Differential Expression of Genes Involved in Saikosaponin Biosynthesis Between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd

Ma Yu1,2, Hua Chen2, Shi-Hang Liu3, Yu-Chan Li2, Chun Sui1, Da-Bin Hou2* and Jian-He Wei1*

1 Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, 2 School of Life Sciences and Engineering, Southwest University of Science and Technology, Mianyang, China, 3 Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China

Radix Bupleuri (roots of *Bupleurum* spp.) is an important medicinal herb. Triterpenoid saponins of saikosaponins generally constitute the main class of secondary metabolites of plants in the *Bupleurum* genus. However, the molecular regulatory mechanism underlying their biosynthesis remains elusive. In this study, we observed significantly different saikosaponin biosynthesis between *Bupleurum chinense* and *Bupleurum scorzonerifolium* at the seedling stage. The sequential and expression characterization of 232 genes in the triterpenoid saponin biosynthetic pathway, which includes the mevalonate (MVA) pathway and methylerythritol phosphate (MEP) pathway, between *B. chinense* and *B. scorzonerifolium* was also investigated. Sixty of these genes may be involved in saikosaponin biosynthesis. Manipulation of these genes, especially those of the β-AS, P450, and UGT families, may improve saikosaponin production.

Keywords: *Bupleurum*, saikosaponin biosynthesis, transcriptome analysis, gene expression, P450

INTRODUCTION

Radix Bupleuri (roots of *Bupleurum* spp.) is one of the most important medicinal herbs in Eurasia and North Africa used as a treatment for fever, chronic hepatitis, nephrotic syndrome, inflammatory diseases, menstrual disorders, and digestive ulcers (Pistelli et al., 1996; Guo et al., 2000; Ikegami et al., 2006; Mabberley, 2008). In addition, this herb has been used for more than 2,000 years in China (Tan et al., 2008). In the Chinese Pharmacopoeia, the official botanical origin of Bupleuri Radix is the roots of *Bupleurum chinense* DC. or *Bupleurum scorzonerifolium* Willd. (Chinese Pharmacopoeia Commission, 2015). Several groups of secondary metabolites have been isolated from *Bupleurum* species, including triterpenoid saponins (saikosaponins), steroidal saponins, lignans, essential oils, and polysaccharides (Ashour and Wink, 2011). Among these, saikosaponins generally represent the main class of secondary metabolites and constitute to up to 7% of the total dry weight of roots of plants in the *Bupleurum* genus (Ashour and Wink, 2011). Owing to their wide range of pharmacological activities, including their immunomodulatory activity, anti-inflammatory activity, antioxidant and hepatoprotective activity, cytotoxicity, antitumor activity, and antiviral activity, these triterpenoid saponins, especially...
saikosaponins a and d, are the most important pharmacological constituent in *Bupleurum* root extracts (Zhao and Xiao, 2007; Lin et al., 2013; Liang et al., 2014).

To date, more than 130 glycosylated oleane-type and ursane-type saponins have been isolated from the genus *Bupleurum* L. (Ashour and Wink, 2011; Wang et al., 2017b; Chelghoum et al., 2018). A previous study on the species *B. chinense*, *B. scorzonerifolium*, and *Bupleurum falcatum* L. demonstrated that saikosaponins are mainly distributed in the tissues of the cork and cortex of roots (Liang et al., 2014). Similar results were reported based on histochemical studies (Tan et al., 2008). In addition, the synthesis and accumulation of saikosaponins is strongly influenced by intrinsic factors, including growth stage, developmental phase, and root structure, and by environmental conditions, such as drought, fertility, and light deficiency (Tan et al., 2008; Zhu et al., 2009a,b; Gong et al., 2017). The combination of cultural practices together with manipulation of the expression of genes involved in triterpenoid saponin biosynthesis may be a more effective way to improve the total yield of saikosaponins.

