5 Breeding and Cultivation of Medicinal Plants

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CONTENTS

5.1 INTRODUCTION

Plants are the source of many important pharmaceuticals. Especially plants rich in secondary metabolites are of interest. These metabolites have been subjected to natural selection during evolution when the presence of particular secondary metabolite conferred an advantage to the species. The secondary metabolites are thought to be beneficial for the plant itself as a physiological active compound, as stress-protecting agents, and by their role in plant resistance against pests and diseases. In addition, some of these secondary metabolites have a beneficial impact on the human health. A main issue related to human health is to optimize the production of the valuable secondary plant metabolites. Considerable effort has been done to generate such metabolites in plant cell or tissue culture. Nevertheless, collection from wild and agricultural production of medical plants still remains the most important supply for plant-derived pharmaceuticals. However, harvesting from wild, especially for species with a high demand, can cause loss of genetic diversity and habitat destruction due to overharvesting. The agricultural cultivation of medicinal plants is an interesting alternative and offers several advantages: reliable botanical identification, less genetic, phenotypic, and phytochemical diversity, availability of well-defined cultivars adapted to the requirements of the stakeholders, better guarantee for appropriate conservation, less extract variability and instability, and a steadier source of raw material.

Agronomic research and development play an essential role to improve the cultivation of medicinal plants by increasing their quality, profitability, and sustainability. In this context, breeding of new cultivars is a key factor allowing us to adapt genotypes to the requirements of the stakeholders. Breeding for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses, and for better homogeneity of the cultivars are important issues. Compared with traditional food crops, breeding of medicinal plants is now in the initial stages, with the advantage that breeders can exploit a high available natural variability within one species. This high genetic variability offers the opportunity to get a high selection response in a relatively short time. Natural variability within one species can be obtained by gathering accessions from wild, from germplasm collections, or from cultivars. For the future, it is important to prevent the loss of genetic diversity, including the diversity within species, *in situ* and *ex situ*. In this context, various policies, plans, and interventions have been recommended worldwide at international, national, and state levels such as the Convention on Biological Diversity defined at the United Nations Conference on Environment and Development in 1992.

The generally high natural variability within medicinal plant species is one of the reasons that classical breeding approaches were mainly used till now. Other reasons are that these methods are relatively cheap and allow a return on investments also with low seed sales quantity. Furthermore, transgenic medicinal plants are not accepted on the European market (Canter et al., 2005; Pank, 2010). At the beginning of a breeding program, it is important to get information about the natural variability of the target traits within a species and its specifics of the flower biology to define the breeding strategy. Breeding a new cultivar needs 5 to 15 years according the species and the selection criteria. To react more quickly to the requirements of the stakeholders, methods to accelerate the breeding procedures must be taken into account such as the use of morphological, phytochemical, and genetic markers at a very early stage in the reproduction cycle, increasing the number of generations per year, as well as rapid and cheap measurement methods of target traits.

To optimize the yield and quality potential of the selected cultivars, research on best cultivation practices are also essential to get information on optimal conditions for seed germination, plant growing (planting, fertilization, and irrigation), harvest, drying, and conservation. These research results are integrated to the guidelines for good agricultural practice for medicinal plants recommending cultivation procedures optimizing the quality of medicinal plants.

The importance of breeding programs and of improving the cultivation procedures to increase the benefit of medicinal plants is shown in the following sections by some projects conducted in Switzerland by Agroscope Changins-Wädenswil Research Station ACW and Mediplant.

