

# Growth and Accumulation of Five Main Bioactive Components in the Roots of *Salvia miltiorrhiza* at Different Growth Stages and Using Different Culture Systems

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**Abstract:** To Growth and accumulation of five main bioactive components in the roots of *Salvia miltiorrhiza* at different growth stages and using different culture systems. We analyzed growth parameters and the accumulation of selected bioactive components in *Salvia miltiorrhiza* that was grown in quartz sand-pot (hydroponic culture), soil-pot, and field culture systems at 3 growth stages (flower, root swelling, and mature). The highest bioactive compound concentrations (danshensu (DSS), 0.618 mg·g<sup>-1</sup>; salvianolic acid B (SAB), 52.5 mg·g<sup>-1</sup>; cryptotanshinone (CTS), 0.617 mg·g<sup>-1</sup>; tanshinone II A (TS II A), 1.11 mg·g<sup>-1</sup>; and total tanshinone (TTS), 2.5 mg·g<sup>-1</sup>, at the mature stage) were present in the roots of plants grown in the hydroponic culture system. These concentrations were significantly higher than those of plants grown in the field system. The highest values for root parameters ( longest root length (LRL), 46.72 cm; largest root diameter (LRD), 14.68 mm; and the number of roots per plant (RN), 9.56), plant biomass (shoot dry weight (SDW), 18.9 g·plant<sup>-1</sup>; root dry weight (RDW), 19.6 g·plant<sup>-1</sup>, at the mature stage), and yield (DSS, 8.36 mg·plant<sup>-1</sup>; SAB, 657 mg·plant<sup>-1</sup>; CTS, 7.95 mg·plant<sup>-1</sup>; TS II A, 15.2 mg·plant<sup>-1</sup>; and TTS, 30.7 mg·plant<sup>-1</sup>, at the mature stage) were obtained from plants grown in the field system. Plants grown in the field culture system had significantly greater plant biomass and higher yields of bioactive compounds than plants grown in the quartz sand-pot (hydroponic culture) and soil-pot systems. Greenhouse hydroponic culture provides sufficient bioactive compound accumulation in the roots, but does not stimulate plant growth and root production. Therefore, the field system could greatly improve plant growth and root production in *S. miltiorrhiza*.

**Keywords:** *Salvia miltiorrhiza*, Culture System, Growth Stages, Bioactive Components, Growth

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## 1. Introduction

Radix *Salvia miltiorrhiza* (Fam. Labiatae), Danshen, is an important medicinal plant that is widely used in traditional Chinese medicine for the treatment of chronic renal failure, bone loss, hepatitis [1-2], menstrual disorders, blood circulation diseases, coronary heart disease, and other cardiovascular diseases [3-6]. In recent years, Danshen has been introduced into Canada, Germany, America, the

Netherlands, South Africa, Russia, South Korea, and other countries and regions as a medicine for cardiovascular disease and to improve immunity [7].

The market demand for Danshen was initially satisfied by the collection of wild plants, but the increasing interest in herbal products has promoted the cultivation of *S. miltiorrhiza* [8]. Furthermore, there is considerable international interest in medicinal and aromatic plant culture [9-11]. This interest is largely due to the growing international interest in traditional

herbal treatments, which has increased demand for natural raw medicinal materials by the pharmaceutical industry [12]. To meet this demand, controlled-environment production systems for medicinal plants have been developed in addition to traditional cultivation in outside fields.

Medicinal plant production is different from the production of other agricultural products in that medicinal plants are grown to produce specific phytochemicals for human use. Different medicinal plants are suited to different culture systems. The traditional outdoor field culture system is often considered to be the optimal culture method for most crops and medicinal plants because it provides the highest root yields and the best marketing attributes [13-14]. In recent decades, some medicinal plants have been produced using soil-less culture systems. Hydroponic production of medicinal and aromatic herbs is a new method for the mass production of these plants that results in high yields of high-quality secondary metabolites [15-19]. Numerous studies have shown that soil-less culture production of plants, particularly medicinal herbs, has many valuable advantages, including the following: high yields; faster crop growth; year-long cultivation when appropriate planting schedules and multiple harvest schemes are used; and high quality raw material that is clean and easy to process due to minimal contamination from herbicide and pesticide residues and microorganisms [9, 20]. At present, different techniques can be used for hydroponic cultivation, including water culture, nutrient film techniques, aeroponics, and container culture in artificial substrates (i.e. quartz sand, vermiculite, or river sand matrix) [21-22]. Among these techniques, container culture in quartz sand requires a relatively low initial investment and has low running costs. This technique is generally used for short-cycle greenhouse cultivation of root vegetables that are marketed as fresh-cut products.

Previous studies suggest that controlled-environment soil-less, soil, and outside field culture systems could be profitably applied to the cultivation of medicinal plants [23]. all 3 systems can be used for plants with roots that are used medicinally and that the best system to use differs between plant species. To date, however, there have been studies that have directly compared these cultivation systems for the production of *S. miltiorrhiza* and its bioactive compounds.

In this study, *S. miltiorrhiza* seedlings were grown in quartz sand-pot, soil-pot, and outside field systems with an adequate supply of nutrients. The growth of plants and the accumulation of bioactive components at different growth stages in the 3 culture systems were evaluated in separate experiments that were conducted in 2015 and 2016.

Danshensu (DSS), salvianolic acid B (SAB), cryptotanshinone (CTS), tanshinone II A (TS II A), and total tanshinone (TTS) are the main bioactive components in extracts of *S. miltiorrhizae* roots [24-26]. We therefore used these as standards for the HPLC analysis of our plant samples.

