GROWTH AND CAMPTOTHECIN CONCENTRATIONS OF 18 CAMPTOTHECA *ACUMINATA* SEED SOURCES FROM ITS NATIVE DISTRIBUTION

Zhijun Guomo Zhou,[2] and Shaoyuan Xu2

Camptotheca acuminata, a deciduous tree indigenous to southern China, contains camptothecin (CPT). FDA approved two CPT derivatives in 1996 for treating ovarian and colorectal cancer. Currently, manufacturing of the two anti-cancer drugs continues to rely on extraction from plant materials that are primarily harvested from naturally grown trees. There is no plantation production in an agricultural setting to supply the plant materials known in the U.S. or in China. The medicinal plant research program at the LSU Agricultural Center initiated a study using *Camptotheca acuminata* to develop a production system. Cultivation of *C. acuminata* not only presents cropping opportunities but also allows the input of effective production management that offers superior quality raw materials to the currently available natural sources. Since 1993, plantations of *C. acuminata* have been grown in southern Louisiana and extensive growth studies have been conducted aimed at finding cultural practices to enhance the levels of CPT. The plantations, however, were established with propagules from a single tree that is perhaps the offspring of an earlier introduction program in USDA. Therefore, the genetic base is narrow. C. *acuminata,* however, has a broad range of natural distribution in China, covering almost all areas south of the Yangtze River. The vast distribution area led the researchers to believe that natural variations in terms of growth and camptothecin concentrations exist. Consequently, a joint project was conducted to identify the variations associated with geographical source of C. *acuminata.*

Within its natural distribution, eighteen seed sources were collected from 10 provinces south of the Yangtze River (Figure 1). Seed-bearing trees aged 20 to 29 years were selected from a large local area of each seed source in November. The collected seeds were air-dried in shade and stored in a well-ventilated room. From each seed source, 200 seeds were randomly selected for seed quality test indicated by their purity, moisture content, thousand-seed weight, soundness percentage, germination rate, germination potential, average germination time, and nursery germination percentage.

In Huzhou City, Zhejiang, China, nursery beds 1 m wide and 18 m long were prepared. The area has a characteristic seasonal weather with an annual precipitation of 1050 to 1850 mm, annual average temperature of 12.2°C to 16.1°C with the coldest month in January (-0.3 to 3.6°C) and extreme low temperature reaching –17.4°C, and 224 - 246 days frost-free. The soils had pH 5.2 to 5.5, bulk density 0.83 to 1.25, a moderate amount of organic matter and nitrogen concentration, rich phosphorus and potassium, and good drainage. Each bed (block) was subdivided into 18 1-meter long mini-plots. Seed sources were randomly assigned to these mini-plots. The experiment was designed as a randomized complete block with 12 blocks (beds) distributed on 5 plots with the same experiment design and arrangement on each plot.

On each seedbed, three rows were drilled at even spacing and seeds were sowed in mid-March at approximately 5-cm intervals within each row. A layer of finely sieved and uncultivated subsoil was placed to cover the seeds 1.5-cm deep. After sowing, a thin layer of straw was used as mulch to cover the seedbeds.

School of Forestry, Wildlife and Fisheries, LSU Agricultural Center, Baton Rouge, La. 70803 USA

[2] Zhejiang Forestry College, Lin'an, Zhejiang 311300, People's Republic of China.

Seedlings were fertilized in late June with undiluted urea (15.0 g N m^{-2}) in combination with soil loosening, in mid-August with compound fertilizer (N-P-K), and in early September with urea (15.0 g N m^{-2}). From March to May the plots were irrigated whenever there was no precipitation for over 15 days. Seedlings were thinned 3 times to space the seedlings to final within-row intervals of 12.5 cm.

Six sample seedlings were selected randomly from each mini-plot (a seed source) of five randomly selected beds. A total of 540 seedlings were thus selected representing 18 seed sources, 6 replicates and 5 blocks. For each sample seedling, periodic increments in stem height and diameter at the base were measured twice a month from May to October. At the end of the growing season, seedlings were harvested for biomass determinations of leaves, shoot (main stem and branches), roots, and bark. Plant materials were oven-dried at 70°C for 48 hours and the dry weight of each component was obtained. For each sample seedling, three leaves from the positions 4, 5, and 6 from the apex were harvested. These leaves were then combined within each block to make a bulk leaf sample representing a seed source for each of the 18 seed sources. The leaves were oven-dried, ground to fine powder and stored in plastic bags until chemical analyses for CPT concentrations.

