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Research Article

Cloning and Expression Analysis of After-Ripening Related Genes in *Panax ginseng* Seed

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Panax ginseng enjoys a wide range of medicinal applications and good economic value, the market demand is large, but *Panax ginseng* seed's after-ripening characteristic seriously restricts development of *Panax ginseng* industry. In this experiment, starting from the main action hormone ABA and GA of *Panax ginseng*, primers of GA and ABA related to *Panax ginseng* seed after-ripening were screened and designed, and the possible relationship between transcription factor WRKY gene and *Panax ginseng* seed after-ripening was probed. Semiquantitative and quantitative real-time PCR of these genes were performed. ABA and GA-related metabolizing enzymes play an important role in the process of *Panax ginseng* seed after-ripening, and WRKY transcription factor regulates gene expression. In this study, we cloned the PgGA2ox gene, which is the key metabolic enzyme of GA, and analyzed the genes by the bioinformatics software, which laid a foundation for studying the molecular mechanism of *Panax ginseng* seed after-ripening.

1. Introduction

Panax ginseng, a valuable medicinal plant, is widely used in human medical treatment and healthcare, and the market demand is on the rise [1]. *Panax ginseng* is a plant of seed propagation, and *Panax ginseng* seeds have not fully developed embryo after natural maturity [2–4]. With the characteristic of postripening, it must be treated by a long period of stratification to break dormancy and germinate. In the main producing areas of *Panax ginseng*, seeds are planted in early August every year and seeded immediately after harvest. Most of the seeds will germinate in the spring of the third year (after 21-22 months) under natural conditions. Many growers cannot promote seed after-ripening, leading to plant failure and seriously affecting economic benefits. The long production cycle restricts large-scale popularization of *Panax ginseng* cultivation to a certain extent, and cultivation process is vulnerable to climate, cultivation conditions, pests, and diseases, resulting in low production of *Panax ginseng* and low economic efficiency. Therefore, it is necessary to find an effective way to improve *Panax ginseng* yield and quality, thereby improving production efficiency [5].

Phytohormone is a minor molecule signal substance, with very small concentration or even close to 0, but plays a very important role in the plant's life cycle. During seed dormancy and germination, hormones regulate the metabolism of proteins and enzymes through signal transduction, regulating seed dormancy and germination [6].

ABA is a positive regulatory factor of dormancy and is involved in induction and maintenance of dormancy, which negatively regulates germination [7–9]. ABA will resynthesize during imbibition of dormant seeds, which is a requirement for seed to remain dormant. ABA produced by the embryo is necessary for dormancy [10]. The decrease of ABA content before germination is due to inhibition of newly synthesized ABA and activation of ABA catabolism.

NCED is the key rate-limiting enzyme in ABA synthesis. There are five types present in *Arabidopsis thaliana*, including AtNCED1, AtNCED3, AtNCED5, AtNCED6, and AtNCED9. AtNCED6 and AtNCED9 are essential ABA biosynthetic enzymes [11, 12]. The expression level of

NCEDs is directly related to ABA content [13]. AtNCED5 and AtNCED6 are highly expressed in the mid to late stages of *Arabidopsis thaliana* embryogenesis [11]. The CYP707A family encodes the ABA 8′-hydroxylase, which is involved in ABA catabolism. CYP707A1 enhances expression at the early stage of seed maturation, while CYP707A2 enhances expression at the late stage of maturation [14]. After seed imbibition, ABA content decreased, CYP707A2 activity increased, and lack of this enzyme will increase ABA content and strengthen dormancy [14, 15].

GA, a positive regulatory factor of germination, can antagonize inhibition of ABA [16]. In the early stage of seed germination, GA can promote embryonic development and material decomposition. According to Kucera, GA exerts two positive effects on seed: enhancing seed vigor and promoting germination; breaking the seed coat bound and weakening the radicle surrounding tissue [17]. Low-temperature stratification can promote GA synthesis, enable ABA/GA changes, and promote dormancy release and seed germination [18].

GA can regulate GA metabolism by controlling the expression of many key enzymes in the GA synthesis pathway through feedforward or feedback regulation. GA synthesis regulation occurs mainly in late course of transduction. GA2-oxidase (GA20ox) can be catalyzed into active GA1, which is a key regulatory enzyme of GA biosynthesis. GA2-oxidase (GA2ox) can catalyze active GA1 to inactive GA34, catalyzing degradation of GA (Henderson et al. [19–21]).

