TAXONOMY AND NODULATING ABILITY OF PAPILIONOID LEGUMES OF BULGARIA WITH POTENTIAL USE IN FORAGE PRODUCTION

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Abstract

The livestock industry plays an important role in the economy of Bulgaria by contributing a large portion of gross agricultural products. Increased demand for livestock products has led to corresponding increases in demand for forage. Considering the importance of legumes in forage production, this study was undertaken to explore nodulating status of Papilionoid legumes of Bulgaria. Nodulating ability was examined in 172 legumes of Bulgaria distributed in 10 Papilionoid tribes. Nodules were observed in 61 legume species growing in the greenhouse or under natural field conditions. Nodulation is reported for the first time in five of the Papilionoid legume species (*Lathyrus alpestris* (Waldst. & Kit.) Kit., *Lathyrus pannonicus* (Jacq.) Garcke, *Medicago rhodopea* Velen., *Onobrychis alba* (Waldst. & Kit.) Desv.), *Pisum sativum* L. var. *elatius* (Steven ex M. Bieb.) Alef.) found in Bulgaria. Nodulating status of 111 legume species was checked as reported in the literature and nodulation has been observed in 104 of these legumes species. Information is missing about nodulation about 7 legume species from Bulgaria and their nodulating status could not be confirmed from the field observations. *Lotus uliginosus* Schkuhr is a lost plant of Bulgarian flora and its nodulating ability could also not be established in the present study. This work is a preliminary step towards exploration of efficient symbiotic rhizobia and their use in the management of grasslands and pastures growing these legumes individually and in association with grasses for enhancing forage production in Bulgaria.

Introduction

Bulgaria is situated on the Black Sea in south-eastern Europe, occupying the north-eastern part of the Balkan Peninsula. The land for agricultural use in Bulgaria is about 6,168,100 ha. As a whole Bulgaria's vegetation can be ranked among the Central European forest region, but the influence of the South Russian and Asia Minor regions is also felt (Petrov and Palamarev, 1989). The vegetation includes shrub lands, grasslands and forests (Assyov et al., 2012; Delipavlov and Cheshmedzhiev, 2003; Jordanon, 1963-1989; Kozkuharov, 1992, 1995; Petrov and Palamarev, 1989). There is a great diversity of herbs (Kozkuharov, 1992, 1995). According to the latest data (Assyov et al., 2012; Tashev, 2007), the higher flora of Bulgaria is distributed in 20 floristic regions (Fig. 1) and comprises of 4000 species belonging to 906 genera and 153 families.

Bulgaria is dominated by rugged mountains, except for the Danube lowland in the north that it shares with Romania. Rich farmland in the Danube Valley, 130 kilometers (80 miles) of sandy beaches on the Black Sea, and mountainous terrain characterize one of Eastern Europe's least densely populated nations. Two mountain ranges and two great valleys mark the topography of Bulgaria. The Maritsa is Bulgaria's principal river, and the Danube also flows through the country.

Topography: The topography of Bulgaria is predominantly hilly and contributes considerably to the water erosion appearance: only 16% of the territory are relatively flat with slopes less than 3°, while more than half (55,3 %) of it has slopes from 3 to 12 %. The main characteristic of Bulgaria's topography is alternating bands of high and low terrain that extend east to west across the country. From north to south, those bands are the Danubian Plateau, the Balkan Mountains (called Stara Planina, meaning old mountains in Bulgarian), the central Thracian Plain, and the Rhodope Mountains. The easternmost sections near the Black Sea are hilly, but they gradually gain height to the west until the western most part of the country is entirely high ground. More than two-thirds of the country is plains, plateaus, or hilly land at an altitude less than 600 meters. Plains (below 200 meters) make up 31% of the land, plateaus and hills (200 to 600 meters) 41%, low mountains (600 to 1,000 meters) 10%, medium-sized mountains (1,000 to 1,500 meters) 10%, and high mountains (over 1,500 meters) 3%. The average altitude in Bulgaria is 470 meters.

The Danubian Plateau extends from the Yugoslav border to the Black Sea. It encompasses the area between the Danube River, which forms most of the country's northern border, and the Balkan Mountains to the south. The plateau slopes gently from cliffs along the river, and then it abuts mountains of 750 to 950 meters. The plateau, a fertile area with undulating hills, is the granary of the country.

