

## Article

# Contribution of Awns to Seed Yield and Seed Shattering in Siberian Wildrye Grown under Irrigated and Rainfed Environments

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**Abstract:** The seed yield of grass species is greatly dependent on inflorescence morphological traits, starting with spikelets per inflorescence and seeds per spikelet, to kernel size, and then to awns. Previous studies have attempted to estimate the contribution of these traits on the harvested yield of major cereal crops, but little information can be accessed on the influence of awns on seed yield of forage grass species. Siberian wildrye (*Elymus sibiricus* L.) is a widely important perennial forage grass used to increase forage production in arid and semi-arid grasslands. The grass has long inflorescences with long awns developed at the tip end of the lemmas in the florets. In order to evaluate the effect of awns on Siberian wildrye seed production, awn excision analyses from 10 accessions were performed at flowering stage under irrigated and rainfed regimes. Overall, awn excision reduced thousand-seed weight and seed size under both irrigated and rainfed regimes, which decreased final seed yield per plant. De-awned plants produced significantly more seeds per inflorescence, but spikelets per inflorescence was not influenced by awn excision in either condition. Moreover, histological analyses showed a high degradation of the abscission layer in the awned plants than de-awned ones, and awn excision evidently improved average seed breaking tensile strength (BTS), and thus decreased the degree of seed shattering. In conclusion, the observed significant impact of awn excision on different yield-related traits mirrored the impact of awns on the performance of Siberian wildrye under diverse growing conditions. These results provide useful information for plant breeders, seed producers, and researchers to efficiently improve seed production in Siberian wildrye.

**Keywords:** abscission layer; awns; *Elymus sibiricus*; grasses; inflorescence morphology; photosynthesis; seed shattering; seed yield; yield components



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## 1. Introduction

Siberian Wildrye (*Elymus sibiricus* L.) is a perennial, self-pollinating allotetraploid bunchgrass species that is important for arid and semi-arid regions [1]. Siberian wildrye has an erect culm ranging from 40 to 90 cm long, and the leaves are basal and cauline. The inflorescence of Siberian wildrye is a nodding spike containing 15–30 spikelets, with 4–5 fertile florets in each spikelet, and bristle, curling awns developed at the tip of the lemmas in the florets, which can reach 20 mm in length. The grass is native to high-altitude regions of western and northern China, and widely distributed in Northern Europe, Northern Asia, and North America [2]. The species plays an important role in maintaining and developing the alpine pastures because of its seedling vigor and resistance to pests and stress conditions. Owing to its forage yield potential and palatability (for

livestock), it is favored by farmers, especially in the Qinghai–Tibet plateau of China, and some cultivars have been developed and distributed in the region [3]. Under natural conditions, however, Siberian wildrye is biologically inefficient at producing seeds and thus cannot meet the growing seed demand for ecological restoration and research purposes. Seed yield fluctuation in this species may be due to insufficient assimilates for developing kernels, especially during the last growth stages [4,5]. Thus, it is critical to develop feasible approaches to improve seed yield of Siberian wildrye, especially under water deficit and high-temperature conditions.

Unlike cereal crops, most forage grass species are neglected for their capacity for seed production, and most breeding programs mainly focus on biomass yield, forage quality, and abiotic and biotic resistance [6]. Thus, the most feasible approach to improving seed yield of forage grass species is to focus on the traits that comprehensively influence seed yield under diverse growing conditions. Under stress-free conditions, the development and filling of kernels depend on the assimilates produced by leaves [7]. However, under unfavorable conditions (water limitation and high temperatures), the contribution of leaves to photosynthesis decreases sharply [8]. Thus, the focus should be whether the photosynthetic capacity of the inflorescence could support carbon assimilation during kernel development after premature leaf senescence. The photosynthetic capacity of grass inflorescence could contribute between 10% and 76% of final seed weight, depending on the species and growing conditions, with awns playing a crucial role [9].

Awns, the bristle-like outgrowths of the lemmas in the florets, are morphological features of several cereal crops such as rice, wheat, and barley, as well as many forage grass species [10]. Awns could function at different plant development stages spanning from seed germination and floral development to grain setting and grain filling [11]. Awns influence seed dispersal and burial, and protect the kernel against animal predators [11,12]. The awn is triangular-shaped at its cross-section, with the base oriented towards the rachis and containing vascular bundles and chlorenchyma cells with the potential to improve the photosynthetic capacity of the canopy [13]. Awns are genetically controlled and are a highly heritable trait with the relatively high broad-sense heritability previously observed ( $H^2 = 0.75$ ) [14]. The connection between awns and yield-related traits such as grains per plant, grain size, and kernel weight has been reported in some cereal crops [15]. Awns could improve yield potential by improving the photosynthetic rate of the canopy, water-use efficiency, and thermo-tolerance [16]. In barley, long awns have resulted in the highest grain yield per plant under well-watered regimes [13]. Under water-stress conditions, however, long awns compete with growing kernels, thus reducing harvested grain yield [7]. In other cereals such as wheat, awn  $\times$  environment interaction is unclear, with some studies reporting that awns could double the net photosynthetic rate of the canopy under favorable growing conditions. For example, Rebetzke et al. [8] observed that awns of wheat are coupled with increased grain size and yield under rainfed conditions, but they considerably decreased grain number under irrigated conditions, which could result in reduced grain yield. Blum [17] reported that awns of wheat contributed to about 50% of the total canopy carbon exchange rate under well-watered regimes. Similarly, Maydup et al. [18] observed that 45% of the yield increased in awned wheat cultivars under water-limited environments. In comparison, the contribution of awns to grain yield of rice varied depending on production systems. In paddy rice genotypes, awns are considered a less important trait for yield potential as several studies observed no significant differences between awned and awnless genotypes [19,20]. In some upland rice landraces, such as *Tipakhiya* and *Sathi*, the removal of awns accelerated water loss in the panicle, which resulted in grain sterility [21].

Seed shattering is an essential trait for the efficient propagation of offspring in nature, yet this may threaten row crop production by increasing yield loss in cultivated species [22]. Thus, a reduction in seed shattering will increase seed yield in grain crops. In monocots, seed shattering is generally induced by the progressive degradation of the abscission layers developed at the attachment point between the lemma and the pedicel [23]. Very few

studies have demonstrated the association of seed shattering and floral morphological structures. A previous study on Siberian wildrye mentioned that seed shattering is induced by seed traits such as kernel weight, seed length, and awns [22]. Another study on wheat indicated that long awns are easily controlled by external dispersal factors such as wind, and their movement may propel the seed into the ground [12,19].

Compared to major cereal crops, very few studies have investigated the impact of awns on seed production of forage grasses. In addition, the association between awns and seed shattering in Siberian wildrye remains unknown. The aim of this study was to assess the impact of awn removal on seed yield, yield components, and seed shattering of Siberian wildrye under irrigated and rainfed regimes. The results of this study will enable Siberian wildrye seed producers, plant breeders, and seed production researchers alike to more effectively understand traits coupled with seed yields.

## 2. Materials and Methods

### 2.1. Plant Materials

The contribution of awns to seed yield, seed shattering, and other seed yield-related traits was analyzed in a total of 10 Siberian wildrye accessions (Table 1). These accessions were selected by evaluating awn length and were screened from 80 Siberian wildrye accessions. In this species, long-awned genotypes usually have awn lengths of about 20 mm, while short-awned genotypes have awns of less than 10 mm in length [4]. To ensure phenotypic variation, both long-awned and short-awned accessions were selected. The seeds of these accessions were obtained from the U.S. Department of Agriculture Germplasm Resources Information Network (GRIN), Sichuan Agricultural University, Sichuan Academy of Grassland Science, and Lanzhou University. Before planting, the seeds were germinated in plastic boxes containing a moistened double layer of blotter paper at 25 °C, 85% relative humidity, and a photoperiod of 12 h light, 12 h dark. Seedlings were grown under greenhouse conditions until they reached 8 weeks.

