Performance of *Artemisia annua* L. Cultivars in Different Locations in Argentina

X. Simonnet[1], M. Quennoz[1], C. Carlen[2], O. Lopez[3], D. Kowalyzyn[3], G. Ciccia[4] and C. Desmarchelier[4]
[1] Médiplant, Research Centre, 1964 Conthey, Switzerland
[2] Agroscope Changins-Wädenswil ACW, 1964 Conthey, Switzerland
[3] Research and Development Division, Garruchos SA, Argentina
[4] Fundacion Mundo Sano, Av. Del Libertador 1146, Buenos Aires, Argentina

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Abstract

Artemisinin, a sesquiterpene lactone endoperoxide isolated from the herb *Artemisia annua* L. (Asteraceae), is a highly potent antimalarial compound, which is efficient against multidrug-resistant strains of *Plasmodium falciparum*. The promotion of artemisinin-based combination therapies (ACTs) by the WHO during the past years lead to strong pressure on the world market of artemisinin. In order to assess the potential of this new culture in Argentina, the 'Artemis' cultivar was grown in 2007 and 2008 in four different locations. On one of the sites, 2 new hybrids were tested to assess their potential. The following parameters were recorded during the growing period: plant height, phenology, biomass production, percentage of dry leaves and artemisinin content. The results obtained with the cultivar 'Artemis' showed that the highest yield of biomass reached at the time of harvest was 2.5 t/ha dry leaves. At the harvesting stage, artemisinin contents ranged from 0.77% to 1.12% depending on the location. The two new hybrids tested showed a similar production of leaves, but a significantly higher level of artemisinin than those observed for 'Artemis'. Future trials are planned to optimise the cultivation of annual wormwood in Argentina.

INTRODUCTION

Artemisinin, a sesquiterpene lactone endoperoxide isolated from the herb *Artemisia annua* L. (Asteraceae), is a highly potent antimalarial compound, which is efficient against multidrug-resistant strains of *Plasmodium falciparum*. The promotion of artemisinin-based combination therapies (ACTs) by the World Health Organisation (WHO) during the past years lead to strong pressure on the world market of artemisinin. Despite the research of new technologies, the extraction from *A. annua* leaves remains the only source of artemisinin (Brisibe et al., 2008). Annual wormwood (*A. annua)* is widely produced in China and also in Vietnam, where a major part of artemisinin is produced and commercialized. In recent years, there have been some efforts for its production in some other countries like in Eastern Africa (Ferreira et al., 2005). However, in South America, where it is also known by the common name of "Artemisia", its agronomic production is rare, with the notable exception of Southern Brazil, where there has been at least one project for its agronomic production.

Recently, Mundo Sano Foundation launched a non-profit project in order to determine the technical and economical feasibility for the production of *A. annua* and artemisinin in Argentina. The aim of this project is to create a local, transparent, and cost-effective artemisinin production venture, according to the guidelines established by the WHO.

As a part of this project, and due to the little agronomical information available for "Artemisia" in the region, the aim of the present study was to test the effects of different locations on the cultivar 'Artemis' within Argentina. A second experiment was focused on the comparison of 2 new hybrids with 'Artemis'. The preliminary information obtained from these investigations will allow to better understand the behaviour of *A. annua* in Argentina and its potential as a future crop in the region, both from a technical and financial point of view.

MATERIALS AND METHODS

The commercial *Artemisia annua* cultivar 'Artemis', an hybrid F_1 created by the Swiss research institute Médiplant was grown in 2007 and 2008 in four different locations (La Coronita, Puerto Valle, El Encuentro and Garruchos) with latitudes between 27°S and 35°S and, different soil and climatic conditions (Table 1). A plot with 400 plants at a density of 15.625 plants/ha was planted on each site. The experimental design, randomized complete block, was conducted with 3 replicates (10 plants per replicate). Seeds were sown in a nursery and transplanted on the field once they reached 10 cm height. No fertilisation was used during the experiment, and weeding and watering of the plants were performed manually, according to local needs. No plant protection treatment was applied. At harvests, plants were cut at the soil level and dried at room temperature in an aired shed. The leaves were then separated from the stems and reduced to powder (≤0.5 mm). The following parameters were recorded every other week during 20 weeks from transplant: plant height, phenology, biomass production, percentage of dry leaves and artemisinin content.

At the site of El Encuentro, in 2008, cultivar 'Artemis' was compared with 2 new hybrids created by Médiplant. Cultural practices are similar to conditions described below for the other trials except for fertilisation (in kg per hectare before plantation: 100 N, 65 P_2O_5, 100 K_2O). The field plantation was done on November 5th 2007 and the harvest 18 weeks later.

The determination of artemisinin content from the dry leaves powder was realised in the laboratory of chemistry of Médiplant by using a thin layer chromatography (TLC) method described by Gaudin and Simonnet (2005).

