Genome-wide identification of *SNARE* gene in plant and expression pattern of *TaSNARE* in wheat

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SNARE (Soluble N - ethylmaleimide - sensitive - factor attachment protein receptor) proteins are mainly mediated eukaryotic cell membrane fusion of vesicles transportation, also play an important role in plant resistance to fungal infection. In this study, 1342 SNARE proteins were identified in 18 plants. According to the reported research, it was splited into 5 subfamilies (Qa, Qb, Qc, Qb+Qc and R) and 21 classes. The number of *SYP1* small classes in Qa is the largest (227), and Qb+Qc is the smallest (67). Secondly, through the analysis of phylogenetic trees, it was shown that the most SNAREs of 18 plants were distributed in 21 classes. Further analysis of the genetic structure showed that there was a large difference of 21 classes, and the structure of the same group was similar except for individual genes. In wheat, 173 SNARE proteins were identified, except for the first homologous group (14), and the number of others homologous groups were similar. The 2000bp promoter region upstream of wheat *SNARE* gene was analyzed, and a large number of W-box, MYB and disease-related cis-acting elements were found. The qRT-PCR results of the *SNARE* gene showed that the expression patterns of the same subfamily were similar in one wheat varieties. The expression patterns of the same gene in resistant/sensitive varieties were largely different at 6h after infection. This results might indicate that early stages of the SNARE protein in pathogen infection play an important role. In this study, the identification and expression analysis of the SNARE protein provides a theoretical basis for future studies on the function of the SNARE protein and wheat resistance to powdery mildew.

Genome-wide identification of *SNARE* gene in plant and expression pattern of *TaSNARE* in wheat

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Abstract

SNARE (Soluble N - ethylmaleimide - sensitive - factor attachment protein receptor) proteins are mainly mediated eukaryotic cell membrane fusion of vesicles transportation, also play an important role in plant resistance to fungal infection. In this study, 1342 SNARE proteins were identified in 18 plants. According to the reported research, it was splited into 5 subfamilies (Qa, Qb, Qc, Qb+Qc and R) and 21 classes. The number of *SYP1* small classes in Qa is the largest (227), and Qb+Qc is the smallest (67). Secondly, through the analysis of phylogenetic trees, it was shown that the most SNAREs of 18 plants were distributed in 21 classes. Further analysis of the genetic structure showed that there was a large difference of 21 classes, and the structure of the same group was similar except for individual genes. In wheat, 173 SNARE proteins were identified, except for the first homologous group (14), and the number of others homologous groups were similar. The 2000bp promoter region upstream of wheat *SNARE* gene was analyzed, and a large number of W-box, MYB and disease-related cis-acting elements were found. The qRT-PCR results of the *SNARE* gene showed that the expression patterns of the same subfamily were similar in one wheat varieties. The expression patterns of the same gene in resistant/sensitive varieties were largely different at 6h after infection. This results might indicate that early stages of the SNARE protein in pathogen infection play an important role. In this study, the identification and expression analysis of the SNARE protein provides a theoretical basis for future studies on the function of the SNARE protein and wheat resistance to powdery mildew.

Key words: Plant SNARE; Disomic Addition Expression Pattern; Wheat; Genome-wide.

1. Introduction

SNARE proteins were employed to any significant vital movement, as they mediate the fusion of membranes of cargo-containing small shuttles, referred to as vesicles, and target membranes (Lipka et al., 2007). It was involved in vesicle membrance fusion and was responsible for the transport of the endomembrance system, as well as for endocytosis and exocytosis. According to their function, SNARE proteins can be divided into vesicle-associated (v-SNAREs) and target-membrane-associated (t-SNAREs) (Söllner et al., 1993). Alternatively, SNAREs can be grouped as Q-SNAREs and R-SNAREs, they have either a conserved glutamine or arginine residue in the center of the SNARE domain, Q-SNAREs can be further subdivided into Qa-SNARE, Qb-SNARE, and Qc-SNAREs (Bock et al., 2001), SNAP-25-like proteins comprise a Qb-SNARE and a Qc-SNARE motif (Schilde et al., 2008); R-SNAREs have either a short or long N-terminal regulatory region, further subdividing them into brevins and longins (Lipka et al., 2007). Previous studies have shown that 60 SNARE protein in *Arabidopsis thaliana*, 57 SNAREs in *Oryza sativa*, 69 SNAREs in *Populus trichocarpa* (Lipka et al., 2007) and 21 Syntaxins in *Solanum lycopersicum* (Bracuto et al., 2017). In addition, Sanderfoot revealed the evolution of eukaryotic SNARE (Sanderfoot, 2007).

The pathway of plant cell endomembrane secretion system plays an important role in the interaction between plant cells and microbes (BA and RL, 1990; Walther-Larsen et al., 1993). Plant cells were capable of identifying the pathogen-associated molecular patterns through surface receptors, and cell surface receptor proteins exist in the signal peptide, it was shown that processing and positioning of these receptors were done through the protein secretion pathway (Wang and Dong, 2011). The autoimmunity of plants to the infiltration of powdery mildew fungi is accomplished by targeting the cell wall with a papillary process, including purine, cytoplasmic, extracellular membrane components and SYP121 / PEN1 (Nielsen et al., 2012). In *Arabidopsis*, *PEN1* (*SYP121*) and its closest homologue, *SYP122*, appear to have a fundamental function in secretion and specific defense-related functions at the plant cell wall (Collins et al., 2003; Assaad et al., 2004). Similarly, HvROR2 (Collins et al., 2003) or SiPEN1 (Bracuto et al., 2017) was associated with the defense against powdery mildew fungi. AtSYP121/AtPEN1-AtSNAP33-AtVAMP-721/722 protein complexes can assist cell emesis at the site of fungal invasion (Douchkov et al., 2005; Wick et al., 2003; Kwon et al., 2008; Lipka et al., 2008). In addition, *AtSEC11* modulates *PEN1*-dependent vesicle traffic by dynamically competing for *PEN1* binding with *VAMP721* and *SNAP33* (Karnik et al., 2013).

MdSYP121 affects the pathogen infection process in apple by regulating the SA pathway and the oxidation-reduction process (He et al., 2018). The SYP4 group regulates both secretory and vacuolar transport pathways and related extracellular resistance to fungal pathogens (Uemura et al., 2012). *NbSYP132* may act as a homologous SNARE protein receptor and positively regulate the exocytosis of vesicles containing antibacterial PR proteins (Kalde et al., 2007). Silencing StSYR1 enhances the resistance of the potato to Phytophthora infestans (Eschen-Lippold et al., 2012).

