

Original Paper

Collaborative Exploration and Collection of Forage Grass Genetic Resources in Bulgaria in 2017

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Summary

A program for the exploration and collection of forage grass genetic resources was carried out in Bulgaria by a collaborative team from two Japanese institutes, Hokkaido Agricultural Research Center, NARO (HARC) and Institute of Livestock and Grassland Science, NARO (NILGS), and two Bulgarian institutes, Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) and the Institute of Plant Genetic Resources 'K. Malkov' (IPGR), from 19th to 30th July 2017. Bulgaria is rich in genetic resources for forage crops as it lies close to their genetic center of origin, the Mediterranean region, and has diverse climate conditions. The aim of this program was to collect wild materials of forage crops with a variety of genetic variations, especially *Dactylis* spp., due to their adaptability to severe environmental conditions including high or low temperatures, in flat and mountainous regions. We collected the seeds of forage grasses, mainly *Dactylis glomerata*, in the Balkan and Rodopi mountain areas. A total of 65 seed accessions of forage grass were collected at 54 sites.

KEY WORDS: *Dactylis*, forage grass, genetic vegetable, Bulgaria

Introduction

Since the stable production of self-supplying roughage is an important problem in Japanese livestock farming, the National Agriculture and Food Research Organization (NARO) has been breeding forage grasses for stable forage production and adaptability to Japanese climatic conditions. The main breeding objective is improvement of winter hardiness for cold regions and tolerance to high temperatures for temperate regions in Japan. We have developed new grass cultivars using genetic resources that were collected and introduced from foreign countries as breeding materials. Forage grass species are not native to Japan, so it is important to increase the genetic diversity of the breeding populations using genetic resources that originate from various environmental conditions.

Hokkaido Agricultural Research Center, NARO (HARC) and the Institute of Livestock and Grassland Science, NARO (NILGS) obtained an opportunity to collaborate with Bulgarian research institutes, the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) and the Institute of Plant Genetic Resources 'K. Malkov' (IPGR) for the collection of grass genetic resources in 2017. Bulgaria is located at around 43 °N which is almost the same as that of Hokkaido, while also being close to the genetic center of origin, the Mediterranean region, of forage species. The climate of Bulgaria is diverse, ranging from a west coast marine climate to a Mediterranean climate. The mountains ranges are more than 2,000 m above sea level and stretch from the central to southern regions of the country. The summer climate is hot and dry in the plain areas, and the winter climate is cold and snowy in the mountainous regions. Stock breeding has been conducted since the dawn of history in the pastures and meadows of the mountainous areas where there exist many different kinds of forage grasses and legume species under various climatic conditions. Genetic resources of forage legumes, such as *Trifolium* spp. and *Medicago* spp., were collected in Bulgaria by the collaborative expedition in 1995 (Kanbe and Gau 1996) and 2006 (Okumura *et al.* 2007). They reported diverse genetic resources, that were resistant to severe environmental conditions including low temperatures and acidic soils, and were generally persistent and existed from the flat to high mountainous regions of Bulgaria.

We collected grass genetic resources, focusing mainly on cocksfoot (*Dactylis glomerata* L.), in this collaborative expedition. Cocksfoot, which is one of the main grass species in cold and temperate regions in Japan for hay or silage production and grazing, has high tolerance to environmental conditions and has good regrowth after cutting. It is expected that the grass genetic resources growing under the severe climate conditions of Bulgaria have environmental tolerance to stresses such as high and low temperatures.

Methods

We explored around Troyan city, the Balkan Mountains and the northern area of Troyan based in RIMSA from 20th to 26th July and explored around the Rodopi Mountains from 28th to 30th July after visiting the IPGR on the 27th July in 2017 (Table 1, Fig. 1). We found pastures, meadows and natural grasslands along the roadside while moving by car or foot, and the panicles of the grasses were collected from more than five plants of each population at each site. We recorded basic geographical data including altitude, latitude, longitude by GPS receiver (GPSMAP62SJ, Garmin Ltd, Japan), and so on for registration of passport data information according to the form of International Board for Plant Genetic Resources (IBPGR).

