Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):171-172, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Morphological and flavonoid pattern variations within some *Euphorbia cyparissias* L. populations

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ABSTRACT The leafy spurge *Euphorbia cyparissias* L. is a very common and adaptive plant species in Europe. It prefers the xeroterm areas. Eleven populations of this plant were studied in Hungary at different ecological habitats: forest and road edge, swamp field and lime stone rock grass. These areas were exposed to sun to various degree and have different base stones and soil types. Populations have different phenotypes: various stem and cyma height, leaf length and number of branches. We detected two main and other flavonoid components of this plant by TLC and HPLC in different quantity. Altogether 18 flavonoid compounds were detected in the populations by HPLC with very different flavonoid patterns. Plants living at shadowy habitats were higher, they had more leafy branches, leaves and more (9-13) flavonoid constituents. Populations living at sunny areas had smaller stem and leaves, and only 4-9 flavonoid compounds. According to the different morphology and flavonoid pattern the populations were classified into ecotype groups which can be related to the studied ecological habitats. These results prove that *Euphorbia cyparissias* L. is a very polymorph plant species.
Acta Biol Szeged 49(1-2):171-172 (2005)

KEY WORDS

populations
flavonoid pattern
polymorphism

Populations of *Euphorbia cyparissias* L. are very common in Europe and in Hungary. In this study some populations were collected from various ecological habitats in the vicinity of Pécs in South Hungary. These habitats are characterised by various base stone, soil type and plant association. We studied 11 populations of this plant species. The investigation aimed at answering the question, if they differ only in their morphology or in flavonoid pattern too, if they are ecotypes of this species adapting to the different ecological habitats. Therefore the morphology and flavonoid pattern of these populations were compared.

Materials and Methods

Plant materials

The 11 habitats of *Euphorbia cyparissias* L. were the followings: 1. pioneer rock grass (Kővágószőlős), 2. degraded rock grass (Cserkút), 3. degraded lime stone rock grass (Tettye 1.), 4. polluted rock grass (Tettye 2.), 5. pine tree forest (Tettye 3.), 6. lime stone rock grass (Csarnóta 1.), 7. edge of arable land (Csarnóta 2.), 8. swamp field (Pellérd), 9. edge of a hornbeam-oak forest (Árpádtető 1.), 10. deforestation field (Árpádtető 2.) and 11. Botanical Garden of University of Pécs. Plants were collected in May 2003 and 2004, then they were dried at room temperature.

Thin layer chromatography (TLC)

Stems, leaves and cyathia (special inflorescence of *Euphorbia* species) were homogenized (0.2-0.2 g each), afterwards 5 ml from the mixture ethanol:water (7:3) was added to them. Extractions were shaken at room temperature for 30 minutes (150 rotation/min, Edmund Bühler KL-2) and then filtered by Whatman filter paper. 7-7 µl of the plant extractions and 3-3 µl of the standards (1 mg/ml methanolous solution of camphorol, quercetin, rutin, hyperosid, chlorogenic acid, caffeic acid and ferulic acid) were studied by TLC. Development was carried out on DC-Kieselgel 60 F_{254} Merck silica gel plates, with the mixture of ethyl-acetate:formic acid:acetic acid:water (100:11:11:27). After development the plates were dried at 105°C and visualised with Naturstoff reagent. Quantitative measurement of the compounds was carried out by CAMAG TLC Scanner II (Switzerland) at 365 nm.

High performance liquid chromatography (HPLC)

Plants were homogenized at room temperature (0.5-0.5 g each), thereafter 5 ml 70% methanol was added to them. Extractions were shaken at room temperature for 20 minutes (200 rotation/min) and then filtered. They were evaporated at 95°C water bath (FALC WB U4), and the evaporation residues were sold in 2 ml 96% ethanol. The study was carried out by Waters Millipore HPLC: 600 Controller, 600 Pump, 2487 Dual&Absorbance Detector, In-line degasser AF, LiChrospher RP-18-column (5 µm, 200x4mm). Developing mixture: acetonitrile:water (5:95) + 0.5% phosphoric acid. Stream speed: 0.8 ml/min. Evaluation was carried out at 254 and 329 nm.