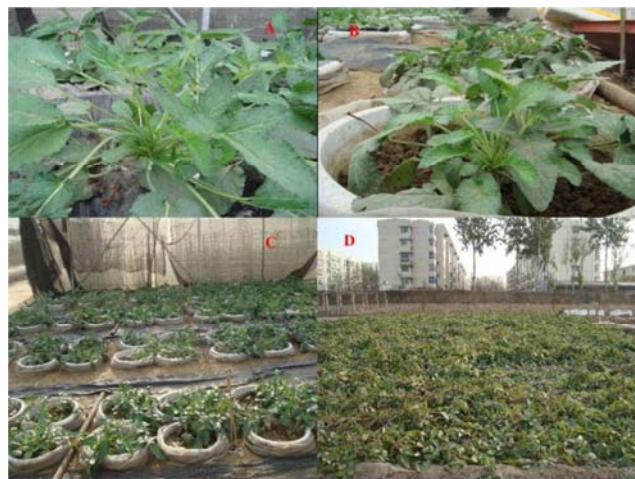
## 2. Materials and Methods

### 2.1. Field Experiment

The field experiment was carried out in a field at the

Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences (CCAMS) and Peking Union Medical College. The soil type is a calcareous alluvial fluvo-aquic soil with a loamy and silty texture (FAO) that is typical of the region. The soil was analyzed before planting. The chemical properties of the 0–40 cm soil layer were as follows: extracted mineral, N 70 kg ha<sup>-1</sup>; pH (H<sub>2</sub>O), 7.6–8.0; soil density, 1.40 g cm<sup>-1</sup>; available P, 18.5 mg kg<sup>-1</sup>; NH<sub>4</sub>Ac-extracted K, 157.5 mg kg<sup>-1</sup>; and organic matter, 20 g kg<sup>-1</sup>. The field was irrigated with a sprinkler a few days before planting and was ploughed just before planting. The water capacity of the soil was maintained at 60% (w/w) after planting. Danshen (*S. miltiorrhiza*) germplasm lines were planted on 28<sup>th</sup> April 2015 using conventional commercial cultivation methods. Danshen was thinned at the seedling stage to a density of 55000 plants·ha<sup>-1</sup>. Urea was added as a fertilizer at a rate of 150 kg ha<sup>-1</sup> N. We applied 30% of the urea fertilizer before planting and the rest as a topdressing after the seedlings reached 15 cm in height. Additionally, 52 kg ha<sup>-1</sup> P (superphosphate) and 55 kg ha<sup>-1</sup> K (potassium chloride) were banded. The experiment had 4 replicates, each with 230 plants. The distance between rows and plants in rows was 0.55 m and 0.33 m, respectively. Border plots were included, and weed growth on plots was controlled using hand planters.

*S. miltiorrhizae* was harvested on 25<sup>th</sup> September, 22<sup>nd</sup> October, and 22<sup>nd</sup> November, 2015. Twenty plants were harvested randomly at each growth stage. We used the same planting time, cultivation methods, and harvest dates in 2016. Precipitation and air temperature during the growing season in 2015 and 2016 are shown in Figure 1, and the water capacity of field soil was maintained about 80% (w/w) with little irrigation.



**Figure 1.** *Radix Salvia miltiorrhiza* (Fam. Labiatae) in quartz sand-pot, soil-pot and outside field cultures (photo credits: X. L. Zhang). A = quartz-sand-pot culture (hydroponics culture), B = soil-pot culture, C = quartz sand-pot and soil-pot cultures in greenhouse, D = outside field culture.