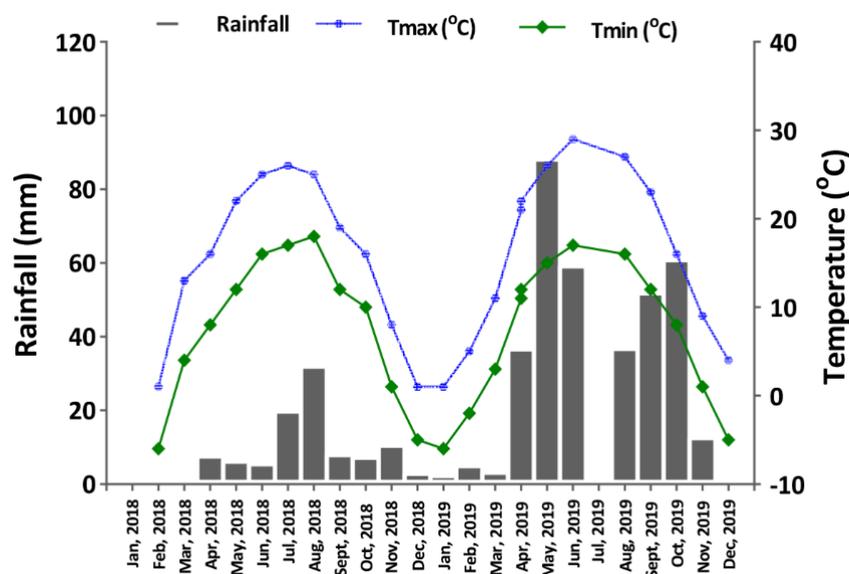
**Table 1.** Siberian Wildrye accessions used in the study.

Running Code	Accessions	Status	Origin
1	PI499456	Wild	Inner Mongolia, China
2	LT02	Wild	Gansu, China
3	PI499615	Wild	Xinjiang, China
4	PI531665	Wild	Beijing, China
5	ZN03	Wild	Gansu, China
6	PI598479	Wild	Altai, Russia
7	PI598788	Wild	Alma Ata, Kazakhstan
8	PI655097	Wild	Alma Ata, Kazakhstan
9	PI659942	Wild	Alma Ata, Kazakhstan
10	HZ03	Wild	Gansu, China

### 2.2. Experimental Conditions and Trial Design

The field trial was carried out at the Yuzhong experimental station of Lanzhou University in Gansu province, China (latitude: 35°34' N, longitude: 103°34' E, elevation: 1720 m). The soil at the site was sandy loam (organic matter content 2.4%, pH 7.6) with a depth of about 0.82 m and a dry bulk density of about 1.42 gcm<sup>3</sup>. The 10 accessions were grown under irrigated and rainfed conditions during two successive growing seasons (2018 and 2019), which denoted as Season 1 and Season 2, respectively. Weather data were collected from Yuzhong meteorological station located at ~2 km from the experimental site (CMA Archives, 2019). The location received an average monthly rainfall of ~9.15 mm and ~31.57 mm in 2018 and 2019, respectively (Figure 1). The maximum temperatures in summer ranged from 22–26 °C in 2018 and from 22–29 °C in 2019. The minimum temperatures in winter ranged from –8 to –5 °C in 2018 and –6 to –2 °C in 2019. In Season 1, compared to Season 2, the rainfall was much less during the vegetative growth stage, which is the

growth between early seedling emergence and the anthesis (May, June, July), than seed development stages. Moreover, the average temperature of both seasons increased during anthesis and seed development stages, and this increment was more pronounced in the second season than the first.



**Figure 1.** Maximum and minimum temperatures and precipitation at the Yuzhong experimental station (Lanzhou, Gansu, China) from January 2018 to December 2019.

The plots were arranged in a randomized complete block design with three replicates. Individual treatment plots were 30 m<sup>2</sup> (6 m long × 5 m wide; 50 cm row spacing and 20 cm inter-plant spacing), and seedlings were transplanted at an optimal 10–15 cm depth. After transplanting, a total of 50 mm of water was applied immediately in both irrigated and rainfed plots and no fertilizers were applied afterwards. A total of 75 mm was additionally added to the irrigated plots between heading to maturity. When plants reached anthesis stage, two treatments involving the presence (awned) and absence (de-awned) of awns were imposed. Within each plot, treatment was replicated three times. Excision of awns was performed for basal, central, and apical spikelets with scissors.

### 2.3. Data Collection and Measurements

#### 2.3.1. Awn Length and Seed Yield Components

Seed-related traits were measured at early maturity stage (21 days after anthesis). Seed samples were oven-dried at 80 °C for 2 days, and clean and dry seed samples were stored in paper bags at 20 °C and 15% relative humidity before being subjected to measurements. For awn length (AL), seed length (SL), and seed width (SW), seeds sampled in the basal, central, and apical spikelets were used; fifty random seeds were chosen in each sample. Seeds were scanned by Epson Perfection V700 Scanner (Seiko Epson Corp., Nagano, Japan). The images were analyzed by Image J software [24]. Thirty random inflorescences were used to evaluate spikelets per inflorescence (SpI), seeds per inflorescence (SI), florets per spikelet (FS), and seeds per spikelets (SSp). All these measurements were measured from the same spikelets. To obtain thousand-seed weight (TSW), thirty random inflorescences from each plant were selected. The TSW was determined by averaging the weight of three thousand seed samples from basal, central, and apical spikelets.

#### 2.3.2. Spike Dry Matter

Spike dry matter (SDM) was estimated at early maturity stage (21 days after anthesis). Ten random tillers of each plant in each treatment were selected, and their spikes were careful hand-cut just below the spike and oven-dried at 80 °C for 2 days [5]. Before

measurement, awns were also removed for awned spikes to avoid differences that could be caused by the presence of awns. The dried spikes were weighed, and data were expressed as g tiller<sup>-1</sup>.

### 2.3.3. Seed Yield

In early August of each year (2018 and 2019) when seeds had fully matured (28 days after anthesis), a total of 10 plants of each treatment were randomly selected, and the heads of each plant were carefully hand-cut. To avoid border effects, seed samples were taken from the center of each plot. Seed samples were harvested when the seed moisture content was approximately 45%. Seed yield per plant (SYP) measurement was carried out after samples were weighed and when the seed moisture content was about 10%.

### 2.3.4. Seed Shattering

#### Breaking Tensile Strength (BTS)

Seed shattering was determined by measuring the minimum breaking tensile strength (BTS) required to detach seeds from pedicels. The spikes were carefully harvested at early maturity (21 days after anthesis). Each spike was attached vertically upside down to a digital force gauge (HANDIPI, China), and forceps were used to pull down each seed. The BTS measured was recorded in gram-force (gf) units. A total of 50 seeds from five spikes representing each plant in each treatment were measured.

#### Scanning Electron Microscope (SEM)

For histological analysis, abscission zone tissues of the two accessions (TZ02 and ZN03) with contrasting awn length and degree of seed shattering were used. Samples were collected from three central spikelets from each inflorescence at early maturity stage (28 days after heading). The samples were fixed in FAA solution [ethylalcohol (70%):glacial acetic acid:formaldehyde (40%) = 90:5:5] at 4 °C. Transverse sections were achieved by hand-cutting through the seed and pedicel junction. The sections were dehydrated in a series of ethanol solutions (50, 70, 90, and 100%), substituted in 3-methylbutyl acetate, and vacuum dried. Sections were then gold plated and observed using a Hitachi S-3000N variable pressure scanning electron microscope.

## 2.4. Statistical Analysis

The statistical analyses were performed using R statistical software (version 4.0.1; R Foundation for Statistical Computing, Vienna, Austria). The data were first checked for normality and error variance heterogeneity. The normality tests showed non-significant error variances (data not shown), and the data were left untransformed. The data of seed yield per plant, yield components, and seed shattering were subjected to analysis of variance (ANOVA). The presence (awned) and absence (de-awned) of awns and growing conditions (irrigated and rainfed) were treated as fixed factors, while block and season were treated as random factors. Means were compared by using the Student's *t*-test and Turkey's HSD post hoc test with a level of significance of  $p < 0.05$ . To ensure reproducibility, all the experiments were replicated at least three times.

