Introduction

Somatic embryos are embryos derived from somatic cells that are capable of developing into young plantlets via a series of morphological changes, a process that closely resembles that of zygotic embryos [1]. Direct somatic embryogenesis (DSE) is a way of developing young plantlets by direct differentiation from explants without an intervening callus phase. During DSE, somatic cells turn into embryogenic cells, and then enter into a differentiation phase. Sometimes, the embryogenic cells do not differentiate directly, but proliferate for a period of time before the proliferated cells start to differentiate. These cells are called the embryogenic cell clusters, and the process is considered a type of DSE because of the direct generation of embryogenic cells. Studies of DSE and embryogenic cell cluster formation have been prevalent [2,3], and cell proliferation is more conductive to increasing the heritability coefficient. One culture cycle can yield a large number of somatic embryos through embryogenic cell cluster process.

Studies identified several genes that play regulatory roles either in specific phases of embryogenesis or during the entire process [4,5], such as BABY BOOM (BBM), which plays a more general role in maintaining cells in an undifferentiated state and which proteins belong to the AP2 subfamily [6]; WUSCHEL (WUS), which promotes somatic embryo development in seedlings when ectopically expressed [7]; AGAMOUS-LIKE15 (AGL15), which promotes somatic embryogenesis in part by controlling ethylene biosynthesis and response [8]; and LEAFY COTYLEDON (LEC), which is essential for induction of somatic embryo development [9]. During embryogenesis, specific remodeling of the cell wall is a crucial process. Xyloglucan endotransglucosylases/hydrolases (XTHs) possess both xyloglucan endotransglucosylase (XET) and xyloglucan endohydrolase (XEH) activities and provide the enzymatic activities responsible for cell wall plasticity. Two XTH-related genes showed a high level of expression during SE induction in Cucumis sativus [10].

Several recent experiments applying expressed sequence tag, microarray, and transcriptional profile analysis were performed to define the molecular events for somatic embryogenesis (SE) [8,11–
1g somatic embryos were transferred to 1/3 MS medium with exchange. For direct embryogenic cell cluster induction (DEC), 1/3 MS medium and cultured for 3 months without medium for 5 days, then transferred to plant growth regulator (PGR)-free medium without PGR for somatic embryo development, and dehiscent seeds were chosen as the culture material. Sterilized seeds were transferred onto 1/3 MS (Murashige and Skoog) medium with 1% sucrose. Cultures were maintained under white fluorescent light [photosynthetic photon flux density (PPFD): 40 μmol·m⁻²·s⁻¹] and long day conditions (16 h light/8 h dark).

For direct embryonic cell induction (DSEI), seeds whose cotyledon had been exposed for 1–3 days were incubated at 40°C (16 h light/8 h dark). Ultrastructural observation showed significant differences of epidermal cells among four lines of Siberian ginseng embryos developed in this study. We report here the screening, isolation, and functional prediction of candidate genes using EST, microarray, and cDNA library construction.

Materials and Methods

Plant materials and growth conditions

Seeds of Siberian ginseng were stratified in moist sand at 15°C for 6 months; dehiscent seeds were chosen as the culture material. After removing the coat, seeds were sterilized in 70% ethyl alcohol for 30 sec followed by 1% NaClO for 10 min, then rinsed 5 times with sterile water. Sterilized seeds were transferred onto 1/3 MS (Murashige and Skoog) medium with 1% sucrose. Cultures were maintained under white fluorescent light [photosynthetic photon flux density (PPFD): 40 μmol·m⁻²·s⁻¹] and long day conditions (16 h light/8 h dark).

For direct embryonic cell induction (DEC), seeds whose cotyledon had been exposed for 1–3 days were incubated at 40°C for 5 days, then transferred to plant growth regulator (PGR)-free 1/3 MS medium and cultured for 3 months without medium exchange. For direct embryonic cell cluster induction (DEC), somatic embryos were transferred to 1/3 MS medium with 1 mg·L⁻¹ 2,4-D (2,4-Dichlorophenoxyacetic acid); this medium possesses the ability to continuously generate embryogenic cell clusters. Embryogenic cell clusters were transferred onto 1/3 MS medium without PGR for somatic embryo development, and mature embryos eventually formed [20].

