

# Genome-wide analysis of the SWEET gene family and its response to powdery mildew and leaf spot infection in the common oat (*Avena sativa* L.)

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## Research Article

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# Abstract

The yield and nutritional quality of oats are usually inhibited by plant diseases such as red leaf, powdery mildew, and leaf spot. SWEETs (Sugars Will Eventually be Exported Transporters) are a newly discovered type of sugar transporter that play important roles in regulating plant growth and development, the abiotic stress response, and the interactions between plants and pathogens. However, members of the *SWEET* gene family and their roles in responding to biotic stresses have not yet been characterized in oats (*Avena sativa*). In this study, 13 *AsSWEET* genes were identified, distributed across nine chromosomes in the oat genome. They all were predicted to contain seven transmembrane regions. Phylogenetic analysis showed that *AsSWEET* proteins clustered into four clades and possessed high homology with *SWEET* proteins in the family Poaceae. *AsSWEET* proteins were predicted to localize to the plasma membrane. The promoter regions of the oat *AsSWEET* genes contained cis-acting elements related to light response, growth and development, and stress response. Furthermore, the expression patterns of *AsSWEET* genes in two oat varieties, 'Yanwang' and 'Mulesi', were analyzed after powdery mildew and leaf spot infections. The relative expression levels of the majority of *AsSWEET* genes were significantly up-regulated or down-regulated after infection with pathogens, including *AsSWEET1a*, *AsSWEET1b*, *AsSWEET2b*, *AsSWEET3a*, *AsSWEET11*, and *AsSWEET16*. These *SWEETs* are potential candidate genes for the response to diseases in oats. In conclusion, this study provides a theoretical basis for future research into the regulatory mechanisms of the *AsSWEETs*, particularly their roles in the resistance to powdery mildew and leaf spot, and may promote further enhancements to the molecular breeding of the oat.

## 1 Introduction

Among nutrients that can be utilized by plants, sugars are the main sources of carbon and energy for organisms<sup>[1]</sup>. Sugars are also known to be important signaling molecules. In mesophyll cells of higher plants, chloroplasts can fix carbon by converting carbon dioxide into organic carbon through photosynthesis<sup>[2-4]</sup>. However, sugars cannot be transported independently through the plant cell membrane systems, requiring the assistance of appropriate sugar transporters<sup>[2]</sup>. Three main types of sugar transport proteins have been discovered thus far, namely monosaccharide transporters (MSTs)<sup>[5]</sup>, sucrose transporters (SUTs)<sup>[6, 7]</sup>, and sugar efflux transporters (SWEETs)<sup>[8]</sup>. In the past twenty years, MSTs and SUTs have been widely studied in plants<sup>[9]</sup>. The SWEET (Sugars Will Eventually be Exported Transporter) family genes play important roles in transporting hexose or sucrose across cell membranes<sup>[10]</sup>, though their specific functions still remain largely unknown.

SWEETs are widely distributed in both eukaryotes and prokaryotes. They are a type of sugar transfer protein that does not depend on environmental pH values. Eukaryotic SWEET proteins typically consist of seven alpha-helical transmembrane domain (7-TMs), which are folded into two 3-TM domains (conserved MtN3/saliva domains) connected by one central TM<sup>[11]</sup>. SWEETs have been reported to be involved in regulating the response of plants to biotic stress. When plants are invaded by bacterial or fungal pathogens, the pathogens obtain the necessary sugars from the plants, thereby triggering the upregulation of SWEET gene expression<sup>[12, 13]</sup>. Studies have found that expression levels of some SWEET family genes were elevated in *Arabidopsis thaliana* after infection by *Pseudomonas syringae*, indicating that SWEETs are involved in the interaction between *A. thaliana* and this pathogen<sup>[14]</sup>. In *Vitis vinifera*, *VvSWEET4* has been found to interact with the fungal pathogen *Botrytis cinerea*<sup>[15]</sup>. The SWEET gene *UPA16* was upregulated in *Capsicum annuum* by infection with *Xanthomonas campestris*<sup>[16]</sup>. Similarly, in *Triticum aestivum*, the expression of a *SWEET* gene is induced by infection with the fungus *Puccinia striiformis*<sup>[17]</sup>. The role of endogenous sugars in mediating plant immune responses and combating pathogen

attacks is receiving increasing attention, leading to the emergence of concepts such as "sweet immunity" or "sugar enhanced defense" [18, 19]. The regulation of these sugar levels will be of great significance for crop improvement.

Oats (*Avena sativa* L.) are an important annual cereal and feed crop in Poaceae [20]. Due to their strong stress resistance, excellent quality, high yield, good palatability, easy harvesting, and easy processing and modulation, oats are a globally cultivated crop [21]. Oats are mainly produced in countries including Russia, Canada, the United States, Australia, Germany, Finland, China, as well as others in the northern hemisphere [22]. The growth of the global population and the increasing demand for nutritious and healthy foods have led to an annual increase in oat production [23]. With changes in climate and environmental conditions, oat cultivation has been threatened by diseases such as powdery mildew, red leaf disease, smut, and leaf spot, which have inhibited the quality and yield of oats in recent years [24, 25]. At present, fungal plant diseases in oats are controlled using methods such as field cultivation management, biological control, chemical control, and the breeding of resistant varieties [26]. However, cultivation management is time-consuming and labor-intensive, and chemical control can easily cause environmental pollution [27]. Thus, the identification and characterization of genes that may be related to disease susceptibility and resistance are necessary to provide a basis for the genetic improvement of crops.

Thus far, numerous studies have shown that SWEET sugar transporters are closely related to biotic and abiotic stress responses in plants [28–30], but few of these studies have characterized the roles of oat SWEETs in response to biotic stress. Therefore, this study aimed to identify the oat *SWEET* family genes and systematically analyze the structural characteristics, conserved protein motifs and structure, and the expression of these *SWEET* genes in oat leaves under different disease stresses. This study provides a theoretical foundation for further understanding the role of *SWEET* genes in regulating the resistance to plant diseases, which will contribute to the exploration of excellent oat gene resources and the breeding of new varieties.

