Comparative investigation on *Hypericum perforatum* L. populations of different origin

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Summary Widespread application for herbal medicines based on *Hyperici herba* has been experienced in the last few years, especially in the treatment of depression syndrome. As the wild origins could not satisfy the market demands neither in quantitative nor in qualitative respect, the necessity of the development of intensive growing methods has been raised. In the course of our investigations we intended to clear up the morphological and chemical variability among and within populations of different origin in order to start a new breeding program.

According to our data, growth dynamics of populations could be characterised by a logistic curve. According to the time of flowering the populations formed early, middle and late groups. Morphological diversity among populations was measurable in differences of flower length, plant height, different leaf types and plant habit, according to which characteristics groups were distinguished. Generally, the accessions were the most homogenous in plant height (CV: 7–15%), followed by inflorescence-lengths (CV: 11–36%) and the least uniform characteristics proved to be the number of flowering shoots (CV: 14–59%). The greatest morphological heterogeneity was experienced in the accessions of wild origin as it has been expected. In the second vegetation period generally a much better homogeneity was obtained, than in the first year. The most outstanding accessions produced 1.2 t of dry flowers and 4 t of dry flowering shoots, calculating to one hectare area. The individual yields varied on a large scale in each population (CV: 18–70%).

The content of hypericin varied between 1,85 and 9,9 mg/g in 1996, and between 0,18 and 2,70 mg/g in 1997, showing high individual divergences. Flavonoid values -including first of all hyperosid, rutin and quercitrin - reached 17–39 mg/g in the first and 15–20 mg/g in the second year, respectively. Individual and seasonal variation was less than in hypericin. A joint high level of these two compound groups seems to have low frequencies.

The superior population were selected for further breeding.

Introduction

Recently, *Hyperici herba* has become one of the most frequently used herbal drugs in the pharmaceutical industry as well as in self-medication system of developed countries (*Brevoort*, 1998). The relevance of this drug, originating from *Hypericum perforatum* L. (St. John's wort), is due to its antidepressant and antiretroviral effects beside its antibiotic activity which was known much earlier (*Bombardelli & Morazzoni*, 1994). Hyperici herba contains hypericin derivatives (0,1–0,5%), flavonoids (1-4%), hyperforin (4–6%), tannins (4–10 %), xantones, etc (*Upton*, 1997). Pharmaceutical preparations with different formulations (capsules, tabets, solutions) are consumed in largest quantities in the USA (70%) and in Germany (30%), mainly in neurotic indications (*Franke*, 1998). These herbal remedies are also available in the Hungarian pharmacies.

An increased market demand for Hyperici herba has been rised in the last few years, which could not be satisfied by collecting the raw material from the wild habitats only, but the cultivation of the species became necessary. The introduction of the plant into the culture has been started some years before, in abroad and in Hungary as well (*Pluhár & Zelnik*, 1994, *Bomme*, 1997). However, to support this cultivation efforts, till now only one Polish cultivar ('Topas') and a German one ('Anthos'), are available (*Pank & Heine*, 1998). For providing homogenous plant material of high productivity and disease resistance, breeding work has been started in many countries of Europe.

Fortunately, *Hypericum perforatum* can be characterized by wide natural variability due to its well known (97%) apomictic characteristics (*Formanowiczowa & Kozlowski*, 1972). It provides a good chance to select accessions of advantageous properties and develop appropriate cultivars for large-scale production. The general requirements for an up to date cultivar are the following: uniform and moderate plant height with numerous flowering branches and shoots, the ability of flowering in the first vegetation cycle and uniform flowering time were required, high production capacity, high level of hypericin derivatives and different flavonoid components, tolerance against fungal infections with special regard to *Hypericum* wilt.

In connection with this, an intensive experimental work had been started in the recent years at our department too. Aim of the investigations was clearing up the morphological and chemical diversity of populations of different origin in order to determine the most appropriate ones as basic material for a breeding process.

Materials and methods

Place of investigations

The open field experiments were carried out at the Research Station of the Department of Medicinal and Aromatic Plants, UHFI, Budapest in 1996 and 1997. The calciferous loose sandy soil of the experimental plots is characterized by fairly good K_2O (378 mg/kg) and P_2O_5 (345 mg/kg) content and by poor accessible N (1,65 mg/kg) supply. The wheater conditions of the growing seasons are shown in *Table 1*.

