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Improvement of medicinal plant quality: a *Hypericum perforatum* literature review as an example

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Abstract

Numerous factors influence the chemical quality of medicinal plants from crop establishment to extraction of raw material. The most important ones are described using the example of *Hypericum perforatum*. Optimization of these factors contributes to the objective of producing a high-quality drug, and a method consisting of three scientific approaches (technological, agronomical, plant breeding) is presented. All data concerning the plant (biology, physiology and environmental impacts) and the active components and by-products (pathway, localization and stability) are useful to adapt and to develop management sequences. Although plant breeding appears to be the principal way of improvement, and gives good results in terms of resistance to pathogens, active component content and yield; the agronomical and the technological approaches are also very important. The technological approach after harvesting is especially important to avoid degradation of the active components and to induce, in some cases, the transformation of by-products to those molecules sought. This integrated method (plant breeding and agronomical and chemical approaches) requires research on different levels of organization from molecule to field, and includes all processing systems from farmers to chemists.

Keywords: agronomy; drug; *Hypericum perforatum*; medicinal plant; plant breeding; quality

Introduction

Studies on medicinal plants are rapidly increasing because of the search for new active molecules, and for the improvement in the production of plants or molecules for the herbal pharmaceutical industries (phytotherapy or allopathy). This requirement of the industry needs a control of the quality drug. Different elements contribute to the quality: purity of the raw material (no adulteration), low bacterial, fungus, pesticide, radioactive or heavy metal contamination, and high active component concentration. This literature review focuses particularly on this last element, and describes the specific approaches useful to all medicinal plants: plant breeding, agronomical and technological approaches. The objective of this paper is to describe, through the example of *Hypericum perforatum*, major factors influencing chemical quality of a medicinal plant and to present strategies aiming to improve and to use all potentiality of the active component.

Numerous factors influence the production of active components by the plant, and their presence in the end product (dry grind herb for tea or capsules, extract, or pure molecules) (Schilter *et al.*, 2003). Each step of production from crop establishment to extraction of the raw material has an impact on the quality and quantity of components (Fig. 1). Choice of the accession and agricultural practices determines the content and the yield of the active components, but also the presence of

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by-products with a close structure to the molecules searched. Post-harvest techniques aim to value the active components and to avoid their loss.

To improve the quality of a medicinal plant requires obviously a precise definition of what is meant by quality. Chemical quality corresponds generally to a clear identification of one or several active components, or to specific markers where they are not known. However, frequently, the complexity of the extract, and the interactions and synergy between constituents, makes it difficult to determine the nature of the active components (Wills *et al*., 2000).

Hyperici herba consists of the dried flowering tops or aerial parts of *H. perforatum* (*Clusiaceae*) (St John's wort) containing a huge number of secondary metabolites: naphtodianthrones, phloroglucinols, flavonoids, xanthones, procyanidins and essential oils (Bombardelli and Morazonni, 1995; Ploss *et al*., 2001). Several clinical and pharmacological studies have demonstrated the activity of *H. perforatum* especially in the treatment of mild to moderate depression, but the mechanism of action is still unknown (Cott, 1997; Butterweck *et al*., 1998, 2003; Nathan, 2001). Furthermore, during fractionation of the extract much of the pharmacological activity is lost. For many years, the red pigment, hypericin, was considered as the main active constituent. Nevertheless, it has now been demonstrated that acylphloroglucinol, hyperforin (Singer *et al*., 1999), together with hypericin, is involved in the antidepressant action (Mennini and Gobbi, 2004).

The control and the optimization of each step of the production will contribute to a good quality drug (Fig. 1). We intend to demonstrate how each of these

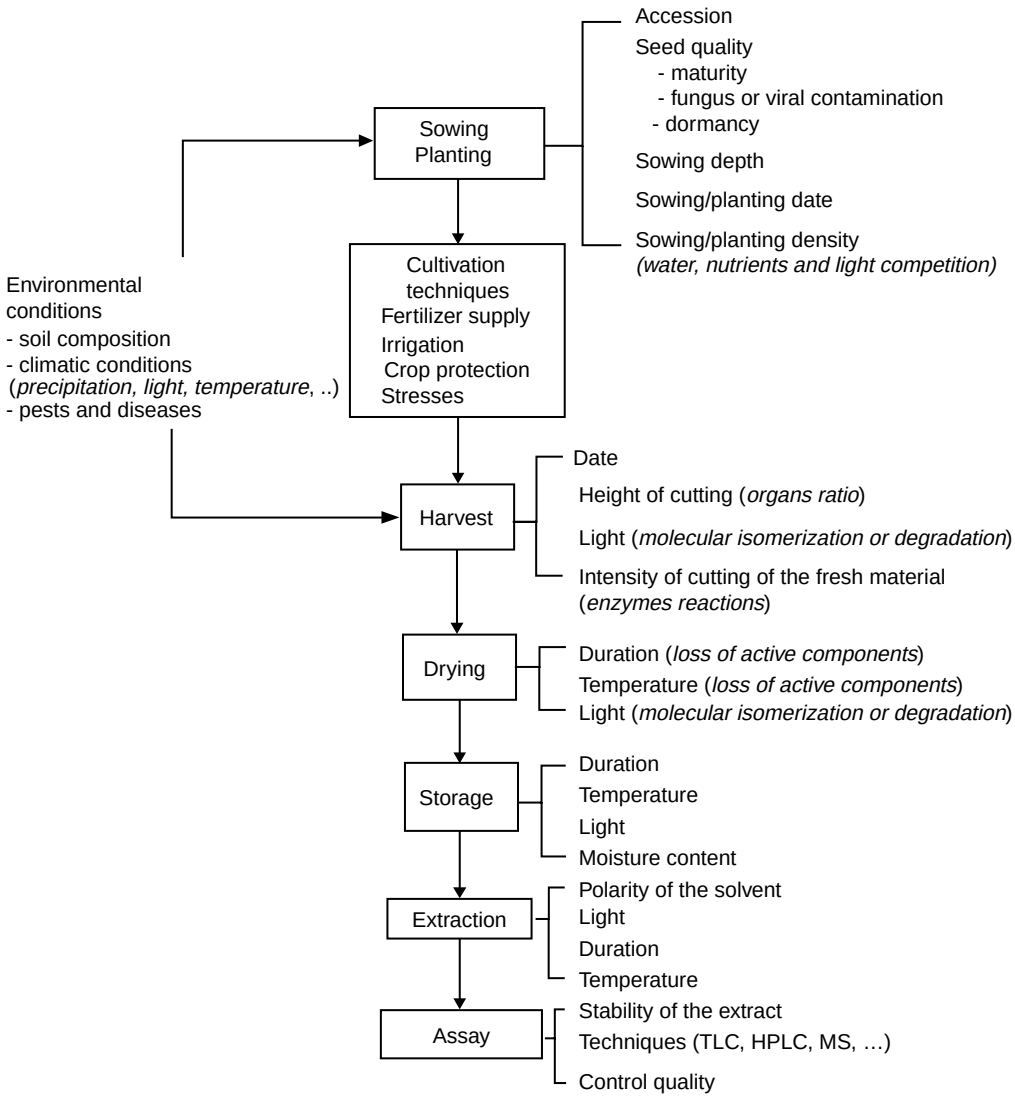

Fig. 1. Factors which could interfere with the active component content and yield of a medicinal plant. TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry.