## 2 Results

### 2.1 Identification and analysis of physicochemical properties of oat *SWEET* genes

Using comparative approaches, 13 *SWEET* genes were identified in the oat genome. These were named *AsSWEET1a*, *AsSWEET1b*, *AsSWEET2a*, *AsSWEET2b*, *AsSWEET3a*, *AsSWEET4*, *AsSWEET5*, *AsSWEET11*, *AsSWEET12*, *AsSWEET13*, *AsSWEET15*, *AsSWEET16*, and *AsSWEET17*. The *AsSWEETs* were distributed across nine chromosomes (Fig. 1). These genes encoded proteins ranging from 231 (*AsSWEET2b*) to 313 (*AsSWEET16*) amino acids in length, with an average of about 267 amino acids and molecular weights ranging from 25.404 kDa to 34.145 kDa (Table 1). Except for *AsSWEET4*, *AsSWEET15*, and *AsSWEET17*, whose theoretical isoelectric points (PIs) were less than 7, the predicted PIs of other *AsSWEET* proteins were greater than 7, indicating that they were alkaline in nature. All *AsSWEETs* were predicted to contain 7 transmembrane helices (TMHs), and all were predicted to localize to the plasma membrane (PM).

Table 1  
Physicochemical properties of AsSWEET proteins in oats.

| Gene Name                          | Number of amino acids | Molecular mass (Da) | Theoretical PI | Instability index | Fat index | Hydrop-hilicity | Transme-mbrane helix number | Subcellular localization |
|------------------------------------|-----------------------|---------------------|----------------|-------------------|-----------|-----------------|-----------------------------|--------------------------|
| AsSWEET1a                          | 265                   | 28627.93            | 8.94           | 28.83             | 115.92    | 0.731           | 7                           | PM                       |
| AsSWEET1b                          | 267                   | 28527.36            | 9.05           | 40.72             | 97.94     | 0.474           | 7                           | PM                       |
| AsSWEET2a                          | 243                   | 26565.72            | 8.95           | 39.97             | 116.38    | 0.809           | 7                           | PM                       |
| AsSWEET2b                          | 231                   | 25530.26            | 8.83           | 42.88             | 112.34    | 0.774           | 7                           | PM                       |
| AsSWEET3a                          | 245                   | 27376.65            | 9.21           | 38.79             | 109.31    | 0.633           | 7                           | PM                       |
| AsSWEET4                           | 233                   | 25404.34            | 6.55           | 36.85             | 130.86    | 0.865           | 7                           | PM                       |
| AsSWEET5                           | 253                   | 27556.95            | 9.47           | 32.02             | 121.38    | 0.713           | 7                           | PM                       |
| AsSWEET11                          | 295                   | 31888.62            | 8.42           | 45.77             | 108.07    | 0.784           | 7                           | PM                       |
| AsSWEET12                          | 308                   | 33799.10            | 6.44           | 47.85             | 116.75    | 0.569           | 7                           | PM                       |
| AsSWEET13                          | 298                   | 32613.50            | 8.81           | 39.35             | 118.39    | 0.605           | 7                           | PM                       |
| AsSWEET15                          | 304                   | 33501.60            | 6.91           | 33.12             | 112.83    | 0.582           | 7                           | PM                       |
| AsSWEET16                          | 313                   | 34145.39            | 9.27           | 37.57             | 104.95    | 0.438           | 7                           | PM                       |
| AsSWEET17                          | 238                   | 25910.76            | 6.71           | 34.96             | 121.64    | 0.867           | 7                           | PM                       |
| Note: PM indicates plasma membrane |                       |                     |                |                   |           |                 |                             |                          |

## 2.2 Predictions of AsSWEET protein secondary and tertiary structures

The secondary structures of all 13 AsSWEET proteins were predicted to contain  $\alpha$ -helices, irregular curls,  $\beta$ -turns, and extension chains, though the proportions of each structure varied (Table 2, Fig. 2). For all AsSWEET proteins,  $\alpha$ -helices were the largest contributors to their secondary structures. The proportions of irregular curly structures ranged from 13.74–25.30%, with an average of 18.98%.  $\beta$ -turn structures were the smallest contributors in all AsSWEET proteins. The proportions of extended chains fluctuated, ranging from 28.68–39.70%. Tertiary structure predictions for the AsSWEET proteins (Fig. S1) indicated that the tertiary structures of AsSWEET1a and AsSWEET1b were highly similar, as were the tertiary structures of AsSWEET2a and AsSWEET2b. The remaining proteins had significant differences in their tertiary structures, though  $\alpha$ -helical structures were the main structural component in all.

Table 2  
Predictions of AsSWEET protein secondary structure.

| Protein Name | $\alpha$ -helical structure (%) | Random curl knot (%) | $\beta$ -turn structure (%) | Extension chain (%) |
|--------------|---------------------------------|----------------------|-----------------------------|---------------------|
| AsSWEET1a    | 50.19                           | 18.87                | 2.26                        | 28.68               |
| AsSWEET1b    | 39.70                           | 16.85                | 3.75                        | 39.70               |
| AsSWEET2a    | 44.03                           | 18.52                | 3.29                        | 34.16               |
| AsSWEET2b    | 51.95                           | 15.58                | 3.46                        | 29.00               |
| AsSWEET3a    | 48.57                           | 14.69                | 1.22                        | 35.51               |
| AsSWEET4     | 38.63                           | 24.89                | 4.29                        | 32.19               |
| AsSWEET5     | 37.94                           | 25.30                | 3.95                        | 32.81               |
| AsSWEET11    | 39.66                           | 23.05                | 2.71                        | 34.58               |
| AsSWEET12    | 40.91                           | 18.83                | 2.92                        | 37.34               |
| AsSWEET13    | 41.61                           | 21.14                | 2.68                        | 34.56               |
| AsSWEET15    | 45.39                           | 15.13                | 3.29                        | 36.18               |
| AsSWEET16    | 52.40                           | 13.74                | 3.83                        | 30.03               |
| AsSWEET17    | 43.70                           | 20.17                | 4.20                        | 31.93               |

## 2.3 Conserved motif analysis of oat AsSWEET protein sequences

Twelve conserved motifs were identified across the 13 oat AsSWEET proteins (Fig. 3), with motifs 1, 2, 3, 4, 5, and 8 identified in all 13 AsSWEETs. Motif 6 was found in AsSWEET1b, 12, 13, 15, and 16, but in different locations within each protein. Motif 7 was found in AsSWEET12, 13, and 16. Motif 9 was found in AsSWEET1b, 12, and 16. Motif lengths ranged between 6–50 amino acids, with motif 1 and motif 2 both 50 amino acids in length (Fig. S2). Motif 11 and motif 12 were both 26 amino acids in length, while motifs 3, 4, 5, 6, 7, 8, 9, and 10 had lengths of 31, 22, 21, 32, 8, 20, 6, and 7 amino acids, respectively. In addition, motifs 3, 4, 5, 6, and 8 contained relatively few conserved amino acid residues. Motifs 1, 2, 7, 9, 10, 11, and 12 contained multiple conserved amino acid residues, suggesting that they may play important roles in sugar transport in oats. According to NCBI's Batch CD-search, online analysis of the conserved domains of oat SWEETs proteins revealed that all SWEETs proteins contain two conserved domains, with six oat SWEETs proteins containing two MtN3\_slv conserved domains, three oat SWEETs proteins containing two PQ loop conserved domains, and four oat SWEETs proteins each containing one MtN3\_slv and one PQ loop conserved domain.

