Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Shanfa Lu *Editor*

The *Salvia miltiorrhiza* Genome

Springer

Compendium of Plant Genomes

Series Editor

Chittaranjan Kole, Raja Ramanna Fellow, Government of India,
ICAR-National Research Center on Plant Biotechnology, Pusa,
New Delhi, India

Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

Interested in editing a volume on a crop or model plant?

Please contact Dr. Kole, Series Editor, at ckoleorg@gmail.com

More information about this series at http://www.springer.com/series/11805

Shanfa Lu
Editor

The *Salvia miltiorrhiza* Genome

Springer

Editor
Shanfa Lu
Institute of Medicinal Plant Development
Chinese Academy of Medical Sciences
& Peking Union Medical College
Beijing, China

ISSN 2199-4781 ISSN 2199-479X (electronic)
Compendium of Plant Genomes
ISBN 978-3-030-24715-7 ISBN 978-3-030-24716-4 (eBook)
https://doi.org/10.1007/978-3-030-24716-4

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

This book series is dedicated to my wife Phullara, and our children Sourav, and Devleena
Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F2 were utilized, and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still, they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, the emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, the sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful both to students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology, physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with a lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to Springer staff particularly, Dr. Christina Eckey and Dr. Jutta Lindenborn, for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav, and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

New Delhi, India Chittaranjan Kole

Preface

Salvia miltiorrhiza is a member of Lamiaceae, the sixth largest family of flowering plants. It is a deciduous perennial plant widely distributed in China. It also grows in other countries, such as Korea, Japan, Vietnam, and Australia. *S. miltiorrhiza* is a well-known traditional Chinese medicine (TCM) material and an emerging model medicinal plant. It was recorded in *Shen Nong's Classic of the Materia Medica*, the oldest materia medica book in China composed in about the second century BC. It was also recorded in many other ancient Chinese books. Nowadays, over 10% of the traditional Chinese patent medicines and simple preparations recorded in the Chinese Pharmacopoeia 2015 edition contain *S. miltiorrhiza*. A huge market has been developed for *S. miltiorrhiza* and its products. Annual consumption of *S. miltiorrhiza* materials has over 16 million kilograms in China.

Although the knowledge on medicinal plants is relatively poor compared with model plants and crops, significant progresses have been achieved in cultivation, bioactive compounds, pharmacological activities, and genes and genomes of *S. miltiorrhiza* after the studies of more than 1000 years, particularly the extensive studies of recent 30 years. With the decoding of *S. miltiorrhiza* genomes, studies on this plant are being accelerated. The information obtained from *S. miltiorrhiza* will be useful for understanding the genomes and bioactive components of other medicinal plants.

This is the first book on the genome of *S. miltiorrhiza*. It dedicates to recent research progresses on molecular maps, whole-genome sequencing, chloroplast and mitochondria genomes, epigenetics, transcriptomics, and functional genomics of *S. miltiorrhiza*. It also describes the taxonomy, distribution, morphology, and growth requirements of this significant medicinal plant and provides useful information on its resources, cultivation, and breeding. The current knowledge of biochemistry and biosynthesis of tanshinones and phenolic acids, two main classes of bioactive components produced in this plant species, is summarized. The technology of hairy root induction, tissue culture, and genetic transformation of *S. miltiorrhiza* is reviewed and discussed. We hope the book will be useful for students, teachers, and scientists in academia and industry interested in medicinal plants and pharmacy. We welcome any criticisms and comments.

We thank the support from the CAMS Innovation Fund for Medical Sciences (CIFMS) (2016-I2M-3-016), the National Natural Science Foundation of China, and other sources of funding. We highly appreciate Series Editor Prof. Chittaranjan Kole for inviting us to prepare this book. We are very grateful to the Springer team for their great assistance.