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factors may be involved in the active component content and yield. Three approaches (Fig. 2) contribute to the improvement of drug quality:

- The 'chemical' or 'technological' approach from harvest to end product.
- The 'agronomical' approach from soil preparation before sowing to harvest.
- The 'genetic' approach.

These three approaches will be described and their contributions discussed, in relation to the active components of *H. perforatum*.

The technological approach

Characteristics of the active components and their precursors and by-products

An understanding of the characteristics of the active components: pathways, structure (glycosylation, methylation, polymerization), localization in the plant (organs, tissues and cells), stability and enzymes involved in their synthesis and their degradation is very important.

In the case of *H. perforatum*, the terms hypericin(s) and hyperforin(s) appear in numerous scientific papers but are not always clearly defined. In this paper, hypericins will be defined as the sum of protopseudohypericin, pseudohypericin, protohypericin and hypericin; and, protohypericins as the sum of protopseudohypericin and protohypericin. The pharmacological activities of the protohypericins have not yet been studied. Hyperforins include hyperforin and adhyperforin. Standardization of St John's wort products has been based mainly on the quantification of the three components, hypericin, pseudohypericin and hyperforin (Chatterjee *et al.*, 1998; Orth *et al.*, 1999; Butterweck *et al.*, 2003).

Protopseudohypericin and protohypericin convert into pseudohypericin and hypericin respectively in the presence of light (Freytag, 1984; Gaedcke, 1997). The localization of synthesis of emodin-anthrone (the precursor of hypericins) and of hyperforins is not yet clearly defined (Pasqua *et al.*, 2003). Hyperforins, a family of antimicrobial phloroglucinols, are particularly unstable in the presence of light, and are rapidly oxidized (Granzow and Holzl, 1998; Sirvent and Gibson, 2002).

Extraction and assay

Various types of extraction can be applied to raw material resulting in significant changes in the quantities and proportions of active components affecting safety and benefits (Schilter *et al.*, 2003). According to the conditions of the extraction: solvent, duration, light conditions and temperature, the secondary metabolite content of the extract will change (Liu, 2000; Melnikova *et al.*, 1998). Therefore, it is almost impossible to compare published results. The flowering tops of St John's wort contain approximately 30% hypericins in the form of protohypericins (Poutaraud *et al.*, 2001a) which could be rapidly phototransformed in the extract, under the action of sunlight, to hypericin and pseudohypericin. However, a 5-min exposure of the crude extract of *H. perforatum* to sunlight (1 E/m^2) induces a 96% loss of hyperforins

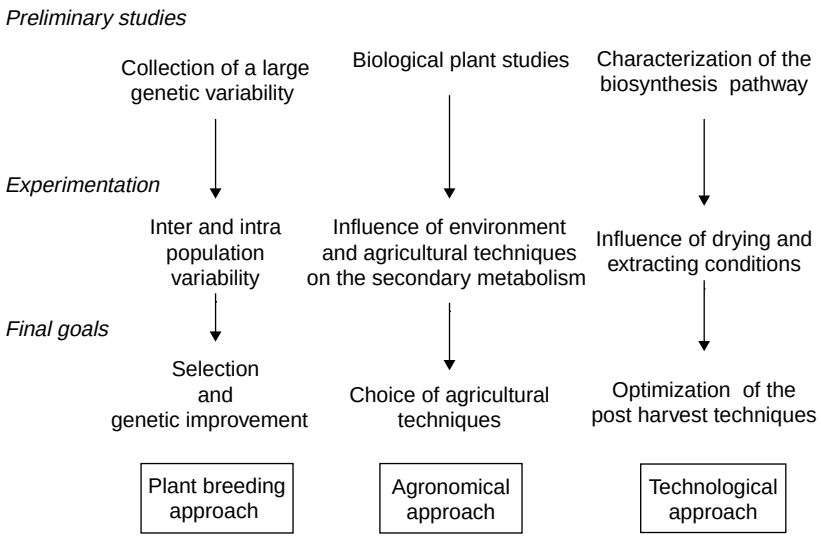

Fig. 2. Three approaches to improve the quality of a medicinal plant.

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(Poutaraud *et al*., 2001b). The phototransformation of the protohypericins without loss of hyperforin could be done by application of 515 nm light on the extract because it is the optimal wavelength of protohypericin phototransformation (Poutaraud *et al*., 2001a) and hyperforins do not absorb at this wavelength. The level of extraction of some compounds could be linked to the presence of other constituents. For example, the solubility of pure hypericin in water increases upon addition of some phenolic constituents typical for *Hypericum* extracts (Jurgenliemk and Nahrstedt, 2003). All these chemical data must be taken into account during extraction and assay to avoid experimental bias and transform by-products into active components.

Storage and drying

There is generally very little data regarding the influence of storage and drying on the quality even if we can expect an eventual loss of active components depending on the duration, and, the temperature and light conditions. Many secondary metabolites are not stable (oxidation, hydrolysis, enzymatic reactions) or are volatile (Bottcher *et al*., 2003). Secondary metabolites are accumulated in special organs or cells to avoid negative toxic contact with other organs or cells, and particular attention must be paid to protect these structures. Hypericins are located in specialized glands which appear as tiny black dots and/or lines on all the above-ground parts, but particularly in flowers and buds (Curtis and Lersten, 1990; Ciccarelli *et al*., 2001). During the drying process, these glands may collapse, inducing an important loss of hypericins. Light during drying reduces slightly the proportion of protohypericins and increases the hypericin and pseudohypericin contents (Poutaraud *et al*., 2001a) whereas the dry plant material loses 20% of hyperforins after 2 h exposure to sunlight (24 E/m^2) (Poutaraud *et al*., 2001b). To maintain the high quality of *H. perforatum*, it is essential to ensure effective ventilation and cooling after harvesting, and to keep the dry plant material protected from light (Bottcher *et al*., 2003).

