Macedonian Genebank: Seed Protein Content of Wild Red Clover (*Trifolium pratense* L.) Accessions

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Summary

During this study, the content of crude protein content in seeds of 23 wild red clover (*Trifolium pratense* L.) populations, collected in East part of Macedonia, district Probistip, was assessed. On the basis of the average crude protein content, the collected accessions were divided into 3 groups with the crude protein content ≥ 15.0% (MKD01527, MKD01530, MKD01531, MKD01539, MKD01540, MKD01542, MKD01543); ≥ 16.0% (MKD01525, MKD01528, MKD01529, MKD0538, MKD01541, MKD01553, MKD01554, MKD01560) and ≥ 17.0% (MKD01536, MKD01537, MKD01544). The highest crude protein content of 17.3% was determined for population MKD01535 and the lowest (14.4 %) for MKD01526. Information on protein content, as an indicator of wild red clover quality, could be used as a selection tool for identifying accessions to be included in red clover breeding programs.

Key words

seed protein content, red clover, genetic resources

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Introduction

Red clover (*Trifolium pratense* L.) has been used for animal feed and as an meadow component that contributes to floristic composition in many parts of the world (Smith, 1983). It plays an important role in cattle and sheep diet on pastures of the Probistip region. It could be an important protein source in areas where few or no alternatives are at disposal (Taylor and Quesenberry, 1996). During the dry period, recognized as a critical part of the year when forage quality and quantity of meadows are limited, mixture of plant mass and seeds could represent a rich protein source (Kratovalieva and Cvetanovska, 2000). Red clover accessions acquired during collecting missions, as one of the leading forage legumes in region particularly of high seedling vigor, fast growth, high quality and yield (Smith and Sharpee, 1992), are a valuable germplasm resources to be used by the public and private breeding programs. Developing red clover cultivars with the elevated seed protein content would provide cultivars with better nutritive value. Underground structures enable better and more efficient use of soil nitrogen (Smith and Kretschmer, 1989; Venuto et al., 1992). The new and novel efforts would be needed to improve the efficiency of red clover breeding programs when selecting for above mentioned desirable traits.

The aim of the present work was to determine the crude seed protein content (CP%) in wild red clover populations collected in the eastern part of the Republic of Macedonia, Mt. Plavica, Probistip region and to assess relationship between CP content and collecting site altitude.

Material and methods

Collecting

Collecting expeditions were organized within the SEED Net framework in collaboration with Slovenian partner during the period July-September 2007 in the eastern part of Macedonia. The area was chosen on the basis of the previous mapping efforts exhibiting red clover rich meadows and grasslands. Close collaboration and experience of local people knowing the region and agricultural practices, was truly helpful. Vertisole and rendzine soil types characterize selected collecting sites. The area covers various altitudes, from 720 to 1435m above the sea level. During the expeditions twenty three red clover population seed samples were collected and consequently prepared for protein analysis. Each collecting site was described and geographical coordinates (longitude, latitude, altitude) using GARMIN 12 GPS receiver recorded. The seed samples of all red clover accessions were prepared for long-term storage and conservation at -18°C in the Macedonian gene bank. Beforehand Eurisco passport data (MCPD) were recorded for each accession.

Chemical analyses

100 grams of seeds per accession were dried at 60°C and milled through a 1 mm sieve for chemical analysis. Nitrogen (N) content was measured by the Khjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N X 6.25 (%/100 g DM).

Statistical analyses

Obtained data were subjected to ANOVA statistical analysis. A simple correlation analysis was used to establish the relationship between seed protein content and collecting site altitude using linear correlation coefficient (Najchevska, 2002).

