcription factor regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. *Front. Plant Sci.* 10, 228 (2019).

32. Duan, Y. B. et al. Identification of a regulatory element responsible for salt induction of rice *OsRAV2* through ex situ and in situ promoter analysis. *Plant Mol. Biol.* 90, 49–62 (2016).

33. Kourielis, J. & van der Heuven, R. A. L. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell***30**, 285–299 (2018).

34. Eshed, Y. & Lippman, Z. B. Revolutions in agriculture chart a course for targeted breeding of old and new crops. *Science***366**, eaaax025 (2019).

35. Huo, N. et al. New insights into structural organization and gene duplication in a 1.75-Mb genomic region harboring the α-gladin gene family in *Aegilops tauschii*, the source of wheat D genome. *Plant J.* 92, 571–583 (2017).

36. Zheng, Y. et al. tAK: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. *Mol. Plant***9**, 1667–1670 (2016).

37. Xie, Z., Nolan, T. M., Jiang, H. & Yin, Y. AP2/ERF transcription factor regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. *Front. Plant Sci.* 10, 228 (2019).

38. Duan, Y. B. et al. Identification of a regulatory element responsible for salt induction of rice *OsRAV2* through ex situ and in situ promoter analysis. *Plant Mol. Biol.* 90, 49–62 (2016).

39. Kourielis, J. & van der Heuven, R. A. L. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell***30**, 285–299 (2018).

40. Eshed, Y. & Lippman, Z. B. Revolutions in agriculture chart a course for targeted breeding of old and new crops. *Science***366**, eaaax025 (2019).

41. Kinoshita, E., Kinoshita-Kikuta, E., Kubota, Y., Takekawa, M. & Koike, T. The Sec-1 duplication in a 1.75-Mb genomic region harboring the α-gladin gene family in *Aegilops tauschii*, the source of wheat D genome. *Plant J.* 92, 571–583 (2017).

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Methods

Plant materials and fluorescence in situ hybridization assay. The Weining rye line used for genome analysis was selfed for 18 generations, and its genome was confirmed to carry seven pairs of chromosomes using fluorescence in situ hybridization (FISH; Supplementary Fig. 5). Jingzhou rye is another population variety cultivated in Hubei, China. Rye plants were grown under greenhouse conditions with day and night temperatures of 25°C and 20°C and a photoperiod consisting of light for 16h and dark for 8h. FISH assays of Weining rye root tip cells were accomplished as detailed previously using the fluorescently labeled probes pSCh19.2 and (AAC)15 (ref. ). The Weining × Jingzhou genetic population was prepared using Weining as the female parent. The F_2 individuals were cultivated on the experimental farm from early March to late June of 2018.

Genome sequencing. Thirty paired-end libraries (150bp) with insert sizes of ~270bp were constructed according to the manufacturer’s instructions. A total of 430 Gb of short reads was obtained for genome survey and polishing. PacBio sequencing libraries were constructed as recommended by Pacific Biosciences. DNA fragments of about 10–50 kb were selected using BluePippin electrophoresis. Next, libraries were constructed and sequenced on the PacBio Sequel system with P6-C4 chemistry. A total of 120 SMRT cells were sequenced, producing 497 Gb of raw data. Hi-C libraries were created using a previously described method . Six Hi-C fragment libraries, including five DpnII and one HindIII libraries, with fragment sizes ranging from 300 to 700bp, were constructed and sequenced on the Illumina X Ten platform. A total of 1,869,066,895 paired reads (560 Gb of raw data) were generated.