The agronomical approach

Cultivation of medicinal plants presents many advantages compared with harvesting from the wild. It avoids the risk of error in identification and of overexploitation and destruction of natural sites, and allows mechanical harvest (Franke *et al*., 1993). Agronomical factors may influence at the same time the quality and quantity of the drug. The concentration of active components in the plant varies with internal factors: organs, age of the plant, development stage or external factors: biotope, climatic conditions, season. *H. perforatum* is difficult to grow (Mayo and Langridge, 2003) and is usually not cultivated for more than 3 years, because of its susceptibility to fungal diseases. These agricultural problems often induce a significant decrease in dry weight yield. Sites of synthesis and storage of active components are different according to the plant and the compounds concerned. Seasonal variations in the content of hypericins were shown (Southwell and Bourke, 2001): hyperforins are mainly located in flowers (pistils) (Repcak and Martonfi, 1997) and fruits (Nahrstedt and Butterweck, 1997; Pietta *et al*., 2001). Translocation of these metabolites within the plant during development could occur, but, for hyperforin and precursors of hypericins, the mechanisms have not been studied.

The biosynthesis of hypericins seems to be strictly linked to the differentiation of the glands (Pasqua *et al*., 2003). The increase in hypericin and hyperforin content, in response to chemical and biotic elicitors, suggests these secondary metabolites are components in the inducible plant defence responses of *H. perforatum* (Sirvent and Gibson, 2002). Their synthesis could perhaps be stimulated by stress as shown with other plant metabolites (Chappell and Hahlbrock, 1984; Chatterjee *et al*., 1988; Zobel *et al*., 1994; Baricevic *et al*., 1999).

Agricultural practices

Generally, very few data on agricultural practices are published, which slows down possible improvements.

Soil properties and fertilization

The soil has a complex influence on plants through its physical, chemical and biological properties. It is not easy to interpret the influences of separate factors (Hornok, 1992). Fertilizers are well known for their influence on primary metabolism and biomass production. Mineral elements are involved in the structure of some secondary metabolites but could also interfere in their regulation. Microelements have been studied in numerous other medicinal plants for inducing synthesis of secondary metabolites (Gasic *et al*., 1978; Hornok, 1992; Wierzchowska-Renke *et al*., 1995). Several studies were performed on the influence of fertilizer on *H. perforatum*, but are contradictory regarding nitrogen supply (Denke *et al*., 1999; Briskin *et al*., 2000; Azizi and Omid-Beigi, 2001; Dias *et al*., 2001). Metal contamination can also change the chemical composition of medicinal plants, thereby seriously impacting the quality, safety and efficacy of natural plant products. Environmental contamination with a common inorganic pollutant like nickel influences the synthesis and the accumulation of

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Table 1. Bibliographic data of St John's wort plants or accessions harvesting at full bloom

Number of plants or accessions	Control	Site of culture/ natural site	Harvest	Plant part	Number of plants and replications	Assay	Molecule	Content	References
Fifty plants		1 SC Germany	First year of cultivation	Flowers		HPLC (Hölzl and Ostrowski, 1987)	Hyperforin Hypericin Pseudohypericin	0.206–0.606 mg per flower 0.00288–0.02281 mg per flower 0.011–0.063 mg per flower	(Hölzl and Ostrowski, 1987)
Five accessions Broadleaved Narrowleaved		1 SC Australia		Flowers, leaves, stems, capsules	3 replications	Spectrophotometry (Southwell and Campbell, 1991)	Hypericins	From 0.220 to 0.519% in flowers From 0.027 to 0.181% in leaves 0.004% in stems From 0.071 to 0.076 in capsules	Southwell and Campbell, 1991
Thirteen plants		1 SC Slovakia	Second year of cultivation	Flowers for analysis, aerial part for dry weight		HPLC (Hölzl and Ostrowski, 1987), modified	Hypericin Pseudohypericin	0.036–0.122% 0.143–0.247%	Oravec Sen *et al.*, 1994
Two accessions	Topaz	1 SC Poland	3 years	Aerial part			Hypericin Pseudohypericin	Topaz: 0.243% Local origin: 0.238%	Seidler-Lozykowska and Dabrowska, 1996
Seven accessions *H. perforatum* *H. maculatum* *H. tetrapterum* Three of them are preselected clones produced by *in vitro* propagation		3 SC Switzerland: Eschikon alt. 550 m Basel alt. 310 m Fougères alt. 480 m	2 years	Flowers for analysis, aerial part for dry weight, 10 cm above the soil surface, divided in two parts	5 flowers per plant, 4 plots of 8 plants per accession and per site	HPLC (Hölzl and Ostrowski, 1987) modified	1 Hyperforin 2 Hypericins	Just peak area From 0.1% to 0.7% the first year function of accessions, site and years	Büter *et al.*, 1996, 1998b
In vitro regenerant R0 Altered ploidy level from Topaz plant		1 SC Slovakia	First year (R0, R1, R2) and second year (R0)	Aerial part	82 plants in R0 generation, 226 plants in R1 generation, 90 plants in R2 generation	Spectrophotometry (Cellarova *et al.*, 1994)	2 Hypericins	0.3–0.4% R0 first year 0.3–0.5% R0 second year 0.35–0.4% R1 0.65–0.9% R2 Higher content in $2n = 16$ than $2n = 24$ or 32	Cellarova *et al.*, 1997

Table 1. *Continued*

Number of plants or accessions	Control	Site of culture/ natural site	Harvest	Plant part	Number of plants and replications	Assay	Molecule	Content	References
Ten accessions (narrow area in Switzerland) Twenty accessions (Europe, Canada)		1 SC Switzerland (near Basel)		Flowers for analysis, aerial part for dry weight	10 plants, 3 replications	HPLC (Hölzl and Ostrowski, 1987), modified	2 Hypericins	3 morphotypes No clear correlation between morphotypes and quantity and quality of the constituents	Büter *et al.*, 1998b
One hundred and fifty-three accessions	Topaz	2 SC Italy, Germany	2 years	Aerial part	4 replications	HPLC (Hölzl and Ostrowski, 1987)	2 Hypericins 1 Hyperforin	0.02–2.1% 2–4% Show a group of accessions with double value of Topaz hypericin content and higher value of hyperforin	Franke *et al.*, 1998; Franke, 1998 oral communication
Seventy-four accessions 21 *H. perforatum* 6 *H. maculatum* *H. hirsutum*, *H. tetrapterum* *H. montanum*, *H. humifusum*		NS Slovenia	1 year	Flowers, green parts		HPLC (Kartnig *et al.*, 1996)	Hypericin	0.169 herbs, 0.520% flowers, *H. perforatum* 0.046% herbs, 0.187% flowers, *H. maculatum*	Umek *et al.*, 1999
							Pseudohypericin	0.249 herbs, 0.522% flowers, *H. perforatum* 0.199% herbs, 0.803% flowers, *H. maculatum*	
							Hyperforin	0.601 herbs, 1.359% flowers, *H. perforatum* 0.000% herbs, 0.000% flowers, *H. maculatum*	
Twenty-four accessions (Switzerland, Germany, Italy, Australia, Canada)	Topaz Hyperimed Elixir	3 SC Switzerland Epines alt. 480 m Fougères alt. 480 m Bruson alt. 1060 m	All accessions in first year 1997 The most interesting accessions in second year	Aerial part	10 plants, 3 replications but only one assay by accession and by site	HPLC	2 Hypericins	From 0.11 to 0.45%	Debrunner *et al.*, 1997 Gaudin *et al.*, 1999