The collected seeds were divided into two subsets: one for the Bulgarian institutes and another for the Japanese institute. After cleaning and quarantine tests at the IPGR, the seeds were transferred to HARC

Table 1. Itinerary of exploration of grass genetic resources in Bulgaria in 2017

Date	Places of visit	Activity
17-Jul	Mon	Move (Sapporo, Tokyo - Paris)
18-Jul	Tue	Move (Paris - Sofia - Troyan)
19-Jul	Wed	Troyan Meeting with RIMSA
20-Jul	Thu	Troyan Exploration around RIMSA
21-Jul	Fri	Troyan Exploration around Troyan
22-Jul	Sat	Lovech Exploration around Lovech
23-Jul	Sun	Cleaning of seed
24-Jul	Mon	Shipkovo Exploration around western part of Troyan
25-Jul	Tue	Pavlikeni Exploration around Pavlikeni based in Soybean Experimental Station Pavlikeni
26-Jul	Wed	Apriltsi Exploration around eastern part of Troyan
27-Jul	Thu	Plovdiv Move (Troyan - Sadovo), Visit to Institute of Plant Genetic Resources 'K. Malkov'
28-Jul	Fri	Plovdiv to Ardino Exploration from eastern to central part of Rodopi mountains
29-Jul	Sat	Ardino to Trigrad Exploration around central part of Rodopi mountains
30-Jul	Sun	Trigrad to Plovdiv Exploration from central to western part of Rodopi mountains
31-Jul	Mon	Sofia Move (Plovdiv - Sofia - Paris)
1-Aug	Tue	Move (Paris - Tokyo, Sapporo)

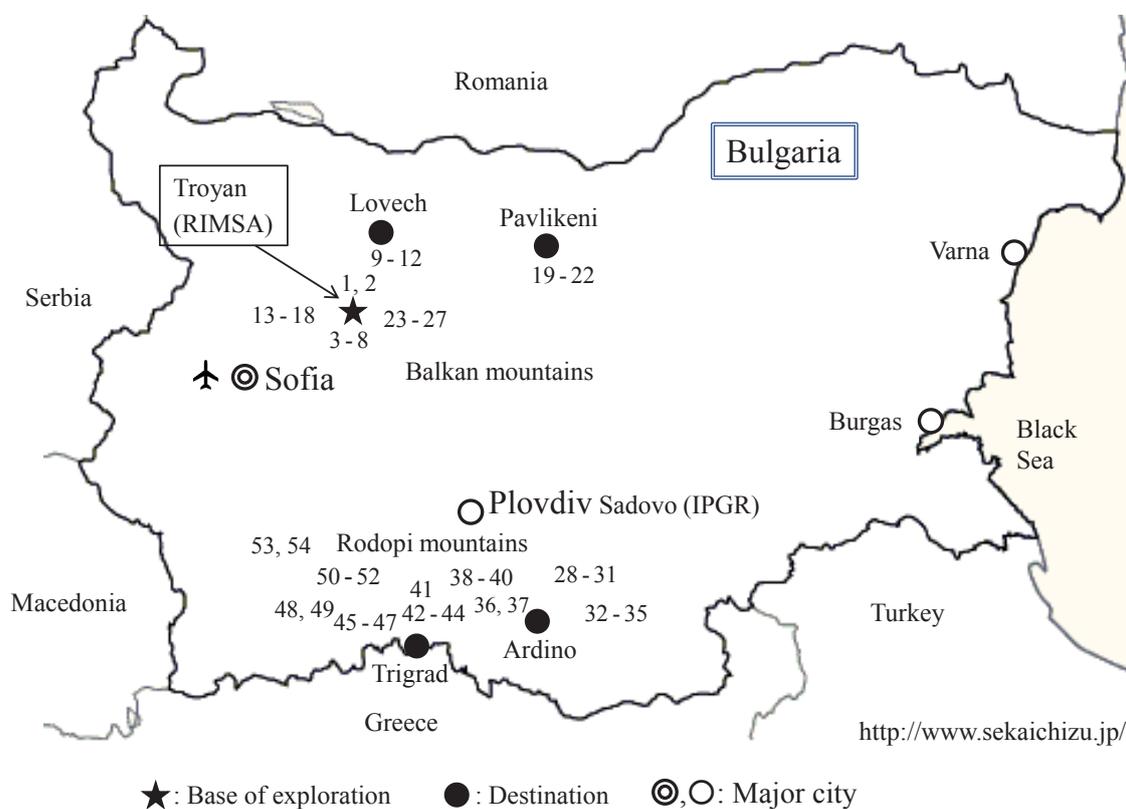


Fig. 1. Collection sites of grass genetic resources in Bulgaria on July 2017. Numbers in figure indicate collection sites.

in Sapporo, Hokkaido, through customs inspection in Japan using a standard material transfer agreement (SMTA) between HARC and IPGR.

Results

The itinerary and route of expedition are shown in Table 1 and Fig. 1. A total of 65 seed accessions of grasses, mainly cocksfoot, were collected at 54 sites in both the mountainous and plain areas of Bulgaria (Table 2). We collected 54, 4, 4, 2 and 1 accessions of cocksfoot, perennial ryegrass (*Lolium perenne*

L.), *Phleum* spp., and tall fescue (*Festuca arundinacea* Schreb.) and quack grass (*Agropyron repens*), respectively (Table 3). Most of the pastures and meadows that we investigated in this expedition consisted of native species and were not artificial grasslands, i.e. not seeded with bred cultivars.

