Macedonian Genebank: Seed Protein Content in Wild Meadow Fescue (*Festuca pratensis* Huds.) Accessions

Suzana KRATOVALIEVA [1(✉)]
Gordana POPSIMONOVA [1]
Sonja IVANOVSKA [2]
Ljupcho JANKULOSKI [2]
Vladimir MEGLIČ [3]

Summary

During several collecting expeditions conducted in the Eastern part of Macedonia, district Probistip, 27 wild meadow fescue (*Festuca pratensis* Huds.) accessions were collected on different natural stands, like meadows and pastures. Accessions were documented, characterized, evaluated and seeds stored at -18°C. One of the traits evaluated was seed crude protein content, which is a desired trait for forage mixture components. The crude protein contents varied from 8.63 % / 100g DM for accession MKD 01514 to 14.6 % / 100g DM for accession MKD01496. Accessions grown on dry stands exhibited SCP content less than 10,0 % / 100g DM, therefore accessions MKD01498, MKD01508 and MKD01514 may be recommended for further use on a stands with a good-water supply. The rest of the populations collected were growing on semi-moist or moist meadows, with the SCP content ≥11.0 % / 100g DM, providing material with a high seed protein content to be included in meadow fescue breeding programs.

Key words

wild meadow fescue, seed protein content, genetic resources

[1] University Ss. Cyril and Methodius, Institute of Agriculture - Skopje, Aleksandar Makedonski blvd. bb, 1000 Skopje, Republic of Macedonia
✉ e-mail: suzanakrat@yahoo.com
[2] University Ss. Cyril and Methodius, Faculty for Agricultural Sciences and Food, Aleksandar Makedonski blvd. bb, 1000 Skopje, Republic of Macedonia
[3] Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia

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Introduction

Macedonia has a great biodiversity potential of forage grasses, but neglected until several year ago, when it got promoted through several collecting missions, both national and international. Eastern part of the country is recognized as the most suitable for livestock grazing on natural pastures and grasslands, characterized with a high nutritive value and optimal grass and legume species composition. If it is not utilized as a pasture, the composition of pasture species starts to change and desired species became less abundant. To preserve disappearing species and especially wild meadow fescue populations for long-term, collecting expeditions were organized through SEEDNet project, to collect the seed material of forage species.

In the early 80's collecting expeditions were organized and seed material was included into the breeding programs, which resulted with new varieties harboring desirable quality traits (Krivoshieva, 1996). Protection and preservation of biodiversity as a world trend did not avoid Macedonia and a great attention has been paid to collecting plant species having economically valuable traits in particular (Kratovalieva et al., 2007a), and to the establishment and the implementation of the National gene bank program in Macedonia (Kratovalieva et al., 2007b). The responsibility for plant germplasm conservation and utilization is divided between the Ministry of agriculture, forestry and water economy, Universities, Institutes and NGO's sector. Despite the lack of national financial support, the most important activities, including seed collecting, were possible due to the SEEDNet financing program.

The aim of this research was to determine the wild meadow fescue seed protein content (SPC) and to assess relationships and correlation between seed protein content and the collecting site altitude.

Material and methods

Sampling

Collecting missions organized within the framework of the SEEDNet project were carried out in the period July-August 2007 on national and international level with the collaboration of the Slovenian partner. Eastern part of Macedonia was chosen since it is covered predominantly by meadows, pastures and grassland abundant with meadow fescue (*Festuca pratensis* Huds.). With close collaboration and discussion with local people who know well the region and agricultural practices, collecting sites were chosen. Vertisol is the predominant soil type on the locations visited. The wider collecting area is located at different altitudes, from 687 m to 1447 m above sea level. A total of 95 grass accessions were collected and 27 seed samples of meadow fescue were used for seed protein analysis. Each collecting site was described by geographical coordinates (longitude, latitude, elevation) using GARMIN 12 GPS receiver. All meadow fescue accessions were equipped with the Eurisco passport descriptor data and stored and conserved in the Macedonian gene bank at -18°C for long-term storage.