Induction of secondary somatic embryos

Somatic embryos from DEC and DSEI were used for generating secondary somatic embryos. The primary somatic embryos were cultured on 1/3 MS medium with 1% sucrose without PGR for secondary embryo induction. Secondary somatic embryos arising from the somatic embryos were used for further proliferation by repeated subculture onto fresh medium of the same composition. Secondary somatic embryos from DEC were transferred into 1/3 MS liquid medium without PGR for ECS and ECB into shaken flask suspension cultures [20] and bioreactor cultures [20], respectively. Embryo explants in both instruments were collected on the fifth day of the culture cycle.

Transmission electron microscope (TEM) observation

Somatic embryo explants generated by DEC, DSEI, ECS and ECB were collected on the 3th day. The samples were pre-fixed in 3% glutaraldehyde in phosphate buffer [pH 7.4] for at least 3 hours at 4°C. After rinsing 3 times with phosphate buffer, samples were fixed with 1% osmium tetroxide for 1–2 hours, dehydrated in an increasing concentration series of acetone, and embedded in epoxy resin. Ultrathin sections were obtained with an ultramicrotome (Leica Ultracut UC7) and contrasted with uranyl acetate and lead citrate. Image collection was performed with a transmission electron microscope (FEI/Philips TCNAI G2) at 50 kV.

cDNA library construction

Total RNA from DEC embryos on the fifth day of culture was extracted by the SDS method. mRNA was isolated using the PolyATtract Isolation System III Kit (Promega, Madison, WI, USA), following the manufacturer’s instructions. A 3 M NaAc aliquot was added to the mRNA at 1/10 of the total volume, and an equal volume of isopropanol was added to precipitate the mRNA at −20°C overnight. Following centrifugation at 15,000 × g for 30 min, the mRNA was rinsed with 70% ethanol, then 100% ethanol, dried, and dissolved in RNase-free water.

Five micrograms of mRNA was used to construct the reference library using the cDNA Library Construction Kit and following the manufacturer’s instructions (Takara, Otsu, Japan). Ten microliters of bacteria containing the unamplified library was diluted 10-, 10⁻²-, and 10⁻³-fold in succession with SOC liquid medium, 10 μL of diluted bacteria liquid were then plated onto LB medium agar plates containing 30 mg/L of Ampicillin (Amp); plates were incubated for 12 h at 37°C, and the titer of the unamplified library was measured. Clones from the cDNA library were randomly selected for sequencing, and expressed sequence tags (ESTs) were used for bioinformatic analysis. Initially, we assembled EST sequences using the Phred/Phrap/Consed package. For annotation, the assembled consensus sequences and singletons were used as queries in searches of the Genebank Nt, Genebank Nr, and SWISSPORT databases performed using BLASTN and BLASTX with an E-value cut-off of 10⁻¹⁰. Singletons were annotated and functionally classified by comparison with sequences in the COG database.

Microarray production

Probes were designed based on the contigs of the Siberian ginseng cDNA library. cDNA from DEC, ECS, ECB and DSEI on the fifth day of culture was hybridized with probes. Two hybridization replicates were performed.

The tissue was homogenized in liquid nitrogen using a mortar and pestle, and total RNA was prepared using the SDS method. After precipitation, Qiagen RNeasy Kit (Qiagen, Valencia, CA, USA) was used for purification. RNA from each sample was reverse transcribed into aminoliquese-labeled cDNA, using 2 μg of total RNA primed with 5 μg of T7 promoter primer, and 200 U of M-MLV reverse transcriptase (Takara). The cDNA was purified using Qiagen RNeasy Mini kit (Qiagen). The cDNA (4 μg) was concentrated to 6.6 μL, mixed with 10 μL DMSO, and added to 3.4 μL of 0.3 M NaHCO₃ [pH 9.0]. The mixture was added into fluorescent dye (Cy3). Excess dye was removed using the Qiagen RNeasy Mini kit (Qiagen). Then, 11 μL of 10× Blocking Agent and 2.2 μL of 25× Fragmentation Buffer were added to the purified Cy3 labeled targets to a final volume of 35 μL, and 55 μL
of 2× GE hybridization buffer was added for fragmentation. The mixture (100 μL) was put on the slide for rolling hybridization at 65°C for 17 h. After hybridization, the slide was washed at 37°C. The slides were dried, and then scanned using an Agilent scanner.