Genome assembly. For processing raw PacBio polymerase reads, sequencing adapters were removed, and reads with low quality and short length were filtered using the PacBio SMRT Analysis package with stringent parameters (readScore, 0.75; minSubReadLength, 500). The obtained 497 Gb of high-quality PacBio subreads were corrected using the error correction module embedded in Canu (version 1.5) with the parameter ‘correctedErrorRate’ set to 0.045. The corrected reads were used for contig assembly by wtdbg (https://github.com/ruanjue/wtdbg) with the ‘wtdbg-cns -m 64 -k 15’ setting, FALCON (version 0.2.2) with ‘wvlp_HPCalignment_option, -v -B128 -e96 -l2400 -s100 -k18 -h1024 -M8’ parameters and MACET (version 1.3) with the settings ‘corOutCoverage=60, corMhapSensitivity=high, correctedErrorRate=0.02’. The assembly results generated by the three assemblers were merged together using the Quickmerge (version 0.2) package with the ‘-hco 5.0 -c 1.5 -l 100000 -ml 500’ setting, using the wtdbg contigs as reference input. For contig polishing, the Illumina paired-end reads (430 Gb) from Weining rye were mapped to the initial contigs using BWA (version 0.7.10-r789); polishing was performed by Pilon (version 1.22) using at least three iterations with the parameter ‘--mindepth 10 --changes --fix bases’. This step corrected 2,171,903 SNPs, 359,604 insertions and 1,145,074 deletions. Subsequently, a pre-assembly was executed for the error-corrected contigs using Hi-C data. Briefly, adaptor sequences in raw Hi-C reads were trimmed with Cutadapt (version 1.0), and low-quality (over 10% N base pairs or Q10 < 50%) paired-end reads were removed, which resulted in 560 Gb of high-quality Hi-C data. Hi-C data were mapped using BWA with the aln method. The uniquely mapped reads with map quality > 20 were retained to perform assembly. Duplicate removal, sorting and quality assessment were performed with HIS-Pro (version 2.8.1) with the command ‘mapped_thc_segments.py -v -S + -100 -11000 -a -f -r -e’. The Hi-C links were aggregated in 50bp bins, normalized to recapitulate the Hi-C HC module was used to correct raw similarity search results to solve the overlap and fragmental problems of TE predictions and to reconstruct nested TEs. The families within each superfamilies were classified using the 80–80–80 rule . For LTR-RTs, the families were clustered based on their LTR sequences. The final set of repetitive sequences in the Weining rye genome was obtained by integrating the ab initio-predicted TEs and those identified by homology through RepeatMasker. Intact LTR-RTs were identified and analyzed using the ‘LTR_retriever’ pipeline (Supplementary Note).

Annotation of protein-coding genes. De novo prediction, homology-based and transcriptome-based strategies were combined to identify and annotate protein-coding genes (Supplementary Fig. 3). To facilitate gene annotation, 25 transcriptomic datasets were generated for Weining rye by performing Illumina RNA-seq on leaf, stem, root and spike samples as well as on developing grain samples harvested at 10, 20, 30, 40 d after anthesis (Supplementary Table 1). The transcripts were assembled, followed by merging and removal of redundancy using HISAT (version 2.0.4) and StringTie (version 1.2.3). Concomitantly, two PacBio RNA-seq experiments were conducted for Weining rye with the Sequel platform using total RNA extracted from mixed organs or mixed grains (Supplementary Table 3). Two libraries with insert sizes ranging from 0.5 to 8 kb were constructed and sequenced, yielding 29 and 31 Gb of sequencing data, respectively. These data were processed using IsoSeq3. Circular consensus sequences were generated with the parameters ‘min_length 300, no_polish TRUE, min_passes 1, min_predicted_accuracy 0.8, max_length 15000’. The transcripts constructed from Illumina and PacBio transcriptome data were merged and aligned to the Weining genome using BLAT (identity ≥ 95%, coverage ≥80%) and coverage ≥95%; minSubReadLength, 500). The obtained 497 Gb of high-quality PacBio reads (430 Gb) from Weining rye were mapped to the initial contigs using BW A (version 0.2) package with the ‘-hco 5.0 -c 1.5 -l 100000 -ml 5000’ setting, using the wtdbg contigs as reference input. For contig polishing, the Illumina paired-end reads (430 Gb) from Weining rye were mapped to the initial contigs using BWA (version 0.7.10-r789); polishing was performed by Pilon (version 1.22) using at least three iterations with the parameter ‘--mindepth 10 --changes --fix bases’. This step corrected 2,171,903 SNPs, 359,604 insertions and 1,145,074 deletions. Subsequently, a pre-assembly was executed for the error-corrected contigs using Hi-C data. Briefly, adaptor sequences in raw Hi-C reads were trimmed with Cutadapt (version 1.0), and low-quality (over 10% N base pairs or Q10 < 50%) paired-end reads were removed, which resulted in 560 Gb of high-quality Hi-C data. Hi-C data were mapped using BWA with the aln method. The uniquely mapped reads with map quality > 20 were retained to perform assembly. Duplicate removal, sorting and quality assessment were performed with HIS-Pro (version 2.8.1) with the command ‘mapped_thc_segments.py -v -S + -100 -11000 -a -f -r -e’. The Hi-C links were aggregated in 50bp bins, normalized to recapitulate the Hi-C HC module was used to correct raw similarity search results to solve the overlap and fragmental problems of TE predictions and to reconstruct nested TEs. The families within each superfamilies were classified using the 80–80–80 rule . For LTR-RTs, the families were clustered based on their LTR sequences. The final set of repetitive sequences in the Weining rye genome was obtained by integrating the ab initio-predicted TEs and those identified by homology through RepeatMasker. Intact LTR-RTs were identified and analyzed using the ‘LTR_retriever’ pipeline (Supplementary Note).

