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Adaptation of *Astragalus membranaceus* varieties to Southeastern United States: Growth, Root Development and Astragaloside IV Content

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Astragalus membranaceus roots have been used in traditional Chinese medicine for thousands of years to treat diseases. A field experiment was established to assess the growth, root development and astragaloside IV concentration of seven varieties of *Astragalus membranaceus* in Auburn, Alabama. Significant differences among varieties were observed in concentration of Mn and Zn. Accessions AM2, AM3, AM4 and AM5 had the best adaptability in terms of shoot growth, root weight and concentration and yield of astragaloside IV in roots. AM7 ranked highest in root length and weight in 13 month plants, but had low astragaloside concentration and yield and the plants did not flower. Large 13-month roots of AM3, AM4 and AM5 had concentrations of astragaloside IV comparable to a commercial sample. These varieties may have potential for cultivation in the southeastern US. However, control of root rot and white fringe beetle are keys to its successful cultivation in this region.

Keyword: *Astragalus membranaceus*, Varieties, Growth, Root development, Astragaloside IV.

1. Introduction

Astragalus membranaceus (Fisch.) Bge. (Fabaceae) is a perennial flowering plant native to the northern parts of China [10]. *Astragalus membranaceus* is also known as Huang-qi or yellow leader, and the root has been used in traditional Chinese medicine for thousands of years to treat various diseases [16]. The active components of its root can stimulate the immune system and many organs of the body, lowering blood pressure and blood sugar levels. Recent research has shown its root can increase the production of interferon and macrophages and thus help restore normal immune function in cancer patients [3, 14]. Astragalus medicinal value lies in the polysaccharides, triterpene glycosides, flavanoids and isoflavanoids found in the taproot [6, 12, 20, 25].

The Climatic condition affects the growth and quality of *A. membranaceus*. In China, the center of origin of wild *A. membranaceus* is the mountain areas in Sichuan province [4, 23], where the climate is moderate, N30-N33° latitude, annual average temperature 4-12 ºC, annual precipitation 500-900 mm, and annual sunlight 1600-2600 hours. Although *A. membranaceus* still exists there, *A. membranaceus* is currently mainly widely distributed in the pine forest region and mountain areas in Northern China, Siberia and Northern Korea.

Plants have the ability to adapt and survive in different climates. *Astragalus membranaceus* has

been successfully introduced and acclimated in moderate regions in some European countries, such as Germany, Poland and Turkey [13]. Successful cultivation should depend on growth performance, flowering, seeding, yield of roots and the content of medicinal component. For instance, the criteria for successful cultivation of *A. membranaceus* in China is that astragaloside IV concentration is greater than 4% [18].

Different varieties of *A. membranaceus* are grown in different areas worldwide. Jiang *et al.* [7] compared the active compounds of three different varieties including wild *A. membranaceus*, cultivated *A. membranaceus* and cultivated *A. membranaceus mongholicus*, all of which grew in the same area. The concentration of astragaloside IV, total flavonoids and polysaccharides in wild *A. membranaceus* variety was higher than that in cultivated *A. membranaceus* and *A. membranaceus mongholicus* [7]. Hybridization has resulted in new varieties with improved yield and higher concentration of active components [19]. Environmental and genetic factors affect accumulation of bioactive compounds in medicinal plants. The effect of water deficient conditions on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), proline and soluble sugar in *A. membranaceus* were compared in three varieties grown outdoors in pots. The order of drought tolerance in three varieties was Mongolia > wild > Hebei [15]. Astragalus grown in different regions has different therapeutic potency. Ma *et al.* [11] analyzed Astragali Radix from different regions of China and concluded Astragali Radix from Shanxi contained significantly higher amounts of isoflavonoids, saponins and polysaccharide than from other locations. This suggests that growing conditions and varieties greatly affect the pharmacological contents.

Based upon the range in climatic conditions under which *A. membranaceus* is cultivated, including South Korea, we hypothesized that it would be possible to cultivate *A. membranaceus* in the southeastern United States. The objective of this study was to assess the suitability of *A. membranaceus* for cultivation in Alabama and to identify varieties of *A. membranaceus* adapted to Alabama.

2. Materials and Methods

2.1. Site Description

The study site was at Auburn, Alabama (32°35'N, 85°29'W). The mean annual temperate is 18 ºC and annual precipitation is 1400 mm. The site had been uncultivated for the last five years and had a cover of grass and weeds. The soil was classified as Marvyn loamy sand (Fine-loamy, kaolinitic, thermic, Plinthic Kanhapludults). Soil pH was 6.6 in 1:1 soil: water (v/v) and Mehlich-1 extractable soil P, K, Mg and Ca concentration were 14, 60, 64 and 59 mg kg^{-1}, respectively.

2.2. Experimental Design

The experiment was a randomized complete block design with 4 replications. Seven varieties of *A. membranaceus* were obtained from the USDA Germplasm collection, commercial seed companies in the United States, as well as one variety from South Korea and on from Hebei, China (Table 1).

