Systematic relationships among the taxa of *Festuca valesiaca* group (*Poaceae*) from the Ukraine as judged by their phenolic compounds' patterns

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Abstract. Thin-layer chromatography (TLC) was employed to analyze the phenolic compounds' patterns of six Ukrainian taxa of the *Festuca valesiaca* group in an attempt to reveal the systematic relationships among them. The results showed that the species *F. pseudodalmatica* was among the most distantly positioned taxa within the studied species. The species *F. valesiaca*, *F. rupicola*, *F. arietina, F. macutrensis,* and *F. galiciensis* demonstrated higher mutual affinities. Generally, all studied species form a closely related group as judged by their phenolic compounds' composition.

Key words: *Festuca, Festuca valesiaca* group, phenolic compounds, TLC, systematic relationships

Introduction

Genus *Festuca* L. is among the largest genera of *Poaceae* and encompasses worldwide more than 500 species (Catalán & al. 2004). Of the 267 species of *Festuca* in Euro+Med Plantbase (Foggi & Müller 2009), more than 20 belong to the *F. valesiaca* group (Pils 1984, Arndt 2008).

Limits of the genus and taxonomic rank of the numerous taxa belonging to the fine-leaved fescues are still disputable. This is mainly due to lack of materials for study from many regions of Europe, including the Ukraine. The studies of Alexeev (1975) and Tzvelev (1976, 2010) which cover the territory of the former USSR are among the main systematic treatments of genus *Festuca* in East Europe. Until recently, the study of Tveretinova (1977) was the only systematic treatment of the genus in the Ukraine. The last revision of genus *Festuca* confirmed 17 species of the subgenus *Festuca* type, section *Festuca,* which occur in the flora of the Ukraine (Bednarska 2007; Tzvelev 2010). This includes all fine-leaved, densely caespitose species of the genus. These species could be divided into two major groups according to the anatomical structure of their leaves. The first group includes species with a sclerenchyma ring, the so called "ovinoid" type structure – *F. ovina* agg. (*F. ovina* L., *F. filiformis* Pourr., *F. airoides* Lam.), *F. glauca* agg. [*F. pallens* Host, *F. psammophila* (Hack. ex Celak) Fritsch] and *F. beckeri* agg. (*F. beckeri* (Hack.) Trautv., *F. polesica* Zapał.]. The 10 remaining species represent the second group: species with 3–5 sclerenchyma bundles, which in some species may be secondarily fused into a ring, the so called "sulcata" type structure (from *F. sulcata* s.l.), which is characteristic for the species of *F. valesiaca* group.

Most problematic are the systematics and taxonomy of species belonging to *F. valesiaca* group. Due to overlapping diagnostic traits and scarcity of small-scale studies, systematic structure and taxonomy of this group are quite disputable (Pils 1984; Arndt 2008). A

study of the Ukrainian taxa of *F. valesiaca* group revealed a very complicated continuum of variability. Many of them deserve species rank as it was proved for *F. galiciensis* Bednarska nom. prov. (Bednarska 2014a).

Along with this, diversity of naturally occurring populations is significantly higher than the number of formally described taxa. For example, in Western Ukraine (Rohatyn Opillia region), *F rupicola* with green and with glaucous leaves may occur frequently within one and the same biotope. Earlier, the latter form was wrongly identified as *F. pseudodalmatica* Krajina ex Domin (Bednarska 2007). However, the results of long-term observations have shown that both forms belong to *F. rupicola* and the glaucous one should be designated as *F. rupicola* f. *glaucescens* (Bednarska 2014a).

Summarizing most of the thin-leaved fescues demonstrates high variability of morphological and anatomical traits, including the diagnostic ones. So, there is a need to apply different new approaches, including biochemical ones, to reveal the systematic structure and phylogenetic relationships of the thin-leaved fescues.

Thin layer chromatography (TLC) is a good choice in applying a chromatographic technique with good selectivity of detection and low cost. Furthermore, TLC can analyze several parallel samples in a single run, which allows for making a precise comparative analysis (Braz & al. 2012). TLC is widely applied in chemosystematic studies.

Phenolic compounds, including flavonoids, are the most useful class of secondary metabolites from a systematic viewpoint, mainly due to their wide spread in the plant kingdom, structural variability and chemical stability. Phenolic profiles have been extensively used in chemotaxonomy of *Poaceae* as a good additional taxonomic character in assessing the species relationships, and species and genus boundaries (Peterson & Rieseberg 1987; Arrieta & Hernández 2005; Míka & al. 2005; Hilu 2007; Kharazian & Rahiminejad 2008, 2009).