5.2 BREEDING FOR INCREASED LEVELS OF VALUABLE COMPOUNDS: *ARTEMISIA ANNUA*

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 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 to develop hybrids with over 1% artemisinin in the dried leaves such as the cultivar "Artemis" (Delabays et al., 1993, 2001). The breeding work continued to get new cultivars richer in artemisinin and well suited to the intertropical zone. Therefore, Mediplant tested hundreds of genotypes, also called clones, for their artemisinin content in the leaves, the leaf dry weight productivity, and the flowering period under field conditions. The best genotypes were then preserved and reproduced *in vitro* according to Lê and Collet (1991) (Figure 5.1). Since the autofertilization is insignificant for *A. annua* (Delabays, 1997), the genotypes were isolated in groups of two to ensure the production of hybrid seeds. Seeds of 45 combinations

FIGURE 5.1 *In vitro* preservation and reproduction of the most interesting genotypes of *A. annua L.*

TABLE 5.1
Yield and Artemisinin Content of the Leaves of Five New Hybrids of *A. annua* of a Field Trial in Conthey-Switzerland in 2005

Hybrids[a]	Dry Leaves Yield (g/m²)	Artemisinin Content (%) (w/w)	Artemisinin Yield (g/m²)
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	ns	ns

Source: From Simonnet, X., Quennoz, M., and Carlen, C. 2008a. *Acta Horticulturae,* 769, 371–373. With permission.
Note: ns, not significant ($P > 0.05$).
[a] Planting date 25 May 2005 and harvesting date 21 September 2005.

were obtained and tested and five promising hybrids among them were then analyzed in a field trial. Their artemisinin contents in the leaves were very high, ranging from
Q1 1.60% to 1.95% (Table 5.1). After 4 months of field cultivation under Swiss climatic conditions with the density of 17,800 plants/ha, these hybrids produced 2.1–2.85 t/ha dry leaves and 40.5–52.0 kg/ha artemisinin. One of the most promising new hybrid, Hybrid 1, showed a similar yield in dry leaves, but much higher content of artemisinin in the leaves and production of artemisinin compared with the cultivar "Artemis" in 2001 (Table 5.2). Although the artemisinin content is the first selection criterion retained, other factors such as the aptitude for *in vitro* conservation, the productivity of leaves, the flowering requirements, and the tolerance to pests and diseases are also considered and, of course, the adaptability in various areas of the intertropical zone which appear to be the main production sites for artemisinin.

TABLE 5.2
Yield and Artemisinin Content of the Cultivar Artemis and the New Reference Hybrid of a Field Trial in Conthey-Switzerland in 2001

Hybrids[a]	Dry Leaves Yield (g/m²)	Artemisinin Content (%) (w/w)	Artemisinin Yield (g/m²)
Artemis	285	1.33b	3.78b
Hybrid 1	289	1.81a	5.20a
	ns	$P < 0.05$	$P < 0.01$

Source: From Simonnet, X., Quennoz, M., and Carlen, C. 2008a. *Acta Horticulturae,* 769, 371–373. With permission.
Note: ns, not significant. Newman–Keuls test: different letters indicate statistically significant differences ($P < 0.05$).
[a] Planting date 16 May 2001 and harvesting date 20 September 2001.

In a recent study in the UK, York, and Norwich, the molecular basis for marker-assisted breeding of *A. annua* was established and QTL related to key traits controlling artemisinin yield were identified (Graham et al., 2010). The presence of a positive artemisinin yield QTL in parents will help to obtain new high-yielding hybrids for the future, helping to answer to the increasing demand in artemisinin. Q2