A. Around Troyan

The grasslands near RIMSA and Troyan city were explored on 20th and 21st July on foot. Natural grasslands consisted of native species that were found in the forest (Col. No. 1; Photo 1). Warm season grasses such as *Chrysopogon* spp. dominated in those grasslands. A small population of cocksfoot with a small number of panicles grew under a tree (Col. No. 6-9; Photo 2). *Lotus corniculatus* grew in the pasture and *Trifolium medium* grew in the natural grassland.

B. Western and eastern part of Troyan

The grasslands in the mountainous area around the western and eastern regions of Troyan city were explored on the 24th and 26th July. The altitude of collection sites ranged from 419 m to 1,011 m. Natural grasslands, pasture and meadows consisted of native species that were found at roadsides and backyards (Photos 3 and 4). Individuals of cocksfoot were scattered in those grasslands in which warm season grasses and native species dominated. A small population of cocksfoot was found in the pasture at the top of mountain (altitude about 1,000 m) in which *Phalalis arundinacea* dominated (Col. No. 18; Photo 5). Timothy (*Phleum pratense* L.) was found in some grasslands and was collected at site 23 (Col. No. 28; Photo 6).

C. Around Lovech

The grasslands around Lovech city were explored on 22nd July. The altitude of the collection sites ranged from 360 m to 506 m. Sunflower and wheat were cultivated on the slope of hills. Natural grasslands and meadow consisted of native species including grasses, such as *Agropyron repens*, *Chrysopogon* spp. and *Andropogon* spp., and leguminous plant such as *Medicago falcata* that were found at roadsides and backyards (Photos 7 and 8). A population of cocksfoot was sampled from alongside a meadow of *Agropyron repens* (Col. No. 10; Photo 7). A large population of cocksfoot with plant heights of more than 120 cm was sampled from the backyard of farmer (Col. No. 14).

D. Around Pavlikeni

We explored around Pavlikeni city based in Soybean Experimental Station Pavlikeni on 25th July. The altitude of the collection sites ranged from 134 m to 419 m. Wheat was mainly cultivated in the field neighboring Pavlikeni city. The population of cocksfoot in the natural grassland adjacent to the wheat

Table 2. A summary of collected materials in Bulgaria

English name	Latin name	Accessions
Cocksfoot	<i>Dactylis glomerata</i> L.	54
Perennial ryegrass	<i>Lolium perenne</i> L.	4
Timothy and relatives	<i>Phleum pratense</i> L. and <i>Phleum</i> spp.	4
Tall fescue	<i>Festuca arundinacea</i> Schreb.	2
Quack grass	<i>Agropyron repens</i> (L.) P. Beauv.	1

field, with plants more than 120 cm in height, was sampled (Col. No. 21; Photo 9). Natural grasslands and meadows in this area consisted of native species including grasses, such as *Agropyron repens* and *Andropogon* spp., and leguminous plant such as *Medicago falcata*. A population of cocksfoot, in the natural grasslands by roadsides, with plant heights lower than 80 cm was sampled (Col. No. 25; Photo 10). Populations of perennial ryegrass, found outside of the natural grasslands, with plant heights below 30 cm were sampled (Col. No. 22 and 26; Photos 9 and 11).

E. Eastern part of Rodopi mountains

We visited to IPGR on 27th July and then explored around the eastern region of the Rodopi mountains on 28th July. The altitude of the collection sites ranged from 275 m to 970 m. A population of cocksfoot was sampled from under a tree in natural grasslands where *Agilops* spp. and *Festuca rubra* L. dominated (Col. No. 33). A small population of cocksfoot was also collected from under the shade of a broadleaf tree in the forest (Col. No. 35; Photo 12). There was a population of cocksfoot in the weeded land outside of the pasture, with panicles that were shorter than (Col. No. 37; Photo 13). A large numbers of samples of cocksfoot were collected at Perperikon, a historical site on a rocky hill (Col. No. 38; Photo 14). *Phleum* spp. was also collected at this site (Col. No. 39; Photo 15).

F. Central part of Rodopi mountains

We explored around the central region of the Rodopi mountains on the 29th July. The altitude of the collection sites ranged from 504 m to 1,539 m. A large number of cocksfoot plants were collected at the roadside to Devil Bridge which was constructed in the early 16th century (Col. No. 42; Photo 16). A small population of cocksfoot was collected in pasture and meadow in which tall oat grass called 'French ryegrass' in Bulgaria, (*Arrhenatherum elatius* (L.) J. & K. Presl), dominated (Col. No. 50; Photo 17). Populations of cocksfoot with immature panicles were collected from a pasture in which the tall oat grass dominated at an altitude above 1,400 m (Col. No. 49-51; Photo 18).

