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Evaluation of St. John's wort (*Hypericum perforatum* L.) collection

Katarzyna Seidler-Łożykowska, Kazimierz Kaźmierczak

Breeding and Agricultural Laboratory
The Research Institute of Medicinal Plants
Libelta 27, 61-707 Poznań, Poland
e-mail: klozykowska@iripz.pl

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ABSTRACT

St. John's wort is a perennial medicinal plant commonly cultivated in Poland. St. John's wort herb, collected at the beginning of its flowering, has a broad spectrum of medical applications. In recent years St. John's wort plantations have been infected by fungi *Colletotrichum gleosporioides* (Penz.), which has caused necrosis of whole plants. In the Research Institute of Medicinal Plants (RIMP) a collection of 10 accessions was gathered to evaluate their morphological traits, secondary metabolites content and resistance to pathogen in comparison with cultivar 'Topaz'. The two-year evaluation of the collection made it possible to select accession nr 7, coming from Czarny Las near Ostrzeszów, whose plants showed *C. gleosporioides* tolerance and a high content of secondary metabolites.

INTRODUCTION

St. John's wort (*Hypericum perforatum* L.) has been used in folk medicine and modern phytotherapy for its medical properties such as: anxiolytic, anti-inflammatory, antivirus, spasmolytic and antidepressive ones (Bombardelli and Morazzoni 1995). Recently, the herb has been more often used as a natural alternative for mild depression treatment. Fresh or dry flowering tops of the herb are used as a raw material. The herb contains hypericin and pseudohypericin, flavonols: hyperoside, quercetin, rutin, flavanols: hyperforin and essential oil, tannins, C and A vitamins, choline, pectin (Bombardelli and Morazzoni 1995). The importance of St. John's wort increased significantly in the last few years and the yearly market for the herb has reached 570 million US dollars worldwide (Becker 2000). The phytopharmaceutical industry, following GMP demands, requires uniform raw material of high quality, which can be obtained only by cultivation. The research done in the last years showed a strong impact of genetic and environmental factors on the yield of the herb and secondary metabolites content (Büter et al. 1998, Umek et al. 1999). Gaudin et al. (2003) reported that in 1995, St. John's wort plants were infected by *C. gleosporioides*, which destroyed plants even in the first year of cultivation. Whole infected plants had a reddish colour and in the later stage were completely dried. The first symptoms of this disease were observed in 1998 on a few plantations in Poland. Lack of infected plants growing in the wild forced the authors of this paper to examine the collection of St. John's wort as a potential source of genetic resistance to this disease. Osińska and Węglarz (1998) have described *Hypericum* populations collected from nine accessions and they have observed the significant differences in the essential oil, hypericin and flavonoids content.

MATERIAL AND METHODS

St. John's wort (*Hypericum perforatum* L.) seeds were gathered from different accessions (1 - 9) in Poland in 2001. The collection was completed by seeds of cv. Topaz coming from maintenance breeding (10) and seed plantation (11). They were used to establish the collection consisting of 10 accessions (in brackets dates of seed collection):

1. Ryczówek near Ogrodzieniec (23.08.2001)
2. Budachów near Krosno Odrzańskie (30.08.2001)
3. Brzeziny near Kalisz (24.08.2001)
4. Jaromierz near Wolsztyn (28.08.2001)
5. Janisławice near Sośnie (10.08.2001)

6. Ryczówek near Ogrodzieniec II accession (23.08.2001)
7. Czarny Las near Ostrzeszów (10.08.2001)
8. Murzynowo near Gorzów (31.08.2001)
9. Plewiska near Poznań: cv. Topaz – maintenance breeding
10. Witowo near Radziejów: cv. Topaz – seed plantation.

On May 21st, 2002 St. John's wort seedlings, obtained in the glasshouse, were planted in the field of the Research Institute of Medicinal Plants Breeding Laboratory in Plewiska. In 2002 and 2003, during the plant growth and after harvest the following traits were evaluated: plant habitat (I year), shape of leaf (I year), time of flowering, height of plant (II year), susceptibility to dieback (II year), share of herb (without stems) and stems in dry raw material, content of hypericin, pseudohypericin and the sum of flavonoids calculated on hyperoside basis. The biometric traits were evaluated on 20 plants of each accession.