2.3. Field preparation and crop establishment

The field measured 18 m × 25 m. The field was disked in order to incorporate surface vegetation in August 2006. To adjust soil pH to 6.5, ground limestone (2469 kg ha^{-1}) was broadcast on the field on September 19, 2006. Triple superphosphate (39 kg P ha^{-1}) and muriate of potash (37 kg K ha^{-1}) were broadcast and incorporated to assure that these nutrients were not limiting factors for the growth of *A. membranaceus*. Because astragalus is a leguminous plant, it was assumed that N would be provided through symbiotic N fixation.

Raised beds measuring 10 cm high and 91 cm wide were created by first creating a ridge using two disk plows on September 20 and then following with a bedder on September 21. Two in-row subsoilers were mounted ahead of the bedder in order to break up the plow pan. That allowed us to plant so that roots penetrate soil loosened by subsoiler shanks. The plot size was 0.91 m × 5.3 m. On September 22, 2006, all the

plots were covered with landscape fabric and twenty-four 5-cm diameter holes were cut in fabric in each plot. The holes were spaced 41 cm and all the holes were located above the subsoiler path.

Table 1: The origin of *A. membranaceus* varieties.

Accession number	Origin of the seeds
AM1	USDA, PI 515968, 89i (South Korea)
AM2	USDA, W6 22350, 2002i (United States)
AM3	Horizon Herbs, LLC, William, OR 97544
AM4	Elixir Farm Botanical LLC, Brixey, MO 65618
AM5	South Korea
AM6	Johnny's selected seeds, Windslow, Maine 04901
AM7	Anguo, Hebei, China (38°25'N, 115°18'E)

The seeds were soaked in warm water for 24 hours before sowing on September 25. The number of seed applied was adjusted based upon germination rate. Three seeds of AM1 and AM2 were applied per hill, four seeds of AM3 and AM4, ten seeds of AM5, five seeds of AM6 and eight seeds of AM7 were applied per hill. Seeds were covered with less than 0.6 mm soil. The plants were watered daily until emergence except on rainy days. Seedlings were thinned to one plant per hill at about three weeks after planting.

Stand counts were taken on October 5, ten days after planting. The hills where there were no Astragalus plants present were reseeded during October 20 and October 31. On November 21, pine bark mulch was spread on landscape fabric. The landscape fabric and mulch were used to control weed in the field. Hand weed was carried out next to plants. Weeds between the beds were controlled by cultivation and by directed spray of glyphosate (1.9 kg a.i. ha^{-1}).

Following reemergence in the spring, missing plants were replaced by 10-day old seedlings started in the greenhouse. As many seedlings disappeared following insect feeding on underground parts, transplanting continued through March and April 2007. Chlorpyrifos (Duraguard ME, 0.4 g (AI) L^{-1}) was sprayed three times to control soil-borne insects in May, 2007.

2.4. Variables Measured: During the growing period, observations included percent emergence as well as qualitative observations on growth, diseases and insect pests. The dates of flowering, fruit development and fruit maturation were recorded. Plant height was measured from soil surface to the tip of main stem on October 20, 2007 before harvesting in November 2007.

The plants were dug out with shovel and harvested in November 2007. The plants that were planted in October 2006 were labeled 13-month plants; plants that were planted in spring 2007 were labeled 7-month plants. Root branches were counted and the diameter of taproots at the crown and root length were measured. After harvest, fresh weight of the shoots and roots were measured. Shoots were dried in an oven at 65 °C for 2 days. Roots were dried in a forced air dryer at 40 °C for 4 days. After drying, dry weight of the shoots and roots were measured. Roots were cut and separated according to root diameter. The roots whose diameter is less than 2 mm were labeled small-root. The roots whose diameter is greater than 2 mm were labeled big-root. Roots were ground to a fine powder with a Wiley Mill.

Soil samples were collected at the end of the trial on December 7, 2007 and analyzed for pH, N, P, and K.

The aboveground parts of 13-month plants were analyzed for N, P, K, Ca, Mg, Cu, Fe, Zn, Mn and B. Total N was determined by the combustion method using a LECO CHN-600 (LECO Corporation, St. Joseph, MI). ICAP

(Thermo Jarrell Ash, Franklin, MA) to determine P, K, Ca, Mg, Cu, Fe, Zn, Mn and B.

2.5. Measurement of Bioactive Compound

Astragaloside IV was determined by high-performance liquid chromatography with UV-visible detector or with evaporative light-scattering detector. Astragaloside IV reference standard (purity of 95%) and digoxin (purity of 95%) were purchased from Chroma Dex (Irvine, CA) and Sigma-Aldrich (St. Louis, MO, USA) respectively. Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was obtained from a Nanopure water system (Barnstead, Newton, MA, USA).

2.5.1. Apparatus

High-performance liquid chromatography (HPLC) analyses were performed on a Waters HPLC system equipped with two 510 pumps, Automated Gradient Controller, 717 Auto sampler, and 746 Data Module Integrator (Waters, Milford, MA, USA). A Sedex 75 ELS detector (Sedex, Alfortville, France) was used.

