New *Artemisia annua* Hybrids with High Artemisinin Content

X. Simonnet, M. Quennoz and C. Carlen
Mediplant, Centre des Fougères
1964 Conthey
Switzerland

Keywords: annual wormwood, breeding, cultivar, malaria

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 a strong pressure on the world market of artemisinin. The scarcity of artemisinin caused a price increase that strongly renewed the interest for *Artemisia annua* culture at a large scale. The use of varieties with high artemisinin content is a key factor for the development of such cultures. The new hybrids recently obtained by Mediplant, with artemisinin contents nearing 2%, are being presented.

INTRODUCTION

Artemisinin, a sesquiterpene lactone endoperoxid isolated from the herb *Artemisia annua* L. (Asteraceae), is a highly potent antimalarial compound, which is also efficient against multidrug-resistant strains of *Plasmodium falciparum* (Alin, 1997). With the support of the WHO, in 2005 over 53 countries have officially adopted artemisinin-based combination therapies (ACTs) as their first line of treatment against malaria (WHO, 2006). The global consumption of ACTs has increased from a few hundred thousands in 2001 and 2002 to tens of millions treatments in 2005. The rapid increase in demand produced a global supply shortage of artemisinin and a price increase. Despite the research of new technologies (Hentschel, 2005), the extraction from *A. annua* leaves remains the only source of artemisinin. Only the distribution of varieties with a high artemisinin production potential allows making this new culture attractive and this way answer an increasing demand for low cost artemisinin (Ferreira et al., 2005). Research conducted for about 15 years by Médiplant on the biology of *A. annua* and the breeding on artemisinin allowed to develop cultivars with over 1% artemisinin in the leaves (Delabays et al., 1993, 2001). The breeding work continues to get new cultivars rich in artemisinin and well-suited to the inter-tropical zone. The present paper presents the results obtained with the latest hybrids created.

MATERIALS AND METHODS

Creation of the Hybrids

Plants issued from our breeding program and preserved in vitro (Lê and Collet, 1991) are raised in greenhouses with controlled day lengths to ensure floral induction. Since the auto-fertilization being insignificant (Delabays, 1997), the plants are isolated in groups of 2 genotypes to ensure the production of hybrid seeds.

Field Evaluation

Seeds obtained from the controlled cross were sown in greenhouses during March, the hybrid seedlings were transplanted on field about mid-May in Conthey, Switzerland (46°13'N latitude, 7°17'E longitude and 485 m asl) according to an experimental system with 3 replications of 20 plants per elementary plots (density 1.78 plants/m^2). Six central plants per elementary plot were harvested around September 20 at vegetative stage and dried at 35°C. The leaves are then separated from the stems and reduced to powder (≤0.5 mm).

Chemical Analysis

The determination of artemisinin content from the dry leaves powder was realised with HPLC at Agroscope Changins-Wädenswil Research Station ACW according to the method described by Delabays (1997) and Zhao and Zeng (1986).

RESULTS AND DISCUSSION

The results obtained with our reference cultivar 'Artemis' during 5 consecutive years are given in Table 1. The values are stable with an average artemisinin content of the leaves of 1.3%. The mean annual production of artemisinin was about 32 kg/ha artemisinin.

For five new hybrids of *A. annua*, the artemisinin contents in their leaves were very high varying from 1.60 to 1.95% (Table 2). After 4 months of field cultivation in Swiss climatic conditions with the density of 17,800 plants/ha, these hybrids produced 2.1 to 2.85 t/ha dry leaves and 40.5 to 52.0 kg/ha artemisinin. One of the most promising new hybrid, the Hybrid 1, revealed a similar yield in dry leaves, showing 36% higher content of artemisinin in the leaves and 37% higher production of artemisinin compared to the cultivar 'Artemis' in 2001 (Table 3).

The breeding work had been conducted by Mediplant since 1989 and was judged to be successful based on the creation of high-yielding clones' hybrids. During the past 9 years, about 45 new hybrids were tested. A continuous breeding for cultivars with a high artemisinin production has allowed this progression of the artemisinin content in the leaves. Although the artemisinin content is the first selection criterion retained, other factors such as the aptitude for in vitro conservation, the strength, the leaves productivity, the flowering earliness and the tolerance to pests and diseases are also considered.

