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Anatomical and Physiological Characteristics of Awn Development in *Elymus nutans*, an Important Forage Grass in Qinghai-Tibet Plateau

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Abstract: Awns are the important structures of inflorescence in many crops that belong to the Poaceae family. In addition, they actively participate in photosynthesis, transpiration, seed dispersal, and self-planting. The *Elymus nutans* Griseb. is an important, self-pollinated, allohexaploid (2n = 6x = 42) and perennial native forage grass in Qinghai-Tibet Plateau that shows variation in awns length. However, the changes in the anatomical structure, physiological traits, and biochemical characteristics during awn development remain unclear in *E. nutans*. Therefore, this study investigated the changes in anatomical structures, enzymatic activities, and hormonal regulations of awns at four developmental stages, i.e., booting, heading, flowering, and maturity stages of three *E. nutans* accessions having different awn lengths. The results showed that the cross-sections of *E. nutans* awns had an acute triangular shape and structural similarities to wheat awns. In addition, the growth of long awns was recorded faster than short awns at the heading stage, but no significant differences in awns lengths were found at the heading, flowering, and maturity stages. The differences in the sizes of barbs and stomata of three accessions were statistically non-significant; however, the accession with long awns had more stomata than the accession with shorter awns at all developmental stages. In addition, the content of cytokinin (CTK), abscisic acid (ABA), and ethylene (ETH), and activities of superoxide dismutase (SOD) and peroxidase (POD) were significantly related to the development of awn. At the flowering stage, the content of CTK, and activities of SOD and POD of long awn accession were significantly higher than the short awn accession. Therefore, the obtained results provide a sound basis for future research on the molecular mechanisms of awn development and their potential role in *E. nutans*.

Keywords: awns; *Elymus nutans*; anatomical structure; hormone; enzyme; awn development

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1. Introduction

Awns are needle-like extensions at the top of lemma or glumes of Poaceae family members and are involved in leaf metamorphosis and long-term adaptation and evolution [1,2]. Awn length is considered an important morphological marker for distinguishing different varieties of cereal crops [3]. In barley (*Hordeum vulgare*), lemma awns that are more than twice the length of spike are long awns, and less than twice the length of spike are short awns. The wheat (*Triticum aestivum* L.) awns are usually irregular, including the long and short awns [4,5]. All awns are slightly coiled, but they may appear straight. Awn twisting is caused by wetting and drying the awn, and it plays an important role in the self-planting mechanism. Generally, the long awns are more "curly" and the short are "straight" [6].

The epidermis of awns contains cells of different shapes, including long and narrow cells, short cells with eggs or square shapes, thick-walled cells, and tapered single cells [7,8]. However, different species have different types of cells, structures, and number of awns. Usually, the tops and bottoms of awns have a large number of cells over the middle

section [9]. In short awns, the middle protion of the structure is also occupied by smaller cells. The awns of rye (*Secale cereale*) and its close relatives lack parenchymal cells, whereas the wheat and barley's parenchymal cells make up a large part of awn tissue [4]. Two bands of stomata are present on the abaxial side of the awn, one on each side of the longitudinal axis [10]. In addition, the basal part of awn contains the highest stomatal density. In barley, wheat, and oat (*Avena sativa*) awns are triangular in diameter, containing one median and two lateral veins separated by two bands of chlorenchyma tissue [9]. In contrast, the awns of rice (*Oryza sativa*) are round in cross-section, with a single vascular bundle and lack of green tissue, which results in less photosynthetic efficiency [11].

Awns play an important role in cereal crop's seed dispersal and germination, seed propagation, photosynthesis, transpiration, and final yield by protecting themselves from predators [12]. A previous report revealed that awns of cereals have stomata that can fix CO_2 and increase photosynthesis [13]. Due to the awn position being at the top, it directly receives sufficient light energy and performs photosynthetic functions [14]. Interestingly, the crops containing awn have approximately 10% higher yield than non-awn crops. Awns affect the seed weight, but not the number of grains per ear [15]. However, the effective role of awns in seed production has not been fully explored in forage grasses. Ntakirutimana's research [16] shows that awn excision reduces the thousand-seed weight and seed size under both irrigated and rainfed regimes, which results in a decreased final seed yield per plant. De-awned plants produce significantly more seeds per inflorescence, but awn excision do not affect spikelets per inflorescence in either condition. However, the long awn growth requires a specific portion of assimilation, which reduces the accumulation of assimilation in the grain, resulting in yield loss under irrigation conditions [17]. In some rice varieties, the effect of long awn on grain yield is neutral under water shortage conditions, while under irrigation conditions, long awn reduces the total grain yield [18]. Awns directly or indirectly increase the grain weight, which has a trade-off with reducing grain number. Therefore, the beneficial effects of awns on yield-related traits are not consistent under different environmental conditions [19].

Elymus nutans is a perennial sparse clump grass of the genus *Elymus* [20]. Globally, it is distributed within Russia, Turkey, Mongolia, India, and the Himalayas [21,22]. In China, *E. nutans* is mainly dominant in western and northern high-altitude areas with high grass yield, good palatability, high nutritional value, and has resistance to drought, cold, diseases, and insect attacks [23]. As a dominant plant in Qinghai-Tibet Plateau, *E. nutans* is particularly useful for soil and water conservation, for a sustainable ecological environment, and for grassland restoration [24]. *E. nutans* germplasm resources have great variations in awns, but the anatomical structure of awns and their developmental mechanisms are largely unexplored yet.

Therefore, the present study investigated the anatomical characteristics of *E. nutans* awns to better understand the changes in morphological traits and physiological mechanisms of awns during different developmental stages. Furthermore, it could be attributed to the potential role of *E. nutans* awns in photosynthesis to improve transpiration efficiency and promote yield formation.

2. Materials and Methods

2.1. Plant Materials and Field Trials

Based on previous awn length measurements, we selected the *E. mutans* accessions for further study. Three *E. nutans* accessions including W610220 (long awns), PI 655186 (medium awns), and PI 619592 (short awns) were used in the present study (Table 1). The healthy and uniform-sized seeds from each accession were germinated on moist filter paper in Petri plates (10 cm width size). Petri plates were placed in an incubator at a constant temperature of 25 °C with 12:12 h light/dark regime and seeds were germinated. After 10 days, young seedlings were transferred to flower pots (20 cm diameter) and grown under greenhouse conditions until they reached 8 weeks old. Later, the healthy seedlings were selected and transplanted to the field conditions in mid-April.