Quantitative RT-PCR

On the fifth day of culture, total RNA from DEC and DSEI was prepared using the SDS method. All of the qRT-PCR reactions, including the validation of upregulated and downregulated genes
in the microarray assays, were carried out on RQ1 RNase-free DNase (Promega). cDNA synthesis from total RNA was performed using the PrimeScript RT reagents Kit (Takara) according to the manufacturer’s instructions. Assays were performed using SYBR Premix Ex Taq II (Takara) kit for quantification. Primers were designed as 17–25 nt oligomers with Tm 60–65 to amplify a product 80–150 bp long (Table S1). Quantification was performed by $2^{\Delta\Delta C(t)}$ using actin and tubulin as housekeeping genes for normalization.

**EsPLT1 and EsXTH1 gene cloning**

Total RNA of DEC was extracted using the SDS method. 5’-RACE-Ready and 3’-RACE-Ready cDNA synthesis were performed using the SMART RACE cDNA Amplification Kit (Takara) and following the manufacturer’s instructions. The full length EsPLT1 was then cloned by 5’/3’-rapid amplification of cDNA ends (5’/3’-RACE). Primers were designed based on EST sequences using the RACE instructions and Primer Premier 5.0. RACE products were cloned into TA plasmids using pGEM-T and pGEM-T Easy Vector Systems (Promega). After sequencing, the 5’-RACE product and 3’-RACE product were aligned and spliced based on the overlap by using CONTIG software. We then designed a pair of primers based on the obtained sequence corresponding to the nucleotide at position ‘ATG’ and ‘TGA’. The PCR product was then cloned into pGEM-T Vector and sequenced to obtain the full-length gene sequence. *EsXTH1* cloning was the same as the above procedure.

**Results**

The induction of four embryogenic culture lines

Mature seeds (Figure 1A) stopped developing upon treatment with high temperature; at the same time, the surface of the endosperm and the edge of cotyledon became slightly brown and somatic embryos developed directly (Figure 1B). These somatic embryos developed into secondary embryos directly after transfer to the original culture environment for DSEI (Figure 1C). When these somatic embryos isolated, they again gave rise to new small somatic embryos in a repetitive manner (Figure 1D, E). Additionally, visible secondary embryos occurred within 7 days on the surface of the original somatic embryos.

When somatic embryos were cultured on medium containing 1 mg/L $2,4$-D, friable embryogenic cell clusters formed from the surface of primary somatic embryos (Figure 1F). DEC developed mature embryos upon culture on PGR-free medium (Figure 1G). We selected one lines of secondary somatic embryos generated embryogenic cell clusters again in a repetitive manner on PGR-free medium (Figure 1H, I). Visible embryos cells also occurred within 7 days from original somatic embryos. Embryogenic capacity could maintenance for more than 5 years by repeated secondary embryogenesis.
When DEC lines were transferred into liquid medium in a shaken flask suspension culture (ECS) and bioreactor culture (ECB), the somatic embryos developed into secondary embryos directly (Figure 1 J, K and L M) [20]. The secondary embryos failed to generate embryogenic cell clusters either in shaken flasks or bioreactor cultures, and did not generate embryogenic cell clusters after being transferred again to induction medium (data not shown).

Observation of somatic embryo epidermal cells by TEM

On the first day of cultivation, TEM of DEC showed epidermal cells completely filled with amyloplasts, protein bodies, and numerous uncoalesced lipid bodies (Figure 2A).

On the 5th day of cultivation, TEM of epidermal cells of DEC, DSEI, ECS and ECB showed significant differences; DEC showed protoplasm with a large nucleus, prominent nucleus, numerous lipid bodies, mitochondria, small, irregular-shaped vacuoles, and glyoxysomes next to the lipid bodies. Accumulation of starch grains was observed in amyloplasts with dense stroma (Figure 2B, C). The cell wall was thicker, and the intercellular gap was larger than other lines of cells.

