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Morphological, chemical and biochemical characterization of St. John's Wort (*Hypericum perforatum* L.) hybrids

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ABSTRACT After several years of inbreeding, we concluded that combination of advantageous properties of Hypericum perforatum lines is necessary. Two hybrids were established by reciproc crossing and their populations have been tested in the second year. Concerning drug production, hybrid F9 exceeded both parents. In the case of hypericin content, crossing resulted in the manifestation of mother's effect. Hypericin content was higher in flowers, while POD accumulated mainly in leaves. Alterations of them in the mentioned parts proved to be inverse: the level of hypericin increased and that of the POD decreased from vegetative to flowering phase. The content of glucose and fructose changed by organs during ontogenesis, their ratio in flowers increased. There were significant differences among taxa investigated: parent 35 and hybrid F16 showed lower values of POD and glucose in average, which may be in connection with their weaker tolerance against wilt disease.
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KEY WORDS

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peroxidase
glucose

An increasing interest have been experienced worldwide for antidepressant herbal remedies containing standard extract of *Hypericum perforatum* L. in the last few years (Brevoort 1998). A great number of research results have already been obtained regarding its morphological and chemical variability (Pluhár et al. 2000), clinical efficacy (Trautmann-Sponsel 1998) and efforts made to select new varieties for production of raw material (Franke et al. 1999). The extract standardized for hypericin content, has been proven to be the most effective form of administration, where hypericin derivatives, flavonoid glycosids, floroglucinols, etc. contribute to the antidepressant action (Anonym 1996).

The varieties bred earlier ("Topas") or recently ("Hyperimed","Hyperixtract", etc.) are not completely able to satisfy the up-to-date requirements of cultivation (e.g. wilt tolerance) and pharmaceutical industries (high level of active ingredients) at the same time (Heine and Eger 2000). During our previous investigations on the natural variability of St. John's Wort, we have experienced the advantage of selection in fixing certain traits, however, we could not find a good inbred line possessing all the expected features. Therefore, to obtain new varieties of *Hypericum perforatum*, the hybridization seems to be the most promising breeding method. Floral biology and combinating ability of this species have been tested only in intergeneric crossings, already (Schulte et al. 1999).

It is well known that peroxidase is one of the most important enzyme using for identification of different species and infraspecific taxa by gelelectrophoresis or isoelectric focusing (Arulsekar and Parfit 1986). Under natural growing conditions, plants are exposed to different kinds of stress and they have evolved various protective mechanisms to eliminate or reduce the generating active oxygen. Thus, peroxidases play an important role in the enzymatic antioxydant system (Hegedűs et al. 2001). Moreover, there have been found correlation among peroxidase enzyme activity, senescence and rooting processes (Takahama 1999).

The aim of our experiments was to establish infraspecific hybrids of St. John's Wort, followed by an indirect determination of the possible origin of inherited properties. Beside the morphological and chemical investigations, biochemical chracteristics have also been detected in two-year-old populations of infraspecific hybrids and of their parent lines, paralelly. Moreover, the accumulation tendencies of these ingredients have also been tested during three phenophases and in different plant parts.

Materials and Methods

Homogenized inbred lines of well-defined and different properties have been developped by us, then two of them (No. 31 and No. 35) have been used as parents for reciproc crossings in phytotron chambers in 1999. Line No. 31 can be characterized by a homogenous compact habit, medium yield and active substance content as well as good tolerability against *Hypericum* wilt (caused mainly by *Colletotrichum gloeosporoides*). Line No. 35 is a taxa of high productivity and of active substance content, while it is quite sensitive for wilt disease. A population of variety "Topas" (No. 10) was applied as a control of high productivity, of medium level of active substances and of moderate tolerance against *Hypericum* wilt. Populations of probable hybrids (F9= 31x35; F16= 35x31) were established in 2000 from isolately collected seeds. In the second year (2001) we measured the morphological properties in full flowering period, then hypericin content according to the actual Hungarian Standard

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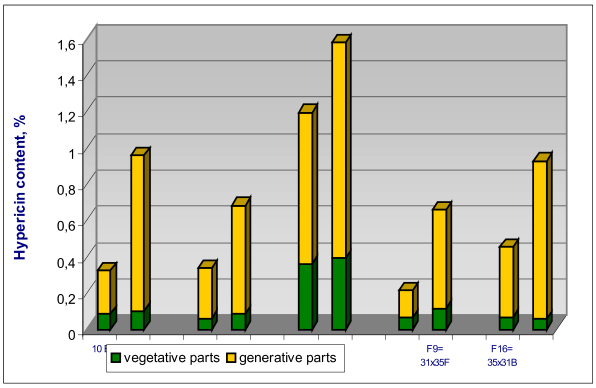
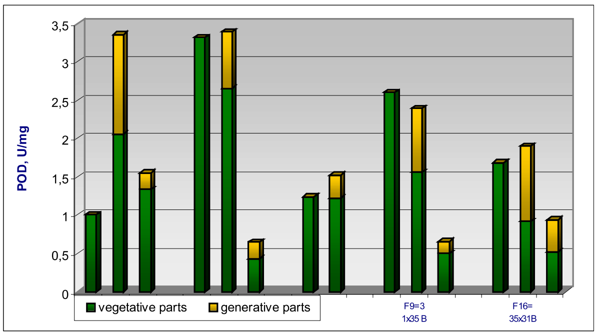




V= vegatative phase, B= phase of yellow buds, F= phase of flowering

Figure 1. Changes of hypericin content (%) by plant parts at two-year-old control (10), parent (31, 35) and hybrid (F9, F16) populations of *Hypericum perforatum*.