In the biosynthetic pathway of triterpenoid saponins in higher plants, the mevalonate (MVA) pathway in the cytosol and the methylerethritol phosphate (MEP) pathway in the plastids are essential biosynthetic processes for formation of the triterpenoid backbone of the five-carbon intermediates isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). In transcriptome studies, genes involved in the biosynthetic pathway of saikosaponins were identified in *Bupleurum kaoi*, *B. chinense*, and *B. scorzonerifolium* (Chen et al., 2007; Sui et al., 2011; Sui et al., 2015). Moreover, genes involved in the biosynthesis of saikosaponins such as squalene epoxidase, β-amylase, cytochrome P450, and uridine diphosphate glycosyl transferases have been cloned in *B. kaoi*, *B. falcatum*, and *B. chinense*, and their expression profiles have been identified (Lin et al., 2006; Kim et al., 2011; Gao et al., 2016). Overexpression of the BcbZIP134 gene in *B. chinense* and BJSS1 in *B. falcatum* has been reported (Kim et al., 2011; Xu et al., 2019). Additionally, cytochrome P450 monoxygenase (P450) of CYP167A1 from *B. falcatum* was combined with oxidoglualene cyclase, P450, and glycosyltransferase genes to construct a synthetic biological platform for the production of bioactive triterpene sapogenins in yeast (Moses et al., 2014). However, the molecular regulatory mechanism underlying triterpenoid saponin biosynthesis remains elusive.

In this study, we investigated the sequential and expression characterization of 232 genes in the MVA pathway and MEP pathway in *B. chinense* and *B. scorzonerifolium* to identify putative genes involved in the biosynthesis of saikosaponins in *Bupleurum* L.

### MATERIALS AND METHODS

#### Plant Materials

The two experimental materials, the commercial varieties Chuanbeichai No. 1 (CBC1) and Chuanhongchai No. 1 (CHC1), which are varieties of *B. chinense* and *B. scorzonerifolium*, respectively, were used. All of the plants were bred via systemic selection and purifying selection from farmholding populations by Dr. Jianhe Wei from the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, and Dr. Da-bin Hou from Southwest University of Science and Technology.

For each genotype, seeds were placed on moist filter paper before germination. Germinated seedlings were then grown in modified Hoagland’s nutrient solution. Five-day-old and 15-day-old CBC1 and CHC1 plants were utilized for isoform sequencing (iso-seq) analysis, transcriptome analysis, and saikosaponin a (SS a) and d (SS d) content assays. For 5-day-old seedlings, whole fresh roots were harvested (S1). For 15-day-old seedlings, 5 mm of the root tip without the region of differentiation (S2) and with the region of differentiation (S3) were harvested separately.

#### Extraction of Saikosaponins and HPLC Analysis

Samples S1, S2, and S3 (three replications each) were dried for 72 h using a freeze-drier (LGJ-18, Beijing Songyuan Huaxing Technology Development Co., Ltd., China). The SS a and SS d content was determined using a Waters HPLC (high-performance liquid chromatography) system (Waters 1525 Binary HPLC Pump, United States) and an ASB-vensil C18 column (250 mm × 4.6 mm, 5 μm). Reference standards of SS a and SS d were purchased from the National Institutes for Food and Drug Control, Beijing, China. The methods and conditions for determination have been reported previously (Xu et al., 2019).

#### Iso-seq and Transcriptome Analyses

The leaf and root samples were mixed and utilized for iso-seq library construction. The iso-seq analysis followed the method published by Wang et al. (2017a). Transcriptome analyses of samples S1, S2, and S3 were performed on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, United States) as previously described (Yang et al., 2018). Both iso-seq and transcriptome analyses were performed at the Novogene Bioinformatics Institute (Novogene, Beijing, China). Three replicates were included in this study.

#### Candidate Gene Selection

Gene sequences of 20 families involved in the MVA pathway and MEP pathway were selected from iso-seq and transcriptome data with annotated gene names. These genes include glycosyltransferases (UGT), P450, β-amyrin synthase (β-AS), squalene synthase (SS), squalene epoxidase (SE), farnesyl diphosphate synthase (FPS), mevalonate-5-pyrophosphate decarboxylase (MVD), phosphomevalonate kinase (PMK), mevalonate kinase (MK), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS), acetyl-CoA C-acyltransferase (AACT), 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), 2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase (CMS), 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol-2,4-cyclophosphate synthase (MCS),
RESULTS

SS a and SS d were detected in the roots of B. chinense plants at the 1-day-old stage, whereas they significantly accumulated in the region of differentiation at the 15-day-old stage (Table 1). In B. scorzonerifolium, no peaks of SS a and SS d were identified in any of the samples during the HPLC analysis. Therefore, the genes showed significantly different expression in SS a and SS d in B. chinense, but insignificant or the opposite expression in B. scorzonerifolium would be interesting.