Phylogeny and divergence time analysis. OrthoMCL (version 1.1.4) was used to identify single-copy orthologous genes conserved in Weining rye and nine other grasses (Os, Bd, Hv, Aet, Tu, Ta, Z. mays, S. bicolor and S. italica). For Ta, the three subgenomes were analyzed separately. All-versus-all BLASTP (E value < 1 x 10^-5) was performed, which led to the identification of 2,517 single-copy orthologous genes. MUSCLE was used to perform multiple alignment of deduced protein sequences, followed by construction of gene trees with BEAST version 2.5.1. The gene phylogenies were calibrated using a Bayesian relaxed clock, implemented in BEAST as previously reported with two priors: (1) the 8d stem node with a normal-distributed prior (44.4 ± 3.53 Ma) obtained from 17 fossil-calibrated analyses and (2) the Aet stem node with normally distributed calibration in the root of the tree (6.55 ± 0.22 Ma). Subsequently, DensiTree was used to generate a superimposed plot of ultrametric gene trees of the 2,517 orthologous genes. Genome divergence for each pair of diploid species or genomes was estimated by (5) the distribution of divergence times of the 2,517 orthologous genes under the multispecies coalescent model .

Synteny analysis between Weining rye and rice or common wheat. To identify syntic gene blocks between rye and rice, barley or common wheat subgenomes, all-against-all BLASTP (E value < 1 x 10^-10, top five matches) was performed for the HC gene sets of each genome pair. Syntetic blocks were defined as based on the presence of at least five synteny gene pairs using the MScXan package with default settings. The adjacent blocks were merged, and large syntic blocks, each with a size over 10Mb, were selected. These large syntic blocks were then used to deduce the chromosome evolutionary scenario of Weining rye as compared with the rice and to investigate the syntic relationships between Weining rye and common wheat.

Analysis of gene duplication. The ‘duplicate_gene Classifier’ program implemented in the MScXan package was employed to classify the HC genes
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For each gene, three biological replicates were analyzed per genotype per DAS time and Jingzhou plants. RT–qPCR assays were performed with the cDNA that was genes (Extended Data Fig. 8) were displayed using the R package pheatmap. quantification of the genes related to heading date. The expression patterns of these Table 11). The resultant transcriptomic data allowed identification and the leaf samples collected from Weining and Jingzhou plants at 4, 7 and 10 DAS, Illumina RNA-seq was conducted for Expression of heading date-related genes.

method and the JTT matrix-based model with 1,000 bootstraps. The phylogenetic tree was constructed using SSP sequences from Weining rye, Aet, Hv and common wheat, Aet and Hv, including those encoding high- and low-molecular weight glutenin subunits, α-, γ-, ω- and δ-gliadins or γ-, B-, C- and D-hordeins, were employed to search the Weining genome assembly using BLASTN (E value < 1 × 10−10) to identify rye SSPs orthologs (identity ≥70% and coverage ≥80%). To annotate the normalized counts of SBRG expression was calculated using Illumina RNA-seq data from root, stem, leaf, spike and developing grain samples the proteins were separated using 12% Phos-tag SDS–PAGE and detected by immunoblotting with the anti-ScFT antibody as described above. This antibody was employed to compare

polyclonal rabbit antibody specific for ScFT was raised using the peptide QLGRQTVYAPGWRQ, conserved in ScFT1 and ScFT2 (Supplementary QLGRQTVYAPGWRQ, conserved in ScFT1 and ScFT2 (Supplementary

