Tropical Life Sciences Research, 22(2), 37–43, 2011

Effects of Environmental Factors on Growth and Artemisinin Content of *Artemisia annua* L.

[1]Bui Thi Tuong Thu, [1]Tran Van Minh, [2]Boey Peng Lim and [3]Chan Lai Keng*

[1]Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
[2]School of Chemical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia
[3]School of Biological Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

Abstrak: Biji benih bagi dua klon *Artemesia annua* yang terpilih, TC1 dan TC2, dicambah dalam rumah hijau. Anak benih berumur empat minggu bagi kedua-dua klon ini ditanam di provinsi Thù Đúc di bandar Ho Chi Minh, pada 2 Januari 2009 dan Đà Lat pada 20 Januari 2009. Dalam jangka masa kajian di provinsi Thù Đúc, yang terletak 4–5 m atas paras air laut, telah mengalami musim tropika yang kering dengan suhu 26.2°C–32.8°C. Manakala Đà Lat yang terletak di 1500–2000 m atas paras air laut mempunyai musim temperat yang kering dengan suhu yang lebih rendah, iaitu 10.5°C–18.0°C. Suhu tinggi dan paras tanah yang rendah di provinsi Thù Đúc telah mengakibatkan kekurangan pertumbuhan vegetatif kesemua tumbuhan kedua-dua klon dan kandungan artemisinin dikurangkan secara bererti. Cuaca persekitaran temperat di Đà Lat menyokong pertumbuhan yang kuat dan sihat dengan ketinggian pokok dan panjang ranting yang 4–5 kali lebih tinggi dan panjang berbanding dengan pokok yang ditanam di provinsi Thù Đúc. Kandungan artemisinin dalam *A. annua* yang ditanam di Đà Lat adalah 3–4 kali lebih banyak daripada pokok yang ditanam di provinsi Thù Đúc. Maka kajian ini telah menunjukkan bahawa kevarianan dalam pertumbuhan pokok dan kandungan artemisinin adalah disebabkan oleh kesan suhu kerana kedua-dua klon yang terpilih adalah homogenus dari segi genetik. Cuaca sejuk di Đà Lat didapati sesuai untuk penanaman *A. annua* berbanding dengan cuaca tropika di provinsi Thù Đúc.

Kata kunci: Artemisinin, Faktor Persekitaran, Klon Terpilih, Pertumbuhan Vegetatif

Abstract: Seeds of two selected clones of *Artemisia annua* L., TC1 and TC2, were germinated in a greenhouse. Four-week-old seedlings from both clones were grown in the Thù Đúc province of Ho Chi Minh City on 2nd January 2009 and Đà Lat on 20th January 2009. During this study period in Thù Đúc province, which is situated 4–5 m above sea level, was experiencing a tropical, dry season with temperatures ranging from 26.2°C–32.8°C. Đà Lat, situated at 1500–2000 m above sea level, was having temperate, dry season with lower temperatures, ranging from 10.5°C–18.0°C. The high temperatures and low elevation in Thù Đúc Province led to slow vegetative growth for all of the plants from the two different clones and the artemisinin contents were significantly reduced. The temperate environment of Đà Lat supported robustly growing plants, with plant heights and branch lengths 4–5 times taller and longer that those planted at Thù Đúc Province. The artemisinin contents of *A. annua* planted at Đà Lat were 3–4 times greater than those cultivated at Thù Đúc Province. Hence, this study indicated that the variations observed in plant growth and artemisinin contents were due to temperature effects because the two

*Corresponding author: lkchan@usm.my

selected clones were genetically homogenous. The cold weather of Đà Lat was suitable for planting of *A. annua* as opposed to the tropical weather of Thù Đúc Province.

Keywords: Artemisinin, Environmental Factor, Selected Clone, Vegetative Growth

INTRODUCTION

Artemisia annua L. (Asteraceae), commonly known as annual wormwood, sweet wormwood or sweet annie, is an annual herb native to Asia and most likely originated from China. Wild populations can be found in China and Vietnam. It has been introduced for cultivation in high-altitude regions or regions with pronounced cool periods, such as Brazil, Cameroon, Ethiopia, India, Kenya, Mozambique, Myanmar, Tanzania, Thailand, Uganda and Zambia (Simon *et al.* 1990).