The present study includes six taxa of the *Festuca valesiaca* group from the Ukraine: *F. valesiaca* Gaud., *F. rupicola* Heuff., *F. macutrensis* Zapał., *F. galiciensis* Bednarska nom. Prov., *F. arietina* Klokov, and *F. pseudodalmatica* Krajina. Excluding the high-mountain *F. saxatilis* Schur, the Crimean *F. callieri* (Hack.) Markgr. and the synantropic species *F. brevipila* Tracey, the above listed species represent 90 % of *F. valesiaca* group in the Ukrainian flora.

The purpose of the present study was to study the phenolic compounds' patterns in an attempt to reveal systematic relationships among the above mentioned taxa of *F. valesiaca* group.

Material and methods

Leaves from natural populations of the above mentioned species were collected in the Ukraine by I. Bednarska (Table 1). Each population sample consisted on the average of 25 plants, which were first identified by their anatomical and morphological traits. A total of

Table 1. Locality list of the studied taxa of genus Festuca from Ukraine.

Taxon	Number LWKS	Locality
F. valesiaca	1598	Zhytomyr region, Berdychiv region, town Berdychiv, 24.07.2010 Leg. О.Орлов, 49°54'0" 28°34'0"
	1533	Zakarpattia region, Vynohradiv district, village Klynovetska Gora, 19.06.2011 Leg. leg. I.Bednarska, I.Danylyk, R.Kish
F. macutrensis locus classicus	1622	Lviv region, Brody district, village Sukhovolya mountain Makitra, 01.07.2013 leg. I.Bednarska, 50° 2'17.32" 25°14'53.75"
F. galiciensis sp. nova locus classicus	1672	Ivano-Frankivsk region., Rohatyn district, v. Kuropatnyky (glaucous plants), 19.07.2014 leg. I.Bednarska, 49°17'2.47" 24°40'8.10"
F. rupicola	1673	Ivano-Frankivsk region., Rohatyn district, v. Kuropatnyky (green plants), 19.07.2014 leg. I.Bednarska, 49°17'1.98" 24°40'8.87"
F. rupicola f. glaucescens	1678	Ivano-Frankivsk region, Rohatyn district, v. Fraga (glaucous plants), 19.07.2014 leg. I.Bednarska, 49°28'2.47" 24°26'49.50"
F. rupicola	1679	Ivano-Frankivsk region., Rohatyn district, v. Fraga (green plants), 19.07.2014 leg. I.Bednarska ,49°28'2.47" 24°26'49.50"
F. rupicola f. glaucescens	1674	Ivano-Frankivsk region, Galych district, village Podilla (glaucous plants), 19.07.2014 leg. I.Bednarska, 49°16'33.46" 24°44'28.45"
F. rupicola	1675	Ivano-Frankivsk region, Galych district, village Podilla (green plants),19.07.2014 leg. I.Bednarska, 49°16'30.88" 24°44'29.95"
F. arietina	1668	Cherkasy region., Kaniv district, between village Keleberda and town Kaniv, 07.07.2014 leg. I.Bednarska, Kostikov, 49°44'22.51" 31°33'53.09"
F. arietina	1445	Belarus. Homiel region., Vietka district, the village Odnopolie, river Sozh, 17.06.2010 leg. I.Bednarska, 52°41'50.70" 30°58'30.66"
F. pseudo-dalmatica	1528	Zakarpattia region, Mukachevo district, town Mukachevo, mountain Lavachka, 16.06.2011 leg. I.Bednarska, I.Danylyk, R.Kish, 48°27' 24.38"N, 22°42'3.62"
F. macutrensis	1526	Lviv region, Zolochiv district, village Chervone, tract Lysa mountain, 11.06.2011 Leg. I.Bednarska , A.Kagalo, 49°47'50" 24°43'38"

330 plants (970 leaf cross sections) were studied. Then leaf population samples were collected. Voucher specimens are deposited at the Institute of Ecology of the Carpathians Herbarium of the Ukrainian National Academy of Sciences (LWKS).

Air-dried powdered aerial parts (0.2 g) of the examined samples were extracted with 1% HCL in methanol at room temperature for 48 hours (Angelov & al. 1988). After evaporation of the solvent, the crude extract was examined by thin layer chromatography (TLC). Acetic acid/water (15:85 v/v) was used as mobile phase on Cellulose F (5574) plates. Chromatograms were viewed under UV = 366 nm light before and after spraying with Naturstoffreagenz A: 1% solution of diphenylboric acid 2-aminoethyl ester complex in methanol. The visualized spots were described by retention factor (Rf): compound migration distance/ solvent front migration distance, and color.

Affinities among the taxa within the studied group were assessed by the Jaccard's coefficient of similarity SI = M/M + N, where M is the number of bands common for the compared taxa, and N – the sum of absent bands in each compared taxon (Angelov & al. 1988).