## 3. Results

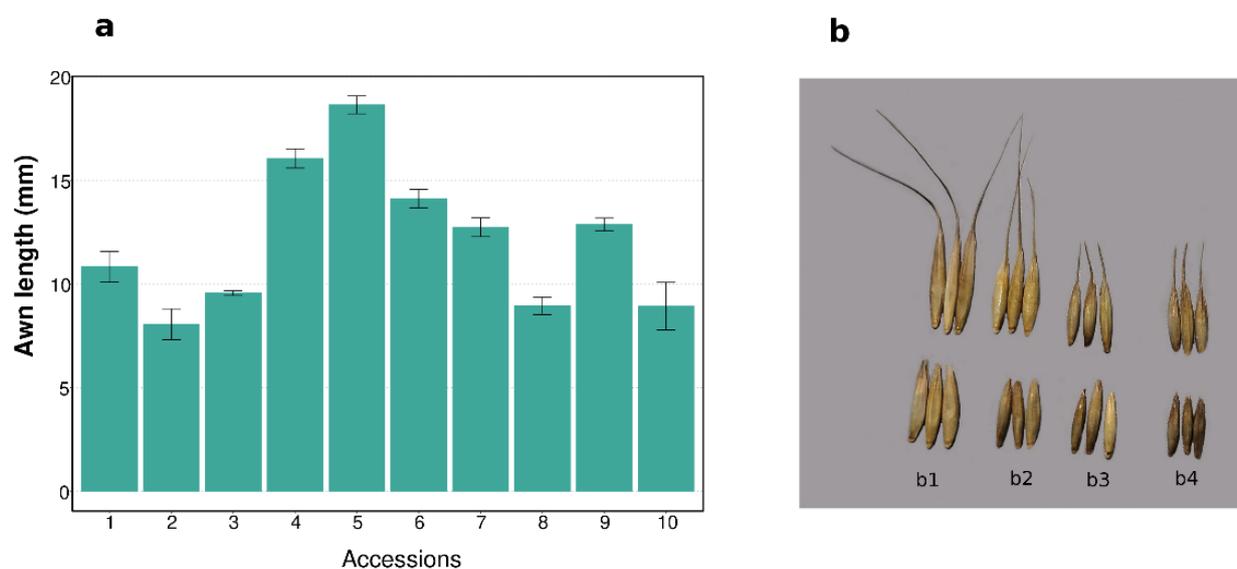
### 3.1. Awn Length and Seed Yield Components

The ANOVA showed that the AL and seed yield components of 10 Siberian wildrye accessions differed in response to awn excision, environment, and their interaction (Table 2). The SL, SW, TSW, and SI were significantly influenced by awn excision. All yield components, except BTS and SP, were significantly affected by the environment. Across irrigated and rainfed conditions, six out of ten accessions, PI531665, ZN03, PI598479, PI598788, PI655097, and PI659942, had an AL longer than 10 mm (Figure 2). ZN03 showed the longest awns (18.64 mm) and TZ02 showed the shortest awns (8.05 mm). The average SL was significantly influenced by awn excision under irrigated (awned, 9.50 mm; de-awned,

9.23 mm;  $p < 0.05$ ) and rainfed (awned, 9.36mm; de-awned, 9.04mm;  $p < 0.01$ ) conditions (Figure 3). The average SW was also decreased by awn excision under irrigated (awned, 2.06 mm; de-awned, 1.89 mm;  $p < 0.001$ ) and rainfed (awned, 1.90 mm; de-awned, 1.75 mm;  $p < 0.001$ ) conditions. Across both environments, the SpI was not significantly affected by awn excision ( $p > 0.05$ ). However, awn excision strongly increased the average SI under both irrigated (awned, 76.70; de-awned, 85.17;  $p < 0.01$ ) and rainfed (awned, 72.73; de-awned, 82.81;  $p < 0.001$ ) regimes.

**Table 2.** Analysis of variance for awns (A), environments (E), seasons (S), and interaction effects on seed yield and yield components. SL, seed length; SW, seed width; TSW, thousand-seed weight; BTS, breaking tensile strength; SpI, spikelets per inflorescence; SI, seeds per inflorescence; SDM, spike dry matter; SYP, seed yield per plant. ns: not significant. \*, \*\*, and \*\*\*: significant at 0.05, 0.01, and 0.001 probability level.

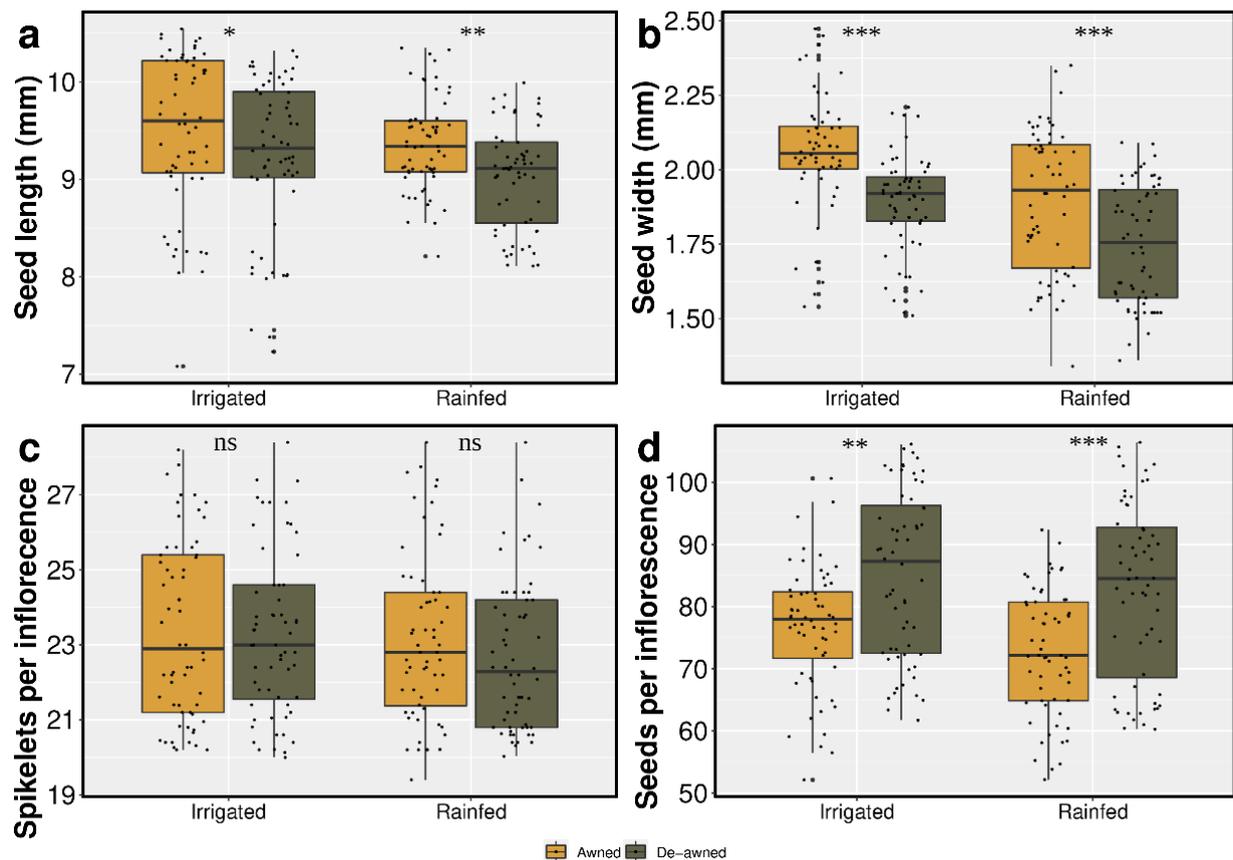
Source of Variation	df	SL	SW	TKW	BTS	SpI	SI	SDM	SYP
Seasons (S)	1	ns	ns	**	ns	***	**	**	ns
Blocks	2	ns							
Awn (A)	1	***	***	***	***	ns	***	**	ns
Environment (E)	1	*	***	*	ns	ns	*	*	*
S × A	1	ns	ns	ns	ns	ns	*	*	ns
S × E	1	ns	ns	ns	ns	ns	ns	*	ns
A × E	1	ns	ns	ns	ns	ns	*	*	*
A × E × S	1	ns							



**Figure 2.** Characterization of Siberian wildrye accessions used in the study. (a) Awn length of 10 accessions used in the study. (b) Seeds of ZN03 and TZ02 accessions. b1 and b2 are awned and de-awned seeds of ZN03 under rainfed and irrigated conditions, respectively. b3 and b4 are awned and de-awned seeds of LT02 grown under rainfed and irrigated conditions, respectively. Each bar represents the mean value  $\pm$  standard deviation.

