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Collaborative Exploration and Collection of Forage Legume Genetic Resources in the Mountainous Areas of Bulgaria in 2006.

Kenji OKUMURA[1)], Taku HAYASHI[2)], Yana GUTEVA[3)] and Tsvetoslay MIHOVSKY[4)]

1)*National Agricultural Research Center for Hokkaido Region*, Hitsujigaoka 1, Toyohira, Sapporo, Hokkaido 062-8555, Japan
2)*Konsen Agricultural Experiment Station*, Asahigaoka 7, Nakashibetsu, Hokkaido, 086-1135, Japan
3)*Research Institute of Plant Genetic Resources*, Bulgaria, 2 Drouzba Str., 4122 Sadovo, Plovdiv, Bulgaria
4)*Research Institute of Mountain Stockbreeding and Agriculture*, 281 Vasil Levski Str., 5600 Troyan, Bulgaria

Summary

A program for the exploration and collection of forage legume genetic resources was carried out in Bulgaria by a collaborative team from two Japanese institutes, the National Agricultural Research Center for Hokkaido Region (NARCH) and the Konsen Agricultural Experiment Station (KAES), and two Bulgarian institutes, the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) and the Research Institute of Plant Genetic Resources (RIPGR), from 21 July to 2 August 2006. Bulgaria is rich in the genetic resources of forage crops since it lies close to their genetic center, the Mediterranean region, and has diverse climate conditions. The aim of this program was to increase the genetic variation of forage crops, especially *Trifolium* species, due to their adaptability to severe environmental conditions including low temperature and acidic soil, by collecting them in mountainous regions. We collected the seeds of forage species, mainly *Trifolium* species, in the Balkan, Strandza, Sakar, Rodopi, and Rila-Pirin mountain areas. A total of 148 seed accessions of forage legume and grass species were collected at 64 sites. In the accessions, 52 of *T. pratense* and 53 of *T. repens* were included.

Introduction

Hokkaido is located in the northernmost part of Japan, extending from approximately 41 to 45 degrees latitude north. In comparison with the main island of Japan, the climate in Hokkaido is dry and cool in summer and cold and snowy in winter. Its cool climate and vast land area have made dairy farming one of Hokkaido's most important industries. More than 80% of Japan's pasture area is in Hokkaido, with the total amount of grassland in Japan being 0.64 million ha

while that of Hokkaido is 0.53 million ha. For this reason we have concentrated the breeding programs of forage legumes and grasses in this area.

Red clover is one of the most important forage legume species in Hokkaido since it was introduced more than one hundred years ago from the United States and European countries. We have developed new cultivars from these introduced cultivars and ecotypes derived from them. This means Japan has no native population or relatives of red clover. Thus, an effort is needed to introduce diverse genetic resources through international collaboration in order to further improve of useful characters.

We have currently been offered the opportunity to collaborate with a Bulgarian research institute, the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA). Bulgaria is located at a latitude of between 41 and 44 degrees, which is almost the same as that of Hokkaido. The climate of Bulgaria is diverse, ranging from continental to Mediterranean zones. One third of its land is mountainous, and stockbreeding has been practiced in the pastures of these mountainous areas since the prehistoric era. In these pastures there exist many kinds of forage grass and legume species, especially *Trifolium* species.

The aim of this exploration in Bulgaria was to collect genetic resources of *Trifolium* and other forage species which are rich in species and genetic diversity. In the last exploration we had tried to collect seeds of forage legumes in flat to low mountain regions of northeastern Bulgaria, but the season was a little earlier than fully matured[1)]. In this program we focused our collection efforts especially on mountainous areas because in such areas we expected to find materials which are resistant to severe environmental conditions including low temperature and acidic soil, and have strong persistence.

Methods

We collected seeds of forage species in the Balkan, Strandza, Sakar, Rodopi, and Rila-Pirin mountains from July 21 to August 2 in 2006 (Figs. 1, 2). The collection season was determined based on information provided by the Bulgarian institutes regarding when the mature seeds of *T. pratense* and *T. repens* could be collected in the central Balkan mountains. During this exploration we searched the collection sites based on information from the RIMSA and RIPGR or from interviews with the inhabitants of these mountainous areas.

We gathered the mature seeds of forage legumes as well as grasses from large numbers of plants throughout the entire area of each site. In addition, we gathered basic geographical data including altitude, latitude, longitude, and so on for registration of passport data information.

