Grain Disarticulation in Wild Wheat and Barley

Mohammad Pourkheirandish*1,2 and Takao Komatsuda2

1 School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Royal Parade, Parkville, Melbourne, VIC, 3010, Australia
2 Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, Shandong 250100, China

*Corresponding author: E-mail, mohammad.p@unimelb.edu.au

(Received 28 January 2022; Accepted 4 August 2022)

Our industrial-scale crop monocultures, which are necessary to provide grain for large-scale food and feed production, are highly vulnerable to biotic and abiotic stresses. Crop wild relatives have adapted to harsh environmental conditions over millennia; thus, they are an important source of genetic variation and crop diversification. Despite several examples where significant yield increases have been achieved through the introgression of genomic regions from wild relatives, more detailed understanding of the differences between wild and cultivated species for favorable and unfavorable traits is still required to harness these valuable resources. Recently, as an alternative to the introgression of beneficial alleles from the wild into domesticated species, a radical suggestion is to domesticate wild relatives to generate new crops. A first and critical step for the domestication of cereal wild relatives would be to prevent grain disarticulation from the inflorescence at maturity. Discovering the molecular mechanisms and understanding the network of interactions behind grain retention/disarticulation would enable the implementation of approaches to select for this character in targeted species. Brittle rachis 1 and Brittle rachis 2 are major genes responsible for grain disarticulation in the wild progenitors of wheat and barley that were the target of mutations during domestication. These two genes are only found in the Triticeae tribe and are hypothesized to have evolved by a duplication followed by neo-functionalization. Current knowledge gaps include the molecular mechanisms controlling grain retention in cereals and the genomic consequences of strong selection for this essential character.

Keywords: Brittle rachis • Domestication • Neo-functionalization

Introduction

Plant domestication was a major pillar in the transition from hunter-gatherer to agriculture-based societies. Domesticated crop species underwent a series of morphological changes that today enable farmers to sustain a population of over 7.2 billion people. As a part of international efforts to bring about a second Green Revolution with a focus on low input and high nutritional output, the domestication of new crops is being considered (Osterberg et al. 2017). Understanding the fundamental morphological changes that historically enabled crops to become domesticated will help to improve our future crops to feed an expanding world population in an era of climate change (Pourkheirandish et al. 2020). Perhaps the most critical event in the process of cereal domestication was the loss of the natural mode of grain dispersal (Salamini et al. 2002). All wild plant species have grain dispersal mechanisms; however, the separation position of grain from the inflorescence varies among different genera (Doust et al. 2014). Grains from wild cereals such as wheat and barley progressively break off along the inflorescence structure, the spike, and scatter on the ground as the plant senesces and dries. During this process, the central axis of the inflorescence, the rachis, becomes brittle and snaps; the wild-type phenotype is therefore referred to as ‘brittle rachis’ (Fig. 1). One of the first steps in domesticating a grain-producing plant was selection against grain shattering. Early farmers naturally selected plants that had lost this trait and retained the grain on spikes, making grains easy to harvest.

Wheat and barley with a stiff (non-brittle) spike have been selected independently several times by early farmers who settled east of the Mediterranean Sea. In barley, independent mutations in two genes responsible for grain disarticulation, called Non-brittle rachis 1 and Non-brittle rachis 2 (brt1, brt2), have been selected during the process of domestication (Takahashi and Yamamoto 1949; Pourkheirandish and Komatsuda 2007). Both functional genes are required for disarticulation. Mutation in either gene can cause cell wall thickening in the grain separation zone, making the rachis non-brittle so as to retain grain (Pourkheirandish et al. 2015). It has been demonstrated that orthologous genes control grain dispersal in wheat (Avni et al. 2017, Pourkheirandish et al. 2018, Nave et al. 2019). These two genes’ orthologs are absent in Arabidopsis, rice and even maize and only recently evolved in a tribe of cereals including wheat and barley, Triticeae. However, the molecular mechanism conditioning the process of grain separation from the spike for brittle rachis genotypes and the role of BTR1 and BTR2 proteins is not well understood.
Fig. 1 Stable harvest is a key factor in crop domestication. (A) In wild rice with panicle inflorescence, seeds break above the glume and shatter at maturity where all rachis remain on the panicle. Non-shattering trait was selected during rice domestication. (B) In wild barley and wheat with spike inflorescence, grain is separated from spike with a rachis segment attached and falls to the ground. The trait is called the brittle rachis. Domesticated barley and wheat retain the grain on the spike, and the whole spike can be easily harvested, also known as non-brittle rachis.