immunoblotting with the anti-ScFT antibody as described above. The selective sweeps potentially related to rye domestication were investigated using DRI (Fr_awsix × fr_awsix), F2 and XP-CLR, following methods in previous studies. The π and F2 values were calculated in 5-Mb windows with 1-Mb steps using VCFtools. The mean and median numbers of SNPs in the 5-Mb sliding windows were 77.8 and 54, respectively (Supplementary Fig. 6c). XP-CLR scores between two populations were obtained using a window size of 0.1 Mb, a grid size of 100 kb and a maximum of 200 SNPs within a window; for the SNPs with a linkage disequilibrium r2 over 0.95, only one of the SNPs was used. Genetic positions of the SNPs were determined using the linkage map generated with the F2 population of the Weining × Jingzhou cross (Supplementary Data 1) by assigning the genetic distances between SNP markers and the likelihood score was calculated in 5-Mb sliding windows with 1-Mb steps across the genome. During scanning for selection sweeps, only the windows with five or more SNPs were used, with the top 5% outliers of the whole-genome rank chosen as initial selection sweep signals. The signals that were ≥1 Mb apart were merged together as a single selection sweep. Only the signals containing at least ten SNPs in the corresponding genomic region were kept as final sets of putative selection sweeps (Supplementary Data 5). To investigate the genes located in the putative selection sweeps, their orthologs in rice or barley were identified using MCScanX as described above. The functional information for syntenic rice genes was obtained from funRiceGenes (https://funricegenes.github.io/). Analysis of ScID1 is described in the Supplementary Note.

Statistical analysis. Spearman’s rank correlation coefficient test statistic was performed using the ‘cor.test’ function in R (parameters, method = ‘spearman’; exact = TRUE). Two-tailed Student’s t-tests were executed using ‘t.test’ in R (parameters, alternative = ‘two.sided’; paired = FALSE).

Reporting Summary. Further information on research design is available in the Natural Resource Reporting Summary linked to this article.

Data availability

The Weining rye genome assembly was deposited in NCBI GenBank under the accession number JADQCU000000000. The raw sequencing data were deposited in the NCBI Sequence Read Archive under the BioProject accession numbers PRJNA680931, PRJNA680499 and PRJNA679094. The assembly and annotation data were also submitted to the Chinese National Genomics Data Center (https:// bigd.big.ac.cn/) under the accession number GWHAYS100000000. The Weining rye genome assembly and annotation are additionally available from the Triticeae Multi-omics Center (http://wheatomics.sdau.edu.cn/). Source data are provided with this paper.

References

52. Cai, X. & Liu, D. Identification of a 1B/1R wheat-rye chromosome translocation. Theor. Appl. Genet. 77, 81–83 (1989).
53. Tang, Z. X., Yang, Z. J. & Fu, S. L. Oligonucleotides replacing the roles of repetitive sequences pASl, pScl192, pT3-55, pT71, CCS1, and pAWRC1 for FISH analysis. J. Appl. Genet. 55, 313–318 (2014).
54. Burton, J. N. et al. Chromosome-scale scaffolding of de novo genome assemblies based on cotranslation interactions. Nat. Biotechnol. 31, 1119–1125 (2013).
55. Sun, X. et al. SLAF-seq: an efficient method of large-scale de novo SNP discovery and genotyping using high-throughput sequencing. PLoS ONE 8, e58700 (2013).
56. Liu, D. et al. Construction and analysis of high-density linkage map using high-throughput sequencing data. PLoS ONE 9, e98555 (2014).
57. Wicker, T. et al. Impact of transposable elements on genome structure and evolution in bread wheat. Genome Biol. 19, 103 (2018).
58. Marcussen, T. et al. Ancient hybridizations among the ancestral genomes of bread wheat. Science 345, 1250992 (2014).
59. Kim, S. J. et al. Post-translational regulation of FLOWERING LOCUS T protein in Arabidopsis. Mol. Plant 9, 308–311 (2016).
60. Zheng, X. et al. Arabidopsis phytochrome B promotes SPA1 nuclear accumulation to repress photomorphogenesis under far-red light. Plant Cell 25, 115–133 (2013).
61. He, F. et al. Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. *Nat. Genet.* **51**, 896–904 (2019).

62. Chen, H., Patterson, N. & Reich, D. Population differentiation as a test for selective sweeps. *Genome Res.* **20**, 393–402 (2010).