Table 1. *Continued*

Number of plants or accessions	Control	Site of culture/ natural site	Harvest	Plant part	Number of plants and replications	Assay	Molecule	Content	References
Nineteen accessions		NS	1 year	Aerial part		HPLC (Girzu *et al.*, 2000)	Hypericin	0.04% in *H. perforatum* 0.02% in *H. maculatum*	Girzu *et al.*, 2000
							Pseudohypericin	0.08% in *H. perforatum* 11% in *H. maculatum*	
One variety (N and P trial)	Topaz	SC near Teheran alt. 1215 m	Second year	15 cm under the inflorescences, aerial part		HPLC-DAD	2 Hypericins 2 Hyperforins	0.11% ± 0.02 1.32% ± 0.15	Dias *et al.*, 2001
Five accessions (USA)		NS	1 year	Flowers, leaves, stems		HPLC	Hypericin	Mean 0.06% (flowers)	Walker *et al.*, 2001
							Pseudohypericin	Mean 0.29% (flowers)	
Five accessions (Italy)		NS	1 year	Flowers, fruits		HPLC-DAD (Pietta *et al.*, 2001)	2 Hypericins	0.8–1.6% (flowers), 0.3–0.9% (fruits)	Pietta *et al.*, 2001
							2 Hyperforins	1.24–2.11% (flowers) 3–4.15% (fruits)	
Seven accessions (Tuscany)		NS	1 year	Flowering top, fruiting top		HPLC-DAD (Bergonzi *et al.*, 2001)	2 Hypericins 4 Hyperforins	0.05–0.11% 1.37–20.8%	Bergonzi *et al.*, 2001
Eighteen accessions (Hungary, Germany)	Topaz Anthos	1 SC Hungary	2 years	?	1 plant, 7–10 replications	HPLC (Hölzl and Ostrowski, 1987), modified	2 Hypericins	0.4–1.58%	Pluhár *et al.*, 2002
Thirty-nine accessions (France, Switzerland)	Topaz	1 SC France	3 years	Flowering top, flowers	6 plants, 4 replications	HPLC-DAD (Poutaraud *et al.*, 2001b)	4 Hypericins 2 Hyperforins 4 Hypericins 2 Hyperforins	0.07–0.30% 0.65%–3% 0.24–0.61% 1.97–5.71%	Poutaraud and Girardin, 2004

alt., altitude; DAD, diode array detector; HPLC, high-performance liquid chromatography; NS, natural site; SC, site of culture.

hyperforin and of pseudohypericin and hypericin (Murch *et al.*, 2003). *H. perforatum* accumulates soil cadmium (Büter *et al.*, 1996), although the interaction with active component synthesis has not been shown. The content of hypericin was slightly increased by tolerated herbicides (Pank, 1990).

Light

Quantity and quality of light is known to interfere with the secondary metabolism, either directly by production of energy/carbohydrates, or indirectly by regulating metabolic pathways (Hornok, 1992). An increase in light intensity for *H. perforatum* induces a continuous increase in the level of leaf hypericins linked to the number of dark glands (Briskin and Gawienowski, 2001). In cultivation, the density of planting can obviously modify the quantity, and to a lesser extent the quality, of light exposure, to each plant.

Harvest

Harvesting techniques are very important because numerous factors can interfere with the quality of the drug. These include climatic conditions, time of year and type of machinery used to harvest. In the case of *H. perforatum* where two different kinds of active components are being researched, the determination of the best date to harvest is a problem. Huge differences in secondary metabolite content were shown according to the stage of the plant growth (Upton, 1997; Tekel'ova *et al.*, 2000). The flowering phase of *H. perforatum* lasts for about 3–4 weeks, and the ripening phase for about 3 weeks (Franke *et al.*, 1998). For hypericins, the highest content is found at full flowering, and then immediately declines (Brantner *et al.*, 1994). For hyperforins, the best harvest date is at the beginning of the fruiting phase (Nahrstedt and Butterweck, 1997). The height of cutting is important, it determines the ratio between organs; the producer must choose between high active component content (high flowers and fruits to stems and leaves ratio) or dry weight yields (low flowers and fruits to stems and leaves ratio). Indeed, if the harvest consists of more than 30 cm of flowering tops, the percentage of stems is too high and dilutes the active components because of the very low content in this organ. Depending on the picking machine, stems can be eliminated (Mohr *et al.*, 1996).

The genetic approach

Genetic variability of medicinal plants is generally very important (Tetenyi, 1991). Wild ecotypes selected for their high dry weight and/or active component contents and/or resistance to pathogens are often used in cultivation (Pank *et al.*, 2003). Several studies have been performed to characterize different accessions (Table 1). Some plants are harvested directly from their natural site (Kartnig *et al.*, 1989; Umek *et al.*, 1999; Girzu *et al.*, 2000; Bergonzi *et al.*, 2001; Pietta *et al.*, 2001; Walker *et al.*, 2001). Their chemical characteristics may be of poor scientific value because the environmental factors which are not accurately defined affect the results, so the best way to compare accessions is to cultivate them on the same site (Southwell and Campbell, 1991; Oravec Sen *et al.*, 1994; Büter *et al.*, 1996, 1998a, 1998b; Seidler-Lozykowska and Dabrowska, 1996; Cellarova *et al.*, 1997; Debrunner *et al.*, 1997; Franke *et al.*, 2000; Pluhár *et al.*, 2002). Study of the accessions on different cultivation sites with different characteristics of soil and climate is also important. Nevertheless, this can be very difficult, and to be useful, accessions must be established at the same date on a homogeneous plot, harvested at a same stage (often difficult to determine) for at least two consecutive years. Then, the accessions must be dried, stored, extracted and assayed according to the same methods. Studies must also be performed on different plant parts. It is a common practice to only study a collection by analysing the organ exhibiting the highest content of active components: flowers in the case of *H. perforatum*. But these data are generally not representative of the accession because the ratio between organs at the harvest stage will determine the real content of active component of the drug. Harvesting the major interesting organs in terms of active component content and of material representative to the drug will give a better evaluation of the accessions. The presence of 'control' varieties such as 'Topas' (Seidler-Lozykowska and Dabrowska, 1996) or 'Hyperimed' for *H. perforatum* is very important for comparing different collection results (Gaudin *et al.*, 2002).

