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Review Article

The Non-Brittle Rachis in Barley: Genetic Insights into a Key Domestication Trait

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Abstract

The non-brittle rachis is a cornerstone trait in the domestication of barley (*Hordeum vulgare* L.) fundamentally enabling efficient grain harvesting by preventing seed shattering. This review integrates current insights into the genetic, molecular and evolutionary foundations underpinning this important characteristic. The non-brittle phenotype is primarily governed by recessive loss of functional mutations in either of two dominant, complementary, and tightly linked genes *Btr1* and *Btr2* located on chromosome 3H. At the molecular level, the functional *BTR1* and *BTR2* proteins are hypothesized to interact as a receptor-ligand pair, influencing cell wall thickness within the abscission zone and thereby regulating rachis disarticulation. The non-brittle phenotype arises from the restoration of robust cell wall architecture passive yet highly effective mechanism. Synthesized archaeobotanical and comprehensive genomic evidence now strongly supports a monophyletic origin of domesticated barley in the "western Fertile Crescent" with the distinct *btr1* and *btr2* haplotypes emerging through sequential events within a single domesticated lineage. While *Btr1* and *Btr2* are the major determinants of rachis brittleness, additional quantitative trait loci (QTLs) have been identified that modulate the degree of rachis tenacity. This complex genetic control presents significant challenges for modern hybrid barley breeding, where crosses between parents with alternative non-brittle mutations can lead to a reversion to the ancestral shattering phenotype in F_1 progeny. Therefore, a deep understanding of this key domestication trait is essential not only for elucidating agricultural history but also for developing future barley improvement strategies.

Keywords: Barley, *Btr1*, *Btr2*, domestication syndrome, hybrid breeding, non-brittle rachis, quantitative trait locus

1. Introduction

Barley belongs to the Poaceae family, a highly versatile and economically critical group of monocotyledonous angiosperms comprising 12 accepted subfamilies (Soreng *et al*., 2017). Evolutionary and phylogenetic studies indicate that the grass family originated approximately 55 to 73 million years ago during the Upper Cretaceous. Early evolutionary divergence gave rise to major sister lineages, prominently the BOP clade (comprising Bambusoideae, Oryzoideae, and Pooideae) and the PACMAD clade. The Pooideae subfamily, representing a massive radiation of cool-season grasses, diverged around 62 to 67 million years ago(Ma *et al*., 2021). This established the genetic foundation from which the *Triticeae* tribe, and ultimately the genus *Hordeum* (*Hordeum vulgare*), emerged. This deep evolutionary timeline underscores

the shared developmental pathways across cereal crops, providing a crucial context for understanding how specific domestication traits, such as inflorescence architecture and seed dispersal mechanisms, evolved from ancestral grass lineages.

Barley (*Hordeum vulgare* L.) represents one of the earliest domesticated and most significant cereals, with archaeological evidence tracing its cultivation back over 10,000 years to the Fertile Crescent (Azhaguvel and Komatsuda, 2007). This profound historical depth establishes barley, alongside wheat, laid the foundation of sedentary farming system and a key founder crop upon which Old World agriculture was built. Barley's remarkable adaptability, including a notable tolerance to salinity and aridity, facilitated its widespread adoption across diverse agro-ecologies starting from Mediterranean basins to Central Asian steppes and high-altitude Himalayan region, cementing its status as a global staple. Today, barley is the fourth most cultivated cereal crop globally by production area, primarily utilized for animal feed and as a critical input material for the malting and brewing industries due to its favourable starch and enzymatic profile (Pankin and von Korff 2017; Badr *et al*., 2000; Brown *et al*., 2009). Although barley's role in direct human consumption has historically been overshadowed by other cereals, its exceptional nutritional profile-including high levels of dietary fibre, essential amino acids, and bioactive compounds is driving a resurgence of interest in health-conscious food markets(Idehen *et al*., 2017).The deep co-evolutionary history of barley and human civilization makes it an exemplary model for investigating the genetic and molecular mechanisms underlying crop domestication.

The transition from wild plants to cultivated crops involved a series of profound morphological and physiological transformations, collectively termed as the "domestication syndrome". This concept, coined by Hammer(1984), describes the suite of traits that emerged under human selection, distinguishing domesticated crops from their wild progenitors. These traits render plants amenable to cultivation while often conferring a selective disadvantage in natural ecosystem. In cereals like barley, these traits include reduced seed dormancy, synchronized tillering and maturity, compact growth habit, increased grain size, and, more importantly the loss of natural seed dispersal mechanisms (Fuller 2007). The selection for these traits was a multifaceted process, where early farmers favoured a package of characteristics that synergistically enhanced the plant's agronomic performance and ultimately its utility. For instance, the selection for a six-rowed spike in barley, which triples seed production per inflorescence compared to the ancestral two-rowed wild barley, significantly amplified the yield advantage conferred by other traits (Brown *et al*., 2009; Zohary *et al*., 2012). This indicates that domestication was not merely the selection of individual, but a co-evolutionary refinement of interrelated set of characteristics that collectively optimized the crop for agriculture.