Protoplasm of DSEI contained large nuclei, prominent nucleoli, nonuniform vacuoles, numerous lipid bodies, and amyloplasts (Figure 2D). Electron dense plate structures were observed in the cytoplasm (Figure 2E). The cell wall of DSEI was unclear and had an irregular edge.

TEM of ECS showed that the nucleus was pushed into the edge of the cell wall by a central vacuole (Figure 2F), and few cell inclusions were observed (Figure 2G).

Epidermal cells of ECB were rich in inclusions and comprised lipid bodies, amyloplasts, protein bodies, mitochondria, and glyoxysomes next to the lipid bodies (Figure 2H, I). The nucleus and nucleolus were clearly observed. Cell walls were clear, but irregular, and the intercellular space was narrowed compared with other cell lines.

cDNA library construction

Siberian ginseng embryonic cell cluster library was generated from somatic embryos of DEC with an unamplified library titer of 2.3×10^6 pfu·mL⁻¹. The range of inserted fragment length was 500–2000 bp, and the average length was 960 bp. Randomly sequenced clones from 5376 cDNA clones and 3691 ESTs were obtained. After removing vector sequences and sequences of inferior quality, 3331 sequences with effective length greater than 100 bp were obtained (library accession # LIBEST_028309).

Then, 2415 unigenes that comprised 497 contigs and 1918 singletons were obtained after assembly of the ESTs. The length of unigenes was 300–1200 bp.

The unigenes were used for annotation through BLASTN and BLASTX searches of the NCBI Nt, NCBI Nr, SWISSPROT and KEGG databases. In total, 1570, 1657, 1188 and 1629 unigenes matched these database entries, respectively. Comparison of unigenes against the COG database resulted in the tentative annotation of 490 unigenes including 7 unigenes of unknown function (Table 1). Sequences encoding proteins putatively involved in translation and ribosomal structure and biogenesis comprised the largest functional group (33.06%; Figure 3). The sequences related to posttranslational modification, protein turnover, and chaperones comprised the second largest category (19.59%), and proteins related to lipid transport and metabolism comprised a considerable portion (19.59%).

Microarray analysis

To find the differentially expressed genes related to development of embryogenic cell clusters, fluorescent probes were synthesized based on the 1948 contigs described above (LIBEST_028309). Total RNA samples were extracted from DEC, DSEI, ECS and ECB lines, and hybridized with array.

The list of expressed genes was significantly different among DEC, DSEI, ECS and ECB lines (Table S2, 3, 4). It was found that 552 transcripts were upregulated and 335 transcripts were downregulated in the DEC line compared with DSEI (Table S2A, B); 40 upregulated and 50 downregulated compared with ECS (Table S3A, B), and 55 upregulated and 44 downregulated compared with ECB (Table S4A, B).

To screen for major genes related to the generation of embryogenic cell clusters, the DEC line was compared with other three lines together, and 7 upregulated genes and 4 downregulated genes were found (Table 1). The functions of these genes were...
predicted using gene ontology analysis to determine in which biological process and cellular component they functioned. In the DEC line, the upregulated genes were involved in stem cell activity, stress resistance, and regeneration of cell wall. In contrast, the commonly upregulated genes in DSEI, ECS and ECB, were involved in plant protective effect, redox sensor mechanisms, and biotic and abiotic stress.

Validation of the expression pattern of genes detected by microarray analysis

To confirm the data of the microarray analysis, quantitative real-time PCR was used to determine the expression level of several selected genes that were differentially expressed between the DEC and DSEI lines (Table 2). PLT, bZIP and LEA stress resistance related genes, XTH cell wall remodeling enzyme related gene, and GLU cell wall protein related gene were all more highly expressed in DEC than in DSEI (Figure 4A). The response to changing environmental conditions for plants with a VQ motif was almost 7-fold higher in DEC than DSEI. The validation of the microarray data by quantitative PCR showed that the results obtained by both methods were consistent with each other.

