Received: 23 June 2023 | Revised: 7 September 2023 | Accepted: 26 October 2023

DOI: 10.1111/gfs.12635

Grass and Forage Science BGS EGF WILEY

REVIEW ARTICLE

Perspectives for reducing seed shattering in ryegrasses

Jenny Kiesbauer[1,2] | Christoph Grieder[1] | Bruno Studer[2] | Roland Kölliker[2]

[1]Fodder Plant Breeding, Division of Plant Breeding, Agroscope, Zurich, Switzerland

[2]Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland

Correspondence
Roland Kölliker, Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Universitaetstrasse 2, 8092 Zurich, Switzerland.
Email: roland.koelliker@usys.ethz.ch

Funding information
Breeding Foundation DSP-BLW

Abstract

In the last decades, the progress in ryegrass (*Lolium* spp.) breeding was mainly on agronomic traits such as biomass yield, forage quality or disease resistance. However, for commercial success, a stable and high seed yield is a prerequisite for any cultivar. The realized seed yield is influenced by many different factors such as non-optimal pollination and fertilization, seed abortion and seed shattering. While seed shattering has been largely eliminated in major cereal crops such as rice, barley or sorghum during domestication, the trait has been largely neglected in ryegrass breeding programs. The close syntenic relationship of cereal and ryegrass genomes offers the opportunity to develop breeding approaches for reducing seed shattering in the latter by transferring knowledge from the former. The objectives of this review are to (1) give an overview on the knowledge of morphology on seed shattering in cereal crops and ryegrasses, (2) compare the genetic background underlying seed shattering in different species, (3) identify putative candidate genes controlling seed shattering in ryegrasses through comparative genomic analysis and (4) give an outlook on new breeding strategies resulting in low seed shattering cultivars of ryegrasses and related forage grass species.

KEYWORDS
candidate genes, forage grasses, seed shattering, seed yield, synteny

1 | INTRODUCTION

Permanent and temporary grasslands cover more than 50% of the European agricultural land area and often contain many different grass, legume and forb species (Food and Agriculture Organization of the United Nations, 2020). For temperate grasslands, the five major grass genera are *Lolium* (ryegrasses), *Festuca* (fescues), *Poa* (bluegrasses or meadowgrasses), *Dactylis* (orchardgrass or cocksfoot [*D. glomerata* L.]) and *Phleum* (timothy [*P. pratense* L.]; Boller et al., 2010). Due to their high yield potential and high forage quality, Italian and perennial ryegrass (*L. multiflorum* and *L. perenne)* are the most important grass species for intensively managed semi-natural grasslands and for temporary grass-clover leys (Humphreys et al., 2006; Jung, 1996).

To provide cultivars adapted to specific management and environmental conditions, targeted breeding efforts are essential. In contrast to major crop species, systematic breeding of ryegrasses (and other forage grasses) started rather recently in the 1970s, with a focus on agronomic performance measured as persistence and biomass yield (Wilkins & Humphreys, 2003). Many ryegrass breeding programs particularly focused on forage quality, resulting in a higher digestibility over the last years in modern cultivars (Casler, 2001). In general, grain bearing tillers have a higher lignin content than leaves, resulting in a lower digestibility. Therefore, selection for higher digestibility may also result in a shift from reproductive to vegetative growth, reducing the ability to produce seeds (Humphreys et al., 2010). However, efficient forage production relies on sward establishment or sward improvement through seeds. Especially for short-term swards, seeds

of high-quality cultivars are indispensable. Therefore, seed yield is economically highly relevant for cultivars to be commercially viable (Casler & Vogel, 2020).

Seed yield is a complex trait influenced by many factors. The potential seed yield is defined as the maximum seed yield achievable under optimal conditions. It accounts for the total number of ovules per area present at flowering time. The total number of ovules is determined by the number of spikes (i.e., culms) per area, spikelets per spike and flowers per spikelet (Falcinelli, 1999). The realized seed yield is influenced by many factors and has been reported to be up to 45% lower than the potential seed for perennial ryegrass, mainly due to non-optimal pollination and fertilization, seed abortion and seed shattering (Falcinelli, 1999; Griffiths et al., 1973). Unsuccessful pollination and fertilization can occur if flowering and pollen distribution by wind is not happening simultaneously (Boelt & Studer, 2010) or if a substantial amount of incompatible pollen is present within the pollen (Studer et al., 2008).

In case of successful pollination and fertilization, seed set starts with the growth of the embryo and the endosperm (Boelt & Studer, 2010). During this stage, the embryo is highly sensitive to environmental influences such as temperature, humidity, or salinity and can potentially be aborted (Boelt & Studer, 2010; Elgersma et al., 1988). In the next phase, the embryo continues to grow, increases its dry weight and accumulates reserves. At the end of this stage, the seed is viable. In the last stage, the seed loses moisture and is finally ripe for harvesting (Boelt & Studer, 2010). A substantial number of seeds may be lost due to seed shattering shortly before or during seed harvest.

1.1 | Seed shattering—A major cause for reduced seed yield in ryegrass

Seed shattering, that is, the displacement of seeds from the stalk, is an adaptive trait in most wild progenitor species as it allows for seed dispersal in natural environments. In domesticated crops, seed retention (i.e., loss of seed shattering) allows for reducing seed loss during harvest and facilitates the simultaneous harvest of a larger number of plants. During early domestication of major cereal crops, farmers have indirectly selected for a non-shattering phenotype during harvesting (Harlan, 1975). Seed retention is one of the most important domestication traits in major cereal crops like barley (*Hordeum vulgare* L.; Pourkheirandish et al., 2015; Schmid et al., 2018), rice (*Oryza sativa* L.; Onishi et al., 2007) and sorghum (*Sorghum bicolor* L. Moench; Lin et al., 2012).

A fully domesticated crop with a non-shattering phenotype can no longer propagate through spontaneous seed dispersal but relies on being sown by the farmer (Fuller & Allaby, 2009). The non-seed shattering phenotype seems to have evolved several times within cereals (Lin et al., 2012; Paterson et al., 1995). For example, Lin et al. (2012) found three different mutations within a gene responsible for seed shattering in sorghum, where each mutation was found in specific sorghum accessions from different origins of domestication. In cereal crops, as seeds are directly used for human or animal nutrition, yield of seeds has always been the main focus of breeding and selection. In ryegrasses, the important part for animal feeding is the green biomass and not the seeds. Consequently, seed shattering has not undergone any strong selection pressure in ryegrasses and still is an issue today (Lüscher et al., 2019; Marshall & Wilkins, 2003), with up to 54% of the ripe seeds being lost through shattering (Maity, Singh, et al., 2021). Seed shattering may be strongly influenced by environmental factors such as temperature (Ji et al., 2006; Thurber et al., 2010), moisture (Maity, Lamichaney, et al., 2021) or wind (Elgersma et al., 1988). Thus, the time of seed harvest plays an important role in seed shattering. Further, if the harvesting date is set too early, the drying cost will increase and the seed quality will be decreased, resulting in a low seedling vigour. If the harvesting date is set too late, many seeds are lost through shattering. The differential ripening stages within the same tiller, where apical seeds are ripening earlier than the basal seeds, makes it even more difficult to find the optimal date for seed harvest. According to our own observations, loss of ripened seeds before harvest by shattering showed to be a major factor reducing seed yield in the breeding material of Agroscope, Switzerland. Indeed, delaying the harvest by only a few days reduced seed yield in material prone to shattering by up to 40% (unpublished data). A preliminary experiment using different Italian ryegrass cultivars in the same environment showed that there is a large variation for seed shattering among different cultivars (Peter et al., 2021). This difference might be due to a different genetic background, which could provide the basis for studying the genetic control of this important trait and to improve seed yield through targeted breeding efforts.

To reduce seed shattering by breeding, a deeper understanding of the genetic architecture of this trait in forage grasses is needed. Reviewing the morphological, physiological and genetical concepts of seed shattering in other major crops can help to address open questions in forage grasses. First, detailed knowledge about systematics and evolutionary relations between species is required. With this knowledge, morphological differences in seed shattering between crop species can be compared. Second, the genetic background underlying seed shattering can be studied in different crops. Third, knowledge on seed shattering genes and their putative function may be transferred into ryegrasses to identify candidate genes for seed shattering. Finally, these findings maybe help to elucidate the genetic mechanism in other forage grass species.

2 | MECHANISMS OF SEED SHATTERING IN MAJOR GRASS SPECIES

Forage grasses, including ryegrasses and fescues (*Festuca* spp.), and cereals such as barley, rice or sorghum belong to the family of Poaceae, which includes between 8000 and 10,000 species (Kellogg, 1998). The family of Poaceae is further divided into 12 subfamilies (Soreng et al., 2017). Forage grasses, as well as wheat (*Triticum aestivum* L.) and barley, are part of the Pooideae subfamily,

whereas rice belongs to the Oryzoideae subfamily. These two subfamilies are separated from each other since the occurrence of a first major radiation in grasses (Kellogg, 1998). A second radiation of grasses led to additional subfamilies, for example the Panicoideae, with species such as sorghum. The subfamily Pooideae is further subdivided into the Triticeae tribe including wheat and barley, while ryegrasses and Brachypodium have their own tribes, Poeae and Brachypodieae, respectively (Soreng et al., 2015). Although all of the most important forage grass species (i.e., ryegrasses, fescues, cocksfoot, timothy, bluegrasses) belong to the Poeae tribe, they differ widely in morphology, physiology and genome constitution (Clayton & Renvoize, 1986; Pellicer & Leitch, 2020). Even though the evolutionary divergence occurred more than 60,000 years ago and the genome size within the Poaceae family can differ by a factor of 40, the genetic structure seems to be largely conserved across genera (Devos & Gale, 2000; Keller & Feuillet, 2000). Indication for such a conserved genetic collinearity was also found in grass species for genes involved in the control of seed shattering (Paterson et al., 1995). Several genes responsible for seed shattering are known in barley, rice and sorghum. Therefore, these closely related species may be used to elucidate the mechanisms and the genetic control of seed shattering in ryegrasses.