The collected seeds were divided into two subsets: one for the Bulgarian institutes and another for the Japanese Genebank. After cleaning and quarantine tests at the RIPGR, the seeds were sent to NARCH in Sapporo, Hokkaido, and quarantined again at the Sapporo Branch of the Yokohama Plant Protection Station. We will start a field evaluation at both NARCH and KAES and seed multiplication at NARCH in 2007.

Results

We collected 148 seed accessions of forage legume and grass species at 64 sites in mountainous areas in Bulgaria (Tables 1, 2). The accessions included at least 20 species,

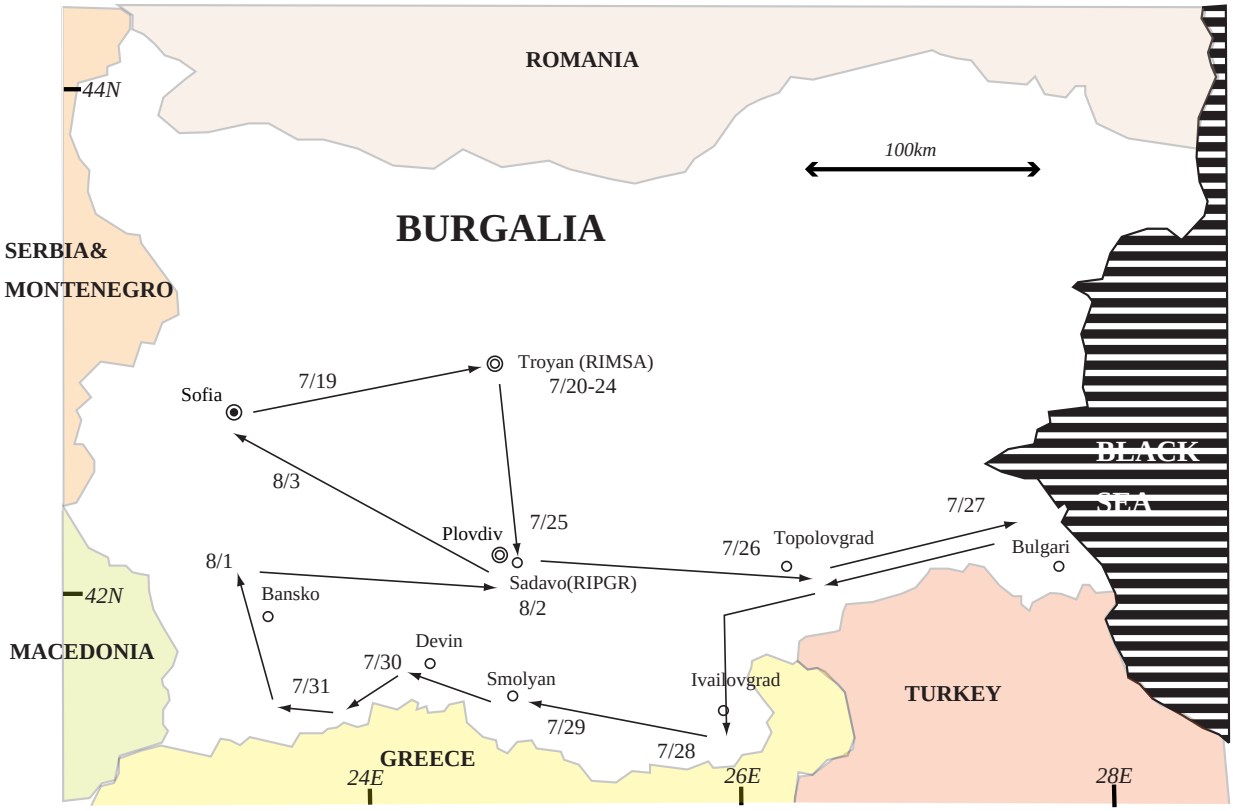

Fig. 1. Exploration route and schedule in Bulgaria in 2006

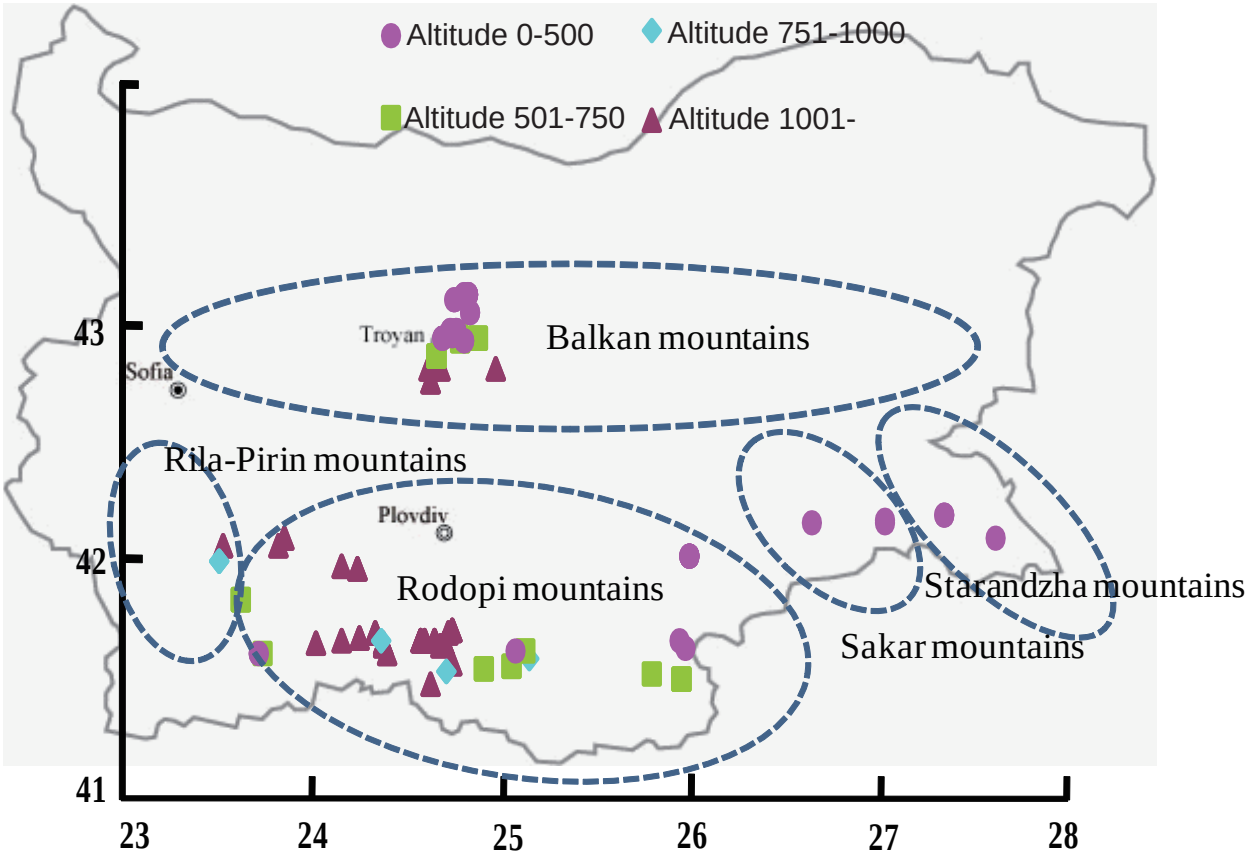

Fig. 2. Collection sites and their geography in Bulgaria in 2006

including red clover (*T. pratense*), white clover (*T. repens*), and other *Trifolium* species.

The main grass species were *Phleum pratense*, *Lolium perenne*, *Festuca pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata*, and *Festuca rubra*. We collected *Arrhenatherum elatius* which is called "French ryegrass" by Bulgarians and has not been cultivated in Japan. We will evaluate the performance of this species in Hokkaido because it shows strong persistence and productivity in Bulgaria. Regarding legumes except *Trifolium*, we collected the seeds of *Onobrychis viciifolia*, *Medicago polymorpha*, *M. minima*, and *Lotus corniculatus*. *Lotus corniculatus* dominates and is an important forage legume in Balkan mountain areas. We also found two interesting species, *Galega officinalis* and *Astragalus cicer*, in these areas, but they did not mature.