Preparation of samples and Chemical analysis

To determine the SPC, the crude samples consisted of matured fescue seeds. The dried samples (dried at 60°C) were milled into the fine powder (passed through 1.0 mm sieve size). The samples were kept in sealed glass containers and stored at +4°C until they were used for the analysis. Each analysis was repeated three times. The values reported for each seed sample correspond to the average of the three replications. Nitrogen was determined by semi-micro Khjeldal method (AOAC, 1990). Protein content was calculated using the nitrogen-conversion factor of 6.25 (N×6.25) and presented as % / 100g DM.

Statistical analysis

Obtained data were analyzed using the one-way analysis of variance (ANOVA). To compare the SCP content and altitude as the main factor, the General Linear Model (GLM) procedure was used (Statistica, 1993). A simple correlation analysis was used to establish the relationship between seed protein content and altitude, using linear correlation coefficient (Najcevska, 2002). Significance between factors was identified using Roemer-Orphal table (Najcevska, 2002). Mean differences were considered significant at P>0.001.

Results

Populations collected in natural habitats were stored for long-term in the gene bank and supplied with the passport data. National (MKD) codes and collecting numbers were assigned (Table 1).

Table 1. List of collected and stored wild meadow fescue populations

NICODE	INSTCODE	ACCENUMB	COLLNUMB	GENUS	SPECIES
MKD	MKD001	MKD01483	SK3-12	*Festuca*	*pratensis*
MKD	MKD001	MKD01484	SK3-13	*Festuca*	*pratensis*
MKD	MKD001	MKD01485	SK3-14	*Festuca*	*pratensis*
MKD	MKD001	MKD01486	SK3-15	*Festuca*	*pratensis*
MKD	MKD001	MKD01487	SK3-16	*Festuca*	*pratensis*
MKD	MKD001	MKD01489	SK3-18	*Festuca*	*pratensis*
MKD	MKD001	MKD01491	SK3-20	*Festuca*	*pratensis*
MKD	MKD001	MKD01492	SK3-21	*Festuca*	*pratensis*
MKD	MKD001	MKD01495	SK3-24	*Festuca*	*pratensis*
MKD	MKD001	MKD01496	SK3-25	*Festuca*	*pratensis*
MKD	MKD001	MKD01498	SK3-27	*Festuca*	*pratensis*
MKD	MKD001	MKD01499	SK3-28	*Festuca*	*pratensis*
MKD	MKD001	MKD01500	SK3-29	*Festuca*	*pratensis*
MKD	MKD001	MKD01501	SK3-30	*Festuca*	*pratensis*
MKD	MKD001	MKD01502	SK3-31	*Festuca*	*pratensis*
MKD	MKD001	MKD01503	SK3-32	*Festuca*	*pratensis*
MKD	MKD001	MKD01505	SK3-34	*Festuca*	*pratensis*
MKD	MKD001	MKD01508	SK3-37	*Festuca*	*pratensis*
MKD	MKD001	MKD01509	SK3-38	*Festuca*	*pratensis*
MKD	MKD001	MKD01511	SK3-40	*Festuca*	*pratensis*
MKD	MKD001	MKD01512	SK3-41	*Festuca*	*pratensis*
MKD	MKD001	MKD01513	SK3-42	*Festuca*	*pratensis*
MKD	MKD001	MKD01514	SK3-43	*Festuca*	*pratensis*
MKD	MKD001	MKD01517	SK3-46	*Festuca*	*pratensis*
MKD	MKD001	MKD01518	SK3-47	*Festuca*	*pratensis*
MKD	MKD001	MKD01519	SK3-48	*Festuca*	*pratensis*
MKD	MKD001	MKD01520	SK3-49	*Festuca*	*pratensis*

NICODE – National Code given by FAO for the country is MKD and is valid for all listed accessions in the table; INSTCODE – Institutional Code given by FAO for the Institution included in Gene Bank which is MKD001 for Institute of Agriculture – Skopje and is valid for all accessions listed in the table

Table 2. Seed protein content for 27 wild meadow fescue populations (SCP % / 100g DM)