2.5.2. Preparation of standard solution

Atragaloside IV (5 mg) was dissolved in methanol in a10-mL volumetric flask. Internal standard (diagoxin, 10 mg) was dissolved in 10 mL methanol. Astragaloside IV and digoxin have been shown to have similar extraction efficiencies in plasma [24]. The calibration curve was prepared from a range of concentrations of astragaloside IV solutions (15-80 µg mL^{-1}), each spiked with a constant concentration (30 µg mL^{-1}) of digoxin. All stock solutions were stored at -20 ºC.

2.5.3. Sample preparation: Finely powdered root (0.5 g) was spiked with I.S. [digoxin stock solution (60 µg)] and extracted with methanol (3 mL). The mixture was vortexed (Fisher), followed by sonication at 25-30 ºC for 15 minutes. Mixtures were centrifuged (1,500 rpm for 10 minutes), and the supernatant collected. Root samples were extracted twice, and and both supernatants combined. Extracts were evaporated (30 ºC) to dryness under nitrogen and dissolved in 2 mL of methanol and filtered through a 0.2 µm nylon filter (Millipore).

2.5.4. Chromatographic conditions

Chromatographic conditions were adapted and modified from Ganzera [5] and Li [8]. For all separations, a SecurityGuard C-18 guard column (Phenomenex, Inc., Torrance, CA, US) preceded separation by a Luna C-18(2) analytic column (150 × 4.6 mm, 5 µm particle size)(Phenomenex, Torrance, CA, US). Mobile phase [acetonitrile (A) and water (B)], was sparged with helium, and pumped as a linear gradient elution: 0-13 min, from 27A/73B to 30A/70B; 13-25 min, from 30A/70B to 38A/62B; 25-35min, from 38A/62B to 70A/30B; 35-40 min, from 70A/30B to 80A/20B; 40-40.5 min, from 80A/20B to 27A/73B; 40.5-45 min, from 27A/73B to 27A/73B. Flow rate of mobile phase was 1 mL/min and injection volume was 10 µL. The ELS detector temperature was 40 ºC, gain was 8 and with a nitrogen pressure of 3.4 bar.

2.5.5. Calibration

Calibration curves were plotted so that peak area ratio equaled peak area of astragaloside IV was divided by peak area of digoxin) versus the concentration of astragaloside IV. Linear regression analysis was calculated by the least squares regression method using the formula Y = a + bx, where Y = peak area ratio and x = concentration of astragaloside IV (µg mL^{-1}). The limit of detection (LOD) and limit of quantification (LOQ) were determined on the basis of response at a signal-to- noise ratio (S/N) of 3 and 10, respectively. The LOD was 6 µg mL^{-1} (60 ng) and LOQ was 12 µg mL^{-1} (120 ng).

2.5.6. Validation

Validation involved calculating precision (intraday variability) determined three times for three samples on one day and accuracy was determined by recovery. Dried powdered root sample was aliquoted into 12 glass vials containing 0.5 g samples. Three replicates were analyzed as described above (each spiked with 60 ug digoxin and extracted twice with 3 ml

methanol) in order to determine the amount of astragaloside IV in the original sample. The remaining 9 aliquots were spiked with astragaloside IV (20, 40 or 60 µg, n=3) and digoxin (60 µg). The three concentrations (in triplicate) resulting from spiking with astragaloside IV were 10, 20, and 30 µg mL^{-1} were used to calculate accuracy and recovery. The variation of precision was evaluated by making one injection from each of the three concentrations in triplicate on a single day. Variations were expressed by the relative standard deviations (R.S.D.) percent, which equals standard deviation divided by mean, multiplied by 100.

The accuracy of this method was determined by measuring recovery of astragaloside IV. Triplicate analyses of three concentrations of astragaloside IV (10, 20, and 30 µg mL^{-1}) was used to calculate recovery. The average recoveries were determined by the following formula: recovery (%) = (measured concentration –original concentration)/spiked concentration × 100%, and R.S.D. values were calculated.

2.5.7. Concentration and Total Content of Astragaloside IV in the Plants

Concentration of astragaloside IV was calculat ed based on the linear calibration curve for astragaloside IV, X = Y + 0.0745/0.0278 (X = concentration of astragaloside IV in the final solution (ug mL-1) ; Y = peak area of astragaloside IV in the final solution. Concentration of astragaloside IV in the plant (µg g^{-1} plant) was calculated according to the formula: concentration of astragaloside IV in the solvent (µg/mL) × 2(mL) weight of sample (g). Total content astragaloside IV (mg $plant^{-1}$) was calculated [(small root weight (mg) × concentration (ug/ml) of astragaloside IV in small root weight) + (big root weight (mg) × concentration (ug/ml) of astragaloside IV in big root weight)] × 0.001.

