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Plant Growth and the Contents of Major Bioactive Compounds of *Salvia miltiorrhiza* Bunge Grown in Mississippi, United States

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Abstract: *Salvia miltiorrhiza* Bunge (danshen) is a traditional herbal medicine and has been widely used to prevent and treat cardiovascular and other diseases. Currently, the majority of medicinal plants, including danshen, used in the United States are imported from foreign countries, which often involves challenges such as inconsistency in contents of bioactive compounds and inadequate supply of high quality plant materials to meet market demand. The objective of this study was to evaluate plant growth and the contents of major bioactive compounds of three selected danshen cultivars and identify cultivars with the greatest potential for commercial production in Mississippi. Results showed that danshen plants can grow well in Mississippi and the three cultivars evaluated had similar growth indices, leaf SPAD values, photosynthetic activities, shoot and root dry weights, and root numbers, but differed in germination rates, maximum root lengths, and maximum root diameters. The major bioactive compounds in danshen root extracts, including tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B, were quantified with no significant difference in their contents among the three cultivars. Results from this study suggested that danshen has the potential to be grown as an alternative crop in Mississippi, USA.

Keywords: danshen; roots; tanshinone I; tanshinone IIA; cryptotanshinone; salvianolic acid B

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1. Introduction

Complementary and alternative medicine (CAM) plays an important role in preventing and treating diseases around the world. The global CAM market was valued at USD 117 billion in 2022 and is expected to expand at an annual growth rate of 25% from 2023 to 2030 [1]. In the United States, the out-of-pocket spending on complementary and alternative medicines and procedures was USD 30 billion in 2016 [2]. One of the main CAMs is traditional herbal medicines (THM). Compounds derived from THM have attracted much attention for their potential use to prevent and treat diseases [3].

Salvia miltiorrhiza Bunge, common name danshen or red sage, is one of the most important and widely used traditional herbal medicinal plants. It belongs to the family Lamiaceae. The genus *Salvia* consists of around 900 species, many of which have been cultivated worldwide for their medicinal and culinary purposes [4–6]. Danshen has been used for many years in China, Japan, and other East Asian countries to prevent and treat ailments including neurological disease, cancer, inflammation, and cardiovascular disease [7–11]. The use of danshen as a natural product has grown substantially in the United States and Europe in recent years [12].

Danshen is native to China and Japan. It prefers a warm humid climate, full sun, a rich, moist, and well-drained sandy soil. Danshen grows well in a wide range of soil pH (4.6–8.5) [13]. The optimum growing temperature for danshen is 20–26 °C and relative humidity is around 80% [14,15]. It is cold-hardy to approximately −10 °C and can grow in USDA cold-hardiness zones 5–9 [16]. Mississippi is located in the subtropical region, USDA cold-hardiness zones 7b–9a [17], which has the potential to grow danshen. Currently, the majority of danshen in the world are produced in China, with small production areas in other countries including Japan and Vietnam [12].

The primary medicinal activity of danshen is in the root. Danshen root contains a variety of chemical components, which are mainly divided into two categories: one is the lipid-soluble component dominated by tanshinone diterpenes, and the other is the water-soluble component dominated by phenolic acid [18]. Among all the compounds, tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B have been demonstrated as major bioactive components by pharmacological studies and clinical trials, and have been considered marker compounds [19,20]. The quality of danshen mainly depends on the contents of these marker compounds [19]. Factors such as cultivar, production region, environment, cultivation practices, harvesting, processing, and storage, could affect the contents of these compounds in danshen [19,21].

Danshen is usually applied clinically in the form of compound preparations, for example, Compound Danshen Dripping Pill or Dantonic® (T89) Capsule, which is a two-herb composition medicine including danshen and sanchi (*Panax notoginseng*) as main ingredients. T89 is the world's first compound Chinese herbal medicine that has passed the US Food and Drug Administration (FDA) regulated multi-center phase 3 clinical trial, and now is in the process of seeking drug approval from FDA [22]. T89 is currently sold in China, Vietnam, South Korea, India, and some other countries as a prescription drug to treat angina and coronary heart diseases [22].