Table 1. *E. nutans* accessions were used in the present study.

Code	Accessions	Status	Origin	Awn Length
1	W610220	Wild	Gilgit, Pakistan	1.78 cm
2	PI 655186	Wild	Gansu, China	1.1 cm
3	PI 619592	Wild	Tibet, China	0.4 cm

The field experiment was conducted at the experimental station of Grassland Agricultural Science and Technology College on the Yuzhong campus of Lanzhou University, Gansu Province, China. The area is located at 35°34′ N and 103°34′ E, with an altitude of 1720 m and an annual average temperature of 6.7 °C. It belongs to a typical temperate semi-arid continental monsoon climate area.

The plots were arranged in a randomized complete block design with three replicates. Individual treatment plots were 30 m^2 (6 m long × 5 m wide; 50 cm row spacing and 20 cm inter-plant spacing), and seedlings were transplanted at an optimal 5–8 cm depth in the soil. The soil 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 g cm^3. After transplanting, a total of 50 mm of water was applied immediately in plots and no fertilizers were applied afterward. The spikes of *E. nutans* were cut at booting, heading, flowering, and maturity stages for further analysis.

2.2. Observation of the Anatomical Structure of the Outer Surface and the Detached Area of Awns

The inflorescence of *E. nutans* was in the form of spikes containing 20 to 30 spikelets (per spike). Most spikelets had 3 to 4 fertile florets in which each floret had a long lemma awn. Awn tissues of three accessions at four developmental stages were sampled. For each accession, four inflorescences were randomly selected from each replicate at each developmental stage. All awns of florets were harvested from four spikelets near the center of each spike for further analyses. Materials were placed in an FAA (alcohol, acetic acid, formalin) fixed solution (absolute ethanol: glacial acetic acid: formaldehyde: distilled water = 60:5:5:30, with a small amount of glycerol added) and stored in a refrigerator at 4 °C. After 24 h, materials were taken out and dried [25], and then the awns were cut out. To observe the sections of awn, the middle of awns were cut (1 mm length) and glued on the metal block. To observe the air holes and spikes, the awns from the bottom were cut, and then the whole section of material were glued to the metal block. All the prepared materials were examined under a JSM-5600LV scanning electron microscope. The length and number of stomata were also measured. Photoshop 2022 was used for enhancing the contrast of the image.

2.3. Determination of Hormones and Enzymes during Four Awn Developmental Stages

For each accession, four inflorescences were randomly selected from each replicate at each developmental stage. The awn length of the primary floret of four central spikelets from each spike was measured, and then samples were collected. After freezing in liquid nitrogen, isolated awns were stored in a −80 °C refrigerator. Later, the antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)), cell wall hydrolase activity (cellulase (CE), polygalacturonase (PG)), amylase (AMS), and endogenous plant hormones (auxin IAA, cytokinin CTK, gibberellin GA, abscisic acid ABA, ethylene ETH, and brassinolate BR) were determined. All indexes were measured with Elisa kit and determined by a company (Shanghai enzyme linked Biotechnology Co., Ltd., Shanghai, China).

2.4. Data Analysis

SPSS 22 was used for analyzing the whole data. The data were subjected to normality, error variance heterogeneity, and log-normalization when necessary. Data were then subjected to analysis of variance (ANOVA). The significant differences among measured

indicators were determined by Tukey's honest significant difference (HSD) test at $p < 0.05$ level. To ensure reproducibility, all the experiments were replicated at least three times.

3. Results

3.1. Anatomical Structure of Awns

The results of the present study depicted that the outer surface of the awns had a large number of protruding barbs and stomata. Therefore, we observed the upper and middle parts of awns of three accessions using a scanning electron microscope with a magnification of 30×. The number of protruding barbs in 2 mm awn length for three accessions at four stages is given in Table 2. At the maturity stage, the number of protruding barbs of long awn and medium awn accessions increased by 18.18% and 22.73%, respectively, as compared to short awn accession (Figures 1 and 2). The length and width of stomata are shown in Figures 3 and 4. The results showed that there were no significant differences in stoma length and width of awns for all accessions at each stage. The number of stomata in long awn accession was significantly larger than in short awn accession (Figure 5). At each of four stages, the number of stomata in long awn accession was 200%, 66.67%, 80%, and 57.14% larger than short awn accession, respectively ($p < 0.05$). In all accessions, the number of stomata was not increased after the heading stage. Stomata are almost absent in the upper part of the awns, whereas a large number of stomata were present in the lower part of the awns. One and two rows of stomata were found in the middle and bottom of the awns, respectively.

Table 2. The number of protruding barbs within the awn length of 2 mm at four stages of three accessions.

Accession	Booting Stage	Heading Stage	Flowering Stage	Maturity Stage
W610220	30	48	46	52
PI 655186	28	42	50	54
PI 619592	26	40	48	44

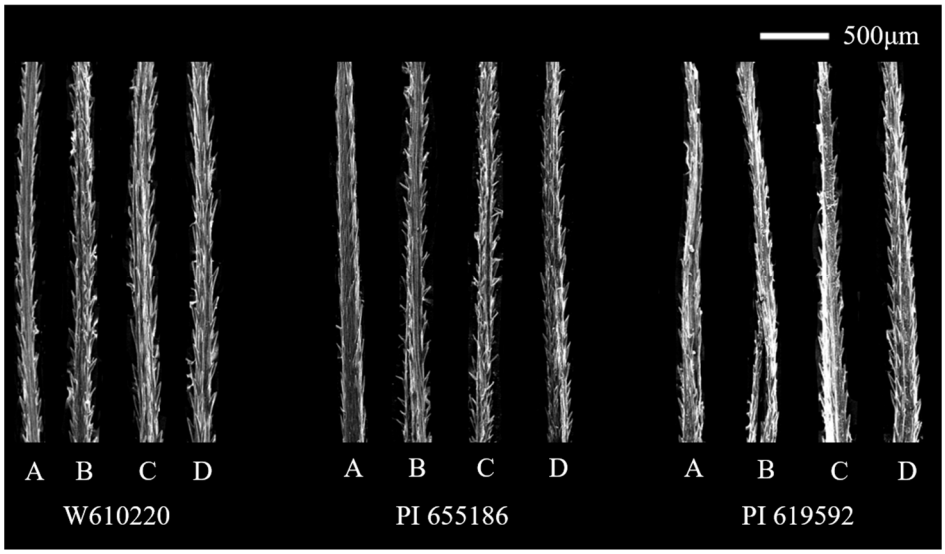


Figure 1. The images of the middle part of awns for three accessions at four developmental stages: (A) booting stage; (B) heading stage; (C) flowering stage; and (D) mature stage. W610220, long awn, PI 655186, medium awn, PI 619592, short awn.