## 2.4 Phylogenetic analysis of SWEET protein sequences in oat, barley, *Arabidopsis*, rice, and maize

Phylogenetic analysis showed that members of the oat AsSWEET protein family were divided into four main clades (Fig. 4). Oat AsSWEETs had high homology with monocotyledonous barley HvSWEETs, rice OsSWEETs, and maize ZmSWEETs, whereas multiple AtSWEETs proteins from the dicotyledonous *A. thaliana* clustered independently of AsSWEETs, HvSWEETs, OsSWEETs, and ZmSWEETs.

## 2.5 Identification of cis-acting elements in *AsSWEET*s

The cis-acting elements identified in promoter regions of the *AsSWEET*s were associated with growth and development, hormone response, and abiotic stress response (Fig. 5). Analysis revealed that all 13 *AsSWEET* genes contained 1 to 7 photoresponsive elements, 10 genes contained 1 to 4 auxin-responsive elements, and 12 genes contained 1 to 4 gibberellin-responsive elements. Several genes contained MeJA-responsive elements, salicylic acid responsive elements, and abscisic acid responsive elements. In addition, a few genes contained cis-acting elements associated with flavonoid biosynthesis. *AsSWEET17* and *AsSWEET12* contained defense and stress response elements. Overall, the promoter regions of these *AsSWEET*s contained various types of cis-acting regulatory elements, indicating their potential involvement in diverse biological processes and regulatory pathways.

## 2.6 Analysis of *AsSWEET* expression under biotic stresses

### 2.6.1 Response to powdery mildew

Expression of *AsSWEET* genes was assayed in two oat cultivars, 'Yanwang' and 'Muleshi', after inoculation with powdery mildew. 'Yanwang' was more susceptible to powdery mildew than 'Muleshi'. The expression levels of several *AsSWEET* genes were significantly up- or down-regulated after infection (Fig. 6). Expression of *AsSWEET1a*, *3a*, *11*, and *16* was significantly up-regulated in leaves of both cultivars after infection. The relative expression of *AsSWEET2a* and *12* was significantly down-regulated in leaves of 'Yanwang' after infection, while they were significantly up-regulated in 'Muleshi' leaves.

### 2.6.2 Response to leaf spot stress disease

The infection with leaf spot was more severe in 'Muleshi' than in 'Yanwang' (Fig. 7). Six days after inoculation, the relative expression levels of *AsSWEET1b*, *2a*, and *2b* in 'Yan Wang' were significantly increased as compared to the control ( $p < 0.05$ ). The expression of *AsSWEET 4*, *5*, *11*, *12*, *13*, and *15* was significantly down-regulated in both cultivars. The expression of *AsSWEET17* was stable in 'Yanwang' after infection, while it was significantly down-regulated in leaves of 'Muleshi'.

## 3 Discussion

Sugar is not only an energy and carbon source for plant growth and cellular metabolism but also an important signaling molecule<sup>[45, 46]</sup>. Members of the SWEET sugar transporter protein family not only control the supply and distribution of sugars inside and outside cells and between organelles, but also play important roles in coordinating plant growth and development under stress<sup>[47, 48]</sup>. A total of 13 oat *AsSWEET* family proteins were identified in this study, all of which were predicted to contain 7 TMH structures. Zhang et al. analyzed the SWEET family in *Poa pratensis* and found that PpSWEET proteins contained 4–7 TMHs, consistent with the results of this study<sup>[49]</sup>.

Phylogenetic analysis indicated that the SWEET proteins were divided into four clades. Previous research has shown that SWEETs on different phylogenetic branches can transport different sugars<sup>[50]</sup>. SWEETs in Clade I (SWEET1, 2, and 3) and Clade II (SWEET4, 5, 6, 7, and 8) are mainly used for transporting glucose and fructose, as well as sucrose. Clade III SWEETs (SWEET9, 10, 11, 12, 13, 14, and 15) are mainly used for the efficient transport of sucrose<sup>[51]</sup>. A recent study showed that SWEET14 and SWEET15 can participate in the regulation of excitatory signaling and regulate the response to gibberellin<sup>[52]</sup>. Clade IV SWEETs (SWEET16 and 17) are vacuolar membrane

transporters, which are primarily used for transporting fructose in leaf and root vacuolar membranes<sup>[53]</sup>. In this study, oat AsSWEETs were also divided into four clades, consistent with previous research results. In general, similar protein structures imply similar functions. Therefore, it can be inferred that different protein members in different clades function to transport different sugars, though the specific functions of each protein need further empirical validation. The oat AsSWEETs identified here have high genetic similarity with other crops in the Poaceae family. Compared to the dicotyledonous *A. thaliana*, Poaceae crops do not have SWEET8 and SWEET9, indicating differences in the evolution of the SWEET gene family in monocotyledonous and dicotyledonous plants.

Salicylic acid can inhibit the activity of plant cell wall degrading enzymes secreted by pathogens, thereby reducing their pathogenicity. Salicylic acid can also induce the expression of disease related genes<sup>[55]</sup>. Several AsSWEET genes, including *AsSWEET1a*, *1b*, *2a*, *2b*, and *16*, were found to contain salicylic acid responsive cis-regulatory elements, and their expression levels were significantly up-regulated or down-regulated after powdery mildew and leaf spot infection stress, indicating that salicylic acid may mediate the resistance of oats to these diseases.