Currently, the majority of medicinal plants, including danshen, used in the United States are imported from foreign countries. However, increasing concerns over quality and safety issues such as inconsistency in contents of bioactive compounds and potential heavy metal and pesticide contamination [23] pose significant challenges to US pharmaceutical and related industries in sourcing quality plant materials at the volume needed to meet market demand. With the projected strong market growth of medicinal plant-derived products, it is critical for US industries to have access to adequate supplies of high quality medicinal plants, which provides a unique opportunity for US producers to incorporate medicinal plants as an alternative crop in their operations. The objective of this study was to evaluate plant growth and the contents of major bioactive compounds of selected danshen cultivars to identify cultivars with the greatest potential for commercial production in Mississippi, USA.

2. Materials and Methods

2.1. Plant Materials and Cultivation

Danshen seeds from three different sources were used in this study. Cultivar V1 was collected from Shandong province and cultivars V2 and V3 were collected from Shaanxi province in China. Shandong and Shaanxi provinces are among the major regions for the production of high quality danshen in China, and are at a similar latitude as Mississippi. Seeds were sown in 128-cell trays, with four trays per cultivar, and placed on a bench under a mist system in a greenhouse at Mississippi State University (MSU, 33°29′ N 88°47′ W) on 14 March 2019 and 8 May 2020, respectively. The experiment was laid out in a randomized complete block design with four replications and cultivar as the experimental factor. Each replication included one tray per cultivar. After germination, the seedlings were transplanted into 0.5 L containers and grown in the greenhouse.

Plants were transplanted into 3.78 L containers on 14 May 2019 and 11.35 L containers on 27 July 2020, respectively, filled with a soilless substrate (Metro-Mix® 852, Sun Gro Horticulture, Agawam, MA, USA). Containers were placed in full sun on a nursery pad at the MSU R. R. Foil Plant Science Research Center in Starkville, MS. Plants were top-dressed with a slow-release fertilizer Osmocote Plus 15N–3.9P–10K (15–9–12, 8–9 months; Scotts Miracle-Grow Co., Marysville, OH, USA) at a rate of 10 g per plant. Drip irrigation was installed, and plants were irrigated as needed. The experiment was laid out in a randomized complete block design with four replications and cultivar as the experimental factor. Each replication included ten plants (subsamples) per cultivar. Plants were harvested on 29 November 2019 and 2 December 2020, respectively.

2.2. Seed Germination Rate and Plant Growth

The number of seeds germinated was counted two weeks after sowing. Germination rate was calculated by (number of germinated seeds/numbers of total seeds) × 100%. Plants were measured for plant height, width 1, and width 2 prior to harvest on 29 November 2019 and 2 December 2020, respectively. Height was measured from the surface of the container substrate to the top of the plant. Width 1 was measured as the greatest width of the plant and width 2 was the perpendicular width to width 1. Plant growth index (PGI) was calculated as the average of height, width 1, and width 2 (PGI = [height + width 1 + width 2]/3). Leaf SPAD readings of three recent fully expanded leaves from each plant were measured using SPAD-502 meter (Konica Minolta, Inc., Osaka, Japan) during the growing season. The SPAD value of each plant was the average of the readings from three measurements [24]. Shoot number was measured after harvesting. Shoot number was counted as the number of primary shoots grown from the root. Root number, maximum root length, maximum root diameter, and root fresh weight were also measured. Root number was counted as the number of roots greater than 2 mm in diameter. Maximum root length was measured as the length of the longest root. Maximum root diameter was measured as the diameter in the upper middle of the tap root [25]. Shoots and roots from each plant were then oven-dried at 60 °C until constant weight, and the dry weights of shoots and roots were measured.