A total of 223 genes with complete open reading frames (ORFs) were identified from the transcriptome database of B. chinense and B. scorzonerifolium (Supplementary Table S1). One to 96 genes were identified for a single gene family whose members are involved in triterpenoid saponin biosynthesis, although the MCS family had zero genes. In the differential expression analysis of B. chinense, 86 genes showed significantly different expression in the S3 sample (Figures 1, 2). Among these genes, 1 to 36 genes were identified for gene families including UGT, P450, β-AS, SS, FPS, GPS, PMK, HMGCR, HMGS, AACT, IDS, HDS, CMS, DXR, and DXS. No gene with significantly different expression was detected for SE, MVD, IDI, MK, and CMK. Five genes involved the gene families β-AS, SS, CMS, HDS, and IDS; these genes exhibited significantly similar expression in B. scorzonerifolium. Seven genes in the FPS, HMGCR, DXS, and P450 gene families showed significantly reversed expression. The BcDXS15975 gene in the DXS family was identified only in B. chinense and was not detected in any of the samples of B. scorzonerifolium.

DISCUSSION

Triterpenoids are derived from C5 isoprene units of IPP and DMAPP through a “head-to-tail” connection (Hillier and Lathe, 2019). Both of these triterpenoid backbones can be synthesized through the MVA pathway in the cytoplasm or the MEP pathway in the plastids. For the MVA pathway, three HMGs genes and one HMGCR gene (BcHMDH15256) showed significantly upregulated expression in S3 of the B. chinense transcriptome, whereas one AACT gene, seven HMGCR genes, and one PMK gene showed significantly downregulated expression. For these genes, the expression differences among S1, S2, and S3 were insignificant in B. scorzonerifolium, with the exception of the BcHMDH1723 gene (Figure 1). In addition, BcHMDH1723 showed the opposite expression trend between B. chinense and B. scorzonerifolium. We did not detect genes with significantly different expression in the MK family or the MVD family.

For the MEP pathway, 12 unigenes encoding enzymes (five DXS genes, three CMS genes, two HDS genes, and two IDS genes) showed significantly downregulated expression in S3 of the B. chinense transcriptome, whereas the expression of two DXS genes, one DXR gene, one CMS gene, one HDS gene, and six IDS genes significantly increased. The CMS gene BcISPD108768, the HDS gene BcISPG26774, and the IDS gene BcISPH3774 showed similar trends in the B. scorzonerifolium transcriptome, whereas the DXS gene BcDXS14138 showed the opposite trend. The DXS gene BcDXS15975 was detected only in the B. chinense transcriptome.

Both the MVA and MEP pathways produce the C5 unit IPP, which can be transformed into its isomer DMAPP by IDI (Xue et al., 2019). In the present study, we did not find that the IDI gene showed significant expression differences among S1, S2, and S3. IPP and DMAPP are assembled into GPP and FPP by GPS and FPS prenyltransferases, respectively. Expression of the GPS gene BcGGPPS5581 increased in S3 of the B. chinense transcriptome, and expression of the FPS gene BcFPSS50324 decreased. SS catalyzes the joining of two units of FPP in a “tail-to-tail” fashion of SQ. Kim et al. (2011) found that overexpression of BfSS1 in B. falcatum more powerfully regulates downstream genes than does MeJA treatment in triterpene and phytosterol biosynthesis. In the present study, three candidate genes in the SS family showed significant expression differences in S3 of the B. chinense transcriptome, and the expression of all these candidate genes among the three samples in the B. scorzonerifolium transcriptome.

---

Table 1: Contents of saikosaponin a and saikosaponin d in the roots of B. chinense and B. scorzonerifolium.

| Species              | Sample | SS a content (µg/g) | SS d content (µg/g) |
|----------------------|--------|---------------------|---------------------|
|                      |        | SD                  | SD                  |
| B. chinense          | S1     | 0                   | 0                   |
|                      | S2     | 0                   | 0                   |
|                      | S3     | 479.77              | 270.64              |
|                      |        | 154.95              | 154.34              |
| B. scorzonerifolium  | S1     | 0                   | 0                   |
|                      | S2     | 0                   | 0                   |
|                      | S3     | 0                   | 0                   |

1http://doua.prabi.fr/software/cap3
2http://smart.embl-heidelberg.de/
3http://www.ncbi.nlm.nih.gov/
4http://web.expasy.org/compute_pi/
5http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/
was not significant. SQ is oxidized by SE to give rise to 2,3-oxidosqualene. We cloned the full-length SE gene in