Soil: The soils vary greatly in Bulgaria. The main types of soil for Northern Bulgaria are the black soil and the grey forest soil, formed under the influence of the moderately continental climate (Kolchakov *et al.*, 2009). To the south, where the transitional continental and sub Mediterranean climate is prevailing, maroon and clay soils are predominant. In the mountains (average altitude 700-800 m above sea level) forest meadow soils are predominant. The following soil types are present in Bulgaria: maroon forest (29.1%), black earth (23.4%), gray forest (17.2%), brown (14.8%), alluvial-meadow (7.0%), clay black earth (5.8%), and forest meadow (1.2%).

The structure of the soil cover of Bulgaria is very complicated and often inadequate to the present climate and vegetation conditions (Koutev and Kolev, 2008). Five types of pedoclimatical regime can be recognized in the country's territory: Crio-Udic, Meso-Udic, Meso-Ustic, Meso-Xeric and Thermo-Xeric (Boyadzhiev, 1994-a). As a result, 20 out of a total 28 FAO soil map units can be found on the relatively small territory of Bulgaria (Boyadzhiev, 1994-b). There are four soil regions: (i) Azmbisol-Podzol-Leptosol with Luvisols; (ii) Chzernozem-Kastenozem-Phaeozem Region with Luvisols; (iii) Luvisol Region with Leptosols and Planosols and (iv) Vertisol Region of Central Bulgaria. Soil erosion is recognized as one of the most serious soil degradation processes on the territory of Bulgaria. About 64% of the country's territory and 78% of the arable land is potentially exposed to risk of water erosion. Agricultural land covers 56.3 % of the whole territory of the country, forest 35.3 % and settlements, industries, transport and infrastructure 6.6, water bodies occupy 1.8%. Cropland is 39.8 %, rangeland and pastures 14.6 %, and permanent crops 1.9%.

Climate: Bulgaria has an unusually variable and complex climate (Nikolova, 2007). The country lies between the strongly contrasting continental and Mediterranean climatic zones. Bulgarian mountains and valleys act as barriers or channels for air masses, causing sharp contrasts in weather over relatively short distances. The climate in Bulgaria is moderately continental with clearly marked four seasons: spring, summer, autumn and winter (Hershkovich, 1984). The continental influence, stronger during the winter, produces abundant snowfall; the Mediterranean influence increases during the summer and produces hot, dry weather. The average annual temperature is 10.5°C. The average January temperature is around 0°C. Average summer temperatures rarely exceed 30°C. Annual precipitation averages comparatively low (698 mm), ranging from 450 mm in the plains to 1200 mm in the high mountains. Snow lasts for an average of 10 days on the Black Sea coast, 20-30 days on the lowlands and more then 200 days in the mountains. Heavy rain pour, severe drought and temperature extremes have resulted in climate change in Bulgaria which has been investigated by many workers (Nikolova and Vasilev, 2006; Nikolova, 2007; Topliiski, 2005; Vekilska and Rathcev, 2000).

The livestock industry plays an important role in the economy of Bulgaria by contributing a large portion of gross agricultural products. Increased demand for livestock products has led to corresponding increases in demand for forage. Within Europe, half of the annual requirement for feed is provided by grasslands. There is a renewed interest in forage legumes for several economical and ecological reasons and European Union strengthen the role of protein rich crops (Carlier *et al.*, 2008). Legumes have world-wide importance for food, fodder, fuelwood, and nitrogen source natural grasslands and agro-ecosystems (Allen and Allen, 1981). The main environmental advantage of legume-based forage production is least dependency on inorganic N fertilizers and vast saving on their manufacture (Amargar, 2001). Considering the importance of legumes in forage production, this study was undertaken to explore nodulating status of Papilionoid legumes of Bulgaria.