2.1 | Breaking of the abscission layer leads to seed shattering

The mechanism of seed disarticulation in the wild crop progenitor species varies in terms of morphological structures. The morphological difference in the mechanism of losing seeds by shattering among grasses may be due to the different inflorescence architecture within the grass family (Bommert et al., 2005). Generally, seed shattering is associated with the formation of cells building an abscission layer (AL; Maity, Lamichaney, et al., 2021; Yu, Leyva, et al., 2020). Structure, formation as well as the anatomical location of the AL seem to be different among plant species (Maity, Lamichaney, et al., 2021). In ryegrasses, the AL contains 4–8 cell layers and its cells are smaller than the surrounding cells (Elgersma et al., 1988). Hydrolases, such as cholesteryl ester (CE) and peptidogylcan (PG) hydrolase, mediate the cleavage of abscission cell wall components (Bunya-atichart et al., 2011). In *Elymus sibiricus*, high seed shattering plants exhibited a smooth fracture edge in the rachilla compared to low seed shattering plants, suggesting a higher activity of hydrolytic enzymes in the AL of high seed shattering plants (Zhao et al., 2017). Further, several hormones, such as gibberellins, abscisic acid, cytokinin, ethylene, and auxin are involved in regulating seed shattering in the AL (Addicott, 1970; González-Carranza et al., 1998). While ethylene was identified to primarily regulate seed shattering, it can be counteracted by auxin, which, at high concentrations, may lead to reduced seed shattering (Patterson, 2001; Roberts et al., 2002).

In the domestication process of cereal crops, modification or loss of the AL led to an interruption of disarticulation and, therefore, to a shift towards seed retention, that is, a non-shattering phenotype (Doust et al., 2014). However, in wild ancestors of cereal crops, the formation of one or several AL(s) in different morphological structures within one spikelet was reported (Doust et al., 2014). The location of the AL varies considerably among different grass species (Figure 1; Doust et al., 2014). The most common and most likely ancestral state of the AL position in grasses is suggested to be located in the rachilla above the glumes (Yu, Leyva, et al., 2020). For several years, an AL was defined by containing small and lignified adjacent cells (Li & Olsen, 2016; Patterson, 2001). However, a recent publication in grasses showed that neither small cells in the AL nor lignification between cells in the AL is required for disarticulation in some grasses (Yu, Leyva, et al., 2020). In addition, the AL anatomy does not seem to be more similar in morphologically similar grass species when compared to morphologically more distinct species (Yu, Hu, et al., 2020). However, despite the considerable variation in AL anatomy, the set of genes in the AL seems to be largely conserved across species (Yu, Hu, et al., 2020).

2.2 | Two main mechanisms lead to seed shattering

Based on the location and the morphological structure of the AL, two main mechanisms are distinguished. The first mechanism is reported as brittle rachis seed shattering and seems to be unique within the Triticeae tribe (Figure 1; Zeng et al., 2020). Brittle rachis seed shattering occurs when the whole spikelet breaks from the rachis. One explanation is that species in the Triticeae tribe produce a spike as their inflorescence without secondary branching. The brittle rachis phenotype in barley was recently investigated and the AL was located either above or below the rachis nodes at grain maturity (Zeng et al., 2020; Figure 1, orange lines). In the wild barley ancestor *H. vulgare* subsp. *spontaneum* (C. Koch) Thell, histological analysis revealed an expansion of five to six cell layers in the AL, which resulted in a disruption of the mature spikelets of the rachis at the AL (Pourkheirandish et al., 2015). Furthermore, morphological studies revealed no reduction in lignin, cellulose or other cell wall polysaccharide content of the AL between brittle rachis and non-brittle rachis barley species. This suggests differences in cell wall thickness rather than differences in the composition of the cell wall to be responsible for the brittle rachis and non-brittle rachis phenotypes in barley (Pourkheirandish et al., 2015).

The second mechanism of seed shattering is referred to as brittle rachilla type of seed shattering and is found in species of the Pooideae subfamily (Sakuma et al., 2011). Here, seed disarticulation occurs in the secondary branches either below or above the glumes or below the rachilla nodes (Zeng et al., 2020; Figure 1, yellow lines). In rice, brittle rachilla seed disarticulation occurs below the lemmas and above the glumes (Yu & Kellogg, 2018; Figure 1). The AL is observed as a band of small cells, which develop before heading (Konishi et al., 2006; Li & Gill, 2006; Lv et al., 2018; Zhou et al., 2012). The AL in rice contains small cells with thin, non-lignified walls surrounding the lignified cells. During ripening of the seed, degradation of the AL cells leads to seed shattering. Lv et al. (2018) showed that a well-

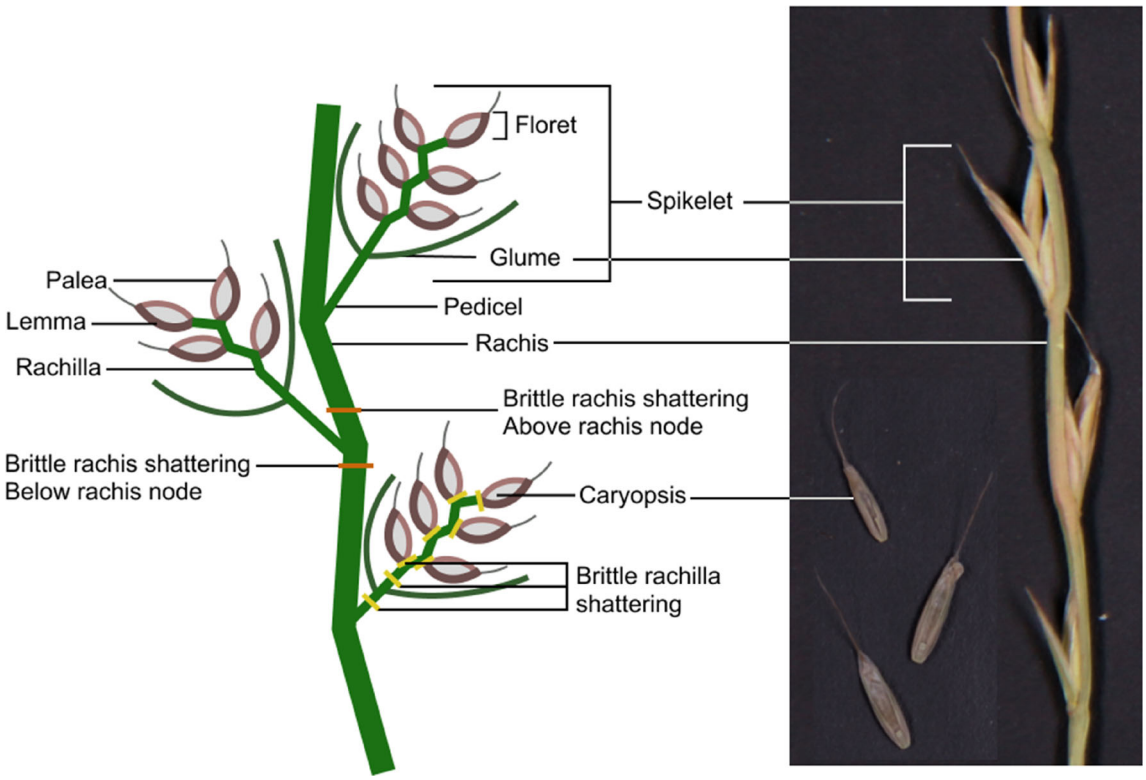

FIGURE 1 Spike morphology of ryegrasses (*Lolium* spp.). The different types of abscission layers indicated with orange and yellow lines illustrate the brittle rachis and the brittle rachilla type of seed shattering, respectively. The brittle rachis seed shattering can either happen below or above the rachis node. This type of seed shattering morphology is found for example in barley (*Hordeum vulgare* L.). The brittle rachilla seed shattering is located in the secondary branches and could happen species dependent below the glumes as in sorghum (*Sorghum bicolor* L. Moench), above the glumes as in rice (*Oryza sativa* L.) or below the florets as in perennial and Italian ryegrass (*L. perenne* L. and *L. multiflorum* Lam., respectively; adapted from Doust et al., 2014).

developed AL is linked to a seed shattering phenotype, while absence of the AL is linked to a non-shattering phenotype in rice. In domesticated sorghum, a reduction of seed shattering is related to the loss of the AL below the glumes (Dong & Wang, 2015; Figure 1). Although brittle rachis and brittle rachilla seed shattering is particularly frequent in particular groups of species, they both are sometimes also observed in the same species (Larson, 2019). For example, in intermediate wheatgrass (*Thinopyrum intermedium*; Altendorf et al., 2021) as well as in hybrid *Leymus* (Triticeae) wildryes (Larson & Kellogg, 2009), brittle rachis and brittle rachilla (floret) shattering were reported and phenotyped separately. A difference in domestication success between the two shattering mechanisms has so far not been observed.