B. chinense (Gao et al., 2016). However, no candidate gene was identified in the differential expression analysis.
In *Bupleurum*, the first committed step in the synthesis of triterpenoid saponins involves the cyclization of 2,3-oxidosqualene by β-AS. Therefore, β-AS is presumed to be the enzyme that catalyzes the first committed step in saikosaponin biosynthesis. A previous study indicated that β-AS in *B. kaoi* and *B. chinense* exhibits tissue-specific responses to both MeJA and PEG (Lin et al., 2013; Zhang et al., 2016). We have also cloned the promoter of β-AS in *B. chinense* and evaluated its activity previously (Gao et al., 2015). We found three β-AS genes (*BcBAMS98705*, *BcBAMS12370*, and *BcBAMS21523*) with significantly increased expression in S3 of *B. chinense*. The *BcBAMS12370* gene showed a similar expression trend in *B. scorzonerifolium*, whereas the expression of the other two genes was non-significant.

For the production of saikosaponins in *Bupleurum*, P450 enzymes catalyze the oxidation of β-amyrin to form 13,28-epoxy and the C11/C12 double-bond structure or two double bonds at C11/C12 and C13/C18 together with hydroxylation at C16 and C23. Previous studies have indicated that the CYP90, CYP72, CYP710, and CYP711 subfamilies of the P450 family may involve triterpenoid-metabolizing enzymes (Ohnishi et al., 2006; Hamberger and Bak, 2013; Yu et al., 2017). We found that 37 P450 genes showed significant expression in the three samples of *B. chinense*, and four of these genes exhibited a significant opposite trend in *B. scorzonerifolium* (Figure 2). These genes are members of 13 families of 7 clans in the P450 family. Among these genes, two unigenes belong to the CYP90 family, five unigenes belong to the CYP72 family, two unigenes belong to the CYP710 family, and the unigene *BcCYP17256* belongs to the CYP711 family. Members of the CYP716 family contribute to the diversification of eudicot triterpenoid biosynthesis (Miettinen et al., 2017). Moses et al. (2014) found that the CYP716Y1 gene from *B. falcatum* catalyzes the C-16α hydroxylation of triterpenes in yeast. However, we did not identify any CYP716 gene that showed significantly different expression in S3 of *B. chinense*.
At the last step of saikosaponin biosynthesis, UGT catalyzes the glycosylation of hydroxylated β-amyrin at C3. Glycosylation contributes to the highly diverse nature of terpenoids in plants (Field and Osbourn, 2008). Therefore, UGT is presumed to be the key enzyme involved in the modification of saikosaponins. A transcriptome analysis of B. chinense revealed that, among 196 UGT genes, three were the most likely candidates involved in saikosaponin biosynthesis (Sui et al., 2011). Three BkUGT85A proteins that contain a highly conserved region of a motif of glycosyltransferases, which are involved in the production of secondary metabolites in plants, were identified in B. kaoi by Lin et al. (2013). In the present study, four UGT genes showed significantly increased expression in S3 of B. chinense, whereas four genes showed the opposite expression trend. The expression differences among all genes in B. scorzonerifolium were non-significant.

CONCLUSION

In this study, unigenes showed significantly different expression in the S3 sample in B. chinense, while insignificant or the opposite expression in B. scorzonerifolium may be involved in saikosaponin biosynthesis. Manipulation of these genes, especially those of the β-AS, P450, and UGT families, may improve saikosaponin production. The combined expression of these genes and reconstituting the synthesis of monoglycosylated saponins in yeast may provide a platform for the production of bioactive saikosaponins.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in NCBI PRJNA645610 for B. chinense (SRR12223465-SRR12223473) and PRJNA662700 for B. scorzonerifolium (SRR12647620-SRR12647628).

AUTHOR CONTRIBUTIONS

MY wrote the manuscript. HC performed the data analysis. S-HL seeded Chuanbeichai No.1 and Chuanhongchai No.1. Y-CL performed the HPLC analysis. CS performed the transcriptome analysis. J-HW bred out the commercial varieties of Chuanbeichai No.1 and Chuanhongchai No.1. J-HW and D-BH funded the whole project and helped MY to complete the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China under grant number 81603223, the China Agriculture Research System under grant number CARS-21, the CAMS Innovation Fund for Medical Sciences (CIFMS) (2016-12M-2-003), the National Transgenic Major Project of China (2019ZX08010004-005), the Natural Science Foundation of Science and Technology Department of Sichuan Province (2019YJ0443 and 2019YFH0072), and Young Elite Scientists Sponsorship Program by CACM (2019-QNRC2-C15).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.583245/full#supplementary-material