G. Western part of Rodopi mountains

We explored around western part of Rodopi mountains on 30th July. The altitude of the collection sites ranged from 265 m to 1,543 m. Populations of cocksfoot were found in the weeded land outside of meadows in which tall oat grass mainly dominated (Col. No. 53-56; Photo 19). Small populations of cocksfoot with immature panicles were collected from the natural grasslands at roadsides at altitudes of higher than 1,500 m (Col. No. 58 and 59). Large populations of cocksfoot were found in meadows that were mixed with timothy, perennial ryegrass, and tall oat grass (Col. No. 61; Photo 20). Small populations of cocksfoot were found growing under the tree at the foot of Rodopi mountains were collected (Col. No. 63 and 64; Photo 21). Two populations of perennial ryegrass were collected at sites 45 and 51 (Col. No. 54 and 62) and tall fescue was collected at site 50 (Col. No. 60; Photo 22).

Discussion

The seeds of cocksfoot collected at altitudes lower than 1,000 m were fully matured, whereas some of them collected at altitude above 1,000 m were immature. The collection time of cocksfoot was thus best in the flat to low mountain regions in comparison to the higher mountainous regions. Timothy was found in some collection sites, but its seeds were immature. The collection time of timothy had improved

several weeks later. Cocksfoot, which did not dominate in most of the collection sites, was found in every grassland, including pastures and meadows, of Bulgaria. Cocksfoot grew with various kinds of native species in the grassland that showed a wide diversity of vegetation. Cocksfoot was generally found under trees in natural grasslands and at the borders between forests and natural grasslands.

We collected various grass genetic resources grown under severe environmental conditions in both flat and mountainous regions. The average of daily minimum temperature in January in Troyan is -5.2 °C which is almost the same as that of Morioka, Japan (-5.6 °C). The temperature of collection site in mountainous region may be lower than that of Troyan. Accessions collected from mountainous region may have cold tolerance and will be used as breeding material for the improvement of winter hardiness traits in northern Japan. The average of daily maximum temperature in July in Plovdiv is 29.3 °C which is almost the same as that of Tokyo (29.2 °C). Accessions collected from the flat regions may have tolerance to higher temperatures and will be used as breeding material for the improvement of summer-stress tolerance traits in the warm regions of Japan.

We will start the evaluation of these genetic resources at HARC and NILGS by using them for breeding material for the improvement of environmental tolerances such as winter-cold-hardiness and summer-heat-stress tolerance. A recent study using simple sequence repeat (SSR) markers revealed that the natural populations of cocksfoot in Bulgaria showed high genetic diversity (Last *et al.* 2013). High genetic diversity was also found among natural populations of cocksfoot collected from areas adjacent to Bulgaria in Turkey (Tuna *et al.* 2003). We are also interested in the analysis of the genetic resources collected from Bulgaria by using molecular markers. It is known that diploid subspecies of *Dactylis* are distributed around Eastern Europe (Borrill 1978; Lumaret 1988), and may be included in the genetic resource collected in Bulgaria; we are planning to investigate the ploidy of the samples.

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References

- Borrill M (1978) Evolution and genetic resources in cocksfoot. *In: Annual Report of the Welsh Plant Breeding Station*. Aberystwyth, pp. 190-209.
- Kanbe M and Gau M (1996) Collaborative exploration for collecting forage crop germplasm in Bulgaria and Greece. *AREIPGR* 12: 65-91.

- Last L, Widmer F, Fjellstad W, Stoyanova S and Kölliker R (2013) Genetic diversity of natural orchardgrass (*Dactylis glomerata* L.) populations in three regions in Europe. BMC Genetics 14: 102. [https://doi.org/10.1186/1471-2156-14-102.].
- Lumaret R (1988) Cytology, genetics and evolution in the genus *Dactylis*. CRC Crit Rev in Plant Sci 7: 55-91.
- Okumura K, Hayashi T, Guteva Y and Mihovsky T (2007) Collaborative exploration and collection of forage legume genetic resources in the mountainous areas of Bulgaria in 2006. AREIPGR 23: 125-135.
- Tuna M, Khadka DK, Shrestha MK, Arumuganathan K and Goldhirsh AG (2004) Characterization of natural orchardgrass (*Dactylis glomerata* L.) populations of the Thrace Region of Turkey based on ploidy and DNA polymorphisms. Euphytica 135: 39-46.