Materials and Methods

A critical search of the existing literature sources on the Bulgarian vascular flora (Assyov *et al.*, 2012; Delipavlov and Cheshmedzhiev, 2003; Jordanon, 1963-1989; Kozkuharov, 1992, 1995; Petrov and Palamarev, 1989) helped building up a database of nodulating ability of Papilionoid legume speices. Some of the legumes were also examined in the field for their nodulation. The legumes examined includes: herbs, shrubs, vines and trees. Nodulation was observed at flowering stage of legumes. At least five plants of each species were examined in greenhouse or in the field conditions by excavation. Wild legumes were examined under natural habitats. Nodules were distinguished from other kinds of root-malformations such as those caused by nematodes, insects or other root-inhabiting parasitic microorganisms (Somasegaran and Hoben, 1994). The nomenclature and classification of legumes follow Polhill and Raven (1981) and author citations follow Brummitt and Powell (1992). Nodulating ability was observed among 172 legumes of Bulgaria distributed in 10 Papilionoid tribes (Table 1). Nodulation was examined in 61 legume species growing in the greenhouse or under natural field conditions. The nodulation results were compared with the existing knowledge summarized for the world by Allen and Allen (1981) and Sprent (2001). The legume listing was also sent to Dr. Joseph Kirkbride, (USDA-ARS, U.S. National Arboretum, Floral and Nursery Plants Research Unit, Washington, DC) for comparison against his world record of legume nodulation.

Results and Discussion

All the species examined were found to be well nodulated. According to these records, nodulation is reported for the first time in five of the Papilionoid legume species (*Lathyrus alpestris* (Waldst. & Kit.) Kit., *Lathyrus pannonicus* (Jacq.) Garcke, *Medicago rhodopea* Velen., *Onobrychis alba* (Waldst. & Kit.) Desv.) and *Pisum sativum* L. var. *elatius* (Steven ex M. Bieb.) Alef.) found in Bulgaria (Table 1). Nodulating status of 111 legume species was checked as reported in the literature (Allen and Allen, 1981; Sprent, 2001) and also by Dr. Joseph Kirkbride and nodulation has been observed in 104 of these legumes species. No information is available about nodulating ability in 7 Bulgarian legume species in literature and it could neither be confirmed from field observations (Table 1). *Lotus uliginosus* Schkuhr is a lost plant of Bulgarian flora and its nodulating ability could not be established in the present study.

Nodulating status was examined in 83 species in Tribe Trifolieae comprising of 19 *Medicago* spp., 4 *Melilotus* spp., 55 *Trifolium* spp., and 5 *Trigonella* spp. The genus *Trifolium* is quite extensive and 67 (51 annual and 16 perennial) of the 237 *Trifolium* species have been reported in Bulgaria (Pederson et al., 1999). They were all nodulated. However, there is no report about nodulation in *Medicago rupestris* M. Bieb., *Trifolium latinum* Sebast and *Trigonella procumbens* (Bess) Reichenb. (Table 1). Tribe Trifolieae was followed by tribe Fabeae (Vicieae) consisted of 59 species containing 23 *Lathyrus* spp., 2 *Lens* spp., 3 *Pisum* spp. and 31 *Vicia* spp. They were all nodulated except *Lathyrus cynaneus* (Steven) K. Koch, *Lathyrus laxiflorus* (Desf.) Kuntze and *Lathyrus panicii* (Juris) Adamovic. Next in line were tribe Hedysareae (10 *Onobrychis* spp.), all of them nodulated and tribe Loteae (7 *Lotus* spp. and one *Securigera* sp.) which were all nodulated except *L. uliginosus*. Tribe Phaseoleae comprised of 4 species and tribe Genisteae 3 species all of them nodulating. Tribe Aeschynomeneae, Cicereae and Galegeae had one species each, all of them bearing root nodules (Fig. 2).