REFERENCES

Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. Genome Biol. 11:R106. doi: 10.1186/gb-2010-11-10-r106 Ashour, M. L., and Wink, M. (2011). Genus : a review of its phytochemistry, pharmacology and modes of action. J. Pharm. Pharmacol. 63, 305–321. doi: 10.1111/j.2042-7158.2010.01170.x Chelghoum, M., Smati, D., Mitaine-Offer, A.-C., Paululat, T., and Lacaille-Dubois, M.-A. (2018). Four new triterpene saponins from Bupleurum rigidum L. Phytochem. Lett. 27, 223–228. doi: 10.1016/j.pytl.2018.07.025 Chen, L.-R., Chen, Y.-J., Lee, C.-Y., and Lin, T.-Y. (2007). MeJA-induced transcriptional changes in adventitious roots of Bupleurum kaoi. Plant Sci. 173, 12–24. doi: 10.1016/j.plantsci.2007.03.013 Chinese Pharmacopoeia Commission (2015). The Pharmacopoeia of the People's Republic of China, 2015 Edition Part I. Beijing: China Medical Science Press. Field, B., and Osbourn, A. E. (2008). Metabolic diversification— independent assembly of operon-like gene clusters in different plants. Science 320, 543–547. doi: 10.1126/science.1154990 Gao, K., Wu, S.-R., Wang, L., Xu, Y.-H., Wei, J.-H., and Sui, C. (2015). Cloning and analysis of β-amyrin synthase gene in Bupleurum chinense. Genes Genom. 37, 767–774. doi: 10.1007/s13258-015-0307-0 Gao, K., Xu, J.-S., Sun, J., Xu, Y.-H., Wei, J.-H., and Sui, C. (2016). Molecular cloning and expression of squalene epoxidase from a medicinal plant. Bupleurum chinense. Chinese Herb. Med. 8, 67–74. doi: 10.1016/s1674-6384(16)60010-2 Gong, J., Liu, M., Xu, S., Jiang, Y., Pan, Y., Zhai, Z., et al. (2017). Effects of light deficiency on the accumulation of saikosaponins and the ecophysiological characteristics of wild Bupleurum chinense DC. in China. Ind. Crops Products 99, 179–188. doi: 10.1016/j.indcrop.2017.01.040 Guo, Y., Matsumoto, T., Kikuchi, Y., Ikejima, T., Wang, B., and Yamada, H. (2000). Effects of a pectic polysaccharide from a medicinal herb, the roots of Bupleurum falcatum L. on interleukin 6 production of murine B cells and B cell lines. Immunopharmacology 49, 307–316. doi: 10.1016/s0162-3109(00)00245-9 Hamberger, B., and Bak, S. (2013). Plant P450s as versatile drivers for evolution of species-specific chemical diversity. Philos. Trans. R. Soc. B-Biol. Sci. 368:20120426. doi: 10.1098/rstb.2012.0426 Hillier, S. G., and Lathe, R. (2019). Terpenes, hormones and life: isoprene rule revisited. J. Endocrinol. 242, R9–R22. doi: 10.1530/JEO-19-00084 Ikegami, F., Sumino, M., Fujii, Y., Akiba, T., and Satoh, T. (2006). Pharmacology and toxicology of Bupleurum root-containing Kampo medicines in clinical use. Hum. Exp. Toxicol. 25, 481–494. doi: 10.1191/096037106het654oa Kim, Y. S., Cho, J. H., Park, S., Han, J.-Y., Back, K., and Choi, Y.-E. (2011). Gene regulation patterns in triterpene biosynthetic pathway driven by overexpression of squalene synthase and methyl jasmonate elicitation in Bupleurum falcatum. Planta 233, 343–355. doi: 10.1007/s00425-010-1292-9 Liang, Z., Oh, K., Wang, Y., Yi, T., Chen, H., and Zhao, Z. (2014). Cell type-specific qualitative and quantitative analysis of saikosaponins in three Bupleurum species using laser microdissection and liquid chromatography– quadrupole/time of flight-mass spectrometry. J. Pharm. Biomed. Anal. 97, 157–165. doi: 10.1016/j.jpba.2014.04.033
Lin, T.-Y., Chiou, C.-Y., and Chiou, S.-J. (2013). Putative genes involved in saikosaponin biosynthesis in Bupleurum species. *Int. J. Mol. Sci.* 14, 12806–12826. doi: 10.3390/ijms140612806

Lin, W.-Y., Peng, F.-H., and Lin, T.-Y. (2006). “Cloning and characterization of beta-amyrin synthase from *Bupleurum kaio*,” in *Proceedings of the 8th International Congress of Plant Molecular Biology*, (Adelaide, SA: Book of Abstracts, ISPMB).