Paralogs of Btr1 and Btr2 were discovered in wheat and barley named Btr1-like and Btr2-like genes. The Btr genes are limited to certain cereal species closely related to wheat and barley, whereas Btr-like genes are present more widely in grass family (Zeng et al. 2020). The biological and molecular functions of Btr-like genes were investigated to infer Btr function and divergence (Cross et al. 2022). This study suggested that Btr and Btr-like genes result from a segmental duplication. Btr-like genes conserved the original function of cell wall development in immature anthers, whereas Btr genes gained a different role related to the cell wall development at the immature rachis nodes, resulting in a novel mechanism of grain disarticulation.

In this review, we aim to leverage the original discoveries of genes controlling grain dispersal (brittle rachis) in wheat and barley to investigate the gene network and suggest further studies to find underlying molecular mechanisms controlling this phenotype crucial to evolution and domestication. Understanding the molecular genetics of grain retention will not only yield fascinating insights into a plant trait that supports human civilization and survival but will also underpin efforts to improve crops through introgression of useful genes from wild species and in the domestication of entirely new cereal crops.

Brittle Rachis Evolved in the Triticeae Tribe

An inflorescence is a cluster of flowers arranged on a stem (rachis). Grass inflorescences are developmentally diverse. Inflorescence branching depends on the developmental fate of the axillary apical meristem and produces various patterns in different species. The final architecture of the inflorescence is the overall sum of the total number of meristems and their arrangement. It is believed that the panicle (a highly branched inflorescence as in rice) is a primitive form from which the spike (an unbranched inflorescence as in wheat) evolved (Judd et al. 1999) (Fig. 1). In wild wheat and barley species, upon maturity, the individual units of the spike break at the rachis between the spikelets and disperse the grain with a segment of the rachis, called the brittle rachis. While a brittle rachis is perfectly suited to optimize grain dispersal in nature, picking up individual grains from the ground makes harvesting difficult and is not desirable in agriculture. In contrast, mature grain
from domesticated cereals is harvested easily and efficiently because the rachis is non-brittle, and the grain remains attached (Fig. 1).

The Triticeae, the wheat tribe of grasses, includes economically important crops such as barley, rye, wheat and their wild relatives (Fig. 2) (Devos 2005, Bernhardt et al. 2017). This tribe harbors more than 350 species (30 genera). Bread wheat is an allopolyploid species that originated by interspecific hybridization of three different diploid species and subsequently contains three ancestral genomes termed; A, B and D. Two of the progenitor species (A and B genomes) exhibit a brittle rachis at maturity, like wild barley. Wild Hordeum (barley), Triticum (wheat), Secale (rye), Taeniatherum and Thinopyrum have spike inflorescences with brittle rachis disarticulation (Fig. 2). There are also several that have spike inflorescences with brittle ‘rachilla’ disarticulation genera, including Australopyrum, Anthosachne, Elytrigia, Elymus and Leymus. The mechanism of grain disarticulation driven by Btr1 and Btr2 is only found in the Triticeae tribe (Sakuma et al. 2011). The exclusive inflorescence structure (spike), unique separation mechanism (brittle rachis) and newly evolved genes (Btr1/Btr2) are common properties only found among the Triticeae (Zeng et al. 2020). These factors collectively support the hypothesis that the brittle rachis evolved relatively recently within the Triticeae tribe.

The non-brittle rachis results from a mutation in either Btr1 or Btr2 in barley. These non-brittle types have arisen at least two times in barley of either a 1-nucleotide deletion at the promoter region of Btr1 or a 11-nucleotide deletion at Btr2 coding sequence resulting in frameshift and a 11-nucleotide deletion at the promoter region of Btr1-B in tetraploid wheat (Avni et al. 2017, Nave et al. 2019).

How Do Btr1 and Btr2 Control Cell Wall Thickness in the Separation Layer?

Previous studies have shown that functional BTR1 and BTR2 proteins are required for disarticulation of spikelets from the spike in both wheat and barley (Pourkheirandish et al. 2015, 2018). However, we do not know ‘how’ they control this process. We know that they promote the formation of thinner cell walls in a so-called separation layer, but we do not understand why or how this is elaborated. However, cytological observations suggest a reprogramming of local primary and/or secondary cell wall formation. Why this occurs only in the cells of the separation layer is unknown. The in silico analysis predicted that BTR1 is a membrane protein containing two transmembrane helices while BTR2 was cytoplasmic (Pourkheirandish et al. 2015). Based on these subcellular locations, we can hypothesize that BTR1 and BTR2 potentially encode a receptor–ligand pair constituting key components of a signal transduction pathway that initiates the highly localized cellular response (Fig. 3A). While this model is likely, we have no ‘proof’ of their subcellular location or whether the receptor–ligand hypothesis is correct. Furthermore, the upstream signal(s) that promote localized, temporal Btr1/2 expression, restricting the control of cell wall thickness strictly to the separation layer, and the consequences of the signal transduction response operating through Btr1/2 are unknown.