An index of group affinity (GA) was calculated for each taxon as a sum of its SI values.

All calculations were done with the STATISTICA 7.0 package (Stat Soft Inc.). The data were analysed by Cluster Analysis based on the coefficient of similarity SI, as well as by some univariate statistical methods.

Table 2. Phenolic compounds' patterns of the studied species of Festuca.

Taxon	Phenolic spots – Rf (rate of flow) and color after spraying with R*												
	0.08	0.16	0.30	0.34	0.37	0.39	0.43	0.50	0.61	0.63	0.65	0.73	0.77
	yellow	yellow	yellow	blue	yellow orange	yellow	brown	blue	brown	blue	green-yellow	blue	yellow
	1	2	3	4	5	6	7	8	9	10	11	12	13
F. valesiaca	+	+	+	+	–	–	+	+	+	–	+	+	+
F. rupicola	+	+	+	+	+	–	+	+	+	+	+	+	–
F. macutrensis	+	+	+	+	–	–	+	+	–	+	+	+	–
F. galiciensis	+	+	+	+	–	+	+	+	–	+	–	+	–
F. arietina	+	+	+	+	–	–	+	+	+	+	+	+	–
F. pseudodalmatica	+	+	+	+	–	–	–	+	+	–	+	–	+

Legend: **R*** 1% solution of diphenylboric acid 2-aminoethyl ester complex in methanol.

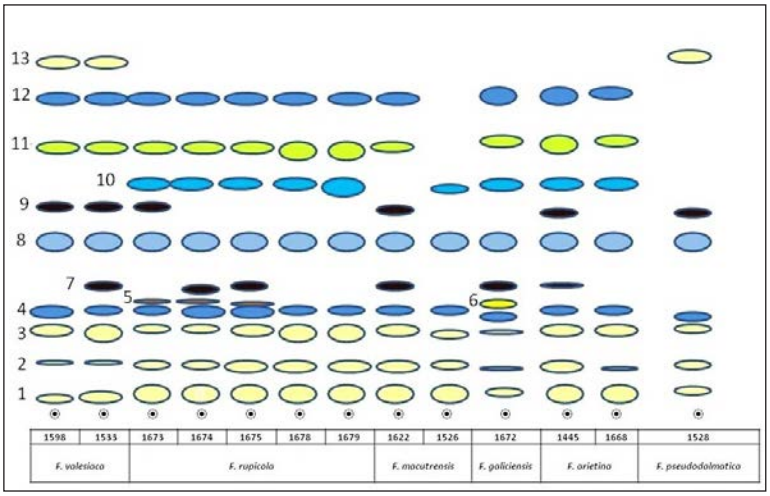

Fig. 1. Schemes of the chromatograms of the studied samples.
Legend: numbers of the schemes correspond to the numbers in Table 1.

Results

Phenolic compounds' patterns of the studied species of *Festuca* are presented in Table 2 and Fig. 1. The samples of *F rupicola* with green (typical form) and with glaucous (*F. rupicola* f. *glaucescens*) leaves showed identical phenolic patterns. Totally, 13 spots (compounds) were chromatographically resolved by TLC. The analysis showed that different phenolic compounds – flavonoids (yellow, yellow-orange and brown spots) and phenolic acids (blue and green-blue spots) – were present in the investigated extract (Kharazian & Rahiminejad 2009). Most of the spots with Rf values of 0.08, 0.16, 0.30, 0.34, 0.50, 0.65 were shared by all investigated species, thus emphasizing their close relationships. Except for *F. pseudodalmatica,* spots with Rf = 0.43 and Rf = 0.73 were also common for the rest of the studied species. A spot with Rf = 0.63 was shared by most of the species, but absent in *F. valesiaca* and *F. pseudodalmatica.* A spot with Rf = 0.37 was species specific for *F. rupicola*, while a spot with Rf = 0.39 was found in *F. galiciensis* only.

The values of coefficient SI varied within a wide range from 0.42 (*F. pseudodalmatica* vs. *F. galiciensis*) to 0.91, where *F. arietina* was contrasted to *F. rupicola* (Table 3). But most SI values were fluctuating in a narrower interval (0.72–0.91), thus confirming the close affinities among the studied species. Within the group, the species *F. arietina* demonstrated the greatest similarity to *F. rupicola* and *F. macutrensis.* The

species *F. pseudodalmatica* was the most distantly positioned taxon within the entire studied group of genus *Festuca,* as its SI values were the lowest ones. Our earlier chemosystematic studies have shown comparable values of coefficient SI for pair-wise comparisons among eight genetically closely related species of the *F. rubra* group (Angelov & al. 1988).