ブルガリアにおけるイネ科牧草遺伝資源の 共同探索・収集，2017年

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和文摘要

2017年7月19日から30日にかけて、ブルガリアにおいて日本の2研究所、農研機構 北海道農業研究センターおよび畜産研究部門、ブルガリアの2研究所、山地家畜育種農業研究所と遺伝資源研究所との共同チームにより、イネ科牧草遺伝資源の探索収集が実施された。ブルガリアは、飼料作物遺伝資源の中心である地中海に近く、多様な気象条件であるため、飼料作物遺伝資源が豊富である。この計画の目的は、イネ科牧草、特に *Dactylis* 属の高温及び低温の厳しい環境条件への適応性について、平地と山地においてこれらを収集することにより、遺伝的変異を拡大することである。我々は、バルカン山脈とロドピ山脈において、オーチャードグラスを主として、イネ科牧草の種子を収集した。54地点において65点のイネ科牧草種子を収集した。

Table 3. List of collected materials by exploration of grass genetic resources in Bulgaria in July 2017

Site No.	Collection No.	Date	Genus & Species	Cultivar name	Location	Latitude N	Longitude E	Altitude (m)	Usage	Topography ¹⁾	Site ²⁾	Stoniness ³⁾	Soil texture ⁴⁾	Characteristics	Note (species observed in grassland and Photo No.)
1	1	20-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/1	Troyan	42°54'37"	24°41'31"	515	Pasture	5	2			Under the tree	<i>Agrostis</i> , <i>Andropogon</i> , <i>Lotus</i> ; Photo 1
2	2	20-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/2	Troyan	42°54'35"	24°41'48"	452	Pasture	5	2		1	Under the tree	
3	3	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/3	Krushak, Troyan	42°51'44"	24°41'35"	448	Pasture	2, 3	1		2		<i>Festuca arundinacea</i> , <i>Trisetum</i>
4	4	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/4	Krushak, Troyan	42°51'35"	24°41'36"	472	Pasture	5	2	1	3		<i>Trifolium repens</i> , <i>Agrostis</i>
4	5	21-Jul	<i>Festuca arundinacea</i>	COL/BG/2017/NARO&RIMSA/5	Krushak, Troyan	42°51'35"	24°41'36"	472	Pasture	6	2	1	3		<i>T. repens</i> , <i>Agrostis</i>
5	6	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/6	Krushak, Troyan	42°51'32"	24°41'42"	502	Natural grassland	6	2				
6	7	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/7	Krushak, Troyan	42°51'21"	24°41'43"	546	Natural grassland	6	2		3		<i>T. medium</i> , <i>Astragalus</i> spp.
7	8	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/8	Dundebsko, Troyan	42°51'15"	24°41'37"	557	Natural grassland	6	2		2, 3		<i>T. medium</i> , <i>Agrostis</i> , <i>Chrysopogon</i>
8	9	21-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/9	Bazikrachol, Troyan	42°50'56"	24°41'40"	694	Natural grassland	6	2		2, 3		<i>Agrostis</i> , <i>Chrysopogon</i> ; Photo 2
9	10	22-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/10	Kakrina, Lovech	43°05'50"	24°53'48"	434	Meadow	5	1		3		<i>Chrysopogon</i> , <i>Agropyron</i> ; Photo 7
9	11	22-Jul	<i>Agropyron repens</i>	COL/BG/2017/NARO&RIMSA/11	Kakrina, Lovech	43°05'50"	24°53'48"	434	Meadow	5	1		3		<i>Chrysopogon</i> , <i>Agropyron</i>
10	12	22-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/12	Prelom, Lovech	43°04'51"	24°51'10"	506	Pasture and meadow	5	1, 2		3		<i>Andropogon</i> , <i>Medicago falcata</i> , <i>Lotus</i> ; Photo 8
11	13	22-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/13	Krapets Dam, Lovech	43°03'57"	24°52'19"	456	Natural grassland	4	1		2	Near lake	<i>T. pratense</i> , <i>T. pannonicum</i>