Brittle rachis is a unique character that evolved in Triticeae; thus, it is necessary to use a Triticeae member such as wheat or barley to study the function of Btr1 and Btr2 genes (Kellogg 2021). These materials will allow direct comparisons between plants that show the brittle rachis phenotype and plants that do not, in the same genetic background (Pourkheirandish et al. 2015) that can illuminate the molecular function of brittle
A split ubiquitin yeast two-hybrid assay would test for interactions between the membrane protein BTR1 and its putative ligand BTR2 (Li et al. 2016). Transgenic barley plants expressing BTR1 and BTR2 proteins with fluorescent tags can be used to validate the yeast two-hybrid results in a native environment. Switch from non-brittle to brittle phenotype in transgenic lines will confirm the biological function of the target proteins. These will allow both functionality and localization to be investigated further. An overlapping signal will be expected if and where the two proteins co-localize. The underlying assumption is that the fluorescent tag does not interfere with protein complex formation and that it can be tested via phenotypic complementation. In the case of fusion protein interference, peptide antibodies can be raised against BTR1 and BTR2 to reveal the subcellular location of the respective proteins.

Co-localization of BTR1 and BTR2 will support the hypothesized molecular function of each protein and confirm their interactions. Finding other proteins involved in the brittle rachis phenomena will shed light on the molecular function of the grain disarticulation in spike. Even though BTR1 and BTR2 are specific to Triticeae, the cell wall thickness adjustment at the separation zone is part of a molecular pathway that can be specific to Triticeae or, more generally, exist in other plant species (Gorskhova et al. 2018, Anderson and Kieber 2020). Potential protein partners involved in this pathway should enable us to map these unique proteins in a functional context within the cell and engineer the pathway to open new research opportunities.

The expression of Btr1 and Btr2 has been detected in the developing barley inflorescence (white anther stage) using quantitative PCR (qPCR), and by using RNA in situ hybridization, Btr2 mRNA has been detected in the abscission zone primordia (Pourkheirandish et al. 2015). However, the Btr1 in situ hybridization was not successful in barley. It is unknown whether the expression of both genes was restricted to the separation layer or whether the separation layer was a region of overlap between wider expression domains of both genes. In Aegilops longissima, one of the wild relatives of wheat, Btr1 was expressed broadly along nodes and internodes, rather than nodes specifically as observed in Btr2 (Zeng et al. 2021). The subcellular protein localization will reveal further hints associated with their molecular operation. Immunohistochemistry can be used to characterize the BTR1 and BTR2 protein localization in wild barley. Transgenic materials mentioned above can be used to validate protein localization.

Based on the receptor–ligand hypothesis, BTR1 and BTR2 will likely exert their effect by triggering a developmental response that leads to altered cell wall structure in the disarticulation zone. Comparing expression profiles between brittle and non-brittle should elaborate on possible downstream genes activated by the BTR1–BTR2 signaling pathway. RNA sequencing of immature spike samples based on the expression time of the Btr2 gene between the wild barley OUH602 (brittle type) compared to its induced mutant M96-1 (non-brittle) will identify potential downstream targets of Btr1/Btr2. This will

The Brittle Rachis Signaling Pathway

Both BTR1 and BTR2 must be functional for grain disarticulation to happen. Given these two proteins’ structural predictions, it is hypothesized that BTR1 and BTR2 physically interact, potentially forming a receptor::ligand pair. The protein–protein interaction hypothesis will demonstrate the molecular interaction between BTR1 and BTR2 and can be tested using complementary approaches to determine if the two proteins directly interact to trigger a common signaling pathway or work independently but are still involved in a shared signaling network (Lampugnani et al. 2018, Zheng et al. 2019). A split ubiquitin
reveal genes triggered by the reconstituted BTR1/BTR2 protein complex. These downstream targets will help understand the BTR initiation and phenotype signaling pathway.

