Bioinformatic Analysis of Strawberry GSTF12 Gene

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Bioinformatic Analysis of Strawberry GSTF12 Gene

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Abstract. GSTF12 has always been known as a key factor of proanthocyanins accumulate in plant testa. Through bioinformatics analysis of the nucleotide and encoded protein sequence of GSTF12, it is more advantageous to the study of genes related to anthocyanin biosynthesis accumulation pathway. Therefore, we chosen GSTF12 gene of 11 kinds species, downloaded their nucleotide and protein sequence from NCBI as the research object, found strawberry GSTF12 gene via bioinformation analyse, constructed phylogenetic tree. At the same time, we analysed the strawberry GSTF12 gene of physical and chemical properties and its protein structure and so on. The phylogenetic tree showed that Strawberry and petunia were closest relative. By the protein prediction, we found that the protein owed one proper signal peptide without obvious transmembrane regions.

1. Introduction
Glutathione S-transferases (GSTs) is a super family in plants, which could be split into 5 kinds named φ (phi, F), τ (tau, U), θ (theta, T), ζ (zeta, Z) and λ (lambda, L) via protein homology and genomic structure. Plant GSTs play an important part in primary metabolism, secondary metabolism, stress tolerance, cell signaling transduction and so on [1]. Certified by gene knockout experiment, some members of GSTs participate in the transportation of anthocyanin. The GSTs reacted to anthocyanin are found in corn firstly named BZ2. Kernels cannot accumulate anthocyanin because of BZ2 mutation [2]. Anthocyanin vanish in petunia on account of AN9 deficiency [3]. Transducing BZ2 and AN9 to carnation (Dianthus caryophyllus L.), petal show color with anthocyanin transported to vacuole [4]. Recent studies show Arabidopsis thaliana GSTF12 (TT19) [5], Perilla frutescens var. crispa PfrGST1[6], Vitis vinifera VvGST4[7], Cyclamen persicum CkmGST3, Senecio cruentus ScGST3[8] and Dianthus caryophyllus DcGSTF2 are connected to anthocyanin transport, too. However, though many GSTs members are isolated from various plant species, the identification of them is limited, according to information analyze of EMBL/DDBJ/GenBank database [9].

Anthocyanin play essential role in coloring plant [10]. The catalytic synthesis of it by the multi-enzyme complex in the cytoplasm [11]. And it is transported and stored in vacuole [12]. The study chosen GSTF12-like gene of 11 kinds species, downloaded their nucleotide and protein sequence from NCBI as the research object, found strawberry GSTF12 gene via bioinformation analyze so as to provide reference for further study and utilization.
2. Result and Analysis

2.1. Strawberry GSTF12 Find and Phylogenetic Tree Construction of GSTF12

Run BLAST in the *Fragaria ×ananassa* database of GDR and Strawberry GARDEN respectively using Arabidopsis thaliana GSTF12 as a sample. Select the sequence with ideal S and C value, showing FAN_iscf00079132.1 likewise. We find that FAN_iscf00079132.1 is strawberry GSTF12.

We have chosen GSTF12-like gene of 11 kinds species (Table 1), downloaded their nucleotide and protein sequence from NCBI as the research object, analyzed sequences via MEGA6.0, constructed phylogenetic tree on the genetic distance base (Figure 1). The relationship between strawberry and petunia is closer than others, because they all contain a lot of anthocyanins probably.

| Species                               | mRNA Accession Number | Protein Accession Number | Amino Acid Number |
|---------------------------------------|-----------------------|--------------------------|-------------------|
| *Arabidopsis thaliana*                | NM_121728             | NP_197224                | 1096              |
| *Zea mays*                            | NM_0011967            | NP_001183661             | 1217              |
| *Perilla frutescens var. crispa*      | AB362191              | BAG14300                 | 976               |
| *Petunia x hybrida*                   | Y07721                | CAA68993                 | 735               |
| *Malus domestic*                      | JN573599              | AEN84869                 | 975               |
| *Cyclamen persicum x Cyclamen purpurascens* | AB682678         | BAM14584                 | 642               |
| *Nicotiana tabacum*                   | KX356542              | ANO39923                 | 1538              |
| *Beckmannia syzigachne*               | KP852061              | ADM26566                 | 681               |
| *Prunus persica*                      | KT312850              | ALE31202                 | 652               |
| *Pyrus pyrifolia*                     | KP965802              | ALF95173                 | 648               |
| *Thellungiella halophila*             | AK353216              | BAJ34302                 | 927               |