WRKY transcription factor regulates seed germination and growth after seed germination. In 2004, ScWRKY1 transcription factor was isolated from wild potato ovule. ScWRKY1 transcription factor was transiently highly expressed in the embryo of torpedo stage. Its expression level was low in the fruit, root, and stem, suggesting that ScWRKY1 might participate in the embryogenesis process. *Arabidopsis thaliana* AtWRKY10 was expressed in pollen and globular embryos and also expressed in binucleate stage of endosperm development to cell stage. New plant with the gene deleted cannot form seed of normal size. The result showed that WRKY transcription factor played an important role in the embryo growth and in reducing early cellularization of endosperm [22].

In this study, beginning from the key hormones ABA and GA directly related to after-ripening characteristic of *Panax ginseng* seed by removing seed dormancy, the related primers were designed. Semiquantitative PCR and real-time quantitative PCR of these genes were performed. As WRKY transcription factor could regulate seed embryo growth to determine its expression level and understand its relationship with other genes can lay a foundation for the study of molecular mechanism of *Panax ginseng* seed postripening. Table 1.

Table 1: Real-time quantitative real-time PCR primers.

Gene	Primers
GA2ox	F-TTCACAGCACAACTGAGGTT
	R-AGAGTATTGGTCGTAACGGC
GA20ox	F-TCGCCCTAATACCCTTGTC
	R-CGATAGTGTTTCTGTGTGAACC
NCED	F-GTGAGCCTTTCTTTCTTCCTTCG
	R-GAAATCCATACGGAACTCTTGACG
CYP707A	F-GGGGAAACTCTTCAGCTCTACTCCA
	R-TCCGGGCTCGACACCATCACA
WRKY6	F-ATAGTCCGACGAGTGAGAT
	R-CTGCCCATATTTTCTCCAAC
WRKY3	F-AGCGAACGCACTGTGGTAT
	R-CTTGGTTTGAAGGCGAGAA
WRKY27	F-TGGAGGAAGTATGGACAAAA
	R-AGGGTGTGTATGATTGTGCTC
5S	F-TATTCTGGTGTCCTAGGCGT
	R-ATCCTGGCGTCGAGCTATTT

2. Materials and Methods

(1) *Panax ginseng* seeds were purchased from the Tonghua Changlong Forest Farm in Jilin Province. The seed was harvested for lamination treatment, washed with 75% ethanol 3 times, rinsed with distilled water 3 times, and wiped with filter paper, to be placed in sterilized sand with carbendazim treatment, with seed: sand = 1 : 3 at lamination. The stratified seeds were sampled at 45-day intervals, 30 seeds at a time, for a total of 5 samples. The samples were washed and frozen with liquid nitrogen and then stored in a refrigerator at −80°C until use.

(2) Total RNA extraction and cDNA synthesis: Total RNA was extracted in accordance with instructions of RNA extraction kit; and then, reverse transcription synthesis of cDNA was performed in accordance with instructions of reverse transcription kit. The kit was purchased from the Dalian Takara Bio Company.

(3) Quantitative real-time PCR primer design: Primers were designed using the Primer Premier 5.0 software. Semiquantitative and quantitative real-time PCR were performed according to the primer sequence in Table 1, and all primers were synthesized by the Beijing Genewiz Company.

(4) Semiquantitative PCR system and procedures: Reaction system: cDNA 1 μl, upstream and downstream primers 1 μl, Ex Taq enzyme 0.2 μl, dNTP 1 μl, 20 × Ex Taq enzyme buffer 2.5 μl, ddH2O 18.3 μl, final volume of 25 μl; Reaction procedure: predenaturation at 94°C for 5 min; denaturation at 94°C for 30 s, 50°C–60°C (according to different primer changes in Tm value) annealing 30 s, 72°C extension for 30 s, 35 cycles; 72°C extension for 5 min; and storage at 4°C.

(5) Quantitative real-time PCR system and procedures: Reaction system: 2 × SYBR Green Mix 10 μl, upstream and downstream primers 1 μl, cDNA 1 μl, ddH2O 7 μl, final volume of 20 μl; Reaction procedure: 95°,C 5 min; 95°C 15 s, 56°C 15 s, and 72°C 30 s single lighting, 40 cycles; 95°C, 1 min; 65°C, 1 min; solubility curve: target temperature 95°C, initial temperature 65°C, constant temperature time 20 s, and step 0.5°C/s; 30°C, 1 min; and end 4°C.