63. Qi, J. et al. A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. *Nat. Genet.* **45**, 1510–1515 (2013).

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**Author contributions**

D.W., J.Y., K.Z. and Q.Y. conceived and designed the project. G.L. and C.J. performed intragenomic and comparative genomic analyses. X.L., G.L., J. Du, H.Z. and C.J. executed genome sequencing and assembly. T.R. and Z.R. developed and provided the initial seeds of the inbred clone of Weining rye and supplied the seeds of Jinzhou rye. L.W., H.H., X.H. and X.W.D. performed transcriptome assays and data analysis. K.Z., F.L. and G.L. developed the F1 genetic population and performed QTL analysis. Z.S., Z.Y. and J. Doležel performed the FISH experiment and estimated the genome size of Weining rye. K.Z., G.L., L.D. and F.L. analyzed SSPs and SBRGs. L.W., H.J., G.L., H.H., X.H., K.Z., Z.W., Y.L. and X. Zhao conducted heading date-related gene analysis. G.L. and H.J. analyzed disease-resistance genes. Z.X., S.Y., C.S., M.Z., G.S. and N.Z. maintained laboratory supplies and greenhouse conditions. X. Zheng and M.G. participated in gene model validation. G.L., D.W. and N.S. wrote the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Pipeline for constructing the seven chromosome assemblies of Weining rye. Multiple sequencing technologies and assembly strategies were applied to construct the genome sequence of Weining rye (Supplementary Tables 1–6). First, 497.01Gb of long reads (62x genome coverage) generated by single molecule real-time sequencing (Pacbio Sequel), together with 430Gb Illumina paired-end clean reads (54x genome coverage), were jointly used to assemble the genome into 63,244 contigs with an N50 size of 480.35 kilobases (kb). Second, six high-throughput chromatin conformation capture (Hi-C) libraries were constructed and yielded 1.87 billion clean paired-end reads, over 90% of which could be mapped to the initial assembly. This three-dimensional proximity information allowed us to correct, order, orientate and anchor PacBio contigs into super-scaffolds, with the seven largest ones selected as candidates of the seven chromosomes of Weining rye (Extended Data Fig. 2 and Supplementary Table 5), which were then subjected to gap filling with corrected PacBio reads and manual adjustment. Third, a genetic map (Extended Data Fig. 3), developed using 295 F2 individuals derived from crossing Weining rye with another rye variety (Jingzhou) (Supplementary Data 1), was used to assign the seven super-scaffolds onto chromosomes, which yielded the final 1R to 7R chromosome-scale assemblies. Approximately 96.02% of the assembly was covered with BioNano molecules (97x genome coverage), and a high consistency was found between the genome assembly of Weining rye and the BioNano genomic reads (Supplementary Fig. 1).
Extended Data Fig. 2 | Hi-C contact map of the seven assembled Weining rye chromosomes (1R - 7R). Abundant intrachromosomal contacts were observed. Interchromosomal contacts were also found but with much decreased intensity.
Extended Data Fig. 3 | Genetic linkage map of Weining × Jingzhou rye varieties. Genetic linkage map (WJ) developed using 295 F2 plants derived from the crossing of two rye varieties (Weining × Jingzhou) (a) and alignment between the WJ linkage map and the physical map of the seven assembled chromosomes of Weining rye (b). In (a) the SNP markers anchored were 2,662, and the total genetic distance covered was 843.8 cM.