Legumes are nodulated by diverse group of bacteria collectively known as rhizobia: *Rhizobium, Bradyrhizobium, allorhizobium, Azorhizobium, Mesorhizobium,* and *Sinorhizobium* (Amarger, 2001; Sebbane *et al.*, 2006; Zakhia, 2004). They are adaptable to adverse environmental, climatic and soil conditions (Zahran, 1999, 2001). Rhizobia form nitrogen-fixing nodules in the leguminous plants. Fodder legumes are extensively cultivated as forage crops in Bulgaria. Most of the Bulgarian legumes studied comprised of either herbs or vines and could potentially be utilized for fodder production (Fig. 3). It is well known that legumes grown alone or in grass mix enhance forage production (Vasileva, 2006, 2009; Vasileva *et al*., 2005, 2011; Vasileva and Vasilev, 2012). Grass-legume mixtures are more sustainable and better overcome unfavorable conditions as compare to their pure cultivation (Vasileva and Vasileva, 2012). They are more productive than pure stands and each species contributes the productivity in varying degrees. Birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis vicifolia* Scop.), clovers (*Trifolium* spp.) and alfalfa (*Medicago* spp.) are valuable forage crops and suitable components of perennial mixtures. Cockfoot (*Datylis glomerata* L.) is a medium to long-term highly productive grass crop. It grows quickly in the spring, but due to the deeper root system grows intensively equally in the dry summer months and autumn and good combination for legume mixer. Cultivation of various grass-legumes mixtures has been tried for forage production in Bulgaria (Vasileva, 2006, 2009; Vasileva *et al.,* 2005, 2011; Vasileva and Vasilev, 2012). Understanding the mechanisms underlying the nutrient flows in ruminants fed on legume-based diets is an essential pre-requist for the achievement of high animal performance together with high efficiency and reduced environmental impact (Carlier *et al*., 2008). It is well known that the presence of the legumes in the sward encourages high level of forage intake by the grazing animal. It is essential that such information is transmitted to the farmers if legumes are to be used in an appropriate way, thereby improving the efficiency of pastures and forage production systems (Carlier *et al.,* 2008; De Vliegher and Carlier 2008). This work is a preliminary step towards exploration of efficient symbiotic rhizobia and their use in the management of grasslands and pastures by growing these legumes individually and in combination with in grasses for enhancing forage production in Bulgaria.

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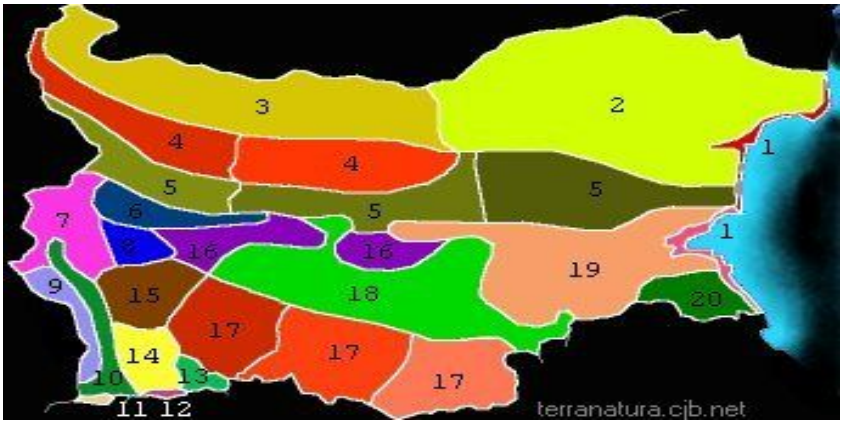

Fig. 1. Floristic regions of Bulgaria.

1. Black Sea Coast	2. Northeast Bulgaria	3. Danube plain	4. Balkan foothill region	5. Stara planina mountain
6. Sofia region	7. Znepole region	8. Vitosha region	9. West frontier mountains	10. Struma valley
11. Belasitsa mountain	12. Slavyanka mountain	13. Mesta valley	14. Pirin mountain	15. Rila mountain
16. Sredna Gora mountain	17. Rhodopes mountains	18. Tracian plain	19. Tundja hilly region	20. Strandja mountain

http://www.terranatura.hit.bg/index.htm?bgflora/bgflora.htm

Reference: Jordanov, D. (ed.). 1963-1989. Flora Republicae Popularis Bulgaricae. tomum I-IX