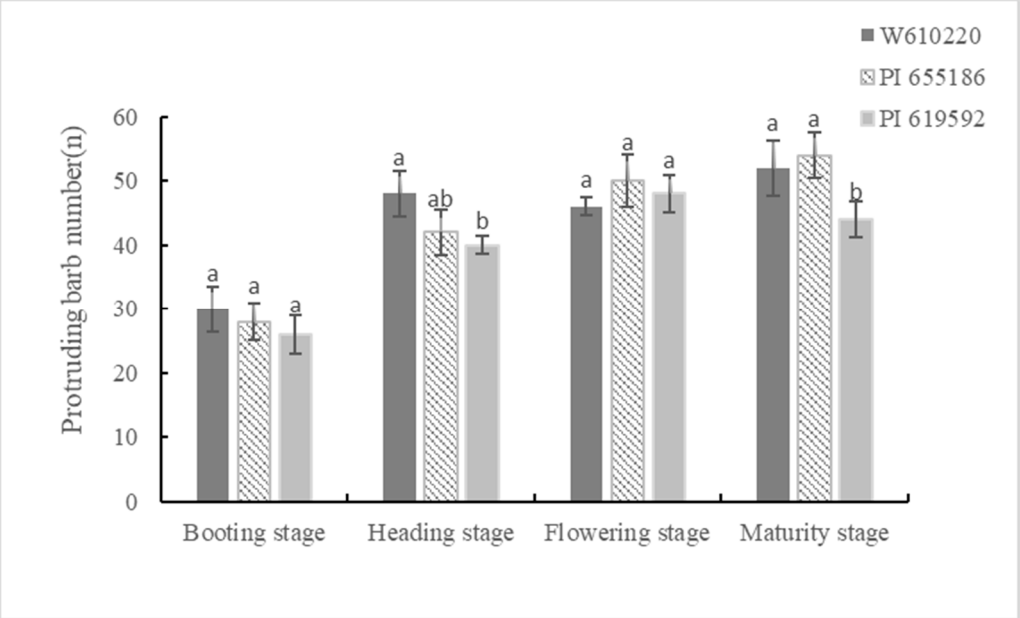


Figure 2. The number of protruding barbs in awns at four stages of three accessions. W610220, long awn, PI 655186, medium awn, PI 619592, short awn. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

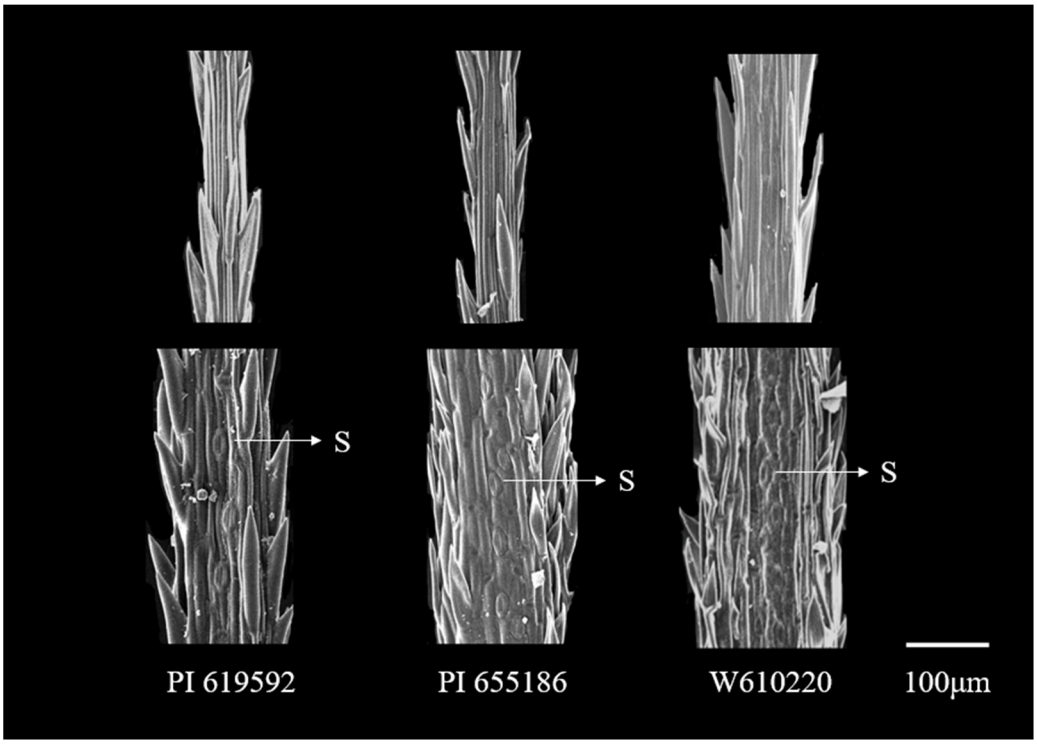


Figure 3. The upper and lower images of the awns of three accessions at heading stage. W610220, long awn, PI 655186, medium awn, PI 619592, short awn. S: Stomas.