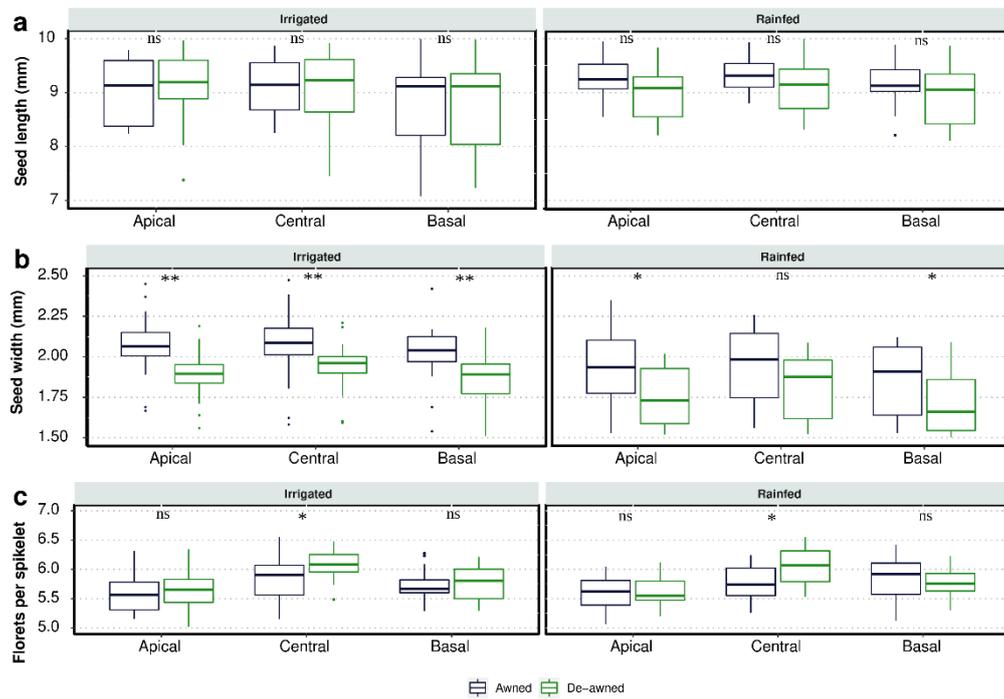
Across both environments, the impact of awn excision on SL was non-significant ( $p > 0.05$ ) at apical, central, and basal spikelet positions (Figure 4). Under irrigated conditions, the average SW for all 10 accessions was significantly reduced by awn excision at apical (awned, 2.07; de-awned, 1.88;  $p < 0.01$ ), central (awned, 2.07; de-awned, 1.94;  $p < 0.01$ ), and basal (awned, 2.03; de-awned, 1.86;  $p < 0.01$ ) spikelet positions. Under the rainfed regime, the average SW was also influenced by awn excision at apical (awned, 1.93; de-awned, 1.78;  $p < 0.05$ ) and basal (awned, 1.83; de-awned, 1.71;  $p < 0.05$ ) spikelet positions. Although no significant difference was observed for the average FS among awned and

de-awned plants in both regimes, a slight but significant increase in FS was observed for central spikelets under awn excision treatments in irrigated conditions (awned, 5.83; de-awned, 6.09;  $p < 0.05$ ) and rainfed conditions (awned, 5.78; de-awned, 6.07;  $p < 0.05$ ). Moreover, awn excision showed a significant effect on SI at the apical (awned, 3.48; de-awned, 4.33;  $p < 0.01$ ) and central (awned, 3.69; de-awned, 4.36;  $p < 0.05$ ) spikelets under irrigated conditions (Figure 5). Under both conditions, the TSW was not significantly impacted by awn excision at all three spikelet positions ( $p > 0.05$ ).

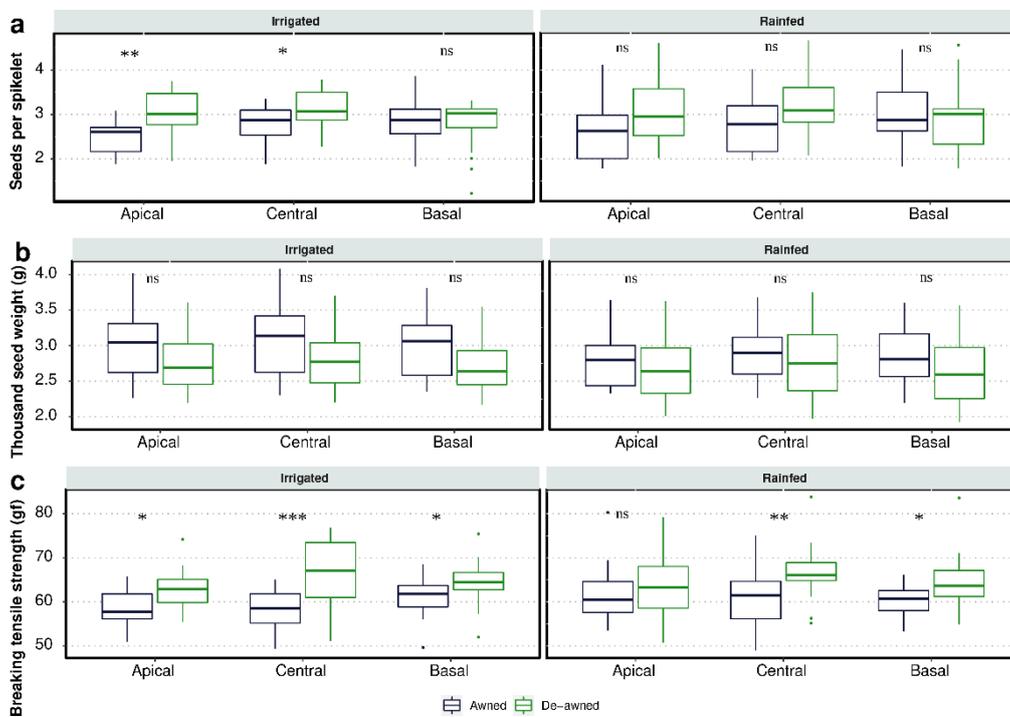


**Figure 3.** Box plots of seed length (a), seed width (b), spikelets per inflorescence (c), and seeds per inflorescence (d), and significance levels of difference between control and awn excision. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Each box plot denotes the average of 10 accessions for corresponding traits. The significances indicate the comprehensive effect of awn excision on different traits of 10 Siberian wildrye accessions.

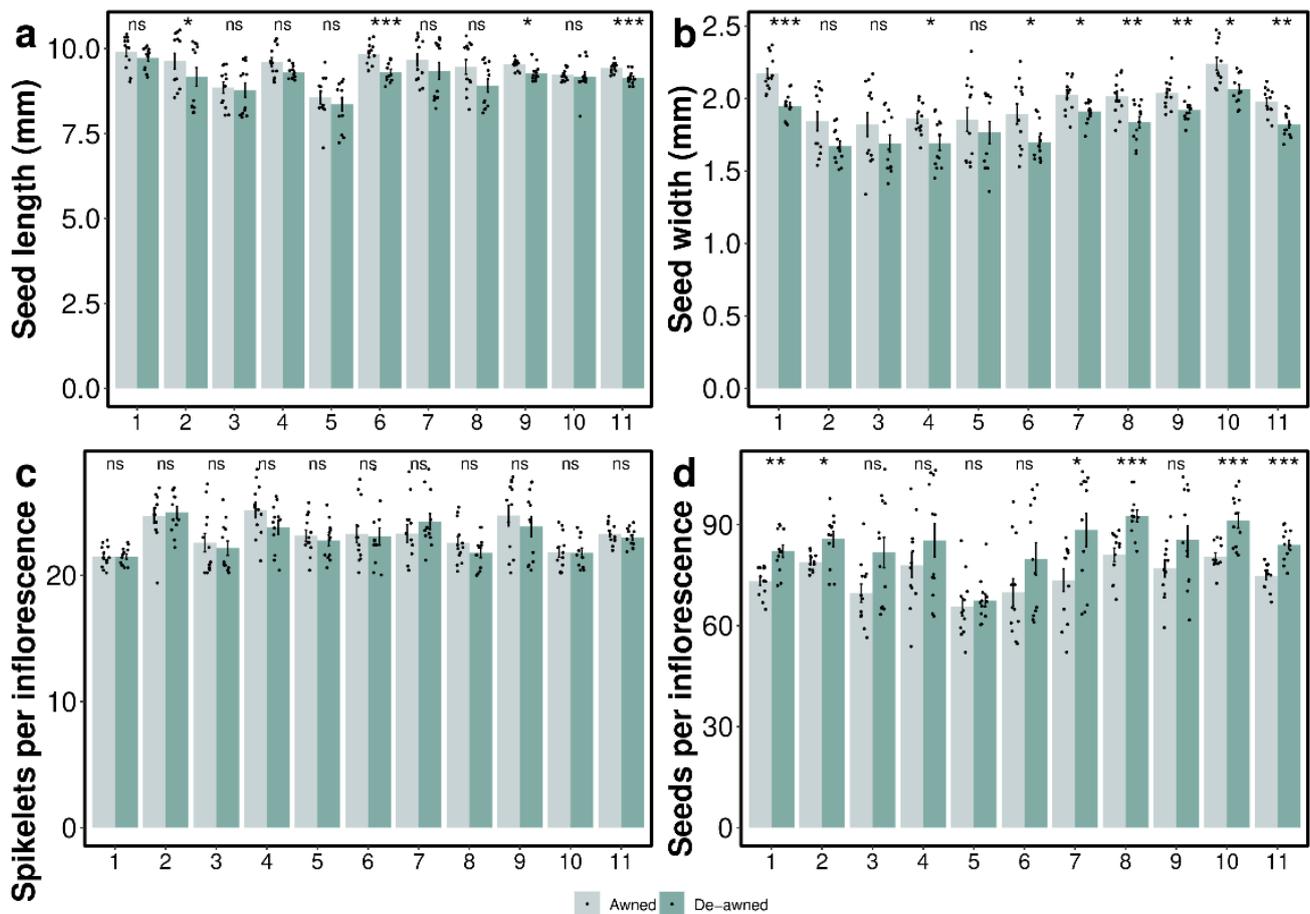
Measurements across both growing conditions revealed that awn excision varyingly influenced seed yield components of 10 Siberian wildrye accessions (Figure 6). For instance, awn excision reduced the SL of all 10 accessions and this reduction was, on average, more pronounced in PI598479 (awned, 9.84 mm; de-awned, 9.31 mm;  $p < 0.001$ ). Awn excision significantly and consistently reduced the SW of seven accessions and this reduction was much higher in PI499456 (awned, 2.17 mm; de-awned, 1.95 mm;  $p < 0.001$ ). The SI of five accessions (PI499456, LT02, PI598788, PI655097, HZ03) was significantly increased by awn excision. The SI was, on average, higher in PI655097 (awned, 81.02; de-awned, 92.59;  $p < 0.001$ ) and HZ03 (awned, 80.48; de-awned, 91.27;  $p < 0.001$ ). Moreover, the contribution of awn excision for SpI, although different in some accessions, was non-significant.