The main energy source for most pathogens is sugar. Plants are known to regulate their sugar contents by up-regulating or down-regulating the expression of the *SWEET* genes in response to various biotic stresses<sup>[54]</sup>. In the rice *SWEET* gene family, *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* are known susceptibility genes to *Candida albicans*<sup>[56]</sup>. In rice varieties containing these genes, the transcription activator-like effectors of *Candida albicans* can bind to the EBE sites in their promoter regions and induce their expression, thus resulting in rice susceptibility<sup>[57–58]</sup>. Zhang et al.<sup>[59]</sup> found that when potato leaves were infected with late blight, the expression of *StSWEET5* was up-regulated, indicating that *StSWEET5* may play a role in resistance to late blight. In this study, significant up-regulation of *AsSWEET5* was observed in both 'Yanwang' and 'Muleshi' cultivars three days after powdery mildew infection, though *AsSWEET5* expression was down-regulated at a comparable timepoint after leaf spot infection. These results indicate that *AsSWEET5* may be involved in the response to both powdery mildew and leaf spot infections, though the response mechanisms may differ. Fan et al.<sup>[60]</sup> found that overexpression of *StSWEET16b* inhibits the expansion of late blight bacteria, thus enhancing disease resistance. In this study, *AsSWEET16* was significantly up-regulated after powdery mildew infection, indicating that *AsSWEET16* may be involved in the regulation of powdery mildew resistance in oats. This study also found that *AsSWEET1a*, *1b*, *2b*, *3a*, *11*, and *16* were either significantly up- or down-regulated after infection with powdery mildew or leaf spot disease. Thus, these genes are promising candidates for the regulation of oat disease resistances.

## 4 Conclusion

In this study, a total of 13 SWEET sugar transporter proteins were identified in the oat, distributed across nine chromosomes in the oat genome. All AsSWEET sugar transporters were predicted to be hydrophobic proteins containing 7 TMHs. Both secondary and tertiary structure predictions indicated  $\alpha$ -helices as the main structural components of the AsSWEET proteins. Phylogenetic analysis indicated that AsSWEETs clustered into four subfamilies, with high homology with the SWEET protein family of multiple crops in the Poaceae family. AsSWEET promoter regions contained cis-acting elements related to light response, growth and development, and stress response. The AsSWEETs actively participated in the response to biological stress. Among them, *AsSWEET1a*, *1b*, *2b*, *3a*, *11*, and *16* were differentially expressed after infection with either powdery mildew or leaf spot, making them important candidate genes for studying oats' response to biological stress.

## 5 Materials and Methods

## 5.1 Materials

Two oat (*Avena sativa* L.) varieties that differ in their susceptibility to powdery mildew and leaf spot infection, 'Yanwang' and 'Muleshi', were used in this study. The pathogenic fungi of powdery mildew (*Blumeria graminis* f.sp. *avenae*) and leaf spot disease (*Drechslera avenacea*) were isolated and stored by our laboratory.

## 5.2 Identification of *AsSWEET* genes

The protein sequences of *SWEET* genes in *Arabidopsis thaliana*, rice (*Oryza sativa*), barley (*Hordeum vulgare*), and corn (*Zea mays*) were directly downloaded from the NCBI database (<https://www.ncbi.nlm.nih.gov/>), and the oat genome file was downloaded from the GrainGenes database (<https://wheat.pw.usda.gov/GG3/content/avena-sang-download>)<sup>[31]</sup>. To identify oat *SWEET* genes, the BLAST wrapper in TBtools<sup>[32]</sup> was used to align known *SWEET* genes to the oat genome.

## 5.3 Characterization of *AsSWEET* proteins

Physicochemical properties of *AsSWEET* protein sequences, including amino acid number, molecular weight, theoretical isoelectric point, instability index, aliphatic index, and total average hydrophilicity, were analyzed using the ExPASy-ProtParam tool (<https://web.expasy.org/protparam/>)<sup>[33]</sup>. The TMHMM tool (<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>) was used to predict transmembrane spiral structures (TMHMM 2.0-DTU Health Tech Bioinformational Services)<sup>[34]</sup>. Subcellular localizations were predicted using ProtComp v. 9.0 (<http://www.softberry.com/>)<sup>[35]</sup>. Predictions of protein secondary structure were made using SOPMA (<https://npsa-prabi.ibcp.fr/cgi-bin/>), and predictions of protein tertiary structure were made using SWISS-MODEL (<https://swissmodel.expasy.org/>)<sup>[36–40]</sup>.

## 5.4 Sequence structure and phylogenetic analyses

Conserved motif analysis of *SWEET* amino acid sequences in the oat were performed using the online tool MEME (<http://meme-suite.org/>)<sup>[41]</sup>. Gene structure and motif distributions were visualized using TBtools<sup>[42]</sup>. The maximum likelihood method in MEGA 11.0 was used to construct a *SWEET* family phylogenetic tree using 13 oat sequences, 16 barley sequences, 17 *A. thaliana* sequences, 21 rice sequences, and 24 maize sequences. The cis-acting elements in the putative promoter regions of the *AsSWEETs*, defined as the 2,000 bp upstream of the genes, were predicted using PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>), and TBtools was used to visualize predicted cis-acting elements<sup>[43]</sup>.

## 5.5 Oat growth conditions and biotic stress treatments

The seeds of two oat cultivars, 'Yanwang' and 'Mulesi', were sown in plastic pots containing a mixed medium (soil and vermiculite, 2:1). The daytime temperature of growth chambers was 25°C, and the nighttime temperature was 20°C. Oats were grown using a 14-hour light: 10-hour dark cycle. Three replicates were generated for each treatment (control and inoculated). Twenty days after planting, the plants were inoculated with *Blumeria graminis* or *Drechslera avenacea* spore solutions (10<sup>4</sup> conidia/mL), with chamber humidity kept above 80%. The control plants were inoculated with an equal volume of sterile distilled water. After inoculation, leaves were sampled at 0 h, 1 d, 3 d, 6 d, 9 d, and 12 d, immediately frozen in liquid nitrogen, and then stored at – 80°C for later RNA extraction.

## 5.6 RNA extraction and real-time quantitative PCR analysis

RNA extraction was performed using the total RNA Extraction Kit (DP419, Tiangen). Then, cDNA was synthesized using the PrimerScript™ RT Reagent Kit with gDNA Eraser (R047A, Takara).

Primers specific for the oat *AsSWEET* gene were designed using Primer BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Primer sequences are shown in Table S1. qRT-PCR was performed using the SuperReal PreMix Plus (SYBR Green) fluorescence quantification kit. The total 20 µL reaction volume consisted of 5 µL of diluted template (20 µL of cDNA diluted 20-fold with 180 µL of ddH<sub>2</sub>O), 3 µL of ddH<sub>2</sub>O, 1 µL of upstream and 1 µL of downstream primers (10 µmol·L<sup>-1</sup>), and 10 µL of 2× SuperReal PreMix Plus (SYBR Green). The reaction process was carried out on a LightCycler®96 instrument. The qRT-PCR reaction procedure was: initial denaturation at 95°C for 15 minutes and then 40 cycles of denaturation at 95°C for 10 seconds and annealing at 58°C for 30 seconds. Three biological replicates were set up for each treatment, and three technical replicates were performed for each biological replicate. The relative gene expression level was calculated using the  $2^{-\Delta\Delta C_t}$  method [44]. *Actin* was used as the reference gene.