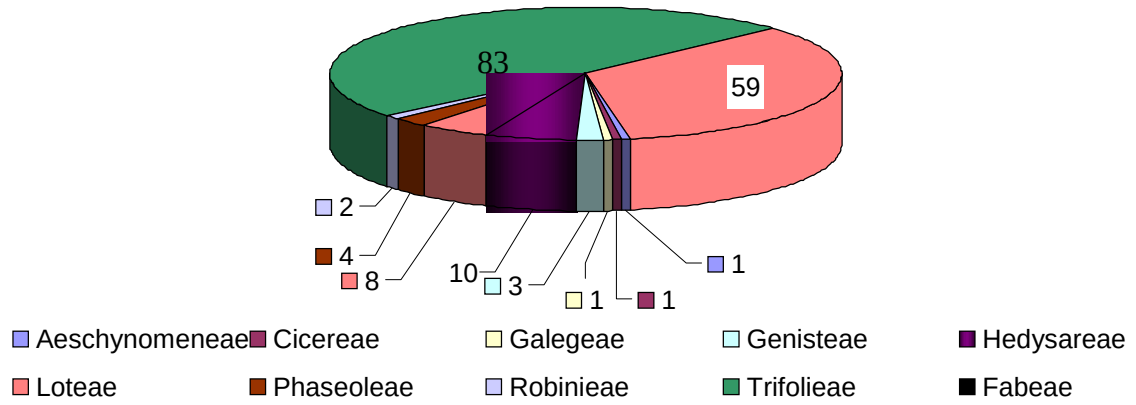

Fig. 2. Classification of legume species of Bulgaria into Papilionoid tribes studied for nodulation.

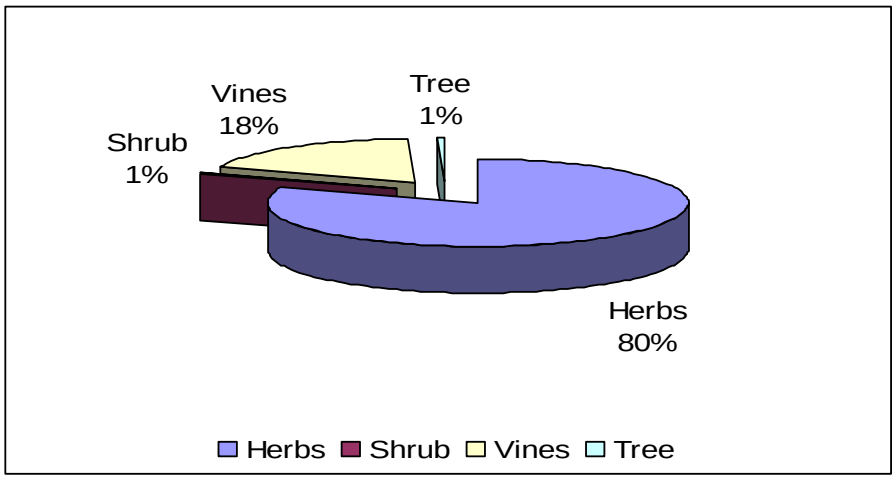

Fig. 3. Plant habit of Papilionoid legume species of Bulgaria studied for nodulation.

Table1. Nodulation in some of the Bulgarian legumes.

Species	Plant habit	Nodule		
		Observed	Reported	No Report
PAPILIONOIDEAE				
Aeschynomeneae				
Arachis hypogaea *L.*	H	+	+	
Cicereae				
Cicer arietinum *L.*	H	+	+	
Galegeae				
Galega officinalis *L.*	H	+	+	
Genisteae				
Lupinus albus *L.*	H		+	
Lupinus angustifolius *L.*	H		+	
Lupinus polyphyllus *Lindl.*	H		+	
Hedysareae				
Onobrychis aequidentata (Sm.) D'Urv.	H		+	
Onobrychis alba *(Waldst. & Kit.) Desv.*	H	+		*
Onobrychis alba *(Waldst. & Kit.) Desv. subsp.* calcarea *(Vandas) P.W. Ball*	H		+	
Onobrychis arenaria *(Kit.) DC*	H	+	+	
Onobrychis arenaria *(Kit.) DC. subsp*. lasiostachya *(Boiss.) Hayek.*	H		+	
Onobrychis caput-galli *(L.) Lam.*	H		+	
Onobrychis degenii Dörfl.	H		+	
Onobrychis gracilis Besser	H		+	
Onobrychis montana DC. subsp. *scardica* (Griseb.) P.W. Ball	H		+	
Onobrychis viciifolia Scop.	H	+	+	
Loteae				
Lotus aegaeus (Griseb.) Boiss.	H		+	
Lotus angustissimus L.	H		+	
Lotus corniculatus L.	H	+	+	
Lotus maritimus L.	H		+	
Lotus strictus Fisch. C.A. Mey.	H		+	
Lotus tenuis Waldst. & Kit. ex Willd.	H		+	
Lotus uliginosus Schkuhr	Lost plant of Bulgarian flora			
Securigera varia (L.) Lassen	H		+	
Phaseoleae				
Glycine max *(L.) Merr.*	H	+	+	
Phaseolus coccineus *L.*	V	+	+	
Phaseolus vulgaris *L.*	V	+	+	
Vigna unguiculata (L.) Walp.	V		+	
Robinieae				
Robinia hispida *L.*	S		+	
Robinia pseudacacia *L.*	T		+	
Trifolieae				
Medicago arabica (L.) Huds.	H	+	+	
Medicago carstiensis Jacq.	H		+	
Medicago coronata (L.) Bartal.	H		+	
Medicago disciformis DC.	H		+	
Medicago hispida Gaertn.	H		+	
Medicago lupulina L.	H	+	+	
Medicago marina L.	H		+	
Medicago medicaginoides (Retz.) E. Small	H	+	+	
Medicago minima (L.) Bartal.	H		+	
Medicago monspeliaca (L.) Trautv.	H		+	
Medicago orbicularis (L.) Bartal.	H	+	+	
Medicago polymorpha L.	H	+	+	
Medicago prostata Jacq.	H		+	
Medicago rhodopea Velen.	H	+		*
Medicago rigidula (L.) All.	H		+	
Medicago rupestris M. Bieb.	H			*
Medicago sativa *L.*	H	+	+	
Medicago sativa *L. subsp.* falcata *(L.) Arcang.*	H	+	+	
Medicago turbinata *(L.) All.*	H		+	