**Figure 4.** Box plots summarizing seed length (a), seed width (b), and florets per inflorescence (c) in the apical, central, and basal spikelets of the spikes. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ . Each box plot denotes the average of 10 accessions for corresponding traits. The significances indicate the comprehensive effect of awn excision on different traits of 10 Siberian wildrye accessions.



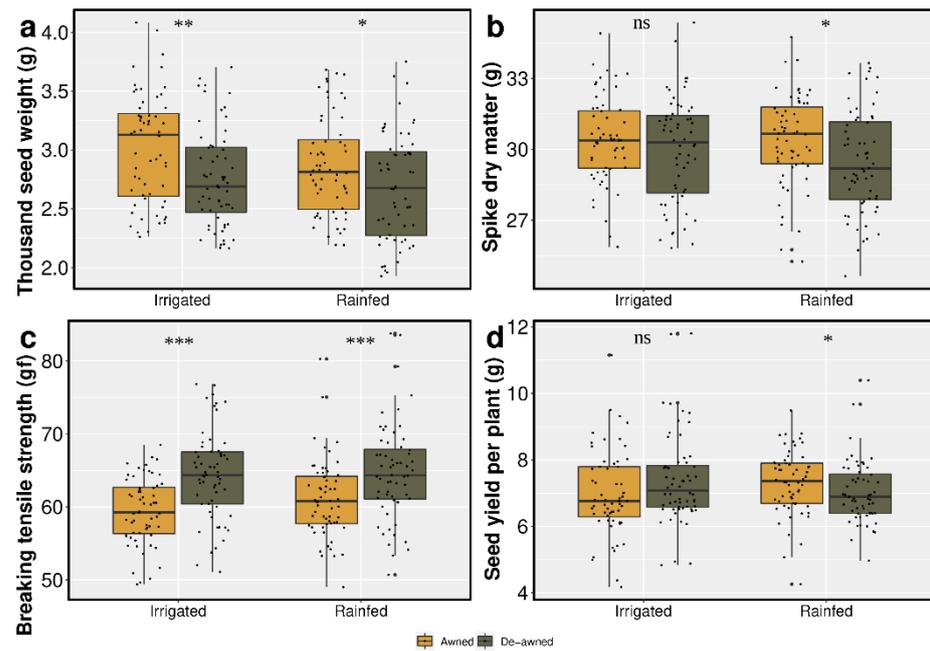
**Figure 5.** Box plots summarizing seeds per spikelet (a), thousand seed weight (b), and breaking tensile strength (c) in the apical, central, and basal spikelets of the spikes. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Each box plot denotes the average of 10 accessions for corresponding traits. The significances indicate the comprehensive effect of awn excision on different traits of 10 Siberian wildrye accessions.



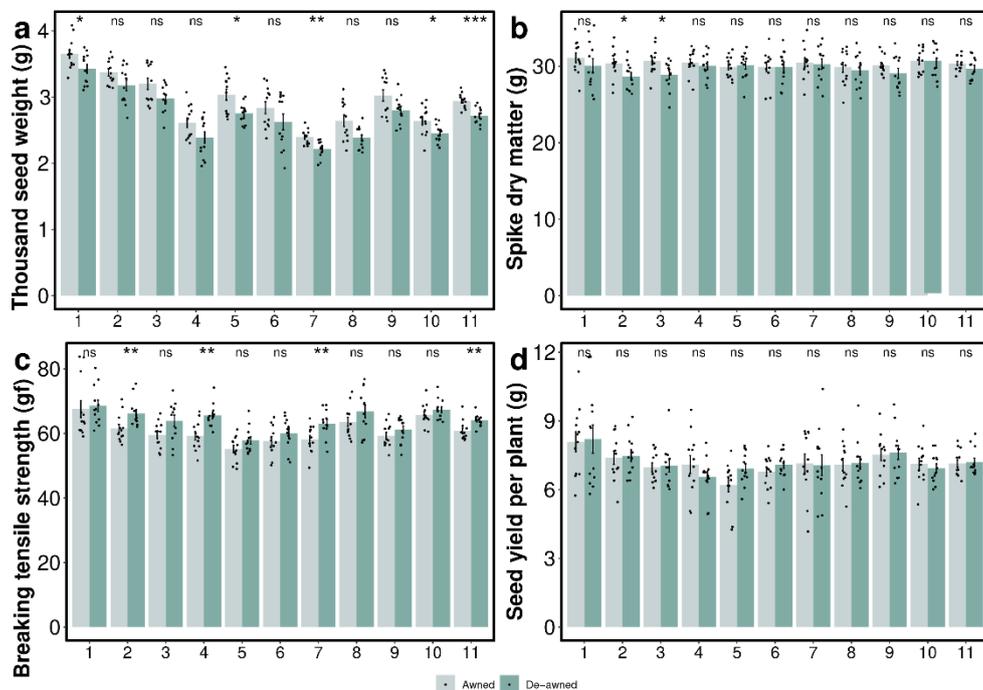
**Figure 6.** Seed length (a), seed width (b), spikelets per inflorescence (c), and seeds per inflorescence (d) of 10 Siberian wildrye accessions (1–10) and averages of accessions (11) under control and awn excision treatments across rainfed and irrigated conditions. Error bars represent mean  $\pm$  standard deviation. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.2. Spike Dry Matter Accumulation

The SDM was significantly influenced by awn excision, the environment, and their interaction (Table 2). The average SDM of awned plants was higher than that of de-awned ones and was significant in rainfed conditions (awned, 30.39 g; de-awned, 29.45 g;  $p < 0.05$ ) (Figure 7). Moreover, awn excision reduced the average SDM of 10 accessions, with significant differences observed in LT02 (awned, 30.40 g; de-awned, 28.70 g;  $p < 0.05$ ) and PI499615 (awned, 30.70 g; de-awned, 28.88 g;  $p < 0.05$ ) (Figure 8).



**Figure 7.** Box plots of thousand-seed weight (a), spike dry matter (b), breaking tensile strength (c), and yield per plant (d), and significance levels of difference between control and awn excision. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Each box plot denotes the average of 10 accessions for corresponding traits. The significances indicate the comprehensive effect of awn excision on different traits of 10 Siberian wildrye accessions.