## 5.7 Data analysis

The Windows version of SPSS 24.0 (SPSS Inc., USA) was used for data analysis, with measurements presented as means with standard errors. Statistical significance between control group and treatment group measurements at different times was assessed using t-tests. Figures were drawn using GraphPad Prism 8.0.2 software.

## Declarations

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Not applicable

### Authors' contributions

KN is expected to have made substantial contributions to the design of the work. YP, PM and JY performed the experiments and acquire the data. YP analyzed the data and wrote the manuscript. ZJ, KN and GZ revised the manuscript. JC, XC and RZ funded this research program. All authors have read and approved the final version of the manuscript.

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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## Figures

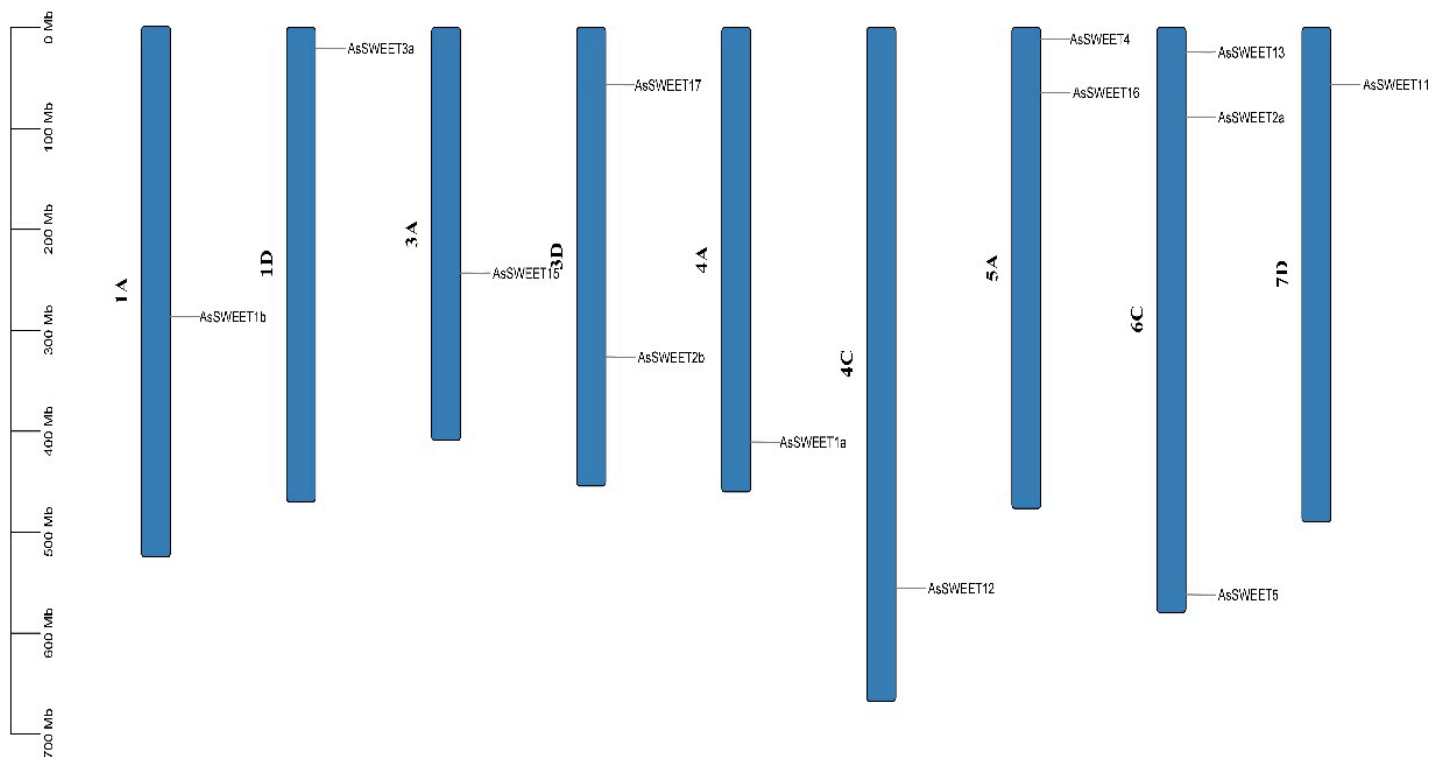


**Figure 1**

The distribution of *AsSWEET*s across oat chromosomes. Blue bars represent nine chromosomes with *AsSWEET* genes. The scale indicates the chromosome length, and the black lines indicate the position of each *AsSWEET* gene.