Beijing, China Shanfa Lu

Contents

1 *Salvia miltiorrhiza*: An Economically and Academically Important Medicinal Plant 1
Shanfa Lu

2 *Salvia miltiorrhiza* Resources, Cultivation, and Breeding 17
Chun Sui

3 Molecular Maps and Mapping of Genes and QTLs of *Salvia miltiorrhiza* 33
Xingfeng Li, Jianhua Wang and Zhenqiao Song

4 The Genome of *Salvia miltiorrhiza* 45
Zhichao Xu

5 The Chloroplast and Mitochondrial Genomes of *Salvia miltiorrhiza* 55
Haimei Chen and Chang Liu

6 *Salvia miltiorrhiza* Epigenetics 69
Xiaoxiao Qiu, Hong Zhou and Shanfa Lu

7 Transcriptome Analysis of *Salvia miltiorrhiza* 83
Hongmei Luo

8 Gene Expression Regulation in *Salvia miltiorrhiza* 97
Caili Li

9 Bioinformatic Tools for *Salvia miltiorrhiza* Functional Genomics 113
Liqiang Wang and Chang Liu

10 Biosynthetic Pathway of Tanshinones in *Salvia miltiorrhiza* 129
Juan Guo and Ying Ma

11 Biochemistry, Biosynthesis, and Medicinal Properties of Phenolic Acids in *Salvia miltiorrhiza* 141
Guoyin Kai, Shucan Liu, Min Shi, Bing Han, Xiaolong Hao and Zhixiang Liu

12 Tissue Culture and Hairy Root Induction of *Salvia miltiorrhiza* 163
Fenjuan Shao and Deyou Qiu

13 Genetic Transformation of *Salvia miltiorrhiza* 173
Meizhen Wang and Shanfa Lu

Contributors

Haimei Chen Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China

Juan Guo State Key Laboratory Breeding Base of Dao-Di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

Bing Han Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Xiaolong Hao Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Guoyin Kai Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Xingfeng Li State Key Laboratory of Crop Biology, Agronomy College, Shandong Agricultural University, Taian, China

Caili Li Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Shucan Liu Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Zhixiang Liu Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Chang Liu Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China

Shanfa Lu Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Haidian District, Beijing, China

Hongmei Luo Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Ying Ma State Key Laboratory Breeding Base of Dao-Di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

Deyou Qiu State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China

Xiaoxiao Qiu Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Fenjuan Shao State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China

Min Shi Laboratory of Medicinal Plant Biotechnology, College of Pharmacy, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China

Zhenqiao Song State Key Laboratory of Crop Biology, Agronomy College, Shandong Agricultural University, Taian, China

Chun Sui Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education and National Engineering Laboratory for Breeding of Endangered Medicinal Materials, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Meizhen Wang Institute of Medicinal Plant Development Chinese Academy of Medical Sciences and Peking Union Medical College, Haidian District, Beijing, China

Jianhua Wang State Key Laboratory of Crop Biology, Agronomy College, Shandong Agricultural University, Taian, China

Liqiang Wang Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China

Zhichao Xu Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China

Hong Zhou Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Salvia miltiorrhiza: An Economically and Academically Important Medicinal Plant

1

Shanfa Lu

Abstract

Salvia miltiorrhiza Bunge is a perennial plant in the genus *Salvia* of the family Lamiaceae. It has a long medicinal history with the first recorded in about the second century BC. *S. miltiorrhiza* has great economic and medicinal value. Over 10% of the traditional Chinese patent medicines and simple preparations contain *S. miltiorrhiza*. Annual consumption of *S. miltiorrhiza* has exceeded 16 million kilograms in China. So far, more than two hundred chemical compounds have been isolated from this plant. *S. miltiorrhiza* and its ingredients have been clinically used for managing vascular diseases and academically shown potential in treating various other diseases. Moreover, *S. miltiorrhiza* has great academic value and is emerging as a model system in medicinal plant biology. This plant has a relatively short life cycle and a relatively small genome size. It is easy to propagate and cultivate. There are whole genome and transcriptome sequences, in vitro tissue culture systems, hairy root and crown gall induction systems and genetic transformation systems available for the research community of *S. miltiorrhiza*. This chapter provides an overview of the taxonomy, distribution, morphology, propagation, growth requirements, chemical constituents, and medicinal and academic significance of *S. miltiorrhiza*.