Breeding programmes

Classical

The majority of accessions under cultivation have been created by conventional selection methods, including individual, mass or special selection methods. But for particularly high-value or problematic plants, breeding programmes could be performed according to the reproductive pathways of the species (allogamous, autogamous, apomictic) to select specific characters (Bernath, 2002).

H. perforatum is considered to be a facultative, pseudogamous, apomictic plant (Mayo and Langridge, 2003). Randomly amplified polymorphic DNA (RAPD) and fingerprinting and restriction fragment length polymorphism (RFLP) are used as tools for research on

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the mode of reproduction of this species (Haluskova and Cellarova, 1997; Arnholdt-Schmitt, 2000; Steck *et al.,* 2001). This plant is thought to be of allopolyploid origin (Martonfi *et al.*, 1996) and most commonly occurs as a tetraploid ($2n = 4x = 32$) (Nielsen, 1924; Brutovska *et al.*, 2000). A relationship between ploidy and hypericin content was shown, with the highest hypericin content found in diploids and the lowest in tetraploids (Cellarova *et al.*, 1997). All apomictic plants were tetraploid and all sexual plants were diploid (Pank *et al.*, 2003). Hybridization, polyploidization and mutation have also been reported (Bernath, 2002). Homogenous populations could be produced either by vegetative propagation from heterozygous genotypes or by generation of homozygous doubled haploid lines (Cellarova *et al.*, 1992) or more easily by apomixis (Pank, 2002). Obligate apomictic plants produce exclusively maternal progenies even though their genetic constitution is heterozygous. Plant breeders are interested now in combining desired characters from different genotypes into new commercial cultivars. However, sexual plants are needed for the generation of genetic variability through crossing (Pank *et al.*, 2003).

For *H. perforatum*, most of the actual cultivars originated in individual plant selection from different ecotypes. No relationship was demonstrated between hypericin and hyperforin contents. It is therefore possible to select accessions containing high levels of hypericins and hyperforins (Poutaraud and Girardin, 2004). Recently, breeding programmes have been started (Matzk *et al.*, 2001) with introgressive hybridization between necessarily sexual genotypes and the available apomicts resistant to *Colletotrichum gloeosporoides* (Pank *et al.*, 2003).

Biotechnological ways

In vitro propagation could be interesting for the production of standardized plant material as required by the pharmaceutical industry. This technique is used on *H. perforatum* for mass clonal propagation (Pretto and Santarem, 2000; Murch *et al.*, 2002; Santarem and Astarita, 2003; Zobayed and Saxena, 2003) and to create genetic variability by somaclonal variation (Cellarova *et al.*, 1994; Kartnig *et al.*, 1996; Brutovska *et al.*, 1998). Numerous studies have been performed to investigate production of secondary metabolites in bioreactors but with very few applicable results as yet (Yu *et al.*, 2001; Zobayed and Saxena, 2003; Zobayed *et al.*, 2003). Synthesis of hypericins and hyperforins hardly occurs in undifferentiated cultures like cell suspensions of calluses, because organ differentiation is required (Greenfield *et al.*, 1998). Moreover, it is necessary that plantlets reach an advanced stage of growth to achieve the biosynthesis of all metabolites required in the drug (Pasqua *et al.*, 2003). *In vitro* synthesis of hypericin in shoot culture can be stimulated by mannan (Kirakosyan *et al.*, 2000). Studies on the enzymes involved in the biosynthetic pathway, and their regulation with genomic tools, is a new and extremely valuable approach (Kosuth *et al.*, 2003; Phillipson, 2003). Transformed cell cultures and genetic engineering based on the overexpression or suppression of genes responsible for biosynthesis of active components offer new possibilities for the investigation of secondary metabolism. A major gene called Hyp-1 encoding for hypericin biosynthesis from emodin was recently cloned and characterized from *H. perforatum* cell cultures (Bais *et al.*, 2003). Some plant cell cultures do not necessarily produce the same secondary metabolite as the plant cultivated outside, and can be manipulated to produce new 'unnatural compounds' (Phillipson, 2003).

Discussion

Control of the production of natural molecules of pharmaceutical interest implies a good understanding of the secondary metabolism of the plant by multidisciplinary research at various organization levels: genomic, for determination of their synthesis and regulation; enzymatic, to study their biosynthesis and their degradation; biochemical, to study the characteristics of these active components and their by-products; cellular and histological, to search their synthesis sites and repartition in the different tissues, and at plant level, to study their production in different organs during the cycle of plant development and the interactions between their synthesis and environmental factors. The process concerns all those involved, from the farmer to the chemist.

The exploitation of the chemical diversity of the plant kingdom is one of the main ways of investigation to address the increasing need for high-quality botanical drugs. If the classical breeding programmes appear to be a good way of improving a medicinal plant, the agronomical and the technological approaches could also contribute in a large part to a better-quality drug. Furthermore, a better understanding of the function of these secondary metabolites in the plant could be of interest for stimulating their synthesis.

For *H. perforatum*, numerous papers have been published on these three approaches of improvement. Classical breeding programmes making use of natural genetic variability have given some good results, but biotechnological methods have not yet allowed the production of high-quality drugs, although this is still an interesting tool for a better understanding of the metabolic pathways involved. Breeding programmes aim to select accessions with high hypericin and hyperforin content, high dry weight flowering top yield, and good wilt resistance. Other characters could be added to overcome technological and agronomical problems: for example maintenance of

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hypericin glands during drying, or low cadmium accumulation. Different stresses could be tested to enhance hypericin and hyperforin synthesis, and macro and micro mineral fertilization needs to be optimized. A suitable control of light during the different steps of processing (drying, extraction) could increase the hypericin content and the phototransformation of the protohypericins in hypericin and pseudohypericin; as long as particular attention is paid to maintain the hyperforin content. The difference between spectral data of these molecules makes possible the phototransformation of protohypericins without degradation of hyperforins.

This method of integrating the three approaches can be used for the improvement of all medicinal plants. Common mechanisms of active component production (regulation of the synthesis, localization, transformation, translocation, degradation) from similar biosynthetic pathways but from different plant species could be expected, facilitating further research programmes for the improvement of medicinal plant quality.