A. annua has been traditionally grown in China for the treatment of fevers. Since artemisinin, a rare sesquiterpene lactone endoperoxide, was isolated from *A. annua*, it has been used for the treatment of malaria, Hepatitis B and parasites that cause schistosomiasis (Mueller *et al.* 2000; Dhingra *et al*. 2000, Utzinger *et al.* 2001). It has also been reported to be effective against various cancer cell lines, including breast, lung and colon carcinomas and leukaemia (Efferth *et al*. 2001; Singh & Lai 2001). At present, artemisinin is the drug recommended by the World Health Organization (WHO) to treat malaria. Combinations of artemisinin and other anti-malarial drugs, such as mefloquine or lumefantrine, have been proven to be highly effective against the multidrug-resistant *Plasmodium falciparum* (Price *et al.* 1996; van Vugt *et al.* 2000). The chemical synthesis of artemisinin is complicated, uneconomical and produces low yields. The complete biosynthetic pathway of artemisinin and some of its precursors are not fully understood at this time. Hence, plant sources remain as the only alternative for obtaining artemisinin. Screening of artemisinin in many *Artemisia* species indicated that only *A. apiacea* and *A. annua* contain artemisinin (Liersh *et al.* 1986). However, the amounts found in these plants were very low and varied according to growing conditions, different seasons and geographic locations (Abdin *et al.* 2003). Variations in artemisinin contents were reported in different plant parts, different stages of vegetative growth and strain origins (Laughlin 2002). *A. annua* from Italy was reported to contain only 0.04% to 0.05% artemisinin dry weight. Artemisinin contents from other European origins were reported to range from 0.03% to 0.22% (Charles *et al.* 1991), while those obtained from China varied from 0.01% to 0.50% dry weight (Klayman 1985). This paper reported the effects of the different environmental conditions in two different locations in the southern part of Vietnam on the vegetative growth and yield of artemisinin in *A. annua* of Vietnamese origin.

MATERIALS AND METHODS

Field Planting and Growth Characteristic Study of *A. annua*

Seeds of two selected high-yielding clones of *A. annua* (TC1 and TC2) were obtained from the Pharmacy Research Centre at Hanoi, Vietnam. Both clones had the same characteristic of late flowering and contained 1%–2% artemisinin. The seeds of TC1 and TC2 were germinated in a greenhouse at both locations. Four-week-old seedlings of uniform sizes were selected and transplanted to the Thù Đúc province of Ho Chi Minh City on 2nd January 2009, and Đà Lat on 20th January 2009. Twenty plants from each clone were planted in each plot with 14 cm spacing between plants and 22 cm between rows. Ten plots were used for each clone and were planted according to two samples paired case. The plants were fertilised with NPK fertiliser (15:15:15) at a concentration of 1.0 g/l twice a week and watered daily to ensure optimal growth. The plant heights, lengths of the second branches from the root systems, total numbers of main branches and numbers of days when the plants started to produce flower buds were determined. The plant heights and branch lengths were measured with a measuring tape to 1 mm accuracy. The aerial plant parts were then harvested and air-dried until constant weights were attained. The collected data were analysed using Student's t-test at $p \leq 0.05$.

Determination of Artemisinin Content

Ten plants from each clonal population from each location were sampled randomly for the determination of total artemisinin. The dried material (100 mg) from each plant (all of the aerial plant parts) was soaked and ultrasonicated in 2 ml n-hexane (analytical grade) at 40°C for 30 min and then extracted twice. Three dried samples from each plant were used for the extraction. The hexane extract was evaporated to dryness in a fume chamber with nitrogen gas. The residue was then redissolved with 5 ml acetonitrile and filtered through a 0.45 µm nylon micro-filter (Millipore Corporation, USA). The samples were analysed, using reverse phase high performance liquid chromatography (RP-HPLC), according to the method described by ElSohly *et al.* (1987) with modifications. The mobile phase, the acetonitrile:sodium acetate buffer (70:30), was used for the gradient elution at a flow rate of 1 ml/min. The artemisinin was detected at 260 nm with a retention time of 10±0.5 min in a chromatogram. The injection volume was 20 µl for each sample with 3 replications. An artemisinin standard (Sigma, USA) was used to prepare the calibration curve for the determination of the artemisinin contents in the tested samples.

RESULTS AND DISCUSSION

The two different clones used in this study, TC1 and TC2, were selected as elite, high-yielding clones from the Pharmacy Research Centre at Hanoi, Vietnam. The two clones exhibited consistently high artemisinin contents (1%–2%) and were genetically homogenous (personal communication). The two chosen study sites

were distinctly different in temperatures and elevation heights above sea level (Table 1).

Table 1: Environmental variation of two different study sites during study (January–May 2009).