An index of group affinity contributed further to revealing the systematic relationships within the examined group of genus *Festuca.* Lower values of index GA mean greater distance for a given taxon, and vice versa, higher values indicate a closer affinity within the group. The species *F. pseudodalmatica* possessed the lowest value of the group affinity index (GA = 2.98) and occupied the remotest position within the studied group of genus *Festuca.* The species *F. galiciensis* (GA = 3.24) was also somewhat distantly positioned in respect to the rest of the taxa. The values of index GA for the remaining species were higher and varied from 3.76 (*F. valesiaca*) to 3.94 (*F. arietina*), and indicated close affinity among them. The systematic relationships among the species of *F. valesiaca* group were graphically demonstrated by a dendrogram based on coefficients of similarity (Fig. 2).

Table 3. Values of coefficient SI for all pair wise comparisons among the studied species of *Festuca*.

Taxon	Coefficient of similarity SI					
	1	2	3	4	5	6
1. *F. valesiaca*	x					
2. *F. rupicola*	0. 75	x				
3. *F. macutrensis*	0.72	0.81	x			
4. *F. galiciensis*	0.58	0.72	0.80	x		
5. *F. arietina*	0.81	0.91	0.90	0.72	x	
6. *F. pseudodalmatica*	0.80	0.58	0.58	0.42	0.60	x

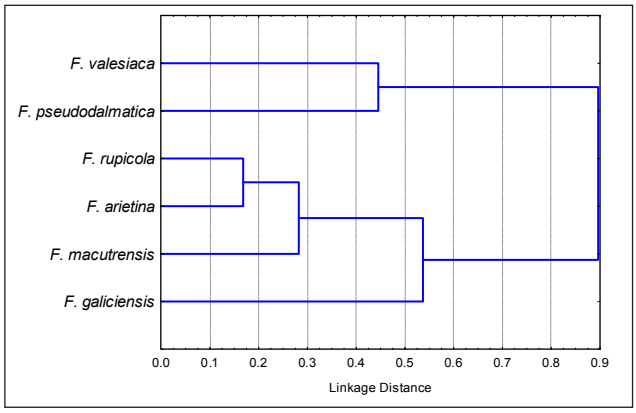

Fig. 2. Dendrogram based on coefficient SI showing the relationships among the studied species of *Festuca.*

Discussion

Summarizing the results of the present chromatographic study, it was evident that the species *F. pseudodalmatica* was among the most distantly positioned taxa within the studied group of genus *Festuca*. But generally, all studied species form a closely related group as judged by their phenolic compounds' composition.

The most stable and reliable diagnostic traits are characteristic for diploid *F. valesiaca* only. All other taxa form a complicated polyploid series, with a spectrum of transitional forms among them. The species *F. rupicola* (2n = 42) has stable 3(–5) sclerenchyma bundles. Out of all taxa in the group, *F. rupicola* has the widest range of distribution which reflects to some extent the variability of its morphological traits. The remaining taxa - *F. arietina* (2n = 42), *F. macutrensis* (2n = 28) and *F. galiciensis* (2n = 28) – have a more restricted distribution, but variability of their leaf anatomical traits is among the highest within the group (Bednarska 2000, 2014a,b; Bednarska & Orlov 2011). In these three taxa, the sclerenchyma may be developed as isolated bundles, or fused to a different ring into the sclerenchyma. According to some authors (Tzvelev 1976; Bednarska 2014a), these taxa are of hybridogeneous origin which explains their high polymorphism. According to the suggestions of the above mentioned authors, one of the parental species of these taxa has a sclerenchyma ring (the "ovinoid" type structure). For *F. arietina* it was *F. polesica*, while for *F.galiciensis, F. pallens* is supposed as a parental species. The species *F. rupicola* is most probably the second parental species. For instance, its morphological traits in Western Ukraine (including indumentum of lemma) are fully overlapping with those of *F. macutrensis*; and the unique population of *F.galiciensis* is entirely surrounded by the locally dominant *F. rupicola*. Both taxa form a mixed population in locus classicus. It is too early yet to discuss definitively the phylogenetic relationships within the studied group. However, the results of this study generally support the theories based on anatomy, morphology, ecological and phytogeographical characteristics of the studied species.

Evolutionary, this group is among the youngest and the process of species differentiation has not been completed yet. In fact, these taxa form a very complex continuum. It is very difficult to divide it into discrete taxa solely on the basis of morphological traits. Just like their morphological, anatomical and ecological peculiarities,

the results of the choromatographic analysis demonstrated that they are closely related taxa and any distinction among them based solely on one set of markers is problematic. Apparently, additional biochemical markers are needed to clarify the systematic relationships among the species of *F. valesiaca* agg.

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