Figure 2. Changes of POD (U/mg) by phenological phases and by plant parts at two-year-old control (10), parent (31, 35) and hybrid (F9, F16) populations of *Hypericum perforatum.*

(Anonym 1991) in bud and full flowering stages. The sample collection for biochemical investigations was carried out in vegetative, in bud and in full flowering phases at three individuals of each populations. In bud and flowering stages we separated the generative and vegetative parts, too.

The total peroxidase activity was measured by a spectrophotometric method at λ= 460 nm by a Varian DMS 100 UV-visible spectrophotometer using H_2O_2 as a substrate and ortodianizidine as a chromogen reagent (ε = 11.3) (Cseke 1991). Protein content was determined by Bradford method (Bradford 1976). Three replications of each experiment were performed. In the case of carbohydrate analysis the glucose and fructose contents were established by OPLC (Sárdi et al, 1996).

Results and Discussion

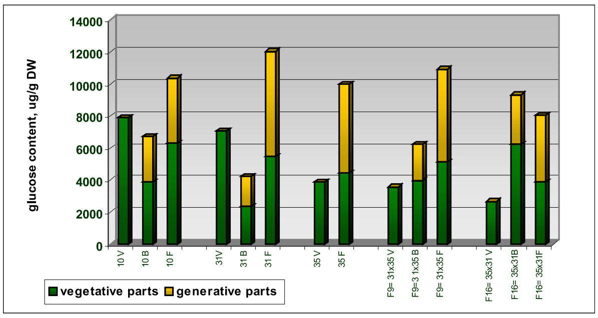
Morphological measurements of hybrid and parent populations in open field resulted that the two parents (31, 35) and hybrid F9 were quite similar in plant height (88-92 cm) and plant size in diameter (67-72 cm), while hybrid F16 remained small (30 and 25 cm). This latter phenomenon may due to the replantation of the F16 population at the beginning of the second vegetation period. F9 showed strong growth and even higher generativity than the control (No. 10).

Concerning fresh and dry mass production, hybrid F9 (fresh: 367 g/plant; dry: 97 g/plant) exceeded both parents (fresh: 240/310 g/plant; dry: 67/71 g/plant) as well as the control (fresh: 285 g/plant; dry: 83 g/plant). Because of their slow development, individuals of F16 populations did not reach considerable yield.

In harmony with the literature data (*e.g.* Repcak and Mártonfi 1997) we have found that the generative parts of the plants contain several times more hypericin (0.152-1.19%) than the vegetative parts (0.06-0.39%) (Fig. 1). Regarding yellow buds, this meant triple or four fold values and in the case of the flowers the level of hypericin is extremely high (4-8 fold) comparing it to that of the vegetative parts. The total amount of this component remained almost constant in the vegetative organs, while increased during flower opening in generative parts at each taxon. The parent 35 accumulated the highest content in both vegetative and generative parts. In hybrids, the hypericin content decreased comparing them to their parents, which was originated possibly from parent 31. The effect of mother could be shown more exactly at F9, containing similar proportion of hypericin as parent (mother) 31. In the case of F16, where mother was 35, only intermediate values were obtained. Therefore, regarding hypericin content, the hybridization has not proven to be an advantageous breeding method. To understand more completely the heritability of active substance accumulation in *Hypericum*, it would be useful to test the changes of flavonoid glycoside contents owing to hybridization.

Comparing to hypericin levels, data concerning peroxidase (POD) activity (Fig. 2), an inverse tendency could be shown: higher values characterized the vegetative organs, where their total amount usually (at 31, F9 and F16) decreased from vegetative to yellow bud and flowering phases. Between bud and flowering phases a significant decrease was observed in generative parts. In average, higher levels of POD were characteristic for control and 31, while hybridization caused negative change due mainly to the effect of mother. Little amounts have been observed at parent 35 and hybrid F16, which may be in connection with their weaker tolerance against wilt disease.

The accumulation course and level of examined monosacharides (glucose and fructose) were similar by taxa. In vegetative phase, the control and the parent 31 contained the highest levels of them (7000-8000 mg/g DW), while the parent 35 and the hybrids showed lower values (Fig. 3). Later on, glucose and fructose have been transported to the buds



V= vegatative phase, B= phase of yellow buds, F= phase of flowering

Figure 3. Changes in glucose content according to plant parts and phenological phases at two-year-old control (12), parent (31, 35) and hybrid (F9, F16) populations of *H. perforatum*.

and flowers. During flowering, higher values could be detected in generative organs (4000-6500 mg/g DW) than in the vegetative ones (3900-6300 mg/g DW). According to earlier experiences (Repcak and Mártonfi 1997), the amounts of flavonglucosids (quercitrin, isoquercitrin) are higher in flowers, while that of the rutin (glucorhamnosid) can be found mainly in green parts of the plant at *Hypericum perforatum*. The latter compound was present in very little amount or absent in parent 35, however, it was quite rich in hyperosid and the other mentioned glucosids accumulating in flowers. This different phenomenon of monosaccharid accumulation and their binding to flavonoid aglica is probably related to the tolerance to *Hypericum* wilt.

On the basis of our preliminary observations on biochemical traits of *Hypericum perforatum* it can be concluded that they have significance in distinction of infraspecific taxa and hybrids. To extend our knowledge regarding correlations between the accumulated ingredients and stress tolerance of the species, it would be useful to investigate the total flavonoid content as well as the ratio of the flavon glycoside compounds in the future.

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