ACCENUMB	COLLNUMB	X	Sx	StDev	CV
MKD01483	SK3-12	11.5	0.01	0.12	1.01
MKD01484	SK3-13	11.6	0.01	0.15	1.31
MKD01485	SK3-14	10.1	0.02	0.21	2.07
MKD01486	SK3-15	10.1	0.02	0.15	1.51
MKD01487	SK3-16	11.3	0.01	0.15	1.36
MKD01489	SK3-18	11	0.02	0.17	1.57
MKD01491	SK3-20	11.8	0.01	0.17	1.47
MKD01492	SK3-21	11	0.03	0.3	2.73
MKD01495	SK3-24	11.4	0.03	0.35	3.04
MKD01496	SK3-25	14.6	0.03	0.4	2.76
MKD01498	SK3-27	9.8	0.03	0.26	2.7
MKD01499	SK3-28	11.5	0.02	0.21	1.82
MKD01500	SK3-29	11	0.02	0.21	1.9
MKD01501	SK3-30	10.6	0.03	0.31	2.89
MKD01502	SK3-31	10.9	0.03	0.36	3.31
MKD01503	SK3-32	11.6	0.03	0.36	3.11
MKD01505	SK3-34	14.1	0.03	0.42	2.95
MKD01508	SK3-37	9.93	0.02	0.15	1.54
MKD01509	SK3-38	11.5	0.03	0.3	2.61
MKD01511	SK3-40	11.1	0.04	0.46	4.13
MKD01512	SK3-41	11.6	0.02	0.21	1.8
MKD01513	SK3-42	11.8	0.02	0.21	1.77
MKD01514	SK3-43	8.63	0.04	0.35	4.07
MKD01517	SK3-46	11	0.04	0.4	3.66
MKD01518	SK3-47	12.1	0.03	0.31	2.43
MKD01519	SK3-48	11.7	0.02	0.25	2.14
MKD01520	SK3-49	11.1	0.04	0.42	3.74

Meadow fescue is one of the important grass forage species as well in Macedonia and is a good carbohydrate source and a valuable component in mixtures (Kratovalieva and Cvetanovska 2000). The aim of this study was to assess the seed crude protein content of 27 wild meadow fescue populations (Table 2). Although accession MKD01514 had the lowest SCP content of 8.63 %/100g DM, there are many populations with similar SCP content ranging between 10.0 to 11.5 %/100g DM (Table 2). The highest SCP content was recorded for accession MKD001496 with 14.6 %/100g DM followed by MKD1505 with 14.1%/100g DM. Only three populations exhibited less than 10.0% SCP/100g DM; MKD01498, MK01508 and MKD01514 grown in less favourable and dry growing conditions on the altitude below 1000 m.

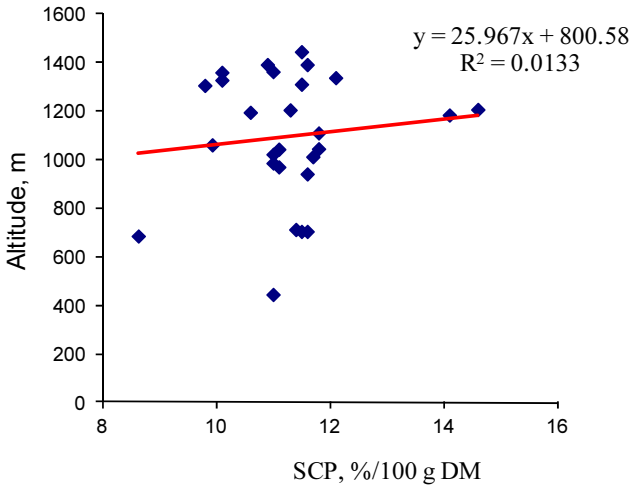

Figure 2. Relationship between altitude and seed protein content (SCP, %/100g DM)

Population MKD1505 with the highest SCP content of 14.1 % / 100g DM was collected on a meadow with a good water supply. The range of SCP values given in Table 2 could be compared to those reported by Kallenbach et al. (2003).

Correlation coefficient (r = 0.11) calculated for SCP content and collecting site altitude is showing that altitude does not affect the crude seed protein content and is not significant ($p<0.01$, $p<0.05$) (Figure 1). There is no significant effect between the seed SCP content and the collecting site altitude based on regression coefficient (Figure 2).