Extended Data Fig. 4 | Evaluation of genome assemblies by LTR Assembly Index (LAI). The x-axes show the chromosomes of each genome. LAI scores, represented by the dots, were calculated using 3 Mb-sliding windows with 300-kb steps. The blue line indicates whole genome average of LAI score. Sc, Secale cereale (Weining rye); Os, O. sativa ssp. japonica (Nipponbare); Tu, T. urartu (G1812); Aet, Ae. tauschii (AL8/78); Hv, Hordeum vulgare (Morex); TaA, TaB and TaD, the A, B and D subgenomes of common wheat (Chinese Spring).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | SDS-PAGE analysis of seed storage proteins extracted from the mature grains of Weining rye. Four types of secalins, that is, HMW-secalins, 75k γ-secalins, ω-secalins, and 40k γ-secalins, were detected. HMW-secalins and 75k γ-secalins are encoded by Sec-3 and Sec-2, respectively, whereas ω-secalins and γ-secalins are specified by Sec-1 and Sec-4. The secalins exhibited anomalous electrophoretic mobilities in SDS-PAGE because of carrying highly repetitive, proline- and glutamine-rich motifs in their proteins (Supplementary Table 19). M, protein size marker (kDa); WN SSP, Weining rye seed storage protein. The data shown were reproducible in three independent experiments.
Extended Data Fig. 6 | Phylogenetic analysis of rye secalins and homologous SSPs in barley (Hv), Ae. tauschii (Aet) and the three subgenomes of Chinese Spring wheat (TaA, TaB and TaD). A typical neighbor joining phylogenetic tree showing five distinct clusters of prolamin seed storage proteins (SSPs), that is, HMW glutenins/secalins/hordeins, α-gliadins, γ-gliadins/secalins/hordeins, ω-gliadins/secalins, and LMW-GSs/B-hordeins. The GenBank accession numbers for the compared SSPs are listed in Supplementary Table 20.
Extended Data Fig. 7 | Distribution patterns of different types of predicted disease resistance associated (DRA) genes along the seven assembled chromosomes of Weining rye (1R-7R). The number of DRA genes on each chromosome is written in blue. In addition, there were 80 DRA genes found in the unassigned scaffolds, bringing the total number of DRA genes to 1,989 (see Supplementary Data 3 for a full list of the 1,989 DRA genes).
Extended Data Fig. 8 | Different expression patterns of the genes related to the genetic control of heading date (flowering time) in Weining (WN, early heading) and Jingzhou (JZ, late heading) rye plants. These differentially expressed genes were identified using the transcriptome analysis data (Supplementary Data 4). 4, 7, and 10 indicate days after sowing.
Extended Data Fig. 9 | Investigation of the molecular mass of ScFT protein and evidence for its phosphorylation. (a) Immunoblotting detection of ScFT in Weining rye leaf tissues at 7 days after sowing. Total leaf proteins (10 μg) were separated using 12% SDS-PAGE, followed by immunoblotting with an antibody against the peptide QLGRQTVYPWQRQ conserved in ScFT1 and ScFT2. Based on the protein size markers, the ScFT protein detected (asterisk) had a molecular mass of ~29 kDa. (b) Detection of ScFT phosphorylation using Phos-tag SDS-PAGE coupled with immunoblotting. Total leaf proteins (20 μg), with or without a treatment by λ protein phosphatase for 30 min at 30 °C, were separated using 12% Phos-tag SDS-PAGE, followed by immunoblotting with ScFT specific antibody. A ScFT phosphoprotein band (arrow) was specifically detected for the sample that was not treated by λ protein phosphatase. The data shown were reproducible in three independent experiments.
Extended Data Fig. 10 | Evolutionary analysis of SciD1.1 and SciD1.2. (a) Alignment of the deduced amino acid sequences of SciD1.1 (ScWN6R01G057200), SciD1.2 (ScWN6R01G057300) and their orthologs in rice (RDI1, LOC Os10g28330), maize (ID1, Zm00001d032922), barley (HORVU4Hr1G043540), T. urartu (TuG1812G060001328), Ae. tauschii (AET6Gv20332200), and the three subgenomes of common wheat (TJrasCS6A02G126000, TJrasCS6B02G154000, and TJrasCS6D02G16300). The nuclear localization signal (NLS), the four zinc-finger domains (ZF1 to ZF4) and the ‘TRDFLG’ motif are labeled. The residues are color coded according to their conservancy. (b) Phylogenetic tree of ID1 proteins from representative grasses as inferred using the Maximum Likelihood method and a JTT matrix-based model in MEGA X website. The tree with the highest log likelihood (−2146.66) is shown. The bootstrap values (1000 times) are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 334 positions in the final dataset.
Reporting Summary