Melilotus albus Medik.	H	+	+	
Melilotus indicus (L.) All. var. *indicus*	H		+	
Melilotus officinalis (L.) Lam.	H	+	+	
Melilotus sulcatus Desf.	H		+	
Trifolium affine C. Presl	H		+	
Trifolium alpestre L.	H	+	+	
Trifolium angustifolium L.	H	+	+	
Trifolium arvense L.	H	+	+	
Trifolium aureum Pollich	H		+	
Trifolium badium Schreb.	H		+	
Trifolium bocconei Savi	H		+	
Trifolium bocconei Savi var. *tenuifolium* (Ten.) Griseb.	H		+	
Trifolium campestre Schreb.	H	+	+	
Trifolium cherleri L.	H		+	
Trifolium dalmaticum Vis.	H		+	
Trifolium diffusum Ehrh.	H		+	
Trifolium dubium Sibth.	H		+	
Trifolium echinatum M. Bieb.	H	+	+	
Trifolium fragiferum L.	H	+	+	
Trifolium globosum L.	H		+	
Trifolium glomeratum L.	H	+	+	
Trifolium grandiflorum Schreb.	H		+	
Trifolium hirtum All.	H		+	
Trifolium hybridum L.	H	+	+	
Trifolium incarnatum L.	H	+	+	
Trifolium lappaceum L.	H		+	
Trifolium latinum Sebast.	H			*
Trifolium leucanthum M. Bieb.	H		+	
Trifolium medium L.	H	+	+	
Trifolium michelianum Savi	H		+	
Trifolium michelianum Savi var. *balansae* (Boiss.) Azn.	H		+	
Trifolium micranthum Viv.	H		+	
Trifolium montanum L.	H	+	+	
Trifolium nigrescens Viv.	H		+	
Trifolium ochroleucon Huds.	H		+	
Trifolium pallescens Schreb.	H		+	
Trifolium pallidum Waldst. & Kit.	H		+	
Trifolium pannonicum Jacq.	H	+	+	
Trifolium patens Schreb.	H		+	
Trifolium pignantii Brong. & Bory	H		+	
Trifolium pratense L.	H	+	+	
Trifolium purpureum Loisel	H	+	+	
Trifolium repens L.	H	+	+	
Trifolium resupinatum L.	H	+	+	
Trifolium retusum L.	H		+	
Trifolium scabrum L.	H		+	
Trifolium sebastianii Savi	H		+	
Trifolium setiferum Boiss.	H		+	
Trifolium spadiceum L.	H		+	
Trifolium striactum L.	H	+	+	
Trifolium strictum L.	H		+	
Trifolium subterraneum L.	H	+	+	
Trifolium suffocatum L.	H		+	
Trifolium sylvaticum Gerard ex Loisel.	H		+	
Trifolium trichopterum Pančič	H		+	
Trifolium uniflorum L.	H		+	
Trifolium variegatum Nutt.	H		+	
Trifolium velenovsky Vandas	H		+	
Trifolium vesiculosum Savi.	H		+	
Trigonella caerulea (L.) Ser.	H		+	
Trigonella foenum-graecum L.	H		+	
Trigonella gladiata Steven ex M. Bieb.	H		+	
Trigonella procumbens (Bess) Reichenb.	H			*
Trigonella spicata Sm.	H		+	