In perennial ryegrass, seed disarticulation was reported as brittle rachilla seed shattering and happens below the rachilla nodes (Elgersma et al., 1988; Figure 1). There, formation of the AL leads to seed shattering. The AL is located above the glumes and below the florets (Figure 1) and can be observed after heading. In contrast to rice (Lv et al., 2018), no morphological difference in the AL between cultivars differing in their level of seed shattering was found (Elgersma et al., 1988). Also, no difference in lignin content of the AL in different cultivars after flowering stage was observed (Fu et al., 2019) and no cell wall degradation was found in seed shattering phenotypes of perennial ryegrass (Elgersma et al., 1988). One explanation for no cell wall degradation could be that seed shattering in ryegrasses is due to mechanical rather than biochemical breaking of the AL. In Italian ryegrass, a seed shattering mechanism with breakages below the rachilla nodes was predominant and, therefore, responsible for the loss of seeds, while brittle rachis seed shattering was not observed (unpublished data).

2.3 | Reducing seed shattering without completely removing the abscission layer

In domesticated rice and sorghum, a reduction of seed shattering always resulted from a loss of the AL (Dong & Wang, 2015). Therefore, in these two species, the presence of the AL seems to be always connected to seed shattering. In forage grasses, different levels of seed shattering were observed in different genotypes, all containing an AL. This could be due to seed size, seed weight and the stability of the glumes and inflorescence (McWilliam, 1980). Therefore, one strategy for forage grasses could be to breed for higher stability of the glumes and inflorescences to reduce seed shattering, rather than to avoid it completely. Whether seed shattering can be reduced without losing the breakage of the AL needs to be investigated. This different approach to reduce, but not completely prevent seed shattering in

forage grasses could have the advantage of still enabling natural dispersal of seeds and its propagation in permanent or semi-permanent grassland.

However, the breeding goal of reducing or completely removing seed shattering also depends on the accessibility of breeding material showing variation for this target trait. To date, breeding for higher glume or inflorescence stability was never investigated in forage grasses and to find an efficient phenotyping method to select for these traits might be very difficult. Therefore, more detailed knowledge of the genetic background of seed shattering will help to design an appropriate breeding scheme in forage grasses.

3 | GENETIC CONTROL OF SEED SHATTERING

The loss of seed shattering as a domestication trait underwent selection several times independently within the same species or also between different crop species (Doust et al., 2014; Lin et al., 2012; Paterson et al., 1995). Nevertheless, the selection led to the same functional changes, namely from a seed shattering to a non-shattering phenotype (Doust et al., 2014).

Knowledge of the genetic control of a trait is beneficial to determine efficient breeding strategies. For seed shattering in grasses, many QTL have been identified and some loci have been shown to be shared among different species (Dong & Wang, 2015). Further analyses in major crops such as barley, rice and sorghum resulted in the identification of genes controlling this trait (Figure 2). In many grass species, not only one major gene, but a rather complex network of several genes seems to be responsible for seed shattering (Dong & Wang, 2015). For example, expression studies in rice proposed the interaction of several genes influencing each other and resulting either in a reduction or a complete loss of seed shattering (Zhou et al., 2012). Although genes having an impact on seed shattering identified in different grass species are slightly different, many of them show a high degree of homology across species.

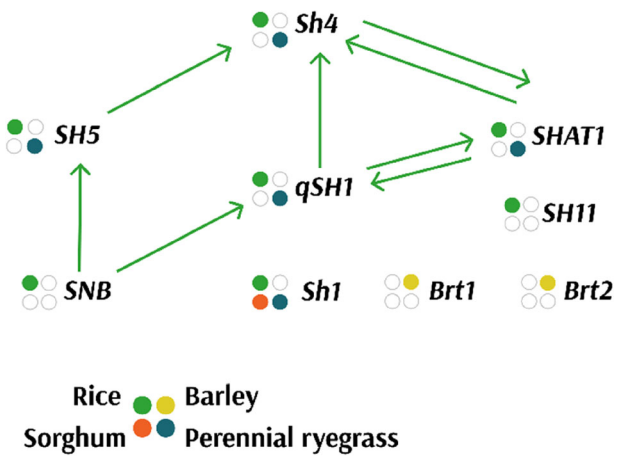

FIGURE 2 Genetic model for seed shattering in rice, sorghum, barley and perennial ryegrass. Green indicates all the genes which were found in rice (Jiang et al., 2019; Konishi et al., 2006; Li et al., 2006; Lin et al., 2007, 2012; Ning et al., 2023; Yoon et al., 2014; Zhou et al., 2012), yellow the genes found in barley (Pourkheirandish et al., 2015), orange the gene found in sorghum (Lin et al., 2012) and blue the genes which are suggested to be involved in seed shattering in perennial ryegrass (Fu et al., 2019). Green arrows indicate suggested relationships between genes in rice (Li & Olsen, 2016; Zhou et al., 2012).

3.1 | *Brt1* and *Brt2* are important for the non-seed shattering phenotype in barley

In barley, the two complementary and tightly linked genes *Brt1* and *Brt2* are known to be involved in the brittle rachis type of seed shattering (Takahashi & Hayashi, 1964). A loss-of-function mutation in either *Brt1* or *Brt2* changed the phenotype from brittle rachis to non-brittle rachis (Pourkheirandish et al., 2015). Either a 1 bp deletion in *Brt1* or an 11 bp deletion in *Brt2* resulted in loss of function and a non-seed shattering phenotype (Civáň & Brown, 2017). The protein functions of both genes are still unknown (Zeng et al., 2020), but the gene action was identified as recessive (Pourkheirandish et al., 2015). Thus, for a non-brittle rachis phenotype, either *brt1* or *brt2* need to be homozygous for the non-brittle rachis allele. In modern barley cultivars with the non-brittle rachis phenotype, either one or the other gene is homozygous, whereas no cultivar containing two copies of the non-shattering allele for both genes has so far been identified (Pourkheirandish et al., 2015). Histological experiments showed that plants with a non-brittle rachis phenotype containing the two recessive alleles of *brt1* or *brt2* do not show an expansion of cell layers (Haberer & Mayer, 2015). The non-brittle rachis genes, *Brt1* and *Brt2*, are conserved orthologues across the Triticeae genomes (Haas et al., 2019). In polyploid wheat, mutations in *Brt1* in the A and B subgenomes seem to be necessary to obtain a non-brittle rachis shattering phenotype (Avni et al., 2017). Furthermore, *Brt1* and *Brt2* were also found in diploid einkorn wheat (*T. monococcum*; Pourkheirandish et al., 2018), but were never mapped in other Pooideaes (Doust et al., 2014; Sakuma et al., 2011). Therefore, Pourkheirandish et al. (2015) speculated that the evolution of the brittle rachis type of seed shattering pathway in the Triticeae tribe follows a different molecular pathway than other cereals.

3.2 | Several genes control seed shattering in rice

For several years, *qSH1 and Sh4* were thought to be the two only genes responsible for the development of the brittle rachilla type of seed shattering (elimination of the AL below the glumes) during rice domestication (Konishi et al., 2006; Li et al., 2006). More recent studies suggested that the control of seed shattering in rice might be more complicated and that additional genes play a role. It was reported that *qSH1*, which encodes a BEL1-type homeobox-containing protein (Konishi et al., 2006), is downstream of *Sh4* and seed shattering abortion 1 (*SHAT1*), and maintains their expression in the AL, resulting in

AL differentiation (Zhou et al., 2012). One single nucleotide exchange in *qSH1* in the rice subspecies *japonica* resulted in a loss of expression of *qSH1* in the AL and explained 69% of the loss-of-seed shattering phenotype (Konishi et al., 2006). A mutation in *Sh4*, creating a reading frame shift, was found in mutagenized wild rice (*O. rufipogon*), resulting in a loss-of-function mutation in *Sh4* with a non-shattering phenotype (Jiang et al., 2019). Furthermore, the loss-of-function mutation in *Sh4* completely removes the AL (Zhou et al., 2012). Therefore, *Sh4* could also be involved in the activation of the disarticulation process (Li et al., 2006; Table 1). It seems that the expression of *Sh4* and *SHAT1* in the AL influences the AL formation during the early spikelet development stage. *Sh4* upregulate the expression of *SHAT1* in the AL (Zhou et al., 2012). Furthermore, an insertion in the gene *Shattering1* (*Sh1*), encoding for a YABBY transcription factor, resulted in a reduced seed shattering phenotype of the rice mutant SR-5 (Fukuta & Yagi, 1998; Lin et al., 2012). *Sh1* as well as *SHAT1* are two genes with a proven conserved role in the AL development not only in rice but across Poaceae (Lin et al., 2012; Lv et al., 2018; Simons et al., 2006; Yu & Kellogg, 2018; Zhou et al., 2012).