CPT concentrations were determined using a high-performance liquid chromatography system (Beckman Instruments, Inc., Fullerton, CA, USA) consisting of a Model 125 pump, a Model 168 photodiode-array detector with a Beckman ODS C_{18} reverse-phase column (25 cm x 4.6 mm), and a Model 502 autosampler. Approximately 1 g of dried leaf material was extracted three times with 50 ml of methanol each for 16 h at 25°C. The 150-m1 extract solution was condensed to 12 ml with a rotary evaporator (Brinkmann Instruments, Inc., Westbury, NY, USA). The condensed crude solution was filtered with a Maxi-clean C_{18} filter (Alltech Associates, Inc., Deerfield, IL, USA) and 0.2 syringeless filters (Whatman Inc., Fairfield, NJ, USA) before being transferred to 2-ml vials. Its specific absorption spectrum between 210 and 250 nm (Figure 2A) and retention time at 6.86 min identified CPT after the elution (Figure 2B). CPT concentration is expressed as a percentage of tissue dry weight.

The height growth followed a typical "S"-shaped pattern. In the beginning of the growing season, height increment was negligible. Next came the slow growth period from June 27 to August 11. In the rapid growth period that followed, stem height increased at an average of 23 cm every half-month, triple the increment rate of the previous growth period. After the rapid growth period, the stem height increment declined and terminal buds set in mid-October.

Significant differences in biomass production were found among the 18 seed sources in the provenance test (Table 1). SS-4 and SS-8 attained greatest whole-plant biomass, whereas SS-3 gained only half of that of SS-4 and SS-8. The two seed sources that produced the greatest shoot biomass were SS-4 and SS-8 (average 22.5 g per seedling), whereas the two smallest were SS-2 and SS-3 (average 10.5 g per seedling). SS-8 produced the most leaves at an average of 19.3 g per seedling, whereas SS-17 and SS-2 produced the least leaf biomass of 8.3 g and 8.0 g, respectively. For root biomass production, SS-4 and SS-9 topped the list among the 18 seed sources attaining an average of over 13 g per seedling, whereas SS-15 and SS-3 produced only half of that amount. Biomass accumulated as bark was also significantly different among the 18 seed sources. More bark was produced by SS-4 and SS-8 (average 4.0 g per seedling) than SS-6 and SS-3 (average 2.5 g and 2.1 g per seedling, respectively).

Leaf CPT concentrations were significantly different among the 18 seed sources tested. The top five seed sources were SS-15, SS-1, SS-14, SS-18, and SS-13 displaying concentrations over 0.11% on a dry weight basis (Table 1). The lowest CPT concentration was found in the leaves of SS-2 seedlings, showing only 0.034% of dry weight, almost 3-fold less. Ten seed sources displayed leaf CPT concentrations over 0.08%, and two seed sources had a leaf CPT concentration around 0.07%.

The products of leaf biomass and CPT concentrations obtain CPT contents in leaves. The top five seed sources that were highest in leaf CPT content were seen in SS-8, SS-15, SS-12, SS-13, and SS-11 (Table 1). The highest leaf CPT content in SS-8 was attributed largely to its greatest leaf biomass production, whereas the second highest leaf CPT content in SS-15 was largely due to its highest CPT concentration. Closely comparing these two seed sources, it is found that SS-15 is 9% less than SS-8 in leaf CPT content per seedlings, but SS-8 needs 34% more biomass to maintain a 9% difference.

Based on the CPT content (mg per seedlings), the top five seed sources were SS-8, SS-15, SS-12, SS-13, and SS-11. The top five seed sources that achieved the greatest whole plant biomass were SS-8, SS-4, SS-11, SS-12, and SS-9. The top five seed sources based on leaf CPT concentration were SS-15, SS-1, SS-14, SS-18, and SS-13. Since leaves are to be used as the target plant materials, basing on the findings of this study, top five seed sources were identified. These seed sources warrant further growth studies to determine the optimal growth conditions for accumulating CPT.

