

## ASL 2: *Artemisia annua* L.: Polyploidy and NIRS, two tools to improve breeding efficiency



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### Abstract

Breeding a new cultivar needs 5 to 15 years according to the species and the breeding objectives from bioprospection up to cultivar registration. This is a very long time for companies developing and trading plant based products. To react more quickly to the requirements of the stakeholders, methods to accelerate the breeding procedures have to be taken into account. Among different possibilities, polyploidy induction and rapid methods to measure target traits with near infrared spectroscopy (NIRS) were tested on *Artemisia annua* L.

Tetraploid progenies were compared to the cultivar Apollon. These tests showed no significant differences between the tetraploid plants and the cultivar Apollon for the artemisinin content, as well for the leaf and the artemisinin yield.

The determination of artemisinin in powder of *Artemisia annua* using a hand-held NIRS device showed accurate results in predicting artemisinin contents. Root mean square error values of cross-validation and prediction of 0.1 % were calculated, in both cases.

Keywords: Artemisinin, *Artemisia annua*, breeding, NIRS, polyploidy

### Introduction

*Artemisia annua* L. is an important medicinal plant for the production of antimalarial drugs based on artemisinin. Artemisinin, a sesquiterpene lactone endoperoxid isolated from the leaves of *A. annua*, 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 the artemisinin-based combination therapies (ACTs) became the first line of treatment against malaria. Despite the research of new technologies, the extraction from *A. annua* leaves remains the only source of artemisinin. Only the distribution of cultivars with a high artemisinin production potential allows making this new culture attractive and this way answering to the increasing demand for low cost artemisinin (FERREIRA et al., 2005). The breeding work conducted by Mediplant since 1989 allowed the development of hybrids with artemisinin contents in the leaves with up to 2 % (SIMONNET et al., 2008). To increase the breeding efficiency, induction of polyploidy and the NIRS-method were tested on *Artemisia annua*.

### Materials and Methods

**Polyploidy:** The increase of chromosomes sets per cell can be artificially induced by applying the molecule colchicine, which leads to a doubling of the chromosome number. To improve the basis for breeding, such a polyploidy induction was made on in-vitro plantlets and seeds of *A. annua*. Different concentration (0 to 2 % colchicin) and different durations (6 to 48 h) were used. The surviving tetraploid plants were used as parental lines to get seeds. The tetraploid plants from this seed were compared in the field with 3 replications (RBC) and 18 plants per replication with the cultivar Apollon.

**NIRS:** This rapid, low-cost method was developed to determine artemisinin content in dry powder of *A. annua* leaves (Camps et al., 2011). A calibration set of 60 samples and a validation set of 40 samples of *A. annua* hybrids with artemisinin contents varying between 0.7 and 1.6 % were used. The NIRS device was a handheld Phazir 1018 (Thermo Scientific, Wilmington, MA, USA).

## Results

**Polyploidy:** The use of colchizin at different concentration and durations allowed to get some tetraploid *A. annua* plants. The crossing of these tetraploide plants was not very successful. Only one combination of two parental lines gave seeds. The progenies of both parental lines were tested individually in comparison with the cultivar Apollon. These tests showed no significant differences between the tetraploid plants and the cultivar Apollon of the artemisinin content in the leaves, as well as of the leaf and the artemisinin yield (Tab. 1).

Tab. 1 Artemisinin content in the leaves and leaf yield of 3 hybrids of *Artemisia annua*. Values in brackets indicate the standard deviation ( $\pm$ sd).

Hybrids	Artemisinin content	
	in dried leaves (%) ( $\pm$ sd)	Dried leaf yield (g per m <sup>2</sup> ) ( $\pm$ sd)
<b>Cultivar Apollon</b>	<b>1.33</b> ( $\pm$ 0.07)	<b>4.46</b> ( $\pm$ 0.03)
<b>Tetraploide A</b>	<b>1.37</b> ( $\pm$ 0.03)	<b>4.56</b> ( $\pm$ 0.29)
<b>Tetraploide B</b>	<b>1.50</b> ( $\pm$ 0.08)	<b>4.89</b> ( $\pm$ 0.41)

**NIRS:** The development of this method is crucial in order to analyse a large number of samples corresponding to hundreds of hybrids in a breeding program. The results obtained showed the feasibility of artemisinin quantification by using NIRS (Fig. 1). Root mean square error values of cross-validation and of prediction of 0.1 % were calculated, in both cases. The accuracy of the prediction is about 0.1 % in a fully external validation. This threshold of precision has been judged as correct for application of the method in a breeding program.

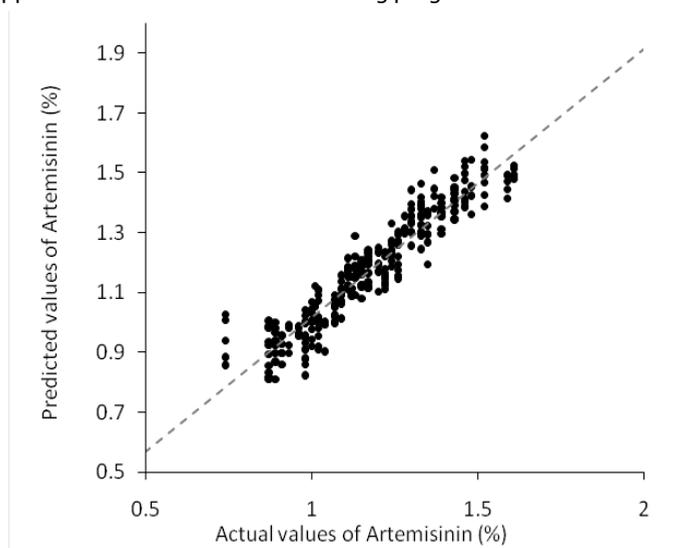


Fig. 1 Actual values (thin layer chromatography) versus predicted values (NIRS) with the partial least squares regressions (Camps et al., 2011).

## References

- 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.
- CAMPS, C., TOUSSIROT, M., QUENNOZ, M., SIMONNET, X., 2011. Determination of artemisinin and moisture content of *Artemisia annua* L. dry powder using a hand-held near infrared spectroscopy device. *J Near Infrared Spectrosc* 19:191—198.
- FERREIRA, J.F.S., LAUGHLIN, J.C., DELABAYS N., MAGALHAES, P.M., 2005. Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. *Plant Genetic Ressources* 3:206-229.
- SIMONNET, X., QUENNOZ, M., CARLEN, C., 2008. New *Artemisia annua* hybrids with high artemisinin content. *Acta Hort* 769:371-373.