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No software was used in data collection.

Data analysis

Canu (version 1.5), WTDDBs (https://github.com/ruanjue/wtdbg), FALCON (version 0.2.2), MECAT (version 1.3), Quickmerge (version 0.2), Pilon (version 1.22), Cutadapt (version 1.0), HIC-Pro (version 2.8.1), LACHESIS (https://github.com/shendurelab/LACHESIS), BWA (version 0.7.10-r789), GATK (version 3.3.0), SAMtools (version 1.9), VCFtools (version 0.1.13), HighMap (http://highmap.biomarker.com.cn/), RefAligner (6700.6902rel), BLASTN (version 2.7.1), BLASTP (version 2.7.1), R (version 3.5.1), BUSCO (version 3.0.2), LTR_retriever (version 2.8), RepeatScout (version 1.0.5), LTR-FINDER (version 1.0.5), MITE-hunter (http://target.iplantcollaborative.org/mite_hunter.html), PILER-DF (version 2.4), Repbase (version 19.06), REPET (version 2.5), CLARITE (https://github.com/daron/CLARI-TE), RepeatMasker (version 1.332), LTRharvest (version 1.5.10), IsoSeq3 (version 3.4.0), GeneWise (version 2.4.1), GeMoMa (version 1.3.1), Gensc (http://hollywood.mit.edu/GENSCinfo.html), Augustus (version 2.4), GlimmerHMM (version 3.0.4), Hsatin (version 2.0.4), Stringtie (version 1.2.3), BLAT (version 350), PASA (version 2.0.4), TopHat (version 2.0.13), Cufflinks (version 2.2.1), Transdecoder (version 2.0), EvidenceModeler (version 1.1.1), GO (Release 20180910), KEGG (Release 87.0), Swiss-Prot (Release 2015_01), TR EM BL (Release 2015_01), trNAscan-SE (version 2.0), miRBase (Release 22), miRDeep2 (https://github.com/rajewsky-lab/mirdeep2), Infernal (version 1.1.2), OrthoMCL (version 1.1.4), MUSCLE (version 3.8.31), BEAST (version 2.5.1), DensiTree (version 2.2.5), MCScanX (http://chibba.pgm.uga.edu/mcscan2/), MScanX (Python version,https://github.com/tanghaibao/cjvc/wiki/MCscan-(Python-version)), iTAK (version 1.7a), RGAu gury (https://bitbucket.org/yannpc/gaugury/wiki/Home), isoCon (version 1.0), MEGAGX (https://www.megasoftware.net/), iTOL (https://itol.embl.de/), R/qtl (version 3.5.3),XP-CLR (version 1.0),funRiceGenes (https://funricegenes.github.io/),DupGen_finder (version 1.0),LTR_FINDER_parallel (https://github.com/oushujun/LTR_FINDER_parallel), pheatmap (version 1.0.12),R (version 3.5.1).

All codes used to generate the results reported in the study are available upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
The Weining rye genome assembly has been deposited in NCBI GenBank under the accession number JADQCU000000000. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) under the BioProject accession numbers PRJNA680931, PRJNA680499 and PRJNA679094. The assembly and annotation data have also been submitted to the Chinese National Genomics Data Center (https://bigd.big.ac.cn/) under the accession number GWHASIY00000000. The Weining rye genome assembly and annotation are additionally available at the Triticeae Multi-omics Center (http://wheatomics.sdau.edu.cn/). Source data are provided with this paper.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical methods were used to determine sample size. We incorporated a population of 295 F2 individuals derived from Weining and Jingzhou rye varieties to assist genome assembly and validation and to detect major QTLs for heading date. A genome wide genotyping-by-sequencing data set of 101 accessions of domesticated rye and wild Secale forms was employed for SNP calling and selection sweep analysis.

Data exclusions

No data were excluded from analysis. Raw sequencing data was quality filtered as described in manuscript.

Replication

Three replicates were executed for the qRT-PCR and immunoblotting experiments presented in Figure 6, Figure 7, Extended Data Figure 5, Extended Data Figure 9, and Supplementary Figure 5. All attempts at replication were successful. Other experiments not mentioned here were also reproducible over at least three separate trials.

Randomization

Plants were randomly allocated in the greenhouse and in the field.

Blinding

Blinding was not relevant for this study and phenotypic data were collected without knowledge of passport records or genetic data.

Reporting for specific materials, systems and methods

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- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Antibodies

A polyclonal antibody specific for rye FT protein was raised in rabbit using the peptide QLGRQTVYAPGWRQ conserved in ScFT1 and ScFT2 amino acid sequences by HuaBio (HuaAn Biotechnology Co., Ltd, Hangzhou, China, https://www.huabio.com). This antibody was used (in 1:2000) to detect the FT protein in rye leaf tissues by immunoblotting. As this antibody was self-made, it had no catalog number. The secondary antibody goat anti-Rabbit IgG H&L (IRDye® 800CW) was purchased from Abcam (https://www.abcam.com, Shanghai, China) and used in 1:5000 dilution in immunoblotting.

Validation

The peptide antibody was validated by its specific recognition of an in vitro expressed rye FT protein. Furthermore, a highly
similar peptide [QGRQTYAPGWRO] has been used successfully to raise an antibody specific for Arabidopsis FT protein (Kim et al., 2016. Post-translational regulation of FLOWERING LOCUS T protein in Arabidopsis. Molecular Plant 9, 308-311).