(6) Cloning the full-length cDNA of PgGA2ox gene: The cDNA of *Panax ginseng* seed was used as template, and PgGA2ox gene was amplified by the following system: 1 μl of cDNA, 1 μl of upstream and downstream primers, 0.2 μl of Ex Taq enzyme, 1 μl of dNTP, 20 × Ex Taq enzyme buffer 2.5 μl, 18.3 μl of ddH_2O, and final volume of 25 μl. PCR reaction annealing temperature was 55°C; the reaction primers were F-ATGGTAGTCTTGCCCAAGCCAACAA and R-TCATGAGGCTGCAATTTTCTCAAAG. PCR products were detected by 1% agarose gel electrophoresis, and extraction of DNA from agarose gel of target amplified fragments was performed according to operation instructions of the DNA purification recovery kit. The recycling products were ligated into pMD18-T vector and transferred into E.*coli* to be cultured on LB medium containing ampicillin. After 12 hours, the colonies were observed. The positive clones were placed in LB medium containing ampicillin for 12 hours' culture before colony PCR amplification. After electrophoresis detection, it was sent to Genewiz for sequencing.

3. Results

3.1. Panax ginseng Embryos in Different Stages of After-Ripening. Dormancy of *Panax ginseng* seed needs to pass through the two stages of morphological after-ripening and physiological after-ripening. Samples were taken 5 times at 45-day intervals and defined as SE1–SE5 stages: SE1, initial phase of morphological after-ripening; SE2, mid-term of morphological after-ripening; SE3, late morphological after-ripening, seed fissure; SE4, initial phase of physiological after-ripening; and SE5, mi-term of physiological after–ripening.

3.2. Semiquantitative PCR Results. The cDNA of seeds of the five stages of *Panax ginseng* embryo after-ripening was used as template, and 5S rRNA was used as internal reference. The seven primers were amplified after the annealing temperature was set based on primer Tm value. Detection was performed with 1% agarose gel electrophoresis, and loading quantity of sample was 5 μl. Picture was taken in the ultraviolet light irradiation after electrophoresis, and gene expression is shown in Figure 1.

From the figure, we can see that most of the genes were in low expression or no expression in the morphological after-ripening stage, GA2ox gene expression in morphological after-ripening stage is higher than that in physiological after-ripening, and CYP707A gene and GA2ox gene expression level tends to be consistent. WRKY transcription factor family showed a completely different expression. WRKY6 expression level in the physiological after-ripening period was higher than that in the morphological after-ripening stage, suggesting that it was associated with seed dormancy. WRKY3 and WRKY27 in morphological and physiological after-ripening stage were in low expression.

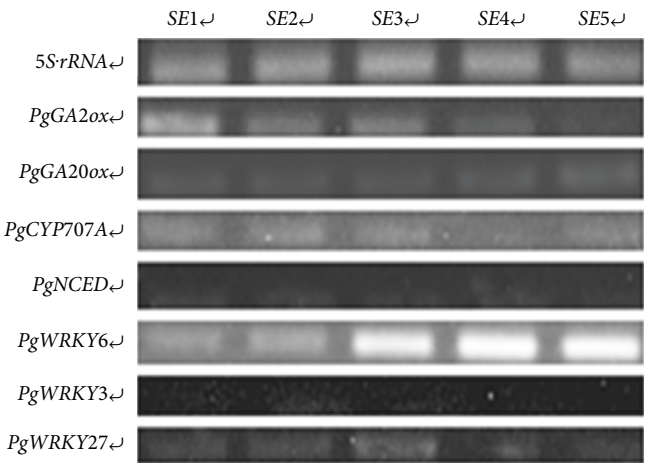

Figure 1: Gel electrophoresis photograph of semiquantitative PCR.

3.3. qRT-PCR Results. With 5S rRNA as internal reference, expression of GA, ABA, and WRKY transcription factor and embryo after-ripening related genes was detected in different period of *Panax ginseng* seed. The experimental data were analyzed by a software that came with quantitative real-time PCR instrument, as shown in Figures 2–4.

GA is a key factor in embryo dormancy and germination, GA2ox is a key gene in GA anabolism and catabolism. The study on action law of these two genes is helpful for the analysis of GA action mechanism in *Panax ginseng* seed after-ripening. The expression of GA20ox gene in physiological after-ripening stage was higher than that in morphological after-ripening stage, and GA20ox gene expression increased gradually in the two periods, which indicated that GA20ox gene played a more important role in physiological after-ripening. The expression level of GA20ox gene decreased in physiological after-ripening stage, quite low in physiological after-ripening stage, but increased slightly at the end of physiological after-ripening stage. It was inferred that the increase of expression level was related to release of seed dormancy.