S. Lu (✉)
Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 151 Malianwa North Road, Haidian District, Beijing 100193, China
e-mail: sflu@implad.ac.cn

1.1 Introduction

Salvia miltiorrhiza Bunge, also known as Danshen, Zi Danshen, Tan Shen, red sage and Chinese sage, is a perennial plant in the genus *Salvia* with great economic and medicinal value and a long medicinal history. *S. miltiorrhiza* was first recorded in the oldest materia medica book in China, *Shen Nong's Classic of the Materia Medica* (Shen Nong Ben Cao Jing), composed in about the second century BC. In this book, it was classified as a top-tier medicine. Afterward, it was recorded in many other ancient Chinese books, such as *Collection of Commentaries on the Classic of the Materia Medica* (Ben Cao Jing Ji Zhu) written over 1500 years ago, *Illustrated Classic of the Materia Medica* (Tu Jing Ben Cao) published in Song Dynasty, *Essentials of Materia Medica Distinctions* (Ben Cao Pin Hui Jing Yao) of Ming Dynasty, and *Compendium of Materia Medica* (Ben Cao Gang Mu) written by Shizhen Li in Ming Dynasty. Nowadays, *S. miltiorrhiza* has become one of the most popular and extensively used traditional Chinese

S. Lu (ed.), *The Salvia miltiorrhiza Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-24716-4_1

medicine (TCM) materials. It is used alone or often mixed with other herbs for treating dysmenorrhoea, amenorrhoea, and cardiovascular diseases in China and other countries, such as Japan, the USA, and European countries (Zhou et al. 2005; Cheng 2007; Xu et al. 2007).

1.2 Taxonomy

S. miltiorrhiza is a member of Lamiaceae, the sixth largest family of flowering plants. Lamiaceae contains more than 7000 species across about 236 genera. In an earlier study, 226 of the 236 genera was classified into seven subfamilies, including Viticoideae, Symphorematoideae, Ajugoideae, Prostantheroideae, Nepetoideae, Scutellarioideae, and Lamioideae, whereas the other ten could not be placed in a subfamily (Harley et al. 2004). Recently, using large-scale chloroplast sequences, Li et al. (2016a) reconstructed the phylogenetic relationship of Lamiaceae species and described three new subfamilies, including Cymarioideae, Peronematoideae, and Premnoideae. Based on this analysis, the Lamiaceae family was divided into ten subfamilies and two unassigned genera (Li et al. 2016a). Among these subfamilies, Nepetoideae, which is characterized by hexacolpate, three-nucleate pollen, an investing embryo and the presence of rosmarinic acid, is one of the most clearly defined subfamilies (Harley et al. 2004; Bräuchler et al. 2010). This subfamily contains about 3400 species across 118 genera and is recovered as monophyletic (Harley et al. 2004; Bräuchler et al. 2010; Li et al. 2016a). Nepetoideae is divided into three tribes, including Elsholtzieae, Ocimeae, and Mentheae, of which tribe Mentheae is the largest and includes many well-known genera, such as *Rosmarinus* (rosemary), *Salvia* (sage), *Mentha* (peppermint), and *Nepeta* (catnip).

Salvia is the largest genus of the family Lamiaceae. It is characterized by the well-known stamina lever mechanism of the flower. Two monothecic stamens are modified to levers with a thin ligament between the connective and the filament to form a joint, which enables a lever-like reversible movement causing pollen transfer (Wester and Claßen-Bockhoff 2007). Filaments are short, horizontal, or erect. Connectives are prolonged, linear, and T-shaped. Upper arms have fertile elliptic or linear anther cells. Lower arms are robust or slender, with fertile or sterile anther cells, separated or connected to each other. Two staminodes are small or absent (Flora of China Editorial Committee 1994).