RESULTS AND DISCUSSION

After 4-5 months of cultivation, yields of dry leaves were highly variable depending on the location, ranging from 1.3 to 3.1 t/ha (Table 2). But if one refers to the GAP edited by WHO (Anonymous, 2006) which recommended a harvest latest at the beginning of budding, results obtained showed that the highest yields of biomass was obtained in Puerto Valle in 2008 with 2.5 t/ha dry leaves. Also note that the dry leaves/total aerial part ratio decreased during the growth to reach about 30% at the harvest (results not shown). This level of performance is consistent with previous reports (Pilloy, 2008). Low yields in 2007 (at budding stage) in Puerto Valle (0.2 t/ha) and La Coronita (0.6 t/ha) were caused by planting too late; increased biomass being particularly important at the end of culture (Fig. 1). At the harvesting stage (budding), artemisinin contents reached 0.73% to 1.12% depending on the location (Table 4). But during the growing period, the dynamic of the artemisinin content appeared different according to locations (Table 3). In La Coronita, El Encuentro and Garruchos, the content of artemisinin increased during the initial weeks and then stabilized. In Puerto Valle, in both 2007 and 2008, a maximum artemisinin level was observed around 10 weeks after transplant, followed by a continuous decrease. In this case, the peak appeared approximately 2 months earlier in 2008 compared to 2007, without apparent link with the phenological stage.

Monitoring the phenology of the cultivar 'Artemis' on these sites has provided interesting results, with flowering appearing always in March/April, but with differences between latitudes. Thereby, budding and flowering in 2007 were earlier in Puerto Valle (lat. 27°S), budding at the end of February and flowering the 3rd week of March, than in La Coronita (lat. 35°S) where flowering occurred two weeks later. Also in 2008, flowering in Puerto Valle and Garruchos (lat. 27/28°S) started two weeks earlier than in El Encuentro (lat. 35°S). When at Puerto Valle, the trial was planted two months earlier in the second year compared to the first year, flowering occurred in both years at the same moment i.e., March. The known sensitivity of *A. annua* to short day length for floral induction (Ferreira et al., 2005) expressed itself well in these trials with the natural day length decrease starting in January. The difference in earliness between the two latitudes can be explained by photoperiod. Eventually the different temperatures at these two latitudes also had an influence (Marchese et al., 2002).

Finally, the last test was conducted in El Encuentro in 2008 to compare the reference variety 'Artemis' to two new *A. annua* hybrids. At the time of harvest, no significant differences were observed in the height of plant (average 1.4 m) or in the yield of dried leaves (average 1 t/ha) when the three hybrids were compared (Table 4). However, new varieties have shown a significantly higher level of artemisinin than those observed for 'Artemis' (1.17 and 1.11% vs. 0.72%). Even if the potential in this test remained limited (poor soil condition), the superiority of these new varieties on 'Artemis' for the content of artemisinin is in line with previous observation reported by Simonnet et al. (2008).

CONCLUSIONS

These first cultural trials in Argentina highlighted the possibility to grow *A. annua* at these latitudes. Harvests with artemisinin content up to 1% are realistic. The field planting has to be done at the latest in October to achieve about 5-month growth before flowering. New varieties still in development should soon provide higher yields. Future trials are planned to optimise the cultivation of annual wormwood in Argentina.

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Tables

Table 1. Soil and weather conditions, flowering and harvest periods at the different production sites under investigation.

Location	Puerto Valle		La Coronita	El Encuentro	Garruchos
Altitude (m asl)	85		89	80	90
Latitude/longitude	27º35S/ 56º41W		35°37S/ 61°22W	35°40S/ 58°57W	28°11S/ 55°38W
Granulometric soil composition	Sandy loam		Sandy loam	Sandy loam	Sandy loam
Soil pH	5.1		6.6	6.2	4.8
Soil organic matter (%)	1.9		2.2	2	3.3
Trial reference	PV 2007	PV 2008	LC 2007	EE 2008	GA 2008
Cultivars tested	Artemis	Artemis	Artemis	Artemis + 2 news hybrids	Artemis
Date of transplanting in the field	15/12/2006	15/10/2007	15/12/2006	5/11/2007	15/10/2007
Flowering start (Artemis cvs.)	3rd week March 2007	1st week March 2008	1st week April 2007	3rd week March 2008	1st week March 2008
Total (cumulated) rainfall from transplant to the last harvest (mm)	953	768	973	414	741
Average temperature from transplant to the last harvest (°C)	25.4	25.4	20.6	18.4	24.3

Table 2. Seasonal variation of the dry leaves yield for the *A. annua* cultivar 'Artemis', grown in four different locations in Argentina (2007 and 2008).