2.6. Statistical Analysis

Analysis of variance was performed on raw data using version 9.1 of SAS to test main effects and interactions (SAS Institute Inc., 2003). Because of the high mortality rate and resulting poor stands, yield was calculated based upon yield per plant, rather than on an area basis. Parameters were analyzed using PROC MIXED as randomized complete block factors. The treatment means were compared using Duncan's multiple range tests. All statistical tests were made at $\alpha = 0.10$ significance level.

3. Results

3.1. Validation of HPLC Method

Astragaloside IV and the internal standard were separated using HPLC-ELSD. Standards of digoxin (30 µg mL^{-1}) and astragaloside IV (40 ug/ml) were eluted at 9.94 and 23.08 minutes. Ground root spiked with digoxin (30 µg mL^{-1}) was eluted at 9.86 minutes and astragaloside IV was eluted at 23.02 minutes in sample extracted with methanol Over the range of 10 to 80 µg mL^{-1}, linearity of calibration curve for astragaloside IV is y=0.0278x – 0.0745, where y = peak area ratio; x = concentration of astragaloside IV (µg mL^{-1}) (R^2=0.9932). The LOD of 6 µg mL^{-1} (60ng) and LOQ of 12 µg mL^{-1} (120 ng) indicate the sensitivity of this method.

Astragaloside IV spiked root samples were performed to evaluate the intra-day reproducibility. R.S.D. (%) were found to be less than 11.21%, 4.69%, and 2.97% for 10, 20, and 30 µg mL^{-1} spiked sample, respectively. The average recovery of astragaloside IV was 93.23% (n=3)

3.2. Plant Growth Measurements

Percent germination after ten days varied among varieties (Table 2). AM3 had highest germination rate (89%) and AM5 had lowest germination rate (4%). During the winter months (December 2006 through February 2007), the above-ground plant parts died due to frost. In the middle of March, 2007 new leaves came out from the crowns and the plants began to grow again. The percent recovery after winter ranged from 81% to 95% for different varieties (Table 2).

Mortality was high in 2007 and was most severe with small seedlings, but was observed

throughout the season. Mortality was about 70% for AM7 and about 50% for the remaining accessions. Soil-borne insects fed on the root and crown of the seedlings which caused the roots to break. However, those plants that were cut above crown by insects still had the ability to produce new shoots. Larvae collected near the roots of Astragalus seedlings were cultured in the lab and identified as white fringe beetles (*Naupactus* spp.) The height of above-ground parts was only about 10 to 20 cm and the roots were still small and slender at that time. Beyond this stage, root rot, caused by *Pythium* and *Phytophthora* fungi, identified by The Auburn University Plant Diagnostic Lab on June 2007, caused aboveground parts to wilt. Because of high mortality rate and poor stands, all the plants were harvested in late October and November. All of the varieties had flowers and produced seeds except AM7.

Table 2: Percent emergence, percent stands and recovery after winter for each variety of *Astragalus membranaceus.*

	Percent emergence 10 days after sowing†	**Hills having seedlings (%)†**	**Percent recovery‡**
AM1	30.2	60.4	81
AM2	48.0	81.0	91
AM3	89.5	94.7	91
AM4	40.3	81.0	92
AM5	3.7	27.1	95
AM6	71.2	98.9	92
AM7	20.1	60.4	85

† Planted on 9/25/2006 and counted on 10/05/2006 (10 days)

‡ Counted on 3/30/2007

Among 7-month plants, shoot height among varieties differed statistically (Pr=0.010). AM5 had highest shoot height (Table 3). Among 13-month plants, shoot height did not differ statistically among varieties by the F-test, however significant differences were observed with the Duncans Multiple Range Test and AM5 was tallest. Shoot weight tested significant among varieties for both 7-month (Pr=0.004) and 13-month plants (Pr=0.020). AM5, AM2, AM1 and AM4 had highest shoot weight in 7-month plants and AM7 had lowest shoot weight (Table 3). Among 13-month plants, AM4, AM5 and AM2 had highest shoot weights and AM3 had lowest shoot weight.

Table 3: Shoot height and shoot weight of seven *A. membranaceus* varieties.

Shoot height (cm)			**Shoot weight (g plant^{-1})**	
Variety	7 month	13 month	7 month	13 month
AM1	49.3b†	67.5bc	29.4abc	57.0bc
AM2	56.6ab	70.7abc	34.3ab	71.0abc
AM3	51.2b	67.0c	20.9c	55.6c
AM4	51.1b	70.7abc	24.9abc	89.1a
AM5	59.3a	75.2a	36.0a	76.5ab
AM6	49.5b	67.3bc	22.9bc	59.2bc
AM7	40.7c	74.2ab	7.8d	60.6bc

† Columns followed by the same letter are not significantly different by Duncan multiple range test (α = 0.1)

Root weight differed statistically among varieties for both 7-month (Pr=0.029) and 13-month (Pr=0.086) plants. In 7-month plants, root weight was greatest in AM5. With 13-month plants, root weight was greatest in AM7, AM5 and AM4 (Table 4). Root length and number of root branches per plant and root length did not differ statistically in either 7-month or 13-month plants although AM7 and AM2 had longer roots than did AM4 as determined by the Duncan's multiple range test (Table 4). Root diameter differed statistically among varieties for both 7-month (Pr=0.014) and 13-month plants (Pr=0.026). AM5 had the biggest root diameter in 7-month plants, and AM5 and AM2 had the biggest root diameter in 13-month plants (Table 4).