Genes that were downregulated in the DEC line and had an extremely large difference in expression were chosen to be validated using quantitative PCR (Table 2). Two SDR short-chain dehydrogenases/reductases genes, the GH antifungal protein related gene (GH, Glucan endo-1,3-beta-glucosidase), the CDF cell growth factor related gene, and a major form of the nucleoside diphosphoglucose gene UDP-glucose pyrophosphorylase (UGPGP) were all upregulated in DSEI compared with DEC. SDR2 and GH were upregulated more than 300-fold in DSEI (Table 2). This result was consistent with the microarray chip.

Relative expression of other genes was higher in DSEI than in DEC (Figure 4B), consistent with the microarray chip.

Cloning of full-length cDNA encoding EsXTH1 and EsPLT1

EsXTH1 (accession no. KF660542), which was upregulated in DEC, was cloned using RACE and sequenced. An 1101-bp cDNA containing 873-bp of coding sequence was obtained, and the sequence was predicted to encode a protein of 290 amino acids (Figure S1A). Conserved domains were found in EsXTH1 by aligning the cDNA sequence to other plant XTH sequences (Figure 5). According to Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/), amino acids 30-211 aligned to glycosyl hydrolyases family 16 (E-value 1.2e-62), and amino acids 236-287 aligned to the C-terminus of xyloglucan endotransglycosylase (E-value 8.63e-33); the conserved domains that were recognized are underlined in Figure 4B. EsPLT1 (accession no. KF660541), which was also upregulated in DEC, was cloned using RACE. The cDNA was sequenced, and a 2026-bp cDNA sequence was obtained that contained a 1650-bp coding sequence, and was predicted to encode a 549-amino acid protein in length (Figure S1B). The cDNA was aligned to other plant PLTs (Figure 6). These include the following domains underlined in Figure 6, which are characteristic of an AP2 subfamily that belongs to the AP2/EREBP family: amino acids 171–243 (E-value 1.0e-29) and amino acids 273–337 (E-value 8.63e-33) constitute the AP2 DNA-binding domain (according to http://smart.embl-heidelberg.de/).

Expression of EsXTH1 and EsPLT1 mRNA

To better understand the gene expression pattern of EsXTH1 and EsPLT1, relative expression of these two genes in the DEC

| GenBank Accession number | Experimental name | Fold change | Up-Down Regulation | BLASTX sequence similarity (accession number) | E value |
|--------------------------|-------------------|-------------|---------------------|-----------------------------------------------|---------|
| JZ513132                 | PLT               | 8.00        | Up                  | PLT2, A. thaliana (NM_103997.3)                 | 2.00E-09 |
| JZ513081                 | bZIP              | 8.62        | Up                  | HBP-1b (c1)-like, V. vinifera (XM_002281994.2) | 3.00E-10 |
| JZ512520                 | XTH               | 3.45        | Up                  | XTH, V. vinifera (XM_003633163.1)              | 2.00E-21 |
| JZ511852                 | LEA               | 3.02        | Up                  | LEA1, A. hypogaea (ADQ91833)                   | 1.00E-33 |
| JZ512706                 | VQ                | 2.66        | Up                  | Structural constituent of cell wall, R. communis (XP_002513957) | 9.00E-08 |
| JZ514398                 | GLU               | 3.19        | Up                  | Endo-1,3/1,4-beta-D-glucanase, V. vinifera (XP_002275697) | 2.00E-44 |
| JZ5143138                | SDR1              | 2.31        | Down                | SDR, R. communis (XM_00259970.1)               | 4.00E-33 |
| JZ512158                 | SDR2              | 431.34      | Down                | Glucose and ribitol dehydrogenase, M. truncatula (XP_003591095) | 4.00E-33 |
| JZ512411                 | GH                | 12.42       | Down                | Glucosidase precursor, R. communis (XM_002510658.1) | 3.00E-12 |
| JZ514265                 | CDF               | 103.26      | Down                | Cell growth factor-2, A. thaliana (NM148494.2) | 1.00E-19 |
| JZ514152                 | UDPGP             | 330.78      | Down                | UDP-glucose pyrophosphorylase, Zea mays (DAA62613) | 1.00E-15 |