Salvia includes approximately 1000 species and is well-known for its medicinal, ornamental, esculent, or hallucinogenic plants (Will and Claßen-Bockhoff 2017). It has radiated extensively in three regions of the world: Central and South America (500 spp.), western Asia (200 spp.), and eastern Asia (100 spp.). The most recent common ancestor (MRCA) of *Salvia* existed in the later Eocene (34 million years ago) to the middle Oligocene (26 million years ago) (Will and Claßen-Bockhoff 2017). *Salvia* is polyphyletic. Walker et al. (2004) and Walker and Sytsma (2007) revealed three distinct evolutionary lineages (Clade I–III). Later, Will and Claßen-Bockhoff (2014) recognized the Clade III described by Walker et al. (2004) and Walker and Sytsma (2007) as two independent lineages (Clades III and IV). The four clades described strongly reflect the geographical distribution of *Salvia* species, with Clade I mainly in Europe, Southwest and Central Asia, and Southern Africa, Clade II mainly in Central and South America, Clade III mainly in Southwest Asia and Northern Africa, and Clade IV mainly in East Asia (Will and Claßen-Bockhoff 2017).

S. miltiorrhiza is a Clade IV species native to China (Will and Claßen-Bockhoff 2017). China is the center of diversity in East Asia, with 84 *Salvia* species (Flora of China Editorial Committee 1994). Among them, 70 species are endemic to China, whereas the other 14 are cosmopolitan species (Wei et al. 2015). The majority of *Salvia* species in China, such as *S. miltiorrhiza*, *Salvia yunnanensis*, *Salvia plebeia*, *Salvia bowleyana,* and *Salvia trijuga*, are members of Clade IV (Will and Claßen-Bockhoff 2017). Few of the others, such as *Salvia deserta* and *Salvia officinalis*, belong to Clade I.

In addition, there are several species introduced from America and Europe, such as *Salvia coccinea*, *Salvia splendens*, and *Salvia sclarea*.

1.3 Distribution

S. miltiorrhiza is widely distributed in China. Wild and domestic *S. miltiorrhiza* can be found in at least nineteen provinces and cities of China. It includes Liaoning, Hebei, Beijing, Tianjin, Jiangsu, Shandong, Shanghai, Anhui, Zhejiang, Jiangxi, Hunan, Hubei, Henan, Shanxi, Shaanxi, Gansu, Sichuan, Guizhou, and Yunnan, covering a total of 192 counties of China (Wei et al. 2015). *S. miltiorrhiza* also grows in Mongolia, Korea, Japan, Vietnam, and Australia (Xu et al. 2007; Sheng et al. 2009; Li et al. 2011; Tung et al. 2017). It grows at about 100–1300 m elevation and prefers sunny and wet places in forests, hillsides, and streamsides (Flora of China Editorial Committee 1994).

1.4 Morphology

S. miltiorrhiza is a deciduous perennial plant with quadrangular, erect, and branched stems (Fig. 1.1). The stems are about 40–80 cm tall and covered with hairs and sticky glands. The cotyledons and the true leaf are simple leaf. Compound leaves developed after the true leaf are odd-pinnate compound with 3–5(–7) leaflets. Each of the leaflets has 1.5–8 × 1–4 cm in size. Leaves are ovate or broadly lanceolate and pilose. Leaf margin is crenate. Leaf apex is acute to acuminate. Petiole is 1.3–7.5 cm in length. Petiolule is 2–14 mm in length. *S. miltiorrhiza* has a verticillaster inflorescence with densely villous or glandular villous (Flora of China Editorial Committee 1994).

The flowers of *S. miltiorrhiza* grow in whorls. Each whorl has 6–many flowers with light purple to lavender blue or white corollas. The corolla is about 2.5 cm in length, glandular pubescent, and two-lipped (Fig. 1.2). The upper lip of corolla is vertical and falcate. The lower lip is shorter, and its terminal is lobulated and trifid. The central lobe is longer and larger than the two side lobes. Corolla tube is exserted with imperfectly pilose annulate inside. The tube is shorter than limb. The calyxes are dark purple and slightly bell-shaped and divided into two parts or lips. The upper lip of calyxes is entire and triangular with three-mucronate apex. The lower lip is almost as long as upper and is two-toothed (Flora of China Editorial Committee 1994).