Every genetic study has reported the Btr locus located on the short arm of chromosome 3 as major genes controlling brittle rachis in barley and wheat. Minor quantitative trait loci (QTL) affecting the brittle rachis trait independent of the Btr locus were also detected in different genetic investigations (Komatsuda and Mano 2002, Kandemir et al. 2004, Komatsuda et al. 2004). Some of these independent loci may act as a molecular partner of Btr genes, and they may operate in the brittle rachis development pathway. The genomic location of historical QTL discovered in association with brittle rachis can be instrumental in studying the molecular mechanism of grain disarticulation.

### What are the Physical and Compositional Changes in the Walls of Cells that Promote Disarticulation?

It has been demonstrated that changes in cell wall thickness are strongly associated with grain dispersal in barley at maturity (Pourkheirandish et al. 2015) (Fig. 3A, B). Histochemical staining of rachis sections revealed that five to six cell layers expand above each rachis node in brittle lines (Btr1Btr2) but not in non-brittle types (btr1Btr2 or Btr1btr2). Fluorescence staining suggested a significant reduction in lignin content in the separation layer (abscission zone) below the rachilla node in species such as wild rice, Bromus spp. and Agropyron spp. that disarticulate below the rachilla node (Pourkheirandish et al. 2015). A follow-up study demonstrated lignin reduction at the rachis node in Aegilops tauschii that disarticulate below the rachis node (Zeng et al. 2020a). No lignin reduction was detected in wild barley. It remains to be examined whether the thinning of the cell wall at the separation zone in wild barley is due to (i) the regulation of primary and/or secondary wall biosynthesis or (ii) changes to specific cell wall components that alter the overall composition of this region. These fundamental questions about whether a specific component is missing in the separation zone or a simple proportional reduction in cell wall thickness responsible for rachis breakage will provide clues about genes involved in the brittle rachis process.

Plant cells are surrounded by a wall of rigid extracellular fibrils embedded in an amorphous matrix. Cell walls typically occur in two forms: a flexible primary wall while the cell is growing and a secondary wall that increases the strength of the wall and reduces flexibility (Evert and Eichhorn 2006). The primary wall has a high matrix polysaccharide content with cellulose but lacks lignin, whereas secondary wall thickening includes depo-sitions of crystalline celluloses, heteroxylans and lignins (Evert and Eichhorn 2006).

Cell wall formation and subsequent modifications in the developing barley spike can be monitored by sampling at different developmental stages starting from the awn primordium stage when the spike is about 5 mm in length to full maturity (Kirby and Appleyard 1981). To observe and quantify cell wall thickness in a spatiotemporal manner comparing brittle and non-brittle types, transmission electron microscopy should be employed to image the cell wall layers at different stages. This fundamental experiment will show when cell wall thickness changes and enable a comparison of the separation zone and non-separation zones between wild type and mutants and the type of cell wall—primary, secondary or both—modified. The study will test the basic question of whether the thin cell wall results from the suppression of cell wall biosynthesis or is the consequence of degradation of developed walls similar to that observed in wild rice (Thurber et al. 2011).

The specific cell wall component changes between brittle (wild type) and non-brittle (induced mutant) types should be further examined. Fluorescence-based immunohistochemical studies using specific antibodies against cell wall polysaccharides, including arabinoxylan (LM11), xyloglucan (LM15), (1,3;1,4)-β-glucan (BG1), de-methylesterified pectin (LM19) and methylesterified pectin (LM20), as well as the cellulose-binding module (CBM3a) can be used at the rachis junction to investigate the wall composition in situ. This will provide a spatiotemporal map of where and when wall changes occur in the rachis junction.

### The Origin of Brittle Rachis

The Btr1 and Btr2 genes controlling grain disarticulation in wild wheat and barley are specific to Triticeae. How the Btr genes evolved and gained the brittle rachis function has been debated (Zeng et al. 2020, Cross et al. 2022). Basic Local Alignment Search Tool searches of the Btr1 and Btr2 sequences against the barley genome detected homology with two further hypothetical proteins termed BTR1-LIKE and BTR2-LIKE (Pourkheirandish et al. 2015). The genomic organization and orientation of Btr1/Btr2 and Btr1-like/Btr2-like gene pairs in barley and wheat are exactly the same (Pourkheirandish et al. 2018), suggesting that the configuration must already have been present in their common ancestor before the divergence of the wheat and barley lineages dating back to more than 8 million years (Middleton et al. 2014).