Results

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

Table 1. List of collected and stored wild red clover populations

Nicode	Instcode	Accenumb	Collnumb	Genus	Species
MKD	MKD001	MKD01524	SK3-53	*Trifolium*	*pratense*
MKD	MKD001	MKD01525	SK3-54	*Trifolium*	*pratense*
MKD	MKD001	MKD01526	SK3-55	*Trifolium*	*pratense*
MKD	MKD001	MKD01527	SK3-56	*Trifolium*	*pratense*
MKD	MKD001	MKD01528	SK3-57	*Trifolium*	*pratense*
MKD	MKD001	MKD01529	SK3-58	*Trifolium*	*pratense*
MKD	MKD001	MKD01530	SK3-59	*Trifolium*	*pratense*
MKD	MKD001	MKD01531	SK3-60	*Trifolium*	*pratense*
MKD	MKD001	MKD01533	SK3-62	*Trifolium*	*pratense*
MKD	MKD001	MKD01534	SK3-63	*Trifolium*	*pratense*
MKD	MKD001	MKD01535	SK3-64	*Trifolium*	*pratense*
MKD	MKD001	MKD01536	SK3-65	*Trifolium*	*pratense*
MKD	MKD001	MKD01537	SK3-66	*Trifolium*	*pratense*
MKD	MKD001	MKD01538	SK3-67	*Trifolium*	*pratense*
MKD	MKD001	MKD01539	SK3-68	*Trifolium*	*pratense*
MKD	MKD001	MKD01540	SK3-69	*Trifolium*	*pratense*
MKD	MKD001	MKD01541	SK3-70	*Trifolium*	*pratense*
MKD	MKD001	MKD01542	SK3-71	*Trifolium*	*pratense*
MKD	MKD001	MKD01543	SK3-72	*Trifolium*	*pratense*
MKD	MKD001	MKD01544	SK3-73	*Trifolium*	*pratense*
MKD	MKD001	MKD01553	SK3-82	*Trifolium*	*pratense*
MKD	MKD001	MKD01554	SK3-83	*Trifolium*	*pratense*
MKD	MKD001	MKD01560	SK3-89	*Trifolium*	*pratense*

Table 2. Seed protein content for 23 wild red clover (*Trifolium pratense* L.) populations (%/100g DM)

Accenumb	Collnumb	X	Sx	StDev	CV
MKD01524	SK3-53	14.8	0.01	0.21	1.01
MKD01525	SK3-54	16.1	0.01	0.20	1.31
MKD01526	SK3-55	14.4	0.03	0.36	2.07
MKD01527	SK3-56	15.0	0.02	0.26	1.51
MKD01528	SK3-57	16.3	0.02	0.38	1.36
MKD01529	SK3-58	16.6	0.01	0.21	1.57
MKD01530	SK3-59	15.1	0.03	0.40	1.47
MKD01531	SK3-60	15.4	0.02	0.32	2.73
MKD01533	SK3-62	14.2	0.03	0.0	3.04
MKD01534	SK3-63	16.7	0.02	0.35	2.76
MKD01535	SK3-64	17.3	0.03	0.47	2.70
MKD01536	SK3-65	17.0	0.01	0.25	1.82
MKD01537	SK3-66	17.2	0.03	0.57	1.90
MKD01538	SK3-67	16.0	0.02	0.25	2.89
MKD01539	SK3-68	15.9	0.03	0.51	3.11
MKD01540	SK3-69	15.5	0.02	0.31	2.95
MKD01541	SK3-70	16.3	0.02	0.31	1.54
MKD01542	SK3-71	15.9	0.02	0.36	2.61
MKD01543	SK3-72	15.6	0.02	0.38	4.13
MKD01544	SK3-73	17.0	0.01	00	1.80
MKD01553	SK3-82	16.3	0.03	5.0	1.77
MKD01554	SK3-83	16.8	0.02	00	4.07
MKD01560	SK3-89	16.0	0.04	0.67	3.66

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

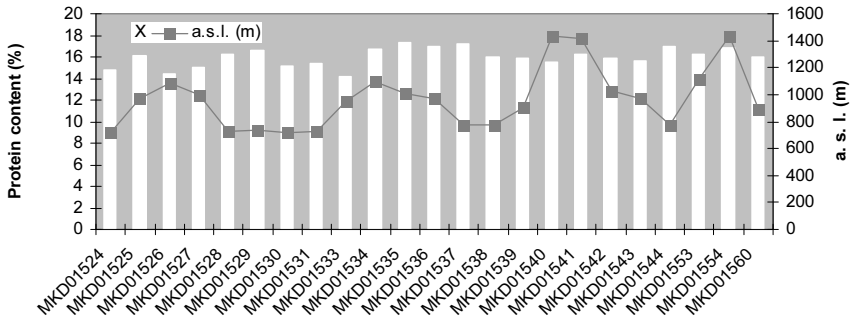

Figure 1.
Seed protein content (%) and collection site altitude (m) for 23 analyzed accessions