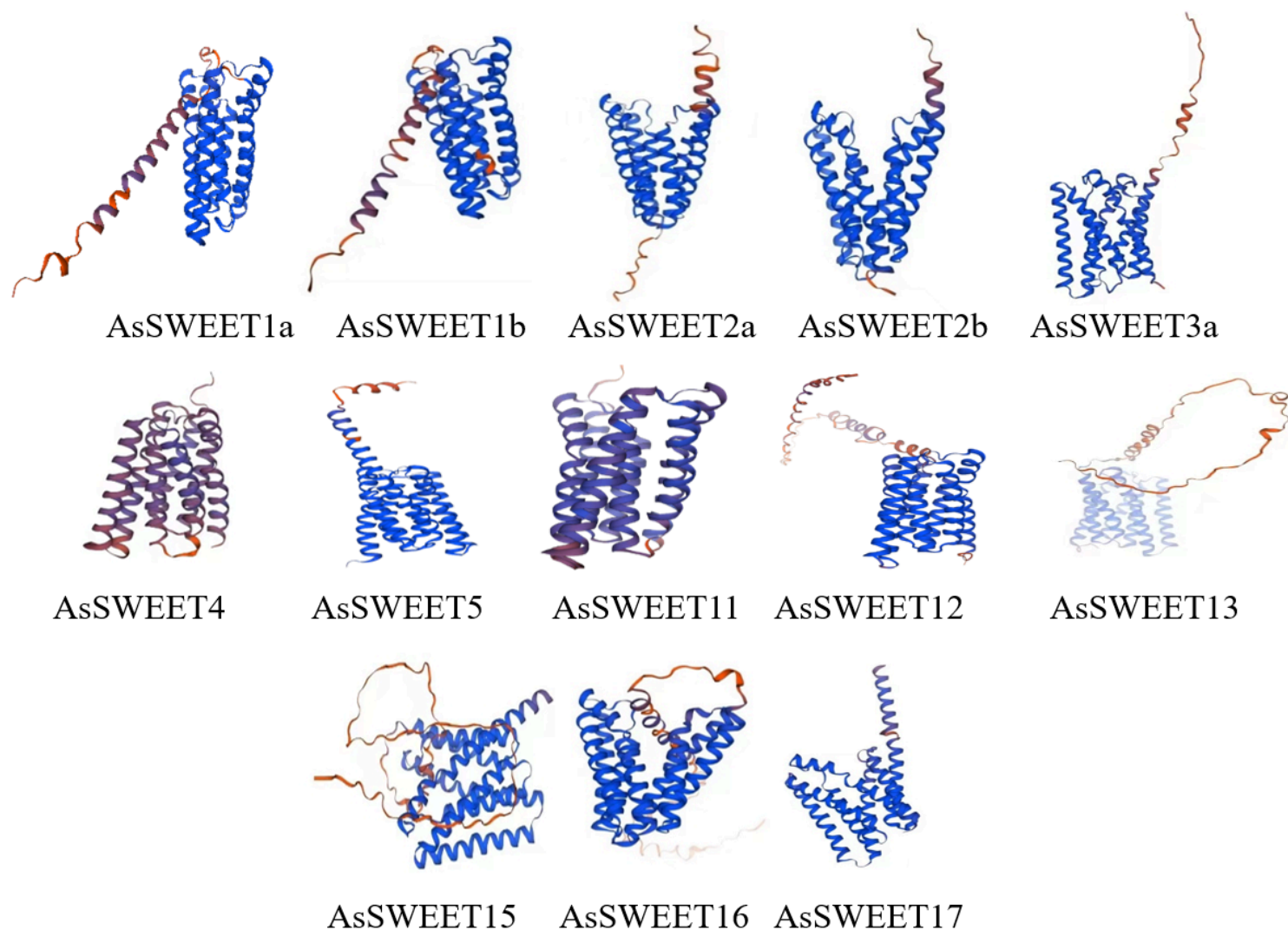


**Figure 2**

Tertiary structure predictions of AsSWEET proteins. Blue indicates  $\alpha$ -helices, green indicates  $\beta$ -folds, yellow indicates irregular curls, and red indicates extension chains.

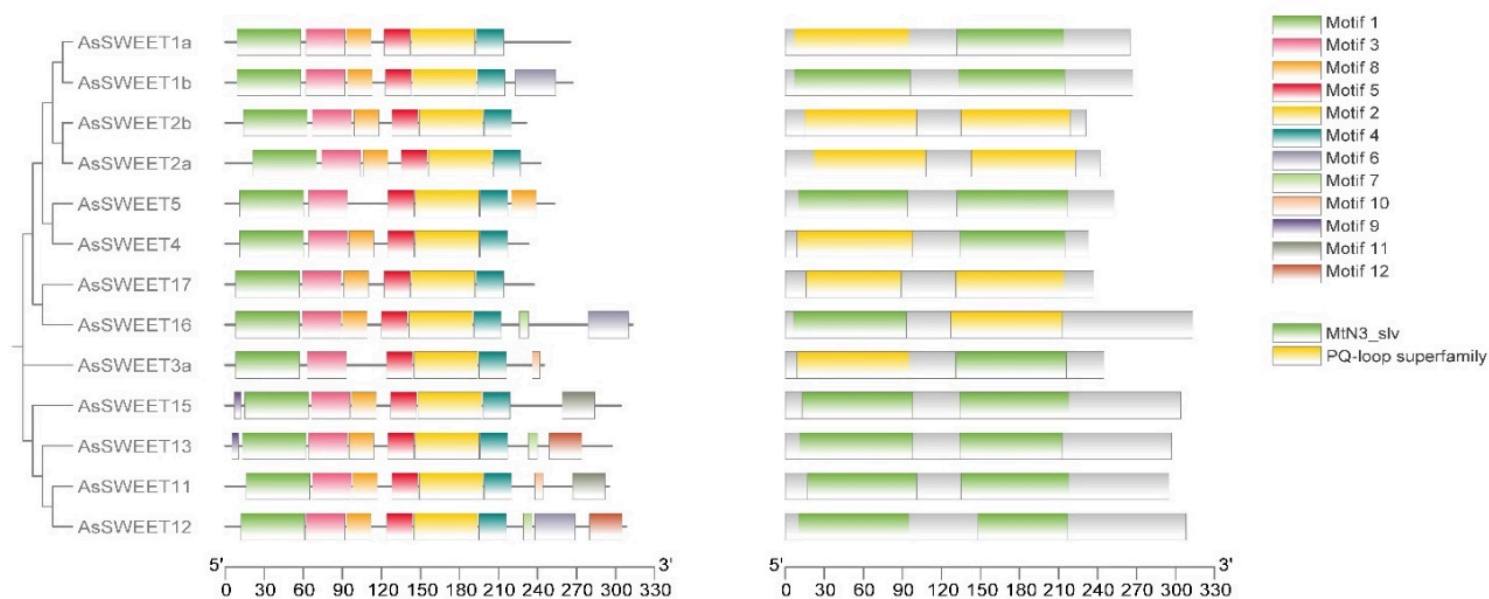


Figure 3

Conserved motif diagrams of oat AsSWEET proteins.