The susceptibility of plants to dieback was observed in the following stages of growth: beginning of flowering, after flowering, seeds ripening. The resistance to dieback was estimated according to the authors own four-point scale (Table 2).

Samples of herb were collected during the stage of flowering. In dry material leaves and flowers were separated from stems to estimate their share. The content of secondary metabolites was determined in the air-dry total herb in the Laboratory of Analysis and Control of the Research Institute of Medicinal Plants.

The following traits were statistically analyzed:

1. analysis of variance: share of dry herb without stems in total herb (I and II year), plant height (II year);
2. coefficient of variation: content of hypericin, pseudohypericin and flavonoids (I and II year).

RESULTS AND DISCUSSION

The observations done in the first year of the growth of the plants (Table 1) showed that the plant habitats were: prostrate (1), erect (1, 2, 4, 6, 9, 10, 11) and fruticose (3, 5, 7). Plant habitat is important in the process of harvesting which is easier when plants are erect (Hannig et al. 1995). Most of the plants had big, broad leaves, except for plants of accession nr 1, 4 and 9. The size of leaf affects the content of secondary metabolites in the herb (Fornasiero et al. 1998). All the plants of the collection bloomed in the first year, but most of them very late (3, 4, 5, 7, 9).

The height of the plant was measured in the second year of cultivation and ranged from 56.0 (10) to 78.0 cm (9) and differences were statistically significant. Too high plants can easily lay over, which is a disadvantage of a good cultivar.

Table 1. Selected traits of St. John's wort plants in the first year of cultivation (2002)

Accession	Habitat	Shape of leaf	Time of flowering	Dry herb without stems [%]	Dry stems [%]	Content of secondary metabolites		
						Hypericin [mg%]	Pseudohypericin [mg%]	Sum of flavonoids [%]
1	50% prostrate	50% narrow	50% early	72	28	0.014	0.025	1.80
	50% erect	50% broad	50% late					
2	erect	broad	very early	58	42	0.011	0.029	2.24
3	fruticose	broad	late	63	37	0.012	0.041	1.50
4	erect	very narrow	late	62	38	0.013	0.039	2.10
5	fruticose	broad	very late	63	37	0.010	0.044	1.95
6	erect	broad	early	63	37	0.010	0.049	2.00
7	fruticose	broad	late	65	35	0.017	0.045	1.95
9	erect	narrow	late	63	37	0.031	0.045	2.56
10	erect	broad	early	68	32	0.023	0.035	2.74
11	erect	broad	early	68	32	0.016	0.035	2.50
Coeffic. of variation [%]						40.59	19.73	16.86
$LSD_{0.05}$				7.34				

The share of dry herb without stems oscillated from 72% (1, I year) and 66% (1, II year) to 58% (2, I year) and 54% (10 and 11, II year). Statistical analysis showed that in the first year the share of herb without stems was significantly higher than in the second year. The most favourable share of the leaf-flower fraction in the herb was found in plants from accession nr 1 in both years, but also nr 7 had a good one. Bomme (1997) and Hannig et al. (1995) showed that the highest content of hypericin and pseudohypericin was noted at a height 20-30 cm of the upper part of the plant, where leaves and flowers were most abundant.