Red clover is one of the most important forage species as well in Macedonia. The aim of this study was to assess seed crude protein content of 23 wild red clover populations (Table 2). The highest CP content was recorded for accession MKD01535 with 17.3 %/100g of dry matter (DM) followed by MKD01537 with 17.2 %/100g DM, MKD01536 and MKD01544 both with 17.0 %/100g DM. Based on the results we can divide populations into three groups according to the CP content: the first with the range from 15.0 to 15.9%/100g DM and seven samples (MKD01527 15.0%/100g DM, MKD01530 15.1%/100g DM, MKD01531 15.4%/100g DM, MKD01540 15.5%/100g DM, MKD01543 15.6%/100g DM and MKD01539 and MKD01542 15.9%/100g DM). The second group encompass nine accessions with CP content from 16.0 to 16.9%/100g DM (MKD01560 and MKD01538 16.0%/100g DM, MKD01525 16.1%/100g DM, MKD01553, MKD01541 and MKD0528 16.3%/100g DM, MKD01529 16.6%/100g DM, MKD01534 16.7%/100g DM, MKD01554 16.8%/100g DM). The rest forms the third group having CP content lover than 16%. The populations with the high CP content could be included in the red clover breeding programs (Taylor and Smith, 1979) to create new varieties of better nutritional quality. As well accessions with not as desirable CP content could be used, if they are carrying other qualitative or quantitative traits (Smith, 1983). Correlation coefficient (r = 0.07) calculated for CP content and collecting site altitude is showing that altitude does not affect crude seed protein content (Figure 1). There is no significant effect between seed CP content and collecting site altitude based on regression coefficient (R^2 = 0.052).

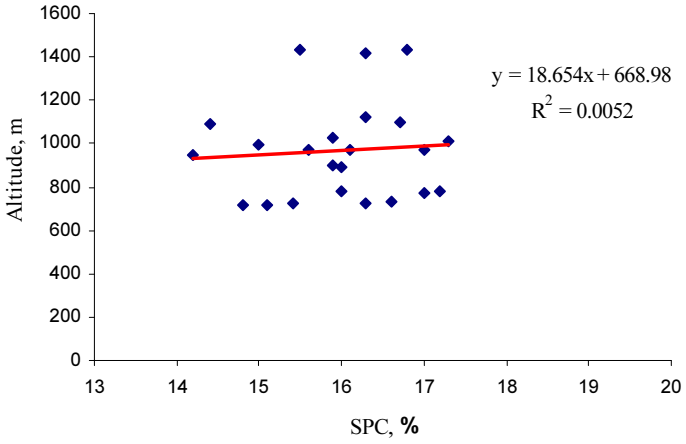

Figure 2. Relationship between collection site altitude and seed protein content (%)

Discussion

Based on results, attention should be put on wild red clover populations with a high seed protein content, >16.0%/100g DM, to be included in further breeding activities.

All populations collected were grown on fertile vertisole and rendzine soil types. Red clover and other legumes vary in the amount of atmospheric nitrogen they can fix. This variation could be due to various soil and environmental conditions. Nitrogen fixation is likely to be lower for stands grown in soils high in naturally available nitrogen. Several populations collected on dry stands, beside the roads or on grassland not properly managed, were exhibiting good qualitative traits and therefore could be included in the future breeding process for better performance and persistence. However, it could be recommended that a future selection of red clover cultivars should be performed in the management system that is similar to one in the nature (Wiersma et al., 1999). Papadopoulos and Mckersie (1983) stated that red clover has 90 % less proteolysis during ensiling than alfalfa, therefore protein utilization by the ruminants should be improved by inclusion of red clover in mixtures. This effect could not be neglected and Jones (1995) reported that red clover enzyme system polyphenol oxidase (PPO) inhibits activity of the plant's proteases in the silo. Among the forage legumes PPO enzyme system is unique to red clover. This important attribute of red clover should be further exploited in breeding programs.

Because of its unique qualities of high seedling vigor, excellent forage quality, importance for soil conservation and ease of establishment and competitiveness with grasses, red clover should continue to play a major role in sustainable agriculture and in utilization of the gene bank accessions in the Macedonian agriculture and in the agriculture of regions with similar growing conditions.

Conclusion

Results of this study demonstrate that CP content in seeds of 23 different wild red clover populations collected in the mountainous region of Probistip varied, with the highest CP content recorded for accessions MKD01535 with 17.3%/100g DM, MKD01537 17.2%/100g DM, MKD01536 and MKD01544 17.0%/100g DM. On average seed CP content is considered moderately high. No correlation was found with the altitude of collecting site and regression coefficient was not significant.

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