Mabberley, D. J. (2008). *Plant-book*: Mabberley’s plant-book: a portable dictionary of plants, their classifications and uses; utilizing Kubitzki’s “The families and genera of vascular plants” (1990-) and current botanical literature; arranged according to the principles of molecular systematics. Cambridge: Cambridge University Press.

Miettinen, K., Pollier, J., Buyst, D., Arendt, P., Csuk, R., Sommerwerk, S., et al. (2017). The ancient CYP716 family is a major contributor to the diversification of eudicot triterpenoid biosynthesis. *Nat. Commun.* 8, 1–13. doi: 10.1038/ncomms14153

Moses, T., Pollier, J., Almagro, L., Buyst, D., Van Montagu, M., Pedreño, M. A., et al. (2014). Combinatorial biosynthesis of sapogenins and saponins in Saccharomyces cerevisiae using a C-16a hydroxylase from *Bupleurum falcatum*. *Proc. Natl. Acad. Sci. U.S.A.* 111, 1634–1639. doi: 10.1073/pnas.1323369111

Ohnishi, T., Sztatmari, A.-M., Watanabe, B., Fujita, S., Bancos, S., Koncz, C., et al. (2006). C-23 hydroxylation by Arabidopsis CYP90C1 and CYP90D1 reveals a novel shortcut in brassinosteroid biosynthesis. *Plant cell* 18, 3275–3288. doi: 10.1105/tpc.106.045443

Pistelli, L., Bertoli, A., Bilia, A. R., and Morelli, I. (1996). Minor constituents from *Bupleurum fruticosum* roots. *Phytochemistry* 41, 1579–1582. doi: 10.1016/0031-1899(95)00749-0

Sui, C., Chen, M., Xu, J., Wei, J., Jin, Y., Xu, Y., et al. (2015). Comparison of root transcripts and expressions of genes involved in main medicinal secondary metabolites from *Bupleurum chinense* and *Bupleurum scorzonerifolium*, the two Chinese official Radix bupleuri source species. *Physiol. Plant.* 153, 230–242. doi: 10.1111/ppl.12254

Xiang, L., Jin, Y., Huang, W., Sun, Q., Liu, F., and Huang, X. (2018). Full-length transcriptome sequences of ephemeral plant *Arabidopsis pumila* provides insight into gene expression dynamics during continuous salt stress. *BMC Genom.* 19:717. doi: 10.1186/s12864-018-5106-y

Xu, J., Tehrim, S., Wang, L., Dossa, K., Zhang, X., Ke, T., et al. (2017). Evolutionary history and functional divergence of the cytochrome P450 gene superfamilies between *Arabidopsis thaliana* and Brassica species uncover effects of whole genome and tandem duplications. *Bmc Genom.* 18:733. doi: 10.1186/s12864-018-4094-7

Zhang, Y., Zhou, Z., Xia, P., Liang, Z., Liu, S., and Liu, Z. (2016). Expression of key enzyme genes and content of saikosaponin in saikosaponin biosynthesis under drought stress in *Bupleurum chinense*. *China J. Chinese Mater. Med.* 41, 643–647.

Zhang, Z., and Xiao, P. (2007). Encyclopedia on Contemporary Medicinal Plants. Shang Hai: World Publishing Corporation.

Zhao, Z., and Xiao, P. (2007). Encyclopedia on Contemporary Medicinal Plants. Shang Hai: World Publishing Corporation.

Zhu, Z., Liang, Z., and Han, R. (2009a). Growth and saikosaponin production of the medicinal herb *Bupleurum chinense* DC. under different levels of nitrogen and phosphorus. *Ind. Crops Products* 29, 96–101. doi: 10.1016/j.indcrop.2008.04.010

Zhu, Z., Liang, Z., and Han, R. (2009b). Saikosaponin accumulation and antioxidative protection in drought-stressed *Bupleurum chinense* DC. plants. *Environ. Exp. Botany* 66, 326–333. doi: 10.1016/j.envexpbot.2009.03.017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Yu, Chen, Liu, Li, Sui, Hou and Wei. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.