With more than 55% amino acid identity between BTR1 and BTR1-LIKE also BTR2 and BTR2-LIKE, they are not simply redundant. A functional Btr1-like gene cannot recover non-brittle rachis caused by mutation at the btr1 locus, and similarly, the mutated btr2 locus cannot be compensated by a functional Btr2-like gene (Pourkheirandish et al. 2015). Btr1-like/Btr2-like pairs are exclusively expressed at the immature anther stage (Cross et al. 2022). Interestingly, the expression time coincides with the Btr1/Btr2 expression, meaning that at the same immature spike stage, Btr1-like/Btr2-like is expressed at anther primordia, whereas Btr1/Btr2 is expressed at separation zone primordia. It is hypothesized that Btr1/Btr2 originated through a segmental duplication followed by divergence at the expression location. This hypothesis is demonstrated in Fig. 4. In situ hybridization of Btr1-like and Btr2-like are required to test
this hypothesis. According to this assumption, Btr1-like/Btr2-like preserved the original biological function related to anther development and Btr1/Btr2 gained a different biological function, which is grain disarticulation. The molecular function of BTR1-LIKE/BTR2-LIKE could be the same as BTR1/BTR2 proteins and the biological divergence could be just due to the shift of expression location. However, BTR1/BTR2 proteins may gain a different molecular function. BTR1/BTR2 may cooperate in dissolving a cell wall component essential for the rigidity of the wall at maturation of the rachis; BTR1-LIKE/BTR2-LIKE may cooperate similarly, but in the anther cell wall surrounding the microspore cells. In contrast, BTR1/BTR2 may interfere with the cell wall biosynthesis at the rachis junction, resulting in a thinner cell wall that can break easier at maturity, while BTR1-LIKE/BTR2-LIKE may act alike in cell wall wrapping microspore cells. Co-expression analysis suggested that Btr-like genes involved in the cell wall biosynthesis and control of cell wall development are synergetic with BTR1/BTR2 operation. Here we hypothesize that Btr and Btr-like molecular networks may overlap, but this requires further investigation. The brittle rachis mechanism evolved through a duplication followed by neo-functionalization, which is described in the case of other genes/traits (Pourkheirandish et al. 2007, Sakuma et al. 2010, Lye and Purugganan 2019).

Back to the Future: Re-domestication of Non-brittle Barley from Wild

Ancient farmers selected non-brittle wheat and barley due to the ease of harvest. Once these mutants were fixed, growers always selected against brittle rachis which has resulted in a strong bottleneck effect at these loci. Plant breeders often intercross domesticated crop species with their wild relatives to bring across one or a few small chromosomal segments from the wild species to the domesticated recipient to improve cultivars with desirable traits (He et al. 2019). However, a huge diversity and genetic potential of wild grasses are left behind (Pourkheirandish et al. 2020). The opposite procedure where the wild progenitor can be used as a recurrent parent with selection against domestication traits has recently been suggested (Langridge and Waugh 2019). In this way, we back to the original resources to re-domesticate barley or wheat, taking advantage of currently known domesticated genes. The knowledge generated by understanding the domestication process could be used to modify wild relatives of grain crops and forage species that are fit for specific environments. Many agronomically adapted forage species are commercially unavailable due to poor seed production characteristics. It will be possible to look for mutations within brittle rachis genes using reverse genetic approaches and to use undirected mutagenesis approaches in the wild species to find mutants that retain grain on the spike. Perennial crop development is an emerging field of research focused on improved environmental sustainability of crop production system. Intact rachis is an incredibly important trait for perennial crops and forages as their flowering time is indeterminant and the grain needs to be retained longer on the earlier maturing heads. There are two pathways to new crop development; hybridization and domestication. Examples of perennial wild grass species that can be hybridized with wheat are members of the Thinopyrum genus. Thinopyrum is well known for high biomass production and high protein content as well as cold and salt tolerance and disease resistance. Species within this genus have already been widely used to cross-hybridize with wheat to transfer chromosomal segments containing genes for these valuable characteristics into wheat (King et al. 1997, Liu et al. 2013, Kuzmanović et al. 2014). An example of a perennial wild grass that proved more difficult to hybridize with wheat is Elymus, which is well known for its drought tolerance that is critical for the Australian environment (Newell et al. 2015). In this case, domestication might be a better approach. An alternative approach would be genome editing, which was recently applied to re-domesticate wild tomato as a proof of concept (Li et al. 2018, Zsögon et al. 2018). However, it is important to mention that currently there are no transformation protocols for most
wild species even though there are efforts to overcome those barriers (Wang et al. 2022).