Table 1 Weather conditions of growing seasons 1996 and 1997

Months	Mean temperature (°C)		Duration of sunshine (h)		Precipitation (mm)	
	1996	1997	1996	1997	1996	1997
III	2,1	6,1	146	210	11	11
IV	12,3	9,0	193	245	27	21
V	17,5	17,0	253	261	102	45
VI	20,5	19,8	280	269	33	39
VII	19,8	18,3	292	228	40	52
VIII	20,8	21,8	217	222	33	37
IX	12,9	17,2	100	256	96	7

Plant material and the growing conditions

18 populations of *Hypericum perforatum* were examined during two successive years. Their origins were the following: a) population of the original Polish cultivar 'Topas' as control (marked as pop. 2). b) seven accessions of 'Topas' origin (marked 3, 10, 14, 16, 17, 20 and 28), c) two Hungarian wild accesions (marked 23 and 24), which were collected in dry grassland communities of Tihany Peninsula and Somlyó Hill, Fót. d) eight selected German materials (marked 30, 31, 32, 34, 35, 36, 37 and 38).

The seeds of populations were sown in greenhouse in February. Pretreated seeds (24 h soaking in 1000 ppm GA_3) were used. Four weeks after sowing the seedlings were picked, then grown in greenhouse, followed by training session in plastic tunnel. 25–30 individuals of each population were planted into open-field plots to plant density of 5 plant/ m^2.

Morpho-phenological observations

The growth of populations was measured in the first vegetation cycle in fortnight periods, continuously. Plant height of five identical plants were determined. In both vegetation periods the time of flowering and the predictable time of harvest were also detected.

The most important morphological characteristics (plant height, plant width, number of shoots, plant habit and leaf type) were measured on ten individuals of each populations in the first autumn and in the blooming period of the second year. The lenght of inflorescence as a determinative element of drug quality was also involved in the measurements.

Evaluation of production characteristics

In the first vegetation cycle fresh and dry mass production of populations were determined by harvesting plants in full blooming. 7–10 identical individuals – selected considering their excellent morphological features – were sampled. In the second vegetation period a more detailed analysis was carried out, weighing dry flower mass and total herb production, parallelly. Yields were expressed in g/m^2 values and converted to unit hectare area.

Chemical analysis

The laboratory analysis was performed at the Central Laboratory of UHFI, Budapest. Hypericin and hyperforin content as well as flavonoid content and composition were determined. Acetone-methanol solvent extraction followed by HPLC analysis was applied according to *Hölz* and *Ostrowski*, 1987. The HPLC system involved a Waters Pump 990 equipped with diode array detector; the column was a Waters Novapack C_{18}. The HPLC instrument was programmed for gradient elution method with mobil phases A: acetonitril: water: H_3PO_4 (19:80:1), B: acetonitril: methanol: H_3PO_4 (59:40:1). Injected volume of 10 µl, flow rate of 1 ml/min, detection wavelenght of UV 254 nm were used. Compounds were identified by means of pure standards.

Statistical analyses

Regression analysis was used to describe the growing feature of different accessions in the first year. Morphological and production properties were evaluated by one-way analysis of variance. Cluster analysis was applied to compare and group individuals according to their active agent content. Homogeneity within populations concerning different characteristics was determined by coefficients of variance (CV%). Statgraphics 5.1 and Microsoft Excel 5.0 programs were used for analysis.

Results

Changes of development and growth

Although *Hypericum perforatum* is a perennial plant, among appropriate weather conditions it tends to bloom in the first year, too. In our experiment most of the accessions, with the exception of two ones (17, 20), exhibited this feature. By the end of the first vegetation cycle 33–83% of the individuals of all populations reached the flowering phase. There were no differences in seed ripening and seed setting percentages. In the second vegetation year, according to the flowering characteristics, the populations could be divided into three distinct groups. The Hungarian accession of wild origin (24) started blooming on June 10 (early type), followed by the German ones and a 'Topas' progeny (28) on June 20 (middle type). The other 'Topas' progenies belonged to the late type group, which started flowering on June 25.

In contrast of the flowering characteristics, there were no remarkable differences in growing dynamics of neither the populations nor the years. The logistic curve fitted with $r^2=0,99679$ and the equation of $35\cdot(1-6,2 \cdot e^{-1,05935x})^{-1}$

characterises the growth type of the species, which had not been described in the literature before (*Figure 1*).