ABA can induce and maintain dormancy, a positive regulatory factor of dormancy, a negative regulatory factor of germination, which acts antagonistically with GA. NCED is the key rate-limiting enzyme in ABA synthesis. The expression of NCED gene is directly related to ABA content. CYP707A gene is involved in ABA catabolism. The expression level of NCED gene decreased gradually at morphological and physiological after-ripening stages, with the former higher than the latter, indicating that the content of ABA was decreased and the dormancy was gradually broken. The expression level of CYP707A gene declined continuously in morphological after-ripening stage and increased at SE5 stage.

WRKY transcription factor has a special zinc finger structure, which plays an important regulatory role in the process of plant response to biological and abiotic stress, plant development (such as seed germination, dormancy, and leaf senescence). The three WRKY genes showed different expression trends in after-ripening stage of *Panax ginseng* seed. Expression levels of WRKY3 and WRKY27

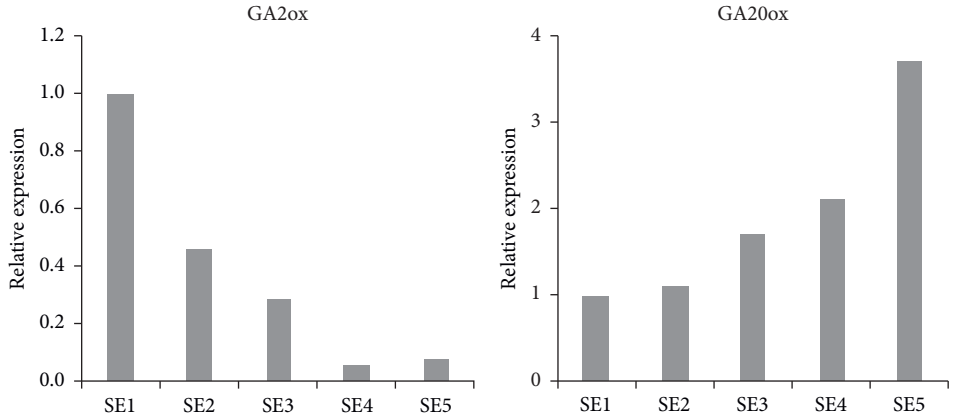

Figure 2: Expression of genes related to GA metabolism in after-ripening stage of *Panax ginseng* seeds.

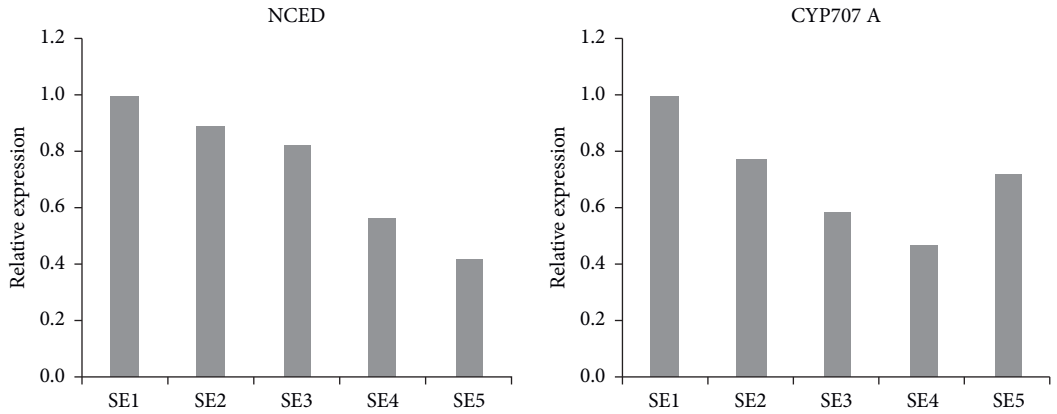

Figure 3: Expression of ABA-related genes in after-ripening stage of *Panax ginseng* seeds.

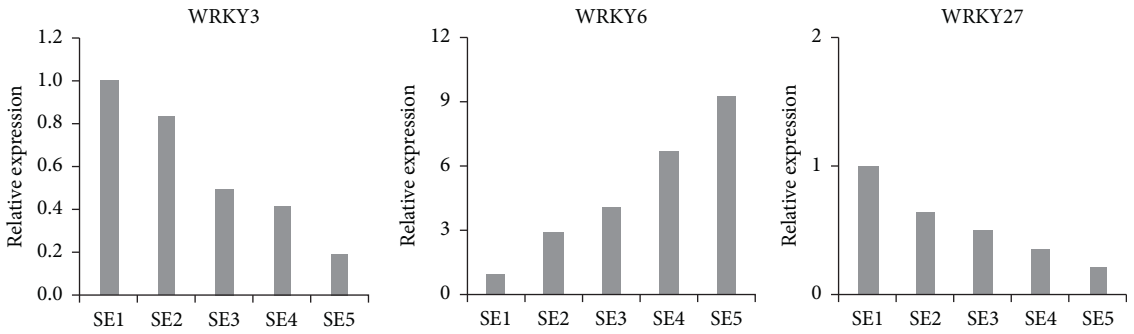

Figure 4: Expression of WRKY transcription factor in after-ripening stage of *Panax ginseng* seeds.