In the mountainous areas we examined, grasslands are used mainly as pastures where forage is harvested for hay production at first cutting, then grazed. Most of meadows and pastures consist of native species without artificial seeding with modern cultivars. Scythes are used for cutting, and gathering hay is also carried out manually. Basic fertilizer has not been applied to the pastures in these areas, and the pH of the soil was quite low[2)]. Thus, their management and environmental conditions are quite different from those in Hokkaido.

The basic features of these areas are as follows.

a. Balkan mountains

The altitude of this area ranges from around 500 m near Troyan to more than 1,000 m in the south. The growing stage of red and white clovers varies from the full-flowered to mature seed stages. We could collect the seeds of both species in most of the sites. *T. medium* dominated over *T. pratense* on the pastures' slopes and on the road sides at some sites. We found *G. officinalis* and *A. cicer* only in this area.

b. Sakar and Strandzha mountains

The Strandzha area has a relative low altitude of 200-300 m and is affected by the Mediterranean climate. In late July most forage plants die because of the hot and dry conditions. Thus it was difficult to find seeds and identify their species. In contrast, Site 0623 in the Sakar area has a slightly higher altitude and a milder climate than those of the Starandzha area. Here we collected white clover and annual *Medicago* species as well as grass species.

c. Rodopi mountains

The Rodopi mountains are huge areas. The eastern side of this area is affected by the Mediterranean climate. These lands were too dry to collect red clover seeds in midsummer. As we traveled west the area gained altitude. In the higher altitude area near Smolyan the growth stage of *T. prantese* was in full flower, allowing us to more easily gather enough seeds in contrast to the lower areas of the Western Rodopi. We found many species of *Trifolium*. We collected the seeds of *T. michelianum* only at one site, Site 0652, near Yagodina. This species is annual and shows strong winter-hardiness and spring growth.

d. Pirin and Rila mountains

We did not have a chance to explore the higher altitudes of these mountains, but did collect specimens at the base of these mountainous areas, at one site in the Pirin mountains and two sites in the Rila mountains.

Conclusion

The genus *Trifolium* is comprised of 237 species, including many important forage legumes[3]. Bulgaria is close to the Mediterranean Sea which is a center of this genus, and harbors 60 of its species [4]. Fourteen species, 23 % of the genus' total number, are perennial. In the present study we focused on the species *T. pratense* and *T. repens* which are perennial species, and collected 52 and 53 seed accessions, respectively. We collected 10 more species, 6 of which are perennial. We could not collect the seeds of some perennial species including *T. badium* which is tolerant to water logging. In addition there are many annual species in Bulgaria. We succeeded in collecting *T. michelianum* which is a winter annual and has the potential to be used as a winter cover crop in northern Japan.

In light of the native diversity of climate and ecological conditions, the populations of each collection site could have specific useful characteristics such as winter hardiness, acidic soil tolerance, and so on. We will begin a field evaluation program in 2007 in Hokkaido. In 2006 we also created a MOU between RIMSA and NARCH for "the improvement of environmental adaptability in forage crops by exchange of cultivars and mutual evaluation." Continuous collaboration with the Bulgarian institutes concerning the continued collection of genetic resources, their evaluation, information exchanges, and publication of the results will be needed for the further genetic improvement of forage crops.

Acknowledgements

This exploration and collection program in Bulgaria was carried out with the collaboration of two Bulgarian institutes: the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) and the Research Institute of Plant Genetic Resources (RIPGR). We would like to express our sincere appreciation to their two Directors, Dr. Martin Todorov, RIMSA, and Dr. Lilia I. Krasteva, RIPGR, for arranging the collaboration. Drs. Makoto Kawase and Kazuto Shirata, and Mr. Keisuke Tanaka were also helpful in arranging and supporting the program. Dr. Sika D. Stoyanova, Director of the National Genebank, also assisted in the seed quarantine and the procedure for material transfer. We wish to express our thanks to them and to other members involved in the program at RIMSA and RIPGRI. This project was funded by the Genebank Project of the National Institute of Agrobiological Science, Japan.