Recently, additional transcription factors controlling the AL were described. For example, a single nucleotide polymorphism (SNP) in the gene *SUPERNUMERARY BRACT* (*SNB*), which encodes for an APETALA2-like transcription factor, caused the change from a seed shattering to a non-shattering phenotype (Jiang et al., 2019). The transcription factor *SNB* is known to regulate the development of floral organs. In addition, *SNB* positively regulates *qSH1* and *SH5*. Like *qSH1*, *SH5* belongs to the BEL1-type homeobox transcription factors and is highly homologous to *qSH1*. *SH5* is suggested to positively regulate *Sh4* and it was found to be highly expressed in the AL (Yoon et al., 2014). *SH5* and *SHAT1* were shown to be important genes for the differentiation of the AL, but their importance in domestication by artificial selection still needs to be clarified (Dong & Wang, 2015). Recently, a knock-out mutation in *SH11*, which is a MYB transcription factor, showed a significant reduction of seed shattering in African rice (Ning et al., 2023). The interaction of several genes and their expression in the AL together with a large variation from non-shattering to almost complete shattering phenotypes, leads to the conclusion that seed shattering in rice is a quantitative trait regulated by many genes, rather than a qualitative trait regulated by just a single gene (Zhou et al., 2012).

3.3 | A single gene seems to control seed shattering in domesticated sorghum

In domesticated sorghum, *Shattering1* (*Sh1*) is suggested to control seed shattering in a dominant manner (Lin et al., 2012; Table 1). *Sh1* encodes a YABBY transcription factor and three different mutations

TABLE 1 Summary of genes reported to be involved in seed shattering of different grass species.

Plant species	Name	Gene category	Mechanism	Phenotypic effect	References
Oryza sativa	*Sh4* *Shattering4*, *SHA1*	MYB Transcription factor (TF)	May activate degradation of the cell wall in AL	Quantitative effect on seed shattering	(Li et al., 2006; Lin et al., 2007; Zhou et al., 2012)
Oryza sativa	*qSH1*	BEL1-type homeobox TF	Development of AL	Quantitative effect on seed shattering	(Konishi et al., 2006)
Oryza sativa	*SH5*	BEL1-type homeobox TF, homologue to *qSH1*	Induces *SHAT1* and *SH4*, expressed in AL	Inhibits lignin biosynthesis, enhances AL development, still needs *qSH1*	(Yoon et al., 2014)
Oryza sativa	*SHATTERING ABORTION1*, *SHAT1*	AP2-like TF	Differentiation of AL	Non-shattering, reduced spikelet fertility, changed morphology of floral parts, fewer primary branches	(Zhou et al., 2012)
Oryza sativa	*Shattering1*, *Sh1*	YABBY TF	Effect on AL unclear	Reduced shattering	(Lin et al., 2007, 2012)
Oryza glaberrima	*SH11*	MYB TF	Repress lignin deposition in AL	Knock out reduce seed shattering	(Ning et al., 2023)
Oryza rufipogon	*SSH1 allele of SNB*	AP2-like TF	Development of AL	Reduced seed shattering	(Jiang et al., 2019)
Sorghum bicolor	*Shattering1*, *Sh1*	YABBY TF	Effect on AL unclear	Non-shattering	(Lin et al., 2012)
Hordeum vulgare	*Brt1*		Unknown	Non-shattering	(Pourkheirandish et al., 2015)
Hordeum vulgare	*Brt2*		Unknown	Non-shattering	(Pourkheirandish et al., 2015)
Lolium perenne	*qSH1*	Putative transcription regulator BEL1-like homeobox TF	Unknown	Unknown	(Fu et al., 2019)
Lolium perenne	*Sh1*	Putative TF (YABBY)	Unknown	Unknown	(Fu et al., 2019)

of the *Sh1* gene in sorghum landraces were found to be associated with a non-shattering phenotype. The distribution of the origin of these landraces suggests three independent loss-of-function mutations in *Sh1* in sorghum. *Sh1* seems to be conserved in several other crops like rice. The *Sh1* orthologs in rice contain mutations, which can lead to a reduction of seed shattering in rice rather than a complete loss like in sorghum (Lin et al., 2012; Paterson et al., 1995). However, Lin et al. (2012) suggested that the *Sh1* genes in sorghum, rice and maize were under parallel selection during domestication.

3.4 | Unknown control of seed shattering in ryegrasses

Genes related to a non-shattering phenotype in ryegrasses are still largely unknown. In one study, all plants of a F_1 population derived from a cross between a genotype of *L. persicum*, (seed shattering) and a genotype of *L. temulentum* (non-seed shattering) displayed a shattering phenotype. The resulting F_2 population segregated in a ratio of 15 shattering to one non-shattering plants. This indicates two recessive genes to be involved in the non-shattering phenotype of *L. temulentum* (Senda et al., 2006). Whether the same mechanisms are in place in *L. multiflorum* or *L. perenne* remains unclear. Another study used the hypothesis that orthologous genes from other Poaceae could play a role in regulating seed shattering of perennial ryegrass. Nine putative candidate genes with a high similarity, among others to *qSH1*, *SHAT1*, *SH1* and *SH5*, were identified using an in-house transcriptome database of *L. perenne* (Fu et al., 2019). From these candidate genes, expression studies, together with histological analysis of the AL at different time points during seed development, revealed candidate genes that are highly similar to *qSH1* and *Sh1* of rice and that might play a role in AL formation in *L. perenne* (Figure 1; Fu et al., 2019).

4 | COMPARATIVE GENOME ANALYSIS TO FIND SEED SHATTERING CANDIDATE GENES IN RYEGRASS AND APPROACHES TO IMPROVE SEED YIELD

Some major cereal crops like barley seem to have developed distinguished molecular pathways for a non-shattering phenotype. Others, like rice and sorghum, although they are not close relatives, share some similarities of the non-shattering phenotype. Doust et al. (2014) showed that non-shattering grasses most probably share a common genetic pathway among various grass species. Furthermore, the hypothesis of a shared common genetic pathway is supported by a recent publication: Abrouk et al. (2020) found a 60 kb deletion within the *Sh1* ortholog from sorghum in several Fonio millet accessions. These accessions have a quantitatively reduced seed shattering phenotype compared to the other accessions without the deletion (Abrouk et al., 2020). Searching for orthologous genes in forage grasses known from major crops could be a promising approach to get a first idea on the seed shattering trait in these species.

4.1 | Comparative analysis revealed six candidate genes putatively involved in seed shattering in perennial ryegrass

With the possibility to cost-efficiently use third generation sequencing methods, the release of high-quality genome assemblies of forage grasses is increasing. This is a prerequisite to establish reverse genetic approaches like comparative analyses. The recently published genome of the doubled haploid *L. perenne* genotype 'Kyuss' (Frei et al., 2021), the genome of the highly homozygous *L. perenne* inbred genotype 'P226/135/16' (Nagy et al., 2022) and the genome of the highly heterozygous *L. multiflorum* genotype 'Rabiosa' (Copetti et al., 2021) are the first high-quality diploid reference assemblies available for ryegrasses. We used the genome sequence of the doubled haploid *L. perenne* genotype 'Kyuss' as reference to conduct a comparative analysis for genes involved in the control of seed shattering in other grass species. For this, candidate genes involved in seed shattering from barley, rice, perennial ryegrass, and sorghum were obtained from published articles (Table 1). The proteins encoded by these target candidate genes were then, for the first time, used to find highly similar genes in the perennial ryegrass reference genome 'Kyuss' (Frei et al., 2021). Each protein sequence was aligned and compared to the perennial ryegrass reference genome using BLASTP 2.9.0+ (Altschul et al., 1997; Schäffer et al., 2001). Candidate genes were identified using the highest bit score (the highest alignment score between the query and the reference sequence), the highest percentage of identity (between the query and the reference sequence) and the highest E-value (the probability that the observed match between the query and the reference sequence is due to chance). With these parameters, our comparative analysis of seed shattering genes from barley, rice and sorghum in perennial ryegrass revealed 11 different putative candidate genes in the 'Kyuss' reference genome (Table 2). In general, the highest hits were found for *qSH1* and *SH5* from rice. Starting with *qSH1*, two highly similar sequences were found in perennial ryegrass with a bit-score of 732 and 674, identities of 72% and 68% and E-values of 0 and 0, respectively. *SH5*, which is highly homologous to *qSH1* in rice (Yoon et al., 2014), was also found in perennial ryegrass with the two highest hits on the same two genes as found for *qSH1* (KYUSt_chr3.34849 and KYUSt_chr1.33748; Table 2), marking these two as particularly interesting putative candidate genes. In *L. perenne*, *qSH1* is predicted to be involved in development and formation of the AL as well (Fu et al., 2019). For *SHAT1 as well as for SSH1*, two similar genes, KYUSt_chr2.53254 and KYUSt_chr4.5079 were found with high identities (74% and 62%, respectively), bit-scores (512 and 338, respectively) and E-values (4.00E-180 and 8.00E-110, respectively) (Table 2). According to Fu et al. (2019), *SHAT1* is regulated downstream of *qSH1* and plays a role in the differentiation of the AL in perennial ryegrass. Lower hit scores were found for *SH4*, *Sh1*, *and*

TABLE 2 Results of the comparative analysis (BLASTP) of known seed shattering genes in *Oryza sativa* and *Sorghum bicolor* (listed in Table 1) aligned to the genome sequence of the doubled haploid *Lolium perenne* genotype 'Kyuss' (Frei et al., 2021).