**Figure 8.** Thousand-seed weight (a), spike dry matter (b), breaking tensile strength (c), and seed yield per plant (d) of 10 Siberian wildrye accessions (1–10) and averages of accessions (11) under control and awn excision treatments across rainfed and irrigated conditions. Error bars represent mean  $\pm$  standard deviation. ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

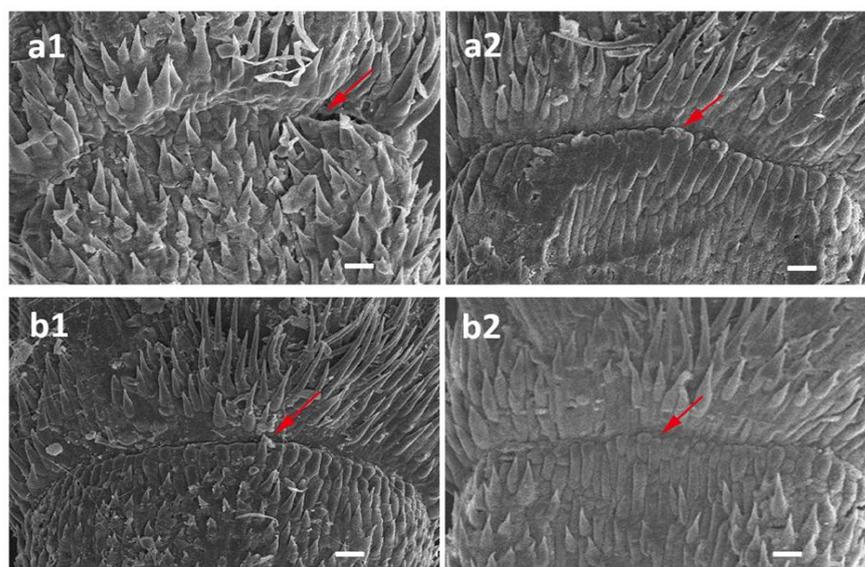
### 3.3. Seed Yield

The ANOVA showed that SYP was not significantly impacted by awn excision but was significantly influenced by the environment and the interaction between awn excision and the environment (Table 2). The influence of awn excision on the average SYP was more pronounced in the rainfed regime (awned, 7.32; de-awned, 7.02;  $p < 0.05$ ) than the irrigated one (awned, 6.98 g; de-awned, 7.40 g;  $p > 0.05$ ) (Figure 7). Assessments across both environments revealed awn excision influenced the SYP of 10 accessions, although this was non-significant (Figure 8). The highest average SYP was found for PI499456 (awned, 6.98 g; de-awned, 7.40 g;  $p > 0.05$ ), while the lowest value was found for ZN03 (awned, 6.20 g; de-awned, 6.92 g;  $p > 0.05$ ).

### 3.4. Effect of Awn Excision on Seed Shattering

There was a significant effect of awn excision on seed shattering (SS) in both growing conditions (Table 2). Awn excision significantly reduced the degree of seed shattering by increasing the average BTS under both irrigated (awned, 59.46 gf; de-awned, 64.37 gf;  $p < 0.001$ ) and rainfed conditions (awned, 61.09 gf; de-awned, 64.75 gf;  $p > 0.01$ ) (Figure 7). Under irrigated conditions, measurements at three spikelet positions confirmed the major impact of awn excision on the average BTS at apical (awned, 58.91 gf; de-awned, 62.85 gf;  $p < 0.05$ ), central (awned, 58.19 gf; de-awned, 65.94 gf;  $p < 0.001$ ), and basal (awned, 61.27 gf; de-awned, 64.33 gf;  $p < 0.05$ ) positions (Figure 5). Under rainfed conditions, awn excision significantly improved the average BTS of Siberian wildrye accessions at central (awned, 60.93 gf; de-awned, 66.67 gf;  $p < 0.01$ ) and basal (awned, 60.45 gf; de-awned, 64.31 gf;  $p < 0.05$ ) spikelet positions. In addition, assessments across both growing regimes indicated that awn excision improved the average BTS of all 10 accessions tested, and this improvement was much higher in LT02, PI531665, and PI598788 (Figure 8).

The structures of the abscission layers of pedicel junctions that were manually excised are shown in Figure 9. Cross-sections showed that the shape and arrangement of abscission layers were similar in the short-awned genotype (TZ02) for awned and de-awned seeds. For the long-awned genotype (ZNO3), the smooth fracture on the rachilla was observed in awned seeds, while a well-defined boundary between the pedicel and the spikelet was observed in de-awned seeds.



**Figure 9.** Histological structures showing variations in abscission formation in awned and de-awned Siberian wildrye accessions. (a1,a2) scanning electron photos of awned (a1) and de-awned (a2) seeds of ZNO3. (b1,b2) scanning electron photos of awned (b1) and de-awned (b2) seeds of LT02. Red arrows indicate the location of the abscission layer.

## 4. Discussion

### 4.1. Impacts of Awns on Seed Yield and Components

The observed differences in seed yield and yield components between awned and de-awned plants were associated to the amount of assimilates partitioned to growing kernels, and were in agreement with several reports on cereal crops [18,25,26]. The removal of Siberian wildrye awns resulted in a reduced kernel weight and harvested seed yield, and this reduction was more obvious under rainfed regimes (Figure 7). Awns improved seed length, seed width, and thousand-seed weight, and this improvement compensated for the effect of reduced seeds per inflorescence. The significant reduction in seed weight for de-awned plants resulted in small and shriveled seeds, and this impact was more obvious in apical and basal spikelets (Figure 4). The variation in seed size between apical, central, and basal spikelets in this species could also be associated with flowering order, which starts in central spikelets, and then the top and bottom spikelets, respectively. The observed inverse association between harvested yield and shorter or non-existent awns under rainfed conditions has also been reported in wheat [27,28], barley [29,30], and rye [31]. Under irrigated conditions, long awns required a particular portion of assimilates for development, which decreased the assimilates accumulated in the kernels and thus led to a yield loss, a trade-off previously documented in several cereal crops such as wheat [8,32], barley [33], and rice [20]. Under irrigated conditions, the present study revealed that awns improved spikelet number, although this was non-significant, compensating for the reduced fertile florets per spikelet (Figure 4). Besides, awn excision treatments significantly reduced seed length and seed width, but this reduction was compensated for by increased fertile spikelets, which directly resulted in a higher frequency of kernels per inflorescence. Thus, small seed yield differences were observed for awned and de-awned plants grown under irrigated conditions.

The contribution of awns on seed yield components varied across seasons. The availability of soil moisture during the growing season promoted plant growth and affected seed yield components. In 2018, rainfall was lower during the vegetative growth stage but increased during anthesis and kernel development stages, resulting in a higher seed yield under the rainfed regime in this season compared to that of 2019. Similarly, Wang et al. [34] indicated that increased rainfall during the anthesis stage contributed to increased seed yield in Russian wildrye. Soil moisture is the key variable for the effective flowering and seed development in Siberian wildrye [35], but the excessive moisture could reduce pollination, which consequently reduces the number of seed sets per spikelet. This illustrates the fluctuations in seed yield per plant observed in 2019, which supports a previous study of Siberian wildrye that observed that excessive precipitation reduced seed setting rate and thus final seed yield [5]. The estimated association of awn length with seed width and seed length was inconsistent across growing conditions, with differences in seed width being greatest in irrigated conditions and differences in seed length being greatest in the rainfed regime (Figure 3). This indicated that, as postulated by Teich [32], moisture could not always decide the impact of awns as the environments also vary in temperature, radiation, and soil fertility [32]. This is in agreement with our previous study that observed that the contribution of awns to seed yield of Siberian wildrye varied across latitudes, longitudes, and altitudes [4]. Thus, opportunity remains in the selection of awn lengths with comparable yields improvements under diverse environment conditions.

### 4.2. Role of Awns on Partitioning of Spike Dry Matter

Dry matter accumulation in the inflorescence, as in leaves and stems, was thought to be associated with nitrogen supply and soil moisture content [5,36]. Although previous studies have indicated that maximum plant dry weights could be obtained by improving the photosynthetic capacity of leaves [37], the present study mirrored the capacity of awns to support dry matter partitioning into the growing kernels, especially under stress-free regimes (Figure 4). The comparisons of Siberian wildrye accessions revealed that spike dry matter was higher in the awned treatments than de-awned ones (Figure 8). Given this

capacity of awns to increase spike assimilates, one could expect that improved spike dry weight by awns is associated with yield increase under irrigated conditions. However, awns develop rapidly under favorable conditions and impose a competing sink with growing kernels, reducing the amount of assimilates allocated to kernel filling and thus causing yield loss. This competition for assimilates between different plant organs and growing florets in the reproductive stage was previously documented in wheat. For instance, potential yield increases by spike dry weights were likely limited by the competition between growing florets and elongating stems, as reported by González et al. [38].