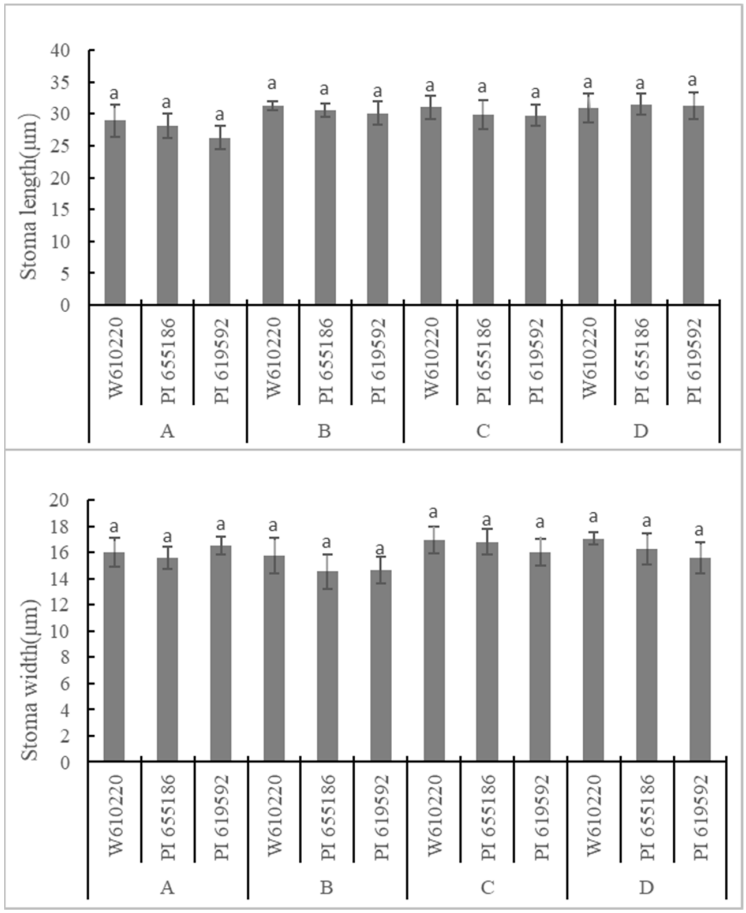


Figure 4. The difference of stoma length and width in awns of three accessions at four developmental stages. W610220, long awn, PI 655186, medium awn, PI 619592, short awn: (A) booting stage; (B) heading stage; (C) flowering stage; and (D) maturity stage. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

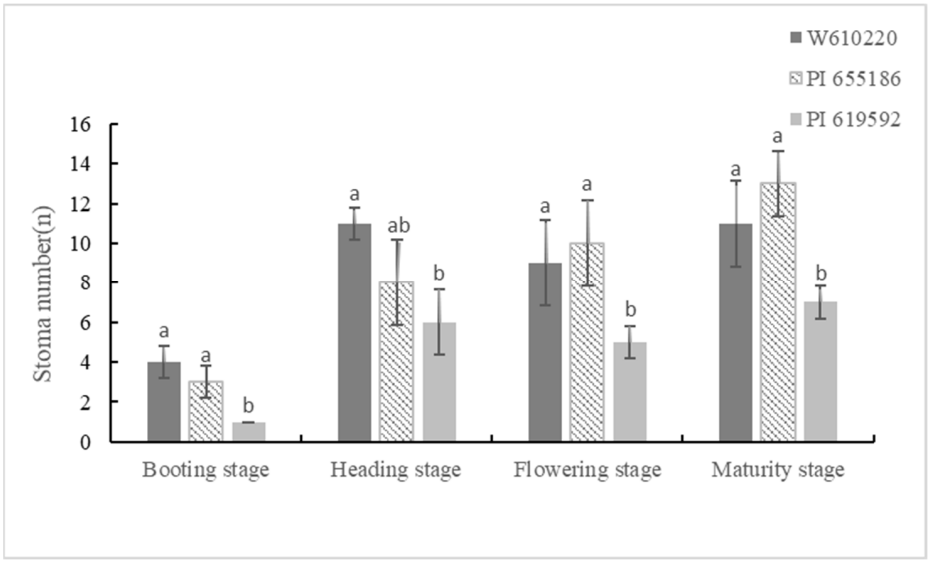


Figure 5. The number of stomas in the lower part of awns of three accessions at four developmental stages. W610220, long awn, PI 655186, medium awn, PI 619592, short awn. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

The results showed that the cross-section of awns in *E. nutans* presents an acute triangular shape. Here, the green cells, parenchyma cells, sclerenchyma cells, and vascular bundles can be seen (Figure 6).

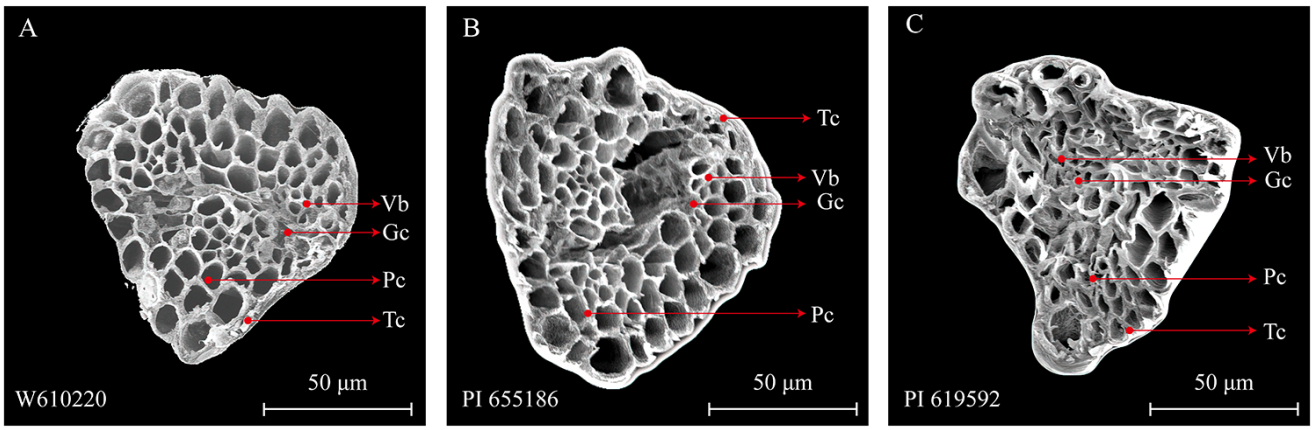


Figure 6. Anatomical structure image of three accessions at heading stage: (**A**) long awn; (**B**) medium awn; (**C**) short awn; Gc: green cells; Pc: parenchymal cells; Tc: thick walled cells; Vb: vascular bundle.