OsVAMP714 can positively regulate the disease resistance to blast in rice, but *OsVAMP7111* can't. Furthermore, *OsVAMP714* overexpression promotes leaf sheath elongation (Sugano et al., 2016). Ectopic expression of *AtBET12* had no inhibition in the general ER-Golgi anterograde transport but had an intracellular accumulation of PR1 (Chung et al., 2018). *GOS12* is an essential host factor for PD targeting of P3N-PIPO protein to defense the Soybean mosaic virus (Song et al., 2016). *AtMEMB12* was targeted by miR393b* to modulate exocytosis of antimicrobial PR1 (Zhang et al., 2011). *AtSyp71* is a host factor essential for successful virus infection by mediating the fusion of the virus-induced vesicles with chloroplasts during *TuMV* infection (Karnik et al., 2013). *OsSEC3A* enhances rice resistance to *Magnaporthe oryzae* by negatively regulating the pathogenesis and expression of SA synthesis-related genes (Ma et al., 2017). *TaNPSN11*, *TaNPSN13*, and *TaSYP132* have diversified functions in the

prevention of *Pst* infection and hyphal elongation (Wang et al., 2014). The previous studies, it has shown that SNAREs are involved in the regulation of diverse aspects of defense fungal disease. The aim of this study was to develop a better understanding of identification, evolution, and expression in SNAREs and explore the relationship between wheat SNAREs and powdery mildew.

2. Materials and Methods

2.1 Identification of plant *SNARE* genes

The plant genomes and annotations (*Triticum urartu, Aegilops tauschii, Brachypodium distachyon, Hordeum vulgare, Sorghum bicolor, Oryza sativa, Solanum lycopersicum, Brassica napus, Vitis vinifera, Chlamydomonas reinhardtii, Zea_mays, Populus trichocarpa, Arabidopsis thaliana, Setaria italica, Glycine max* and *Physcomitrella patens*) were obtained from the newest ensemblplant database (https:/ http://plants.ensembl.org/index.html), and the Triticum aestivum database were used newest IWGSC v1.0 (https://wheat-urgi.versailles.inra.fr/Seq-Repository). The Hidden Markov Model (HMM) of the SNARE(PF05739), Syntaxin (PF00804), longin (PF13774), Synaptebrevin (PF00957), SEC20 (PF03908), V-SNARE-C (PF12352), V-SNARE (PF05008) and USE1 (PF09753) motifs were downloaded from the Pfam database(http://pfam.sanger.ac.uk/). All plant SNARE protein sequences were analyzed with the HMMER 3.0 (http://hmmer.janelia.org/) as the query and default parameters ($E < 0.01$). All presumptive *SNARE* genes were retained and confirmed using the Pfam database and the NCBI conserved domain database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Multiple alignments of SNARE proteins were performed using the ClustalW (Larkin et al., 2007) in MEGA 7.0 (http://www.megasoftware.net/). Phylogenetic analyses were using N J(neighbor-joining) method in MEGA 7.0 (Kumar et al., 2016) with 1000 bootstrap resampling, the Jones-Taylor-Thornton (JJT) model (Jones et al., 1992), and pairwise deletion option.

2.2 Exon/intron structure analysis and conserved motif identification

The gene structure provides important information, including disaggregated and evolutionary relationships among gene families. The SNARE genomic sequences and CDS sequences extracted from the plant database were compared with gene structure display server programmes to determine the exon/intron organization of *SNARE* genes. Default parameters were used for the Multiple Em for Motif Elicitation (MEME) (http://meme-suite.org/) programme for the identification of conserved protein motifs and a maximum number of 10 motifs.

2.3 Analysis of cis-acting elements

According to the genome sequences of the *Triticum aestivum* published database, we cut out the 2000 bp of the 5′ sequence as the promoter domain of the *SNARE* gene to analyze the cis-acting elements using the online software New Place (https://sogo.dna.affrc.go.jp).

2.4 Fungus and Wheat materials

The wheat-*Ae. geniculata disomic* addition line NA0973-5-4-1-2-9-1 (CS-SY159 DA 7M^{g}, (CS)/*Ae. geniculata* SY159//CS)) (Wang et al., 2016). Shanyou 225 was the powdery mildew susceptible control variety. The wheat-*Ae. geniculata* disomic addition line TA7661 (CS-AEGEN DA 7M^{g}) were kindly provided by Dr. Friebe BR and Dr. Jon Raupp of the Department of Plant Pathology (Friebe et al. 1999), Throckmorton Plant Sciences Center, Kansas State University, Manhattan, USA. Powdery mildew isolates E09 was maintained on susceptible wheat ‘Shaanyou 225’. All plants were cultured in an

incubator with soil at 18°C under a 16 h light/8 h dark photoperiod. Half of the 14-day-old seedlings were inoculated with powdery mildew conidia from 'Shaanyou 225' seedlings infected 10 days previously.

2.5 RNA-seq expression analysis of SNARE genes

To further understand the function of the *SNARE* gene, we investigated the reported RNA-seq data, including developmental timecourse in five tissues (Choulet et al., 2014), grain layers (Pearce et al., 2015), grain layer developmental timecourse (Pfeifer et al., 2014), senescing leaves timecourse (Pearce et al., 2014), photomorphogenesis for DV92 and G3116(Fox et al., 2014), drought and heat(Liu et al., 2015). Data were analyzed using MeV (Multi Experiment Viewer) software (Saeed et al., 2006). Data obtained from the RNA-seq expression atlas were normalized based on the mean expression value of each gene in all tissues/organs analyzed and clustered by hierarchical clustering method.

Developmental time course in five tissues include all of the wheat stage (Zadoks et al., 1974): seeding (first leaf through coleoptile, Zadoks Scale 10(Z10)), three leaves (3 leaves unfolded, Z13), three tillers (Main shoot and 3 tillers, Z23), Spike at 1 cm (pseudostem erection, Z30), two nodes(2nd detectable node, Z32), meiosis (flag leaf ligule and collar visible, Z39), anthesis (1/2 of flowering complete, Z65), 2 days after anthesis (DAA) (Kernel (caryopsis) watery ripe, Z71), 14 DAA(medium Milk, Z75), 30 DAA (soft dough, Z85). Grain layers contain three parts in 12 days post anthesis (DPA): outer pericarp, inner pericarp, and endosperm. Grain layer developmental timecourse contains seven stages: 10 DPA whole endosperm, 20 DPA whole endosperm, 20 DPA starchy endosperm, 20 DPA transfer cells, 20DPA aleurone, 30 DPA starchy endosperm, 30 DPA aleurone plus endosperm. Senescing leaves timecourse contains three stages: heading date (HD), 12 DAA or 22 DAA. Photomorphogenesis for the wild winter wheat *T. monococcum ssp. aegilopoides* (accession G3116) and the domesticated spring wheat *T. monococcum ssp. monococcum* (accession DV92). Drought and heat contain seven treatments: control, drought 1hr, drought 6hr, heat 1hr, heat 6hr, drought plus heat 1hr, drought plus heat 6hr.

2.6 RNA extraction and real-time quantitative PCR

Total RNA was extracted from samples of fungi inoculated leaves at the specified time points using the Trizol reagent (BioFlux, Hang Zhou) method with a few modifications pertaining to DNase digestion and RNA purification. Oligo (dT)-magnetic beads were used to enrich the mRNA, which was then broken into fragments with fragmentation buffer. First-strand cDNA synthesis using reverse transcription-PCR system (Promega, Madison, WI, USA).