The capacity of awns to improve spike dry matter was non-significant under rainfed conditions. According to Martínez et al. [39], this might be linked to limited moisture and high temperatures. Similarly, in wheat, Wang et al. [5] observed that leaf, stem, and spike dry matter contents were greatly affected by air temperature and soil moisture content. However, in the present work, the decrease in spike dry weights did not compromise the capacity of awns to improve seed yield under rainfed conditions. This was probably related to the reduction in awn lengths observed for several plants grown under rainfed conditions, reflecting a reduction in photosynthates allocated to those awns. The increase in assimilates allocated to growing kernels was associated with a greater increase in kernel size, especially in apical and basal spikelets (Figure 4). Overall, given the growing pressure to improve harvested yields of grass species, future yield gains should be targeted by improving spike dry matter accumulation [8]. Developing cultivars with optimum awn length will be an attractive option to improve the inflorescence dry weights, and thus the harvest index, in grass species.

#### 4.3. The Impact of Awns on Seed Shattering

Seed shattering is an essential adaptive trait for the efficient propagation of offspring in wild plants [40]. Breeding initiatives in grasses have primarily focused on improving grain yield and biomass production rather than reducing their shattering trait, even though seed shattering has greatly hindered harvested yield in many cultivated grass species [22]. Seed shattering is a result of the progressive deterioration of the abscission layers [41], causing mature seeds to detach from the pedicel at these layers [42]. In the present study, analyses of pedicel junctions of awned plants confirmed the presence of an abscission with two small cell layers (Figure 9), controlling cell separation and breakage. Moreover, the evaluation of seed shattering of awned and de-awned plants showed that awned seeds displayed a shattering trait with a complete abscission zone between the seed and the pedicel. However, the removal of awns induced a deficiency in the formation of the abscission zone near the vascular bundle, which caused the seeds to stay on the plant at maturity. These results indicate that long-awned species are more susceptible to shattering before harvest since awns increase seed weight and increase the axis of where the weight is concentrated, exerting additional stress on the abscission layer [22,43].

Our analyses indicated the discrepancies in the breaking tensile strength of several awned and de-awned plants (Figure 8). This illustrated how seed shattering is a highly-coordinated trait not only associated with morphological traits but also with changes in cell structure, metabolism, and gene expression [44]. Previously, a study revealed that abscission is associated with a rapid increase in cellulose and polygalacturonase activity [22]. However, how awns interact with these hormones is not clear and remains a subject of intensive investigation. The study of Magwa et al. [45] on rice indicated a possible interaction between the awn development gene (*An1*) and the grain-shattering-related QTL (*sh4-1*) on chromosome 1. Furthermore, a number of studies also showed that the expression of seed shattering may be controlled by environmental factors such as moisture and temperature [22,44]. Similarly, in this work, we observed significant differences in seed breaking tensile strength among awned and de-awned plants across irrigated and droughted conditions (Figure 7), indicating the role of the deficit and surplus of water in the expression of seed shattering in Siberian wildrye. Overall, a wide range of germplasm collections, assessments with multiple location and year testing, and both

outdoor and greenhouse trials would be vital to better understand the influence of awns on seed shattering.

## 5. Conclusions

This study attempted to identify the influence of awns on harvested seed yield of Siberian wildrye. The presence of awns slightly increased the seed yield per plant through increased seed size and kernel weight. Awn excision improved seeds per inflorescence and was associated with a reduction in seed shattering. These results revealed the advantages of growing awned species in conditions with water shortages and high temperatures, and show the existing opportunities of growing awnless grass species under stress-free conditions. Plant breeders should focus on increasing spikelet per inflorescence and the number of fertile florets through awn length manipulation. Moreover, this study revealed the possibility of improving seed production by increasing seed retention through awn length optimization. In the future, a greater number of genotypes, large field experiments, and a divergent selection of multi-location trials and multi-year testing will facilitate the identification of the further impacts of awn traits on the performance of awned grass species.

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## References

1. Zhang, Z.; Xie, W.; Zhao, Y.; Zhang, J.; Wang, Y. EST-SSR marker development based on RNA-sequencing of *E. sibiricus* and its application for phylogenetic relationships analysis of seventeen *Elymus* species. *BMC Plant Biol.* **2019**, *19*, 235. [CrossRef]
2. Barkworth, M.E. Flora of North America: North of Mexico: Magnoliophyta: Commelinidae (In Part): Poaceae, Part 1. 2006, Volume 24. Available online: <http://floranorthamerica.org/volumes> (accessed on 20 July 2020).
3. Xie, W.; Zhang, J.; Zhao, X.; Zhang, Z.; Wang, Y. Transcriptome profiling of *Elymus sibiricus*, an important forage grass in Qinghai-Tibet plateau, reveals novel insights into candidate genes that potentially connected to seed shattering. *BMC Plant Biol.* **2017**, *17*, 78. [CrossRef] [PubMed]
4. Ntakirutimana, F.; Xiao, B.; Xie, W.; Zhang, J.; Zhang, Z.; Wang, N.; Yan, J. Potential effects of awn length variation on seed yield and components, seed dispersal and germination performance in Siberian wildrye (*Elymus sibiricus* L.). *Plants* **2019**, *8*, 561. [CrossRef]
5. Wang, M.; Hou, L.; Zhu, Y.; Zhang, Q.; Wang, H.; Xia, F.; Chen, L.; Mao, P.; Hannaway, D.B. Siberian wildrye seed yield limited by assimilate source. *Field Crop Res.* **2018**, *218*, 18–23. [CrossRef]
6. Capstaff, N.M.; Miller, A.J. Improving the Yield and Nutritional Quality of Forage Crops. *Front. Plant Sci.* **2018**, *9*, 535. [CrossRef] [PubMed]
7. Guo, Z.; Schnurbusch, T. Costs and benefits of awns. *J. Exp. Bot.* **2016**, *67*, 2533–2535. [CrossRef]
8. Rebetzke, G.J.; Bonnett, D.G.; Reynolds, M.P. Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. *J. Exp. Bot.* **2016**, *67*, 2573–2586. [CrossRef] [PubMed]
9. Merah, O.; Monneveux, P. Contribution of different organs to grain filling in durum wheat under Mediterranean conditions I. Contribution of post-anthesis photosynthesis and remobilization. *J. Agron. Crop Sci.* **2015**, *201*, 344–352. [CrossRef]