Among the suite of domestication traits, the alteration of the seed dispersal mechanism is arguably the most pivotal. In its wild progenitor, *Hordeum vulgare* subsp. *spontaneum*, the rachisthe central axis of the spikeis inherently brittle (Pourkheirandish *et al*., 2015; Balda *et al.,* 2025). At maturity, this brittleness facilitates spontaneous disarticulation of the spike into individual dispersal units, a phenomenon known as shattering (Maity *et al*., 2021; Gupta *et al., 2025;* Salamini *et al*., 2002). While this trait is an essential adaptive mechanism for seed dispersal and species propagation in natural ecosystems, it poses a significant challenge in agricultural contexts, leading to substantial grain loss before and during harvest. Consequently, the evolution of a tough, non-brittle rachis that retains grains until harvest was a critical milestone in the domestication of barley (Zeng *et al*., 2020). This genetic transformation represents a pivotal, human-mediated shift in the plant's reproductive strategy transforming it from a self-propagating wild grass into an efficiently harvestable crop and laying the foundation for settled agriculture and cereal based food systems.

Fig 1. demonstrates the phenotypic comparison between brittle (left) and non-brittle (right) barley rachis structures. The brittle rachis sample shows the characteristic loose attachment and easy separation of individual spikelets, facilitating natural seed dispersal in wild barley (*H.spontaneum*). In contrast, the non-brittle rachis displays intact, tightly bound spikelets that remain attached during harvest, representing the key domestication trait that enabled efficient grain collection by early farmers.

The genetic and molecular basis of the non-brittle rachis trait has been the focus of extensive research,

Fig 1. Comparative morphology of brittle versus non-brittle barley rachis

offering insights into crop evolution and the mechanisms underlying morphological change. This review aims to present a comprehensive and updated synthesis of the current understanding of this key domestication trait in barley. It will delve into the genetic architecture, from the major complementary genes to the network of minor modifying loci; explore the molecular and cellular mechanisms that confer rachis tenacity; trace the evolutionary trajectory of the trait from its origins in the Fertile Crescent; and examine its profound and ongoing implications for modern crop improvement strategies, with a particular focus on the challenges and opportunities it presents for hybrid barley breeding.

2. The genetic architecture of rachis brittleness

2.1. The major complementary loci: btr1 and btr2

Decades of genetic research have firmly established that rachis brittleness in barley is primarily governed by the complex interaction of two dominant, complementary, and tightly linked genes: *Brittle rachis 1* (*Btr1*) and *Brittle rachis 2* (*Btr2*). These loci are located in close proximity on the short arm of barley chromosome 3H (3HS) (Takahashi and Hayashi, 1964; Komatsuda and Mano, 2002; Pourkheirandish *et al*., 2015; Zeng *et al*., 2020). In wild barley (*H. spontaneum*), the presence of functional, dominant alleles at both loci (*Btr1 Btr1 Btr2 Btr2*) is necessary to produce the brittle rachis phenotype that facilitates natural seed dispersal. In contrast, the non-brittle rachis defining trait of all cultivated barley, results from recessive loss of function mutations in either of these two genes. This means that a genotype of either *btr1 btr1 Btr2 Btr2* or *Btr1 Btr1 btr2 btr2* will exhibit a tough, non-shattering spike (Komatsuda and Mano, 2002; Pourkheirandish *et al*., 2015; Fernández-Calleja *et al*., 2020; Mukesh *et al.,* 2024). The complementary nature of these genes has profound implications for modern agriculture, as a cross between parents carrying alternative mutations (e.g., a *btr1* type and a *btr2* type) will produce an F_1 hybrid with the genotype *Btr1 btr1 Btr2 btr2*. Because a functional allele is restored at each locus, this hybrid reverts to the ancestral brittle phenotype, creating significant challenges for grain retention in hybrid breeding programs. Molecular analyses have identified the specific genetic lesions responsible for the most common

non-brittle phenotypes. The *btr1* allele typically arises from a single base-pair deletion (1 bp deletion) in the *Btr1* coding sequence, while the *btr2* allele is most commonly caused by an 11-bp deletion in the *Btr2* coding sequence. Both of these frame shift mutations result in non-functional proteins, thereby preventing the development of a fragile rachis (Pourkheirandish *et al*., 2015; Zeng *et al*., 2020; Mukesh *et al.,* 2025).