As the other species of the genus *Salvia*, *S. miltiorrhiza* is also characterized by the well-known stamina lever mechanism of the flower (Fig. 1.3) (Wester and Claßen-Bockhoff 2007). The filaments of *S. miltiorrhiza* are 3.5–4 mm, and the connectives are 1.7–2 cm. Both filaments and connectives are much exserted (Flora of China Editorial Committee 1994).

S. miltiorrhiza plants bloom from April to August and fruit from September to October. The nutlets of *S. miltiorrhiza* are ellipsoid with about 3.2 × 1.5 mm in size (Flora of China Editorial Committee 1994). *S. miltiorrhiza* roots are the medicinal parts of the plant and are well-known TCM materials. It consists of taproot and lateral roots. The taproot is thickened, succulent, and usually scarlet outside (Flora of China Editorial Committee 1994).

1.5 Propagation

S. miltiorrhiza can be propagated from seeds, cuttings, and root segments. It can also be propagated through tissue culture.

S. miltiorrhiza seeds are small, dark brown nutlets. It cannot be stored for a long time at room temperature. Seed germination rate will decrease significantly after three-month storage at room temperature (Li et al. 2016b). Under normal circumstances, the optimal storage temperature is 4 °C. For long-term storage, it requires −20 °C (Li et al. 2016b). Except for temperature, water content is the other key factor affecting the vitality of stored seeds. The optimal water content is about 5% for storage of *S. miltiorrhiza* seeds (Li et al. 2016b). In practice, the seeds are usually sown from late March to early April in the spring or late September in the

Fig. 1.1 *S. miltiorrhiza* plants and its Chinese name

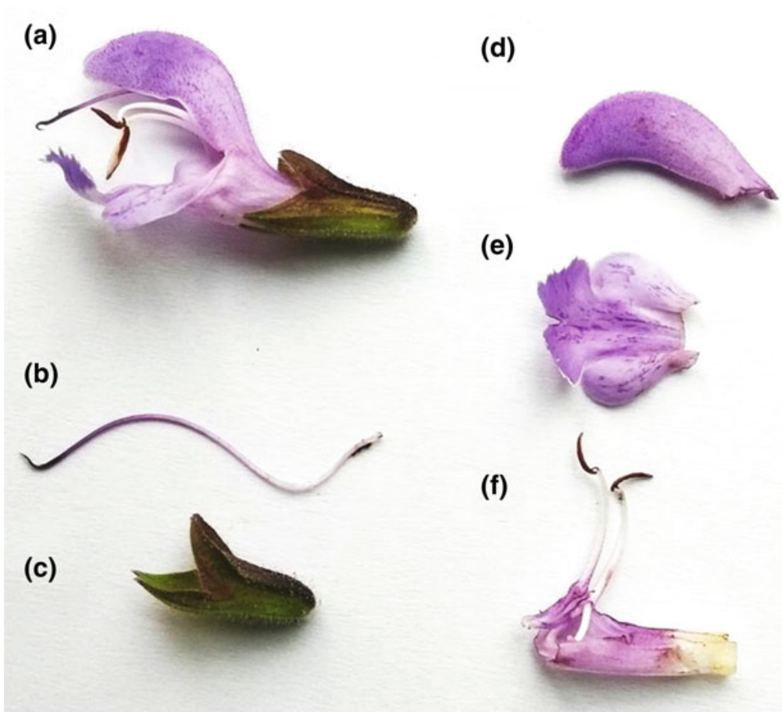

Fig. 1.2 Flowers of *S. miltiorrhiza*. The entire flower (**a**), style (**b**), calyx (**c**), upper lip of corolla (**d**), lower lip of corolla (**e**), corolla tube, and lever-like stamens (**f**) are shown