2.3. Photosynthetic Activities

Plant photosynthetic activities of one randomly selected plant in each replication were measured in June 2019 using a portable photosynthesis system (LI-6400 XT; LI-COR Biosciences, Lincoln, NE, USA). For each plant, one recent fully expanded leaf was enclosed into a 2 cm^2 leaf chamber for measurement. Photosynthetically active radiation (PAR) and reference CO_2 concentration inside the leaf chamber were maintained at 1500 µmol m^{-2} s^{-1} and 400 µmol mol^{-1} during measurement, and the leaf chamber block temperature was maintained the same as the air temperature [26]. Leaf transpiration rate (Trmmol), net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), leaf-to-air vapor pressure deficit (VPD), and stomatal conductance (g_s) were measured.

2.4. Preparation of Danshen Extract

Dried root samples were ground to pass a 40-mesh (0.425 mm) sieve using a Wiley mill (Thomas Scientific, Thorofare, NJ, USA). A total of 0.5 g of the danshen powder was added into 50 mL 75% methanol aqueous solution and extracted for 30 min in an ultrasonic bath at room temperature. After ultrasonic extraction, the extracted solution was filtered through a filter paper (Grade 1, GE Healthcare Bio-Sciences Corp., Marlborough, MA, USA) using a vacuum pump, and then fixed volume to 50 mL with 75% methanol solution in volumetric flasks [26,27].

2.5. HPLC Analysis

Tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B contents in danshen extract were analyzed using high performance liquid chromatography (HPLC) (1260 Infinity II series; Agilent Technologies, Wilmington, DE, USA). Danshen extract was filtered through a 0.22 µm membrane. HPLC analyses were performed using a diode array detector (G1315C Diode-array Detector, Agilent Technologies, Wilmington, DE, USA) with an injection volume of 10 µL, flow rate of 1 mL min^{-1}, controlled oven temperature of 30 °C, and a C18 column [Agilent TC-C18 (2), 4.6 mm × 250 mm, 5 µm; Agilent Technologies, Wilmington, DE, USA]. Mobile phase A was 100% acetonitrile, mobile B was 0.02% phosphoric acid. The program for tanshinone I, tanshinone IIA, and cryptotanshinone was 0–6 min: 61% A; 6–20 min: 61–90% A; 20–20.5 min: 90–61% A; 20.5–25 min: 61% A. Chromatograms were recorded at 270 nm. This procedure was modified slightly based on the method described in Chinese pharmacopoeia [28]. The program for salvianolic acid B was 0–20 min: 5–20% A; 20–30 min: 20–30% A; 30–40 min: 30–40% A. Chromatograms were recorded at 280 nm. This method was modified slightly based on Ren's study [29].

Standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, water, and phosphoric acid of chromatographic grade were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.6. Statistical Analysis

Significance of the main effect was determined by analysis of variance (ANOVA) using the PROC GLM procedure. Where indicated by ANOVA, means were separated by Tukey's honestly significant difference test at $p \leq 0.05$. All statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Germination Rate, Plant Growth Index, and Leaf SPAD Values

Danshen cultivars varied in germination rates ranging from 30.5% in V3 to 71.1% in V2 in 2019 (Table 1). V1 and V2 had similar, but higher germination rates than V3. In 2020, the trend of germination rates among cultivars was similar to 2019, ranging from 22.7% in V3 to 66.7% in V2. V3 had the lowest germination rates in both years. Seed germination rate is an important indicator of seed quality and can show the activity of seed embryos [30,31]. Cultivars with higher germination rates normally have higher survival rates after germination. Factors such as seed age can affect germination rate, with older seeds normally having lower germination rates [32]. Previous research comparing several danshen cultivars reported that certain cultivars had higher germination rates than others [33]. In our study, V1 and V2 had higher germination rates than V3 in both years, suggesting cultivars V1 and V2 would be better choices when considering germination rate.

Table 1. Germination rate, plant growth index (PGI), and leaf SPAD value of three danshen cultivars grown in Mississippi, United States in 2019 and 2020.