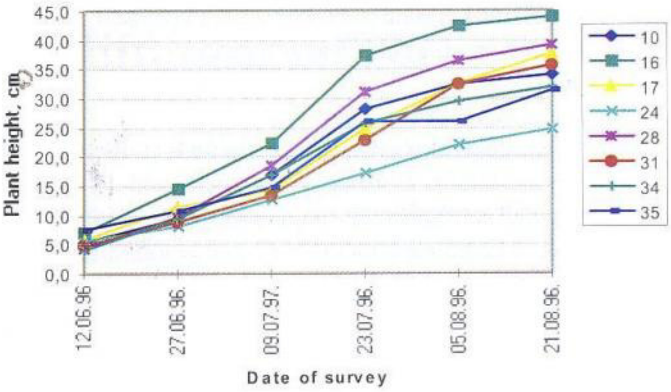

Figure 1 Growth dynamic of *Hypericum perforatum* populations in the first vegetation period

Morphological diversity of populations

Leaf type has been already determined in the first vegetation period. Four different types were distinguished and established, that the narrow-leaved plants occurred in highest abundance among our accessions (*Table 2*).

The heights of plants varied between 25 and 44 cm in 1996, 55 and 80 cm in 1997, respectively. Population of Hungarian wild origin was the smallest and generally 'Topas' progenies were the tallest. In 1996 in the average 1-8 pieces and in 1997 11-22 pieces of flowering shoots were grown by the surveyed plants, respectively. 11,5 cm was the maximum length of inflorescences in the first year, while it was 22,9 cm in the second growing period (*Table 3*).

Ratios of plant height to plant width showed values between 1,1-1,5, which meant that plant height always exceeded plant width. The lowest values were found in accessions No. 31 and 35 as well as in 'Topas' progenies, those could be described by compact plant shape.

In the first growing year about half of our accessions possessed a compact habit with numerous erect shoots, which is considered to be the ideal form of the bush, because it may enable an easy harvest. However, in the second vegetation period due to the more intensive growth, plants were standing closer to each other, and a much better homogeneity was obtained. Plants formed erected shoots, full with flowers (*Figure 2*).

Figure 2 *Hypericum perforatum* accessions of different origin in flowering phase in the second vegetation cycle

The uniformity was outstanding in the 'Topas' progenies, considering both the plant habit (high density bush) and leaf type (narrow leaves), respectively. Among the other populations, a Hungarian one (No. 24) is to be mentioned, where a single individual was found showing the ideal morphological features, such as compact habit with numerous flowering branches, located in the same level.

Generally, the accessions were the most homogenous in plant height (CV%: approx. 7-15), followed by inflorescence-lengths (CV%: 10,5-36) and the least uniform characteristics proved to be the number of flowering shoots (CV%: approx.14-59). The greatest morphological heterogeneity was experienced in the accessions of wild origin as it has been expected.

Table 2 Leaf types and plant habit occuring at the H. perforatum accessions in the first year

Origin	Leaf type					Plant habit				
	very narrow	narrow	medium	broad	varying	dense high	dense small	loose high	loose small	varying
2		+				+				
3		+				+				
10		+				+				
14		+				+				
16		+				+				
17		+				+				
20		+				+				
23		+				+				
24	+						+			
28			+					+		
30			+						+	
31			+				+			
32			+						+	
34					+					+
35				+					+	
36				+					+	
37		+					+			
38			+					+		

Table 3 Morphological characteristics of different Hypericum perforatum L. accessions in the first and in the second vegetation periods

Acces-sions	Average plant height, cm			Average number of shoots, pcs			Average length of inflorescences, cm		
	1996	1997	$CV\%_{97}$	1996	1997	$CV\%_{97}$	1996	1997	$CV\%_{97}$
2	33,00	63,60	13,05	3,75	12,40	28,50	6,50	19,40	16,52
3	44,00	70,60	8,56	8,00	15,20	27,88	9,30	22,90	15,60
10	32,25	53,60	10,38	4,75	17,40	27,52	7,00	13,40	22,30
14	42,00	71,10	7,03	5,00	15,50	33,36	11,50	16,30	15,85
16	41,50	73,90	8,73	5,50	19,27	20,25	11,50	18,30	12,37
17	35,50	80,00	8,56	2,75	15,60	26,20	7,00	21,10	14,56
20	36,50	74,00	14,32	4,00	15,40	36,27	4,70	22,50	16,53
23	32,75	59,10	10,95	6,00	11,56	58,86	8,50	16,00	18,78
24	25,50	59,20	10,82	3,25	14.40	43.52	5,70	13,00	29,32
28	34,25	62,30	14,57	1,75	16,00	21,42	8,50	19,10	22,82
30	38,25	68,30	8,70	2,25	17,78	13,98	7,00	19,80	36,01
31	36,75	54,90	9,62	1,25	17,50	18,90	5,70	18,90	15,06
32	32,75	60,60	7,83	1,50	22,10	18,16	6,00	19,50	14,76
34	29,00	62,70	13,13	3,50	20,00	20,14	5,70	18,80	12,98
35	32,50	67,60	8,48	3,00	20,90	21,82	6,00	18,90	10,54
36	38,25	73,00	12,09	2,25	16,89	44,84	6,30	19,20	14,75
37	23,75	63,10	10,59	7,00	18,40	13,80	7,00	12,00	15,60
38	37,75	72,70	7,60	2,75	17,20	21,68	7,00	19,20	19,26
$LSD_{5\%}$	11,32	10,84	–	4,85	7,08	–		5,28	–