5.3 BREEDING FOR REDUCED LEVELS OF UNWANTED COMPOUNDS: *ARTEMISIA UMBELLIFORMIS*

Genepi is the name of five species of alpine plants. All of them are protected in Switzerland and their picking is prohibited. Their floral stems have digestive properties and are mainly used to prepare a highly prized liqueur characterized by a bitter taste and a specific flavor. These properties have been traced to the bitter compounds from the class of sesquiterpenic lactones, flavonoids and a complex essential oil mainly containing terpenes such as thujone (Bicchi et al., 1982; Rey and Slacanin, 1997). To avoid collecting from wild, a domestication program was started by Agroscope with the species *A. umbelliformis* Lam. This species was chosen for its erect growth and its relatively high yield potential compared with the other four alpine *Artemisia* species (Rey and Slacanin, 1997). In a second step, the objective was to breed two cultivars of *A. umbelliformis* both with a very erect growth but with different thujone contents in the essential oil. On the one hand, thujone is considered in higher concentrations as neurologically toxic in alcoholic beverages, but on the other hand is appreciated due to its aromatic properties in liquors. The breeding program started with the collection of different accessions from wild from several sites to analyze the natural variability of this trait. Plants were obtained from these seeds and grown in an open field. The yield and the phytochemical composition were analyzed as well as the resistance against different soil and airborne diseases. The Mattmark origin showed a relatively low mortality, high yield, and nearly no thujone, whereas the Simplon origin showed a very high content with about 70% thujone in the essential oil (Table 5.3). Several highly erect clones were selected from the two populations of Mattmark and Simplon and multiplicated *in vivo* and *in vitro*. The selected clones from Mattmark were isolated from the ones from Simplon. Due to the flower biology of this species with combined self-pollination of the hermaphrodite flowers and a cross-pollination of the "female" flowers (flowers without pollen formation), open pollination between the selected clones allowed us to get relatively homogenous cultivars (Rey and Slacanin, 1997). The seeds obtained from the Mattmark clones give the cultivar RAC 12 (nearly no thujones) and from the Simplon clones the cultivar RAC 10 (60–70% thujones in the essential oil). This example shows that a great variability can exist in nature within one species concerning the phenotype, the tolerance to diseases, and the phytochemical profile. This high variability is important for successful breeding.

5.4 BREEDING FOR ABIOTIC AND BIOTIC TOLERANCE: *HYPERICUM PERFORATUM*

Cultivars tolerant to abiotic and biotic stress highly increase the supply stability. As an Q3
example, *H. perforatum* L., recommended in plant therapy for its antiviral, vulnerary,

TABLE 5.3
Mortality after 3 Years of Cultivation, Yield of Two Harvest in the Second and Third Year and Composition of the Essential Oil of Four Accessions of *A. umbelliformis* Lam. in a Field Trial in Bruson (1100 m) in Switzerland

	Accession from Different Alpine Sites in Switzerland			
Analysed Parameters	**Mattmark**	**Simplon**	**Gornergrat**	**Valsorey**
Mortality (%)	19	41	98	55
Yield (dw, g/m^2)	155	79	30	96
Essential oil composition (%)				
β-Pinene	31	0	3	2
1,8-Cineole	17	3	1	23
α-Thujone	0	72	0	0
β-Thujone	0	0	0	0
Borneol	5	3	0	20
Terpinen-4-ol	2	0	2	3
Trans-caryophyllene	2	0	1	2

Source: Adapted from Rey, C. and Slacanin, I. 1997. *Revue suisse de viticulture, d'arboriculture et d'horticulture*, 29, I–VIII.

and antidepressive properties (Schaffner et al., 1992), badly suffers from a wilt disease, an anthracnose caused by *Colletotrichum* cf. *gloesporioides* (Debrunner et al., 2000). Therefore, an important breeding aim is the resistance or the tolerance to this anthracnose. In a project of Mediplant, the commercial cultivars Topas, Hypermed, and Elixir and 21 wild accessions collected from Switzerland, Germany, Italy, and Canada were compared. The tests were made in three locations in Switzerland at different altitudes with natural infestations of this anthracnose. The accessions were very different with regard to anthracnose resistance (Table 5.4), flowering time, plant morphologies, flower and drug yields, and phytochemical profiles. The accession P7 was selected because of its low susceptibility to this disease. Furthermore, yield and the phytochemical profile (hypericines and flavonoides) were similar to those of the standard cultivar Topas and met the industrial requirements. Thanks to the flower biology of this species, a pseudogamous apomictic reproduction, seeds of the accession P7 were relatively easy to obtain. Apomictic means that seed formation occurs without fertilization of the egg cells by the sperm cells. Breeders can use the apomictic behavior for fast genetic fixation of aimed trait expression (Pank et al., 2003) because seed progenies have the same genetic code as the mother plants. The accession P7 is registered now as the cultivar "Hyperivo 7."