Cultivar	Germination Rate (%) [2]		PGI (cm) [3]		SPAD	
	2019	2020	2019	2020	2019	2020
V1	68.5 ± 4.0 a [1]	62.0 ± 2.3 a	49.5 ± 9.0 a	39.9 ± 4.2 a	25.8 ± 6.0 a	20.0 ± 8.7 a
V2	71.1 ± 5.7 a	66.7 ± 1.7 a	41.7 ± 9.3 a	37.1 ± 10.2 a	32.0 ± 4.4 a	22.4 ± 6.4 a
V3	30.5 ± 5.6 b	22.7 ± 5.6 b	43.9 ± 6.6 a	46.2 ± 12.5 a	30.4 ± 4.7 a	25.0 ± 5.5 a

[1] Different lower-case letters within a column suggest significant differences among cultivars indicated by Tukey's HSD test at $p \leq 0.05$. [2] Germination rate was calculated by (number of germinated seeds/numbers of total seeds) × 100%. [3] Plant growth index (PGI) was calculated as the average of plant height, width 1 (widest points apart), and width 2 (perpendicular to width 1), (plant height + width 1 + width 2)/3.

There were no significant differences for plant growth indices in 2019 or 2020 (Table 1), suggesting the three cultivars are comparable in aboveground plant size. Leaf SPAD values were similar among cultivars in both years (Table 1). Leaf SPAD value measures the relative chlorophyll content in leaves, which often positively correlates to plant nitrogen (N) status [34]. In this study, the three cultivars had similar SPAD values, indicating they were similar in chlorophyll and N status.

3.2. Shoot and Root Characteristics

Among the three cultivars tested, there were no significant differences in shoot number and shoot dry weight in 2020 (Table 2). Danshen cultivars originating from different regions may have different growth due to their long term adaptations to local environments. For example, one study evaluating eight cultivars collected from the main danshen production regions in China reported that cultivars originating from Shandong, Shaanxi, and Hebei provinces had similar plant height, number of shoots, but parameters were lower compared to the cultivar from Henan province [15]. Differences in genotype and environmental factors such as light, temperature, humidity, water, and nutrients could all contribute to the differences in plant growth.

Table 2. Shoot number, shoot dry weight, root number, maximum root length, and maximum root diameter of three danshen cultivars grown in Mississippi, United States in 2020.

Cultivar	Shoot Number (Per Plant)	Shoot Dry Weight (g Per Plant)	Root Number [1]	Maximum Root Length (cm) [2]	Maximum Root Diameter (mm) [3]
V1	10.6 ± 1.8 a [4]	34.6 ± 8.8 a	57.2 ± 12.2 a	53.0 ± 5.5 a [4]	7.6 ± 1.8 b
V2	8.2 ± 2.2 a	31.2 ± 7.3 a	60.6 ± 28.1 a	40.6 ± 8.3 b	11.5 ± 3.8 a
V3	8.6 ± 1.7 a	34.6 ± 6.5 a	51.8 ± 7.8 a	46.4 ± 8.7 ab	7.8 ± 0.8 b

[1] Root number was counted as the number of roots greater than 2 mm in diameter. [2] Maximum root length was measured as the length of the longest root. [3] Maximum root diameter was measured as the diameter of the thickest root. [4] Different lower-case letters within a column suggest significant differences among cultivars indicated by Tukey's HSD test at $p \leq 0.05$.

Shoot growth is closely related to root growth. Shoot growth relies on the water and mineral nutrients taken up by roots and then transported in the xylem to the shoots, and root growth relies on the photosynthates produced in the shoot system and transported in the phloem to the roots [35]. Similar shoot growth among the three cultivars in this study implies that they might produce similar amount of photosynthates to support root growth.

Root growth is important in danshen cultivation since major bioactive compounds are concentrated in roots, which is harvested for medicinal purpose [36]. Root number, root length, and diameter are good indicators of root growth. In this study, the number of roots (>2 mm in diameter) was similar among the three cultivars (Table 2). Other research reported that cultivars from different origins could vary in root numbers. For example, danshen from Shaanxi province had a greater number of roots compared to those from Henan and Hebei, but similar to the ones from Shandong [15].