Table 4: Root weight, root length, root diameter and root branch of seven *A. membranaceus* varieties.

	Root weight (g plant^{-1})		Root length (cm)		Root diameter (mm)		Root branch (number plant^{-1})	
Variety	7 month	13 month	7 month	13 month	7 month	13 month	7 month	13 month
AM1	14.7bc†	23.4b	38.1a	54.0ab	11.2b	18.2c	10.5a	10.0a
AM2	18.4ab	26.6b	39.9a	60.2a	11.7b	21.9ab	10.2a	9.4a
AM3	14.2bc	24.6b	40.4a	53.5ab	11.1b	18.7c	9.3a	9.8a
AM4	15.9bc	28.3ab	41.2a	51.0b	12.1b	19.4bc	11.1a	9.0a
AM5	24.2a	29.6ab	41.4a	57.2ab	15.5a	23.6a	12.5a	10.1a
AM6	16.3bc	20.6b	43.7a	58.6ab	11.2b	20.0bc	11.8a	8.9a
AM7	9.6c	37.9a	37.6a	60.8a	7.7c	20.4bc	8.6a	8.9a

†Columns followed by the same letter are not significantly different by Duncan multiple range test (α = 0.1)

Table 5: Concentration of elements in above-ground portion of 13-month plants of *Astragalus membranaceus* varieties.

Variety	N %	P %	K %	Ca %	Mg %	Cu mg kg^{-1}	Fe mg kg^{-1}	Mn mg kg^{-1}	Zn mg kg^{-1}	B mg kg^{-1}
AM1	1.98a†	0.15a	1.37a	1.00a	0.28a	10.13a	343.20a	34.08b	18.9ab	7.9a
AM2	1.49a	0.12a	1.15a	051a	0.14a	6.99a	63.13a	17.24c	14.76bcd	1.93a
AM3	1.54a	0.12a	1.18a	0.55a	0.14a	12.39a	70.67a	27.10bc	16.06bc	8.01a
AM4	1.65a	0.13a	1.20a	0.59a	0.16a	5.32a	69.04a	21.40c	13.73cd	1.77a
AM5	1.60a	0.14a	1.10a	0.72a	0.21a	7.92a	88.23a	35.29b	19.04ab	7.72a
AM6	1.46a	0.10a	1.15a	0.57a	0.17a	3.62a	68.81a	19.23c	11.62d	2.85a
AM7	2.10a	0.16a	1.57a	0.56a	0.17a	8.56a	195.35a	51.13a	21.05a	6.63a

†Columns followed by the same letter are not significantly different by Duncan multiple range test (α = 0.1)

No significant differences among varieties were observed in concentration of N, P, K, Ca, Mg, Cu, Fe, and B in above-ground plant parts (Table 5). Concentration of Mn and Zn differed significantly among varieties, at Pr=0.001 and Pr=0.062, respectively (Table 5). AM7 had highest concentration of Mn and Zn. AM2 had lowest concentration of Mn and AM6 had lowest concentration of Zn.

3.3. Astragaloside IV content: Significant differences among varieties were observed in concentration of astragaloside IV in big and small roots of both 7-month and 13-month plants

(Table 6). In big roots of 7-month plants, AM4 had significantly higher concentration of astragaloside IV than other varieties with 64.5 µg g^{-1}, and AM7 had lowest concentration of astragaloside IV with 22.1 µg g^{-1}. In big roots of 13-month old plants, AM3 had significantly higher concentration of astragaloside IV than other varieties with 172.9 µg g^{-1}, while AM7 had the lowest concentration of astragaloside IV with 21.3 µg g^{-1}. In small roots of 7-month plants, AM4 had highest concentration of astragaloside IV with 204.3 µg g^{-1} and AM7 had lowest concentration of astragaloside IV with 34.0 µg g^{-1}. AM4 did not differ significantly from AM1, AM3 and AM5. In small roots of 13-month plants, AM3 and AM2, with 320.8 µg g^{-1} and 297.5 µg g^{-1} had significantly higher concentration of astragaloside IV than did AM6 and AM7. AM7 had lowest concentration of astragaloside IV with 100.1 µg g^{-1} (Table 6).

Table 6: The effect of variety on concentration of astragaloside IV.