The SYBR Green Premix Ex Taq™ II quantitative PCR system (Takara, Dalian) was used for qPCR analysis. All experiments involving q-PCR were performed on a Q7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using primers described in Table S2. The actin gene was used as the reference gene. PCR reaction comprised 5 μL of an enzyme, 3 μL of cDNA product, 1 μL of primer mix containing both upstream and downstream primers, and 1 μL of DNase/RNase-free water (a total reaction volume of 10 μL). The quantitative PCR thermal cycler programme included 95°C for 10 s, followed by 40 cycles at 95°C for 5 s and 60°C for 31 s. All primers synthesized by the same company (AoKe, yangling) (Table S3).

3. RESULTS

3.1 Identification of the SNARE protein in plants

To identify SNARE proteins in 18 plants, the HMMER profile was implemented to identify the plants' genomes. The results showed that 1342 (Table 1) hypothesis SNARE genes (Table 1) were characterized by 18 plant databases. Qa, Qb, Qc, Qb+Qc and R SNAREs subfamilies have 418 (31%), 250 (19%), 278 (21%), 68 (5%) and 328 (24%) respectively (Fig.1). Among all 21 subfamilies in 18 plants, SYP1 has a maximum of 227 proteins, and VAMP72 was the next 165 proteins.

Qa, Qb, Qc Qb+Qc and R percentage in total *SNARE* genes was 15.4% (*Chlamydomonas reinhardtii*)-38.6% (*Solanum lycopersicum*), 14.0% (*Solanum lycopersicum*)-25%(*Aegilops tauschii*), 15.5%(*Physcomitrella patens*)-30.8% (*Chlamydomonas reinhardtii*), 0% (*Chlamydomonas reinhardtii*)-9.1% (*Aegilops tauschii*) and 20.8% (*Triticum urartu*)-34.6% (*Chlamydomonas reinhardtii*). On average, Qa had the largest proportion of *SNARE* genes; Qb, Qc and R had the similar proportion. Qb+Qc had the least proportion. Interestingly, *Chlamydomonas reinhardtii* had only 1 *SYP1* gene, in other 17 plants, the number of *SYP1* gene was at least 8.

Generally speaking, most SNAREs of 18 plants were distributed in 21 classes, except for *SYP2* and *SNAP* in *Chlamydomonas reinhardtii*, *SYP3* in *Triticum urartu*, *SYP3* and *SYP4* in *Aegilops tauschii*, *MEMB* in *Hordeum vulgare*, *SFT1* in *Physcomitrella patens.*

3.2 Analysis of the *SNARE* gene phylogenetic, structure and motif

To gain further insight into the phylogenetic relationship among Qa, Qb, Qc, Qb+Qc and R *SNARE* genes, to identify all of 18 plants SNAREs sequences were used to conduct a multiple sequence alignment and construct a phylogenetic tree. The results showed that these proteins were divided into 5 main clusters labeled (SYP1,2,3,4,8) in Q a(Fig S1), 5 clusters labeled (GOS1, VTI1, NPSN1, SEC20) in Qb (Fig S2); 6 clusters labeled (BET1, SFT1, USE1, SYP5,6,7) in Qc (Fig S3); 4 main clusters labeled (VAMP71, VAMP72, YKT6, SEC22) in R (Fig S5).

The gene structure analysis showed that the same clades have similar structures, including the number of exons/introns. In Qa subfamily (Fig S1): SYP1 mainly has1, 2 or 13 exons (*SYP13* class contains multiple introns and most are located on the ORF (Sanderfoot, 2007)); *SYP2* mainly has 1, 6 or 7 exons, with a maximum of 9 (TRIUR3_19543); *SYP3* mainly 5 or 6 exons, at least 1 (PGSC0003DMTD400047329); *SYP4* mainly 8 exons, with a maximum of 11 (TRIUR3_01356); SYP8 mainly has 7 exons, at most 11 (CDY33863)and at least 3 (GLYMA17G29392). In Qb subfamily (Fig S2): *NPSN1* mainly has 10 exons, at least 5 (EDP04002); SEC20 mainly has 7 exons, at most 12 (TRIUR3_20340); *GOS1* mainly has 5 or 6 exons, at least 1 (HORVU5HR1G065530); *MEMB* mainly has 4 exons, at most 7 (EDP03091)and at least 2 (PP1S11_26V6); *VTI1* mainly has 5 exons, at least 2 (AT3G29100). In Qc subfamily (Fig S3): *BET1* mainly has 5 exons, at least 1 (Zm00001d01850); *SFT1* mainly has 4 exons, at most 7 (TRIAE_CS42_2AS_TGACv1_113035_AA0350270); *USE1* mainly has 7 exons, at most 12 (TRIUR3_18313)and at least 1 (PP1S26_171V6); *SYP5* mainly has 5 or 6 exons, at most 11 (CDX87371); SYP6 mainly has 5 or 7 exons, at most 12 (EMT10766); *SYP7* mainly has 8 or 9 exons, at least 5 (CDY50077). In the Qb+Qc subfamily (Fig S4), it mainly has 5 exons and at most 16 (EMT24031). In R subfamily (Fig S5): *SEC22* mainly has 5 exons, at most 8 (EMT12128); *YKT6* mainly has 6 exons, at least 2(EDP00430); *VAMP71* mainly has 1 or 4 exons, at most 8 (EDO099593); *VAMP72* mainly has 5 exons.

There were 10 motifs in the Qa, Qb, Qc, Qb+Qc and R subfamily. In the Qa subfamily, motif 4 and motif 9 were the SNARE domain (Fig S1). In Qb subfamily, motif 1 was the SNARE domain (Fig S2). In Qc subfamily, motif 6 was the SNARE domain (Fig S3). In Qb+Qc subfamily, motif 1 and motif 5 were SNARE domain (Fig S4). In R subfamily, motif 5 and motif 7 were the SNARE domain (Fig S5).

3.3 Identification of the SNARE protein in wheat

All the sequences were divided into 64 groups of wheat (Table S1). Of them, 38 groups representing 114 genes consisted of three genes from each of the different sub-genomes that were regarded as orthologous copies of a single *SNARE* gene named triplet. 5 groups were on different homoeologous but from the same homeologous group (e.g., *TaSYP43-4AL*, *TaSYP43-7AS* , and *TaSYP43-7DS*). 8 groups were containing two genes (e.g., *TaSYP131-2BS* and *TaSYP131-2DS*). The remaining 8 groups consisted of only one gene (e.g., *TaGOS12-6BS*). 5 groups have four genes, among of them, 4 groups had tandemly repeated genes(e.g., *TaSNAP1-2A1*, *TaSNAP1-2A2*, *TaSNAP1-2B*, and *TaSNAP1-2D*).

3.4 Chromosomal locations of *SNARE* gene family members in wheat

The chromosomal distribution of the *SNARE* gene family of *Triticum aestivum* was analyzed. The results revealed 173 *SNARE* genes in the chromosome location information (Fig 1). All of 21 wheat chromosomes have several SNARE gene family members: wheat 1 to 7 homoeologous groups had 14 (1A=5,1B=4,1D=5), 22 (2A=7, 2B=8, 2D=7), 31 (3A=12, 3B=9, 3D=10), 25 (4A=9, 4B=9 4D=7), 23 (5A=8, 5B=9, 5D=6), 26 (6A=9, 6B=9, 6D=8) and 27 (7A=11,7B=8,7D=9) *SNARE* genes and 4 had no chromosome location. In addition to the homologous group 1, the *SNARE* genes are evenly distributed in the wheat genome, and the number of genes on each chromosome is similar. The most striking result to emerge from figure 2 was that the triplets, which from different sub-genomes, were similar in relative position to their chromosomes.