5.5 BREEDING FOR HIGHER HOMOGENEITY: *THYMUS VULGARIS*

Thymus vulgaris L. is especially well known for its aromatic and therapeutic properties due to the essential oil of its leaves. The essential oil is used as a flavor additive,

TABLE 5.4
Susceptibility (% of Plants with Symptoms: Diseased and Dead Plants) of 24 Accessions of *Hypericum Perforatum* Against a Wilt Disease (Anthracnose Caused by *Colletotrichum cf. Gloesporioides*) after 2 Years of Cultivation in 1998 at Three Different Sites with Natural Infestation

	Sites in Switzerland		
Cultivars, Accessions (A) Parameters	Fougères (Conthey, 480 m)	Epines (Conthey, 480 m)	Bruson (Bagnes, 1100 m)
A01 (cv. "Topas")	70b	64ab	0a
A02 (cv. "Hyperimed")	93b	100b	30ab
A03 (cv. "Elixir")	96b	100b	17ab
A04	100b	87b	20ab
A05	100b	100b	97c
A06	100b	100b	100c
A07	17a	50a	0a
A08	100b	87b	93c
A09	100b	100b	100c
A10	100b	100b	100c
A11	100b	100b	100c
A12	90b	100b	73c
A13	100b	100b	100c
A14	100b	100b	100c
A15	100b	100b	100c
A16	87b	100b	64c
A17	100b	73ab	0a
A18	83b	100b	0a
A19	100b	100b	20ab
A20	87b	100b	0a
A21	77b	100b	0a
A22	100b	100b	70b
A23	100b	100b	40b
A24	100b	100b	3a
Mean	89	94	51

Source: Adapted from Gaudin, M. et al. 1999. *Revue suisse de viticulture, d'arboriculture et d'horticulture,* 31, 335–341.

Note: Newman–Keuls test: different letters indicate statistically significant differences ($P < 0.05$).

as well as an antimicrobial and antioxidative product (Mewes et al., 2008). For *T. vulgaris* as well as for all medicinal plants, it is important to have good-quality cultivars with a high level of homogeneity. Homogenous cultivars are very important for the whole supply chain allowing to standardize better the production and extraction. Rey (1993), Pank and Krüger (2003) and Rey et al. (2004) showed that crossing sterile male (MS) with fertile male (MF) plants to breed hybrids is an adequate approach to improve the homogeneity of thyme cultivars, for example. Therefore, a breeding program was conducted by Agroscope to optimize the quality, the yield performance, and the homogeneity of *T. vulgaris* by exploiting the natural gynodioecy of its flowers (MS and hermaphroditic plants). The breeding program yielded 56 new hybrids obtained by crossing MS and MF clones (Carlen et al., 2010). Some of them showed very promising results; in particular Hybrid 3 (cultivar "Varico 3"), obtained by crossing two accessions from the Agroscope ACW breeding material, showed a high essential oil content with 4.4% average over five harvests and a high leaf yield. In addition, the parents of this hybrid are well synchronized in their flowering period and have quite a good seed production potential.

The new hybrid cultivar "Varico 3" was then compared with two other thymol-chemotype cultivars "Varico 2" and "Deutscher Winter." "Varico 3" and "Varico 2" showed higher yields over 2 years and higher content of the essential oil with 4.9% and 3.5%, respectively (Table 5.5). Furthermore, the two hybrid cultivars showed a higher homogeneity than the population cultivar "Deutscher Winter" (Table 5.6). These results confirm the usefulness of hybrid breeding for the improvement of homogeneity for thyme (Pank and Krüger, 2003). In addition, other breeding programs conducted by Agroscope with *Salvia officinalis* L. (cultivar "Regula"), with *Origanum* sp. (cultivar "Carva") and with *Leontopodium alpinum* Cass. (cultivar "Helvetia") showed that crossing MS with MF plants to breed hybrids is a well-adapted breeding strategy to improve the homogeneity of a cultivar. The exploitation of male sterility allowing controlled pollination will become more and more important in the future (Carlen et al., 2010; Pank, 2010).