The circular genetic map of barley chromosomes1H-7H by Maurer and Pillen (2021) showing the chromosomal distribution of major genes and QTLs. The *BTR1* and *BTR2* genes are specifically highlighted on chromosome 3H, illustrating their tight linkage on the short arm (3HS). The heat map sections on the outer edge indicate allele fixation percentages, with red representing Hv allele fixation and blue representing Hsp allele fixation. This comprehensive mapping demonstrates the genomic context of the non-brittle rachis loci and their relationship to other important barley genes.

2.2. Allelic diversity, haplotypes, and geographic distribution

While the 1-base-pair and 11-base-pair deletions in *Btr1* and *Btr2* are predominant, additional research has uncovered greater allelic diversity, pointing to a more complex evolutionary history of the brittle rachis trait. A point mutation in *Btr1*, leading to a single amino acid substitution, has been identified as a third independent origin of non-brittleness. This allele, designated *btr1b*, has been found in landraces from Serbia, Greece, and the Gaziantep region of southeast Türkiye, suggesting that multiple mutational events leading to the same desirable phenotype were independently selected by early farmers (Civáň and Brown, 2017). A critical finding with significant implications for understanding barley's dispersal is the distinct geographical distribution of the primary non-brittle haplotypes. The *btr1* mutation (*btr1 btr1 Btr2 Btr2*) is predominantly found in barley cultivated in Europe and other western regions (Pourkheirandish *et al.,* 2015; Fernández-Calleja *et al*., 2020; Tan *et al.* (2025). In contrast, the *btr2* mutation (*Btr1 Btr1 btr2 btr2*) is more frequent in eastern accessions, including those from East Asia. This clear geographical pattern provides a powerful genetic signature that has been instrumental in tracing the complex migration and diversification pathways of cultivated barley following its initial domestication (Azhaguvel and Komatsuda 2007; Tan *et al*., 2025).

2.3. The polygenic nature of rachis fragility: role of minor loci and quantitative trait loci (QTLs)

While the two-gene complementary model cantered on *Btr1* and *Btr2* effectively explains the primary genetic switch between brittle and non-brittle phenotypes, evidence suggests that rachis fragility is governed by a more complex, polygenic inheritance (Fernández-Calleja *et al*., 2020; Komatsuda *et al*., 2004). Breeding experiments have revealed substantial variation in rachis toughness even among non-brittle genotypes. For example, hybrids such as *btr1* x *btr1* or *btr2* x *btr2* often display a higher percentage of rachis disarticulation under mechanical stress compared to their inbred parents, indicating the influence of additional modifying genes. This points to a hierarchical system of genetic control of the trait. The major *Btr1* and *Btr2* genes function as a binary "on/off switch" for the shattering phenotype; a recessive loss-of-function mutation in either gene is sufficient to deactivate the shattering mechanism, thereby preventing catastrophic grain loss. However, the degree of rachis tenacity appears to be controlled by a network of minor loci functioning as "dimmer switches" or rheostats. This model explains why a hybrid between two *btr1* parents may be more fragile than either parent: each parent may carry different set of favourable alleles at these minor loci, and the hybrid's allelic combination may be suboptimal for maximum rachis strength (Pourkheirandish and Komatsuda 2022). Quantitative trait locus (QTL) mapping has begun to identify these modifying loci (Table 1). Secondary QTLs with smaller but significant effects on rachis brittleness have been detected on barley chromosomes 5H and 7H (Table 1). Furthermore, Kandemir *et al.* (2000) mapped a major QTL associated with the "weak rachis" (*Hst-3*) trait to the centromeric region of chromosome 3H, distinct from the *Btr1/Btr2* locus. This complex inheritance pattern, involving at least five interacting genes, highlights that while the major domestication event was the fixation of a non-functional *btr* allele, subsequent selection and breeding efforts have likely targeted this network of minor loci to enhance harvestability across diverse genetic backgrounds and environments. For barley breeders, this polygenic architecture is essential to consider securing a non-brittle genotype at the major loci alone may not

suffice to ensure optimal grain retention, particularly when developing hybrids from genetically diverse germplasm (Jiang *et al*., 2014).

QTL mapping positions on barley chromosomes 1H, 2H, 3H, and 5H showing key loci relevant to grain size and malt extract traits in a *Triticeae* species. The identification of major loci (e.g., on chromosome 3D, an ortholog to 3H) and minor loci on other chromosomes that contribute to the phenotype, demonstrating the polygenic nature of the trait. (Wang *et al*., 2021).