Environmental variation (January to May)	Location	
	Thù Đúc Province	Đà Lat
Temperature (°C)	26.2–32.8	10.5–18.0
Type of weather	tropical dry season	temperate dry season
Rainfall (mm/month)	36–120	69–100
Average relative humidity (%)	75–79	77–79
Elevation from sea level (m)	4–5	1500–2000

Results obtained from our study (Table 2) clearly indicate that temperature affected the growth and artemisinin content of *A. annua.* Obvious effects on the vegetative growth of the *A. annua* plants could be observed. The high temperatures and low elevation of Thù Đúc Province caused the vegetative growth of all of the plants from the two different clones to slow, and they were much shorter in height with fewer, short branches compared to the same clones cultivated at Đà Lat. The possible explanation for the slowing in vegetative growth at the Thù Đúc Province was that the high temperatures and hot weather resulted in strong transpiration effects, and thus, more water was lost in the *A. annua* plants. To overcome these effects, root growth normally increased to aid in water absorption, as explained by Wang *et al*. (2007). Unfortunately, we did not obtain the root biomass to confirm this. Both locations had similar amounts of rainfall and relative humidities during the study period, from January to May 2009, but the hot weather at the Thù Đúc Province caused the plants to require significantly more water to overcome the strong transpiration effects. This hot weather also induced earlier flowering. At the Thù Đúc Province, both of the clones, TC1 and TC2, produced their first flower buds within 98–105 days. The same two clones that were cultivated at Đà Lat produced their first flower buds after 147–154 days of planting. Đà Lat is situated 1500–2000 m above sea level and was considered to have a temperate dry season during the study period with lower temperatures (10.5°C–18.0°C) than the Thù Đúc Province (Table 1). This indicated that the cold weather of Đà Lat was suitable for the planting of *A. annua* as opposed to the tropical weather of the Thù Đúc Province. Because the two clones were genetically homogenous, the variation observed could likely be due to environmental factors. Klayman (1989) had also reported that the humid tropics were not suitable for the cultivation of *A. annua* because long days are required for the plants to reach maturity before flowering, which is induced by short days; hence, *A. annua* is classified as a short-day plant. Environmental variations, such as light, temperature and availabilities of water and salt, were reported to alter the artemisinin yield (Weathers *et al*. 1994).

The temperate weather of Đà Lat induced significantly elevated artemisinin levels in *A. annua.* The high artemisinin levels correlated well with the robustness of the plants, which grew in the cold weather of Đà Lat with heights and branch lengths that were 4 to 5 times taller or longer and more branching (two times) than those cultivated at the Thù Đúc Province (Table 2). Elhag *et al*. (1992) also found that the high artemisinin-producing clones were characteristically tall plants, producing long internodes, dense leaves, open branching and thick stems.

From this study, four plants were selected based on their reasonably good vegetative growth and artemisinin contents. The selection was performed by choosing one clone for TC1 and TC2 from the 10 plants from each location, and these were used to determine the artemisinin content (Table 3). These four clones will be used for future in vitro studies of *A. annua.*

CONCLUSION

Our study strongly indicates that the growth of the *A. annua* plants and the variations in artemisinin contents were attributed more strongly to environmental factors than to genetic factors because two similar clones were used at the two different study sites.

Table 2: Growth characteristics and artemisinin contents of *A. annua* grown in two locations with different environmental factors.

Parameters	TC1 clone		TC2 clone	
	Thù Đúc Province	Đà Lat	Thù Đúc Province	Đà Lat
Plant height (cm) ± s.d.	48.9±5.5a	255.5±16.8b	56.6±8.2a	258.8±37.1b
No. of main branches	26.6±7.4a	70.5±15.9b	35.6±8.3a	72.3±11.2b
Length of 2nd branch (cm) ± s.d.	31.1±4.9a	198.7±13.3b	36.8±7.5a	199.7±39.4b
Duration of vegetative growth until onset of first flower buds (weeks)	14–15	21–22	14–15	21–22
Artemisinin content (µg/g DW) ± s.e.	2595.2±274.1a	11202.8±659.1b	3213.01±255.3a	10845.3±356.6b
Total artemisinin (%) ± s.e.	0.259±0.025a	1.120±0.066b	0.321±0.026a	1.085±0.036b

Notes: Mean values for each parameter of each clone between two different locations followed by different letters were significantly different (Student's t-test at $p \leq 0.05$).

Table 3: Four selected clones of *A. annua* from the two study sites, Thù Đúc Province and Đà Lat.

