NEW HYBRID LINES OF THE ANTIMALARIAL SPECIES Artemisia annua L.

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Abstract

Malaria is still one of the greatest causes of mortality in the world; in Brazil there are over 500,000 reported cases each year. Malaria, caused by the protozoon Plasmodium, has been aggravated by the increasing resistance of Plasmodium to the traditional drugs chloroquine and mefloquine. The study of new drugs resulted in the identification of anti-malarial activities of an endoperoxide sesquiterpene lactone, called Qinghaosu or Artemisinin, extracted from the leaves of Artemisia annua L., from the Asteraceae family. The research work developed at MEDIPLANT (Switzerland) and CPQBA-UNICAMP (Brazil) involved the selection and breeding of genotypes rich in artemisinin and presenting high biomass followed by a second selection to adaptation to Brazilian climatic conditions. Through controlled hybridization between selected genotypes from China and Vietnam, genetic gain was obtained as artemisinin and population uniformity. Through the increase of biomass and artemisinin content (estimated by analytical monitoring), it was possible to increase the artemisinin production of 5 Kg/ha for the base population to approximately 25 Kg/ha for the genetically bred population. In the cultivation carried out in Brazil, 3 hybrid lines, \(2/39 \times 1V, 1V \times 2/39\), and \(Ch \times Viet.55\), produced respectively 21.38, 19.27 and 15.80 Kg of artemisinin/ha. The cultivation of these new hybrid lines in Brazil is technically viable and highly competitive, due to the production obtained.

1. Introduction

1.1. General

Artemisinin, a sesquiterpene lactone endoperoxide extracted from the leaves of Artemisia annua is a very potent antimalarial (White, 1994). As this molecule is very difficult and expensive to synthesize, the agricultural production of Artemisia annua has been studied as an economical way to obtain artemisinin (Woerdenbag et al., 1990).

Artemisia annua L., one of nearly 400 species of the genus in Asteraceae (Compositae) is native to Asia, most probably China. A. annua occurs naturally as part of a steppe vegetation in the northern parts of Chahar and Suiyuan Provinces (40°N, 109°E) in China, at 1,000 to 1,500 m above sea level (Wang, 1961). The plant now grows wild in many other countries such as the former Yugoslavia, Hungary (where it is cultivated for its aromatic oil), Bulgaria, Romania, Turkey, the former Soviet Union, Argentina, Italy, France and Spain (Klayman, 1989, Klayman, 1993).

The first essays to grow A. annua in Brazil had presented heterogeneous results, due to photoperiodic influence, making mechanized cultivation difficult and obtaining low yields with most plants presenting early flowering. Since existing large genetic variability occurs in the species (Delabays, 1992) the research could be forwarded to
selection and breeding, looking for genotypes of late flowering, high artemisinin content and high leaves biomass yield. This could only be started after the basic studies on reproduction biology of this species.

In fact, the process of domestication of new species requires several biological and agronomic trials, besides characterization of variation on active principle.

1.2. The biology of Artemisia annua L.

A. annua L. is an herbaceous-arbustive plant that can reach more than 2.0 m in height. It has alternate, deeply dissected leaves ranging from 2.5 to 5.0cm in length, which tend to be replaced by tiny yellow capitula only 2 to 3mm across, displaced in loose panicles containing numerous bisexual flowers in the center and pistillate marginal flowers. The chromosome number is 2n=18 (Ferreira, 1994). The plant is naturally cross pollinated by insect and wind action, and dies after seeds are mature. A. annua is mainly outcrossing with less than 20% of seeds obtained by selfing (Delabays et al., 1992). On the other hand, a large quantity of seeds (ca. 1mm in length) are produced by cross pollination. The seeds germinate easily. Glandular biseriate trichomes composed of two columns of five cells each were reported in leaves of A. annua (Delabays et al., 1992). The subcuticular spaces of the glandular trichomes are the sites of accumulation of artemisinin and artemisitene, both produced by A. annua. Daylengths above 13 hours or under 11 hours promote only vegetative development (Duke et al., 1994). Flowering occurs between 11 and 13 hours of daylength. Besides, the area chosen for the crop should consider adequate latitude or period of year to avoid early flowering and as consequence low yields.

2. Material and methods

2.1. The hybrid line processes

The processes developed by MEDIPLANT to obtain an hybrid of A. annua, began with a rigorous genotype selection into one basic population, looking for high artemisinin content, biomass productivity and good agronomic features. The selected genotypes are cloned by cuttings or tissue culture (Lê et al., 1991) and put in controlled environment to induce flowering. Each pair of progenies selected is then isolated for crossing, using normally a topcross mating design with a rich artemisinin Chinese genotype (Delabays, 1992). This program produced several hybrid lines, a lot of them presenting more than 1% of artemisinin. In parallel with this breeding program of A. annua L., another selection was made to select genotypes with late flowering on short day length, as occurs in intertropical regions as in Brazil (Magalhães and Delabays, 1996). The last step puts the late flowering genotypes selected together, in order to promote its crossing and obtaining in this way a new hybrid line which could be adapted to Brazilian conditions of photoperiod (Magalhães, 1996).