3.5 Phylogenetic, motif and structure analysis of the SNARE family genes

Further to analyze the phylogenetic, motif and structure of *TaSNAREs*, we selected one protein (A chromosome priority selection) from each group respectively in 64 groups and we got 64 SNAREs. The results showed that these proteins were primarily divided into 5 clades (Fig 2). Most have three homologous proteins in the same branch, and these three homologous proteins come from three chromosomes in the same homologous group.

It is apparent from Figure 3 that SNAREs in different subfamilies had a different motif. Qa had motif 1, 2, 5, 8, 11 and 13. Qb had motif 6, 7, 10, 12 and 13. Qc had motif 5, 9 and 13. Qb+Qc had motif 9 and 12. R had motif 3, 4, 13 and 14. It showed that motif 6, 8, 10, 11, 13 and 15 were no predicted. Motif 1 was the SNARE domain; Motif 2 and 5 were syntaxin domain; Motif 3 was the synaptobrevin domain; Motif 4 and 14 were longin domain; Motif 7 was the SEC20 domain; Motif 12 was the V-SNARE-C domain. Qa, Qb, Qc, and R had the motif 13, which located in C-terminal, association SNAREs with lipid bilayers named transmembrance (TM) domain (Lipka et al., 2007).

Analyze the structure in TaSNAREs. It showed that the results have a number of similarities with the other SNAREs in 17 plants. Qa had 1-13 exons. Qb had 4-10 exons. Qc had 4-10 exons. Qb+Qc had 5 or 6 exons. R had 4-7 exons.

3.6 Cis-acting elements of TaSNARE genes

Further to analysis *TaSNARE* gene 5' upstream 2000bp promoter. It contains 9 types of resistance-related cis-acting elements (Table S2), include W-box (Cis-I), Germs-related (Cis-II), MYB (Cis-III), SA responsible (Cis-IV), Eth responsible (Cis-V), EIRE (Cis-VI), G-box (Cis-VII), H-box (Cis-VIII)and IAA responsible (Cis-IX).

As shown in Fig 3, Cis I-IX include 2230, 5054, 1647, 882, 170, 225, 152, 118 and 309 elements in all 173 *SNARE* genes promoter. A, B and C make up 82.92% of all disease-related elements. In A element, *TaUSE12-7A* was the largest 36. In B element, TaSEC222-5B was the largest 87. In C element, *TaSEC222-5A* was the largest 19.

In one triplet, the promoter of the resistance-related element, the number was similar. But there are exceptions, TaSFT11-2A/B/D had 5/21/16 A elements; TaNPSN12-4A/B/D had 71/17/21 B elements; TaSYP222-6A/B/D had 3/11/1 D elements.

3.7 Expression analysis of TaSNARE genes from RNA-seq

To further understand the function of the *SNARE* genes, we extracted 54 genes expression information from six published RNA-seq databases (Fig 4).

As shown in Fig 5, in the growth period of wheat, the SNARE gene is expressed in roots, stems, leaves, seeds and spike, low in seeds and leaves, and high in roots, stems, and spike. In the seeds of Z75, the expression levels of most genes (45) were very low, and in Z71-Z75-Z85, a high-low-high expression pattern was exhibited. Many genes (36) were most expressed in the 20 DPA aleurone layer during seed development. Most SNARE genes (49) are expressed under light conditions compared to dark conditions. Among them, SYP122-6A in the DV92 light is higher than the dark expression, but G3116 is the opposite. Compared with the control, the expression of 22 genes was up-regulated 6 h after stress (drought 6h, heat 6h or drought plus heat 6h) and 9 genes expression patterns were the opposite. In the process of leaf senescence, 42 genes have the highest expression in 12DAA. More than half of the genes (33) have the following expression patterns in the grain layers: outer pericarp > inner pericarp > endosperm.

3.8 Expression patterns of TaSNARE genes in powdery mildew treatment

We selected one gene from each class respectively in 21 classes and we got 21 *TaSNARE* genes (*TaYKT6* was no signal) to the designed primer (Table S3). As can be seen from Figure 5 that the expression patterns of different *SNARE* genes in the same sample and subfamily were similar. Most of the *TaSNARE* gene had a similar expression pattern in 7M US and CS, but 7M CH had a different expression pattern. The majority of *TaSNARE* genes in 7M CH had a high expression at 6h. *TaSYP4*, TaSYP8 *TaMEMB* and *TaSEC22* in the Shanyou225 had the high expression at 6h, but the other wheat not.

In the Qa subfamily: the expression of all genes changed little at each time point in CS. *SYP121*, *SYP221*, and *SYP3* were upregulated in the 7M CH 6h sample but downregulated in Shanyou225. *SYP4* and *SYP8* were upregulated in the Shanyou225 6h sample but no change in 7M CH. *QaSNARES* expression was similarity in 7M US and Shanyou225.

In Qb subfamily: *GOS12* expression patterns, upregulated at 6h and then downregulated, in 4 wheat varieties were a similarity. *MEMB* expression was upregulated in Shanyou225 6h, 24h, and 48h but the contrast to CS; There was no significant difference in the expression of time points in 7M CH and 7M US. *VTI12* expression patterns were similarity to *MEMB* in Shanyou225 and 7M CH. *NPSN11* in 4 wheat varieties were similarity, downregulated at 6h-48h, except upregulated at 24-48h in 7M CH. SEC203 were downregulated at 6h-48h in 7M US and CS; These were downregulated at 12h-24h and upregulated at 48h in Shanyou225; In 7M CH, *SEC203* downregulated at 24h and the others upregulated.

In Qc subfamily: all *QcSNARE* in the same variety were a similarity. In CS and 7M US, most genes were downregulated at 6-24h. In 7M CH, genes downregulated at 24h and the others upregulated. In Shanyou225, genes downregulated at 12h-24h and upregulate at 6h and 48h.

In Qb+Qc subfamily: *SNAP1* were upregulated at 6h in all varieties and downregulated in12-48h; Except in Shanyou 225 at 48h, SNAP1 upregulated.

In R subfamily: In CS, *VAMP712* were upregulated at 6h and others were no change; In Shanyou225, *VAMP712* were downregulated at 6h and 24h, other times were no change; In 7M CH, *VAMP712* were upregulated at 6h,12h,48h and no change at 24h. In 7M US, *VAMP712* were upregulated at 24h and other times downregulated. No signal of *VAMP723* was detected in shanyou225. In 7M US, *VAMP723* were

upregulated at 24h and downregulated at other times. In 7M CH, *VAMP723* were upregulated at 12h and downregulated at 24h 48h. In CS, *VAMP723* were upregulated at 6h and downregulated at 48h. SEC222 were upregulated at all time in Shanyou225 and other varieties no significant difference.

4. DISCUSSION

SNAREs are mainly involved the membrance-related life activities. It is apparent from previous reports that rarely described SNAREs from the perspective of gene families. For the first time, we identified 1,340 proteins in 18 plants using bioinformatics methods and analyzed them in several ways.