### Negative Consequences Associated with Selection for Grain Retention

Non-shattering was critical for barley domestication; as mutations in either Btr1 or Btr2 can result in grain retention, there were at least two domestication paths in barley. Despite the ability of mutations in either loci to result in grain retention, genetic diversity around the Btr1 and Btr2 loci is limited to one or two alleles at each locus (Pourkheirandish et al. 2015, Civan and Brown 2017). Low diversity around the Btr region is exceptional compared to other independent loci in barley. For example, six-row spike locus vrs1 has at least eight alleles, including two-row and six-row type results from several allele exchanges between wild and cultivated forms at this locus (Saisho et al. 2009). The bottleleneck effect at Btr loci can lead to two issues for barley improvement. Firstly, the introduction of novel diversity by crosses with wild barley has been successful for many regions and traits but has been impractical for the regions surrounding Btr due to strong selection for the mutant alleles. This probably hindered the introduction of beneficial alleles at linked loci. Secondly, negative traits associated with loci linked to the Btr loci remained fixed in cultivated barley. Several studies demonstrated QTL from wild barley in the vicinity of the Btr loci with positive effect in agronomic characteristics, including grain number per ear (Saade et al. 2016), grain elements such as B, K and Zn (Herzig et al. 2019), resistance against net blotch (Vatter et al. 2017) and microbiota composition in the rhizosphere (Escudero-Martinez et al. 2022). Other studies have indicated that linkage drag associated with key domestication or adaptive traits can significantly impact the overall crop performance (Dreissig et al. 2020).

It remains to be answered if selection for grain retention carries detrimental alleles through to cultivated barley. We know that btr1 and btr2 would have been under intense selection during domestication. They reside in a low-recombining region of the barley genome, potentially limiting the local diversity available to breeders (Pourkheirandish et al. 2015). Previously, we were able to discover recombination between Btr1 and Btr2 in a large segregating population (four recombinants out of 14,000 F2) (Pourkheirandish et al. 2015). Recombination also detected between Btr genes among the natural population of agricorithon barley that has a six-row and brittle phenotype (Pourkheirandish et al. 2018a). These indicate that recombination between and around Btr1 and Btr2 is possible but at a low frequency. Comparing these recombinants will assess the implication of fixation on genetic diversity and develop options to bring novel variations into cereal breeding programs.

### Data Availability

New data were not generated in this research.

### Funding

Australian Research Council Discovery Project (DP200102271 to M.P.); FY2020 Japan Society for the Promotion of Science (JSPS) Invitational Fellowships for Research in Japan (S20034 to M.P.); JSPS (18H02184 to T.K.).

### Acknowledgements

We thank T. Brown and R. Kutcher for reading the manuscript. M.P. acknowledges the ongoing support from the University of Melbourne, Faculty of Veterinary and Agricultural Sciences, Start up grant and Internal Foundations, and T.K. acknowledges Leading Talent Research Startup Fee of SAAS (CXC2021B01) and the Double hundred talent program of Shandong Province (WST2020012).

### Disclosures

The authors have no conflicts of interest to declare.

### References

Andersen, C.T. and Kieber, J.J. (2020) Dynamic construction, perception, and remodeling of plant cell walls. Annu. Rev. Plant Biol. 71: 39–69.

Arni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S.O., Gundlach, H., et al. (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357: 93–97.

Bernhardt, N., Brassac, J., Kilian, B. and Blattner, F.R. (2017) Dated tribe-wide whole chloroplast genome phylogeny indicates recurrent hybridizations within Triticeae. BMC Evol. Biol. 17: 161.

Civan, P. and Brown, T.A. (2017) A novel mutation conferring the nonbrittle phenotype of cultivated barley. New Phytol. 214: 468–472.

Cross, A., Li, J.B., Waugh, R., Golitz, A. and Pourkheirandish, M. (2022) Grain dispersal mechanism in cereals arose from a genome duplication followed by changes in spatial expression of genes involved in pollen development. Theor Appl Genet. 135: 1263–1277.

Devois, K.M. (2005) Updating the ‘Crop circle.’ Curr. Opin. Plant Biol. 8: 155–162.

Doust, A.N., Mauro-Herrera, M., Francis, A.D. and Shand, L.C. (2014) Morphological diversity and genetic regulation of inflorescence abscission zones in Grasses. Am. J. Bot. 101: 1759–1769.

Dreissig, S., Maurer, A., Sharma, R., Milne, L., Flavell, A.J., Schmutzer, T., et al. (2020) Natural variation in meiotic recombination rate shapes introgression patterns in intraspecific hybrids between wild and domesticated barley. New Phytol. 228: 1852–1863.

Escudero-Martinez, C., Coulter, M., Alegria Terrazas, R. et al. (2021) Identifying plant genes shaping microbiota composition in the barley rhizosphere. Nat Commun. 13: 3443.

