

Germplasm Release of Four High-artemisinin Clones of *Artemisia annua* L.

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Artemisia annua L., a native of China, is now widely naturalized and cultivated as an antimalarial medicinal plant. It is known under the name of qinghao in China and annual wormwood or sweet Annie in the United States. The tall shrubby annual plant blooms under short days and produces tiny flowers and seeds. Glandular trichomes, abundant in leaves and inflorescences, are the source of the nonvolatile sesquiterpene artemisinin A, best known as artemisinin or qinghaosu, effective against drug-resistant *Plasmodium falciparum*, responsible for the often-fatal cerebral malaria. Artemisinin combination therapies (ACTs) are presently the first-line treatment for malaria in the tropics. Yields of artemisinin from seed-grown plants average 0.7% in Africa and Asia. *Artemisia* has not been commercially cultivated as a medicinal crop in the United States, but trials have been performed in Washington state to determine its use as an alternative coxidiostat to replace organic arsenic use in poultry. Furthermore, trials are currently underway in Kentucky in an attempt to produce raw material for ACTs in fields previously used to grow tobacco.

Four clones of *Artemisia* with artemisinin concentrations varying from 1.81% to 2.16% have been identified based on research carried out at Purdue University and at the University of Georgia. This material could be the source of improved germplasm, and clonal propagation through tissue culture or cuttings (already used in African plantations) could be practical methods for field production of homogeneously high-artemisinin clones.

Artemisia annua seed obtained from China and Brazil and their open pollinated progeny were grown in a greenhouse and field at Purdue to research artemisinin (Ferreira, 1994; Ferreira and Janick, 1994, 1996; Ferreira et al., 1995). In 2007, plants and seed from this material were sent to the

University of Georgia for studies of floral anatomy, micropropagation, and selection (Wetzstein et al., 2014, 2018). Selections were made over successive generations at the University of Georgia from 2007 to 2014, based on agronomic characteristics such as leaf area, shoot biomass, flowering time, and artemisinin concentration. Selections were cloned by cuttings and maintained in a greenhouse under long days. Cloned material was used in field evaluation trials.

Description

The performance of our target selections was evaluated in field plots that were located at the University of Georgia campus in Athens, GA, and at the University of Georgia Horticulture Farm in Watkinsville, GA. Studies were conducted using a randomized complete block design in 2012, where seven genotypes were compared using four replications (rep or reps), with each containing eight plants per rep (i.e., 32 plants per genotype). In 2013, a large-scale field plot study was conducted to evaluate six genotypes containing eight reps with eight plants per rep (i.e., 64 plants per genotype). Additional guard-row plants were used along the perimeter of plots. Spacing was at one plant per square meter. No supplemental irrigation or fertilization was provided, nor were herbicides or pesticide applied. Plots were hand weeded as needed.

Availability

Four clones, C1, C10, B6, P137, are freely released by the Agriculture Experiment Stations of Purdue University and the University of Georgia. Cuttings can be obtained by contacting Jules Janick (janick@purdue.edu). A FedEx account number must be provided.

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Table 1. Artemisinin concentration per plant and kg/ha from six selected clones of *Artemisia annua*.

Genotype	Artemisinin (%)			Plant ht (cm) ^z	Plant width (cm) ^z	Stem dry wt (kg) ^z	Leaf dry wt ^z		Artemisinin (kg/ha) ^z
	2012	2013	Avg				(kg)	(t/ha)	
C1	2.13	2.19	2.16 a	179 a ^y	113 a	0.69 a	0.32 ab	3.22	70.6
C10	1.93	2.13	2.03 ab	188 a	106 ab	0.77 a	0.28 ab	2.81	59.9
B6	1.65	2.21	1.93 ab	181 a	112 ab	0.67 a	0.26 ab	2.61	57.8
P137	1.93	1.68	1.81 bc	192 a	111 ab	0.81 a	0.39 a	3.88	65.2
P63	1.81	1.53	1.67 c	144 b	100 bc	0.44 b	0.25 b	2.46	37.7
B4	1.83	1.37	1.60 c	140 b	89 c	0.34 b	0.27 ab	2.65	36.3

^z2012 data.

^yMean separation Tukey test, $\alpha = 0.05$.

Artemisinin productivity is based on leaf dry weight, 1 plant/m², and without considering a commercial extraction loss of 25%. Means within the same column followed by the same letter are not significantly different.

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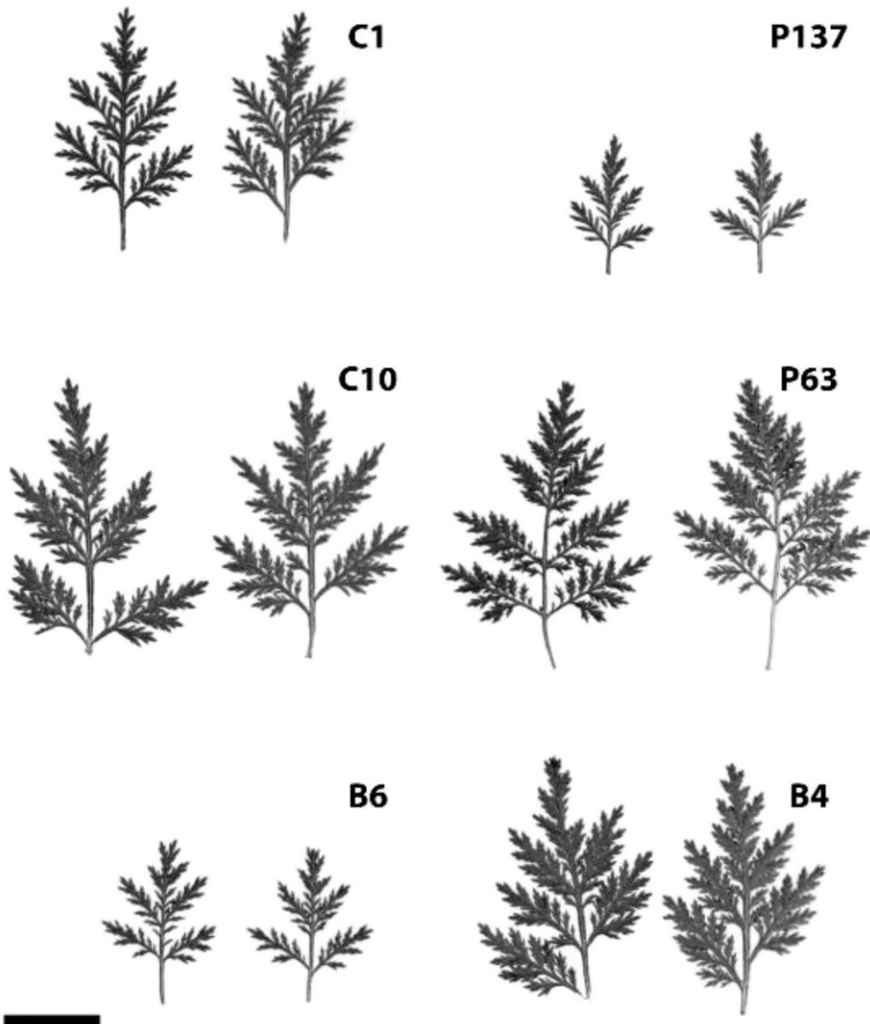


Fig. 1. Variation in the leaf size of six selected clones of *Artemisia annua* grown under field conditions (bar = 2 cm).

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