3.2. Developmental Dynamics of Awns

In all three accessions, the awn length reaches its final expansion at heading stage (Figure 7). The length of long awn accession at heading stage was 30.7% longer than booting stage, and short awn accession at heading stage was 25.8% longer than booting stage, respectively ($p < 0.05$). In addition, it was noticed that long awn accession elongated faster than short awn accession.

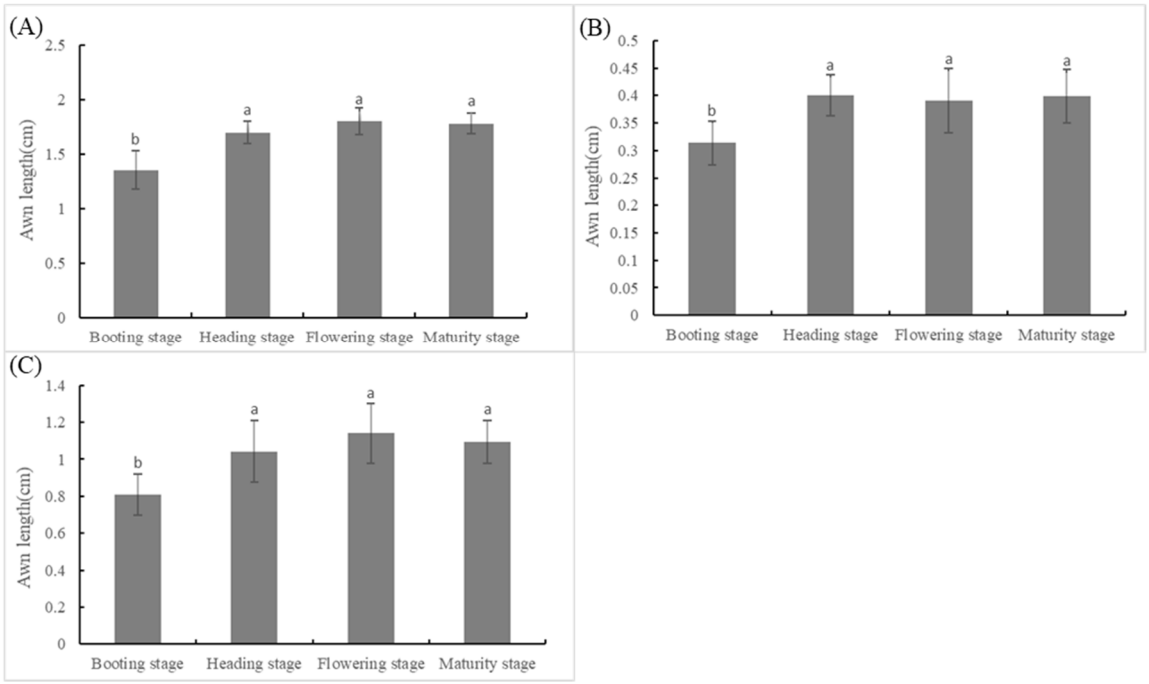


Figure 7. The difference of awn length of three accessions at developmental stages: (**A**) W610220, long awn, (**B**) PI 655186, medium awn, (**C**) PI 619592, short awn. Error bars represent mean ± standard deviation. Different normal letters mean significant difference among different treatments at 0.05 level.

3.3. The Difference in Plant Hormone Activity in Awns at Four Different Stages

During the four developmental stages, the content of indol acetic acid (IAA) of all accessions was firstly increased and then decreased, and later reached to a maximum at the heading stage (Figure 8A). The IAA content of long awn accession at heading stage was 17.5% higher than that of short awn accession ($p < 0.05$). A similar increasing and decreasing trend of gibberellin (GA) content was noted at all stages and found maximum at the heading stage (Figure 8B). The GA content of long awn accession at heading stage was 14.82% higher than that of short awn accession ($p < 0.05$). Similar to IAA, the abscisic acid (ABA) content of all accessions was firstly increased then decreased, and later reached the maximum at the flowering stage. The ABA content of long awn accession at heading stage was 9.2% lower than that of short awn accession ($p < 0.05$) (Figure 8D). On the other hand, the cytokinin (CTK) content of three accessions was firstly decreased and then increased. The lowest CTK content of long awn accession and medium awn accession was recorded at the heading stage, and short accession at flowering time, respectively (Figure 8C).