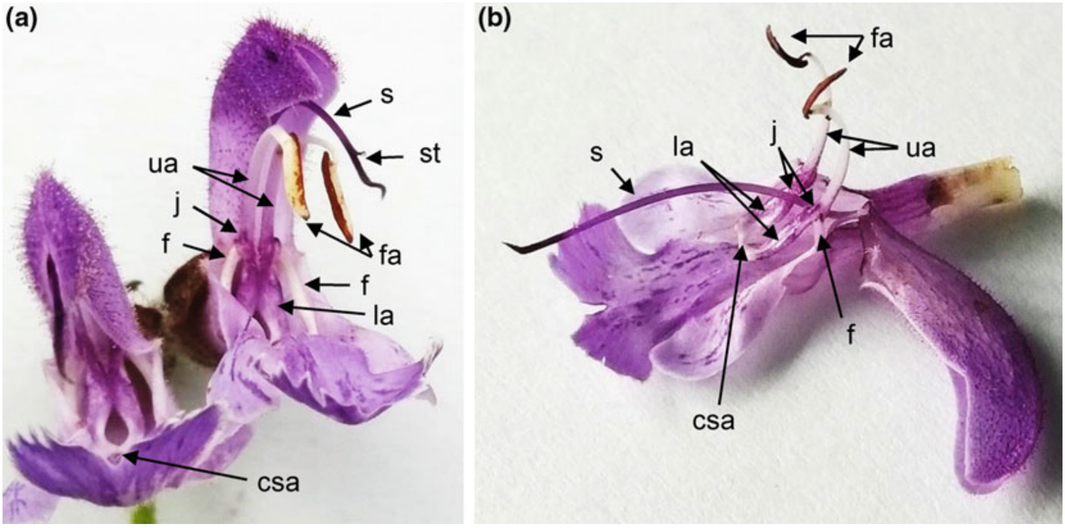

Fig. 1.3 Lever-like stamens of *S. miltiorrhiza*. **a** The entire flowers; **b** A flower with the upper lip of corolla removed. f, filament; fa, fertile anther; j, joint between filament and connective; la, lower lever arm of stamen; s, style; csa, connected sterile anthers; st, stigma; ua, upper lever arm of stamen

autumn and can be sown into the soil immediately after harvest (He et al. 2014).

To propagate *S. miltiorrhiza* from cuttings, select healthy and strong stems of vigorous plants during growing season, cut them into about 10 cm segments, excise the entire leaves on the lower part and half of the leaves on the upper part, and slantingly insert the cuttings about one-third to half of their length into moist and free-draining soil (Xu 2002; Zhang 2018; Wang 2018). Arrange the cuttings with space between each one to avoid overcrowded. Provide some shade and water regularly to keep the soil consistently moist. Avoid letting the soil dry out or waterlogging the cuttings. Once the roots form, transfer them to the field.

Propagation from root segments is one of the most popular propagation methods for *S. miltiorrhiza*. To propagate using this method, select healthy and strong roots with about 1.0 cm in diameter, cut them into about 5 cm segments, put the segments into pre-prepared soil holes, keep the segments slantingly with the upper part upward, and then cover the segments with about 2 cm of soil (Xu 2002; Zhang 2018; Wang 2018). Root segment propagation can be done in February or March. The roots used for propagation can be harvested in November and stored in damp sand until use in February or March of the next year (Zhang 2018). Alternatively, it can be kept in the soil until the next February or March (Xu 2002; Zhang 2018; Wang 2018). Middle and upper parts of the roots and one-year-old roots will be greater for shoot emergence (Xu 2002). In addition, rootstalks can also be used for propagation (Zhang 2018).

Tissue culture is the other useful method for *S. miltiorrhiza* propagation (Jia et al. 2016). It includes suspension cell culture, hairy root culture, crown gall tissue culture, in vitro shoot culture, and callus culture. Tissue culture technology can be widely used for rapid propagation and elite breeding of *S. miltiorrhiza*, industrial production of active compounds, and academic purposes, such as functional characterization of genes and mutant library construction (Zhang et al. 1995; Lee et al. 2008; Cui et al. 2011; Li et al. 2017a).