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和文摘要

ブルガリアでは有史以前から牧畜が営まれ，多種多様な牧草種が利用されてきている．また標高 1500 mを超える山間地でも牧草が利用されており，多様性に富んだ牧草遺伝資源を有するとされている．そこで，（独）農業生物資源研究所のジーンバンク事業の一環として，不良環境耐性等に優れる遺伝資源の集積を目的に，山岳地帯を中心にマメ科牧草，とくにクローバ類を中心に探索収集を実施した．

探索収集は 2006 年 7 月 21 日から 8 月 2 日まで，ブルガリアの植物遺伝資源研究所 (RIPGR) ならびに山地家畜育種・農業研究所 (RIMSA) の両機関と共同で探索収集を実施した．収集地域は大きく分けて，標高 500 ～ 800m 程度の中標高で気候が穏やかなバルカン山脈中央部，地中海性気候で夏期には高温干ばつとなるサカールおよびストランジャ山地など南東部，ならびに標高 1000m を超える高標高のロドピおよびピリン・リラ山地などトルコ・ギリシア国境に近い南部の 4 地域である．収集地は草地を対象として，地点毎に可能な限り広い面積から種子を採集した．また，緯度，経度，標高，植生，開花登熟のステージ，利用法などの情報を合わせて記録した．今回の収集時期は，南東部では夏期の干ばつで既に多くの牧草は枯れ上がってやや遅く，一方，南部高標高地では開花盛期でやや早すぎたものの，多くの地点で採種が実施できた．収集場所の多くは現在も利用されている採草地や放牧地および耕作放棄地であり，全て造成播種を行っていない自生の草地であった．収集点数は，マメ科牧草ではアカクローバ 52 点，シロクローバ 53 点，その他の *Trifolium* 属を含むマメ科牧草 14 種 21 点，（未同定種を除く），イネ科牧草ではペレニアルライグラス 10 点，オーチャードグラスやフェスク類など計 8 点であった．ブルガリアの土壌の特徴は pH が 5 以下の強酸性で生産性が低く，緯度は北海道と同程度であるが標高や気象条件は変異に富み，多様なエコタイプを形成している可能性がある．そこで，今後これらの収集材料を活用するために，北海道における特性評価試験を 2007 年より開始する．

Table 1. The list of accessions and species

Species	No. accessions	Species	No. accessions
***Trifolium* species**		**Forage legume and their relatives**	
Trifolium pratense L.	52	*Onobrychis viciifolia* Scop.	1
Trifolium repens L.	53	*Medicago polymorpha* L.	1
Trifolium hybridum L.	1	*Medicago minima* (L.) Bart.	1
Trifolium medium L.	3	*Lotus corniculatus* L.	2
Trifolium montana L.	1		
Trifolium incarnatum L.	1	**Forage grass species**	
Trifolium heldreichianum Hausskn.	2	*Lolium perenne* L.	10
Trifolium alpestre L.	2	*Festuca pratensis* Huds.	2
Trifolium spadiceum L.	1	*Arrhenatherum elatius* (L.) Beauv.	2
Trifolium aureum Poll.	1	*Dactyris glomerata* L.	4
Trifolium pannonicum Jacq.	3		
Trifolium michelianum Savi	1		
Trifolium spp.	3		
Number of *Trifolium* accesssions	124	Total of all accessions	148