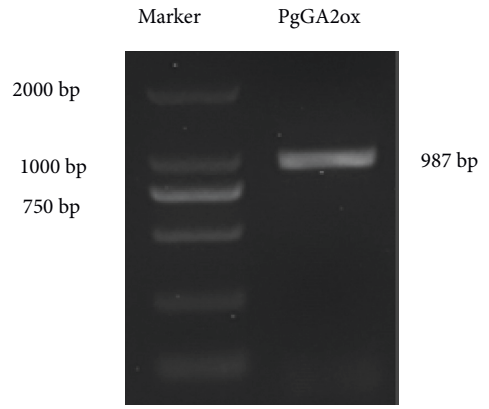

Figure 5: Gel electrophoresis photograph of cloned PgGA2ox gene.

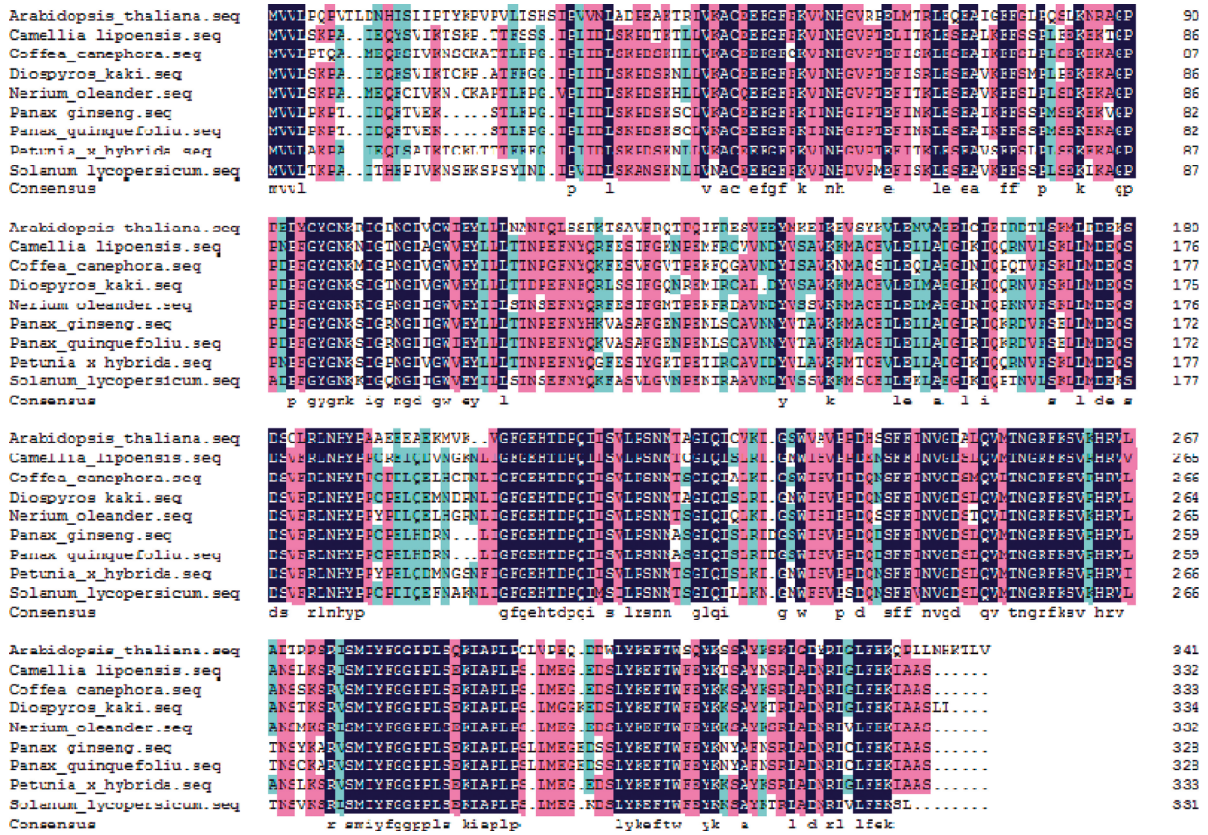

Figure 6: Comparison of amino acid sequence similarity between PgGA2ox and other species.

were low, which decreased in morphological and physiological after-ripening stages. But expression level of WRKY6 was opposite, which gradually increased in physiological after-ripening stage. It is inferred that it is related to release of embryo dormancy and accelerated seed germination.