Variety	Big 7-month µg g^{-1}	Big 13-month µg g^{-1}	Small 7-month µg g^{-1}	Small 7-month µg g^{-1}
AM1	37.2bc†	40.6cd	139.8ab	250.0ab
AM2	47.4b	44.2cd	119.0b	297.5a
AM3	45.1b	172.9a	148.1ab	320.8a
AM4	64.5a	85.6bc	204.3a	237.2ab
AM5	28.8cd	113.1b	161.0ab	179.8ab
AM6	41.7b	48.8cd	103.5bc	269.3bc
AM7	22.1d	21.3d	34.0c	100.1c

† Columns followed by the same letter are not significantly different by Duncan multiple range test (α = 0.1)

Table 7: The effect of variety on total content of astragaloside IV per plant.

Variety	7-month (mg $plant^{-1}$)	13-month (mg $plant^{-1}$)
AM1	0.482bc†	1.58ab
AM2	0.931ab	1.91ab
AM3	0.824abc	3.17a
AM4	1.24a	2.76ab
AM5	0.637bc	1.11ab
AM6	0.875abc	1.79ab
AM7	0.417c	0.84b

†Columns followed by the same letter are not significantly different by Duncan multiple range test (α = 0.1)

The mean total content of astragaloside IV in 7-month plants differed statistically. AM4 had highest total content of astragaloside IV, followed by AM2 and AM3, and AM7 had lowest total content of astragaloside IV (Table 7). The average of total content of astragaloside IV in 13-month plant did not differ statistically as determined by the F test. However, the Duncan test revealed that AM3 had significantly higher total content of astragaloside IV than did AM7. AM7 had lowest total content of astragaloside IV (Table 7).

4. Discussion

The seeds of *A. membranaceus* are very tiny, about 2-5 mm long and the germinated seeds had

difficulty emerging if more than 0.7 cm soil covered them. Frequent irrigation was necessary to facilitate emergence. Although poor germination rate for some varieties was compensated in part by a higher seeding rate it was inadequate to achieve acceptable stands of this variety. The low germination rate of AM5 (4%) was probably because the seeds, obtained from South Korea, may have been stored for a long time. In spring 2007, seedlings were established from fresh seeds and transplanted in the field. At harvest, there were many more plants of AM5 that were 7-month old than 13-month old plants.

Although *A. membranaceus* belongs to the legume family and can fix nitrogen through nodules, we did not find nodules on roots in the field. It is apparent that rhizobia compatible with *A. membranaceous* were not present in the soil. Limited publications were found about the nodulation of *A. membranaceus*. Weir [18] inoculated *A. membranaceus* with *Mesorhizobium* spp for other Astragalus species that had never been tested on A. *membranaceus* and obtained effective nodules.

For leguminous plants, the critical value of nitrogen is 3 to 4.25 percent, phosphorus is 0.20 to 0.25 percent, potassium is 1.75 to 2.00 percent [1]. The concentration of N, P and K in variety (all the seven varieties) was lower than those critical values and the concentrations of Ca, Mg, Cu, Fe, Mn, B and Zn were in the normal ranges (Table 5).

In this study, only phosphorus and potassium fertilizer were applied in field and no nitrogen was applied, because we assumed that *A. membranaceus* would be self-sufficient in N through N fixation. Zhao [23] reported that the best ratio of nitrogen, phosphorus and potassium in applied fertilizer was in the range of 1:0.8-1.2 for N: P_2O_5 and 1:1.2-1.8 for N:K2O, which indicated that nitrogen application was recommended for the cultivation of *A. membranaceus*. The nutrients in the field were unbalanced for the growth of *A. membranaceus*. According to the Liebig's Law of the Minimum, yield is proportional to the amount of the most limiting nutrient. Nitrogen was probably the most deficient nutrient element for the growth of *A. membranaceus*, so its deficiency limited root growth and shoot growth. Nitrogen deficiency may have affected uptake of other elements.

In 2007, we inoculated seed in the greenhouse with (*Rhizobium* spp.) specific to *Astragalus* genus. After 45 days, we observed nodules with pink centers on the seedling roots. This suggests that *Rhizobium* bacteria appropriate to *Astragalus* was not present in the field in Auburn, but that N fixation may be achieved with inoculation with appropriate Rhizobia.

Mortality was high and about 50-60% plants died in spring and summer 2007. Root rot, caused by *Pythium* and *Phytophthora* fungi and these fungal organisms were often found where soil is kept continually wet. This suggests that *A. membranaceus* has poor adaptation to wet soils and care should be taken to avoid overwatering after the seedlings have been established.

High quality roots should have long root length, big root diameter and few branches [9]. No variety in this experiment possessed all three root characteristics. Among 7-month plants, AM5 had greatest and AM7 had lowest shoot weight, root weight and root diameter. Among 13-month plants, AM7 had greatest root weight and root length and AM5 had biggest root diameter (Table 6). Within 13-month plants, AM4 had lowest root length and AM1 had lowest root diameter (Table 4).