Table 1: Genetic loci associated with rachis brittleness in barley and related cereals

Gene/Locus Name	Chromosomal Location	Nature of Effect	Phenotypic Effect	Reference(s)
Btr1	3HS	Major Gene, Dominant (wild), Recessive (non-brittle)	Controls primary rachis brittleness; loss-of-function mutation leads to non-brittle rachis.	Pourkheirandish *et al.* (2015)
Btr2	3HS (linked to *Btr1*)	Major Gene, Dominant (wild), Recessive (non-brittle)	Complementary to *Btr1*; loss-of-function mutation leads to non-brittle rachis.	Pourkheirandish *et al.* (2015)
Hst-3	3H (centromeric)	Major QTL	Associated with "weak rachis" and head shattering.	Kandemir *et al.* (2000)
Minor QTL	5HL	Minor QTL	Modifies rachis fragility, particularly in *btr2* backgrounds.	Komatsuda *et al.* (2004)
Minor QTL	7H	Minor QTL	Modifies rachis fragility; may have an unlinked inhibitor gene (*D*).	
Qbr.sau-3D	3DS (Wheat)	Major QTL (likely *Br1* ortholog)	Explains ~39% of phenotypic variation for brittle rachis in Tibetan semi-wild wheat.	Jiang *et al.* (2014)
Qbr.sau-5A	5AL (Wheat)	Major QTL (pleiotropic)	Explains ~18% of variation for brittle rachis and ~35% for threshability in wheat.	

3. Molecular and cellular mechanisms of rachis tenacity

3.1. The btr1 and btr2 proteins: a hypothesized receptor-ligand interaction

At the molecular level, the *Btr1* and *Btr2* genes encode relatively small proteins, consisting of 196 and 202 amino acids, respectively (Zeng *et al*., 2020). While their exact biochemical functions remain under investigation, structural predictions and genetic evidence have led to a compelling hypothesis for their mode of action (Pourkheirandish *et al*., 2015). It is proposed that *BTR1*, which contains predicted transmembrane domains, functions as a membrane-bound receptor, while *BTR2* acts as its corresponding extracellular ligand (Kandemir *et al*., 2000). The functional presence and physical interaction of both proteins are believed to be essential for initiating a downstream signalling cascade that ultimately leads to the development of a fragile rachis node. This

receptor-ligand model provides clear explanation for the genetic complementarity, where the absence of either the "receptor" (*btr1*) or the "ligand" (*btr2*) disrupts the signalling pathway, thereby preventing rachis disarticulation. A significant aspect of these proteins is their evolutionary specificity. Homologs of *Btr1* and *Btr2* are found exclusively within the *Triticeae* tribe, which includes cereals such as barley, wheat, and rye. This indicates that the *BTR1/BTR2*-mediated grain shedding mechanism represents a distinct evolutionary innovation within this agriculturally critical group of grasses, employing a different molecular mechanism employed by more distantly related cereals such as rice (Kandemir *et al*., 2000; Pourkheirandish *et al*., 2015; Idehen *et al*., 2017).

Model of the interaction between *BTR1* and *BTR2* proteins depicted that *BTR1* acts as a receptor and *BTR2* as a ligand. The *BTR1–BTR2* interaction results in thin cell walls and grain dispersal at maturity. Loss of function of either protein impairs the interaction and results in thick cell walls and an intact spike even at maturity (Pourkheirandish and Komatsuda 2022).

3.2. Evolutionary origin: gene duplication and neofunctionalization of btr-like paralogs

The evolutionary history of the *Btr* genes exemplifies a classic mechanism of trait evolution *via* gene duplication followed by functional divergence. Within the barley genome, paralogous genes-*Btr1-like* and *Btr2-like-* located in close proximity to the *Btr* loci on chromosome 3HS (Pourkheirandish and Komatsuda, 2022; Pourkheirandish *et al*., 2015; Zeng *et al*., 2020) Unlike *Btr1* and *Btr2*, these *"like"* genes are more broadly conserved across the Poaceae family, indicating that they likely represent the ancestral gene forms. Critically, these paralogs are not functionally redundant with *Btr1* and *Btr2,* and are unable to complement the non-brittle mutations in cultivated barley. The prevailing evolutionary model suggests that *Btr1* and *Btr2* originated from a local gene duplication event of their ancestral *Btr-like* counterparts that occurred specifically within the Pooideae lineage after its divergence from other grasses (Pourkheirandish *et al*., 2018). Following duplication, the newly formed *Btr1* and *Btr2* genes underwent neofunctionalization, acquiring a novel, spike-specific expression pattern and evolving a new role in promoting rachis disarticulation (Pourkheirandish *et al*., 2015). Meanwhile, the ancestral *Btr-like* copies likely retained their original function, which is hypothesized to involve processes such as pollen development (Pourkheirandish and Komatsuda, 2022). This evolutionary pathway enabled the emergence of a new adaptive trait-seed shattering– without disrupting existing essential function, providing a clear molecular blueprint for the evolution of this key wild characteristic, thereby shaping its domestication trajectory.