Species	Gene annotation name	Common name	Score (bits)	E-value	Identity (%)	Gene name in *L. perenne* Kyuss	Reference
Oryza sativa	LOC_Os01g62920	*qSH1*	732	0.00E+00	72%	KYUSt_chr3.34849	(Konishi et al., 2006)
			674	0.00E+00	68%	KYUSt_chr1.33748	
Oryza sativa	LOC_Os05g38120	*SH5*	804	0.00E+00	81%	KYUSt_chr1.33748	(Yoon et al., 2014)
			630	0.00E+00	64%	KYUSt_chr3.34849	
Oryza sativa	LOC_Os04g55560	*SHAT1*	512	4.00E-180	74%	KYUSt_chr2.53254	(Zhou et al., 2012)
			338	8.00E-110	62%	KYUSt_chr4.5079	
Oryza sativa	LOC_Os04g57530	*Shattering1*, *SH1*	173	1.00E-50	34%	KYUSt_chr4.15900	(Fukuta & Yagi, 1998)
Oryza sativa	ORGLA04G0254300	*SH4*	168	3.00E-49	34%	KYUSt_chr4.15900	(Li et al., 2006)
			107	4.00E-26	82%	KYUSt_chr2.55506	
Oryza rufipogon		*SSH1 allele of SNB*	440	1e-149	66%	KYUSt_chr4.5079	(Jiang et al., 2019)
			306	7e-101	77%	KYUSt_chr1.2807	
			263	3e-83	81%	KYUSt_chr2.53254	
Oryza glaberrima		*SH11*	330	4e-114	68%	KYUSt_chr4.25513	(Ning et al., 2023)
			317	5e-109	65%	KYUSt_chr5.7779	
Sorghum bicolor	Sobic.001G152901	*SH1*	108	2.00E-29	84%	KYUSt_chr6.15457	(Lin et al., 2012)
			108	5.00E-29	66%	KYUSt_chr2.44485	

SH11 (from rice and sorghum). *Brt1* and *Brt2* from barley do not show any remarkable similarity in the *L. perenne* reference genome investigated.

4.2 | Prospects for breeding low seed shattering forage grass cultivars

The natural variation for seed shattering observed in ryegrasses (Maity et al., 2021; Peter et al., 2021) provides the basis for improvement of seed shattering through targeted selection. Since phenotyping of seed shattering is rather laborious and requires the plants to be grown to seed maturity, genomics assisted breeding tools have the potential to make breeding for seed shattering more efficient.

The 11 putative seed shattering regulatory genes found in our comparative analysis (Table 2) may be used for functional validation. For example, gene expression studies in genotypes with varying degrees of shattering conducted after flowering until seed ripening could further indicate which of the putative candidate genes might play a role in seed shattering. A first expression study in perennial ryegrass indicated that *Sh1* and *qSH1* could be involved in differentiation of the AL and, therefore, regulating seed shattering in perennial ryegrass (Fu et al., 2019). In particular, *Sh1* and *qSH1* are suggested to play a role in abscission layer formation during seed development.

In our comparative analysis, we found a gene highly similar to *qSH1* from rice, but not for *Sh1* (Table 2). One explanation for the lack of similarity with *Sh1* might be that different cultivars could have different genes responsible for regulating shattering and that the genes depend on the origin of the breeding material. To address whether *Sh1* is involved in seed shattering in some genotypes but not in others, further comparative analyses with different references assemblies or targeted expression studies in selected germplasm are essential. Thanks to initiatives like the International *Lolium-Festuca* Pangenome Consortium (ILFPC), the number of available reference assemblies in forage grasses is expected to increase in the near future (Studer et al., 2021). This will enable additional comparative studies to detect a potential involvement of *Sh1* in seed shattering. In contrast to *Sh1*, *qSH1* was identified in our comparative analysis in perennial ryegrass and is, therefore, highly likely to be involved in seed shattering. Thus, *qSH1* is a valuable candidate for functional validation through knock-out mutagenesis.

Using forward genetic approaches (i.e., identifying the genetic basis behind a specific phenotype), additional evidence for the function of potential candidate genes may be gained. In addition, these approaches may also be used to identify other candidate genes for seed shattering in addition to *Sh1* and *qSH1*. First, bi-parental QTL mapping is, especially in outcrossing, self-incompatible forage grasses, frequently and successfully used to elucidate the genetic basis of several traits. These include vernalization response (Jensen et al., 2005), flowering time and its morphological constituents (Byrne et al., 2009), heading date (Armstead et al., 2004) or lodging resistance (Inoue et al., 2004). Although bi-parental QTL mapping populations are easy to establish, these populations are only based on two paternal plants and, therefore, do not reflect the allele diversity of a breeding population. Furthermore, due to the low number of recombination events in an F_1 bi-parental population of limited size, the resolution of the mapping is usually limited and not useful for pinpointing individual genes controlling the target trait.

Second, genome-wide association studies (GWAS) can cover the whole diversity of the breeding gene pool, but have the drawback to potentially miss genes represented at a low frequency within the population. Third, multi-parent populations like nested association mapping (NAM) populations could help to overcome that obstacle and result in higher resolution and higher statistical power, particularly useful for quantitative traits. However, independent of the type of mapping population (bi-parental, GWAS panel, or multi-parent population), presence of significant phenotypic variation is a prerequisite in all forward genetic approaches. In comparison to major cereals, the domestication history of forage grasses is very short and, to the best of our knowledge, there is yet no genotype of a commonly bred *Lolium* or *Festuca* species that shows a clearly non-shattering phenotype. Hence, as most breeding materials that might serve as parents for a potential mapping population are still showing a relatively high degree of seed shattering, this limited variation towards the non-shattering phenotype might reduce the success in finding major genes controlling seed shattering. Nevertheless, such studies might still be helpful to identify minor effect genes that reduce seed shattering via pathways different not involving the AL, like for example, stability of glumes and inflorescence (McWilliam, 1980). An alternative to increase phenotypic variation in bi- or multi-parental mapping populations might be the inclusion of exotic materials or other species such as *L. temulentum*, the latter showing a non-shattering phenotype (Senda et al., 2006).

If available variation for a target trait in the relevant breeding material is a limiting factor but potential candidate genes are known, reverse genetic approaches might be a suitable option. In such approaches, the candidate genes identified (through the comparative analyses presented or through additional mapping approaches) could be used as targets for TILLING (Targeting Induced Local Lesions IN Genomes) or genome editing. Thereby, a gene of interest is disrupted by either random (TILLING, e.g., x-rays, ultraviolet irradiation or chemical treatment) or targeted (genome editing, e.g., using CRISPR/Cas9) mutagenesis. The mutated plants are subsequently phenotyped to demonstrate the effect of the mutation, which is commonly known as a functional validation. If the phenotype resulting from a disrupted gene is also the one favoured by breeding, these approaches are also useful to produce suitable phenotypic variation that can be used for further breeding activities. This is clearly the case in the context of seed shattering, where the knockout of a gene responsible for forming the AL would, for example, result in a less- or non-shattering phenotype.

TILLING could be useful to transfer the knowledge of genes of model systems to other crops. TILLING has been implemented in many crops such as wheat (Slade et al., 2005; Uauy et al., 2009), rice (Suzuki et al., 2008; Till et al., 2003), barley (Caldwell et al., 2004; Talamè et al., 2008) or sorghum (Xin et al., 2008). In forage grasses, three TILLING populations consisting of 550, 1350 and 1700 M_1 plants were successfully established (Manzanares et al., 2016). As regulations on genetically modified crops, strongly limiting their use in European countries, do not apply to plants derived from TILLING treated with classical mutagens, these populations could be directly used to identify advantageous mutations and to introgress them into breeding programs. On the other hand, genome editing could potentially also be used to validate candidate genes for seed shattering and to transfer reduced seed shattering into elite cultivars. However, there are only very few records successful genome editing in ryegrass so far (Grogg et al., 2019; Kumar et al., 2022; Zhang et al., 2020) and most other forage grass species lack adapted transformation and gene editing systems. In addition, the current regulation in Europe does not allow for using plants derived from genome editing in breeding programs.

In conclusion, by reviewing the literature on seed shattering mainly in major grass species such as barley, rice and sorghum, and by combining the insights with a comparative genetic analysis in perennial ryegrass, we identified a number of candidate genes involved in seed shattering in *L. perenne*. These candidate genes not only provide targets for future functional validation, but may already be used for marker assisted selection approaches in existing breeding programs. Another, more immediate approach to reduce seed shattering in existing breeding programs would be through direct phenotypic selection for plants with reduced seed shattering. For this, as well as for elucidating the genetic control of seed shattering in forage grasses, a suitable phenotyping method is indispensable and urgently needs to be developed.

ACKNOWLEDGEMENTS

The authors would like to thank Anjo Elgersma for her support as research peer and Linda Helene Schlatter for proof-reading the manuscript. This study was funded through the Breeding Foundation DSP-BLW project 'Improving Seed Yield in Swiss Bred Italian Ryegrass (IMPSEED)'.