Weeks after transplant	Dry leave yield (kg/ha)				
	PV 2007	PV 2008	GA 2008	LC 2007	EE 2008
2	6 c	68 e	83 e	nr	nr
4	19 c	203 e	240 e	nr	nr
6	45 c	448 de	677 de	119 e	182 b
8	34 c	625 de	1130 cd	174 e	154 b
10	265 c *	1026 cd	1270 bcd	635 d	260 b
12	964 bc	1083 cd	1698 abc	624 d *	396 b
14	1307 b **	1620 c	2276 a	958 c	396 b
16	2088 a	2495 b *	2052 ab *	1052 c **	750 ab *
18	2708 a	3177 a	2547 a	1328 b	750 ab
20	nr	nr **	nr **	1744 a	1302 a **
	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Different letters indicate significant differences between weeks (Newman-Keuls test, α=0.05).
nr=not recorded; * beginning of budding; **beginning of flowering.

Table 3. Seasonal variation of artemisinin content for the *A. annua* cultivar 'Artemis', grown in four different locations in Argentina (2007 and 2008).

Weeks after transplant	Artemisinin content (% w/w)				
	PV 2007	PV 2008	GA 2008	LC 2007	EE 2008
2	0.47	0.44	0.48	nr	nr
4	0.72	0.85	0.64	nr	nr
6	0.64	1.07	0.81	0.64	0.41
8	0.84	0.99	0.85	0.82	0.68
10	0.98 *	1.01	0.81	0.79	-
12	0.89	1.04	0.91	0.77 *	0.77
14	0.69 **	0.86	0.76	0.76	0.94
16	0.53	0.73 *	1.12 *	0.79 **	nr *
18	0.49	0.86	0.85	0.82	0.90
20	0.20	0.63 **	nr **	0.75	0.92 **

[1]only one replication.
nr=not recorded; * beginning of budding; **beginning of flowering.

Table 4. Comparison of three hybrids of *Artemisia annua* (El Encuentro, 2008).

Hybrids	Plant height (cm)	Dry leave yield (kg/ha)	Artemisinin content (% w/w)
Artemis	129	1036	0.72 b
Hybrid 1	143	1098	1.11 a
Hybrid 2	140	1019	1.17 a

Different letters indicate significant differences between hybrids (Newman-Keuls test, α=0.05).

Figures

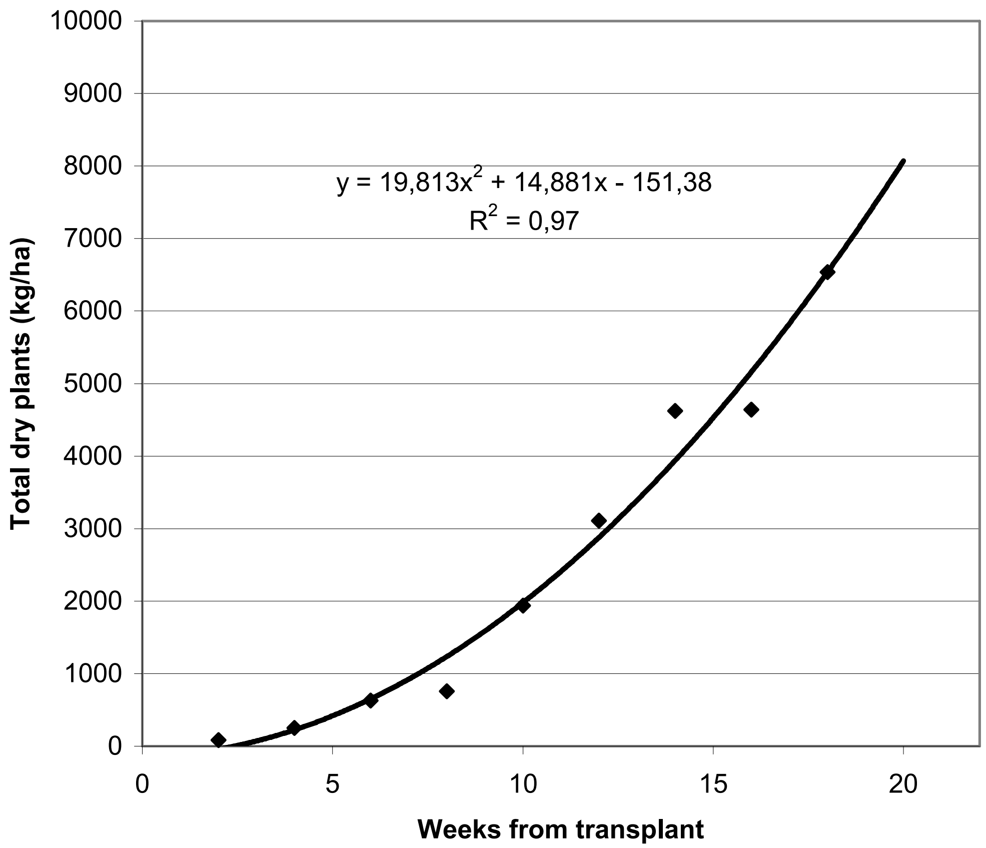

Fig. 1. Dynamic of the dry biomass increase of total above ground plants of *Artemisia annua* during a period of 20 weeks in the field. Mean of 2 growing years for the cultivar ‘Artemis’ (Puerto Valle, 2007-2008).