3.2.1. Evolutionary development and structural analysis of barley spike architecture

The development of the barley inflorescence is an intricate, highly orchestrated progression of meristematic differentiation. To fully appreciate the impact of rachis mutations, one must examine the fundamental architectural transitions of the spike. The evolutionary trajectory of grass inflorescence structures reveals a theoretical transition from highly complex, heavily branched ancestral compound arrangements to the highly simplified, linear spike formation characteristic of modern *Triticeae* species.

In wild-type barley, developmental progression follows a strict sequence: initial triple mound formation, followed by the development of the glume primordium, the emergence of the lemma primordium and finally, the formation of the stamen primordium. However, genetic mutations can fundamentally and catastrophically alter this architecture. Analysis of mutants, particularly the *compositum 1* (*com1.a*) mutant, reveals dramatically altered, heavily branched spike structures at maturity that contrast sharply with normal wild-type morphology. In such mutants, compromised central spikelet meristem (CSM) identity triggers the aberrant formation of small, spike-like branch structures emerging directly from central spikelet positions (Poursarebani *et al.,* 2020).These structural deviations are profound, involving the absence of basal pulvinus structures and significantly altered palea architecture. Histological cross-sections and transmission electron microscopy (TEM) confirm that these morphological differences manifest at the ultrastructural cellular organization level shortly before anthesis. Understanding these broader architectural constraints and the role of meristem identity is absolutely critical when breeders attempt to alter *Btr* loci or engineer new spike traits, as the metabolic energy redirected from abscission zone formation must be seamlessly and efficiently integrated

into overall spike development without triggering adverse, yield-destroying pleiotropic effects.

3.3. The cellular basis of non-brittleness: a failure of fragility, not an active abscission

The phenotypic distinction between brittle and non-brittle rachises in barley arises from distinct structural modifications at the cellular level. In wild barley, a specialized zone of weakness, often called a "constriction groove," forms at each rachis node (Pourkheirandish and Komatsuda, 2022; Pourkheirandish *et al*., 2015). Histological studies reveal that this zone consists of five to six layers of enlarged cells whose walls are underdeveloped and thin (Azhaguvel and Komatsuda, 2007). The cell walls in this disarticulation zone are only about 25% as thick as those in the non-brittle rachis of domesticated barley, showing reduction in both primary and secondary wall thickness (Jiang *et al*., 2014).

Importantly, the mechanism underlying rachis disarticulation in barley is fundamentally different from the enzymatic degradation of a pre-formed abscission layer that facilitates leaf or fruit drop in many other plant species (Pourkheirandish and Komatsuda, 2022; Pourkheirandish *et al*., 2015). Instead of active shedding, rachis brittleness is a passive process resulting from a programmed developmental failure. The *BTR1/BTR2* signalling pathway appears to interfere with the normal process of cell wall construction, specifically inhibiting the formation of the second and third wall layers (Azhaguvel and Komatsuda, 2007). This leads to an inherently fragile structure that readily breaks under minimal physical stress once the spike matures and dries.

The non-brittle phenotype of domesticated barley is not achieved through activation of a new inhibitory pathway to prevent shedding. Rather, it is the result of a simple restoration of the default developmental program: building a structurally sound cell wall. The loss-of-function mutations in *btr1* or *btr2* disrupt the signal that induces fragility, thereby allowing the cell walls at the rachis node to develop to their full, robust thickness (Azhaguvel and Komatsuda, 2007; Pourkheirandish *et al*., 2015).This process often referred as "domestication by subtraction" represents a highly efficient evolutionary strategy from a bioenergetic standpoint. Instead of expending metabolic energy on a new mechanism to hold its seeds, the plant simply ceases to expend energy on the pathway that causes them to shatter. This genetic simplicity contributed to the rapid and complete fixation of the non-brittle trait during early agricultural development, offering a low-cost genetic change with an immense agricultural benefit.