As Table 1 shows, the absolute number of SNARE genes associated in wheat, soybean (ancient tetraploid (Shoemaker et al., 1996)) and edible rape (2), the ratio of *SNARE* gene number to each genome is comparable, imply the expanded *SNARE* genes might be due to whole-genome duplication events during the plant evolution. 18 plants we selected ranged from the most primitive algae to the highest woody plants. Most SNAREs of 18 plants were distributed in 21 classes. Except for *SYP2* and *SNAP* in *Chlamydomonas reinhardtii*, *SYP3* in *Triticum urartu*, *SYP3* and *SYP4* in *Aegilops tauschii*, *MEMB* in *Hordeum vulgare*, *SFT1* in *Physcomitrella patens*, indicating that these genes had been produced before the differentiation of monocotyledon and dicotyledon plants. According to previous reports, *SYP7* and *NPSN* are members of the plant-specific SNARE family (Sanderfoot, 2007). All plants contain 21 SNARE subfamilies, but this was not found in this paper, which may be due to sequencing splicing or insufficient depth.

The completion of the wheat genome sketch work is helpful for us to analyze the key genes and agronomic traits of the wheat from the perspective of the genome. However, sequencing work moves slowly because of the huge genome and too many repeated sequences. In this paper, 173 non-redundant *SNARE* genes were obtained from the newly published IWGSC 1.0 wheat genome reference sequence. Common wheat is a heterogenous hexaploid crop, and it usually contains three paranormal homologous genes from groups A, B, and D, which can be called triplets. But in our study, 16 out of 64 groups did not appear as a triplet (Table S1). This could be explained by the loss of these genes in long-term evolution, or it could be due to insufficient sequencing depth or incomplete splicing. There are also some triplets in which A/B/U occurs, possibly because the difficulty in splicing leads to the inability of genes to located in their chromosomes.

Evident from the analysis of cis-acting elements, genes in the same triad are mostly alike in their components. But there are still a few differences, may lead to some bias in the expression of these homologous genes in some physiological state. In addition, depending on the composition of cis-acting elements found, it mainly contains W-box, disease-related and MYB. This suggested that SNAREs resistant function in plants may mainly be regulated by transcription factors such as WRKY, MYB and other disease-resistant genes.

Some interesting information was obtained by analyzing the RNA-seq data. Because these RNA-seq databases are older, the genetic information used is a wheat genetic sketch. We compare the TGAC v1.1 data to a sketch database to find the corresponding *SNARE* gene in the sketch. It can be seen from Table S4 that the expression patterns of the same group of triplet genes are very similar, and in photomorphogenesis, most of the triplets do not have gene expression data of the B and D genomes, so we select the A genome in the triplet. The gene is analyzed, and if there is no group A gene, the gene of group B or D is used.

Members of the same class as the sub-families, their role in the same life activities are diverse. In Arabidopsis, severe male gametophytic defects occur only when *syp123*, *syp125*, and *syp131* are

simultaneously mutated (Slane et al., 2017). Arabidopsis *SCYL2B* and *CHC1* undergo vesicle transport through *VTI11* or *VTI12* for plant growth (Jung et al., 2017). On the other hand, homologous genes may also play different roles. Overexpression of *OsVAMP7111* did not enhance rice resistance to rice blast, while overexpression of *OsVAMP714* increased. This suggests that VAMP714 is potentially specifically for resistance to rice blast (Sugano et al., 2016). *PEN1* in plants forms the SNARE complexes during defense against powdery mildew fungi with VAMP721 and 722, it also forms SNARE complexes in vitro with VAMP724 and VAMP727 which are not related to plant immunity (Kwon et al., 2008). PVA31 is involved in SA-associated apoptosis by interacting with VAMP721/722/724 but not VAMP711/727 to combat pathogen infection (Ichikawa et al., 2015). In wheat, silencing *TaNPSN11/13* reduced resistance to CYR23, whereas silencing *TaNPSN12* did not (Wang et al., 2014).

In the RNA-seq data. It has also appeared that homologous genes in the same evolutionary branch appear in many different expression patterns under the same conditions. *VTI11/12/13/14* and *GOS11/12*, but some were different. Such as *NPSN11/13* were in one class, and *NPSN12* was not clustered with NPSN11/13. In the senescing leaves timecourse, NPSN11 and NPSN13 exhibited a low-high-low expression pattern, while *NPSN12* showed no difference in expression at each stage. In photomorphogenesis, the expression of NPSN12 and NPSN13 in the dark was higher than that in the light, and NPSN11 was no difference. In the heat and drought treatments, both *NPSN12/13* were down-regulated compared to the control, and there was no change in expression from 1 to 6 h after treatment. On the other hand, after treatment, *NPSN11* was up-regulated with drought and up-regulated at high temperature, and up-regulated at 1-6 h after treatment. In another group of subfamily genes, SNAP, the expression patterns between the three members differed a lot. The expression level of *SNAP3* (FPKM) is higher than *SNAP1/4* in each period and process. In the developmental timecourse of the wheat stage, most of *SNAP3* showed up-regulation in all tissues at various developmental stages, while *SNA4* was down-regulated and *SNAP1* expression was low. In the gain layers, expression of *SNAP1* was endosperm> outer pericarp>inner pericarp, *SNAP2* was endosperm=inner pericarp>outer pericarp, *SNAP4* was outer pericarp>inner pericarp >endosperm. In the heat and drought and senescing leaves timecourse, only *SNAP3* expression is higher, while the other FPKM values were less than 1.

CS is the parent of A and B, and their other parents are different varieties of *Ae. geniculata*. But their resistance to powdery mildew is quite different. We chose these two materials to try to explain the effects of exogenous chromosomes on endogenous gene expression from a genomic perspective. It can be obviously seen in the Fig S6 that mycelium has appeared on the 3 days after 7M US infection with powdery mildew, and a large number of mycelia have appeared on the fifth day. And 7M CH did not have a large amount of mycelium on day 5. It can be seen from figure 5 that 7M US and CS expression patterns are similar after infection by powdery mildew, but 7M CH is very different from them. This suggests that our exogenous chromosomes have some effect on endogenous gene expression and may lead to differences in resistance. It has been reported that after the introduction of exogenous chromosomes, genes on exogenous $7M^g$ chromosomes mainly affect the homologous genes on their homologous chromosomes. Whether the resistant gene carried on $7M^g$ will affect the expression of the *SNARE* related gene. Therefore, we have two kinds of speculations. The first one is that the resistance gene of exogenous $7M^g$ could achieve the purpose of resisting powdery mildew by participating in the disease resistance pathway of wheat. Secondly, it might that the exogenous $7M^g$ chromosome achieves resistance to powdery mildew by affecting the expression of the endogenous seventh homoeologous gene.

In conclusion, we identified 1342 SNAREs in 18 plants, which laid a foundation for further studies on the function of SNARE genes. In addition, it also helpful for the study of wheat powdery mildew resistance.

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Table 1(on next page)

SNARE protein in 18 plants

1 Chlorophyta 2 Bryophyta 3 Monocot 4 Dicotyledonous Weed 5 Dicotyledonous Tree *: The percentage of Qa, Qb, Qc, Qb+Qc and R in the total *SNARE* genes.