Significant differences among populations in morphological traits were proved especially between accessions in the 'Topas' group and when comparing members of this group with German accessions (*see LSD5% values in Table 3*).

Production aspects

Evaluating the total dry-mass production, generally about five-fold higher production values were measured in the second vegetation cycle, than in the first one. The dry-mass production of individuals varied between 30 g/m^2 (No. 24) and 160 g/m^2 (No. 3) in 1996; between 171 g/m^2 (No. 24) to 399 g/m^2 (No. 36) in 1997. The most prominent accesions with more than 300 g/m^2 dry-mass production in the second season were the ones No. 14, 16, 17, 20, 35, 36, 38 ('Topas' progenies and German origins).

Flower mass varied from 60 to 122 g/m^2, where the highest average values occurred at populations No. 14, 16, 17, 36 and 38, usually in positive correlation with the mass of whole flowering shoots (*Table 4*).

The ratio of flower mass to herba mass varied between 1: 2.5 and 1:4.5. Topas progenies, especially No. 16, 17 and some German ones (30 and 31) were the most excellent ones from this point of view. The mentioned 'Topas' progenies were superior to the control strain in each aspect of production.

The individual yields varied on a large scale in each population (high CV values), only the population No. 37 was moderately homogenous. This feature is the direct consequence of the heterogeneity experienced at the number of flowering shoots, which influence mostly the production.

Accumulation of active ingredients

In the evaluated accessions, it is an overall phenomenon, that higher levels of hypericin could be obtained in the first vegetation cycle. The population average varied between 1,85 and 9,9 mg/g in 1996, while between 0,18 and 2,70 mg/g in 1997 (*Table 4*). The extremely high hypericin content in the first year was possibly based on the higher proportion of flowers, leaves and fine stems. In the second year the aerial part of the plant contains robust woody stems of enormous weight. Its presence decreases the drug quality with respect to hypericin content.

Flavonoid content have been proved to be a more stable characteristics comparing to hypericin content. However, its values decreased from 17-39 mg/g (1996) to 15-20 mg/g (1997) too, as a result of vegetation cycle.

To find accession with equally high flavonoid and hypericin content, in the case of *Hypericum perforatum*, seems to be rather difficult, as the investigated accessions were found to be rich either in hypericin (3, 10, 16 and 30) or in flavonoids (32, 34 and 37). It was supported by Cluster analysis, dividing individuals into three groups, which justify that none of the groups can be characterised by high hypericin and flavonoid content at the same time (*Figure 3*). „A" group contains only two plants with high level (3-4 mg/g) of hypericin and quite low level of flavonoids, the „B" group gathers the greatest part of the individuals with medium values of both active agents, while the members of „C" class are poor in both hypericin and flavonoids.

Within populations, the hypericin level proved to be varying (high CV values). Regarding flavonoid content, most of the accessions possessed great or moderate uniformity. These results suggest, that flavonoid content is less influenced by environmental conditions and/or physiological aspects than hypericin level.

Composition of flavonoids was also determined by HPLC analysis. Hyperosid (5–15 mg/g), rutin (1–7 mg/g) quercitrin (2–6 mg/g) were found in considerable levels within the flavonoid complex (*Figure 4*). Certain taxa (24 and 35) exhibited an extraordinary low rutin level in both years consequently, while at the same time hyperosid (quercetin-galactosid) accumulates in extremely high amount.