FUNDING INFORMATION

Breeding foundation DSP-BLW.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest with the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Jenny Kiesbauer https://orcid.org/0009-0000-4784-9376
Christoph Grieder https://orcid.org/0000-0003-0359-285X
Bruno Studer https://orcid.org/0000-0001-8795-0719
Roland Kölliker https://orcid.org/0000-0003-1959-0402

REFERENCES

Abrouk, M., Ahmed, H. I., Cubry, P., Šimoníková, D., Cauet, S., Pailles, Y., Bettgenhaeuser, J., Gapa, L., Scarcelli, N., Couderc, M., Zekraoui, L., Kathiresan, N., Čížková, J., Hřibová, E., Doležel, J., Arribat, S., Bergès, H., Wieringa, J. J., Gueye, M., & Krattinger, S. G. (2020). Fonio millet genome unlocks African orphan crop diversity for agriculture in a changing climate. *Nature Communications*, *11*(1), 4488. https://doi.org/10.1038/s41467-020-18329-4

Addicott, F. T. (1970). Plant hormones in the control of abscission. *Biological Reviews*, *45*(4), 485–524.

Altendorf, K. R., DeHaan, L. R., Larson, S. R., & Anderson, J. A. (2021). QTL for seed shattering and threshability in intermediate wheatgrass align closely with well-studied orthologs from wheat, barley, and rice. *The Plant Genome*, *14*(3), e20145. https://doi.org/10.1002/tpg2.20145

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389–3402.

Armstead, I. P., Turner, L. B., Farrell, M., Skøt, L., Gomez, P., Montoya, T., Donnison, I. S., King, I. P., & Humphreys, M. O. (2004). Synteny between a major heading-date QTL in perennial ryegrass (*Lolium perenne* L.) and the Hd3 heading-date locus in rice. *Theoretical and Applied Genetics*, *108*(5), 822–828. https://doi.org/10.1007/s00122-003-1495-6

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., Hale, I., Mascher, M., Spannagl, M., Wiebe, K., Jordan, K. W., Golan, G., Deek, J., Ben-Zvi, B., Ben-Zvi, G., Himmelbach, A., Maclachlan, R. P., Sharpe, A. G., Fritz, A., & Distelfeld, A. (2017). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science*, *357*(6346), 93–97. https://doi.org/10.1126/science.aan0032

Boelt, B., & Studer, B. (2010). Breeding for grass seed yield. In B. Boller, U. K. Posselt, & F. Veronesi (Eds.), *Fodder crops and amenity grasses* (pp. 161–174). Springer. https://doi.org/10.1007/978-1-4419-0760-8_7

Boller, B., Posselt, U. K., & Veronesi, F. (Eds.). (2010). *Fodder crops and amenity grasses*. Springer. https://doi.org/10.1007/978-1-4419-0760-8

Bommert, P., Satoh-Nagasawa, N., Jackson, D., & Hirano, H.-Y. (2005). Genetics and evolution of inflorescence and flower development in grasses. *Plant and Cell Physiology*, *46*(1), 69–78. https://doi.org/10.1093/pcp/pci504

Bunya-atichart, K., Ketsa, S., & van Doorn, W. G. (2011). Ethylene-sensitive and ethylene-insensitive abscission in dendrobium: Correlation with polygalacturonase activity. *Postharvest Biology and Technology*, *60*(1), 71–74. https://doi.org/10.1016/j.postharvbio.2010.11.006

Byrne, S., Guiney, E., Barth, S., Donnison, I., Mur, L. A. J., & Milbourne, D. (2009). Identification of coincident QTL for days to heading, spike length and spikelets per spike in *Lolium perenne* L. *Euphytica*, *166*(1), 61–70. https://doi.org/10.1007/s10681-008-9831-1

Caldwell, D. G., McCallum, N., Shaw, P., Muehlbauer, G. J., Marshall, D. F., & Waugh, R. (2004). A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.). *The Plant Journal*, *40*(1), 143–150. https://doi.org/10.1111/j.1365-313X.2004.02190.x

Casler, M. D. (2001). Breeding forage crops for increased nutritional value. In *Advances in Agronomy* (Vol. 71, pp. 51–107). Academic Press. https://doi.org/10.1016/S0065-2113(01)71012-7

Casler, M. D., & Vogel, K. P. (2020). Forage breeding. In *Forages* (pp. 553–565). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119436669.ch30

Civáň, P., & Brown, T. A. (2017). A novel mutation conferring the nonbrittle phenotype of cultivated barley. *New Phytologist*, *214*(1), 468–472. https://doi.org/10.1111/nph.14377

Clayton, W. D., & Renvoize, S. A. (1986). Genera Gaminum. In W. D. Clayton & S. A. Renvoize (Eds.), *Grasses of the World* (Vol. 36). Kew Royal Botanical Gardens.

Copetti, D., Yates, S. A., Vogt, M. M., Russo, G., Grieder, C., Kölliker, R., & Studer, B. (2021). *Evidence for high intergenic sequence variation in heterozygous Italian ryegrass (* Lolium multiflorum *Lam.) genome revealed by a high-quality draft diploid genome assembly* [preprint]. *bioRxiv*, 2021.05.05.442707. https://doi.org/10.1101/2021.05.05.442707

Devos, K. M., & Gale, M. D. (2000). Genome relationships: the grass model in current research. *Plant Cell*, *12*(5), 637–646. https://doi.org/10.1105/tpc.12.5.637

Dong, Y., & Wang, Y.-Z. (2015). Seed shattering: From models to crops. *Frontiers in Plant Science*, *6*, 476. https://doi.org/10.3389/fpls.2015.00476

Doust, A. N., Mauro-Herrera, M., Francis, A. D., & Shand, L. C. (2014). Morphological diversity and genetic regulation of inflorescence abscission zones in grasses. *American Journal of Botany*, *101*(10), 1759–1769. https://doi.org/10.3732/ajb.1400186

Elgersma, A., Leeuwangh, J. E., & Wilms, H. J. (1988). Abscission and seed shattering in perennial ryegrass (*Lolium perenne* L.). *Euphytica*, *39*(S3), 51–57. https://doi.org/10.1007/BF00043367

Falcinelli, M. (1999). Temperate forage seed production: Conventional and potential breeding strategies. *Journal of New Seeds*, *1*(1), 37–66. https://doi.org/10.1300/J153v01n01_04

Food and Agriculture Organization of the United Nations. (2020). "FAOSTAT Statistical Database.". https://www.fao.org/faostat/en/#data/RL.

Frei, D., Veekman, E., Grogg, D., Stoffel-Studer, I., Morishima, A., Shimizu-Inatsugi, R., Yates, S., Shimizu, K. K., Frey, J. E., Studer, B., & Copetti, D. (2021). Ultralong oxford nanopore reads enable the development of a reference-grade perennial ryegrass genome assembly. *Genome Biology and Evolution*, *13*(8), evab159. https://doi.org/10.1093/gbe/evab159

Fu, Z., Song, J., Zhao, J., & Jameson, P. E. (2019). Identification and expression of genes associated with the abscission layer controlling seed shattering in *Lolium perenne*. *AoB Plants*, *11*(1), ply076. https://doi.org/10.1093/aobpla/ply076

Fukuta, Y., & Yagi, T. (1998). Mapping of a shattering resistance gene in a mutant line SR-5 induced from an indica rice variety, Nan-jing11. *Breeding Science*, *48*(4), 345–348.

Fuller, D. Q., & Allaby, R. (2009). Seed dispersal and crop domestication: Shattering, germination and seasonality in evolution under cultivation. In *Annual Plant Reviews Volume 38: Fruit Development and Seed Dispersal* (pp. 238–295). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444314557.ch7

González-Carranza, Z. H., Lozoya-Gloria, E., & Roberts, J. A. (1998). Recent developments in abscission: Shedding light on the shedding process.

Trends in Plant Science, *3*(1), 10–14. https://doi.org/10.1016/S1360-1385(97)01132-1

Griffiths, D. J., Roberts, H. M., & Lewis, J. (1973). "The seed yield potential of grasses.". 117–123 [Annual Report].

Grogg, D., Studer, B., & Broggini, G. A. L. (2019). Regeneration of suspension cultures for genome editing in perennial ryegrass. *Grassland Science in Europe*, *24*, 253–255. https://doi.org/10.3929/ETHZ-B-000353838.