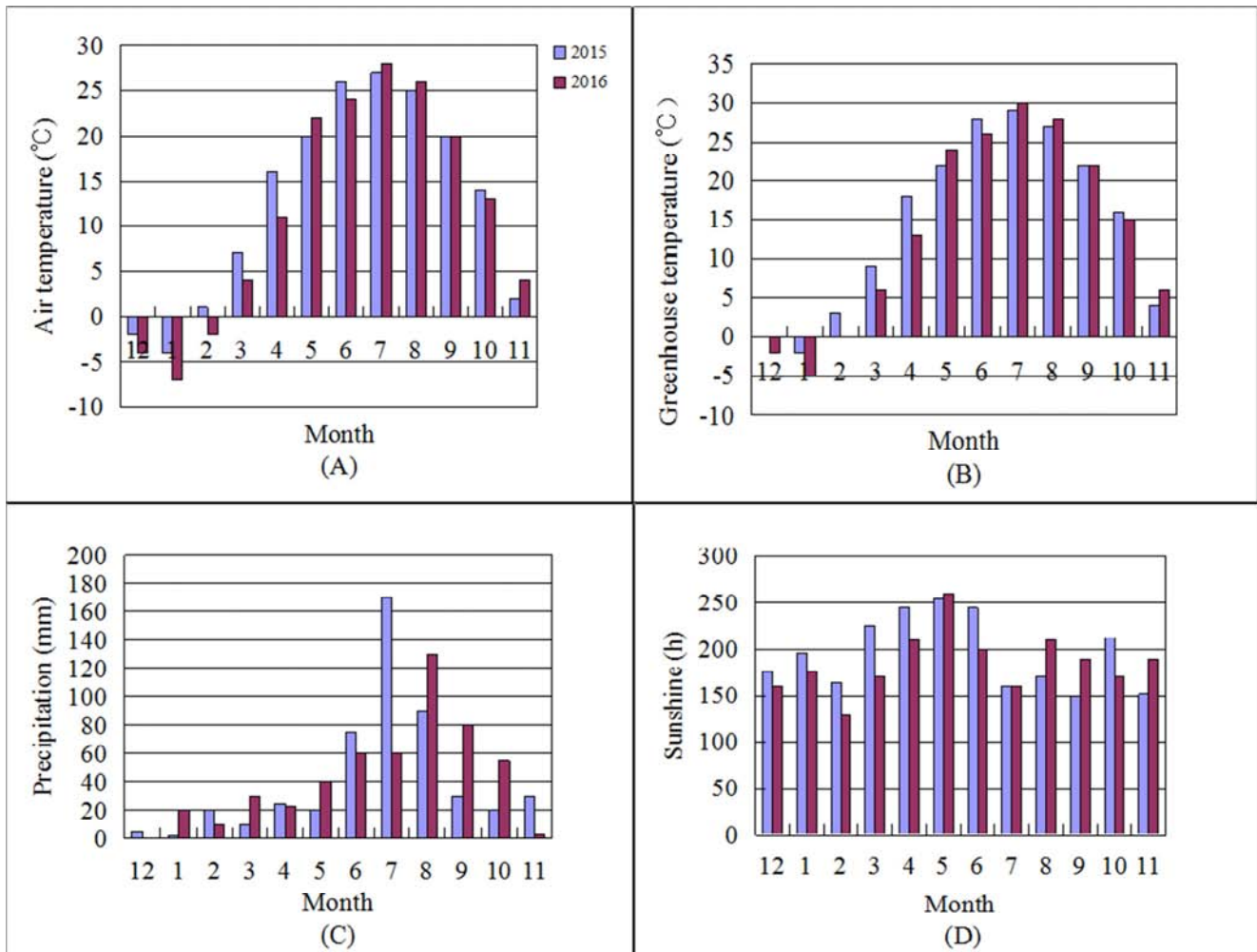
### 2.2. Greenhouse Experiments

*S. miltiorrhizae* germplasm lines were planted in a field at IMPLAD, CAMS, Beijing, China using conventional cultivation methods on 28<sup>th</sup> April 2015 and 28<sup>th</sup> April 2016. *S.*

*multiorrhizae* seedlings at the 4-leaf stage were transplanted into each pot used in greenhouse experiments.

*S. multiorrhizae* was harvested at different growth stages, on 30<sup>th</sup> September, 30<sup>th</sup> October, and 30<sup>th</sup> November, 2015, and

on the same dates in 2016. Ten pots were harvested at each growth stage in the quartz-sand-pot and soil-pot experiments. The greenhouse temperatures are shown in Figure. 2.



**Figure 2.** Temperature in the greenhouse (A), outside field (B), precipitation (C), and incident sunlight (D) in Beijing, China in 2015 and 2016.

### 2.2.1. Quartz Sand-Pot Experiment

*S. multiorrhizae* seedlings were transplanted into 30 plastic pots (25 cm diameter and 40 cm height), each pot had 30 kg of quartz sand (0.25–0.5mm diameter), and a total of 900 kg quartz sand was used in this experiment. Plants were watered with nutrient solution every other day for 10 days during the seedling stage, as described by Hoagland and Arnon (1950) [27]. The pH of the nutrient solution was adjusted to 6.0. nutrients were in excess of plant requirements containing 3 g N, 0.8 g P, 2.5 g K per plant, the total amounts of each nutrient were supplied to the plants during the entire growth period. Small holes in the bottom of the pots allowed drainage. Evaporation from the pots was minimized by covering the exposed surface of the quartz sand with a layer of black plastic film. White filter paper was placed over the plastic film in order to avoid temperature increases due to incident sunlight. The plants were grown in a greenhouse at IMPLAD, CAMS, Beijing. The water capacity of sand in the pots was maintained

at 60–80% (w/w) during the experiment.

### 2.2.2. Soil-Pot Experiment

We filled 30 plastic pots (25 cm diameter and 40 cm height) with a mixture of soils (30 kg soil in each pot, for a total of 900 kg soil in the experiment). The soils included basic fertilizers (the fertilizers which were applied and mixed with soils before transplanting the seedlings) and low-nutrient river sand-soil. In the river-washing sand-soil, the soil to sand ratio was 3:1 (v/v). The sand-soil was passed through a 0.5-cm-mesh sieve prior to placement in the pots. The chemical properties of the original sand-soil were as follows: organic matter content = 10.16 g·kg<sup>-1</sup>, available N = 26.35 mg·kg<sup>-1</sup>, available P = 12.85 mg·kg<sup>-1</sup>, and available K = 65.20 mg·kg<sup>-1</sup>. Pots were placed in a flat membrane greenhouse (Figure. 1). Plants were kept under a rain shelter with sufficient sunlight. The soil in the pots was maintained at a water capacity of 80% (w/w) during the experiment.



## 2.3. Plant Measurements and Active Component Analysis

### 2.3.1. Plant Measurements

Representative examples of experimental sites and *S. miltiorrhiza* plants harvested from field, quartz sand-pot and soil-pot culture experiments are shown in Figure. 1. Shoots (leaves and stems) and roots were separated and the longest root length (LRL), largest root diameter (LRD), number of roots (RN), shoot dry weight (SDW), root dry weight (RDW), and total plant dry weight (PDW) were recorded. Shoots and roots were dried at 60°C for 72 h prior to weighing. Shoots and roots were then ground until they were able to pass through a 0.3-mm sieve.

### 2.3.2. Chemicals and Standards

HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (NJ, USA). Deionised water was purified using the Milli-Q system (Millipore, Bedford, MA, USA). Analytical grade phosphoric acid and ethanol were purchased from Beijing BeiHua Fine Chemicals Co. Ltd. (Beijing, China). Authentic standards of danshensu, salvianolic acid B, tanshinone IIA and cryptotanshinone were purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China).

### 2.3.3. HPLC Analysis

CTS, TSIIA, DSS, and SAB were measured using HPLC. The analysis method referenced Lu *et al.* (2015) [28].

### 2.3.4. UV Spectrophotometry

TTS was analyzed using UV spectrophotometry. Individual samples (0.500 g) were accurately weighed and soaked in 5 ml ethanol at 4°C overnight. Samples were then extracted in an ultrasonic bath at room temperature for 20 min. After centrifugation at  $2856 \times g$  for 20 min, 0.5 ml of the resulting supernatant was diluted with 3.5 ml ethanol and analyzed using an ultraviolet spectrophotometer (UV1100-vis;

Shanghai Tianmei Instrument Co., Ltd., Shanghai, China) at a detection wave length of 270 nm.