12	14	22-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/14	Gorno Pavlikeni, Lovech	43°05'49"	24°47'42"	360	Natural grassland	4	1		3	Mr. Htistov's garden (backyard)	
13	15	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/15	Gorno Trape, Troyan	42°52'05"	24°38'33"	539	Pasture	3, 4	1,2			Road side	Photo 3
14	16	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/16	Gorno Trape, Troyan	42°52'41"	24°37'56"	557	Pasture	3	1		3	Road side	
15	17	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/17	Terziisko, Troyan	42°52'48"	24°36'01"	611	Pasture	5	1,2		3		<i>Agrostis</i>
16	18	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/18	Prohod Ribaritsa, Troyan	42°51'60"	24°32'21"	1,011	Pasture	5, 6	2, 3		2, 3	Partially immature panicle	<i>Phalaris arundinacea</i> , <i>Phleum pratense</i> , <i>Lotus</i> , <i>T. medium</i> ; Photo 5
17	19	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/19	Chiflik, Troyan	42°49'39"	24°33'55"	690	Natural grassland	6	2		2, 3		<i>Agrostis</i> , <i>T. medium</i>
18	20	24-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/20	Beli Osm, Troyan	42°51'31"	24°39'15"	516	Natural grassland	4, 6	1, 2		3		<i>T. campestre</i> , <i>Panicum</i>
19	21	25-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/21	Letishteto, Pavlikeni	43°15'40"	25°18'35"	169	Natural grassland	3, 4	1		2	Road side, wheat field	Photo 9
19	22	25-Jul	<i>Lolium perenne</i>	COL/BG/2017/NARO&RIMSA/22	Letishteto, Pavlikeni	43°15'40"	25°18'35"	169	Natural grassland	4	1		2	Road side, wheat field	Photo 9
20	23	25-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/23	Pchelina, Pavlikeni	43°16'04"	25°17'37"	134	Meadow	4	1, 2		2		
21	24	25-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/24	Vishovgrad, Pavlikeni	43°09'34"	25°17'56"	318	Pasture and meadow	4,5	1, 2				<i>Agropyron</i>
22	25	25-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/25	Dobromipka, Sevlievo	43°04'11"	25°17'12"	419	Natural grassland	4, 5	1		2		<i>Andropogon</i> , <i>Medicago</i> ; Photo 10
22	26	25-Jul	<i>Lolium perenne</i>	COL/BG/2017/NARO&RIMSA/26	Dobromipka, Sevlievo	43°04'11"	25°17'12"	419	Natural grassland	5	1		2		<i>Andropogon</i> , <i>Medicago</i> ; Photo 11
23	27	26-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/27	Drashkova Polyana, Apriltsi	42°53'48"	24°49'19"	448	Pasture and meadow	4, 5, 6	1,2		2		<i>F. arundinacea</i> , <i>P. pratense</i>
23	28	26-Jul	<i>Phleum pratense</i>	COL/BG/2017/NARO&RIMSA/28	Drashkova Polyana, Apriltsi	42°53'48"	24°49'19"	448	Pasture and meadow	5	1		2		<i>F. arundinacea</i> ; Photo 6
24	29	26-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/29	Velchevo, Apriltsi	42°53'15"	24°51'02"	419	Meadow	4, 6	1, 2		2		<i>Phalaris</i> spp., <i>P. pratense</i> ; Photo 4
25	30	26-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/30	Apriltsi	42°51'48"	24°52'01"	455	Meadow	6	2		2		<i>P. pratense</i>