References

Arnholdt-Schmitt B (2000) RAPD analysis: a method to investigate aspects of the reproductive biology of *Hypericum perforatum* L. *Theoretical and Applied Genetics* 100: 906–911.

Azizi M and Omid-Beigi R (2001) Effects of different levels of nitrogen and phosphorus fertilizer on growth, yield and hypericin content of St John's wort. *Iranian Journal of Agricultural Sciences* 32: 720–725.

Bais HP, Vepachedu R, Lawrence CB, Stermitz FR and Vivanco JM (2003) Molecular and biochemical characterization of an enzyme responsible for the formation of hypericin in St John's wort (*Hypericum perforatum* L.). *Journal of Biological Chemistry* 278: 32413–32422.

Baricevic D, Umek A, Kreft S, Maticic B and Zupancic B (1999) Effect of water stress and nitrogen fertilization on the content of hyoscyamine and scopolamine in the roots of deadly nightshade (*Atropa belladonna*). *Environmental and Experimental Botany* 42: 17–24.

Bergonzi MC, Bilia AR, Gallori S, Guerrini D and Vincieri FF (2001) Variability in the content of the constituents of *Hypericum perforatum* L. and some commercial extracts. *Drug Development and Industrial Pharmacy* 27: 491–497.

Bernath J (2002) Strategies and recent achievements in selection of medicinal and aromatic plants. *Acta Horticulturae* 576: 115–128.

Bombardelli E and Morazonni P (1995) *Hypericum perforatum*. *Fitoterapia* 116: 43–67.

Bottcher H, Gunther I and Kabelitz L (2003) Physiological postharvest responses of Common Saint-John's wort herbs (*Hypericum perforatum* L.). *Postharvest Biology and Technology* 29: 343–351.

Brantner A, Kartnig T and Quehenberger F (1994) Comparative phytochemical investigations of *Hypericum perforatum* L. and *Hypericum maculatum* Crantz. *Scientia Pharmaceutica* 62: 261–276.

Briskin DP and Gawienowski MC (2001) Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. *Plant Physiology and Biochemistry* 39: 1075–1081.

Briskin DP, Leroy A and Gawienowski M (2000) Influence of nitrogen on the production of hypericins by St John's wort. *Plant Physiology and Biochemistry* 38: 413–420.

Brutovska R, Cellarova E and Dolezel J (1998) Cytogenetic variability of *in vitro* regenerated *Hypericum perforatum* L. plants and their seed progenies. *Plant Science* 133: 221–229.

Brutovska R, Kusnirikova P, Bogyiova E and Cellarova E (2000) Karyotype analysis of *Hypericum perforatum* L. *Biologia Plantarum* 43: 133–136.

Büter B, Soldati A, Schaffner W and Berger K (1996) Site specific dry matter and concentrations of hypericin, biflavons and cadmium in different *Hypericum* ssp. accessions (first year results). Paper presented at Breeding Research on Medicinal and Aromatic Plants, Quedlinburg, Germany.

Büter B, Orlacchio C, Soldati A and Berger K (1998a) Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum*. *Planta Medica* 64: 431–437.

Büter KB, Schraner E, Schaffner W and Büter B (1998b) Genetic diversity within and among *Hypericum perforatum* populations collected in a geographical narrow and wide area. Paper presented at the 46th Annual Congress of the Society for Medicinal Plant Research—Quality of Medicinal Plants and Herbal Medicinal Products, Vienna.

Butterweck V, Petereit F, Winterhoff H and Nahrstedt A (1998) Solubilized hypericin and pseudohypericin from *Hypericum perforatum* exert antidepressant activity in the forced swimming test. *Planta Medica* 64: 291–294.

Butterweck V, Christoffel V, Nahrstedt A, Petereit F, Spengler B and Winterhoff H (2003) Step by step removal of hyperforin and hypericin: activity profile of different Hypericum preparations in behavioral models. *Life Sciences* 73: 627–639.

Cellarova E, Kimakova GM and Brutovska R (1992) Multiple shoot formation and phenotypic changes of Ro regenerants in *Hypericum perforatum* L. *Acta Biotechnologica* 12: 445–452.

Cellarova E, Daxnerova K, Kimakova K and Haluskova K (1994) The variability of hypericin content in the regenerants of *Hypericum perforatum*. *Acta Biotechnologica* 14: 267–274.

Cellarova E, Brutovska R, Daxnerova Z, Brunakova K and Weigel RC (1997) Correlation between hypericin content and the ploidy of somaclones of *Hypericum perforatum* L. *Acta Biotechnologica* 17: 83–90.

Chappell J and Hahlbrock K (1984) Transcription of plant defence genes in response to UV light or fungal elicitor. *Nature* 311: 76–78.

Chatterjee SK, Nandi RP, Bharati P, Yonjan B and Yonzon MK (1988) Improvement studies on some alkaloid yielding medicinal plants. *Acta Horticulturae* 188: 39–46.

Chatterjee SS, Noldner M, Koch E and Erdelmeier C (1998) Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. *Pharmacopsychiatry* 31: 7–15.

Ciccarelli D, Andreucci AC and Pagni AM (2001) Translucent glands and secretory canals in *Hypericum perforatum* L. (Hypericaceae): morphological, anatomical and histochemical studies during the course of ontogenesis. *Annals of Botany* 88: 637–644.

Cott JM (1997) *In vitro* receptor binding and enzyme inhibition by *Hypericum perforatum* extract. *Pharmacopsychiatry* 30: 108–112.

https://doi.org/10.1079/PGR200567 Published online by Cambridge University Press

Curtis JD and Lersten NR (1990) Internal secretory structures in *Hypericum* (Clusiaceae): *H. perforatum* L. and *H. balearicum* L. *New Phytologist* 114: 571–580.

Debrunner N, Darbellay C, Slacanin I and Ryser A (1997) Selection of *Hypericum perforatum* L. populations resistant to fungal pathogens. Paper presented at the World Congress on Medicinal and Aromatic Plants for Human Welfare, ICMAP ISHS SAIPA, Mendoza, Argentina.

Denke A, Schempp H, Mann E, Schneider W and Elstner EF (1999) Biochemical activities of extracts from *Hypericum perforatum* L.—4th communication: influence of different cultivation methods. *Arzneimittel—Forschung—Drug Research* 49: 120–125.

Dias ACP, Majid A and Reza O (2001) Effect of nitrogen and phosphorus fertilisation in the cultivation of *Hypericum perforatum* variety Topas. Paper presented at the International Congress and 49th Annual Meeting of the Society of Medicinal Plant Research, Erlange, Germany.