### 2.3.5. Inductive Coupled Plasma (ICP) Analysis

Concentrations of P and K in plant parts were determined using inductively couple plasma (ICP) emission spectroscopy (ICPE-9000; Japan, Tsujima). N was determined using an automatic Kjeltex Distilling system (Kjeltex 8400; Germany). Approximately 0.2 g dried and ground samples were ashed in a muffle furnace at 600°C for 4 h. The ash was digested by adding 1.0 mL 6 M HCl for 1 h and in 40 mL of a double-acid solution containing 0.0125 M H<sub>2</sub>SO<sub>4</sub> and 0.05 M HCl for an additional 1 h. the digested solution was then filtered using 2V Whatman filter paper (Shanghai, China) and analysed for N, P and K.

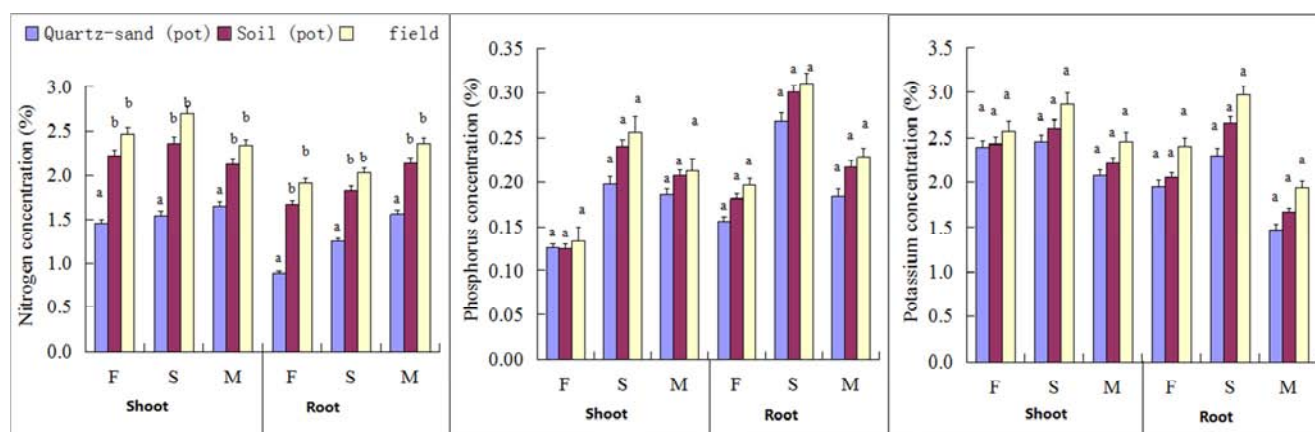
## 2.4. Statistical Analysis

Treatment effects were determined by one-way analysis of variance (ANOVA). Differences between treatments were confirmed using least significant difference tests (LSDTs). SPSS 13.0 software was used for all statistical analyses (SPSS Inc, USA).

## 3. Results

### 3.1. N, P, and K element Concentration in Plant Tissue

Although *S. miltiorrhiza* was supplied with the same amount of nutrients in all 3 culture systems, the N, P, and K concentrations in plants (root and shoot) grown in quartz sand-pots (hydroponic culture system) were all lower than the concentrations in plants grown in soil-pot and field systems. Plants grown using the hydroponic culture system had significantly less N than plants grown using soil-pot and field systems ( $p < 0.05$ ; Figure. 3).



**Figure 3.** Nitrogen, phosphorus and potassium concentrations in shoots and roots of *S. miltiorrhiza* at 3 growth stages, flowering (F), root swelling (S), and mature (M), grown in quartz sand-pot (blue bars), soil-pot (red bars), and outside-field cultures (yellow bars). Values are means ( $\pm$ SE;  $n = 20$ ). For each metabolite, different letters within cultures indicate significant differences, as determined by the least significant difference test (LSDT).

### 3.2. The Effect of Culture System on Root Growth and Bioactive Components of Roots at Different Growth Stages

#### 3.2.1. Plant Growth

The LRL, LRD, SDW, RDW, and PDW of plants grown in an outside field system were higher than those of plants grown using hydroponic culture and soil-pot systems (Table 1). Compared with plants grown using a hydroponic culture system, plants grown in the outside field system had average LRL, LRD, RN, SDW, RDW, and PDW that were 57%, 46%, 48%, 185%, 164%, and 178% greater at the flowering stage, respectively. Compared with the soil-pot system, plants grown in the outside field system had LRL, LRD, SDW,

RDW, and PDW that were 14%, 12%, 60%, 44%, and 54% greater at the flowering stage, respectively. At the root swelling stage, plants grown in the field system had LRL, LRD, RN, SDW, RDW, and PDW that were 48%, 46%, 65%, 210%, 112%, and 154% greater than those of plants grown using a hydroponic system, respectively. Compared with plants grown in the soil-pot system, these values were 9%, 16%, 15%, 94%, 68%, and 83% greater, respectively. At the flowering and root swelling stages, the LRL, LRD, and RN of plants grown using the hydroponic culture system were significantly lower than those of plants grown in the field and soil-pot systems ( $p < 0.01$ ). SDW, RDW, and PDW differed significantly between all 3 culture systems ( $p < 0.01$ ).

**Table 1.** Root parameters and dry biomass of *Salvia miltiorrhiza* plants grown in quartz-sand-pot, soil-pot and outside field cultures at 3 different growth stages.