Table 1. CPT yield as a product of leaf biomass and CPT concentration in 18 C. acuminata seed sources collected in southern China.

Seed source	Biomass/Rank (g)	CPT concentration (% dry wt)	CPT content (mg/seedling)
SS-1	11.19 (11)	**0.114** (2)	12.76 (7)
SS-2	7.96 (18)	0.034 (17)	2.71 (18)
SS-3	8.96 (16)	0.072 (14)	6.45 (17)
SS-4	**15.50** (5)	0.081 (13)	12.56 (9)
SS-5	9.97 (15)	0.076 (14)	7.58 (16)
SS-6	10.86 (14)	0.090 (10)	9.77 (14)
SS-7	10.91 (13)	0.095 (7)	10.36 (13)
SS-8	**19.33** (1)	0.086 (11)	**16.62 (1)**
SS-9	**16.04** (4)	0.069 (16)	11.07 (11)
SS-10	11.96 (10)	0.092 (8)	11.00 (12)
SS-11	**16.71** (3)	0.083 (12)	**13.87 (5)**
SS-12	**16.81** (2)	0.086 (11)	**14.46** (3)
SS-13	13.61 (7)	**0.102 (5)**	**13.88** (4)
SS-14	11.08 (12)	**0.112** (3)	12.41 (10)
SS-15	12.71 (8)	**0.119 (1)**	**15.12** (2)
SS-16	14.45 (6)	0.091 (9)	13.15 (6)
SS-17	8.27 (17)	0.097 (6)	8.02 (15)
SS-18	12.41 (9)	**0.103** (4)	12.78 (8)

Figure 1. Eighteen seed sources of C. *acuminata* were collected from 10 provinces south of Yangtze River. Provenance tests were conducted in Huzhou City, Zhejiang Province, China.

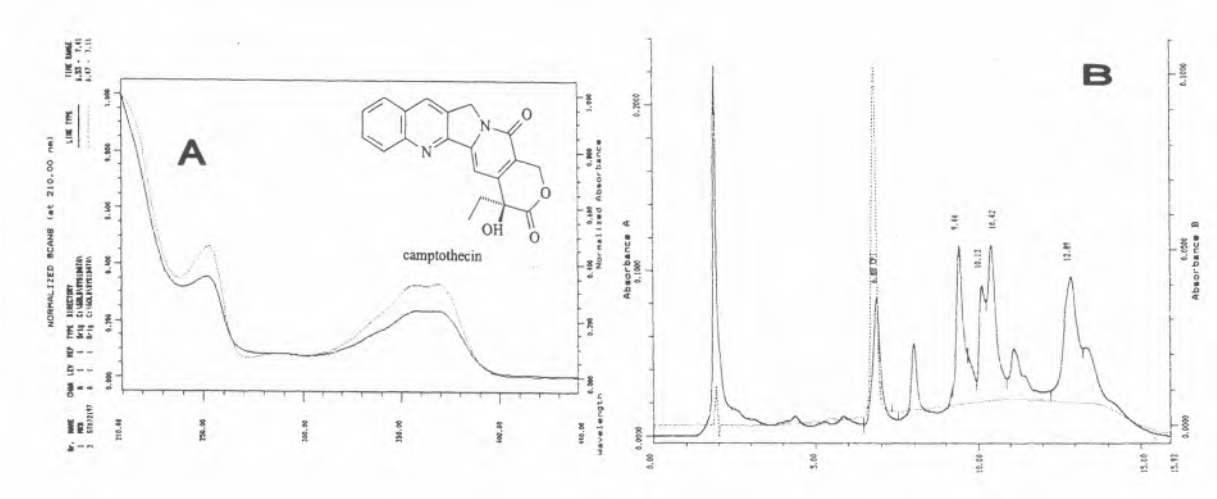

Figure 2. CPT absorption spectra between 210 and 450 nm in standard (dotted line) and sample (solid line) solutions (A) and HPLC trace illustrating the retention time of CPT at 6.86 min for standard CPT (dotted line) and a leaf sample (solid line) of C. *acuminata (B).*