SNARE type	Qa	Qa percent*						Qb	Qb percent*						Qc	Qc percent*							Qb+Qc	Qb+Qc percent*	R	R percent*					Total
SNARE subfamily			SYP1	SYP2	SYP3	SYP4	SYP8			MEMB	GOS1	VTI1	NPSN1	SEC20			BET1	SFT1	USE1	SYP5	SYP6	SYP7	SNAP				VAMP71	VAMP72	YKT6	SEC22	
Triticum aestivum[3]	48	27.7 %	33	6	3	3	3	37	21.4 %	3	5	13	9	7	39	22.5 %	3	3	7	7	13	6	13	7.5 %	36	20.8 %	9	15	6	6	173
Triticum urartu[3]	17	32.1 %	13	2	0	1	1	12	22.6 %	1	2	4	1	4	10	18.9 %	1	1	2	3	2	1	3	5.7 %	11	20.8 %	3	4	2	2	53
Aegilops tauschii[3]	11	25.0 %	9	1	0	0	1	11	25.0 %	1	1	5	2	2	7	15.9 %	1	0	2	2	1	1	4	9.1 %	11	25.0 %	3	4	2	2	44
Brachypodium distachyon[3]	14	25.0 %	8	3	1	1	1	12	21.4 %	1	2	4	3	2	12	21.4 %	2	2	1	2	3	2	4	7.1 %	14	25.0 %	3	7	2	2	56
Hordeum vulgare[3]	18	35.3 %	11	3	1	2	1	9	17.6 %	0	2	2	3	2	10	19.6 %	1	1	2	2	3	1	3	5.9 %	11	21.6 %	2	3	2	4	51
Sorghum bicolor[3]	16	28.6 %	8	3	1	2	2	10	17.9 %	1	2	2	3	2	12	21.4 %	2	2	1	2	3	2	3	5.4 %	15	26.8 %	3	8	2	2	56
Oryza sativa[3]	14	24.6 %	8	2	1	1	2	10	17.5 %	1	3	2	3	1	16	28.1 %	2	2	5	2	2	3	2	3.5 %	15	26.3 %	3	7	2	3	57
Solanum lycopersicum[4]	22	38.6 %	11	5	2	2	2	8	14.0 %	1	2	2	2	1	10	17.5 %	2	1	2	2	1	2	3	5.3 %	14	24.6 %	2	7	1	4	57
Brassica napus[4]	71	32.9 %	34	17	11	5	4	40	18.5 %	2	10	10	14	4	42	19.4 %	6	4	4	8	11	9	8	3.7 %	55	25.5 %	8	32	6	9	216
solanum tuberosum[4]	22	37.3 %	9	6	3	2	2	9	15.3 %	2	2	2	2	1	12	20.3 %	3	2	1	3	1	2	3	5.1 %	13	22.0 %	2	7	1	3	59
Vitis vinifera[4]	18	33.3 %	9	3	3	2	1	9	16.7 %	1	2	3	2	1	13	24.1 %	2	1	3	3	2	2	2	3.7 %	12	22.2 %	3	5	2	2	54
Chlamydomonas reinhardtii[1]	4	15.4 %	1	0	1	1	1	5	19.2 %	1	1	1	1	1	8	30.8 %	1	1	1	1	2	2	0	0.0 %	9	34.6 %	3	4	1	1	26
Zea_mays[3]	24	34.3 %	13	5	2	2	2	13	18.6 %	1	3	3	3	3	12	17.1 %	2	2	3	2	2	1	2	2.9 %	19	27.1 %	3	12	3	1	70

Populus trichocarpa[5]	26	33.8%	12	3	6	3	2	12	15.6%	0	4	4	3	1	15	19.5%	3	2	1	4	2	3	4	5.2%	20	26.0%	5	10	2	3	77
Arabidopsis thaliana[4]	19	30.6%	9	4	2	3	1	12	19.4%	2	2	4	3	1	12	19.4%	2	2	2	2	1	3	3	4.8%	16	25.8%	4	8	2	2	62
Setaria italica[3]	15	28.8%	9	3	1	1	1	9	17.3%	1	1	2	3	2	12	23.1%	2	2	2	2	3	1	3	5.8%	13	25.0%	3	7	1	2	52
Glycine max[4]	39	32.2%	20	6	6	4	3	20	16.5%	2	5	7	4	2	27	22.3%	5	3	4	3	6	6	6	5.0%	29	24.0%	6	17	2	4	121
Physcomitrella patens[2]	20	34.5%	10	5	2	2	1	12	20.7%	1	4	4	2	1	9	15.5%	2	0	1	2	3	1	2	3.4%	15	25.9%	3	8	2	2	58
Total	418	31.1%	227	77	46	37	31	250	18.6%	22	53	74	63	38	278	20.7%	42	31	44	52	61	48	68	5.1%	328	24.4%	68	165	41	54	1342

1

Figure 1

Chromosomal locations of *SNARE* genes in wheat

A total of 173 *SNARE* genes were localized to *Triticum aestivum. Qa SNARE*□ Green. *Qb SNARE:* Fuchsia. *Qc SNARE:* Brown. *Qb+Qc SNARE*: Red. *R SNARE*: Blue.