Stages	Growth parameters	Cultivation systems		
		Quartz Sand-pot	Soil-pot	Field
F <sup>1</sup>	The longest root length (cm)	<sup>2</sup> 18.36 ± 0.82 aA	25.16 ± 1.00 bB	28.76 ± 1.06 bB
	The largest root diameter (mm)	5.17 ± 0.20 aA	6.72 ± 0.31 bB	7.54 ± 0.34 bB
	The number of roots (n)	4.25 ± 0.18 aA	6.85 ± 0.26 bB	6.30 ± 0.30 bB
	Biomass dry matter (g·plant <sup>-1</sup> )	Shoot	4.39 ± 0.19 aA	7.83 ± 0.30 bB
		Root	2.23 ± 0.09 aA	4.08 ± 0.15 bB
		Total plant	6.61 ± 0.29 aA	11.91 ± 0.45 bB
	Root/Shoot	0.53 ± 0.02 aA	0.52 ± 0.02 aA	0.47 ± 0.02 aA
S <sup>1</sup>	The longest root length (cm)	28.82 ± 1.21 aA	39.27 ± 1.50 bB	42.66 ± 1.82 bB
	The largest root diameter (mm)	6.66 ± 0.28 aA	8.40 ± 0.36 bB	9.73 ± 0.43 bB
	The number of roots (n)	5.14 ± 0.22 aA	7.38 ± 0.34 bB	8.49 ± 0.30 bB
	Biomass dry matter (g·plant <sup>-1</sup> )	Shoot	8.02 ± 0.34 aA	12.83 ± 0.49 bB
		Root	6.94 ± 0.30 aA	8.77 ± 0.34 bB
		Total plant	15.60 ± 0.70 aA	21.60 ± 0.83 bB
	Root/Shoot	0.87 ± 0.04 aA	0.69 ± 0.03 bA	0.60 ± 0.03 bA
M <sup>1</sup>	The longest root length (cm)	37.59 ± 1.56 aA	42.86 ± 1.70 bB	46.72 ± 2.12 bB
	The largest root diameter (mm)	7.89 ± 0.34 aA	9.90 ± 0.39 bB	12.18 ± 0.63 cC
	The number of roots (n)	6.22 ± 0.27 aA	8.58 ± 0.34 bB	10.06 ± 0.41 cC
	Biomass dry matter (g·plant <sup>-1</sup> )	Shoot	6.56 ± 0.26 aA	9.21 ± 0.37 bB
		Root	8.20 ± 0.33 aA	11.27 ± 0.37 bB
		Total plant	14.76 ± 0.59 aA	20.48 ± 0.79 bB
	Root/Shoot	1.25 ± 0.05 aA	1.23 ± 0.05 aA	1.04 ± 0.15 aA

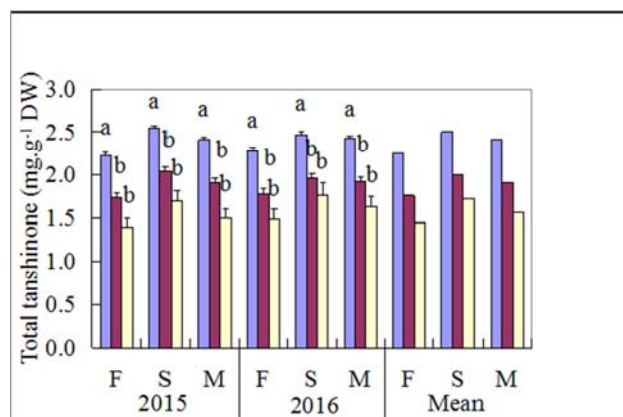
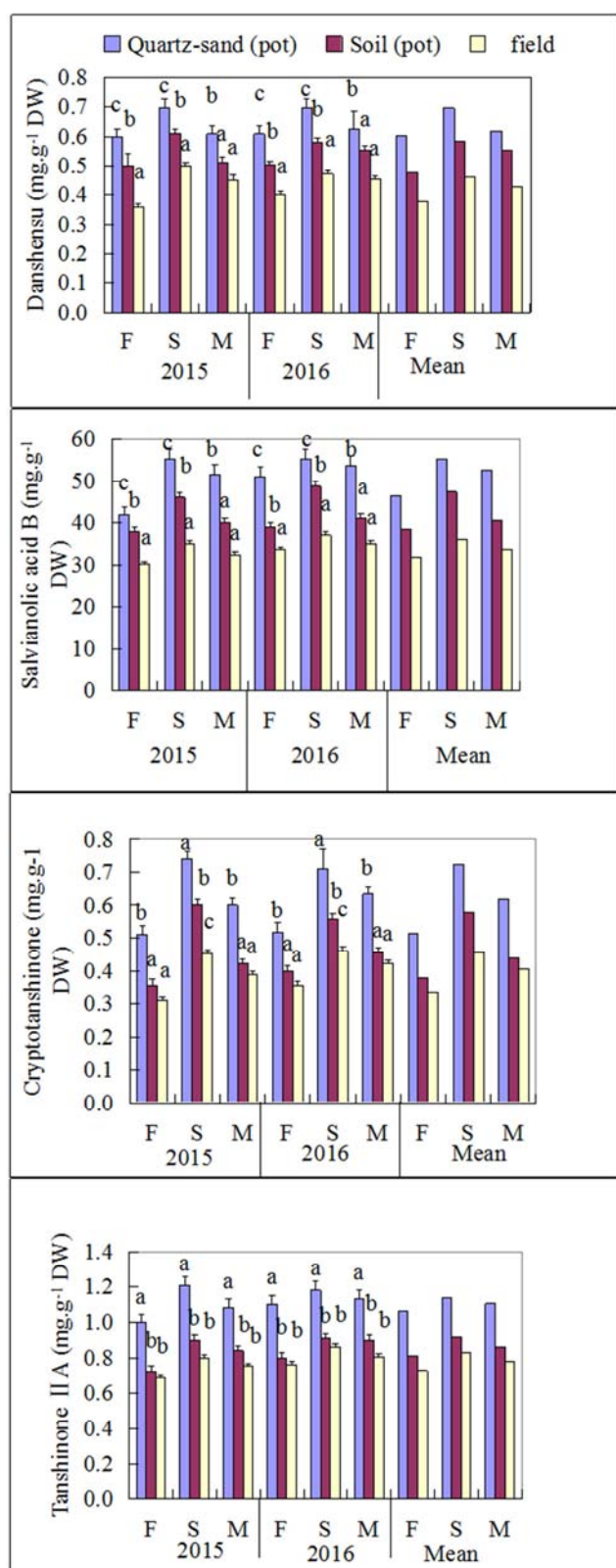
F = plants in flowering stage, S = plants in root swelling stage, M = plants in maturing stage, <sup>1</sup>the average results from culture experiments conducted in 2015 and 2016, <sup>2</sup>mean values (± SE; n = 20). For root parameters and biomass, distinct letters within columns indicate different significant values, lowercase for 5% and uppercase for 1%.