While the root numbers were similar among the three cultivars, the maximum root length and maximum root diameter differed (Table 2). The maximum root length varied from 40.6 cm in V2 to 53 cm in V1, and V1 had significantly higher maximum root length than V2. The maximum root diameter varied from 7.6 mm in V1 to 11.5 mm in V2. V1 and V3 had similar but lower maximum root diameter than V2. Previous research reported that cultivars from different origins could differ in maximum root length and diameter. Some varieties have greater root length and diameter, while others have greater root length but smaller root diameter [15,37,38]. For example, cultivars from Shaanxi had greater root length and diameter than those from Shandong, Henan, and Hebei [16]. In this study, we found that V1 had higher maximum root length but lower maximum root diameter than V2.

The diameter of danshen root has been traditionally used in herbal medicine trade as one of the criteria to classify danshen into different grades. Danshen root diameter >1 cm was considered as top grade and diameter between 0.4–1.0 cm was considered as second-class. However, research found that the fine roots with diameters of 0.4 cm contain higher contents of bioactive compounds than the roots with 1.0 or 1.8 cm diameters [39]. It was reported that the epidermis and phloem of danshen root mainly contain lipid-soluble component tanshinones, and the xylem of the root mainly contain the water-soluble component [40]. Therefore, roots with larger diameter may contain more salvianolic acid B, and roots with smaller diameter may contain higher content of tanshinones because they have more surface area of epidermis and phloem than larger diameter roots.

There were no significant differences in fresh or dry root weights among three cultivars in both years (Table 3). For each cultivar, the fresh and dry root weights were higher in 2020 than in 2019, possibly due to the larger container size used in 2020. Container size is an important factor affecting plant growth. Larger containers contain more soil which can hold more water and nutrients, facilitating more root growth. As container size decreases, the amount of media pore space decreases, reducing both media water holding capacity and aeration [41], thus reducing root growth [42,43].

Table 3. Root fresh weight and dry weight of three danshen cultivars grown in Mississippi, United States in 2019 and 2020.

Cultivar	Root Fresh Weight (g Per Plant)		Root Dry Weight (g Per Plant)	
	2019	2020	2019	2020
V1	210.2 ± 27.7 a [1]	493.7 ± 57.1 a	33.6 ± 4.9 a	100.2 ± 12.4 a
V2	199.8 ± 39.6 a	481.4 ± 68.2 a	33.1 ± 5.9 a	98.5 ± 18.0 a
V3	184.4 ± 41.0 a	438.3 ± 65.3 a	29.4 ± 8.3 a	96.2 ± 13.8 a

[1] Different lower-case letters within a column suggest significant differences among cultivars indicated by Tukey's HSD test at $p \leq 0.05$.

3.3. Photosynthetic Activities

The three cultivars did not vary significantly in net photosynthetic rate (Pn), stomatal conductance (g_s), intercellular CO_2 concentration (Ci), leaf transpiration rate (Trmmol), and leaf-to-air vapor pressure deficit (VPD) (Table 4).

Table 4. Photosynthetic activities of three danshen cultivars grown in Mississippi, United States in 2019.

Cultivar	Pn [2] (μmol m^{-2} s^{-1})	g_{s} [3] (mol m^{-2} s^{-1})	Ci [4] (μmol mol^{-1})	Trmmol [5] (mmol m^{-2} s^{-1})	VPD [6] (kPa)
V1	15.13 ± 10.08 a [1]	0.37 ± 0.29 a	279.24 ± 68.32 a	6.91 ± 4.68 a	2.10 ± 0.42 a
V2	24.87 ± 1.95 a	0.38 ± 0.03 a	269.25 ± 13.10 a	6.76 ± 0.08 a	1.81 ± 0.15 a
V3	16.57 ± 6.32 a	0.22 ± 0.13 a	250.28 ± 26.94 a	4.58 ± 2.10 a	2.13 ± 0.25 a

[1] Different lower-case letters within a column suggest significant differences among cultivars indicated by Tukey's HSD test at $p \leq 0.05$. [2] Net photosynthesis (Pn). [3] Stomatal conductance (g_s). [4] Intracellular CO_2 concentration (Ci). [5] Leaf transpiration rates (Trmmol). [6] Leaf-to-air vapor pressure deficit (VPD).