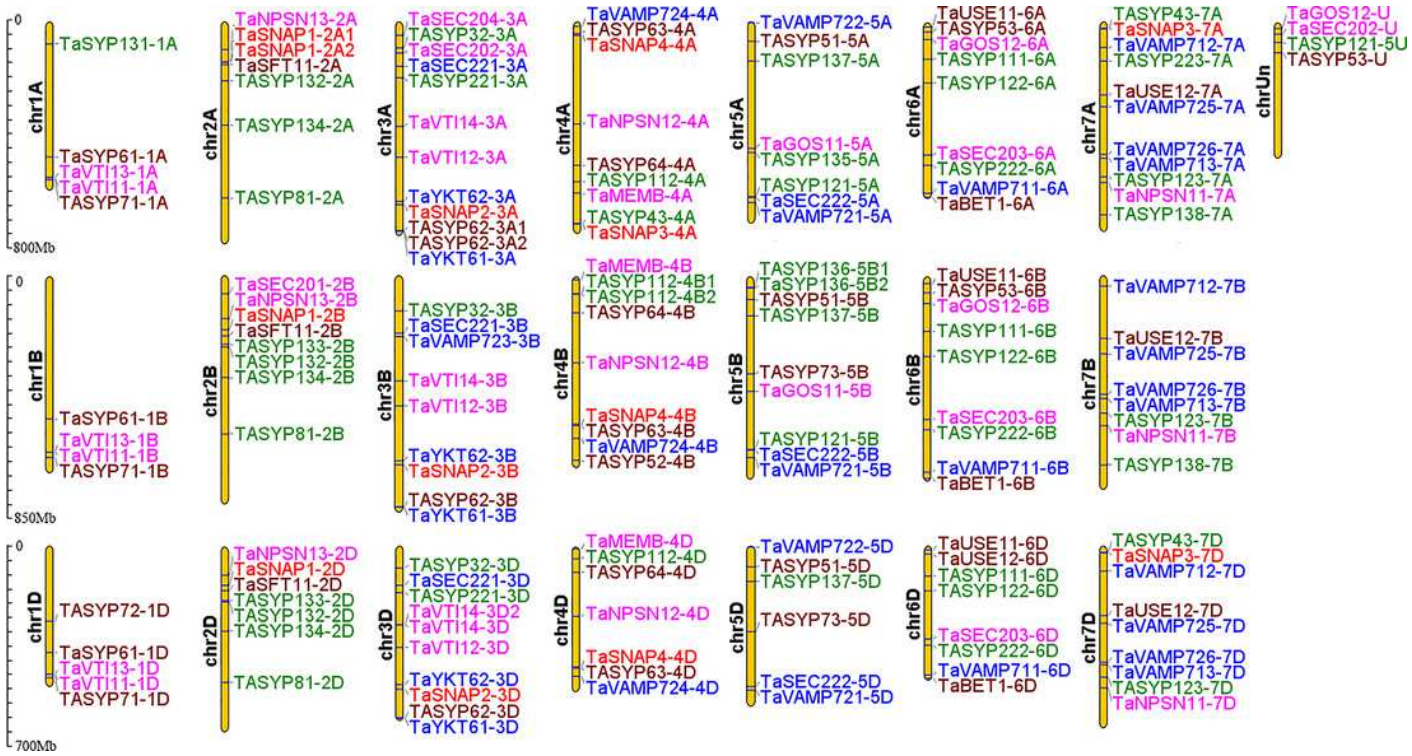

Figure 2

Fig 2 Phylogenetic analysis, gene structure, domain location and motif compositions of the *SNARE gene* family in wheat.

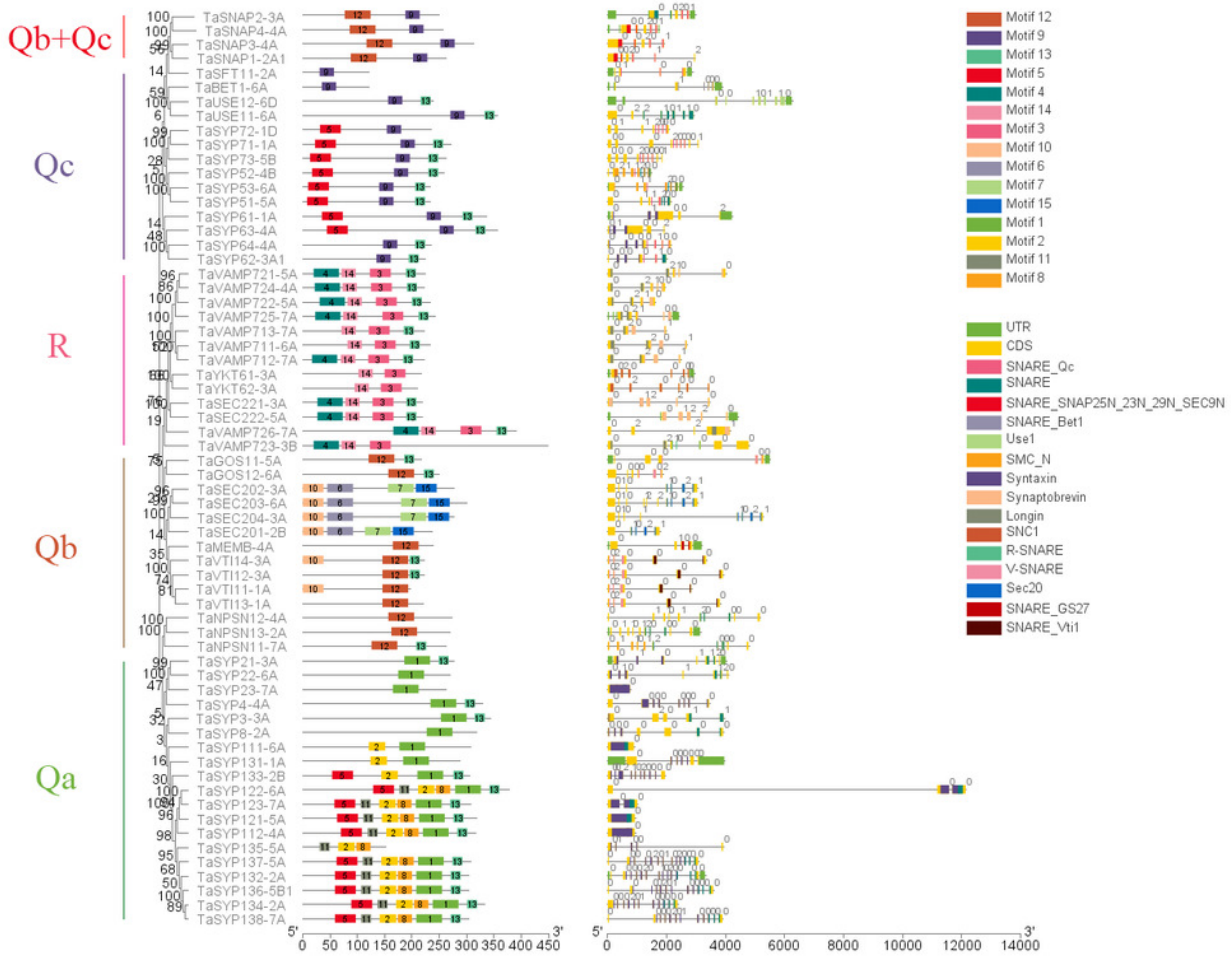

Figure 3

The number and proportion of 9 disease-related cis-acting element in SNARE genes promoter

Cis-I:W-box; Cis-II:Germs-related; Cis-III:MYB; Cis-IV:SA responsible; Cis-V:Eth responsible; Cis-VI:EIRE; Cis-VII:G-box; Cis-VIII:H-box; Cis-IX:IAA responsible.