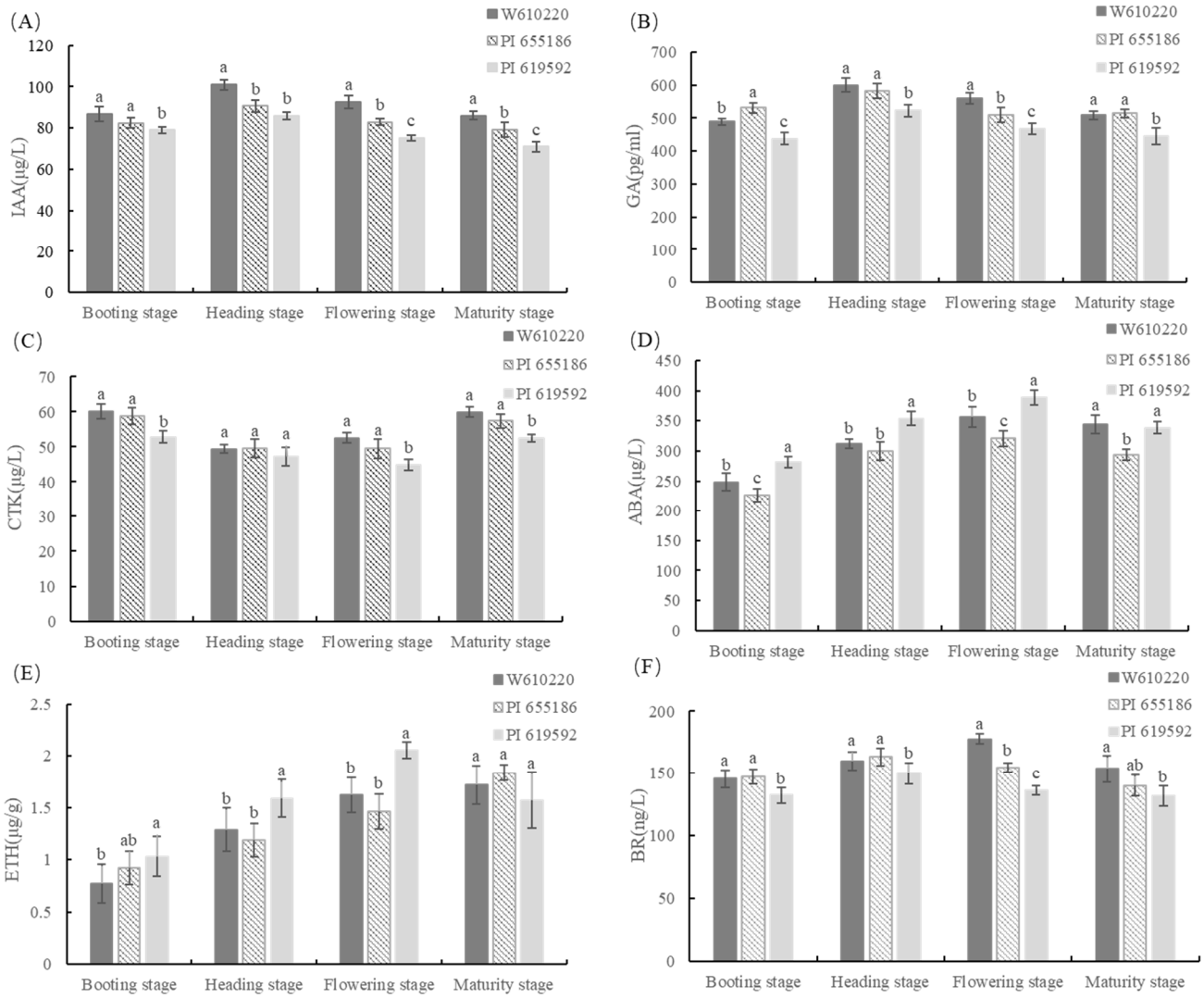


Figure 8. Hormonal changes of awns of three accessions at four developmental stages: (**A**) Auxin, (**B**) Gibberellin, (**C**) Cytokinin, (**D**) Abscisic acid, (**E**) Ethylene, (**F**) Brassinolide. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

The ethylene (ETH) content in long awn accession and medium awn accession increased gradually during four developmental stages. The ETH content of the long awn

accession and medium awn accession reached the maximum at maturity stage. The ETH content of short awn accession was firstly increased and then decreased, and reached the maximum at flowering stage (Figure 8E). In the case of brassinolate (BR), its content gradually decreased and long awn accession reached the maximum at flowering stage, while medium awn accession and short awn accession reached to the maximum at heading stage (Figure 8F).

Overall, the IAA, GA, CTK, and BR contents of long awn accession were higher than that of short awn accession, whereas ABA and ETH contents were lower than that of short awn accession.

3.4. The Difference in Antioxidant Enzyme Levels in Awns at Four Developmental Stages

The superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity of all accessions were firstly increased and then gradually decreased. The SOD, CAT, and POD contents of long awn accession reached the maximum at the flowering stage. The SOD content of medium awn accession reached the maximum at heading stage, while CAT and POD were at flowering stage. The SOD and CAT contents of short awn accession reached the maximum at heading stage while POD was at flowering stage. The contents of these three enzymes in long awn accession were significantly higher than those in short awn accession in most growth stages (Figure 9). The SOD, CAT, and POD contents of long awn accession at flowering stage were 26.77%, 10.66%, and 19.71% higher than that of short awn accession, respectively ($p < 0.05$).

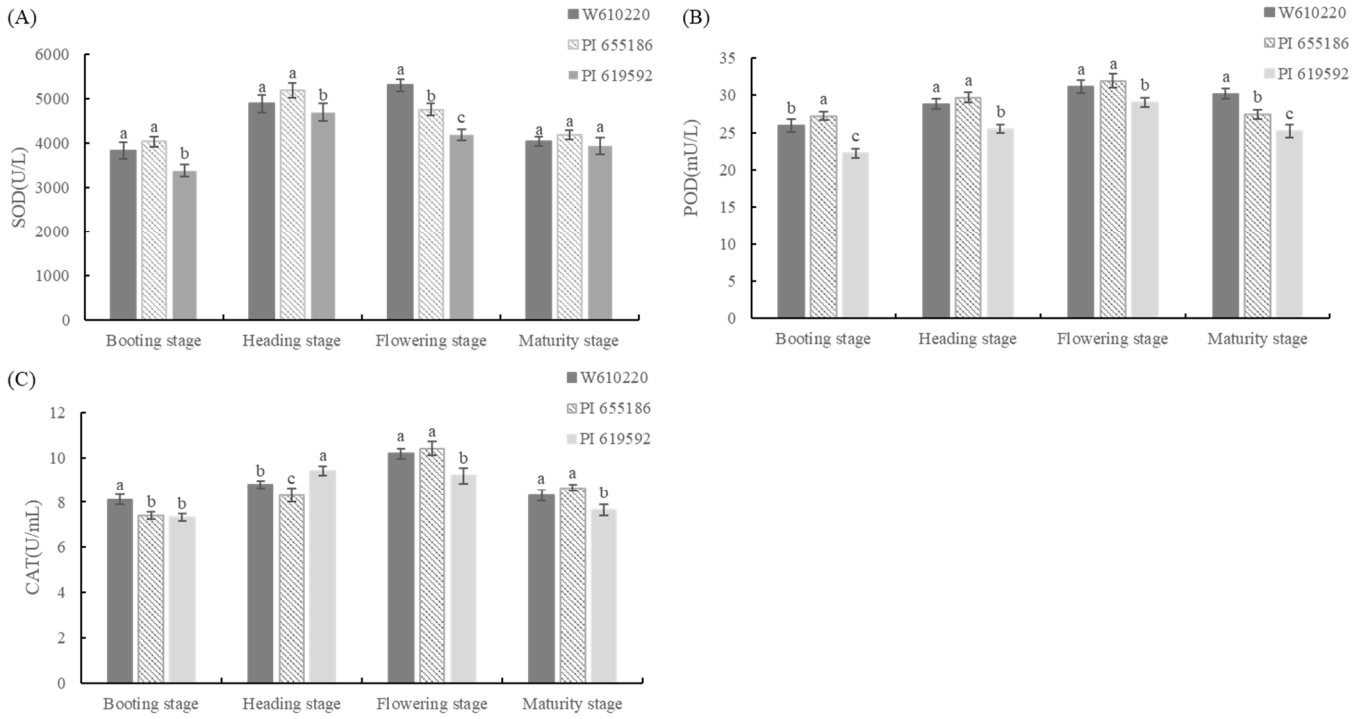


Figure 9. Changes of antioxidant enzyme content in awns of three accessions at four developmental stages: (**A**) Superoxide dismutase, (**B**) Catalase, (**C**) Peroxidase. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