Franke R, Heisig W and Muggenburg D (1993) Quality variation in some medicinal plants. *Acta Horticulturae* 333: 123–127.

Franke R, Schenk R and Bauermann U (1998) Variability in *Hypericum perforatum* L. breeding lines. Paper presented at the 46th Annual Congress of the Society for Medicinal Plant Research—Quality of Medicinal Plants and Herbal Medicinal Products, Vienna.

Franke R, Schenk R and Bauermann U (2000) Variability in *Hypericum perforatum* L. breeding lines. Paper presented at Wocmap—2: Second World Congress on Medicinal and Aromatic Plants for Human Welfare.

Freytag WE (1984) Bestimmung von, Hypericin un Nachweis von Pseudohypericin in *Hypericum perforatum* L. durch HPLC. *Deutsche Apotheker Zeitung* 124: 2383–2386.

Gaedcke F (1997) Herstellung, Qualitat, Analytik und Anwendung von Johanniskraut-Extraken. *Zeitschrift für Arzneimittel aus Gewürzpflanzen* 2: 63–72.

Gasic O, Petrovic M and Cank N (1978) Determination of microelement in soil and the investigation of their correlation with the quantity of total isolated alkaloids and colchicine in *Colchicum autumnale* L. collected from various regions. *Planta Medica* 33: 276.

Gaudin M, Simmonet X and Debrunner N (1999) Sélection d'une variété de millepertuis productive et peu sensible au dépérissement. *Revue Suisse de Victiculture, Arboriculture et Horticulture* 31: 335–341.

Gaudin M, Simmonet X and Debrunner N (2002) Breeding for a *Hypericum perforatum* L. variety both productive and *Colletotrichum gloeosporoides* (penz.) tolerant. *Journal of Herbs, Spices and Medicinal Plants* 9: 107–120.

Girzu MA, Carnat A, Fraisse D, Carnat AP and Lamaison JL (2000) Flavonoid and dianthranoid levels of the St John's Wort flowering tops. *Annales Pharmaceutiques Francaises* 58: 341–345.

Granzow D and Holzl J (1998) Isolation of hyperforin from *Hypericum perforatum* and its stability in organic solvents and extracts. Paper presented at the 46th Annual Congress of the Society for Medicinal Plant Research—Quality of Medicinal Plants and Herbal Medicinal Products, Vienna.

Greenfield AJ, Mead DJ, Pwer JB, Lowe KC and Davey MR (1998) Toward hypericin production from fermenter grown cultures of *Hypericum perforatum*. *Abstracts—Plants and Plant Cell Topics C2* 11: 89.

Haluskova J and Cellarova E (1997) RFLP analysis of *Hypericum perforatum* L. somaclones and their progenies. *Euphytica* 95: 229–235.

Hölzl J and Ostrowski E (1987) Johnanniskraut (*Hypericum perforatum* L.). HPLC-Analyse der wichtigen Inhalsstoffe und deren Varibialität in einer Population. *Deutsche Apotheker Zeitung* 23: 1227–1230.

Hornok L (1992) *Cultivation and Processing of Medicinal Plants*. Chichester: John Wiley and Sons.

Jurgenliemk G and Nahrstedt A (2003) Dissolution, solubility and cooperativity of phenolic compounds from *Hypericum perforatum* L. in aqueous systems. *Pharmazie* 58: 200–203.

Kartnig T, Gruber A and Sauer H (1989) Comparative phytochemical investigations of *Hypericum* species. *Planta Medica* 55: 215.

Kartnig T, Gobel I and Heydel B (1996) Production of hypericin, pseudohypericin and flavonoids in cell culture in various *Hypericum* species and their chemotypes. *Planta Medica* 62: 51–53.

Kirakosyan A, Hayashi H, Inoue K, Charchoglyan A and Vardapetyan V (2000) Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. *Phytochemistry* 53: 345–348.

Kosuth J, Koperdakova J, Tolonen A, Hohtola A and Cellarova E (2003) The content of hypericins and phloroglucinols in *Hypericum perforatum* L. seedlings at early stage of development. *Plant Science* 165: 515–521.

Liu FF (2000) Optimization of extraction conditions for active components in *Hypericum perforatum* using response surface methodology. *Journal of Agricultural and Food Chemistry* 48: 3364–3371.

Martonfi P, Brutovska R, Cellarova E and Repcak M (1996) Apomixis and hybridity in *Hypericum perforatum*. *Folia Geobotanica & Phytotaxonomica* 31: 389–396.

Matzk F, Meister A, Brutovská R and Schubert I (2001) Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. *The Plant Journal* 26: 275.

Mayo GM and Langridge P (2003) Modes of reproduction in Australian populations of *Hypericum perforatum* L. (St John's wort) revealed by DNA fingerprinting and cytological methods. *Genome* 46: 573–579.

Melnikova V, Vainshtein V and Shikov A (1998) New appoaches to the technology of oil and water-alcohol extracts of *Hypericum perforatum* L. Paper presented at the 46th Annual Congress of the Society for Medicinal Plant Research—Quality of Medicinal Plants and Herbal Medicinal Products, Vienna.

Mennini T and Gobbi M (2004) The antidepressant mechanism of *Hypericum perforatum*. *Life Sciences* 75: 1021–1027.

Mohr T, Hecht H and Eichhorn H (1996) Comparative examinations of an improved picking machine for the flower harvest of *Chamomilla recutita* L. (St. Rauschert), *Calendula officinalis* L. and *Hypericum perforatum* L. *Drogenreport* 9: 15–23.

Murch SJ, Rupasinghe HPV and Saxena PK (2002) An *in vitro* and hydroponic growing system for hypericin, pseudohypericin, and hyperforin production of St John's wort (*Hypericum perforatum* CV new stem). *Planta Medica* 68: 1108–1112.

Murch SJ, Haq K, Rupasinghe HPV and Saxena PK (2003) Nickel contamination affects growth and secondary metabolite composition of St John's wort (*Hypericum perforatum* L.). *Environmental and Experimental Botany* 49: 251–257.

Nahrstedt A and Butterweck V (1997) Biologically active and other chemical constituents of the herb from *Hypericum perforatum* L. *Pharmacopsychiatry* 30: 129–134.

Nathan PJ (2001) *Hypericum perforatum* (St John's Wort): a non-selective reuptake inhibitor? A review of the recent

advances in its pharmacology. *Journal of Psychopharmacology* 15: 47–54.

Nielsen N (1924) Chromosome numbers in the genus *Hypericum*. *Hereditas* 5: 372–382.

Oravec Sen V, Oravec Jun V, Matonfi P and Repcak M (1994) Variability pattern in different cultivated *Hypericum perforatum* types. Paper presented at Cultivation and Improvement of Medicinal and Aromatic Plants, Trento.