4. Evolutionary trajectory and agricultural significance

4.1. Archaeobotanical evidence from the fertile crescent

The archaeological record provides a robust timeline for the emergence of the non-brittle rachis trait, firmly placing the domestication of barley in the Fertile Crescent of Western Asia approximately 10,000 years ago (Allaby 2021, Avni *et al*., 2017). Evidence from early sites such as Ohalo II in Israel, dated to approximately 17,000 years before present (BP), indicates that humans were harvesting wild barley well before its formal domestication (Azhaguvel and Komatsuda, 2007). The pivotal transition to agriculture is marked by the appearance of barley remains exhibiting a tough, non-brittle rachis. Clear archaeobotanical evidence of domesticated, non-brittle, two-rowed barley has been obtained from sites such as Tell Abu Hureyra in Syria, dating approximately 10,200–9,550 calibrated years BP. By 8000 BC, non-brittle barley became dominant in cultivated populations across the region, highlighting the intense and rapid selective pressure exerted by early farming practices (Tan *et al.,* 2025; Hillman *et al*., 2001; Zohary *et al*., 2012).

4.2. Reconciling the evidence: a monophyletic origin with sequential haplotype emergence

The origin of domesticated barley has been a subject of extensive debate, with historical hypothesis proposing for either a single (monophyletic) or multiple (polyphyletic) domestication events (Azhaguvel and Komatsuda, 2007; Kharub *et al.,* 2025; Zeng *et al*., 2020; Tan *et al.* (2025). Initial botanical research and genetic studies focusing on the distinct geographical distribution of*btr1* and *btr2* alleles, supported polyphyletic model, suggesting at least two independent domestication events from different wild populations (Morrell and Clegg, 2007; Pankin *et al*., 2018; Tan *et al*., 2025). However, recent comprehensive studies, utilizing large sample sizes and genome-wide markers, have provided unequivocal evidence for monophyletic origin (Tan *et al*., 2025). Phylogenetic reconstructions consistently show that all cultivated barley gene pool branches off from a specific group of wild progenitors located in the Israel-Jordan region of the West Fertile

Table 2: Chronology of key archaeobotanical and genetic events in barley domestication

Approximate Date (before present/ before Christ BP/BC)	Site / Region	Evidence Type	Significance	Reference(s)
~17,000 BP	Ohalo II, Israel	Archaeological (grains)	Pre-domestication harvesting of wild barley.	Kislev *et al.* (1992)
~10,200–9,550 cal. BP	Tell Abu Hureyra, Syria	Archaeological (grains)	Emergence of non-brittle, two-rowed domesticated barley.	Hillman *et al.* (2001)
~10,000 years ago,	Fertile Crescent	Archaeological / Genetic	Initiation of barley domestication from *H. spontaneum.*	Badr *et al.* (2000)
~9,700 BP	Israel-Jordan	Phylogenetic Dating	Separation of the monophyletic domesticated lineage from its wild progenitor in the specific region.	Tan *et al.* (2025)
~8000 BC	Fertile Crescent	Archaeological	Widespread cultivation and fixation of non-brittle forms.	Zohary *et al.* (2012)
Post-domestication	Zagros Mtns, Anatolia	Archaeological / Genetic	Rapid eastward and westward spread of domesticated barley from the West Fertile Crescent.	Zohary *et al.* (2012)
Not earlier than 3,500 BP	Turkey / Iran	Phylogenetic Dating	Re-emergence of modern two-rowed barley, likely derived from six-rowed domesticated types.	Tan *et al.* (2025)

Crescent (Badr *et al.*, 2000; Tan *et al.* (2025) This new evidence provides a reconcilable framework that explains the existence of two major non-brittle haplotypes without invoking multiple domestication events. The most plausible scenario is that these haplotypes arose from two distinct chronological events occurring along the same evolutionary lineage (Pourkheirandish *et al.*, 2015; Tan *et al.* (2025). The initial domestication event in the West Fertile Crescent likely involved the selection of a non-brittle mutation (possibly *btr2*) in a two-rowed background. As this early domesticated form spread, a second mutation (*btr1*) occurred and was selected, possibly in a six-rowed background, which later became predominant in other geographical regions, particularly during the westward expansion into Europe (Pourkheirandish *et al.*, 2015; Komatsuda *et al.*, 2007; Zohary *et al.*, 2012).

4.3. Selection pressures and the global dispersal of a critical trait

The non-brittle rachis trait conferred a profound agricultural advantage and was a primary driver of its rapid fixation during barley domestication. By preventing premature seed shattering, this trait dramatically increased the proportion of grain that could be successfully harvested, stored, and consumed; transforming barley from wild grass into a dependable food source (Fuller, 2007; Pourkheirandish *et al.*, 2015). This shift from incidental gathering of shed seeds to the systematic harvesting of intact spikes represented a technological revolution that fundamentally reshaped the human-plant relationship. The efficiency gains associated with non-brittleness would have been immediately evident to early cultivators, creating an intense selective pressure

in favour of plants that retained their seeds until harvest (Fuller, 2007; Hillman *et al*., 2001; Zohary *et al*., 2012). This single trait was a catalyst for societal change, enabling the production of reliable food surpluses that supported the transition from nomadic hunter-gatherer lifestyles to settled agricultural communities ultimately facilitating the rise of complex civilizations (Salamini *et al*., 2002; Zohary *et al*., 2012). Following its fixation in the Fertile Crescent, domesticated barley and its associated farming technologies spread rapidly across continents, with the distinct *btr1* and *btr2* haplotypes serving as genetic footprints tracing these ancient migration routes across Asia, Europe, and Africa (Salamini *et al*., 2002; Morrell and Clegg, 2007; Azhaguvel and Komatsuda, 2007).