Table 3. (Continued).

Site No.	Collection No.	Date	Genus & Species	Cultivar name	Location	Latitude N	Longitude E	Altitude (m)	Usage	Topography ¹⁾	Site ²⁾	Stoniness ³⁾	Soil texture ⁴⁾	Characteristics	Note (species observed in grassland and Photo No.)
26	31	26-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/31	Ostrets, Apriltsi	42°50'14"	24°57'19"	632	Natural grassland	5, 6	2		1	Road side, Pine forest	
27	32	26-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/32	Oreshak - Troyan	42°53'29"	24°45'22"	444	Pasture	5, 6	1	1	2	Road side	<i>T. hybridum</i> , <i>T. pratense</i> , <i>T. medium</i> , <i>Phalaris</i> spp.
28	33	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/33	Asenovgrad	41°59'15"	24°54'59"	275	Natural grassland	4, 5	1, 2		2	Under the tree	<i>Aegilops</i> , <i>Festuca rubra</i>
29	34	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/34	Novakovo, Asenovgrad	41°53'46"	25°03'28"	449	Natural grassland	5	2		2		
30	35	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/35	Nochevo, Chernochene	41°52'16"	25°09'04"	747	Natural grassland	6	2		2	Under the tree, Forest	Photo 12
31	36	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/36	Panichkovo, Chernochene	41°51'19"	25°10'22"	725	Pasture	6	2		3	Near tobacco field	<i>P. pratense</i>
32	37	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/37	Chernochene	41°46'20"	25°21'10"	461	Pasture	5	2		2	Small panicle, grazing, dry area	Weed; Photo 13
33	38	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/38	Perperikon, Kardzhali	41°43'01"	25°28'10"	358	Natural grassland	3	3	4	3	Road side	Photo 14
33	39	28-Jul	<i>Phleum</i> spp.	COL/BG/2017/NARO&RIMSA/39	Perperikon, Kardzhali	41°43'01"	25°28'10"	358	Natural grassland	3	3	4	3	Road side	Photo 15
34	40	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/40	Chubrika, Ardino	41°35'49"	25°13'32"	717	Natural grassland	6	3		2	Felling areas	<i>Agrostis</i>
35	41	28-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/41	Sinchets, Ardino	41°34'21"	25°10'41"	970	Natural grassland	6	3			Road side, under the tree	
36	42	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/42	Devil's Bridge, Ardino	41°37'38"	25°07'19"	504	Natural grassland	6	2			Road side to Devil bridge	Photo 16
37	43	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/43	Ardino	41°34'21"	25°06'40"	663	Natural grassland	6	2		2	Small population	
37	44	29-Jul	<i>Phleum</i> spp.	COL/BG/2017/NARO&RIMSA/44	Ardino	41°34'21"	25°06'40"	663	Natural grassland	6	2		1, 2	Small population	
38	45	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/45	Vehtino, Madan	41°32'58"	25°00'11"	576	Meadow	6	2		1, 2		<i>Arrhenatherum elatius</i>
39	46	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/46	Leshtak, Madan	41°32'16"	24°55'50"	650	Meadow	6	2		1, 2		<i>Chrysopogon</i>
40	47	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/47	Taran, Smolyan	41°33'52"	24°51'24"	746	Natural grassland	6	2		2	Near Phaseolus and corn field (small)	<i>A. elatius</i>
40	48	29-Jul	<i>Phleum</i> spp.	COL/BG/2017/NARO&RIMSA/48	Taran, Smolyan	41°33'52"	24°51'24"	746	Natural grassland	6	2		2	Immature panicle	<i>A. elatius</i>
41	49	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/49	Smolyan	41°37'10"	24°39'45"	1,495	Pasture	6	2		1	Immature panicle	<i>P. pratense</i>
42	50	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/50	Stoikite, Smolyan	41°38'40"	24°38'47"	1,539	Pasture	6	3			Immature panicle	<i>P. pratense</i> , <i>Phalaris</i> spp, <i>T. montanum</i> ; Photo 17
43	51	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/51	Stoikite, Smolyan	41°38'43"	24°38'05"	1,438	Pasture and meadow	6	2				<i>A. elatius</i> , <i>T. alpinum</i> ; Photo 18
44	52	29-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/52	Grohotno, Devin	41°42'31"	24°24'46"	737	Pasture	6	1, 2		1	Road side, arid	
45	53	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/53	Borino	41°40'33"	24°20'08"	1,049	Meadow	6	2	2	1	Road side	
45	54	30-Jul	<i>Lolium perenne</i>	COL/BG/2017/NARO&RIMSA/54	Borino	41°40'33"	24°20'08"	1,049	Meadow	6	2	2	1	Road side	
46	55	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/55	Borino	41°40'39"	24°19'24"	1,118	Meadow	5, 6	2		2	Good meadow	<i>A. elatius</i> , <i>P. pratense</i>
47	56	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/56	Borino	41°40'09"	24°15'54"	1,176	Meadow	5	2			After cutting	Photo 19
48	57	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/57	Dospat	41°39'09"	24°13'14"	1265,	Meadow	6	2			Road side, Pumpkin field	<i>P. pratense</i> , weed
49	58	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/58	Shiroka Polyana Dam, Dospat	41°46'23"	24°08'44"	1,543	Natural grassland	6	3	3		Near wet land, immature	
50	59	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/59	Beglika Dam, Dospat	41°49'42"	24°07'43"	1,511	Natural grassland	5	1			Road side	
50	60	30-Jul	<i>Festuca arundinacea</i>	COL/BG/2017/NARO&RIMSA/60	Beglika Dam, Dospat	41°49'42"	24°07'43"	1,511	Natural grassland	5	1			Large population	Photo 22

Table 3. (Continued).

Site No.	Collection No.	Date	Genus & Species	Cultivar name	Location	Latitude N	Longitude E	Altitude (m)	Usage	Topography ¹⁾	Site ²⁾	Stoniness ³⁾	Soil texture ⁴⁾	Characteristics	Note (species observed in grassland and Photo No.)
51	61	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/61	Batak	41°55'39"	24°11'08"	1,233	Meadow	4, 6	1, 2			Large population	<i>A. elatius</i> , <i>P. pratense</i> , <i>Lolium perenne</i> , <i>T. repens</i> ; Photo 20
51	62	30-Jul	<i>Lolium perenne</i>	COL/BG/2017/NARO&RIMSA/62	Batak	41°55'39"	24°11'08"	1,233	Meadow	4, 6	1, 2			Large population	<i>A. elatius</i> , <i>P. pratense</i> , <i>Dactylis glomerata</i> , <i>T. repens</i>
52	63	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/63	Peshtera	42°01'35"	24°14'19"	470	Natural grassland	6	2		3	Road side, arid	Photo 21
53	64	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/64	Peshtera - Byaga, Bratsigovo	42°03'10"	24°20'20"	321	Natural grassland	5	1		1	Under the tree, very hot	
54	65	30-Jul	<i>Dactylis glomerata</i>	COL/BG/2017/NARO&RIMSA/65	Byaga - Ispelihovo, Bratsigovo	42°04'04"	24°23'17"	265	Natural grassland	5	2		3	Hot and dry	Wild berry, <i>Eragrostis</i>