Table 2. List of collection site and species

Collection site number	Date	Species*	Usage	Near Town/Village	Area	Altitude (m)	Latitude	Longitude
0601	7/21/2006	1	meadow	Gorno Pavlikene	Balkan mountain range	388	N 43 ° 05 ′ 39.8 ″	E 24 ° 47 ′ 08.9 ″
0602	7/21/2006	1,2	meadow	between G. Pavlikene and Kukrina	Balkan mountain range	485	N 43 ° 06 ′ 33.0 ″	E 24 ° 50 ′ 11.0 ″
0603	7/21/2006	1,2,4,6	meadow	Kukrina	Balkan mountain range	488	N 43 ° 06 ′ 58.6 ″	E 24 ° 52 ′ 02.0 ″
0604	7/21/2006	1,2	meadow	Malinovo	Balkan mountain range	475	N 43 ° 02 ′ 09.3 ″	E 24 ° 52 ′ 40.1 ″
0605	7/22/2006	1,2,4,5	meadow	Belish, Radevska	Balkan mountain range	402	N 42 ° 55 ′ 40.7 ″	E 24 ° 43 ′ 09.5 ″
0606	7/22/2006	1,5,7,8,12	road side	Margatina	Balkan mountain range	597	N 42 ° 56 ′ 19.4 ″	E 24 ° 46 ′ 29.5 ″
0607	7/22/2006	1,2	meadow	Zlatesko (near Margatina)	Balkan mountain range	580	N 42 ° 55 ′ 25.7 ″	E 24 ° 47 ′ 03.2 ″
0608	7/22/2006	1,2	meadow	Gumostnik	Balkan mountain range	422	N 42 ° 55 ′ 08.8 ″	E 24 ° 50 ′ 21.0 ″
0609	7/22/2006	1,2	abandoned	Lakarevo	Balkan mountain range	688	N 42 ° 55 ′ 50.5 ″	E 24 ° 52 ′ 43.4 ″
0610	7/22/2006	1,2,6	meadow	Debnevo	Balkan mountain range	442	N 42 ° 57 ′ 41.8 ″	E 24 ° 47 ′ 53.7 ″
0611	7/23/2006	1,2	abandoned	Zla Reka	Balkan mountain range	461	N 42 ° 57 ′ 41.5 ″	E 24 ° 46 ′ 13.6 ″
0612	7/23/2006	1,2,6	pasture	Radina Livada Novo Selo	Balkan mountain range	535	N 42 ° 50 ′ 40.0 ″	E 24 ° 53 ′ 38.5 ″
0613	7/23/2006	1,2,6	meadow	Marinska Vidima	Balkan mountain range	566	N 42 ° 49 ′ 25.2 ″	E 24 ° 54 ′ 30.9 ″
0614	7/24/2006	1,2,10	abandoned	Haidusko Sbriste	Balkan mountain range	597	N 42 ° 51 ′ 20.6 ″	E 24 ° 39 ′ 36.3 ″
0615	7/24/2006	1,2	meadow	road to Bekleme I	Balkan mountain range	1041	N 42 ° 46 ′ 49.1 ″	E 24 ° 58 ′ 10.0 ″
0616	7/24/2006	1,2	wild	road to Bekleme II	Balkan mountain range	1079	N 42 ° 48 ′ 34.9 ″	E 24 ° 38 ′ 25.5 ″
0617	7/24/2006	1,2,9	wild	Bekleme	Balkan mountain range	1256	N 42 ° 43 ′ 53.1 ″	E 24 ° 37 ′ 40.0 ″
0618	7/24/2006	1	wild	Tchutchul (Below the pass)	Balkan mountain range	1414	N 42 ° 46 ′ 57.9 ″	E 24 ° 37 ′ 23.3 ″
0619	7/24/2006	1,2	road side	Tchutchul cottage	Balkan mountain range	1390	N 42 ° 47 ′ 03.0 ″	E 24 ° 40 ′ 48.2 ″
0620	7/24/2006	2	road side	between Tchutchul and the pass	Balkan mountain range	1542	N 42 ° 46 ′ 44.0 ″	E 24 ° 40 ′ 44.2 ″
0621	7/24/2006	1,2	wild	Parking to the Monument (Troyan pass)	Balkan mountain range	1528	N 42 ° 46 ′ 43.3 ″	E 24 ° 36 ′ 34.8 ″
0622	7/26/2006	2	road side	30km to Topolovgrad	Sakar mountains	319	N 42 ° 00 ′ 46.9 ″	E 26 ° 03 ′ 09.0 ″
0623	7/26/2006	2,6,10,19,20,21	road side	Sakar Hotel	Sakar mountains	416	N 42 ° 00 ′ 44.8 ″	E 26 ° 03 ′ 08.7 ″
0624	7/27/2006	6	meadow	near Goryamo Krushevo	Strandzha mountains	132	N 42 ° 09 ′ 04.7 ″	E 26 ° 42 ′ 04.6 ″
0625	7/27/2006	1	meadow	near Fakiya	Strandzha mountains	193	N 42 ° 09 ′ 05.8 ″	E 27 ° 05 ′ 50.9 ″
0626	7/27/2006	21	wild	near Fakiya (Yabokovo)(Close to Site25)	Strandzha mountains	213	N 42 ° 09 ′ 51.2 ″	E 27 ° 06 ′ 05.2 ″
0627	7/27/2006	2	wild	Bulgari	Strandzha mountains	313	N 42 ° 05 ′ 34.3 ″	E 27 ° 41 ′ 39.1 ″
0628	7/27/2006	1,2	road side	near Byala voda	Strandzha mountains	348	N 42 ° 11 ′ 18.4 ″	E 27 ° 24 ′ 43.9 ″
0629	7/28/2006	21	meadow	Debovech	Rodopi mountains	189	N 41 ° 39 ′ 50.9 ″	E 25 ° 59 ′ 49.6 ″
0630	7/28/2006	10	road side	after Debovech	Rodopi mountains	402	N 41 ° 37 ′ 38.7 ″	E 26 ° 01 ′ 33.3 ″
0631	7/28/2006	2,11	road side	3km to Felezino	Rodopi mountains	546	N 41 ° 30 ′ 02.1 ″	E 25 ° 56 ′ 43.0 ″
0632	7/28/2006	2	wild	Perunika	Rodopi mountains	675	N 41 ° 31 ′ 18.6 ″	E 25 ° 47 ′ 27.5 ″
0633	7/28/2006	1	road side	Belibrezi(Kardzali-Ardino)	Rodopi mountains	928	N 41 ° 34 ′ 26.7 ″	E 25 ° 09 ′ 58.2 ″
0634	7/28/2006	2	road side	Hotel detorina	Rodopi mountains	835	N 41 ° 34 ′ 35.9 ″	E 25 ° 09 ′ 18.1 ″