3.4. Cloning of PgGA2ox Gene of Panax ginseng Seed. The PCR cloning of designed primers was performed using *Panax ginseng* seed cDNA as the template, obtaining a sequence with length of 987 bp. The amplified gene was named PgGA2ox and the nucleotide sequence was submitted to NCBI. GenBank accession number obtained was KT692958.1. The electrophoregram is shown in Figure 5.

3.5. GA2ox Coded Amino Acid Sequence Similarity Analysis and Construction of Phylogenetic Tree. BLASTN analysis was performed on NCBI. It was found that nucleotide sequence of PgGA2ox was similar to gene sequence of *Panax quinquefolius* (KJ802836.1), *Petunia x hybrida* (GU059939.2), and *Camellia lipoensis* (KJ502290.1), with similarity at 99%, 81%, and 80%, respectively. Amino acid sequences coded by PgGA2ox were compared using BLASTP, finding that in terms of amino acid sequence similarity, it was 99% similar to *Panax quinquefolius* (AIL25679.1) GA2ox, 79%, 79%, 79%, 77%, 76%, 73%, 72%, 71%, and 55%, respectively, similar to *Coffea canephora* (CDP06089.1), *Diospyros kaki* (AID65071.1), *Camellia lipoensis* (AHZ13201.1), *Nerium oleander* (AAT92094.1), *Petunia x hybrida* (AFH56955.1)

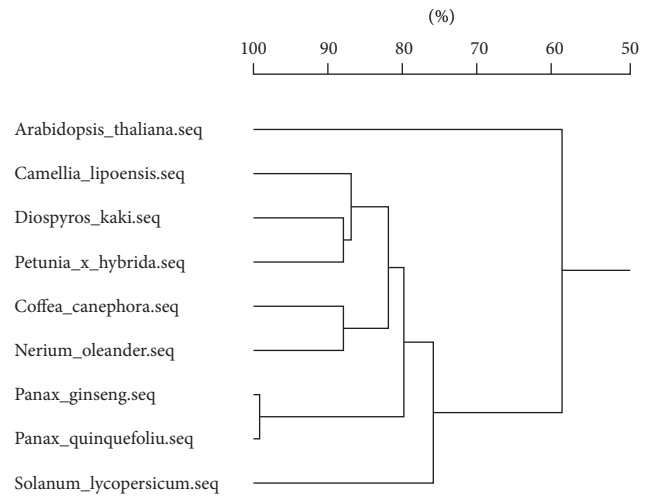

Figure 7: Evolutionary analysis of PgGA2ox.

GA2ox, *Cucurbita maxima* (CAC83090.1), *Solanum lycopersicum* (NP_001234752), *Helianthus annuus* (CAS03784.1), and *Arabidopsis thaliana* (NP_174296.1) gene encoding.

GA2ox is a dioxygenase and 2-ketoglutarate substrate in the GA metabolic pathway. The PgGA2ox protein in the *Panax ginseng* contains a conserved binding amino acid site shared by other plants, i.e., a R residue and a S residue bounded to 2-ketoglutarate, located at positions 226 and 228, respectively, and two H residues and one D residue bound to Fe (Figure 6).

In order to analyze the evolution of PgGA2ox encoded proteins, a phylogenetic tree was constructed using the DNAMAN software (Figure 7). According to the phylogenetic tree, PgGA2ox is most closely related to *Panax quinquefolius*, close to persimmon and *Nerium indicum*, which is consistent with BLASTP result of amino acid sequence.

4. Conclusion

The seed after-ripening is an adaptation of the *Panax ginseng* to the external environment in the long-term evolutionary process. In natural conditions, it helps *Panax ginseng* pass through adverse environment, so that its race multiply, but it brings difficulties to cultivation of mankind. In order to expand cultivation area of *Panax ginseng* and further improve economic benefits of *Panax ginseng*, research on after-ripening mechanism of *Panax ginseng* seed is an urgent problem to be solved. *Panax ginseng* seed after-ripening process is very complex, so current domestic and foreign scholars' study on *Panax ginseng* seed after-ripening process focuses on physiological and biochemical dynamic changes within the seed, seed embryo development changes, and changes in hormones and isoenzyme content in the after-ripening period, but little is reported on the after-ripening mechanism of *Panax ginseng* seed.