In order to validate their production potential, these new promising hybrids will be tested over several years in Switzerland as well as in other various areas of the inter-tropical zone which appear to the main production sites for artemisinin.

CONCLUSION

After the registration in 1999 of a first cultivar 'Artemis' with 1.3% artemisinin content in the leaves, the latest results obtained by the cultivar breeding program let foresee at short term the launching of a new cultivar with a productivity gain of more than 30%.

ACKNOWLEDGEMENTS

The analysis were realised by the chemistry laboratory of the research station Agroscope Changins-Wädenswil ACW.

Literature Cited

Alin, M.H. 1997. In vitro susceptibility of Tanzanian wild isolates of *Plasmodium falciparum* to artemisinin, chloroquine, sulfadoxine/pyrimethamine and mefloquine. Parasitol. 114:503-506.

Delabays, N., Benakis, A. and Collet, G. 1993. Selection and breeding for high artemisinin (Qinghaosu) yielding strains of *Artemisia annua*. Acta Hort. 330:203-207.

Delabays, N. 1997. Biologie de la reproduction chez l'*Artemisia annua* et génétique de la production en artémisinine. Thèse de doctorat: Uni. de Lausanne.

Delabays, N., Simonnet, X. and Gaudin, M. 2001. The genetics of artemisinin content in *Artemisia annua* L. and the breeding of high yielding cultivars. Current Medicinal Chemistry 8:1795-1801.

Ferreira, J.F.S., Laughlin J.C., Delabays, N. and Magalhaes, P.M. 2005. Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. Plant Genetic Resources 3:206-229.

Hentschel, C. 2005. ACT pipeline & synthetic artemisinins. Coartem Advisory Board Meeting, 10-11 March 2005, Dakar, Senegal.

Lê, C.L. and Collet, G.F. 1991. The in vitro culture of *Artemisia annua* L. Schweiz.

Landw. Fo. 31:111-116.
Zhao, S.S. and Zeng, M.Y. 1986. Application of precolumn reaction to high-performance liquid chromatography of Qinghaosu in animal plasma. Anal. Chem. 58:289-292.
WHO. World Health Organisation. 2006. www.who.int.

Tables

Table 1. Yield and artemisinin content in the leaves of 'Artemis' for 5 consecutive years (Conthey, 1998-2002).

Year	Planting date	Harvesting date	Dry leaf yield (g/m^2)	Artemisinin content (%) (w/w)	Artemisinin yield (g/m^2)
1998	May 25th	Sept. 21th	285	1.20	3.41 ab[1]
1999	May 19th	Sept.13th	235	1.43	3.33 ab
2000	May 16th	Sept.19th	211	1.29	2.73 b
2001	May 16th	Sept.20th	285	1.33	3.78 a
2002	May 23th	Sept.19th	236	1.28	3.00 ab
			ns[2]	ns	P<0.05
Mean	-	-	250	1.31	3.25

[1]Newman-Keuls test; [2] ns: not significant (P>0.05).

Table 2. Yield and artemisinin content in the leaves of 5 new hybrids of *Artemisia annua* (Conthey, 2005).

Hybrids[1]	Dry leaves yield (g/m^2)	Artemisinin content (%) (w/w)	Artemisinin yield (g/m^2)
Hybrid 1	268	1.87	4.89
Hybrid 2	236	1.71	4.06
Hybrid 3	302	1.72	5.20
Hybrid 4	253	1.60	4.05
Hybrid 5	254	1.95	4.95
	ns[2]	ns	ns

[1]planting date 25.05.2005 and harvesting date 21.09.2005; [2]ns: not significant (P>0.05).

Table 3. Yield and artemisinin content of the cultivar Artemis and the new reference hybrid (Hybrid 1) (Conthey, 2001).

Hybrids[1]	Dry leaves yield (g/m^2)	Artemisinin content (%) (w/w)	Artemisinin yield (g/m^2)
Artemis	285	1.33 b[2]	3.78 b[2]
Hybrid 1	289	1.81 a	5.20 a
	ns[3]	P<0.05	P<0.01

[1]planting date 16.05.2001 and harvesting date 20.09.2001; [2]Newman-Keuls test; [3]ns: not significant (P>0.05).