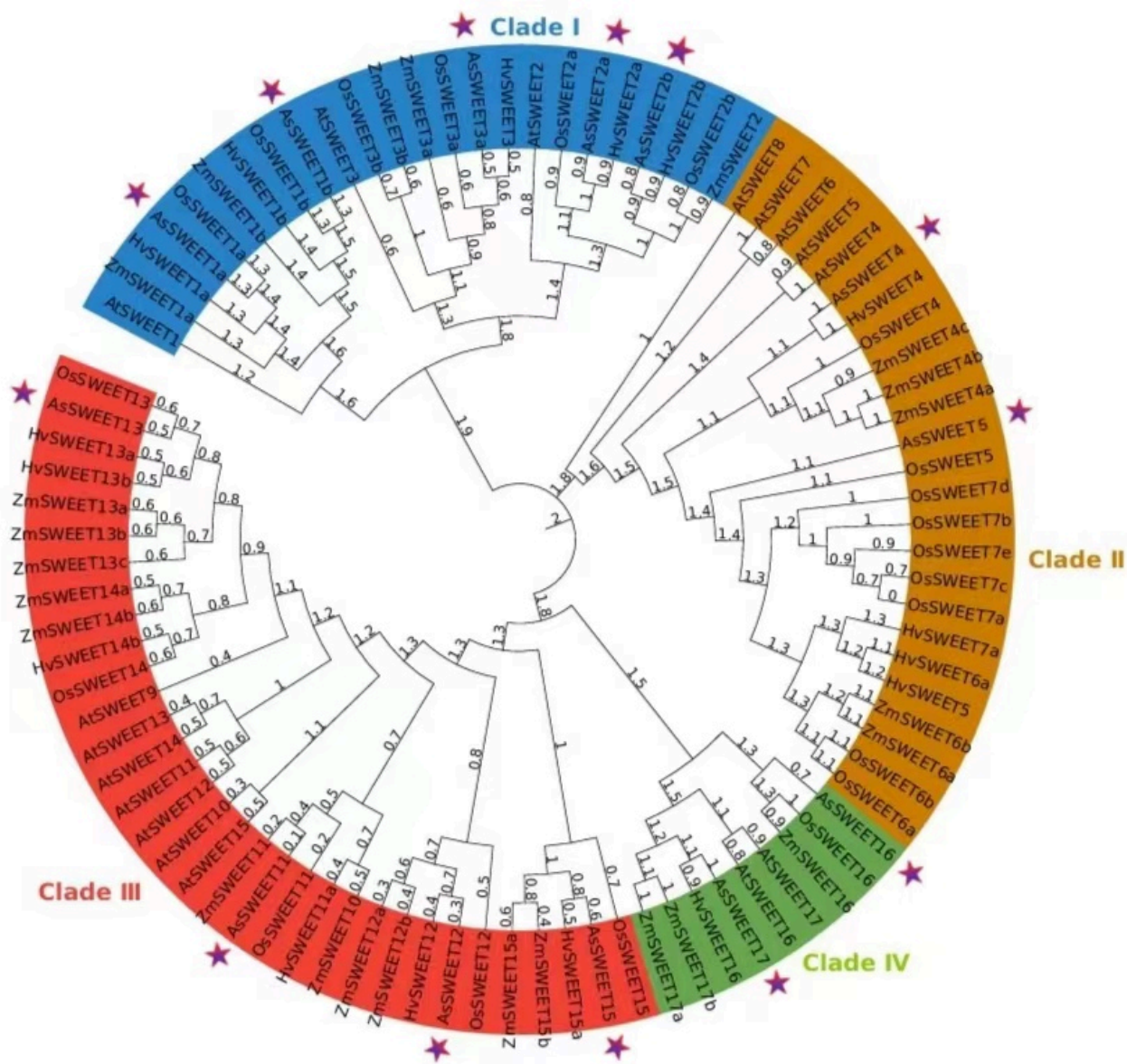


Figure 4

Phylogenetic analysis of SWEET sugar transporter sequences in oat, barley, A. thaliana (L.) Heynh, rice, and maize. SWEET proteins were divided into four major clades.

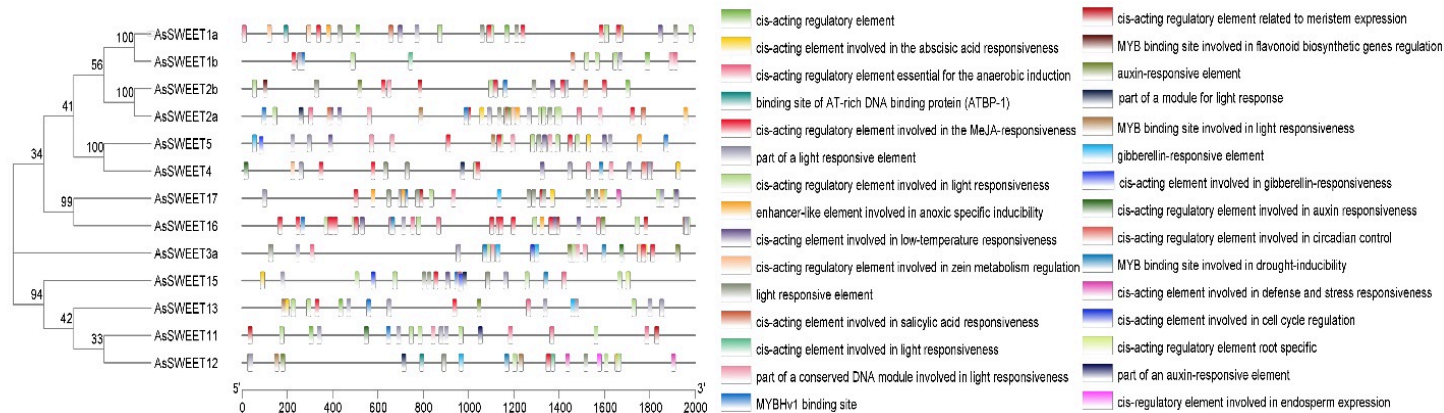


**Figure 5**

The cis-acting regulatory elements contained in the 2 kb promoter regions of the *AsSWEET* genes. Different cis-regulatory elements are indicated by different colored rectangles, with relative positions within the promoters indicated.