5. Implications for modern barley improvement

5.1. The hybrid breeding conundrum: reversion to shattering in F_1 progeny

Although the non-brittle rachis trait was fixed early in domestication, its underlying genetic architecture poses a significant and unexpected challenge for modern barley breeding, particularly in the development of hybrid cultivars (Pourkheirandish *et al*., 2015). Hybrid breeding aims to exploit heterosis (hybrid vigor) by crossing genetically distinct parental lines, often originating from different geographical regions. In barley, this strategy intersects directly with the geographical distribution of non-brittle alleles. European barley germplasm predominantly carries the *btr1* mutation, whereas germplasm from other parts of the world, particularly the East, is typically of the *btr2* type (Longin & Reif, 2014; Morrell and Clegg, 2007; Azhaguvel and Komatsuda, 2007). The complication arises from the complementary dominant nature of the *Btr* genes. When a *btr1 btr1 Btr2 Btr2* parent is crossed with a *Btr1Btr1btr2btr2* parent, the resulting F_1 hybrid has the genotype *Btr1 btr1 Btr2 btr2*. Because a functional, dominant allele is present at both the *Btr1* and *Btr2* loci, the genetic pathway for brittleness is restored, and the hybrid spike reverts to the ancestral shattering phenotype (Takahashi and Hayashi, 1964; Pourkheirandish *et al*., 2015; Fernández-Calleja *et al*., 2020). This reversion can lead to significant grain loss at maturity, jeopardizing the efficient harvest of the hybrid crop and potentially negating the yield gains from heterosis (Fernández-Calleja *et al*., 2020; Maity *et al*., 2021).

5.2. Strategic management of btr genotypes in breeding programs

The risk of producing brittle hybrids remains a significant constraint in barley breeding, effectively limiting the scope of potential crosses and hindering the full exploitation of global genetic diversity (Fernández-Calleja *et al*., 2020; Longin and Reif, 2014). Promising heterotic patterns, such as those between elite European (*btr1*) and Asian (*btr2*) germplasm pools, may be rendered inaccessible due to the risk of spike shattering in the resultingF_1 progeny (Morrell and Clegg, 2007; Pourkheirandish *et al*., 2015). To mitigate this challenge, successful hybrid barley breeding demands the strategic management of *Btr* genotypes within parental pools. Breeders must genotype parental lines to determine their non-brittle allelic status (Pankin *et al*., 2018). The most straightforward approach involves defining heterotic groups based on their *btr* background and restrict crosses to within the same allele type (e.g., *btr1* × *btr1* or *btr2* × *btr2*) to avoid reconstitution of the brittle phenotype (Longin and Reif, 2014). A more proactive, though resource-intensive, strategy involves pre-breeding efforts to introgress a common non-brittle allele into elite germplasm from different pools. For example, marker-assisted backcrossing could be used to convert a high-performing *btr2* line to a *btr1* background, thereby enabling compatibility with the European gene pool without the risk of producing brittle F_1 progeny (Pankin *et al*., 2018). This scenario underscores the necessity of integrating molecular genetics into practical breeding, enabling breeders to navigate historical genetic constraints and unlock the full potential of heterotic diversity in barley (Fernández-Calleja *et al*., 2020).

5.3. Linkage drag and opportunities for fine-tuning rachis tenacity

Intense selection for the *btr1* and *btr2* loci during domestication triggered a strong selective sweep in the surrounding genomic region on chromosome 3H (Maurer and Pillen, 2021; Pankin *et al*., 2018). This process resulted in "linkage drag," whereby alleles at loci physically close to the selected gene whether beneficial or detrimental were co-inherited (Pourkheirandish *et al*., 2015). As a result, modern cultivated barley may have inadvertently lost beneficial alleles from its wild progenitors that were linked to the ancestral brittle alleles, while simultaneously fixing

potentially unfavourable alleles linked to the non-brittle mutations (Pankin *et al*., 2018).