Haas, M., Schreiber, M., & Mascher, M. (2019). Domestication and crop evolution of wheat and barley: Genes, genomics, and future directions. *Journal of Integrative Plant Biology*, *61*(3), 204–225. https://doi.org/10.1111/jipb.12737

Haberer, G., & Mayer, K. F. X. (2015). Barley: From brittle to stable harvest. *Cell*, *162*(3), 469–471. https://doi.org/10.1016/j.cell.2015.07.023

Harlan, J. R. (1975). Crops and man. In *Crops and man* (pp. 147–170). American Society of Agronomy, Madison. https://doi.org/10.1002/9780891186342.ch6

Humphreys, M., Feuerstein, U., Vandewalle, M., & Baert, J. (2010). Ryegrasses. In B. Boller, U. K. Posselt, & F. Veronesi (Eds.), *Fodder crops and amenity grasses* (pp. 211–260). Springer. https://doi.org/10.1007/978-1-4419-0760-8_10

Humphreys, M. W., Yadav, R. S., Cairns, A. J., Turner, L. B., Humphreys, J., & Skøt, L. (2006). A changing climate for grassland research. *New Phytologist*, *169*(1), 9–26. https://doi.org/10.1111/j.1469-8137.2005.01549.x

Inoue, M., Gao, Z., & Cai, H. (2004). QTL analysis of lodging resistance and related traits in Italian ryegrass (*Lolium multiflorum* lam.). *Theoretical and Applied Genetics*, *109*(8), 1576–1585. https://doi.org/10.1007/s00122-004-1791-9

Jensen, L. B., Andersen, J. R., Frei, U., Xing, Y., Taylor, C., Holm, P. B., & Luebberstedt, T. (2005). QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat VRN1. *Theoretical and Applied Genetics*, *110*(3), 527–536. https://doi.org/10.1007/s00122-004-1865-8

Ji, H.-S., Chu, S.-H., Jiang, W., Cho, Y.-I., Hahn, J.-H., Eun, M.-Y., McCouch, S. R., & Koh, H.-J. (2006). Characterization and mapping of a shattering mutant in rice that corresponds to a block of domestication genes. *Genetics*, *173*(2), 995–1005. https://doi.org/10.1534/genetics.105.054031

Jiang, L., Ma, X., Zhao, S., Tang, Y., Liu, F., Gu, P., Fu, Y., Zhu, Z., Cai, H., Sun, C., & Tan, L. (2019). The APETALA2-like transcription factor SUPERNUMERARY BRACT controls rice seed shattering and seed size. *The Plant Cell*, *31*(1), 17–36. https://doi.org/10.1105/tpc.18.00304

Jung, G. A., Van Wijk, A. J. P., Hunt, W. F., & Watson, C. E. (1996). Ryegrasses. In *Cool-season forage grasses* (pp. 605–641). John Wiley & Sons, Ltd. https://doi.org/10.2134/agronmonogr34.c19

Keller, B., & Feuillet, C. (2000). Colinearity and gene density in grass genomes. *Trends in Plant Science*, *5*(6), 246–251. https://doi.org/10.1016/S1360-1385(00)01629-0

Kellogg, E. A. (1998). Relationships of cereal crops and other grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(5), 2005–2010. https://doi.org/10.1073/pnas.95.5.2005

Konishi, S., Izawa, T., Lin, S. Y., Ebana, K., Fukuta, Y., Sasaki, T., & Yano, M. (2006). An SNP caused loss of seed shattering during rice domestication. *Science*, *312*(5778), 1392–1396. https://doi.org/10.1126/science.1126410

Kumar, R., Kamuda, T., Budhathoki, R., Tang, D., Yer, H., Zhao, Y., & Li, Y. (2022). Agrobacterium and a single Cas9-sgRNA transcript system-mediated high efficiency gene editing in perennial ryegrass. *Frontiers in Genome Editing*, *4*, 960414. https://doi.org/10.3389/fgeed.2022.960414

Larson, S. (2019). Genome mapping of quantitative trait loci (QTL) controlling domestication traits of intermediate wheatgrass (*Thinopyrum intermedium*). *Theoretical and Applied Genetics*, *27*, 2325–2351.

Larson, S., & Kellogg, E. A. (2009). Genetic dissection of seed production traits and identification of a major-effect seed retention qtl in hybrid *Leymus* (Triticeae) wildryes. *Crop Science*, *49*(1), 29–40. https://doi.org/10.2135/cropsci2008.05.0277

Li, C., Zhou, A., & Sang, T. (2006). Rice domestication by reducing shattering. *Science*, *311*(5769), 1936–1939. https://doi.org/10.1126/science.1123604

Li, L.-F., & Olsen, K. M. (2016). Chapter three—to have and to hold: Selection for seed and fruit retention during crop domestication. In V. Orgogozo (Ed.), *Current topics in developmental biology* (Vol. 119, pp. 63–109). Academic Press. https://doi.org/10.1016/bs.ctdb.2016.02.002

Li, W., & Gill, B. S. (2006). Multiple genetic pathways for seed shattering in the grasses. *Functional & Integrative Genomics*, *6*(4), 300–309. https://doi.org/10.1007/s10142-005-0015-y

Lin, Z., Griffith, M. E., Li, X., Zhu, Z., Tan, L., Fu, Y., Zhang, W., Wang, X., Xie, D., & Sun, C. (2007). Origin of seed shattering in rice (*Oryza sativa* L.). *Planta*, *226*(1), 11–20. https://doi.org/10.1007/s00425-006-0460-4

Lin, Z., Li, X., Shannon, L. M., Yeh, C.-T., Wang, M. L., Bai, G., Peng, Z., Li, J., Trick, H. N., Clemente, T. E., Doebley, J., Schnable, P. S., Tuinstra, M. R., Tesso, T. T., White, F., & Yu, J. (2012). Parallel domestication of the Shattering1 genes in cereals. *Nature Genetics*, *44*(6), 720–724. https://doi.org/10.1038/ng.2281

Lüscher, A., Grieder, C., Huguenin-Elie, O., Klaus, V., Reidy, B., Schneider, M. K., Schubiger, F., Suter, D., Suter, M., & Kölliker, R. (2019). Grassland systems in Switzerland with a main focus on sown grasslands. *Grassland Science in Europe*, *24*, 3–16. https://doi.org/10.3929/ETHZ-B-000353583

Lv, S., Wu, W., Wang, M., Meyer, R. S., Ndjiondjop, M.-N., Tan, L., Zhou, H., Zhang, J., Fu, Y., Cai, H., Sun, C., Wing, R. A., & Zhu, Z. (2018). Genetic control of seed shattering during African rice domestication. *Nature Plants*, *4*(6), 331–337. https://doi.org/10.1038/s41477-018-0164-3

Maity, A., Lamichaney, A., Joshi, D. C., Bajwa, A., Subramanian, N., Walsh, M., & Bagavathiannan, M. (2021). Seed shattering: A trait of evolutionary importance in plants. *Frontiers in Plant Science*, *12*, 1180. https://doi.org/10.3389/fpls.2021.657773

Maity, A., Singh, V., Martins, M. B., Ferreira, P. J., Smith, G. R., & Bagavathiannan, M. (2021). Species identification and morphological trait diversity assessment in ryegrass (*Lolium* spp.) populations from the Texas Blackland prairies. *Weed Science*, *69*(3), 379–392. https://doi.org/10.1017/wsc.2021.18

Manzanares, C., Yates, S., Ruckle, M., Nay, M., & Studer, B. (2016). TILLING in forage grasses for gene discovery and breeding improvement. *New Biotechnology*, *33*(5 Pt B), 594–603. https://doi.org/10.1016/j.nbt.2016.02.009

Marshall, A. M., & Wilkins, R. W. (2003). Improved seed yield in perennial ryegrass (*Lolium perenne* L.) from two generations of phenotypic selection. *Euphytica*, *133*(2), 233–241. Scopus. https://doi.org/10.1023/A:1025593808010

McWilliam, J. R. (1980). The development and significance of seed retention in grasses. In P. D. Hebblethwaite (Ed.), *Seed production*. Butterworths.

Nagy, I., Veeckman, E., Liu, C., Bel, M. V., Vandepoele, K., Jensen, C. S., Ruttink, T., & Asp, T. (2022). Chromosome-scale assembly and annotation of the perennial ryegrass genome. *BMC Genomics*, *23*(1), 505. https://doi.org/10.1186/s12864-022-08697-0

Ning, J., He, W., Wu, L., Chang, L., Hu, M., Fu, Y., Liu, F., Sun, H., Gu, P., Ndjiondjop, M.-N., Sun, C., & Zhu, Z. (2023). The MYB transcription factor seed shattering 11 controls seed shattering by repressing lignin synthesis in African rice. *Plant Biotechnology Journal*, *21*(5), 931–942. https://doi.org/10.1111/pbi.14004

Onishi, K., Takagi, K., Kontani, M., Tanaka, T., & Sano, Y. (2007). Different patterns of genealogical relationships found in the two major QTLs causing reduction of seed shattering during rice domestication. *Genome*, *50*(8), 757–766. https://doi.org/10.1139/G07-051

Paterson, A. H., Lin, Y.-R., Li, Z., Schertz, K. F., Doebley, J. F., Pinson, S. R. M., Liu, S.-C., Stansel, J. W., & Irvine, J. E. (1995). Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science*, *269*(5231), 1714–1718. https://doi.org/10.1126/science.269.5231.1714

Patterson, S. E. (2001). Cutting loose. Abscission and dehiscence in *Arabidopsis*. *Plant Physiology*, *126*(2), 494–500. https://doi.org/10.1104/pp.126.2.494

Pellicer, J., & Leitch, I. J. (2020). The plant DNA C-values database (release 7.1): An updated online repository of plant genome size data for comparative studies. *The New Phytologist*, *226*(2), 301–305. https://doi.org/10.1111/nph.16261

Peter, J., Kölliker, R., Studer, B., & Grieder, C. (2021). Italian ryegrass—higher seed yield through targeted selection for low seed shattering. In *Exploiting genetic diversity of forages to fulfil their economic and environmental roles: Proceedings of the 2021 meeting of the fodder crops and amenity grasses section of EUCARPIA* (pp. 117–120). Palacký University. https://doi.org/10.5507/vup.21.24459677.28