In this study, we analyzed the expression rules of GA and ABA hormone-related genes and WRKY transcription factor in *Panax ginseng* seed after-ripening, which is the first of its kind in study of *Panax ginseng* seed dormancy.

GA-related genes: Gibberellin acid synthesis regulation occurs mainly in the late transduction pathway. GA20ox can catalyze GA12 and GA53 into active GA1 and GA4. GA2ox, by β-hydroxylation, can convert active GA1 and GA4 into inactive GA13 and GA14 [23]. The expression level of GA20ox (GA synthetic gene) in the physiological after-ripening stage was higher than that in the morphological after-ripening stage, which proved that low temperature could promote GA synthesis and increase expression of GA synthetic gene, which is consistent with the results of Derkx [24]: low temperature can enhance expression of AtGA20ox1 and AtGA20ox1.

ABA-related genes: C40 carotenoid zeaxanthin was oxidized to violaxanthin, which, after forming 9-cis-epoxy carotenoids, turned to C15 xanthohumol after NCED pyrolysis, a key step in ABA synthesis. CYP707A belongs to P450 monooxygenase family and encodes ABA 8′-hydroxylase, which is involved in catabolism of ABA. The expression level of NCED gene and CYP707A gene was not high in the after-ripening stage of *Panax ginseng* seed, both of which decreased gradually during the whole after-ripening stage, which indicated that endogenous ABA synthesis pathway was closely related to seed after-ripening.

WRKY transcription factor: WRKY gene family is a multifunctional gene family. Molecular biological function of its family members is very rich, including regulation of plant resistance to biological stress and abiotic stress, regulation of plant growth and development, morphogenesis, metabolic regulation, and so on. In addition, WRKY transcription factor is also involved in plant low-temperature response. *Panax ginseng* seeds need to go through low-temperature environment in the after-ripening process. The expression level of WRKY3 and WRKY27 decreased during seed ripening, and WRKY6 was highly expressed in the process of *Panax ginseng* seed after-ripening. Therefore, we speculated that WRKY transcription factor could respond to low temperature regulation, then participate in regulation of metabolism, regulation of gene expression, and finally complete *Panax ginseng* seed after-ripening, which can be further verified in subsequent relevant experiments.

In this study, we cloned for the first time the key gene PgGA2ox for gibberellin acid metabolism during the process of *Panax ginseng* seed after-ripening and obtained GenBank accession number KT692958.1.

The results of this study not only provide a basis for molecular function analysis of PgGA2ox gene but also provide a basis for study on mechanism of after-ripening of *Panax ginseng* seeds.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

[1] D. Q. Dou, L. Jin, and Y. J. Chen, "Research progress and prospect of chemical composition and pharmacological activity of Panax ginseng," *Journal of Shenyang Pharmaceutical University*, vol. 16, no. 2, pp. 151–156, 1999.

[2] T. Sadler, *The Influence of Climate, Dormancy and Seed Germination in Understanding the Commercial Limitations of Growth of Panax Ginseng C.A. Meyer and Panax Quinquefolius L. And the Mass Micropropagation of These Species*, Panax ginseng, Australia, 2004.

[3] C. P. Zhang, *Mechanism of Different Exogenous Substances Improving Stress Resistance of Coptis Chinensis Seeds and Seedlings under Salt Stress*, Southwest University, Texas, 2012.

[4] Y. Y. Hu, *Study on Seed Germination Characteristics and Seedling Technology of Fritillaria Sichuanensis*, Sichuan Agricultural University, China, 2008.

[5] L. S. Wang and H. L. Sun, "Seed germination and preservation of medicinal plants," *Plant Magazine*, vol. 17, no. 2, pp. 12–21, 1990.

[6] S. Y. Chen, C. T. Chien, J. M. Baskin, and C. C Baskin, "Storage behavior and changes in concentrations of abscisic

acid and gibberellins during dormancy break and germination in seeds of Phellodendron amurense var. wilsonii (Rutaceae)," *Tree Physiology*, vol. 30, no. 2, pp. 275–284, 2010.

[7] J. Lozanojuste, M. Masi, and A. Cimmino, "The fungal sesquiterpenoid pyrenophoric acid B uses the plant ABA biosynthetic pathway to inhibit seed germination," *bioRxiv*, vol. 36, pp. 241–243, 2019.