Among the most promising hybrid lines from MEDIPLANT, (Ch x Viet.55) the hybrid 2/39 x 1V and its inverse 1V x 2/39, were evaluated in the field in Brazil.

On 14.6.96 the 3 hybrid lines obtained from seeds were sown in greenhouse. After 45 days, all the seedlings were transferred to the field in Campinas, Brazil (lat. 22°48'S, long. 47°07'W) in plots of five rows at 100 cm with 60 cm between plants (25 plants/plot). The experiment was set out in a randomized block with ten replications and was irrigated by overhead sprinklers. Weeds were controlled by hand. Nitrogen fertilizer was applied 20 days after the transfer as ammonium sulphate at 90 kg N/ha. At harvest on 7.1.97, when the plants were at early bud, all nine internal plants from each plot were

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cut and the large stem part separated, leaving it at field. The leaves and small stems were sun dried. Leaves and stems were separated by hand after drying. For artemisinin analysis, around 50 leaves were collected from each plot (9 plants) from the top quarter of the plant and oven dried at 40°C. The analyses were carried out by TLC with densitometric quantification (Delabays et al., 1994).

3. Results

All hybrids presented homogeneous and good agronomic features. Besides the hybrid Ch x Viet.55, chosen as standard due to its excellent yields and just evaluated previously, we had high yields also with another two hybrids obtained by crossing with the genotype 1V, rich in biomass, late flowering and medium-high artemisinin content. From these breedings, the hybrids 2/39 x 1V and 1V x 2/39, where 2/39 are selected genotype from improved Vietnam population, presented “maternal genetic effect” and both higher productivity to the standard hybrid Ch x Viet. 55. This last one is the best hybrid when one considers the leaves/stems ratio. This is an important parameter for industrial processing because the high wax content of the stems renders artemisinin isolation and purification difficult (see table 1).

Table 2 shows the average yields from various fractions of the hybrids as parameters to estimate some important activities on harvest and post-harvest. In fact, one could ascertain the harvest to cut 45 ton/ha of green arbusitive plant; processing the separation of 13 ton/ha of large stems; take off 27 ton/ha of water in drier or other system; following the separation of 2 ton/ha of small stems and 3 ton/ha of dry leaves.

4. Discussion

The process of domestication of Artemisia annua has reached the stage of agrotechnology of cultivate and plant breeding and a new level of yields to artemisinin. Through improved genetics, conventional selection and breeding, high yielding hybrids were obtained. The increase from 5 Kg/ha of artemisinin to 25 Kg/ha opens prospects for the production of anti-malarial drugs. These hybrids could be grown in Brazil with the same yields as in Switzerland where such hybrids were first developed. In fact, with the additional advantage that two harvest can be grown per year in the same area. Furthermore, as subsequent steps, the system of sowing, cultivating, harvesting and drying could be improved to reduce the agricultural costs. It can be concluded that Brazil is a competitive place for the production of anti-malarial drugs from Artemisia annua.

5. References


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**Table 1**  - Leaves and stem yields, rate leaves/stems, artemisinin contents and yields of 3 hybrid lines of *A. annua* L., grown in the field in Brazil. (average of 10 replications)

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Dried leaves (Kg/ha)</th>
<th>Dried small stems (Kg/ha)</th>
<th>Leaves/ Stems (%)</th>
<th>Artemisinin (Kg/ha)</th>
<th>Artemisinin Yield (Kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/39 x 1V</td>
<td>2056.05</td>
<td>1312.20</td>
<td>1,56</td>
<td>1,04</td>
<td>21.38</td>
</tr>
<tr>
<td>1V x 2/39</td>
<td>2141.10</td>
<td>1468.80</td>
<td>1,46</td>
<td>0,90</td>
<td>19.27</td>
</tr>
<tr>
<td>Ch x Viet.55</td>
<td>1975.05</td>
<td>1426.95</td>
<td>1,38</td>
<td>0,80</td>
<td>15.80</td>
</tr>
</tbody>
</table>

**Table 2** - Agricultural yields from various parts of the new hybrid of *Artemisia annua* L.

<table>
<thead>
<tr>
<th>Parts of Plant</th>
<th>Yields (ton/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete aerial plant</td>
<td>45</td>
</tr>
<tr>
<td>All humidity</td>
<td>27</td>
</tr>
<tr>
<td>Large dry stems</td>
<td>13</td>
</tr>
<tr>
<td>Small dry stems</td>
<td>2</td>
</tr>
<tr>
<td>Dry leaves</td>
<td>3</td>
</tr>
</tbody>
</table>

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Figure 1 - Molecule of artemisinin

Figure 2 - Artemisia annua L. (hybrid) at CPQBA-UNICAMP, 1996