Although AM7 had greatest root weight and root length, its mortality was higher than other varieties in the field (70% vs 50%). AM7 was susceptible to the disease caused by *Pythium* and *Phytophthora* and only 4 or 5 plants of AM7 were left alive at harvest in some plots, which suggests that AM7 had lower adaptability than other varieties to southeast US. AM7 originated from Anguo, Hebei (38°25'N, 115°18'E), China, where the annual precipitation is 300-800 mm, average January temperature is -7 ºC and July temperature is 18-27 ºC. The annual precipitation in Auburn is 1340 mm, average January temperate 8.5 ºC and July temperature 26 ºC. Especially the summer in Auburn is humid with high temperature. Those differences of temperature and humidity probably made AM7

less able to adapt to the new environment. AM7 also failed to produce flowers and pods and therefore cannot reproduce in this environment.

AM5 was purchased from South Korea and grew very well in Auburn, Alabama. South Korea is in monsoonal region, summer is hot and humid and rainfall is over 1000 mm, which is similar to the summer in Auburn. AM5 achieved high yield and superior root characteristics, such as root diameter compared to other varieties tested. Considering all of the study results, AM2, AM3, AM4 and AM5 had good adaptability, root weight (yield) and root quality.

A higher concentration of astraloside IV was found in 13-month plants than in 7-month plants, such as the big roots of AM1, AM3, AM4, AM5 and AM6 varieties. The concentration of astragaloside IV was higher in roots of 13-month plants than in roots of 7-month plant, is consistent with Zhang *et al.* [24], who reported that concentration of astragaloside IV increased with age. This suggests that astragaloside content would have been higher had the experiment been extended.

The higher concentrations of astragaloside IV in small roots than in big root for the same growing period and variety is consistent with Anetai et al [2], who assayed the taproots, and thick and thin lateral roots of *A. membranaceus* for astragaloside IV concentration by HPLC. They reported that the concentration of astragalosides IV was much higher in thin (small) roots and the thin (small) lateral root than taproot (big) and thick (big) lateral roots [2]. However, the small root only accounted for a small part (about 12.9% for 7-month plant and 10.7% for 13-month plant) of total root weight and the content of astragaloside IV in small roots did not markedly affect total content of astragaloside IV in Astragalus roots.

The concentration of astragaloside IV in the commercial sample was 134 μg g^{-1}. The commercial sample was sliced from big roots (diameter > 2 mm), therefore it should be compared with the large roots from our sample. Although the concentration of astragaloside IV in the commercial sample was higher than in the big roots of 7-month plants of all the varieties and most varieties at 13 months, it was lower than the concentration of astragaloside IV in AM3 at 13 months (Table 6). However, AM4 (85.6 μg g^{-1}) and AM5 (113.1 μg g^{-1}) also had acceptable concentration of astragaloside IV, based on the commercial sample. It is noteworthy that the concentration of astragaloside in small roots of most varieties was higher than the concentration in the commercial sample (Table 6). These results imply AM3, AM4 and AM5 have the potential to produce significant amounts astragaloside IV in the roots.

The total content of astragaloside IV was highest in AM3 in 13-month plants and 7-month plants was highest inAM4. AM7 had the lowest total content of astragaloside IV in both 7- and 13-month plants. After one year growth, AM3 had higher concentration and content of astragaloside IV than did other varieties. Therefore, AM3 had the best root quality in terms of astragaloside content.

5. Conclusions

The main constraints for cultivation of *Astragalus membranceous* in Alabama related to soil-borne insect pests and diseases, and also lack of N-fixing rhizobium specific to *Astragalus* species. The latter can easily be addressed with inoculants appropriate to *Astragalus* spp. No physiological problems were observed with growing *A. membranaceous* in Alabama. Plant mortality was most severe with small seedlings when white fringe beetles were feeding on roots and crowns, but happened throughout the season as plants succumbed to root rot. Under high soil moisture and wet conditions, *Astragalus* is susceptible to root rot caused by fungi, which is the main constraint to cultivation in the southeastern U.S. Land must be well drained for *Astragalus*. Loose soil and raised beds can be used to control soil moisture. The soil structure of Coastal Plain soils is also of concern with a hard pan and poor soil structure, especially when a rototiller is used to prepare the seedbed.

Despite many problems, this experiment demonstrated that astragaloside concentration as well as adaptation may be improved through selection. The varieties, AM2, AM3, AM4 and

AM5 had the best adaptability in terms of root weight (yield) and root quality relatively high concentration of astragaloside IV in the roots and and have potential for cultivation in the southeastern US. However, overcoming root rot disease and control of the white fringe beetle is necessary before *Astragalus membranceous* may be considered as a suitable crop for the Southeastern U.S