Table 2. (Continued).

Collection site umber	Date	Species*	Usage	Near Town/Village	Area	Altitude (m)	Latitude	Longitude
0635	7/29/2006	1	meadow	Devil's bridge(10km from Ardino)	Rodopi mountains	441	N 41 ° 37 ′ 13.9 ″	E 25 ° 06 ' 53.9 "
0636	7/29/2006	2	pasture	between Ardino and Devil's bridge	Rodopi mountains	584	N 41 ° 36 ′ 57.5 ″	E 25 ° 07 ' 27.9 "
0637	7/29/2006	1,2	wild	Saralia (Near Ardino)	Rodopi mountains	582	N 41 ° 33 ′ 28.8 ″	E 25 ° 02 ' 58.5 "
0638	7/29/2006	1,2	meadow	Ravnishte (Near Smolyan)	Rodopi mountains	679	N 41 ° 32 ′ 26.1 ″	E 24 ° 54 ' 35.1 "
0639	7/29/2006	1,2	meadow	Monasteri (south of Smolyan)	Rodopi mountains	1107	N 41 ° 32 ′ 42.9 ″	E 24 ° 44 ' 27.2 "
0640	7/29/2006	1,2	meadow	Chokmanovo	Rodopi mountains	990	N 41 ° 31 ′ 11.7 ″	E 24 ° 43 ' 43.3 "
0641	7/29/2006	1,2	abandoned	Gorna Arda(Mogilitza, near bordar)	Rodopi mountains	1046	N 41 ° 27 ′ 22.4 ″	E 24 ° 37 ' 32.8 "
0642	7/30/2006	1,2	wild	Smolyan Lakes	Rodopi mountains	1496	N 41 ° 37 ′ 15.8 ″	E 24 ° 40 ' 41.8 "
0643	7/30/2006	1,2,12,13	wild	Pomporovo	Rodopi mountains	1625	N 41 ° 37 ′ 52.6 ″	E 24 ° 42 ' 07.2 "
0644	7/30/2006	1,2,15	wild	Progled	Rodopi mountains	1396	N 41 ° 40 ′ 42.2 ″	E 24 ° 43 ' 00.9 "
0645	7/30/2006	1,2,14,15	meadow	Rojen	Rodopi mountains	1941	N 41 ° 41 ′ 18.8 ″	E 24 ° 44 ' 37.9 "
0646	7/30/2006	1,2,3,6	meadow	Stoikite	Rodopi mountains	1488	N 41 ° 38 ′ 35.9 ″	E 24 ° 38 ' 34.3 "
0647	7/30/2006	1,2,9,15,17,18	wild	Gela	Rodopi mountains	1516	N 41 ° 38 ′ 29.4 ″	E 24 ° 34 ' 07.2 "
0648	7/31/2006	1,2,15	meadow	Solishta	Rodopi mountains	1323	N 41 ° 38 ′ 55.4 ″	E 24 ° 35 ' 35.6 "
0649	7/31/2006	1,11	wild	before Trigrad	Rodopi mountains	941	N 41 ° 39 ′ 18.9 ″	E 24 ° 22 ' 53.0 "
0650	7/31/2006	1,2	wild	after Trigrad	Rodopi mountains	1219	N 41 ° 35 ′ 05.4 ″	E 24 ° 23 ' 35.6 "
0651	7/31/2006	1,2	meadow	between Trigrad and Yagodina	Rodopi mountains	1336	N 41 ° 37 ′ 23.6 ″	E 24 ° 22 ' 25.3 "
0652	7/31/2006	1,2,16	meadow	Yagodina	Rodopi mountains	1233	N 41 ° 37 ′ 43.7 ″	E 24 ° 22 ' 11.2 "
0653	8/1/2006	1,2,6	road side	near Borino	Rodopi mountains	1072	N 41 ° 40 ′ 40.3 ″	E 24 ° 20 ' 00.4 "
0654	8/1/2006	1,2,10	road side	10km from Borino	Rodopi mountains	1252	N 41 ° 39 ′ 05.0 ″	E 24 ° 14 ' 52.0 "
0655	8/1/2006	1,2	road side	Dospat	Rodopi mountains	1188	N 41 ° 38 ′ 16.8 ″	E 24 ° 09 ' 14.4 "
0656	8/1/2006	1,2	meadow	Satovcha	Rodopi mountains	1230	N 41 ° 37 ′ 56.1 ″	E 24 ° 01 ' 22.1 "
0657	8/1/2006	1,2,6	meadow	Gotze Delchev	Rodopi mountains	542	N 41 ° 36 ′ 44.0 ″	E 23 ° 44 ' 26.4 "
0658	8/1/2006	1,2,6	road side	Eleshnitza	Pirin mountains	710	N 41 ° 49 ′ 55.8 ″	E 23 ° 37 ' 07.8 "
0659	8/1/2006	1,2,5	wild	between Beliza and Semkovo	Rila mountains	936	N 41 ° 58 ′ 49.3 ″	E 23 ° 31 ' 54.0 "
0660	8/1/2006	1	wild	Semkovo	Rila mountains	1588	N 42 ° 02 ′ 34.5 ″	E 23 ° 31 ' 27.5 "
0661	8/2/2006	1,2	wild	5km to Yundola (Ablalovo)	Rodopi mountains	1252	N 42 ° 02 ′ 20.7 ″	E 23 ° 49 ' 01.7 "
0662	8/2/2006	1,2	meadow	Yundola	Rodopi mountains	1378	N 42 ° 04 ′ 05.7 ″	E 23 ° 51 ' 16.9 "
0663	8/2/2006	1,2	wild	Tsigov Tchark	Rodopi mountains	1134	N 41 ° 57 ′ 13.2 ″	E 24 ° 09 ' 00.1 "
0664	8/2/2006	1,2,18	meadow	Batak	Rodopi mountains	1013	N 41 ° 56 ′ 49.8 ″	E 24 ° 14 ' 00.0 "