At the mature stage, plants grown in a field system had LRL, LRD, RN, SDW, RDW, and PDW that were 24%, 54%, 62%, 188%, 139%, and 161% greater than those grown using the hydroponic culture system, respectively. These plants had LRL, LRD, RN, SDW, RDW, and PDW that were 9%, 23%, 17%, 105%, 74%, and 88% greater than those grown in a soil-pot system, respectively. At the mature stage, the LRL of plants grown using a hydroponic culture system were significantly lower than those of plants grown in field and soil-pot systems ( $p < 0.01$ ). There was a significant difference in LRD, RN, SDW, RDW, and PDW between the 3 culture

systems ( $p < 0.01$  for all except for differences between field and soil-pot LRD and RN, for which  $p < 0.05$ ).

In all 3 culture systems, the ratio of root to shoot (R/S) increased gradually as the *S. miltiorrhiza* grew. The R/S ratio of plants grown in the hydroponic culture system showed the greatest increase, followed by that of plants grown in the soil-pot system. At the root swelling stage, the R/S ratio of plants grown in the hydroponic culture system was significantly higher than those of plants grown in soil-pot and field systems ( $p < 0.05$ ) (Table 1).

### 3.2.2. Bioactive Components



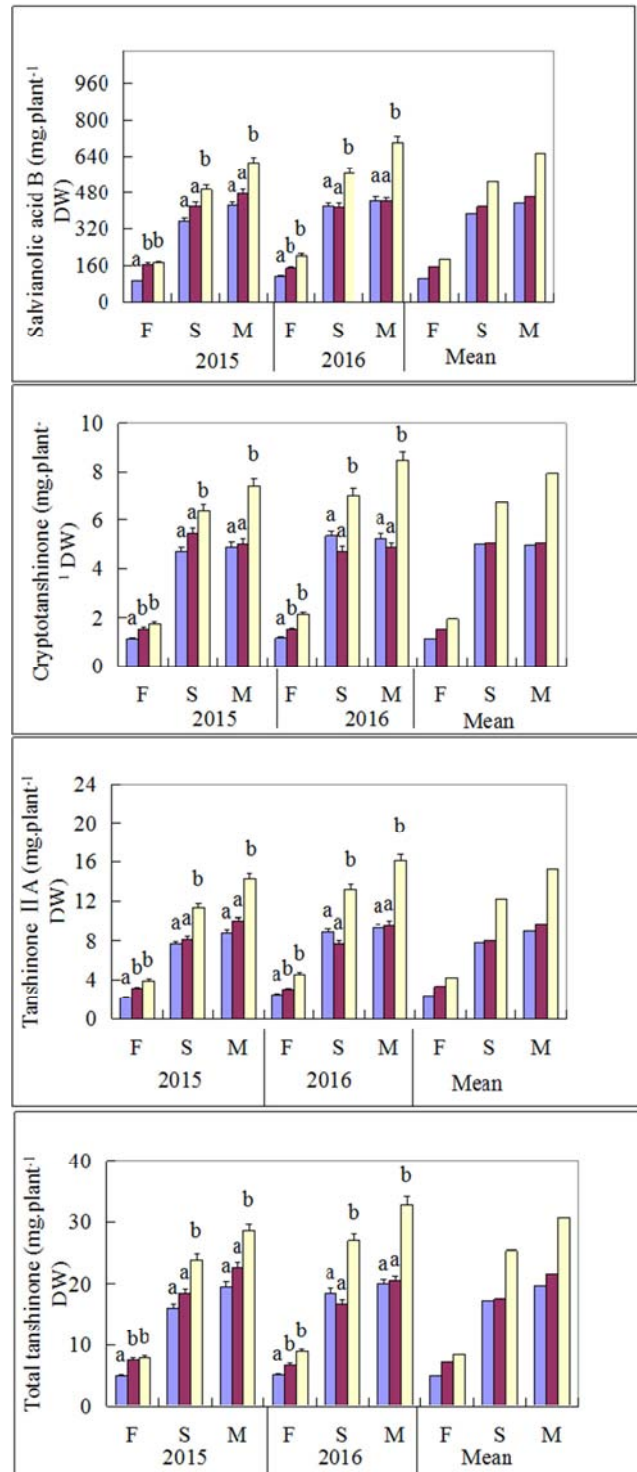
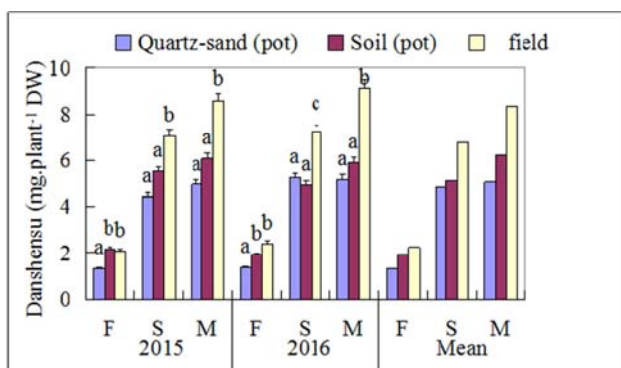
**Figure 4.** Concentration of 5 bioactive compounds in the roots of *S. miltiorrhiza* at 3 growth stages, flowering (F), root swelling (S), and mature (M), grown in quartz sand-pot (blue bars), soil-pot (red bars), and outside-field cultures (yellow bars). Values are means ( $\pm$ SE;  $n = 20$ ). For each metabolite, different letters within the cultures indicate significant differences, as determined by least significant difference test (LSDT).

