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SOME PHYSIOLOGICAL PARAMETERS IN MIXTURES OF COCKSFOOT AND TALL FESCUE WITH SUBTERRANEAN CLOVER

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Abstract

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Some physiological parameters (leaflet area, photosynthetic pigments) of subterranean clover, cocksfoot and tall fescue were studied in a field trial in the Institute of Forage Crops, Pleven, Bulgaria (2011-2014). Three subspecies of subclovers – i.e. *Trifolium subterraneum ssp. brachycalicinum* (cv. Antas), *Trifolium subterraneum ssp. yaninicum* (cv. Trikkala) and *Trifolium subterraneum ssp. subterraneum* (cv. Denmark); and two grasses, i.e. cocksfoot (*Dactylis glomerata* L.) (cv. Dabrava) and tall fescue (*Festuca arundinacea* Schreb.) (cv. Albena) were grown purely and in mixture (a ratio of grass to subclovers 50:50%). From pure grown subclovers most leaflet area formed *Trifolium subterraneum ssp. brachycalicinum* (2.11 cm^2), followed by *Trifolium subterraneum ssp. yaninicum* (1.98 cm^2) and *Trifolium subterraneum ssp. subterraneum* (1.42 cm^2). For the plastid pigments content subclovers were arranged as follows: *Trifolium subterraneum ssp. brachycalicinum* (300.18 mg/100 g FW), *Trifolium subterraneum ssp. subterraneum* (283.53 mg/100 g FW) and *Trifolium subterraneum ssp. yaninicum* (283.35 mg/100 g FW). Leaflet area of subclover was greater in mixtures with cocksfoot as compared to tall fescue. Total plastid pigments content (chlorophyll a+b and carotenoids) was found be higher in both components of the mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (by 16.2% and 10.6%, respectively).

Key words: leaflet area; plastid pigments; subterranean clover; grasses; mixtures

Introduction

Mixed crops between legumes and grasses have an essential role in building a system of sustainable and organic farming (Luscher et al., 2014; Kusvuran et al., 2014). They are more effective than pure grown in using environmental resources better withstand adverse conditions and are more productive (Porqueddu et al., 2003; Albayrak et al., 2011; Chourkova, 2014).

One of the most important factors determining productivity is the photosynthesis process by which green plants accumulate organic matter and energy (Nichiporovich 1971; Smirnova et al., 2013). Ingestion and transformation of solar energy is achieved by photosynthetic pigments – chlorophyll a and b, and carotenoids. Major chlorophyll is chlorophyll a and it provides higher efficiency of the conversion of carbon dioxide and of water in the organic compounds. Carotenoids also have a protective function – prevent destructive photo oxidation of organic compounds of protoplasm in the presence of free oxygen. The content of photosynthetic pigments is an indication of the response of plants to changes in environmental factors and the degree of adaptation to new environmental conditions (Titova, 2010; Nurmakova, 2013; Smirnova et al., 2013).

In mixtures involving different components that come in competition, and productivity and quality of the forage depends on their physiological status.

In the present study traditional pasture forage crops (cocksfoot and tall fescue) were included, the use of which in the pastures of temperate countries is known practice (Vučković, 2004).

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Subterranean clover (*Trifolium subterraneum* L.) is widely distributed in pastures of temperate climatic regions of Middle and Northern Europe and America (Nichols et al., 2012). Studies in recent years showed that it has the practical applicability of the climatic conditions of Bulgaria (Vasilev, 2006, Vasilev and Vasileva, 2012; Ilieva et al., 2015).

The aim of this work was to study some physiological parameters – leaflet area and the plastid pigments content (chlorophylls and carotenoids) in plants of subterranean clover, cocksfoot and tall fescue grown for forage – pure and in mixtures at a ratio of grass to legume 50:50%.

Materials and Methods

The trial was conducted at the experimental field (43° 23'N, 24° 34'E, 230 m altitude) of the Institute of Forage Crops, Pleven, Bulgaria (2011-2014) on podzolized soil subtype without irrigation. Long plot method was used with size of plots 70 m^2 four replicated. Three subclover subspecies, i.e. *Trifolium subterraneum ssp. brachycalicinum* (cv. Antas), *Trifolium subterraneum ssp. yaninicum* (cv. Trikkala) and *Trifolium subterraneum ssp. subterraneum* (cv. Denmark); and two grasses – cocksfoot (*Dactylis glomerata* L.) (cv. Dabrava) and tall fescue (*Festuca arundinacea* Schreb.) (cv. Albena), pure grown (100%) and in mixtures with grasses (a ratio of grass to subclovers 50:50%) were tested.

The sowing was done in autumn of 2011, between row space was 11.5 cm and sowing rates: tall fescue – 25 kg/ha, cocksfoot – 25 kg/ha, subterranean clover – 25 kg/ha. No fertilizers and pesticides were applied during the vegetation. The swards were harvested for forage and leaflet area of subterranean clover, pure and in mixtures from the first cut during the three experimental years (2012-2014) was calculated. For leaflet area – length and breadth of the middle leaflet of clovers were measured on three representative leaves. Leaflet area was calculated according to formulae of Pecetti and Piano (1998): (length x breadth) x 0.730 for *Trifolium subterraneum ssp. subterraneum*; (length x breadth) x 0.769 for *Trifolium subterraneum ssp. brachycalicinum* and (length x breadth) x 0.645 for *Trifolium subterraneum ssp. yaninicum*.