3.5. Differences of Hydrolase and Amylase Levels in Awns at Four Developmental Stages

It was noted that cellulase (CE) and polygalacturonase (PG) contents of long awn accession during four developmental stages were firstly increased and then decreased, and later gradually increased to maximum. The maximum CE and PG contents in long awn accession were found at heading stage. On the other hand, CE and PG contents of short awn accession were gradually increased at each developmental stage. The CE and PG

contents of the short awn accession reached the maximum at maturity stage. At booting and heading stage, the CE and PG contents of long awn accession were significantly higher than those of short awn accession.

The amylase (AMS) content of long awn accession and medium awn accession were found to have gradually increased during the four developmental stages. The AMS content of the long awn accession and medium awn accession reached to a minimum at flowering stage, while the AMS content of short awn accession was found maximum at heading stage (Figure 10).

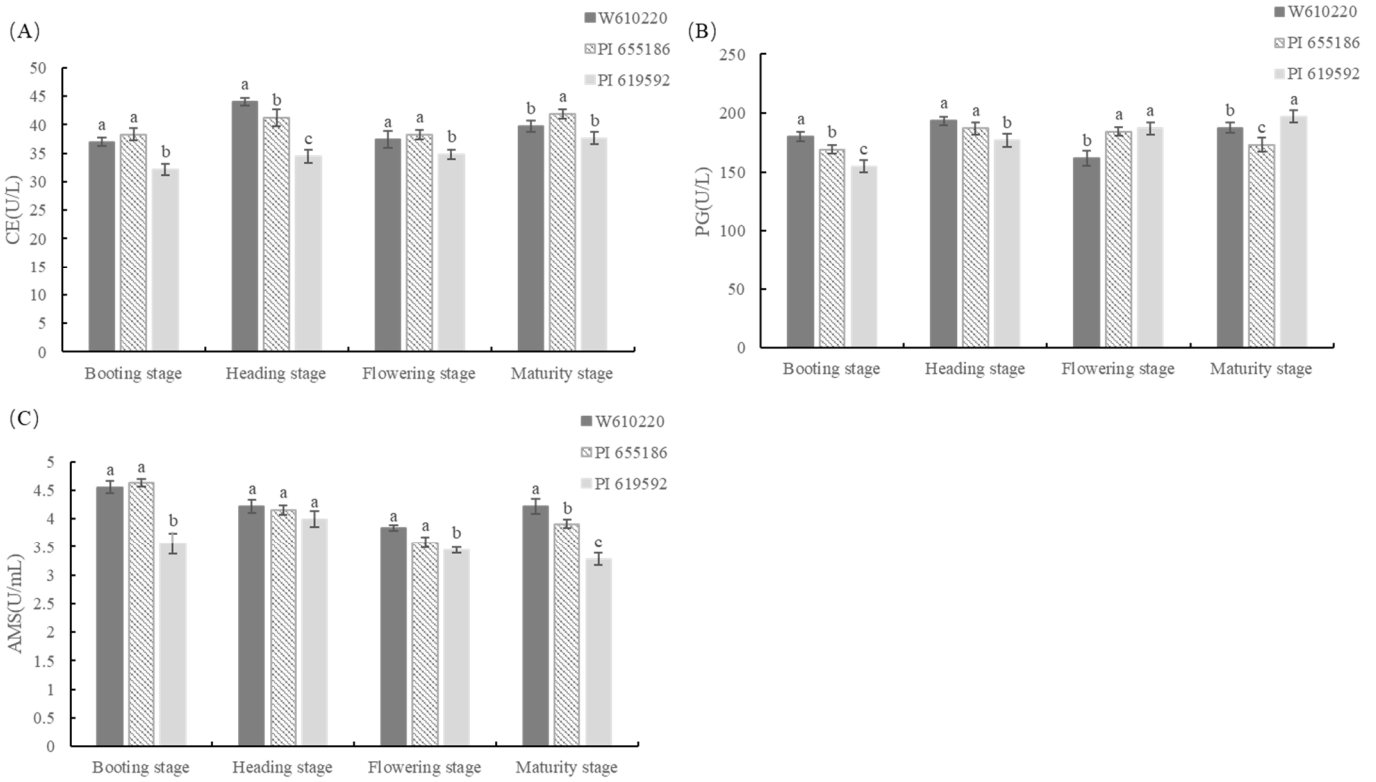


Figure 10. Changes of cell wall hydrolase and amylase contents in awns of three accessions at four growth stages: (**A**) Cellulase, (**B**) Polygalacturonase, (**C**) α-Amylase. Error bars represent mean ± standard deviation. Different normal letters mean significant differences among different treatments at 0.05 level.

3.6. Correlation Analysis of the Developmental Dynamics (Length Change) of Awns with Hormones and Enzymes

In the three accessions at four stages, the CTK of medium awn accession was negatively correlated with the length change of awns ($r = -0.928$; $p < 0.05$). The ABA of long awn accession was positively correlated with the length change of awns ($r = 0.996$; $p < 0.01$). There was a significant positive correlation between the ETH of long awn accession and the length change of awns ($r = 0.975$; $p < 0.05$). Moreover, the SOD of short awn accession was positively correlated with the length change of awns ($r = 0.979$; $p < 0.05$), and the POD of long awn accession and medium awn accession was significantly positively correlated with the length change of awns ($r = 0.99$, $p < 0.01$; $r = 0.997$, $p < 0.01$) (Table 3).

Table 3. Correlation analysis of the length change of awns with hormones and enzymes in three accessions at four stages.