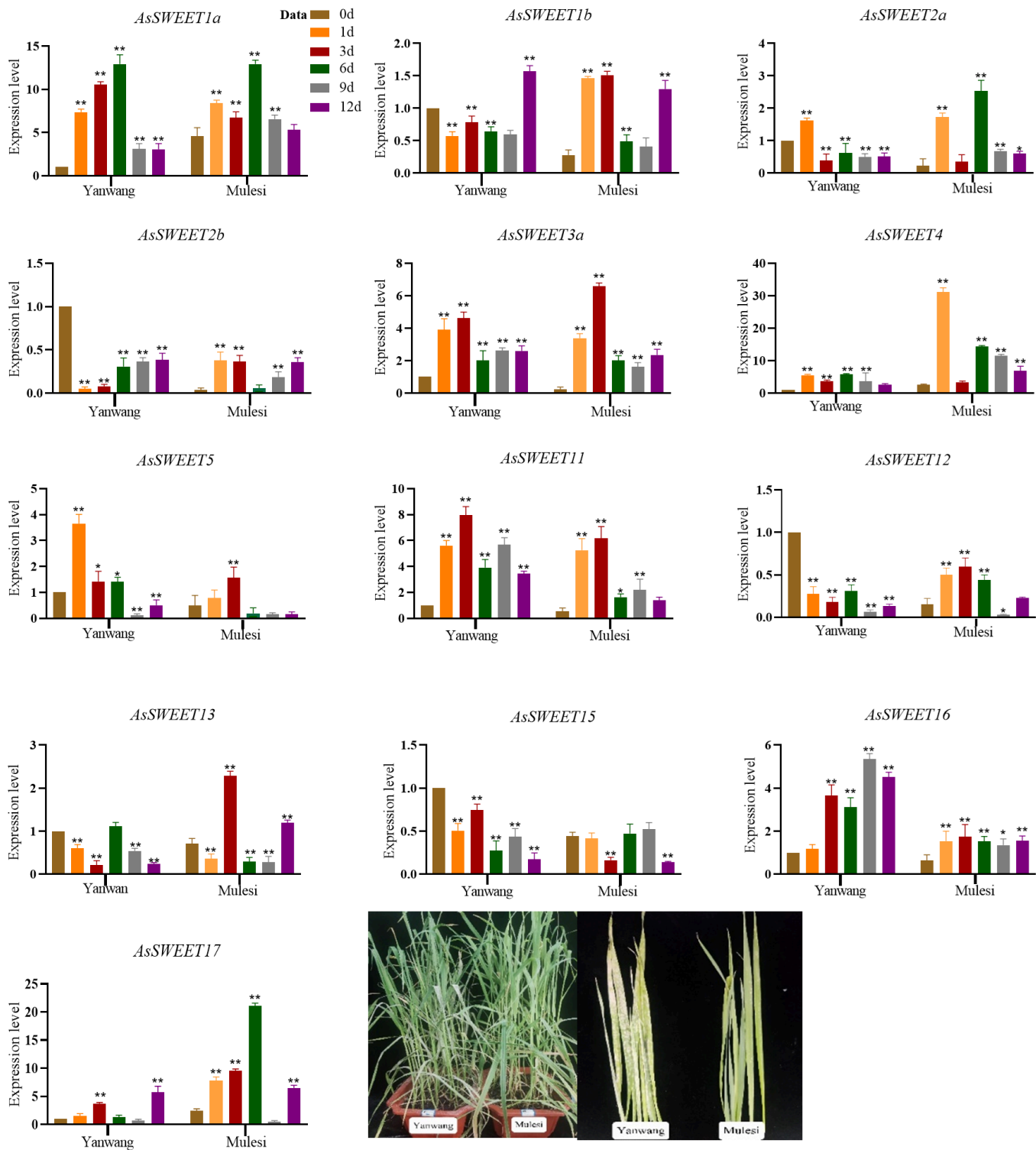


**Figure 6**

Expression of *AsSWEET* genes in two cultivars of oat, 'Yanwang' and 'Mulesi', after infection with Powdery mildew. Significant differences are indicated with asterisks (\* $p < 0.05$ , \*\* $p < 0.01$ ).

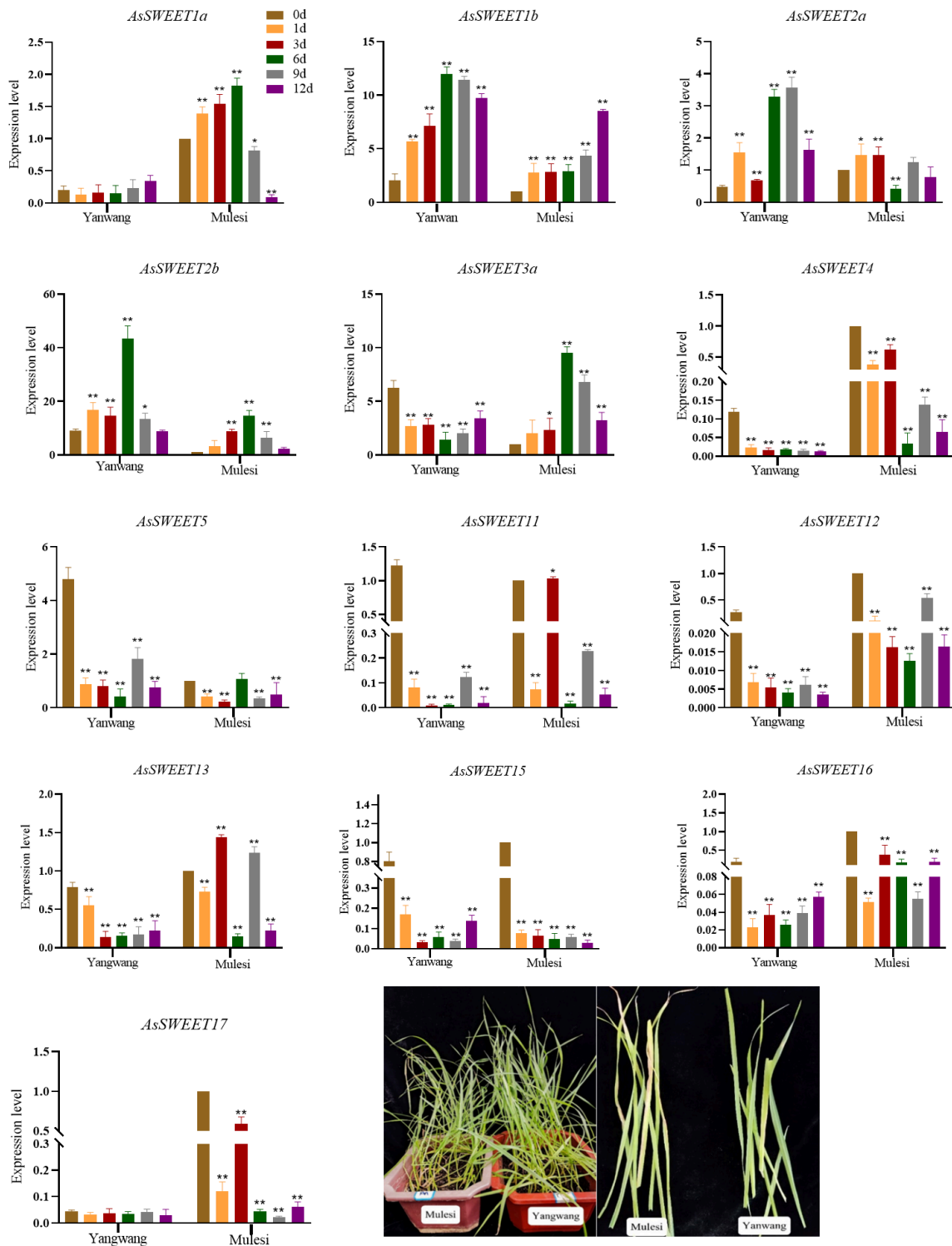


**Figure 7**

Expression of *AsSWEET* genes in two cultivars of oat, 'Yanwang' and 'Mulesi', after infection with leaf spot. Significant differences are indicated with asterisks (\* $p < 0.05$ , \*\* $p < 0.01$ ).

## Supplementary Files

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