Table 4 The most important production characteristics of Hypericum perforatum accessions

Populations	Average dry herba mass, g/m²			Average dry flower mass, g/m²		Total hypericin content, mg/g			Total flavonoid content, mg/g		
	1996	1997	CV%97	1997	CV%97	1996	1997	CV%97	1996	1997	CV%97
2	95,00	265,50	45,04	76,50	52,26	1,88	0,82	89,14	22,50	19,12	8,15
3	160,00	256,00	41,29	77,50	69,77	1,85	2,42	40,53	17,44	17,17	9,29
10	50,00	175,00	35,61	66,00	33,46	3,84	2,70	29,40	25,10	14,82	7,50
14	152,50	305,50	34,80	97,50	36,12	2,31	1,61	78,14	19,28	14,93	9,64
16	152,50	341,00	24,66	122,50	24,55	2,16	2,64	19,61	19,90	15,55	8,52
17	–	377,50	37,84	120,00	35,95	–	1,17	79,74	–	16,58	7,45
20	–	319,00	30,56	93,50	40,57	–	0,30	62,09	–	16,83	12,00
23	46,00	280,00	48,63	72,50	51,43	2,55	1,54	35,74	22,96	17,01	16,04
24	30,00	171,00	49,83	43,50	65,93	2,90	0,18	102,08	23,04	17,61	24,07
28	94,00	285,00	23,54	75,00	37,42	2,16	1,14	39,95	22,06	18,22	11,78
30	90,00	180,00	30,76	75,00	32,66	3,90	2,68	19,28	23,26	19,32	12,18
31	63,50	241,50	24,22	90,00	30,43	3,20	1,15	43,63	29,10	13,99	25,14
32	55,00	240,00	19,54	75,00	28,28	3,48	0,95	19,23	24,68	20,39	10,92
34	40,00	297,00	38,23	73,50	37,81	4,25	1,38	45,55	21,36	20,12	18,55
35	53,50	362,00	26,78	77,00	32,59	9,90	1,44	98,77	27,86	18,92	22,82
36	64,00	399,00	48,14	96,00	41,14	3,62	0,40	167,71	39,40	17,50	14,75
37	77,50	230,00	18,16	60,00	17,68	8,40	0,67	20,57	24,78	20,23	10,05
38	80,00	348,00	38,54	96,00	44,90	3,91	0,74	62,74	20,18	15,96	16,86
$LSD_{5\%}$	–	148,9	–	46,9	–	–	1,27	–	—	4,57	–

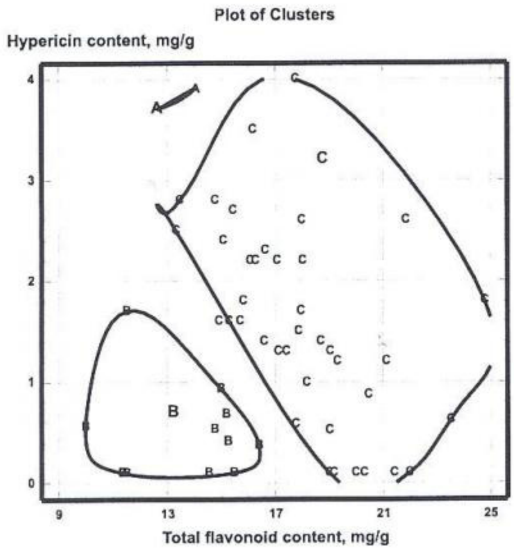

Figure 3 Results of cluster analysis regarding total hypericin and flavonoid levels

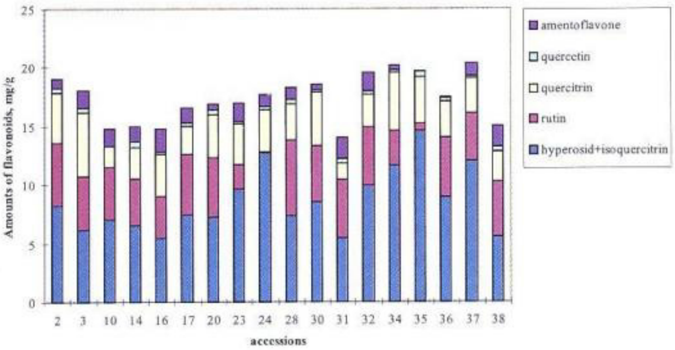

Figure 4 Flavonoid content and composition of different *Hypericum perforatum* accessions (1997)

According to our results – taking into consideration of cultivation requirements - some populations seems to be superior. Thus, considerable selection advance can be expected by more detailed investigation and selection of 'Topas' progenies No. 17 and 28, or by the German accessions 34 and 35 which proved to have a high biomass production capacity, exhibited high content of the flavonoids and acceptable level of hypericin.

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