Pourkheirandish, M., Dai, F., Sakuma, S., Kanamori, H., Distelfeld, A., Willcox, G., Kawahara, T., Matsumoto, T., Kilian, B., & Komatsuda, T. (2018). On the origin of the non-brittle rachis trait of domesticated einkorn wheat. *Frontiers in Plant Science*, *8*, 2031. https://doi.org/10.3389/fpls.2017.02031

Pourkheirandish, M., Hensel, G., Kilian, B., Senthil, N., Chen, G., Sameri, M., Azhaguvel, P., Sakuma, S., Dhanagond, S., Sharma, R., Mascher, M., Himmelbach, A., Gottwald, S., Nair, S. K., Tagiri, A., Yukuhiro, F., Nagamura, Y., Kanamori, H., Matsumoto, T., & Komatsuda, T. (2015). Evolution of the grain dispersal system in barley. *Cell*, *162*(3), 527–539. https://doi.org/10.1016/j.cell.2015.07.002

Roberts, J. A., Elliott, K. A., & Gonzalez-Carranza, Z. H. (2002). Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology*, *53*(1), 131–158. https://doi.org/10.1146/annurev.arplant.53.092701.180236

Sakuma, S., Salomon, B., & Komatsuda, T. (2011). The domestication syndrome genes responsible for the major changes in plant form in the Triticeae crops. *Plant and Cell Physiology*, *52*(5), 738–749. https://doi.org/10.1093/pcp/pcr025

Schäffer, A. A., Aravind, L., Madden, T. L., Shavirin, S., Spouge, J. L., Wolf, Y. I., Koonin, E. V., & Altschul, S. F. (2001). Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Research*, *29*(14), 2994–3005.

Schmid, K., Kilian, B., & Russell, J. (2018). Barley domestication, adaptation and population genomics. In N. Stein & G. J. Muehlbauer (Eds.), *The barley genome* (pp. 317–336). Springer International Publishing. https://doi.org/10.1007/978-3-319-92528-8_17

Senda, T., Hiraoka, Y., & Tominaga, T. (2006). Inheritance of seed shattering in *Lolium temulentum* and *L. persicum* hybrids. *Genetic Resources and Crop Evolution*, *53*(3), 449–451. https://doi.org/10.1007/s10722-005-6096-6

Simons, K. J., Fellers, J. P., Trick, H. N., Zhang, Z., Tai, Y.-S., Gill, B. S., & Faris, J. D. (2006). Molecular characterization of the major wheat domestication gene *Q*. *Genetics*, *172*(1), 547–555. https://doi.org/10.1534/genetics.105.044727

Slade, A. J., Fuerstenberg, S. I., Loeffler, D., Steine, M. N., & Facciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature Biotechnology*, *23*(1), 75–81. https://doi.org/10.1038/nbt1043

Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Teisher, J. K., Clark, L. G., Barberá, P., Gillespie, L. J., & Zuloaga, F. O. (2017). A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *Journal of Systematics and Evolution*, *55*(4), 259–290. https://doi.org/10.1111/jse.12262

Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., Filgueiras, T. S., Davis, J. I., & Morrone, O. (2015). A worldwide phylogenetic classification of the Poaceae (Gramineae). *Journal of Systematics and Evolution*, *53*(2), 117–137. https://doi.org/10.1111/jse.12150

Studer, B., Jensen, L. B., Hentrup, S., Brazauskas, G., Kölliker, R., & Lübberstedt, T. (2008). Genetic characterisation of seed yield and fertility traits in perennial ryegrass (*Lolium perenne* L.). *Theoretical and Applied Genetics*, *117*(5), 781–791. https://doi.org/10.1007/s00122-008-0819-y

Studer, B., Rohner, M., Manzanares, C., Cropano, C., Vogt, M., Grogg, D., Manzanares, C., Yates, S., & International Lolium-Festuca Pangenome Consortium. (2021). *Understanding forage grass genomes beyond single nucleotide variation—examples for reproductive traits* (p. 2). Palacký University. https://doi.org/10.3929/ETHZ-B-000528427

Suzuki, T., Eiguchi, M., Kumamaru, T., Satoh, H., Matsusaka, H., Moriguchi, K., Nagato, Y., & Kurata, N. (2008). MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. *Molecular Genetics and Genomics*, *279*(3), 213–223. https://doi.org/10.1007/s00438-007-0293-2

Takahashi, R., & Hayashi, J. (1964). Linkage study of two complementary genes for brittle rachis in barley. In *Harlan's crops and man* (Vol. 12, pp. 99–105). Ber. Ohara Inst. Landw. Biol., Okayama Univ. https://doi.org/10.1002/9780891186342.ch6

Talamè, V., Bovina, R., Sanguineti, M. C., Tuberosa, R., Lundqvist, U., & Salvi, S. (2008). TILLMore, a resource for the discovery of chemically induced mutants in barley. *Plant Biotechnology Journal*, *6*(5), 477–485. https://doi.org/10.1111/j.1467-7652.2008.00341.x

Thurber, C. S., Reagon, M., Gross, B. L., Olsen, K. M., Jia, Y., & Caicedo, A. L. (2010). Molecular evolution of shattering loci in U.S. weedy rice. *Molecular Ecology*, *19*(16), 3271–3284. https://doi.org/10.1111/j.1365-294X.2010.04708.x

Till, B. J., Reynolds, S. H., Greene, E. A., Codomo, C. A., Enns, L. C., Johnson, J. E., Burtner, C., Odden, A. R., Young, K., Taylor, N. E., Henikoff, J. G., Comai, L., & Henikoff, S. (2003). Large-scale discovery of induced point mutations with high-throughput TILLING. *Genome Research*, *13*(3), 524–530. https://doi.org/10.1101/gr.977903

Uauy, C., Paraiso, F., Colasuonno, P., Tran, R. K., Tsai, H., Berardi, S., Comai, L., & Dubcovsky, J. (2009). A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. *BMC Plant Biology*, *9*(1), 115. https://doi.org/10.1186/1471-2229-9-115

Wilkins, P. W., & Humphreys, M. O. (2003). Progress in breeding perennial forage grasses for temperate agriculture. *The Journal of Agricultural Science*, *140*(2), 129–150. https://doi.org/10.1017/S0021859603003058

Xin, Z., Li Wang, M., Barkley, N. A., Burow, G., Franks, C., Pederson, G., & Burke, J. (2008). Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. *BMC Plant Biology*, *8*(1), 103. https://doi.org/10.1186/1471-2229-8-103

Yoon, J., Cho, L.-H., Kim, S. L., Choi, H., Koh, H.-J., & An, G. (2014). The BEL1-type homeobox gene *SH5* induces seed shattering by enhancing abscission-zone development and inhibiting lignin biosynthesis. *The Plant Journal*, *79*(5), 717–728. https://doi.org/10.1111/tpj.12581

Yu, Y., Hu, H., Doust, A. N., & Kellogg, E. A. (2020). Divergent gene expression networks underlie morphological diversity of abscission zones in grasses. *New Phytologist*, *225*(4), 1799–1815. https://doi.org/10.1111/nph.16087

Yu, Y., & Kellogg, E. A. (2018). Inflorescence abscission zones in grasses: Diversity and genetic regulation. In *Annual Plant Reviews Online*

(pp. 497–532). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119312994.apr0619

Yu, Y., Leyva, P., Tavares, R. L., & Kellogg, E. A. (2020). The anatomy of abscission zones is diverse among grass species. *American Journal of Botany*, *107*(4), 549–561. https://doi.org/10.1002/ajb2.1454

Zeng, X., Mishina, K., Jia, J., Distelfeld, A., Maughan, P. J., Kikuchi, S., Sassa, H., & Komatsuda, T. (2020). The brittle rachis trait in species belonging to the Triticeae and its controlling genes *Btr1* and *Btr2*. *Frontiers in Plant Science*, *11*, 1000. https://doi.org/10.3389/fpls.2020.01000

Zhang, Y., Ran, Y., Nagy, I., Lenk, I., Qiu, J., Asp, T., Jensen, C. S., & Gao, C. (2020). Targeted mutagenesis in ryegrass (Lolium spp.) using the CRISPR/Cas9 system. *Plant Biotechnology Journal*, *18*(9), 1854–1856. https://doi.org/10.1111/pbi.13359

Zhao, X., Xie, W., Zhang, J., Zhang, Z., & Wang, Y. (2017). Histological characteristics, Cell Wall hydrolytic enzymes activity and candidate genes expression associated with seed shattering of Elymus sibiricus accessions. *Frontiers in Plant Science*, *8*, 606. https://doi.org/10.3389/fpls.2017.00606

Zhou, Y., Lu, D., Li, C., Luo, J., Zhu, B.-F., Zhu, J., Shangguan, Y., Wang, Z., Sang, T., Zhou, B., & Han, B. (2012). Genetic control of seed SHATTERING in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. *The Plant Cell*, *24*(3), 1034–1048. https://doi.org/10.1105/tpc.111.094383

How to cite this article: Kiesbauer, J., Grieder, C., Studer, B., & Kölliker, R. (2023). Perspectives for reducing seed shattering in ryegrasses. *Grass and Forage Science*, 1–13. https://doi.org/10.1111/gfs.12635