Evert, R.F. (2006) Esau’s Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. New Jersey: John Wiley and Sons, Inc.

Gorshkova, T., Chernova, T., Mokshina, N., Ageeva, M. and Mikshina, P. (2018) Plant ‘muscles’: fibers with a tertiary cell wall. New Phytol. 218: 66–72.

He, F., Pasam, R., Shi, F., Kant, S., Keeble-Gagnere, G., Kay, P., et al. (2019) Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. Nat. Genet. 51: 896–904.
Herzig, B., Backhaus, A., Seiffert, U., von Wieren, N., Pillen, K. and Maurer, A. (2019) Genetic dissection of grain elements predicted by hyperspectral imaging associated with yield-related traits in a wild barley NAM population. *Plant Sci.*, 285: 151–164.

Judd, W.S., Campbell, C.S., Kellogg, E.A. and Stevens, P.F. (1999) Plant Systematics: A Phylogenetic Approach. Sinauer, Sunderland, Massachusetts.

Kandemir, N., Yildirim, A., Kudrna, D.A., Hayes, P.M. and Kleinhofs, A. (2004) Marker assisted genetic analysis of non-brittle rachis trait in barley. *Hereditas* 141: 272–277.

Kellogg, E.A. (2021) The rachis cannot hold, plants fall apart. A commentary on: The unique disarticulation layer formed in the rachis of *Aegilops longissima* likely results from the spatial co-expression of Btr1 and Btr2. *Ann. Bot.* 127: vi–vii.

King, J.P., Forster, B.P., Law, C.C., Cant, K.A., Orford, S.E., Gorham, J., et al. (1997) Introggression of salt-tolerance genes from *Thinopyrum bessarabicum* into wheat. *New Phytol.* 137: 75–81.

Kirby, E.J.M. and Appleyard, M. (1981) Cereal Development Guide. Cereal Research Centre, National Agricultural Centre, Stoneleigh, Kenilworth, Warwickshire, UK.

Komatsuda, T. and Mano, Y. (2002) Molecular mapping of the intermediate spike-c (int-c) and non-brittle rachis 1 (btr1) loci in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 105: 85–90.

Komatsuda, T., Maxim, P., Senthil, N. and Mano, Y. (2004) High-density AFLP map of nonbrittle rachis 1 (btr1) and 2 (btr2) genes in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 109: 986–995.

Kuzmanović, L., Gennaro, A., Benedettelli, S., Dodd, I.C., Quarrie, S.A. and Ceoloni, C. (2014) Structural-functional dissection and characterization of yield-contributing traits originating from a group 7 chromosome of the wheatgrass species *Thinopyrum ponticum* after transfer into durum wheat. *J. Exp. Bot.* 65: 509–525.

Lampugnani, E.R., Wink, R.H., Persson, S. and Somssich, M. (2018) The toolbox to study protein–protein interactions in plants. *Crit. Rev. Plant Sci.* 37: 308–334.

Langridge, P. and Waugh, R. (2019) Harnessing the potential of germplasm collections. *Nat. Genet.* 51: 200–201.

Li, J., Gao, J., Han, L. et al. (2016) Development of a membrane-associated ligand and receptor yeast two-hybrid system for ligand-receptor interaction identification. *Sci. Rep.* 6: 35631.

Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., et al. (2018) Domestication of wild tomato is accelerated by genome editing. *Front. Plant Sci.* 9: e85761.

Liu, W., Danilova, T.V., Rouse, M.N., Bowden, R.L., Friebe, B., Gill, B.S., et al. (2013) Development and characterization of a compensating wheat-*Thinopyrum intermedium* Robertsonian translocation with Sr44 resistance to stem rust (*Ug99*). *Theor. Appl. Genet.* 126: 1167–1177.

Iye, Z.N. and Purugganan, M.D. (2019) Copy number variation in domestication. *Trends Plant Sci.* 24: 352–365.

Middleton, C.P., Senerchia, N., Stein, A., Akhunov, E.D., Keller, B., Wicker, T., et al. (2019) Accelerating the domestication of new crops: feasibility and approaches. *Trends Plant Sci.* 22: 373–384.

Pourkheirandish, M., Dai, F., Sakuma, S., Kanamori, H., Distelfeld, A., Willcox, G., Kawahara, T., Matsumoto, T., Kilian, B. and Komatsuda, T. (2018) On the Origin of the Non-brittle Rachis Trait of Domesticated Einkorn Wheat. *Front. Plant Sci.* 8: 2031.