Table 1. Basic information of GSTF12 gene in different species

![Figure 1. Phylogenetic Tree of Strawberry GSTF12.](image)
2.2. **Strawberry GSTF12 Protein Physical Analysis**
Analyze strawberry GSTF12 protein physical feature using ProtPram. Result shows as following: the protein is composed by 1592bp, molecular weight is 132276.52, theoretical pI is 5.02, electric neutrality, molecular formula is C_{4916}H_{8242}N_{1592}O_{2077}S_{293}. Because the N-terminal of the sequence considered is A (Ala), the estimated half-life is 4.4 hours in mammalian reticulocytes (*in vitro*), 20 hours in yeast (*in vitro*) and 10 hours in *Escherichia coli* (*in vitro*). The instability index (II) is computed to be 42.27, so the protein is unstable. The aliphatic index of the protein is 29.59. Grand average of hydropathicity (GRAVY): 0.693.

Amino acid composition result is settings as following (Table 2): the peptide chain consists many Ala as 29.6%.

| Amino Acid | Count | Percentage |
|------------|-------|------------|
| Ala (A)    | 471   | 29.6%      |
| Cys (C)    | 293   | 18.4%      |
| Gly (G)    | 344   | 21.6%      |
| Thr (T)    | 484   | 30.4%      |

### Table 2. Strawberry GSTF12 gene amino acid composition

2.3. **Strawberry GSTF12 Protein Signal Peptide Forecast**
Forecast strawberry GSTF12 protein signal peptide using SignalP 4.1. Result shows as following (Figure 2). There is one signal peptide in the GSTF12 between 21th base and 22th base.

![Figure 2. Signal peptide forecast of strawberry GSTF12.](image)

2.4. **Strawberry GSTF12 Protein Transmembrane Domain Forecast**
Forecast strawberry GSTF12 protein transmembrane domain using TMHMM Server. Result shows as following (Figure 2). There isn’t transmembrane domain in the strawberry GSTF12, thus the protein is not cytomembrane receptor or located on cytomembrane.
3. Materials and methods
Sequences access from NCBI (https://www.ncbi.nlm.nih.gov/). BLAST run in GDR (https://www.rosaceae.org/), and Strawberry GARDEN(http://strawberry-garden.kazusa.or.jp/). Phylogenetic tree is constructed via MEGA6.0, forecast strawberry GSTF12 protein signal peptide using Signa IP 4.1 (http://www.cbs.dtu.dk/services/SignalP/), forecast strawberry GSTF12 protein transmembrane domain using TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM/).

4. Conclusion
GST is a multifunctional protease that catalyzes the electrophilic substitution of hydrophobic and electrophilic compounds with glutathione, presenting in animals and plants and microorganisms widely. The genes coding GST present multi-family phenomenon universally. There are 53 GSTs genes in Arabidopsis, where Phi and Tau are of the same type and quantity. Anthocyanins in the vacuole synthesis, through some biochemical mechanism of transport to the vacuoles in the storage, by the bubble in the different ion concentration, conditions and show different colors. GST is a key enzyme for transporting anthocyanins from cytoplasm to vacuoles, which is identified in a variety of plants are involved in the transport of anthocyanins [13]. Later applied to the study of anthocyanin transport in specific species, the members of the GST family should be screened, and the gene can be studied for specific genes due to GSTs diversity. The way could vary such as filtering from database, constructing local database to BLAST, aligning allied sequence and so on. The exploration on strawberry TT12, which is forward gene to strawberry GSTF12, have been attempt in our lab, we would focus on the interaction between strawberry TT12 and GSTF12 so as to provide reference for further study and utilization.

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