5.6 OPTIMIZING THE CULTIVATION PROCEDURE OF SELECTED CULTIVARS

To optimize the yield and quality potential of the selected cultivars, research on best practices of their cultivation is fundamental. The objectives are to get information on

TABLE 5.5
Comparison of Three Cultivars of Thymol-Chemotype *Thymus Vulgaris* L.: Yields. Leaf Propositions and Essential Oil Contents Correspond to the Sum/Mean of Three Harvests (2007–2008) in Arbaz with Four Replications

Cultivar	Total dw Yield (t/ha) 2007–2008	Leaf Proportion (%), $\bar{x}$, 2007–2008	Leaf dw Yield (t/ha) 2007–2008	Essential Oil Content of Leaves (% v/w), $\bar{x}$, 2007–2008	Essential Oil Yield (l/ha), 2007–2008
"Varico 3"	6.16a	63b	3.86a	4.9a	191a
"Varico 2"	6.63a	63b	4.17a	3.5b	146b
"Deutscher Winter"	4.91b	67a	3.31b	2.9c	97c

Source: Adapted from Carlen, C. et al. 2010. *Acta Horticulturae,* 860, 161–166.
Note: Tukey test: different letters indicate statistically significant differences ($P < 0.05$).

TABLE 5.6
Comparison of Different Plant Traits Between the Three Cultivars of Thymol-Chemotype *T. Vulgaris* L. at the Second Harvest in September 2008 in Arbaz. The Values Correspond to the Mean of 12 Measurements per Replication and Four Replications. The Coefficient of Variation (CV) was Calculated from 48 Measurements per Cultivar

Cultivar	Stem Length (mm)	CV (%)	Length of Internode (mm)	CV (%)	Leaf Length (mm)	CV (%)
"Varico 3"	157a	17	8.0a	18	5.8ab	11
"Varico 2"	148a	14	7.3ab	23	6.2a	13
"Deutscher Winter"	141ab	21	7.6ab	43	5.5ab	18

Note: Tukey test: different letters indicate statistically significant differences ($P < 0.05$).

best seed germination conditions, planting schemes, growth conditions, fertilization and irrigation treatments, harvest period, as well as harvest and drying techniques. Some examples show the importance of getting information about the requirements of the medicinal plants.

For medicinal plants, especially for new cultivated species, there is often a lack of knowledge about the specific requirements for seed germination. As an example, for *Verbena officinalis* L., the seed germination is a recurring problem for seedlings producers. Tests in Petri dishes allowed defining the optimal stratification conditions: more than 2 weeks at 3°C increased the germination rate up to two or three times compared with the control treatment without stratification (Carron et al., 2009). Q4
As the alteration in temperatures plays a decisive role in germination for this species, a new stratification method in an incubator was developed: 5 h cycles (17°C in light/2°C in the dark) allowed reducing the duration of stratification up to five times. Q5
This technique will allow considerable time-saving for seedling producers. However, treatments with gibberellic acid were not beneficial for *V. officinalis*. Another example with difficulties in seed germination is *Podophyllum hexandrum* Royle. Germination tests conducted by Mediplant suggest that a post-harvest seed maturation for a few months in combination with gibberellic acid treatment was essential to increase the germination rate from about 20% to 80% (Simonnet et al., 2008b). The optimization of the germination conditions can favor the cultivation of this endangered species in Himalayan sites due to overharvesting.

Optimizing the growing conditions can improve the quality of medicinal plants. This could be shown for different *Lamiaceae* species covered by an agrotextile during the growing period. Yield and essential oil content were generally improved by the microclimate generated under the agrotextile fabric, especially in the spring and at the beginning of summer (Carron et al., 2008a,b). For *Melissa officinalis*, mainly Q6
the essential oil content sensibly increased. However, the essential oil composition was not significantly influenced (Table 5.7).