1.6 Growth Requirements

S. miltiorrhiza seeds can germinate at about 10–30 °C. The optimal germination temperature is around 20–25 °C (He et al. 2014; Li et al. 2016b). The seeds usually take about two weeks to germinate. *S. miltiorrhiza* begins to grow when soil temperature reaches to 10 °C. Adventitious buds start to develop on root division when soil temperature reaches to 15–17 °C in spring (Xu 2002). *S. miltiorrhiza* plants prefer sunny and wet soil and relatively tolerate cold, but they are susceptible to drought and waterlog. The optimal air temperature and relative humidity for *S. miltiorrhiza* plant growth are 20–26 °C and 80%, respectively. The plants grow vigorously during growing season and take about 200 days from transplanting to harvesting. The aerial parts of *S. miltiorrhiza* plants start to wither when the air temperature falls below 10 °C in autumn. The underground parts can overwinter safely even if the temperature drops to 15 °C below zero (Xu 2002).

1.7 Chemical Constituents

S. miltiorrhiza mainly contains lipophilic diterpenoid tanshinones and hydrophilic phenolic acids, which are two major classes of bioactive compounds. According to the Chinese Pharmacopoeia 2015 edition (Pharmacopoeia Commission of People's Republic of China 2015), total content of tanshinone IIA, cryptotanshinone, and tanshinone I should not be less than 0.25% and salvianolic acid B should not be less than 3% in dry *S. miltiorrhiza* medicinal materials. In addition to tanshinones and phenolic acids, *S. miltiorrhiza* also contains monoterpenes, sesquiterpenes, triterpenes, alkaloids, flavonoids, anthocyanidins, sterols, saccharides, quinones, and so on. So far, more than two hundred chemical compounds have been isolated from this plant (Wang et al. 2017a; Mei et al. 2019).

Diterpenoid tanshinones are the representatives of lipophilic components in *S. miltiorrhiza*. They can be divided into three types, including diterpenoid tanshinones, tricyclic diterpenoid tanshinones, and royleanone tanshinones (Luo2015; Wang et al. 2017a). The basic structure of diterpenoid tanshinones contains a 1,2-*o*-naphthoquinone mother nucleus with a furan or dihydrofuran ring. Many bioactive tanshinones, such as tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, and dihydrotanshinone (Zhang and Lu 2017), belong to this type. Tricyclic diterpenoid tanshinones are characterized by ligation of an isopropyl group, instead of a furan or dihydrofuran ring, to the naphthoquinone mother nucleus. Miltirone, miltirone I, dehydromiltirone and methylene miltirone are typical tricyclic diterpenoid tanshinones. Royleanone tanshinones are characterized by 1,4-*p*-quinone. Compounds included in this type are isotanshinone I, isotanshinone IIA, isotanshinone IIB, isocryptotanshinone, 2-hydroxyisodihydrotanshinone, danshenxinkun A, danshenxinkun B, and danshenxinkun C. Besides these diterpenoid tanshinones, there are other diterpenes in *S. miltiorrhiza*, such as ferruginol, sugiol, neotanshinlactone, danshenol A and danshenol B (Mei et al. 2019). Recently, new diterpenoid compounds, including salmiltiorins A–F, normiltirone, and isosalviamides F–H, have been identified from *S. miltiorrhiza* (Wei et al. 2017; Ngo et al. 2019). In total, about 90 diterpenoid compounds have been isolated from *S. miltiorrhiza* (Wei et al. 2017; Mei et al. 2019; Ngo et al. 2019).

Water-soluble phenolic acids are the other major class of bioactive compounds in *S. miltiorrhiza*. It includes more than forty compounds, which can be classified into single phenolic acids and polyphenolic acids (Mei et al. 2019). Single phenolic acids include danshensu, caffeic acid, ferulic acid, isoferulic acid, protocatechuic aldehyde, protocatechuic acid, and *β*-(3,4-dihydroxyphenyl) lactic acid. Many of them contain a core skeleton of C6–C3. Polyphenolic acids includes salvianolic acids A–G, salvianolic acids I–L, salvianolic acid N, salvianolic acid C1, lithospermic acid B, 8″-*epi*-lithospermic acid C, and rosmarinic acid, most of which are depsides of danshensu and derivatives or dimer of caffeic acid (Zhang et al. 2017; Mei et al. 2019). Rosmarinic acid is a simple depside formed by the