6. References

1. AESL Plant Analysis Handbook - Nutrient Content of Plant. Agricultural and Environmental Services Laboratory, University of Georgia. http://aesl.ces.uga.edu/publications/plant/Nutrient.asp. Verified 1/1014.
2. Anetai M, Kanetoshi A, Shibata T, Iida O, Hatakeyama Y. Chemical evaluation of astragali radix prepared from Astragalus mongholicus BUNGE cultivated in Hokkaido. Natural Medicines 1996; 50(2):163-169.
3. Block KI, Mead MN. Immune system effects of echinacea, ginseng, and astragalus: a review. Integrative Cancer Therapies 2003; 2(3):247-67.
4. Chen SL, Wei JH, Sun CZ, Liu ZQ, Zhao RH. Development of TCMGIS-I and its application in suitable producing area evaluation of *Astragalus membranaceus*. World Science and Technology/Modernization of Traditional Chinese Medicine and Material Medica 2006; 8(3):47-53.
5. Ganzera M, Bedir E, Calis I, Khan IA. Separation of Astragalus saponins by reversed phase high performance liquid chromatography and evaporative light scattering detection. Chromatographia 2001; 53(3/4):131-134.
6. He ZQ, Findlay JA. Constituents of *Astragalus membranaceus*. Journal of natural products 1991; 54(3):810-815.
7. Jiang WX, Ge KR, Xue BY. Study on comparing different kinds of efficiency component extracted from three kinds of Astragalus. Journal of Harbin University of Commerce (Natural Sciences Edition) 2004; 20(4):387-389.
8. Li W, Fitzloff J. Determination of Astragaloside IV in Radix Astragali (Astragalus membranaceus var. monghulicus) using high performance liquid chromatography with evaporative light-scattering detection. Journal of Chromatographic science 2001; 39:459-462.
9. Liu J, Wang LX. Study on interrelation concerning the species and soil condition to Radix Astragali Root's pharmacognosical properties. Chinese Wild Plant Resource 1996; 3(4):1-4.
10. Ma XQ, Duan JA, Zhu DY, Dong TX, Tsim KWK. Species identification of Radix Astragali (Huangqi) by DNA sequence of its 5S-rRNA spacer domain. Phytochemistry 2000a; 54:363-368.
11. Ma XQ, Duan JA, Zhu DY, Dong TX, Tsim KWK. Chemical comparison of Radix Astragali (Huangqi) from different regions of China. Natural Medicine 2000b; 54(5):213-218.
12. Ma XQ, Shi Q, Duan JA, Dong TX, Tsim KWK. Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations. Journal of Agricultural and Food Chemistry 2002; 50(17):4861-4866.
13. Matkowski A, Wozniak D, Lamer-Zarawska E, Oszmianski J, Leszczynska A. Flavonois and phenol carboxylic acids in the oriental plant *Astragalus membranaceus* acclimated in Poland. Z Naturforsch 2003; 58:602-604.
14. McKenna D, Hughes K, Jones K. Astragalus. Alternative Therapies 2002; 8(6):34-40.
15. Tan Y, Liang ZS, Shao HB, Du F. Effect of water deficits on the activity of anti-oxidative anzymes and osmoregulation among three different genotypes of Radix Astragali at seedling stage. Colloids and surfaces B: Biointerfaces 2006; 49:60-65.
16. Thwe AA, Thanh MNT, Li X, Kim Y, Kim YB, Uddin MR *et al*. Production of astragaloside and flavones from adventitious root cultures of *Astragalus membranaceus* var. mongholicus. Plant Omics Journal 2012; 5(5):466-470.
17. The State Pharmacopoeia Commission of P.R. China. Pharmacopoeia of People's Republic of China. Chemical industry press. Beijing, China. 2000, 161.
18. Weir B. Systematics, Specificity, and Ecology of New Zealand Rhizobia. Ph.D. dissertation. University of Auckland, Auckland, New Zealand. 2006.
19. Xie XL, Wang XS, Zhao L, Wang L, Li Y. Review of studies on germplasm resources of Radix Astragali. Journal of Anhui Agri Sci 2005; 33(1):121–123.
20. Yu ZK, Liu XJ. Studies of activity constituents of *Astragalus membranaceus*. Journal of plant resources and environment 1993; 2(4):40-43.
21. Zhang SY, Piao HS, Song CY. Study on the relation between duration of cultivation of plant

and content of chemical components in Astragalus. Journal of Medical Science Yanbian University 2005; 28(2):87-89.
22. Zhao YZ. Taxonomy and floristic geographical distribution of the Chinese medicinal Huangqi. Bulletin of Botanical Research 2006; 26(5):532-538.
23. Zhao YZ, Yin HG, Zhang HP. The bulletin on the N, P, K fertilizer for HuangQi. Chinese Agriculture Science Bulletin 2002; 18(4):113-116.
24. Zhang W, Zhang C, Liu R, Li H, Zhang J, Mao C. Quantitative determination of Astragaloside IV, a natural product with cardioprotective activity, in plasma, urine, and other biological samples by HPLC coupled with tandem mass spectrometry. Journal of Chromatography 2005; 822( ):170-177.
25. Zheng ZR, Liu D, Song CQ, Cheng CX, Hu ZB. Studies on chemical constituents and immunological function activity of hairy root of *Astragalus membranaceus*. Chinese Journal of Biotechnology 1998; 14(2):93-97.