Fabeae (Vicieae)				
Lathyrus alpestris (Waldst. & Kit.) Kit.	H	+		*
Lathyrus annuus L.	H	+	+	
Lathyrus aphaca L.	H	+	+	
*Lathyrus aureus (*Steven ex Fisch. & C.A. Mey.) Brandza	H		+	
Lathyrus cicera L.	H	+	+	
Lathyrus cynaneus (Steven) K. Koch	H			*
Lathyrus inconspicuus L.	H		+	
Lathyrus hierosolymitanus Boiss.	H		+	
Lathyrus hirsutus L.	H		+	
Lathyrus laxiflorus (Desf.) Kuntze	H			*
Lathyrus ochraceus Kitt.	H		+	
Lathyrus niger (L.) Bernh.	H	+	+	
Lathyrus nissolia L.	H		+	
Lathyrus palustris L.	H		+	
Lathyrus panicii (Juris) Adamovic	H			*
Lathyrus pannonicus (Jacq.) Garcke	H	+		*
Lathyrus pratensis L.	H	+	+	
Lathyrus sativus L.	H	+	+	
Lathyrus setifolius L.	H		+	
Lathyrus sphaericus Retz.	H	+	+	
Lathyrus tuberosus L.	H	+	+	
Lathyrus sylvestris L.	H		+	
Lathyrus vernus (L.) Bernh.	H		+	
Lens culinaris *Medik.*	H	+	+	
Lens nigricans *(M. Bieb) Godr.*	H	+	+	
Pisum sativum *L.*	V	+	+	
Pisum sativum L. var. *arvense* (L.) Poir.	V	+	+	
Pisum sativum L. var. *elatius* (Steven ex M. Bieb.) Alef.	V	+		*
Vicia abbreviata Fisch. ex Spreng.	V		+	
Vicia articulata Hornem.	V		+	
Vicia barbazitae Boiss.	V		+	
Vicia bithynica (L.) L.	V		+	
Vicia cassubica L.	V		+	
Vicia cracca L.	V		+	
Vicia cracca L. subsp. *incana* (Gouan) Rouy	H		+	
Vicia dumetorum L.	V		+	
Vicia ervilia (L.) Willd.	H		+	
Vicia faba L.	H	+	+	
Vicia grandiflora Scop.	V	+	+	
Vicia hirsuta (L.) Gray	V		+	
Vicia incisa M. Bieb.	V		+	
Vicia lathyroides L.	V		+	
Vicia melanops Sm.	V		+	
Vicia monantha Retz.	V		+	
Vicia onobrychioides L.	V		+	
Vicia pannonica Crantz	V	+	+	
Vicia peregrina L.	V		+	
Vicia pisiformis L.	V		+	
Vicia pubescens (DC.) Link	V		+	
Vicia sativa L.	V	+	+	
Vicia sativa L. var. *nigra* (L.) Ehrh.	V	+	+	
Vicia sepium L.	V		+	
Vicia serratifolia Jacq.	V		+	
Vicia sparsiflora Ten.	V		+	
Vicia pannonica Crantz subp. *striata* (M. Bieb.) Nyman	V		+	
Vicia tenuifolia Roth	V		+	
Vicia tetrasperma (L.) Schreb.	V	+	+	
Vicia villosa Roth	V	+	+	
Vicia villosa Roth subsp. *varia* (Host) Corb.	V	+	+	

Plant habit: Herb (H), shrub (S), vine (V), tree (T).
Nodule observed: Personal observation by Dr. VilianaVasileva about nodulation.
Nodulation report: Positive (+) or no (*) report about nodulation in literature as checked by Dr. Joseph Kirkbride, USDA-ARS, Washington, DC.

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