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Table 1. Components of the flavonoid fraction of the *Euphorbia cyparissias* populations (9. and 11.: two main flavonoids)

Components of the flavonoid fraction →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. edge of hornbeam-oak forest (Árpádtető 1.)	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+
2. deforestation field (Árpádtető 2.)	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
3. edge of arable land (Csarnóta 2.)	+	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-	+	-
4. lime stone rock grass (Csarnóta 1.)	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-
5. degraded grass (Cserkút)	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-
6. pioneer grass (Kővágószőlős)	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
7. degraded rock grass (Tettye 1.)	+	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-
8. polluted rock grass (Tettye 2.)	+	-	-	+	+	+	+	-	+	-	+	-	-	+	-	-	+	-
9. pine tree forest (Tettye 3.)	-	+	-	+	-	-	+	-	+	+	+	-	-	+	+	-	+	-
10. swamp field (Pellérd)	+	-	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	-
11. Botanical Garden	-	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	+	-

Results and Discussion

Morphological characteristics of the studied populations of *Euphorbia cyparissias* L. were different in the studied years. We detected higher plants with long leaves and many leafy branches at 5 populations, and smaller plants in 6 habitats. These morphological results proved to be constant characteristics of the populations every year.

Each part of the plants (stem, leaf, cyathia) had 2 main flavonoids: camphorol-3-glucuronide (R_f=0.7) and quercetin-3-glucuronide (R_f=0.57; Stadtmann and Pohl 1966; Hegnauer 1989).

Beside the 2 main flavonoids three other compounds were detected in the extractions of cyathia of some populations by TLC: compound with R_f =0.92 in the cyathia of plants from pine tree forest (Tettye 3.), swamp field (Pellérd) and deforestation field (Árpádtető 2.). Constituent with R_f=0.51 was found only in the extractions of cyathia from edge of arable land (Csarnóta 2.) and Botanical Garden, and a flavonoid with R_f=0.97 in the plant samples from edge of arable land and lime stone rock grass (Csarnóta 1-2.).

Two constituents of the leaves were detected beside the main flavonoids: one with R_f=0.81 and another with R_f=0.92 (plants from pioneer rock grass in Kővágószőlős, deforestation field in Árpádtető and pine tree forest in Tettye).

Stem extractions of the populations contained other 2 compounds: one with Rf=0.51 (plants from degraded rock grass in Cserkút, deforestation field in Árpádtető, edge of a hornbeam-oak forest and degraded rock grass in Tettye). Another flavonoid (R_f=0.81) was found in the plant stems from deforestation field, degraded rock grass in Tettye and Csarnóta, and edge of arable land.

Results of the study by HPLC showed 18 various flavonoid compounds of the populations. The 11 "fingerprint patterns" of flavonoids differred to a great extent, with 4-16 compounds/plant samples (Table 1). Flavonoids are synthetised by sunlight, therefore we detected difference in the quantity and quality of the flavonoids in the populations living at various habitats. Populations living at shadowy areas have 4-9 type of flavonoids (plants from rock grass in Cserkút, polluted rock grass in Tettye, pine tree forest and Botanical Garden). Plants living at sunny habitats have more (9-13 type) flavonoid constituents (plants from edge of hornbeam-oak forest, deforestation field, edge of arable land and rock grass in Csarnóta, degraded rock grass in Tettye and swamp field). According to these flavonoid patterns the populations were divided into ecotype groups.

Populations of this plant species can be separated clearly from each other according to their morphology and flavonoid pattern. Differences were detected in the quantity and quality of the flavonoids in the studied 11 populations living at various ecological habitats. These results verify the polymorphism of *Euphorbia cyparissias* L.

Acknowledgements

We are grateful for the help with plant collection to Péter Lőrincz and to Ágnes Farkas for her assistance.

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