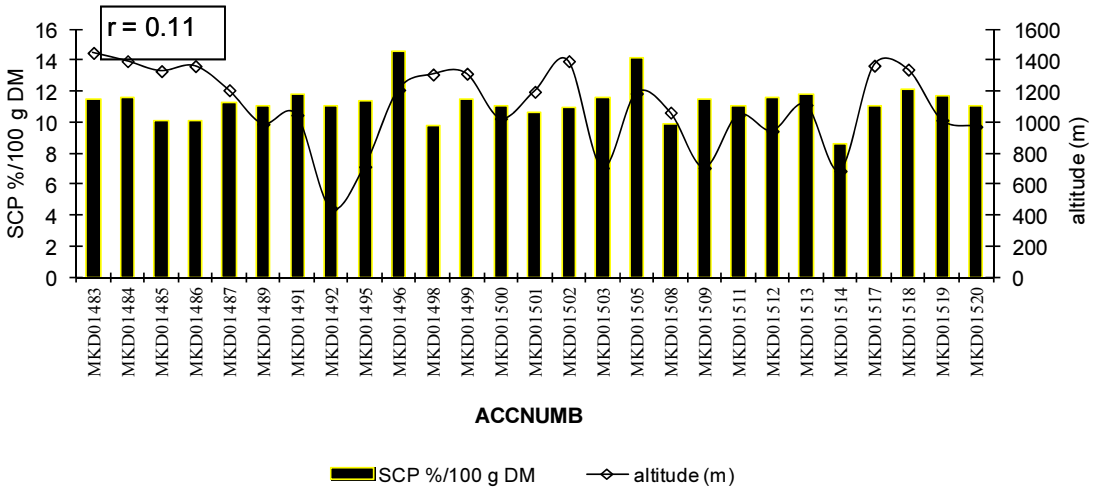

Figure 1. Seed protein content (SCP % / 100g DM) and collection site altitude (m) for 27 analyzed accessions

Discussion

There was a considerable range in the SCP content between meadow fescue populations studied. The SCP content is an important factor to determine nutritive value of feeding mixtures. Meadow fescue contributed to floristic composition in >50 % of visited sites (natural meadows and grassland) offering a valuable forage for cattle and sheep. Results of this study pointed out the possibility to use the collected material for breeding purposes when selecting for higher nutritive value. Collected populations could be placed in three groups based on SCP content determined in collected seeds; the first one including 18 populations with the SCP values from 11.0 % / 100g DM to 12.0 % / 100g DM including accession MKD01518 with 12.1 %/100g DM; the second group with 4 accessions ranging in SCP values from 10.0 % / 100g DM to 10.9 % / 100g DM; the third with three populations with SCP values less than 10.0 % / 100 g DM. The results of the first group are consistent with those reported by Eck al. 1981 and could be recommended to be included in further breeding program (Volenec and Nelson 2007). This group could be amended with two accessions with a very high SCP content 14.1 % / 100g DM and 14.6 % / 100g DM. Present study clearly showed that the regression equation used for SCP predictions of meadow fescue populations collected in different growing conditions (type of vegetation, water supply, altitude) resulted in large differences and had not a significant effect. The altitude has no effect for prediction of SCP content for collected populations and seed protein content is as well not affected by this factor. The caution has to be exhibited when comparing SCP content results for populations grown on sites with different growing conditions and water supply available.

Conclusion

Results of this study pointed out the possibility to use the collected material for breeding purposes. The 18 populations evaluated contained a high SCP content ranking from 11.0%/100g DM to 12.0%/100g DM including accession MKD01518 with 12.1%/100g DM. Populations MKD01496 and MKD01505 contained >14.0 % / 100g DM; only three populations are characterized with SCP content <10.0 % / 100g DM. Correlation between SCP content and altitude is very weak (r=0.11) and not significantly ($p<0.01$, $p<0.05$). The altitude could not be used for prediction of SCP content. On the other hand one has to be cautious when comparing SCP content results for populations grown on sites with different growing conditions and water supply available.

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