Awn Length	IAA	GA	CTK	ABA	ETH	BR	SOD	POD	CAT	CE	PG	AMS
LCL	0.312	0.486	−0.404	0.996 **	0.975 *	0.752	0.641	0.99 **	0.638	0.238	−0.232	−0.866
LCM	0.471	0.081	−0.928 *	0.803	0.317	0.655	0.746	0.997 **	0.866	−0.258	0.862	−0.755
LCS	0.274	0.823	−0.71	0.83	0.742	0.743	0.979 *	0.684	0.857	0.494	0.64	0.415

LCL, length change of long awns; LCM, length change of medium awns; LCS, length change of short awns; * $p < 0.05$, ** $p < 0.01$.

4. Discussion

4.1. The Anatomical Structure and Function of Awns

Awns are the important structural and functional tissues of cereal crops that bear stomata. Stomata are particularly involved in the exchanges of gases in and outside the tissues [26]. The stomata of an awn could adjust its opening and closing according to fluctuations in the external environment that may help in obtaining the required amount of CO_2 without loss of water [27]. CO_2 is necessary for photosynthesis, and the sizes and number of stomata significantly influence the CO_2 fixation and, ultimately, photosynthesis. Previous reports suggested that, longer awns accessions significantly improved the photosynthesis and seed yield in barley and *E. sibiricus* [1,16]. However, in barley, this feature only plays a role in plants that grow under harsh conditions. In moderate climates, awns have no clear advantage over awnless barley [28]. However, the present study showed non-significant differences in the sizes of stomata between long and short awn accessions, whereas the number of stomata of long awn accession was higher than short awn accession. Thus, long awn accession may actively contribute to improving transpiration, water use efficiency, photosynthetic efficiency, seed yield, and seed dispersal rate than short awn accession in *E. nutans.* Although the breeding of grasses using awns has remained unreported, use of the awn length trait could add efficiency to the breeding process. Therefore, the advantages and disadvantages of awn traits in different environmental conditions could be helpful to screen out the most favorable breeding materials for seed production in forage grasses.

4.2. Changes in the Endogenous Hormones during Awn Development

Cytokinin is an important plant hormone involved in cell division, chloroplast development, and delay leaf senescence [29]. The CTK content in awns was firstly decreased and then gradually increased in all accessions. The CTK content of awns was increased during flowering and maturity, which may be due to its involvement in chloroplast development in awns. The CTK content of long awn accession was higher than that of short awn accession at different developmental stages, which was also consistent with the development of awns.

Moreover, the present study depicted that ABA and ETH contents were found to first increase and then gradually decrease in awns. The ABA and ETH contents of long awn accession were positively correlated with the development of awns (ABA: r = 0.996; $p < 0.01$; ETH: r = 0.975; $p < 0.05$). An increase in ABA and ETH leads to enhanced plant/leaf senescence and abscission in different species [30,31]. The higher content of ABA was found at the flowering stage, which reflected the development of chloroplast near the grain filling stage of awns, the main site for ABA biosynthesis [32]. The results of the present study showed that the ABA content of long awn accession was significantly lower than that of short awn accession in most developmental stages, which was simply the opposite to that of awn development. A previous study reported that [33] ABA content of the fruits had risen during senescence and remained higher than that of EHT during both ripening and senescence. Compared with ETH, ABA directly affected fruit ripening and senescence, and ETH worked as a cofactor [29]. Our study also showed a maximum ABA content at the flowering stage for all accession, while the maximum ETH content of medium awn and short awn accessions did not appear at all. This indicated that ABA may have a direct role in promoting awn development and senescence.

Although there was no significant correlation between the development dynamics of awns and other indicators, their "r" values could be greater than 0.4, which indicate that they were closely related to the development dynamics of awns. The growth of awn length stopped between the heading stage and the flowering stage. Therefore, it can be suggested that the hormone values at maturity stage cannot be significantly correlated between awn development and other indicators.

4.3. Changes in Antioxidant Enzymes during the Development of Awns

Antioxidant enzymes including SOD, CAT, and POD are also crucial in regulating internal metabolic processes in plant cells. SOD is the first line of defense that catalyzes oxygen radicals and converts them into H_2O_2. Moreover, CAT and POD dissociate H_2O_2 into water and oxygen. Usually, they are involved in the decomposition of peroxide and hydrogen peroxide produced by reactive oxygen species (ROS) and free radical oxidation, respectively, to reduce oxidative damage and production of malondialdehyde (MDA). These three enzymes formed a complete anti-oxidation chain [34,35]. In the normal metabolism of plants, reactive oxygen species could be produced in many ways and perform signaling functions, for example, plant photosynthesis and respiration [36,37]. Results of the present study showed an increase in POD content at booting stage, and later it decreased, which reached to a maximum at flowering stage. The change of POD content had the highest correlation with the development dynamics of awns (long awns: $r = 0.99$, $p < 0.01$; medium awns: $r = 0.997$, $p < 0.01$). Some studies have shown that POD play a role in respiration, photosynthesis, and auxin oxidation, and could be used as a physiological indicator of tissue aging [38]. Therefore, the change in POD content in *Elymus nutans* was more closely related to its development dynamics.

4.4. Changes of Cell Wall Hydrolase and Amylase during Awn Development

CE and PG are two major cell wall degrading enzymes that are involved in the breakdown of cellulose and pectin, respectively [39]. Moreover, AMS belongs to the enzyme family that has several members with different catalytic characteristics. Among them, α-amylase randomly acts on the non-reducing end of starch, maltose, maltotriose, dextrin, and other sugars and reduces the viscosity of starch slurry [40]. At booting and heading stages, the contents of all of these enzymes (CE, PG, and AMS) of long awn accession were higher than that of short awn accession. It suggested that long awn accession has faster and more dynamic metabolic processes, including cell division at all developmental stages. This is consistent with the development of awns.

Therefore, the present study could be used as a reference for further investigations to explore the role of awn development in forage grasses at the genetic level, as well as possible seed dispersal and final seed yield.

5. Conclusions

This study revealed the anatomical structure of *E. nutans* awns, which complements the anatomical characteristics of awns in Gramineae. It provides the physiological basis of awn development with enzyme and hormone regulations at different developmental stages in three different accessions. The results showed that CTK, ABA, ETH, SOD, and POD were closely related to awn development. Subsequent research can further investigate the role of hormones, enzymes, metabolites, and candidate genes that are involved in the development of awns in *E. nutans* and other related grass species.

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