10. Gu, X.Y.; Kianian, S.F.; Foley, M.E. Phenotypic selection for dormancy introduced a set of adaptive haplotypes from weedy into cultivated rice. *Genetics* **2005**, *171*, 695–704. [[CrossRef](#)] [[PubMed](#)]
11. Bommert, P.; Whipple, C. Grass Inflorescence Architecture and Meristem Determinacy. *Semin. Cell Dev. Biol.* **2017**, *79*, 6429. [[CrossRef](#)]
12. Elbaum, R.; Zaltzman, L.; Burgert, I.; Fratzl, P. The role of wheat awns in the seed dispersal unit. *Science* **2007**, *316*, 884–886. [[CrossRef](#)] [[PubMed](#)]
13. Yuo, T.; Yamashita, Y.; Kanamori, H.; Matsumoto, T.; Lundqvist, U.; Sato, K.; Ichii, M.; Jobling, S.A.; Taketa, S. A SHORT INTERNODES (SHI) family transcription factor gene regulates awn elongation and pistil morphology in barley. *J. Exp. Bot.* **2012**, *63*, 5223–5232. [[CrossRef](#)]
14. Liller, C.; Walla, A.; Boer, M.P.; Hedley, P.; Macaulay, M.; Effgen, S.; Korff, M.V.; Esse, G.V.; Koornneef, M. Fine mapping of a major QTL for awn length in barley using a multiparent mapping population. *Theor. Appl. Genet.* **2017**, *130*, 269–281. [[CrossRef](#)] [[PubMed](#)]
15. Ntakirutimana, F.; Xie, W. Unveiling the actual functions of awns in grasses: From yield potential to quality traits. *Int. J. Mol. Sci.* **2020**, *21*, 7593. [[CrossRef](#)] [[PubMed](#)]
16. Motzo, R.; Giunta, F. Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat. *Aust. J. Agric. Res.* **2002**, *53*, 1285–1293. [[CrossRef](#)]
17. Blum, A. Photosynthesis and transpiration in leaves and ears of wheat and barley varieties. *J. Exp. Bot.* **1985**, *36*, 432–440. [[CrossRef](#)]
18. Maydup, M.L.; Antonietta, M.; Graciano, C.; Guiamet, J.J.; Tambussi, E.A. The contribution of the awns of bread wheat (*Triticum aestivum* L.) to grain filling: Responses to water deficit and the effects of awns on ear temperature and hydraulic conductance. *Field Crops Res.* **2014**, *167*, 102–111. [[CrossRef](#)]
19. Furuta, T.; Komeda, N.; Asano, K.; Uehara, K.; Gamuyao, R.; Angeles-Shim, R.B.; Nagai, K.; Doi, K.; Wang, D.R.; Yasui, H. Convergent loss of awn in two cultivated rice species *Oryza sativa* and *Oryza glaberrima* is caused by mutations in different loci. *G3 Genesgenetics* **2015**, *5*, 2267–2274. [[CrossRef](#)] [[PubMed](#)]
20. Jin, F.X.; Kim, D.M.; Ju, H.G.; Ahn, S.N. Mapping quantitative trait loci for awnness and yield component traits in isogenic lines derived from an *Oryza sativa*/*O. rufipogon* cross. *J. Crop Sci. Biotechnol.* **2009**, *12*, 9–15. [[CrossRef](#)]
21. Singh, S.; Singh, T.N.; Chauhan, J.S. Architectural engineering of rice panicle for increased productivity: A powerful Biological Tool for Combating Agricultural Water Crisis. *J. Crop Improv.* **2009**, *23*, 451–466. [[CrossRef](#)]
22. Zhao, X.; Xie, W.; Zhang, J.; Zhang, Z.; Wang, Y. Histological Characteristics, Cell Wall hydrolytic enzymes activity and candidate genes expression associated with seed shattering of *Elymus sibiricus* accessions. *Front. Plant Sci.* **2017**, *8*, 606. [[CrossRef](#)]
23. Konopatskaia, I.; Vavilova, V.; Blinov, A.; Goncharov, N.P. Spike morphology genes in wheat species (*Triticum* L.). *Proc. Latv. Acad. Sci.* **2016**, *70*, 345–355. [[CrossRef](#)]
24. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [[CrossRef](#)]
25. Duwayri, M. Effect of flag leaf and awn removal on grain yield and yield components of wheat grown under dryland conditions. *Field Crops Res.* **1984**, *8*, 307–313. [[CrossRef](#)]
26. Huo, X.; Wu, S.; Zhu, Z.; Liu, F.; Fu, Y.; Cai, H.; Sun, X.; Gu, P.; Xie, D.; Tan, L. *NOG1* increases grain production in rice. *Nat. Commun.* **2017**, *8*, 1497. [[CrossRef](#)] [[PubMed](#)]
27. Chhabra, A.K.; Sethi, S.K. Inheritance of cleistogamic flowering in durum wheat (*Triticum durum*). *Euphytica* **1991**, *55*, 147–150. [[CrossRef](#)]
28. Khaliq, I.; Irshad, A.; Ahsan, M. Awns and flag leaf contribution towards grain yield in spring wheat (*Triticum aestivum* L.). *Cereal Res. Commun.* **2008**, *36*, 65–76. [[CrossRef](#)]
29. Chen, G.D.; Li, H.B.; Zheng, Z.; Wei, Y.M.; Zheng, Y.L.; McIntyre, C.L.; Zhou, M.X.; Liu, C.J. Characterization of a QTL affecting spike morphology on the long arm of chromosome 3H in barley (*Hordeum vulgare* L.) based on near isogenic lines and a NIL-derived population. *Theor. Appl. Genet.* **2012**, *125*, 1385–1392. [[CrossRef](#)] [[PubMed](#)]
30. Jiang, Q.Z.; Roche, D.; Durham, S.; Hole, D. Awn contribution to gas exchanges of barley ears. *Photosynthetica* **2006**, *44*, 536–541. [[CrossRef](#)]
31. Grundbacher, F.J. The physiological function of the cereal awn. *Bot. Rev.* **1963**, *29*, 366–381. [[CrossRef](#)]
32. Teich, A.H. Interaction of awns and environment on grain yield in winter wheat (*Triticum aestivum* L.). *Cereal Res. Commun.* **1982**, *10*, 1–15. Available online: <https://www.jstor.org/stable/23781379> (accessed on 15 May 2021).
33. Bort, J.; Febrero, A.; Amaro, T.; Araus, J. Role of awns in ear water-use efficiency and grain weight in barley. *Agronomie* **1994**, *14*, 133–139. [[CrossRef](#)]
34. Wang, Q.; Zhang, T.; Cui, J.; Wang, X.; He, Z.; Han, J.; René, G.; Fuller, D.Q. Path and ridge regression analysis of seed yield and seed yield components of Russian Wildrye (*Psathyrostachys juncea* Nevski) under Field Conditions. *PLoS ONE* **2011**, *6*, e18245. [[CrossRef](#)] [[PubMed](#)]
35. Wang, H.; Li, Z.Z. Seasonal characteristics of soil water and biomass of siberian wildrye grass (*Elymus sibiricus* L.) in Bashang Plateau. *Agric. Res. Arid Areas* **2009**, *27*, 90–95.
36. Keller, M.; Koblet, W. Dry matter and leaf area partitioning, bud fertility and second season growth of *Vitis vinifera* L.: Responses to nitrogen supply and limiting irradiance. *Vitis* **1995**, *34*, 77–83.

37. Sulpice, R.; Nikoloski, Z.; Tschoep, H.; Antonio, C.; Kleessen, S.; Larhlimi, A.; Selbig, J.; Ishihara, H.; Gibon, Y.; Stitt, F.M. Impact of the carbon and nitrogen supply on relationships and connectivity between metabolism and biomass in a broad panel of *Arabidopsis* accessions. *Plant Physiol.* **2013**, *162*, 347–363. [[CrossRef](#)]
38. González, F.; Miralles, D.J.; Slafer, G.A. Wheat floret survival as related to pre-anthesis spike growth. *J. Exp. Bot.* **2011**, *62*, 4889–4901. [[CrossRef](#)] [[PubMed](#)]
39. Martinez, D.E.; Luquez, V.M.; Bartoli, C.G.; Guiamét, J. Persistence of photosynthetic components and photochemical efficiency in ears of water-stressed wheat (*Triticum aestivum*). *Physiol. Plant.* **2003**, *119*, 519–525. [[CrossRef](#)]
40. Yang, D.; Wang, Y.Z. Seed shattering: From models to crops. *Front. Plant Sci.* **2015**, *6*, 476. [[CrossRef](#)]
41. Ji, H.; Kim, S.; Kim, Y.; Kim, H.; Eun, M.; Jin, I.; Cha, Y.; Yun, D.; Ahn, B.; Lee, M. Inactivation of the CTD phosphatase-like gene *OsCPL1* enhances the development of the abscission layer and seed shattering in rice. *Plant J.* **2010**, *61*, 96–106. [[CrossRef](#)]
42. Li, W.; Gill, B.S. Multiple genetic pathways for seed shattering in the grasses. *Funct. Integr. Genom.* **2006**, *6*, 300–309. [[CrossRef](#)] [[PubMed](#)]
43. Thomson, M.J.; Tai, T.H.; McClung, A.M.; Lai, X.H.; Hinga, M.E.; Lobos, K.B.; Xu, Y.; Martinez, C.P.; McCouch, S.R. Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theor. Appl. Genet.* **2003**, *107*, 479–493. [[CrossRef](#)] [[PubMed](#)]
44. Zhou, Y.; Lu, D.; Li, C.; Luo, J.; Zhu, B.F.; Zhu, J.; Shangguan, Y.; Wang, Z.; Sang, T.; Zhou, B. Genetic control of seed shattering in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. *Plant Cell* **2012**, *24*, 1034–1048. [[CrossRef](#)]
45. Magwa, R.A.; Zhao, H.U.; Wen, Y.; Xie, W.; Lin, Y. Genomewide association analysis for awn length linked to the seed shattering gene *qSH1* in rice. *J. Genet.* **2016**, *95*, 639. [[CrossRef](#)] [[PubMed](#)]