¹⁾ Topography: 1 Swamp, 2 Food plain, 3 Plain level, 4 Undulating, 5 Hilly, 6 Mountainous, 7 Others

²⁾ Site: 1 Level, 2 Slope, 3 Summit, 4 Depression

³⁾ Stoniness: 1 None, 2 Low, 3 Medium, 4 rocky

⁴⁾ Soil texture: 1 Sand, 2 Loam, 3 Clay, 4 Silt, 5 Highly organic



Photo 1. Collection site of cocksfoot in pasture near RIMSA in Troyan city (Site 1, Col. No. 1, N 42°54'37", E 24°41'31", altitude 515 m)



Photo 2. Collection site of cocksfoot in natural grassland in mountainous area of Troyan city (Site 8, Col. No. 9, N 42°50'56", E 24°41'40", altitude 694 m)



Photo 3. Collection site of cocksfoot in pasture in the western area of Troyan (Site 13, Col. No. 15, N 42°52'05", E 24°38'33", altitude 539 m)



Photo 4. Collection site of cocksfoot in natural grassland in the eastern area of Troyan (Site 24, Col. No. 29, N 42°53'15", E 24°51'02", altitude 419 m)



Photo 5. Collection site of cocksfoot in pasture in the western area of Troyan (Site 16, Col. No. 18, N 42°51'60", E 24°32'21", altitude 1,011 m)



Photo 6. Collection site of timothy in natural grassland in the eastern area of Troyan (Site 23, Col. No. 28, N 42°53'48", E 24°49'19", altitude 448 m)



Photo 7. Collection site of cocksfoot in meadow around Lovech
(Site 9, Col. No. 10, N 43°05'50", E 24°53'48", altitude 434 m)



Photo 8. Collection site of cocksfoot in meadow around Lovech
(Site 10, Col. No. 12, N 43°04'51", E 24°51'10", altitude 506 m)



Photo 9. Collection site of cocksfoot and perennial ryegrass in natural grassland at Pavlikeni city
(Site 19, Col. No. 21 and 22, N 43°15'10", E 25°18'35", altitude 169 m)



Photo 10. Collection site of cocksfoot in natural grassland around Pavlikeni
(Site 22, Col. No. 25, N 43°04'11", E 25°17'12", altitude 419 m)



Photo 11. Collection site of perennial ryegrass in natural grassland around Pavlikeni
(Site 22, Col. No. 26, N 43°04'11", E 25°17'12", altitude 419 m)



Photo 12. Collection site of cocksfoot in the forest in the eastern part of the Rodopi mountains
(Site 30, Col. No. 35, N 41°52'16", E 25°09'40", altitude 747 m)



Photo 13. Collection site of cocksfoot in pasture in the eastern part of the Rodopi mountains (Site 32, Col. No. 37, N 41°46'20", E 25°21'10", altitude 461 m)



Photo 14. Collection site of cocksfoot at Perperikon in the eastern part of the Rodopi mountains (Site 33, Col. No. 38, N 41°43'01", E 25°28'10", altitude 358 m)



Photo 15. Collection site of *Phleum* spp. on the roadside of Perperikon in the eastern part of the Rodopi mountains (Site 33, Col. No. 39, N 41°43'01", E 25°28'10", altitude 358 m)



Photo 16. Collection site of cocksfoot on the roadside to Devil Bridge in the central part of the Rodopi mountains (Site 36, Col. No. 42, N 41°37'38", E 25°07'19", altitude 504 m)



Photo 17. Collection site of cocksfoot in pasture in the central part of the Rodopi mountains (Site 42, Col. No. 50, N 41°38'40", E 24°38'47", altitude 1,539 m)



Photo 18. Collection site of cocksfoot in pasture and meadow in the central part of the Rodopi mountains (Site 43, Col. No. 51, N 41°38'43", E 24°38'05", altitude 1,438 m)



Photo 19. Collection site of cocksfoot in meadow in the western part of the Rodopi mountains (Site 47, Col. No. 56, N 41°40'09", E 24°15'54", altitude 1,176 m)



Photo 20. Collection site of cocksfoot and perennial ryegrass in meadow in the western part of the Rodopi mountains (Site 51, Col. No. 61 and 62, N 41°55'39", E 24°11'08", altitude 1,233 m)



Photo 21. Collection site of cocksfoot on the roadside in the western part of the Rodopi mountains (Site 52, Col. No. 63, N 42°01'35", E 24°14'19", altitude 470 m)



Photo 22. Collection site of tall fescue in natural grassland in the western part of the Rodopi mountains (Site 50, Col. No. 60, N 41°49'42", E 24°07'43", altitude 1,511 m)