Selected plant code (code no.)	Plant height (cm)	No. of main branches	Length of 2nd branch (cm)	Artemisinin content (µg/g DW)	Total artemisinin (%)
TC1.8	60	42	40	4482.06	0.448
TC2.1	66	44	45	4087.95	0.408
TC1.136	275	87	192	14700.23	1.470
TC2.29	267	58	231	12977.02	1.298

Notes: TC1.8 – selected plant of TC1 clone from Thù Đúc Province; TC2.1 – selected plant of TC2 clone from Thù Đúc Province; TC1.136 – selected plant of TC1 clone from Đà Lat; TC2.29 – selected plant of TC2 clone from Đà Lat.

ACKNOWLEDGEMENT

We thank the Institute of Tropical Biology, Vietnam for the field study, the Malaysian Government for the research funding and Universiti Sains Malaysia for the use of their research facilities.

REFERENCES

Abdin M Z, Israr M, Rehman R U and Jain S K. (2003). Artemisinin, a novel anti-malarial drug: Biochemical and molecular approach for enhanced production. *Planta Medica* 69(4): 289–299.

Charles D J, Cebert E and Simon J E. (1991). Characterization of the essential oil of *Artemisia annua* L. *Journal of Essential Oil Resource* 3: 33–39.

Dhingra V, Rao V M and Narasu L M. (2000). Current status of artemisinin and its derivatives as anti malarial drugs. *Life Sciences* 66(4): 279–300.

Efferth T, Dunstan H, Sauerbrey A, Miyachi H and Chitambar C R. (2001). The anti-malarial artesunate is also active against cancer. *International Journal of Oncology* 18(4): 767–773.

Elhag H M, El-Domiaty M M, El-Feraly F S, Mossa J S and El-Olemy M M. (1992). Selection and micropropagation of high artemisinin producing clones of *Artemesia annua* L. *Phytotheraphy Research* 6(1): 20–24.

ElSohly H N, Croom E M and ElSohly M A. (1987). Analysis of the anti-malarial sesquiterpene artemisinin in *Artemesia annua* by high-performance liquid chromatography (HPLC) with post-column derivation and ultraviolet detection. *Pharmaceutical Research* 4(3): 258–260.

Klayman D L. (1989). Weeding out malaria. *Natural History* 10: 18–26.

_____. (1985). Quinghaosu (artemisinin): An antimalarial drug from China. *Science* 228(4703): 1049–1055.

Laughlin J C. (2002). Post-harvest drying treatment effects on anti-malarial constituents of *Artemesia annua* L. *Acta Horticulturae* 576: 315–320.

Liersh R, Soicke H, Stehr C and Tullner H U. (1986). Formation of artemisinin in *artemesia annua* during one vegetation period. *Planta Medica* 52(5): 387–390.

Mueller M S, Karhagomba I B, Hirt H M and Wemakor E. (2000). The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: Agricultural, chemical and clinical aspects. *Journal of Ethnopharmacology* 73(3): 487–493.

Price R N, Nosten F, Luxemburger C, ter Kuile F O, Paiphun L, Chongsuphajaisiddhi M D and White N J. (1996). Effect of artemisinin derivatives on malaria transmissibility. *The Lancet* 347(9016): 1654–1658.

Simon J E, Charles D, Cebert E, Grant L, Janick J and Whipkey A. (1990). *Artemesia annua* L.: A promising aromatic and medicinal. In J Janick and J E Simon (eds.). *Advances in new crops*. Portland, Oregon: Timber Press, 522–526.

Singh N P and Lai H. (2001). Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. *Life Sciences* 70(1): 49–56.

Utzinger J, Xiao S, N'Goran E K, Bergquist R and Tanner M. (2001). The potential of artemether for the control of schistosomiasis. *International Journal of Parasitology* 31(14): 1549–1562.

van Vugt M, Looareesuwan S, Wilairatana P, McGready R, Villegas L, Gathmann I, Mull R, Brockman A, White N J and Nosten F. (2000). Artemether-lumefantrine for the treatement of multidrug-resistant falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 94(5): 545–548.

Wang M L, Jiang Y S, Wei J Q, Wei X, Qi X X, Jiang S Y and Wang Z M. (2007). Effect of irradiance on growth, photosynthetic characteristic, and artemisinin content of *Artemisia annua* L. *Phytosynthetica* 46(1): 17–20.

Weathers P J, Cheetham R D, Follansbee E and Theoharides K. (1994). Artemisinin production by transformed roots of *Artemisia annua. Biotechnology Letters* 16(12): 1281–1286.