[8] E. Zdunek-Zastocka and A. Grabowska, "The interplay of PsABAUGT1 with other abscisic acid metabolic genes in the regulation of ABA homeostasis during the development of pea seeds and germination in the presence of H2O2," *Plant Science*, vol. 285, pp. 79–90, 2019.

[9] H. Nonogaki, "Seed germination and dormancy: the classic story, new puzzles, and evolution," *Journal of Integrative Plant Biology*, vol. 61, no. 5, pp. 541–563, 2019.

[10] J. Pan, H. Wang, Y. Hu, and D Yu, "Arabidopsis VQ18 and VQ26 proteins interact with ABI5 transcription factor to negatively modulate ABA response during seed germination," *The Plant Journal*, vol. 95, no. 3, pp. 529–544, 2018.

[11] G. Q. Song, W. Li, S. J. Zhang et al., "Analysis of the relationship between TaNCED1 gene expression and ABA accumulation in wheat under drought and rehydration," *Journal of Triticeae Crops*, vol. 39, no. 4, pp. 400–406, 2019.

[12] S. Z. Ma, Z. W. Sha, Y. N. Lu, X. L. Zhang, L. X. Zhou, and M. D. Bian, "Functional analysis of *Arabidopsis thaliana* cryptochrome in ABA regulated seed germination," *Journal of Jilin Agricultural University*, vol. 40, no. 3, pp. 264–269, 2018.

[13] Z. Wang, D. Chen, C. Yue, H. L. Cao, and Y. L. Guo, "Cloning and expression analysis of CsNCED2 gene in tea plant," *Acta Botanica Boreali-Occidentalia Sinica*, vol. 38, no. 6, pp. 994–1002, 2018.

[14] A. J. Matilla, N. Carrillobarral, and M. D. C. Rodríguez-Gacio, "An update on the role of NCED and CYP707A ABA metabolism genes in seed dormancy induction and the response to after-ripening and nitrate," *Journal of Plant Growth Regulation*, vol. 34, no. 2, pp. 274–293, 2015.

[15] Y. Zheng, Y. Huang, W. Xian, J. Wang, and H. Liao, "Identification and expression analysis of the Glycine max CYP707A gene family in response to drought and salt stresses," *Annals of Botany*, vol. 110, no. 3, pp. 743–756, 2012.

[16] H. Y. Ma, D. D. Zhao, Q. R. Ning et al., "A multi-year beneficial effect of seed priming with gibberellic acid-3 (GA3) on plant growth and production in a perennial grass, leymus chinensis," *Scientific Reports*, vol. 8, no. 1, 14 pages, Article ID 13214, 2018.

[17] D. Koryznienė, S. Jurkonienė, T. Žalnierius et al., "Heracleum sosnowskyi seed development under the effect of exogenous application of GA," *PeerJ*, vol. 7, no. 5, pp. 88–95, 2019.

[18] G. Gogoi, P. K. Borua, and D. Chetia, "GA3 assisted seed dormancy breaking in abelmoschus moschatus medik. subsp. moschatus," *Cercetari Agronomice in Moldova*, vol. 49, no. 4, pp. 27–34, 2016.

[19] J. T. Henderson, H. C. Li, S. D. Rider et al., "*PICKLE* acts throughout the plant to repress expression of embryonic traits and may play a role in gibberellin-dependent responses," *Plant Physiology*, vol. 134, no. 3, pp. 995–1005, 2004.

[20] X. Z. Huang, C. F. Jiang, and L. L. Liao, "Research progress on molecular basis and regulation mode of gibberellin acid mechanism," *Chinese Bulletin of Botany*, vol. 23, no. 5, pp. 499–510, 2006.

[21] A. L. Hauvermale, T. Ariizumi, and C. M. Steber, "Gibberellin signaling: a theme and variations on della repression," *Plant Physiology*, vol. 160, no. 1, pp. 83–92, 2012.

[22] D. Q. Yu, L. G. Chen, and Y. R. Hu, "AtWRKY8 regulates multiple stress signaling pathways in plants," *Chinese Society of Genetics National General Assembly and academic seminar*, vol. 167, 2013.

[23] Y. B. Li, M. Y. Zhu, X. R. Yang et al., "Advances on gibberellin 2-oxidases gene in higher," *Plants Acta Horticulturae Sinica*, vol. 45, no. 9, pp. 1844–1856, 2018.

[24] T. Boonkaew, C. Mongkolsiriwatana, A. Vongvanrungruang, K. Srikulnath, and S. Peyachoknagul, "Characterization of GA20ox genes in tall and dwarf types coconut (Cocos nucifera L.)," *Genes & Genomics*, vol. 40, no. 7, pp. 735–745, 2018.