Photosynthesis is a fundamental but complex physiological process in plants [44–46]. Photosynthetic activities can be affected by many factors including cultivars and environment. In our study, we did not find any differences in photosynthetic activities among the three cultivars. Similar amount of shoot growth (Table 2) indicates that the leaf area available for photosynthetic activity might be similar, and similar amount of root growth (Table 3) indicates that the roots might take up similar amounts of water and nutrients to transport to leaves, resulting in similar photosynthetic activities among the three cultivars.

3.4. Tanshinone I, Tanshinone IIA, Cryptotanshinone, and Salvianolic Acid B

Tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B were analyzed using high performance liquid chromatography (Figure S1). There was no significant difference among the three cultivars with respect to the contents of tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B (Table 5). Table 2 showed that V1 and V3 had lower maximum root diameter than V2, indicating V1 and V3 might have higher content of tanshinones but lower content of salvianolic acid B than V2. However, Table 5 showed that the contents of the bioactive compounds were similar among the three cultivars. This may be due to the reason that our root samples for bioactive compound analysis were prepared using the whole root system which includes the fine roots, instead of separating the roots based on diameters.

Table 5. Content of Tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B in roots of three danshen cultivars grown in Mississippi, United States in 2019 and 2020.

Cultivar	Tanshinone I (%)		Tanshinone IIA (%)	
	2019	2020	2019	2020
V1	0.077 ± 0.012 a [1]	0.073 ± 0.010 a	0.349 ± 0.031 a	0.335 ± 0.013 a
V2	0.069 ± 0.009 a	0.076 ± 0.007 a	0.334 ± 0.036 a	0.334 ± 0.022 a
V3	0.074 ± 0.01 a	0.077 ± 0.013 a	0.345 ± 0.035 a	0.363 ± 0.035 a
Cultivar	**Cryptotanshinone (%)**		**Salvianolic acid B (%)**	
	2019	**2020**	**2019**	**2020**
V1	0.163 ± 0.03 a	0.131 ± 0.031 a	3.863 ± 0.292 a	3.953 ± 0.397 a
V2	0.146 ± 0.029 a	0.137 ± 0.029 a	3.875 ± 0.292 a	3.943 ± 0.292 a
V3	0.133 ± 0.031 a	0.153 ± 0.031 a	3.873 ± 0.267 a	4.172 ± 0.342 a

[1] Different lower-case letters within a column suggest significant differences among cultivars indicated by Tukey's HSD test at $p \leq 0.05$.

More than 200 compounds have been isolated and identified from danshen [47]. Tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B are the main bioactive compounds in danshen [48–50]. Tanshinone I, tanshinone IIA, and cryptotanshinone are the primary bioactive compounds in tanshinones, and salvianolic acid B is a major compound in phenolics (Figure S2) [51]. Tanshinones are a group of abietane diterpenes isolated from danshen and related species, and tanshinone IIA is the most abundant tanshinone with significant pharmacological effects and gives the red color for danshen root [52].

Due to its high medicinal value, danshen has been included in many pharmacopoeias including the Chinese pharmacopoeia, the United States pharmacopeia, European pharmacopoeia, British pharmacopoeia, and Japanese pharmacopoeia [12]. The quality of danshen is determined by the contents of the marker compounds including tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B [53]. The quality standard varies slightly among different pharmacopoeias. For example, the Chinese Pharmacopoeia requires no less than 0.20% of tanshinone IIA, 0.25% of the total content of tanshinone I, tanshinone IIA and cryptotanshinone, and 3% salvianolic acid B. The United States Pharmacopeia defines the minimum standard as containing 0.20% tanshinones, 0.10% tanshinone IIA, and 3.0% salvianolic acid B. In our study, the contents of these marker compounds in all three cultivars met the minimum standards in both Chinese and the US Pharmacopoeias.