Nevertheless, the polygenic nature of rachis tenacity offers new opportunities for crop improvement. The identification of minor QTLs on other chromosomes that modulate the degree of brittleness provides targets for fine-tuning the trait (Komatsuda *et al*., 2004; Pourkheirandish *et al*., 2015). Even in non-brittle hybrids (*btr1* × *btr1*), a slight increase in fragility compared to the parents has been observed, likely due to the combination of alleles at these minor loci (Kandemir *et al*., 2000). By identifying and selecting favourable alleles at these modifier QTLs, breeders can enhance the overall structural integrity of the rachis (Fernández-Calleja *et al*., 2020). This would not only improve harvestability and reduce grain losses in conventional varieties but also offer an additional safeguard against shattering in hybrid combinations ultimately contributing to a more robust and resilient production system in barley (Komatsuda *et al*., 2004; Maurer and Pillen, 2021).

6. Conclusion and future prospects

6.1. Summary of genetic and molecular insights

The non-brittle rachis is a defining trait of barley domestication, essential for its establishment as a foundational crop for human civilization. Its genetic basis is now well characterized primarily governed by recessive loss of function mutations in one of two complementary dominant genes, *Btr1* or *Btr2*, both located-on chromosome 3H. This primary genetic switch is further modulated by a network of minor QTLs that subtly influence rachis tenacity, revealing a complex, polygenic architecture. At the molecular level, the transition to non-brittleness did not arise from the evolution of a novel function, but rather the disruption of an ancestral developmental pathway unique to the *Triticeae* tribe, which causes developmental fragility. The resulting loss of function mechanism, which restores robust cell wall construction at the rachis node, offers a bio-energetically efficient solution that was rapidly fixed by early human selection.

6.2. Reiteration of the trait's foundational importance

The journey of understanding the non-brittle rachis from early genetic studies to modern genomics and archaeobotanical offers a powerful model for exploring the mechanism of crop evolution. Recent findings strongly support a monophyletic origin for domesticated barley in the West Fertile Crescent, with different non-brittle haplotypes arising sequentially within this single lineage. This knowledge not only resolves long-standing debates about agricultural history but also carries profound implications for contemporary breeding programs. The genetic legacy of this domestication event continues to shape modern breeding efforts, most notably in the development of hybrid barley, where the complementary nature of the *Btr* genes presents a significant obstacle that must be managed through molecular-informed strategies.

6.3. Future research directions

Despite significant progress, several key areas warrant further investigation to fully leverage our understanding of the non-brittle rachis trait for future crop improvement.

- **Experimental validation of molecular mechanisms:** A key priority should be the experimental validation of the hypothesized receptor-ligand interaction between the *BTR1* and *BTR2* proteins (Pourkheirandish *et al*., 2015; Zeng *et al*., 2020). Techniques such as split-ubiquitin yeast two-hybrid assays, co-immunoprecipitation, and advanced co-localization studies using fluorescently tagged proteins *in planta* are needed to confirm this interaction and elucidate the downstream signalling pathway (Pourkheirandish and Komatsuda, 2022).
- **Characterization of minor QTLs:** While several minor QTLs influencing rachis tenacity have been identified, their underlying genes remain unknown (Komatsuda *et al*., 2004; Kandemir *et al*., 2000). Fine-mapping and gene cloning will provide novel targets for breeders aiming to fine-tune rachis strength and enhance yield stability, particularly in the context of hybrid breeding (Fernández-Calleja *et al*., 2020; Wang *et al*., 2021).
- **Functional analysis of ancestral paralogs:** A deeper investigation into the biological roles of the ancestral *Btr1-like* and *Btr2-like* paralogs is required (Pourkheirandish *et al.,* 2015). Understanding their proposed role in processes like pollen development may offer deeper evolutionary insights and potentially reveal novel avenues for manipulating other agronomically important traits (Zeng *et al.,* 2020; Pourkheirandish and Komatsuda, 2022).

- **Application of genomic editing:** Cutting-edge genomic editing technologies, particularly CRISPR-Cas9, hold immense potential for addressing hybrid breeding challenges (Pankin *et al*., 2018). These tools could be used to precisely and efficiently convert elite parental lines to a common *btr* background (e.g., converting a *btr2* line to *btr1*), thereby breaking the genetic linkage between heterotic groups and non-brittle haplotypes (Fernández-Calleja *et al*., 2020). This would unlock previously inaccessible genetic diversity, accelerating the development of higher-yielding, resilient hybrid barley cultivars (Longin and Reif, 2014).

By continuing to integrate fundamental genetic and molecular research with applied breeding objectives, the scientific community can build upon the legacy of the barley agriculturalists– further optimizing this ancient crop to meet the challenges of global food security in the 21st century.

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