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and DSEI lines was measured over a culture cycle. *EsXTH1* was more highly expressed in DEC than in other lines throughout the duration of the culture cycle (Figure 7A). The change in overall gene expression in ECS, ECB and DSEI lines was approximately the same, for example, gene expression in ECB was higher than in ECS and DSEI and lower than in DEC throughout the culture cycle. Gene expression in DSEI and ECS was almost the same. *EsPLT1* in DEC had increased expression with increased culture duration, but remained at a low expression level in other lines (Figure 7B). At the beginning of the culture stage, *EsPLT1* was expressed at a very low level in all four lines, but at the following stage, expression increased sharply only in DEC.

**Discussion**

Four repetitive somatic embryogenesis lines of *E. senticosus* were obtained through different inductive conditions. The different characteristics of DEC, DSEI, ECS and ECB lines allow for different utilities of each line, however, the ability of the DEC line to produce a large number of embryogenic cell clusters is more beneficial for propagation of Siberian ginseng.

TEM was used to observe the ultrastructural differences in epidermal cells among DEC, DSEI, ECS and ECB lines. It was found that DEC epidermal cells had dense cytoplasm, prominent nuclei, notable amounts of reserve compounds, and slightly large mitochondria, as verified in embryogenic cells in other species.
It is generally known that reserve compounds are enriched in the initial stage of SE. Our study found only lipids and proteins in the epidermal cell of Siberian ginseng. Before the start of the formation of the protuberances, proteins and lipids became smaller and were partially or totally consumed. This time coincides with the period where epidermal cells acquire meristematic characteristics [23]. Starch grains were substituted for proteins and lipids in the initial stages of the embryogenic process. A high amount of starch has been observed during this time in different species [21,24,25]. Ultrastructural characteristics were notably different in DEC compared with ECS and DSEI; similarities were observed between DEC and ECB. Abundant inclusions in ECB, as in DEC, may be the reason for the rapid growth of somatic embryos; therefore, this culture method may be useful for industrial production of Siberian ginseng. Plasmodesmata were not observed in all embryos; only in DEC lines were more regularly spherical cells observed, a characteristic of embryogenic cell formation [26]. This regular spherical shape may be what caused the cells in the DEC line to separate more easily and generate the embryogenic cell clusters. Golgi apparatus were found near the cell walls in the DEC line, which provided polysaccharide for thickening the cell wall. In the early stage of embryogenic cells, signals have to be transferred through plasmodesmata for embryogenic cells to acquire the information for their differentiation and division [27]. The disappearance of plasmodesmata and the thickening of the cell wall provided a physiological isolation between embryogenic cells and other surrounding cells, releasing the embryogenic cells from the control of the surrounding cells and allowing them to have totipotency [21,26].

The significant differences in ultrastructure suggested that the involvement of various physiological pathways was different among DEC, DSEI, ECS and ECB in Siberian ginseng, and the pathway regulating somatic embryo development in ECS and ECB changed when DEC lines were transferred into either liquid medium or bioreactor cultures, respectively. During construction of the Siberian ginseng cDNA library (LIBEST_028309), 1948 expressed sequence tags (ESTs) were obtained. A considerable portion of these ESTs corresponded to genes involved in translation, ribosomal structure and biogenesis, post translational modification, protein turnover, and chaperones. These biological processes indicated that gene reprogramming occurred [26,28].

Genes involved in lipid transport and metabolism were also highly expressed (Figure 5). We found robust lipid bodies present at the initial development of somatic embryos (Figure 2A), which became smaller and less numerous as development progressed (Figure 2B). The increased activity of genes related to lipid transport and metabolism could help explain this ultrastructural change.