The changes between growth stages in the concentration of 5 active constituents of *S. miltiorrhiza* are shown in Figure 4. *S. miltiorrhiza* contained more SAB than any other bioactive compound tested. At every growth stage, plants grown in the hydroponic culture system had higher concentrations of all 5 bioactive compounds than plants grown in soil-pot and field systems. In the flowering and root swelling stages, DSS and SAB content differed significantly between the 3 culture systems ( $p < 0.05$ ). At the mature stage, plants grown in the hydroponic culture system had a significantly higher SAB content than plants grown in the soil-pot and field systems ( $p < 0.05$ ). There was no significant difference in the bioactive component contents of plants grown in soil-pot and field. The CTS content of plants grown in the hydroponic culture system was significantly higher than those of plants grown in soil-pot and field systems at all 3 growth stages ( $p < 0.05$ ). The CTS content of plants grown in the soil-pot system was higher than that of plants grown in the field system at all 3 growth stages; however, this difference was only significant at the root swelling stage ( $p < 0.05$ ). Throughout the entire growth cycle, plants grown in the hydroponic cultural system had significantly higher TS II A and TTS content than plants grown in the soil-pot and field systems ( $p < 0.05$ ). Plants grown in the soil-pot system and those grown in the field system did not differ significantly in TS II A and TTS content. Within each culture system, the concentrations of bioactive compounds (DSS, SAB, CTS, TS II A, and TTS) were highest at the root swelling stage, and lowest at the flowering stage. The bioactive compound content of plants was significantly lower at the flowering stage than at the root swelling and mature stages.

### 3.3. Yield of Bioactive Components

The bioactive component yield was determined by multiplying the root concentration by root dry weight (Figure 5). At all 3 growth stages, the yield of the 5 bioactive components from plants grown in the field system

were the highest (average dry weight DSS, 8.36 mg·plant<sup>-1</sup>; SAB, 657 mg·plant<sup>-1</sup>; CTS, 7.95 mg·plant<sup>-1</sup>; TS II A, 15.2 mg·plant<sup>-1</sup>; and TTS, 30.7 mg·plant<sup>-1</sup>, at the mature stage), followed by the yield from plants grown in the soil-pot system (DSS, 6.25 mg·plant<sup>-1</sup>; SAB, 457 mg·plant<sup>-1</sup>; CTS, 5.06 mg·plant<sup>-1</sup>; TS II A, 9.7 mg·plant<sup>-1</sup>; and TTS, 21.6 mg·plant<sup>-1</sup>, at the mature stage), then by the yield from plants grown in the hydroponic culture system (DSS, 5.07 mg·plant<sup>-1</sup>; SAB, 430 mg·plant<sup>-1</sup>; CTS, 4.95 mg·plant<sup>-1</sup>; TS II A, 9.1 mg·plant<sup>-1</sup>; and TTS, 19.8 mg·plant<sup>-1</sup>, at the mature stage). In comparison to the yield from plants grown in the soil-pot and hydroponic systems, plants grown in the field system had a DSS yield that was 14% and 66% greater, an SAB yield that was 19% and 81% greater, a CTS yield that was 27% and 71% greater, a TS II A that was 29% and 80% greater, and a TTS yield that was 18% and 69% greater at the flowering stage, respectively. In comparison to the yield from plants grown in the soil-pot and hydroponic systems, plants grown in the field system had a DSS yield that was 32% and 40% greater, an SAB yield that was 28% and 38% greater, a CTS yield that was 33% and 34% greater, a TS II A yield that was 52% and 55% greater, and a TTS yield that was 45% and 46% greater at the root swelling stage, respectively. In comparison to the yield from plants grown in the soil-pot and hydroponic systems, plants grown in the field system had a DSS yield that was 34% and 65% greater, an SAB yield that was 44% and 53% greater, a CTS yield that was 60% and 57% greater, a TS II A yield that was 56% and 68% greater, and a TTS yield that was 42% and 55% greater at the mature stage, respectively. In each culture system, the yield of all 5 bioactive components was greatest at the mature stage, followed at the root swelling stage, and was lowest at the flowering stage. The yield of all 5 bioactive components from plants collected at the root swelling and mature stages from the 3 systems were significantly greater than those from plants collected at the flowering stage ( $p < 0.01$ ). However, the differences between the yields from plants collected at the root swelling stage and those collected at the mature stage were not significantly different.



**Figure 5.** Yield of 5 bioactive compounds in roots of *S. miltiorrhiza* at 3 growth stages, flowering (F), root swelling (S), and mature (M), grown in quartz sand-pot (blue bars), soil-pot (red bars), and outside-field cultures (yellow bars). Values are means ( $\pm$ SE;  $n = 20$ ). For each metabolite, different letters within cultures indicate significant differences as determined by least significant difference test (LSDT).

## 4. Discussion

The medically active compounds in *S. miltiorrhiza*, and medicinal plants in general, are produced by plant secondary metabolism in response to environmental stress [29-31]. The production of high-quality medicinal plant tissues requires the optimization of growing environments for consistent production of specific metabolites. However, information about the effect of culture methods on plant growth and phytochemical yield is limited. The main objective of the

current study was to evaluate 3 culture systems in controlled and outside environments in order to optimize phytochemical production in plant biomass, phenolic and tanshione concentrations, and active component yield were considered. The experimental results from 2015 and 2016 differed somewhat in root parameters (including RDW, LRL, and LRD) and bioactive concentration; these differences were not statistically significant. The results from both years showed the same trends, and any differences in results may be due to slight differences in climactic conditions between years.

**Table 2.** Approximate concentration range of 5 bioactive components in the roots of *Salvia miltiorrhiza* from 3 culture experiments, and spontaneous collection, and previous field cultivation.