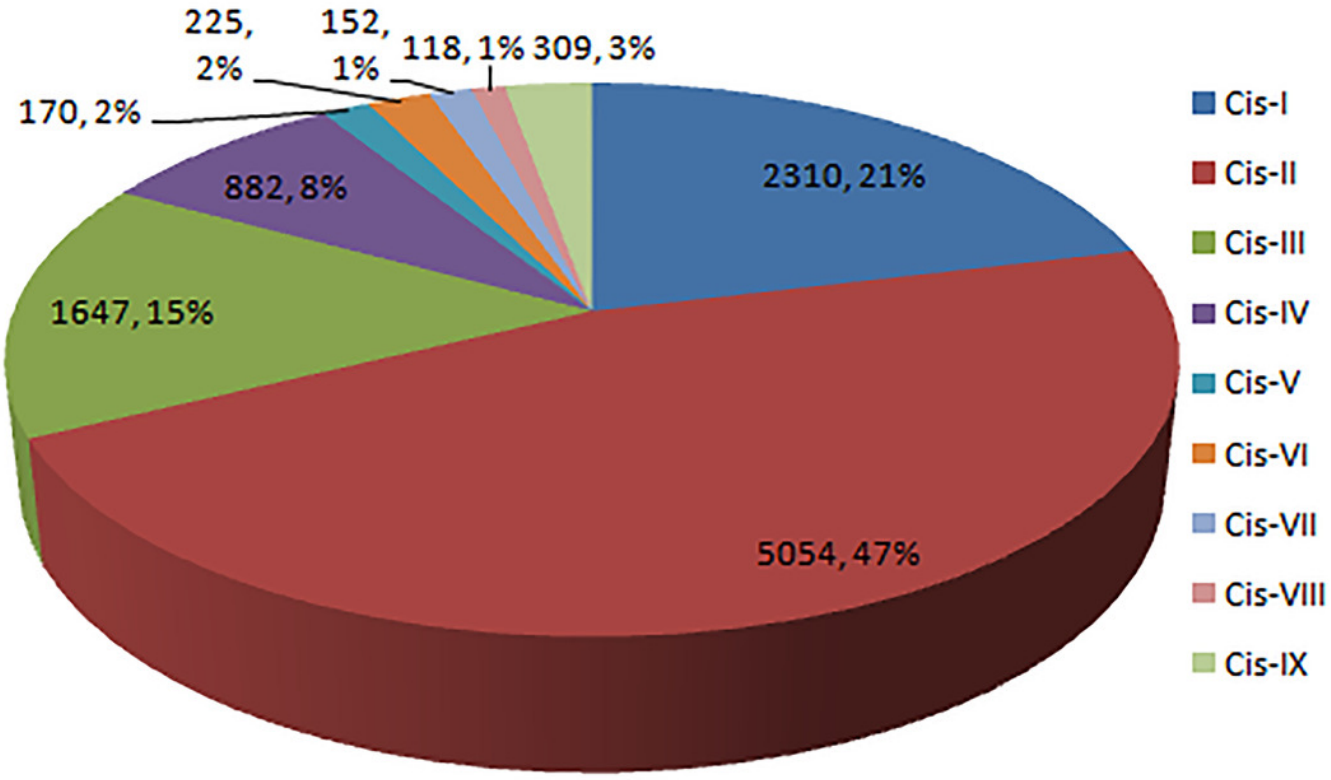

Figure 4

The expression profiles of *TaSNARE* genes in deferent treatment and stage

Developmental time course: Z10-Z85. Grain layers in 12 DPA: outer pericarp, inner pericarp and endosperm. Grain layer developmental timecourse : 10 DPA whole endosperm, 20 DPA whole endosperm, 20 DPA starchy endosperm, 20 DPA transfer cells, 20 DPA aleurone, 30 DPA starchy endosperm, 30 DPA aleurone plus endosperm. Senescing leaves timecourse: HD, 12 DAA and 22 DAA. Photomorphogenesis for DV92 and G3116. Drought and heat: control, drought 1hr, drought 6hr, heat 1hr, heat 6hr, drought plus heat 1hr, drought plus heat 6hr.

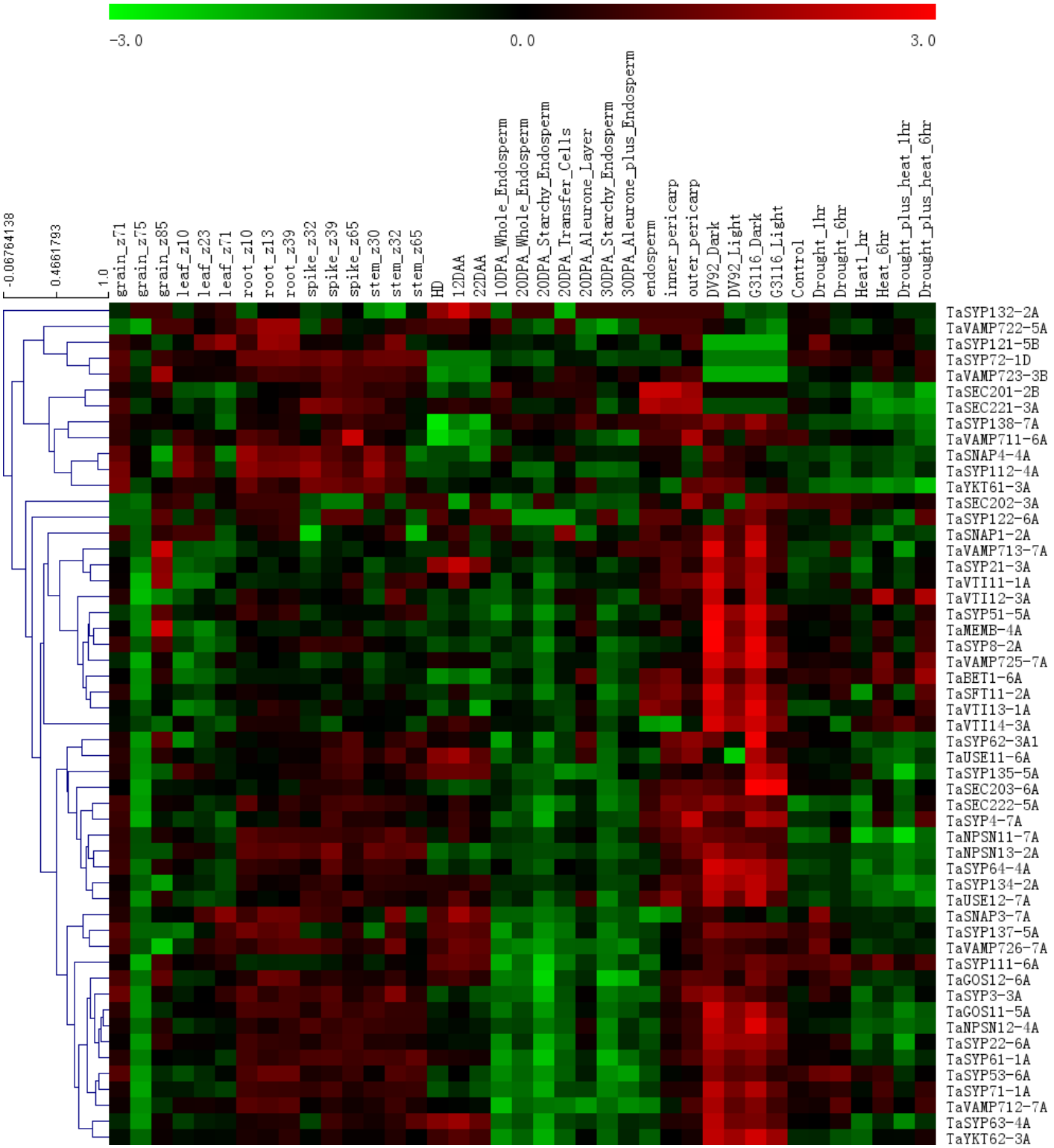

Figure 5

TaSNARE genes expression patterns infected by Bgt E09

CS: susceptible, Chinese spring. 7M CH: resistant, NA0973-5-4-1-2-9-1 (CS-SY159 DA 7M^{g} , (CS)/ *Ae. geniculata* SY159//CS)). 7M US: susceptible, TA7661 (CS-AEGEN DA 7M^{g}). Shanyou 225: susceptible, wheat cultivate variety Shanyou225.