During embryogenesis, the cell wall remodeling process is a crucial step. Polysaccharides, cell wall components, need to be disassembled and remodeled because of cell enlargement [29]. Xyloglucan is one type of polysaccharide present, and is the predominant hemicellulose in the cell walls of most dicotyledons. It forms a network with cellulose that strengthens the cell wall. Xyloglucan endotransglucosylase/hydrolases (XTHs), belonging to sixteen family of glycoside hydrolases, take part in the remodeling step, and are essential for this process [30]. It is worth mentioning that XET (most XTHs possess this activity) was identified as the enzyme responsible for splitting xyloglucan chains and linking the newly-generated reducing end to the non-reducing ends of another xyloglucan chain, resulting in the wall-loosening required for plant cell expansion [30,31]. Two XTH-related genes were identified as having increased expression during SE in Cucumis sativus [10]. One XTH-related gene named EsXTH1 (accession no. KF660541) was found in Siberian ginseng, and was expressed more highly in the DEC line than in the ECS, ECB, and DSEI lines that do not generate embryogenic cell clusters. Among the upregulated genes in DEC were many genes related to cell wall modification. This finding implies the active role of cell wall modifying enzymes during Siberian ginseng development, particularly during embryogenesis. Our result suggests that cell wall remodeling is more likely to occur in DEC, potentially explaining why embryogenic cells develop more easily in that line.

The genes belonging to the AP2/ERF family have roles in undifferentiated cell proliferation and differentiation of stem cell niches within meristems [32], for example, BBM encodes an AP2 domain transcription factor and expressed in developing embryos and seeds, maintains cells in an undifferentiated state [6]. Besides, PLT1 and PLT2 genes of Arabidopsis were identified to encode AP2-family putative transcription factors. These genes are essential for quiescent center (QC) specification and stem cell activity [33]. It was found that the transcribed activity of PLT genes is dependent on auxin accumulation and auxin response transcription factors. Conceptually, the embryogenic cell is similar to a stem cell. Both possess the abilities of cell division and cell totipotency. In the initial stage of SE, certain responsive cells have the potential to activate genes involved in generating embryogenic cells [5]. Auxin may be a factor that mediates the signal transduction cascade to activate those certain responsive cells. This embryogenesis process is similar to the generation of stem cells. In this study, an AP2 gene that was upregulated in DEC of Siberian ginseng showed considerable similarity compared with PLT genes of other species (Figure 6). In comparing the relative gene expression of the AP2 gene between DEC and DSEI of Siberian ginseng, expression was observed to be higher in DEC than in DSEI throughout the cultivation cycle (Figure 7B). Interestingly, the expression of PLT genes in DEC gradually increased over the duration of cultivation. In the same way, two PLT genes described in the Arabidopsis root have been shown to maintain the activity of stem cells [33], and subsequent research showed promotor activity and PLT activities are largely additive and dosage-dependent [34]. In this study, EsPLT1 was expressed throughout cultivation of DEC, increasingly and gradually; in contrast, this phenomenon did not occur in DSEI, ECS, and ECB. Meanwhile, embryogenic cell clusters increased continually and exponentially app:cedwrd:exponentialy in quantity during cultivation. Therefore, we conclude that the EsPLT1, through its continually increasing level, gives DEC the ability to generate embryogenic cells. These results also demonstrated that embryogenic cells were identical to stem cells.

Embryogenic cell cluster induction is more beneficial for SE because of the rapid development and massive somatic embryos. It

Figure 6. Amino acid sequence alignment of EsPLT1 with PLT from other plant species. Black represents 100% homology; gray represents ≥ 50% homology. Amino acid sequences of PLT from tomato (Solanum lycopersicum; XM 004250817.1), poplar (P. trichocarpa, XM 002308352.1), grape (V. vinifera, XM 002855933.1), soybean (G. max, XM 003539733.1), peach (Prunus persica, KF69090.1), Arabidopsis (A. thaliana, NM 103997.3), cucumber (Cucumis sativus, XM 004142079.1), and alfalfa (M. truncatula, XM 00360710.1) revealed the existence of conserved functional domains of AP2 (lined section).

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is worth researching on a molecular level to identify key genes involved in this process. This study suggested that EsXTH1 and EsPLT1 might be the essential genes that provide embryogenic ability for generation of embryogenic cell cluster in Siberian ginseng.

Supporting Information

Figure S1  The nucleotide and amino acid sequences of EsXTH1 and EsPLT1. A showed conserved functional domains of Glyco_hydro_16 (gray section) and XET_C (lined section). B showed conserved functional domains of AP2 (gray section).

Table S1  Primer sequences information.

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