Content of secondary metabolites in total herb is presented in Tables 1 and 2. In the first year the content of hypericin ranged from 0.010 (6) to 0.031 mg% (9). Pseudohypericin content oscillated from 0.025 (1) to 0.049 mg% (6) and the total content of flavonoids: 1.50% (3) – 2.74% (10). In the second year the content of hypericin ranged from 0.016 (6) to 0.068 mg% (9). Pseudohypericin content oscillated from 0.065 (4) to 0.134 mg% (7) and the total content of flavonoids: 1.62% (11) – 2.61% (2). The value of coefficient of variation showed that hypericin content (in I and II year) was more variable than the content of flavonoids. The similar amounts of hypericin, pseudohypericn were reported by Seidler-Łożykowska et al. (1999). The highest content of the sum of hypericins was found in plants of accessions nr 9 and 7 and this content can be considered high. Büter et al. (1998) found that the content of secondary metabolites in the first year was lower in comparison to the second year (hypericin – 40%, pseudohypericin – 37%, biapigenin – 25%, quercetin – 23%, hyperoside – 17% of second year content), which was confirmed in this experiment. Pluhar et al. (2001) observed the broad range of variability of hypericin and pseudohypericin content, which was also found in the present collection.

In the first year, symptoms of dieback were not observed, therefore, the resistance was estimated during the second year of cultivation (Table 2). There were no symptoms of this disease on the plants coming from accession nr 7 and the half of plants from nr 1. Up to 10% of the plants from accessions nr 3, 5, 10, 11 were infected, but the most infected ones were in the group of plants from accession nr 1. While testing cultivars and different accessions for dieback susceptibility, Gaudin et al. (2003) also found plants collected in the wild, which were resistant in the laboratory inoculation.

Table 2. Selected traits of St. John's wort plants in the second year of cultivation (2003)

Accession	Height of plants [cm]	Dieback resistance *	Dry herb without stems [%]	Dry stems [%]	Content of secondary metabolites		
					Hypericin [mg%]	Pseudohypericin [mg%]	Sum of flavonoids [%]
1	56.7	50% - 1 50% - 4	66	34	0.017	0.091	1.64
2	72.7	3	57	43	0.027	0.117	2.61
3	68.0	2	63	37	0.034	0.096	2.08
4	77.7	3	55	45	0.023	0.065	2.03
5	76.7	2	58	42	0.025	0.089	1.92
6	57.0	3	63	37	0.016	0.073	2.55
7	69.0	1	63	37	0.045	0.134	2.19
9	78.0	3	56	44	0.068	0.117	2.09
10	56.0	2	54	46	0.032	0.117	1.67
11	62.3	2	54	46	0.027	0.112	1.62
Coeffic. of variation [%]					46.4	20.6	16.2
$LSD_{0.05}$	6.66		7.34				

* scale of resistance to dieback:
1 – very high (100% healthy plants)
2 – high (0-10% infected)
3 – medium (10-30% infected)
4 – susceptible (over 30% infected)

CONCLUSIONS

The two-year examination of St. John's wort collection made it possible to estimate the wild accessions of the plant and to compare them with Polish cultivar 'Topaz'. The plants from accession nr 7 had a high hypericin content and showed resistance to dieback. In next years the evaluation should be continued to prove the ability to resist dieback. The accession nr 7 can provide the starting material for breeding program to obtain new Polish cultivar of St. John's wort resistant to dieback.

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OCENA KOLEKCJI DZIURAWCA ZWYCZAJNEGO (*HYPERICUM PERFORATUM* L.)

Streszczenie: Dziurawiec zwyczajny (*Hypericum perforatum* L.) jest wieloletnią rośliną leczniczą powszechnie uprawianą w Polsce. Ziele dziurawca, zebrane na początku kwitnienia ma działanie ściągające, spazmolityczne, antywirusowe, tonizujące (przeciwdepresyjne). W ostatnich latach plantacje dziurawca zostały porażone przez grzyb *Colletotrichum gleosporioides* (Penz.), który powoduje nekrozę całych roślin. W Instytucie Roślin i Przetworów Zielarskich zgromadzono kolekcję pochodzącą ze stanu naturalnego w celu oceny cech morfologicznych, chemicznych oraz odporności na porażenie tym grzybem. Dwuletnia ocena kolekcji pozwoliła na wybranie do dalszych badań roślin pochodzących z Czarnego Lasu koło Ostrzeszowa (7), które charakteryzowały się odpornością na porażenie antraknozą oraz wysoką zawartością substancji czynnych.

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