Previous research reported that many factors including production region and genotypes could affect the medicinal properties and efficacy of many traditional herbal medicines (THM) [54]. The quality of many THM is inconsistent, which may be mainly due to the varying sources of origin for THM [55]. Environmental factors such as temperature, precipitation, humidity, light, and soil may differ by production region, so the quality of medicinal materials may vary from place to place [56]. Before the 1970s, danshen used in traditional Chinese medicine was mainly harvested from the wild [57]. Wild danshen is generally considered to have superior quality compared to cultivated danshen [58]. However, wild danshen resources have decreased significantly since the 1970s due to extensive harvesting to meet the increased market demand. Currently, almost all the danshen in the market are from cultivated danshen, and the annual market demand for danshen is around 20,000 tons [59].

Danshen is cultivated across most of China. Shandong, Shaanxi, Sichuan, Henan, and Hebei provinces are recognized as the traditional primary production regions producing high quality danshen [15]. Studies reported that danshen grown in Yinan in Shandong, Zhongjiang in Sichuan, and Lushi in Henan provinces had tanshinone IIA contents of 0.44–0.73%, three to four times higher than contents from some of the secondary production areas [15,60]. Some superior regional danshen cultivars have high levels of bioactive compounds. For example, Shangluo danshen mainly grown in Shangluo district of Shaanxi province has tanshinone IIA content ranging from 0.36–0.48% [15,61], Yu Danshen mainly grown in Fangcheng county of Henan province has tanshinone IIA content of 0.68% and 0.37–0.45% in wild and cultivated Yu Danshen, respectively [15].

Climatic factors play an important role in danshen quality. One study using the same danshen seedlings planted in 18 different ecological regions along a latitudinal gradient in eastern and western China found that the main climatic factors including the air temperature, precipitation, atmospheric vapor pressure, and sunshine duration strongly influenced the contents and compositions of main components in tanshinones and phenolic acids, leading to a specific geospatial distribution of the active components [60]. Temperature was the primary factor affecting tanshinone content, while water greatly influenced the phenolic acid content [60]. Adverse climatic conditions, especially extreme temperature, excessive rainfall, and excessive sunshine could strongly influence the accumulation and proportion of active components in danshen [60]. The increase in temperature promoted the accumulation of tanshinones, scarce or excessive water stimulated the accumulation of phenolic acids, while excessive sunshine did not improve the accumulation of these compounds [60,61].

Mississippi is in the subtropical climate region. It has relatively mild winters and long, hot, sunny summers, with relative humidity around 73% [62]. The annual average temperature is 18 °C, with an average low 11°C and average high 24 °C [63]. The low temperatures rarely drop below −10 °C, while high temperatures above 32 °C occur over 100 days every year. The average annual precipitation is about 1422 mm. Mississippi (32°21′ N) is at a similar latitude as some of the main danshen production regions in China such as Sichuan (30°39′ N), Anhui (31°51′ N), Jiangsu (32°3′ N), Shaanxi (34°15′ N), and Henan (34°45′ N) [64]. The weather conditions in Mississippi are proven suitable for danshen production. However, Mississippi is also subject to periods of drought, flood, high summer temperatures, which could affect plant growth and chemical compositions and contents. In our study, the contents of tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B in all three cultivars met the minimum standards required in both Chinese and the US Pharmacopoeias; however, these are lower than some danshen samples being reported [15,60,61]. Considering the limited number of cultivars evaluated in this study, additional cultivars from diverse sources warrant further investigation.

4. Conclusions

In summary, the three cultivars evaluated in this study differed in germination rate, maximum root length, and maximum root diameter, but had similar plant growth indices, leaf SPAD values, photosynthetic activity, shoot and root fresh and dry weights, and root number. The contents of bioactive compounds including tanshinone I, tanshinone IIA, cryptotanshinone, and salvianolic acid B were similar among the three cultivars and met the minimum standards required in both Chinese and the US Pharmacopoeias. Results suggest that danshen has the potential to be grown as an alternative crop in Mississippi, USA.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030310/s1, Figure S1: HPLC chromatograms of danshen.; Figure S2: Chemical structure of the four major chemical compounds in danshen.

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