Pourkheirandish, M., Golicz, A.A., Bhalla, P.L. and Singh, M.B. (2020) Global Role of Crop Genomics in the Face of Climate Change. *Front. Plant Sci.* 11: 922.

Pourkheirandish, M., Hensel, G., Kilian, B., Senthil, N., Chen, G., Sameri, M., et al. (2015) Evolution of the grain dispersal system in barley. *Cell* 162: 527–539.

Pourkheirandish, M., Kanamori, H., Wu, J.Z., Sakuma, S., Blattner, F.R. and Komatsuda, T. (2018a) Elucidation of the origin of ‘agricrion’ based on domestication genes questions the hypothesis that Tibet is one of the centers of barley domestication. *Plant J.* 94: 525–534.

Pourkheirandish, M. and Komatsuda, T. (2007) The importance of barley genetics and domestication in a global perspective. *Ann. Bot.* 100: 999–1008.

Pourkheirandish, M., Wicker, T., Stein, N., Fujimura, T. and Komatsuda, T. (2007) Analysis of the barley chromosome 2 region containing the six-rowed spike gene vrs1 reveals a breakdown of rice-barley micro collinearity by a transposition. *Theor. Appl. Genet.* 114: 1357–1365.

Saade, S., Maurer, A., Shahid, M. et al. (2016) Yield-related salinity tolerance traits identified in a nested association mapping (NAM) population of wild barley. *Sci. Rep.* 6: 32586.

Saisho, D., Pourkheirandish, M., Kanamori, H., Matsumoto, T. and Komatsuda, T. (2009) Allelic variation of row type gene Vrs3 in barley and implication of the functional divergence. *Breed. Sci.* 59: 621–628.

Sakuma, S., Pourkheirandish, M., Matsumoto, T., Koba, T. and Komatsuda, T. (2010) Duplication of a well-conserved homeodomain-leucine zipper transcription factor gene in barley generates a copy with more specific functions. *Funct. Integr. Genomics* 10: 123–133.

Sakuma, S., Salomon, B. and Komatsuda, T. (2011) The domestication syndrome genes responsible for the major changes in plant form in the Triticeae crops. *Plant Cell Physiol.* 52: 738–749.

Salaminii, F., Ozkan, H., Brandolini, A., Schafer-Pregl, R. and Martin, W. (2002) Genetics and geography of wild cereal domestication in the near east. *Nat. Rev. Genet.* 3: 429–441.

Takahasahi, R. and Yamamoto, J. (1949) Studies on the classification and the geographic distribution of barley varieties. 8. *Nogaku Kenkyu* 38: 41–43.

Thurber, C.S., Hepler, P.K. and Caicedo, A.L. (2011) Timing is everything: early degradation of abscission layer is associated with increased seed shattering in U.S. weedy rice. *BMC Plant Biol.* 11:14.

Vatter, T., Maurer, A., Kopahnke, D., Perovic, D., Ordon, F. and Pillen, K. (2017) A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres f. teres*) in wild barley. *PLoS ONE* 12: e0186803.

Wang, K., Shi, L., Liang, X., Zhao, P., Wang, W., Liu, J., et al. (2022) The gene TaWOX5 overcomes genotypic dependency in wheat genetic transformation. *Nat. Plants* 8: 110–117.

Zeng, X., Chen, G., Wang, L., Tagiri, A., Kikuchi, S., Sassa, H., et al. (2021) The unique disarticulation layer formed in the rachis of *Aegilops longissima* probably results from the spatial co-expression of Btr1 and Btr2. *Ann. Bot.* 127: 297–304.

Zeng, X., Mishina, K., Jia, J., Distelfeld, A., Maughan, P.I., Kikuchi, S., Sassa, H. and Komatsuda, T. (2020) The Brittle Rachis Trait in Species Belonging to the Triticeae and Its Controlling Genes Btr1 and Btr2. *Front. Plant Sci.* 11:1000.

Zeng, X., Tagiri, A., Kikuchi, S., Sassa, H. and Komatsuda, T. (2020a) The ectopic expression of Btr2 in *Aegilops tauschii* switches the disarticulation layer from above to below the rachis node. *Front. PlantSci.* 11: 582622.

Zheng, B., Bai, Q., Wu, L., Liu, H., Liu, Y., Xu, W., et al. (2019) EMS1 and BRI1 control separate biological processes via extracellular domain diversity and intracellular domain conservation. *Nat. Commun.* 10: 4165.

Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S., et al. (2018) De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* 36: 1211–1216.