TABLE 5.7
Influence of Agrotextile Covering on the Yield, Proportion of Leaves, Essential Oil Content and Yield of *Melissa Officinalis* L. Cultivated in the Field in Bruson (1100 m)

	Field Cultivated Since 3 Years (2004)		Field Cultivated Since 1 Year (2006)	
Parameters	**Control**	**Agrotexile Cover**	**Control**	**Agrotexile Cover**
Number of harvests	3	4	2	2
Yield (dw, t/m^2)	5.1	5.1	3.9b	4.7a
Leaf proportion (%)	62	62	70a	62b
Leaf essential oil content (% v/w)	0.15b	0.34a	0.18b	0.48a
Yield of essential oil (L/ha)	4.9b	10.7a	5.1b	14.2a

Source: Adapted from Carron, C. A., Baroffio, C., and Carlen, C. 2008a. *Revue suisse de viticulture, d'arboriculture et d'horticulture*, 40, 125–130; Carron, C. A., Baroffio, C. A., and Vouillamoz, J. 2008b. *Revue suisse de viticulture, d'arboriculture et d'horticulture*, 40, 195–199.

Note: Tukey test: different letters indicate statistically significant differences ($P < 0.05$) between the control and the agrotextile treatment within 1 year.

An important issue for almost all medicinal plants is the optimal harvest period. The harvest period influences not only the yield but also the phytochemical profile. For example, for *A. umbelliformis*, a very strong influence of the harvest period on the essential oil concentration in the floral trusses was highlighted (Table 5.8). At the beginning of flowering, the essential oil content of the variety RAC 12 exceeded

TABLE 5.8
Yields, Essential Oil Content, and Costunolide Content in the Floral Stems of *A. Umbelliformis* Lam. Depending on 5 Harvest Stages (Harvests 2002 and 2003)

Harvest Stages	Floral Trusses Yield (g dw/m^2)		Essential Oil Content (mL/100 g dw)		Costunolide Content (g/100 g dw)	
	2002	**2003**	**2002**	**2003**	**2002**	**2003**
Stage 1	48ab	43b	1.31ab	1.46b	2.72a	2.91a
Stage 2	37b	45b	1.53a	1.76a	3.00a	2.76a
Stage 3	93ab	64b	1.08b	0.71c	2.78a	0.93b
Stage 4	87ab	73ab	0.61c	0.41c	1.26b	0.75b
Stage 5	102a	94a	0.43c	0.41c	1.16b	0.56b

Source: From Simonnet, X. et al. 2009. *Acta Horticulturae,* 826, 31–34. With permission.

Notes: Harvest stages: Stage1, just before flowering; Stage2, beginning of flowering; Stage 3, full flowering; Stage 4, end of flowering; Stage 5, flowering over. Newman–Keuls test: different letters indicate statistically significant differences ($P < 0.05$) between harvest stages.

1.5%. After 7–9 days, the essential oil content dropped 30% in 2002 and even 60% in 2003. However, no significant variation in the chemical composition was observed in relation to the harvesting stages (Simonnet et al., 2009). The average value of the components remained similar for the years 2002 and 2003, except for borneol (7.3% in 2002 and 15.6% in 2003), indicating that also the year could influence the quality. The dynamics of costunolide content is very similar to that of the essential oil, with a maximum at the beginning of flowering and a quick drop toward full flowering (Table 5.8). At its maximal concentration, the costunolide content was very high with nearly 3%. The floral stems yield doubled between the beginning and the end of the flowering period, reaching up to 100 g/m^2 (Table 5.8). Harvesting from the full flowering onward allows better yields. In conclusion, harvesting *A. umbelliformis* at the beginning of flowering is recommended to ensure obtaining a product of quality. However, the yield is lower than at the post-flowering stages. Therefore, price for *A. umbelliformis* harvested at the beginning of flowering must be higher than at later stages.

5.7 CONCLUSIONS

With the increasing demand for herbal medicines, cultivation of medicinal plants becomes more and more important. Conventional plant breeding and development of best cultivation practices for the selected cultivars can improve both agronomic and medicinal traits. However, one of the main obstacles to bring successfully more cultivated medicinal plants in the supply chain is the relatively long period of 5 to 15 years for bringing new species and new cultivars on the market. In the future, methods to accelerate the breeding process must be taken into account to allow react more quickly to the requirements of the stakeholders.

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