Orth H, Rentel C and Schmidt P (1999) Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. *Journal of Pharmacy and Pharmacology* 51: 193–200.

Pank F (1990) The influence of chemical weed control on quality characters of medicinal and aromatic plants. *Herba Hungarica* 29: 51–58.

Pank F (2002) Three approaches to the development of high performance cultivars considering the differing biological background of the starting material. *Acta Horticulturae* 576: 129–137.

Pank F, Matzk F, Kastner U, Bluthner WD, de Garcia EF, Meister A, Ryschka U and Schumann G (2003) Reproductive diversity and strategies for breeding in St John's wort (*Hypericum perforatum* L.). *Euphytica* 134: 77–84.

Pasqua G, Avato P, Monacelli B, Santamaria AR and Argentieri MP (2003) Metabolites in cell suspension cultures, calli, and *in vitro* regenerated organs of *Hypericum perforatum* cv. Topas. *Plant Science* 165: 977–982.

Phillipson JD (2003) Fifty years of medicinal plant research—every progress in methodology is a progress in science. *Planta Medica* 69: 491–495.

Pietta P, Gardana C and Pietta A (2001) Comparative evaluation of St John's wort from different Italian regions. *Il Farmaco* 56: 491–496.

Ploss O, Petereit F and Nahrstedt A (2001) Procyanidins from the herb of *Hypericum perforatum*. *Pharmazie* 56: 509–511.

Pluhár Z, Bernáth J and Németh E (2002) Investigations on the infraspecific variability of *Hypericum perforatum* L. *Journal of Herbs, Spices and Medicinal Plants* 9: 83–88.

Poutaraud A and Girardin P (2004) Agronomic and chemical characterisation of 39 *Hypericum perforatum* L. accessions between 1998 and 2000. *Plant Breeding* 123: 480–484.

Poutaraud A, Di Gregorio F, Chan Fook Tin V and Girardin P (2001a) Effect of light on hypericins contents in fresh flowering top parts and in an extract of St John's Wort (*Hypericum perforatum*). *Planta Medica* 67: 254–259.

Poutaraud A, Lobstein A, Girardin P and Weniger B (2001b) Improved procedure for the quality control of *Hypericum perforatum* L. *Phytochemical Analysis* 12: 355–362.

Pretto FR and Santarem ER (2000) Callus formation and plant regeneration from *Hypericum perforatum* leaves. *Plant Cell, Tissue and Organ Culture* 62: 107–113.

Repcak M and Martonfi P (1997) The localization of secondary substances in *Hypericum perforatum* flower. *Biologia* 52: 91–94.

Santarem ER and Astarita LV (2003) Multiple shoot formation in *Hypericum perforatum* L. and hypericin production. *Brazilian Journal of Plant Physiology* 15: 43–47.

Schilter B, Andersson C, Anton R, Constable A, Kleiner J, O'Brien J, Renwick AG, Korver O, Smit F and Walker R (2003) Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. *Food and Chemical Toxicology* 41: 1625–1649.

Seidler-Lozykowska K and Dabrowska J (1996) Topaz—the Polish variety of St John's wort (*Hypericum perforatum* L.). *Herba Polonica* 42: 140–143.

Singer A, Wonnemann M and Muller WE (1999) Hyperforin, a major antidepressant constituent of St John's Wort, inhibits serotonin uptake by elevating free intracellular Na^+. *Journal of Pharmacology and Experimental Therapeutics* 290: 1363–1368.

Sirvent T and Gibson D (2002) Induction of hypericins and hyperforin in *Hypericum perforatum* L. in response to biotic and chemical elicitors. *Physiological and Molecular Plant Pathology* 60: 311–320.

Southwell IA and Bourke CA (2001) Seasonal variation in hypericin content of *Hypericum perforatum* L. (St John's Wort). *Phytochemistry* 56: 437–441.

Southwell IA and Campbell MH (1991) Hypericin content variation in *Hypericum perforatum* in Australia. *Phytochemistry* 30: 475–478.

Steck N, Messmer M, Schaffner W and Bueter KB (2001) Molecular markers as a tool to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*. *Planta Medica* 67: 384–385.

Tekel'ova A, Repcak M and Zemkova E (2000) Quantitative changes of dianthrones, hyperforin and flavonoids content in the flower ontogenesis of *Hypericum perforatum*. *Planta Medica* 66: 778–780.

Tetenyi P (1991) Biological preconditions for cultivation and processing of medicinal plants. In: *The Medicinal Plant Industry*. Boca Raton, FL: CRC Press, pp. 33–41.

Umek A, Kreft S, Kartnig T and Heydel B (1999) Quantitative phytochemical analyses of six *Hypericum* species growing in Slovenia. *Planta Medica* 65: 388–390.

Upton RE (1997) St John's Wort quality control analytical and therapeutic monograph. *HerbalGram* 40: 1–32.

Walker L, Sirvent T, Gibson D and Vance N (2001) Regional differences in hypericin and pseudohypericin concentrations and five morphological traits among *Hypericum perforatum* plants in the northwestern United States. *Canadian Journal of Botany* 79: 1248–1255.

Wierzchowska-Renke K, Zobel AM and Glowniak K (1995) Effect of fertilizing with copper and manganese on content of coumarin in *Pasticana sativa* L. 43rd Annual Congress on Medicinal Plant Research. G: Cultivation of Plants (abstract G3), Halle (Saale), Germany, p. 45.

Wills RBH, Bone K and Morgan M (2000) Herbal products: active constituents, modes of action and quality control. *Nutrition Research Reviews* 13: 47–77.

Yu K, Hahn E and Paek K (2001) Effects of NH^{4+}: NO^{3-} ratio and ionic strength on adventitious root growth and ginsenoside production in bioreactor culture of *Panax ginseng* C.A. Meyer. *Acta Horticulturae* 560: 259–262.

Zobayed SMA and Saxena PK (2003) *In vitro*-grown roots: a superior explant for prolific shoot regeneration of St John's wort (*Hypericum perforatum* L. cv 'New Stem') in a temporary immersion bioreactor. *Plant Science* 165: 463–470.

Zobayed SMA, Murch SJ, Rupasinghe HPV and Saxena PK (2003) Elevated carbon supply altered hypericin and hyperforin contents of St John's wort (*Hypericum perforatum*) grown in bioreactors. *Plant Cell, Tissue and Organ Culture* 75: 143–149.

Zobel AM, Crellin J, Brown SA and Gloniak K (1994) Concentration of furanocoumarins under stress conditions and their histological localization. *Acta Horticulturae* 381: 510–516.