*Species, 1:*Trifolium prantense*, 2:*T.repens*, 3:*T. hybridum*, 4:*Lotus corniculatus*, 5:*T. medium*, 6:*Lolium perenne*, 7:*T. montana*, 8:*T. incarnata*, 9:*Arrhenatherum elatius*, 10:*Dactyris glomerata*, 11:*T. heldreichianum*, 12:*T. alpestre*, 13:*T. spadiceum*, 14:*T. aureum*, 15:*T. pannonicum*, 16:*T. michelianum*, 17:*Onobrychis viciifolia*, 18:*Festuca pratensis*, 19:*Medicago polymorpha*, 20:*M. minima*, 21:*T.* spp

Photo 1. Pasture of the site No. 0601 near Gorno Pavlikene in the Balkan mountain area (Flowering of *T. pratense*).

Photo 2. Pasture of the site No. 0629 near Debovech in the East Rodopi mountain area (*T.* spp.).

Photo 3. Pasture of the site No.0652 near Yogodina in the West Rodopi mountain area (*T. michelianum*).

Photo 4. Diversity of genus *Trifolium* in Bulgaria (A: *T. fragiferum*, B: *T. pannonicum*, C: *T. medium*, D: *T. aureum*).

Photo 5. Harvesting the hay on a meadow in the Rodopi mountain area.

Photo 6. Member of the team (Drs. T. Hayashi, K. Okumura, Y. Guteva and T. Mihovsky).