Growing systems	DSS (mg g <sup>-1</sup> DW)	SAB (mg g <sup>-1</sup> DW)	CTS (mg g <sup>-1</sup> DW)	TS II A (mg g <sup>-1</sup> DW)	TTS (mg g <sup>-1</sup> DW)
Quartz sand-pot <sup>a</sup> (hydroponics)	0.4–0.6	30–40	0.40–0.6	0.5–0.7	1.2–1.6
Soil-pot <sup>a</sup>	0.6–0.8	38–50	0.5–0.7	1–1.2	2–2.4
Field <sup>a</sup>	0.6–1.1	40–65	0.5–0.8	1–2.1	2.2–2.6
Spontaneous collection or previous field cultivation	0.24–0.48 <sup>b</sup> , 0.34–3.56 <sup>c1</sup> 0.37–3.03 <sup>c2</sup>	13.5–22 <sup>b</sup> , 11–52 <sup>c1</sup> , 21–41 <sup>c2</sup>	0.06–1.84 <sup>b</sup> , 0.57– 4.55 <sup>c1</sup> 0.40–2.40 <sup>c1</sup>	0.14–6.67 <sup>b</sup> 0.705–8.357 <sup>c1</sup> 0.799–7.371 <sup>c2</sup>	0.83–11.2 <sup>b</sup>

<sup>a</sup>results from current experiment (n = 20 each condition); <sup>b</sup>Li *et al.* (2008; n = 30); <sup>c</sup>Ma *et al.* (2004; n<sub>c1</sub> = 23; n<sub>c2</sub> = 7) [14, 39].

Because plants grown in each culture system were provided with the same amount of nutrients, the observed difference in plant nutrient absorption between culture systems is probably due to some other aspect of the culture systems. The results related to the bioactive components implies that *S. miltiorrhiza* is capable of bioactive compound biosynthesis and accumulation at low nutrient concentrations. Nutrient levels not only determine plant growth, but also the amount of secondary metabolites in plant tissues, as nutritional deficiency greatly stimulates the production of secondary metabolites [32-33]. Our results are consistent with studies that indicate low N, P, and K supply significantly increase secondary metabolites in medicinal plant tissues [34-36]. Under low nutrient conditions, plants may intensify metabolic and biochemical activity in order to increase the absorptive capacity of roots. This results in an increase in the production of secondary metabolites. However, in the current study, the field system resulted in the greatest root production and the highest yield of bioactive components, followed by the soil-pot system, and then by the hydroponic culture system. In the field system, plant roots always had sufficient water, nutrients, dissolved oxygen (as measured during the experiment, data not shown) and a large elongation space. These conditions may have been more favourable to plant growth than conditions in the other 2 culture systems tested.

The yield of bioactive compounds was significantly greater in the field system than in the hydroponic culture and soil-pot systems, but this difference was highly dependent on the difference in root biomass between culture systems. Zhu *et al.* (2009) found a similar relationship between growth and saikosaponin production in the medicinal herb *Bupleurum chinense* DC [30]. Throughout the literature, the reports indicate that significant increases in the bioactive compound yield of plants is the result of increases in biomass, not bioactive compound concentration. Therefore, biomass production is more important than bioactive component

concentration for maximizing the hectare yield of *S. miltiorrhiza*.

In all 3 growth stages, the R/S ratio of plants grown in the hydroponic culture system was the greatest, followed by that of plants grown in the soil-pot system, and then by that of plants grown in the field system. This indicates that plants grown in the hydroponic culture system had significantly reduced growth, compared to plants grown in the other 2 culture systems, according to its lower root biomasses.

The concentration of individual phenolic and tanshinone compounds is reported to be related to the growth stage of plants [37]. In the current study, plant growth parameters and bioactive compounds differed between culture systems at all stages of growth. Plants grown in the field system increased root biomass and decreased the concentration of bioactive components in their roots to a greater degree than plants grown in either the hydroponic culture system or in the soil-pot system. In controlled environments such as hydroponic culture and soil-pot culture systems, the space, water, air, temperature, and nutrients available for plant growth are controlled to a greater degree than is possible in a field system. This control environment influences the ability of plant tissues to grow freely, particularly roots [38].

Compared with plants grown in the soil-pot and field systems, *S. miltiorrhiza* plants grown in the hydroponic culture system had a higher or similar concentration of medicinal metabolites. This is consistent with previous studies of both cultivated and wild-harvested *S. miltiorrhiza* plants (Table 2). TS II A and SAB are the markers commonly used for the chemical standardization and quality assessment of *S. miltiorrhiza* roots, even though other bioactive compounds contained in *S. miltiorrhiza* tissues have stronger pharmacological activities (e.g. danshensu and cryptotanshinone) [14]. In hydroponic cultural experiments, most samples contain the minimum concentration of SAB and TSA required to meet quality standards for the production of



standardized extracts with a final concentration of SAB and TS II A of  $30 \text{ mg} \cdot \text{g}^{-1}$  and  $2 \text{ mg} \cdot \text{g}^{-1}$ , respectively (CPA, 2010) [41]. In field and soil-pot cultural experiments, the level of TS II A was lower than this minimum concentration (Table 2). However, root biomass is significantly greater in field systems than in hydroponic systems, resulting in a much larger yield of bioactive components than is obtained from hydroponic systems. Our results are not entirely consistent with previous reports of greenhouse hydroponic culture stimulating plant growth, root production, and secondary metabolites in some medicinal plants [15-16, 40-41, 20]. This may result from differences between our study and previous studies on medicinal plant varieties, growing environments, or hydroponic culture substrate and technology.

## 5. Conclusion

The hydroponic culture system facilitated the accumulation of bioactive components in plants, but was not favourable for plant growth and root production. However, the field culture system resulted in significantly greater plant growth and root production than the hydroponic culture system. Further studies are needed in order to develop appropriate growing procedures for large-scale *S. miltiorrhiza* production, and to provide high plant biomass with satisfactory accumulation of bioactive components in the roots of this important medicinal plant.

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