In fresh plant samples taken from the first cut during the three experimental years plastid pigments content (chlorophyll a, chlorophyll b, carotenoids, and total) (mg/100 mg FW) was determined according Zelenskii and Mogileva (1980). For the mixtures samples were taken from every component. Ratios chlorophyll a/chlorophyll b and chlorophyll a+b/carotenoids were calculated. The samples from the crops pure grown were used for the control. Data were averaged and statistically processed using SPSS (2012).

Results and Discussion

Leaflet area

Leaflet area is an important indicator of the quality and uptake of forage, but there are also essential for the competitiveness of subterranean clover (Smart et al., 1998; Pecetti and Piano, 1998; Evers and Newman, 2008; Østrem et al., 2013). It is related to the morphology of the different subspecies. Our data indicates that from the pure grown subclovers, most leaf-

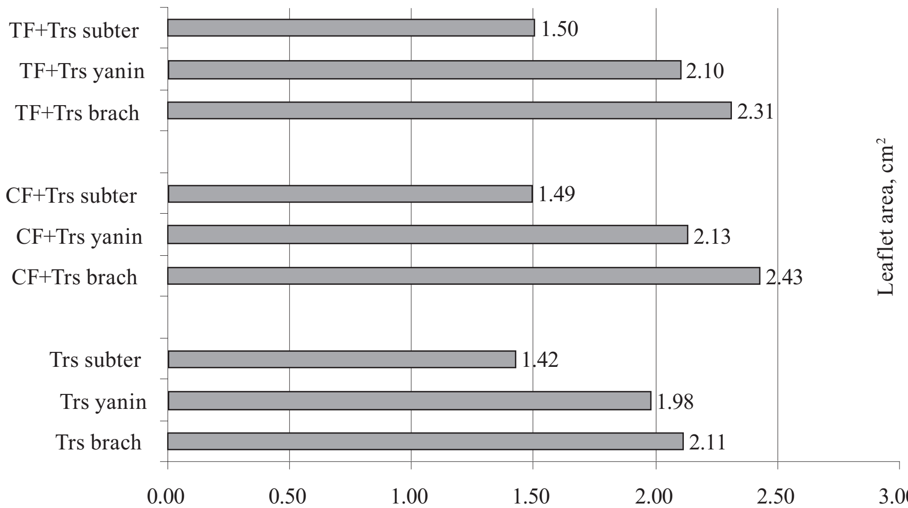

Fig. 1. Values of leaflet area of subterranean clover (pure and mixtures) (forsubclovers pure grown, ±SD = 0.37, SE (P = 0.05) = 0.21; for subclovers in mixture with cocksfoot, ±SD = 0.48, SE (P = 0.05) = 0.27; for subclovers in mixture with tall fescue, ±SD = 0.42, SE (P = 0.05) = 0.24

let area formed *Trifolium subterraneum ssp. brachycalicinum*, followed by *Trifolium subterraneum ssp. yaninicum* and *Trifolium subterraneum ssp. subterraneum* (Figure 1).

Leaflet area that subterranean clover formed in mixtures with cocksfoot is greater than as a pure crops, namely for *Trifolium subterraneum ssp. brachycalicinum* (2.43 vs. 2.11), *Trifolium subterraneum ssp. yaninicum* (2.13 vs. 1.98) and *Trifolium subterraneum ssp. subterraneum* (1.49 vs. 1.42). The difference between leaflet area formed in mixtures with cocksfoot and the pure crops was the largest for *Trifolium subterraneum ssp. brachycalicinum* (by 15.2%) and less (from 4.9 to 7.6%) for the other two subspecies.

In mixtures with tall fescue leaflet area of subterranean clover was also more than as a pure crops, i.e., *Trifolium subterraneum ssp. brachycalicinum* (2.31 vs. 2.11); *Trifolium subterraneum ssp. yaninicum* (2.10 vs. 1.98) and *Trifolium subterraneum ssp. subterraneum* (1.50 vs. 1.42). In *Trifolium subterraneum ssp. brachycalicinum* difference, similar to the mixture with cocksfoot was greatest in comparison with pure grown subclover (by 9.5%). The leaves of *Trifolium subterraneum ssp. brachycalicinum* were with larger size (morphological trait), making it more tolerant of shading and more competitive with compaining component.

The larger leaflet area formed from the subterranean clover in mixtures was due to the higher nitrogen use efficiency. The components included in the mixtures tested have different type of nitrogen metabolism. Nitrogen in grasses is the result solely of nitrate nitrogen assimilation through the

Table 1
Plastid pigments content in cocksfoot and tall fescue in mixtures with subterranean clover

Treatments	Chl. a	Chl. b	Chl. a/b	Chl. a+b	Carotenoids	Chl. a+b/ carotenoids	Total	% to pure
	mg/100 g FW							
Trs brach (100%)	144.94	107.44	1.35	252.05	47.80	5.27	300.18	-
Trs yanin (100%)	141.24	94.94	1.49	236.18	47.17	5.01	283.35	-
Trs subter (100%)	138.14	99.91	1.38	238.05	45.48	5.23	283.53	-
Average	141.44	100.76	1.41	242.20	46.82	5.17	289.02	
SE (P = 0.05)	1.96	3.63	0.04	5.00	0.69	0.08	5.58	
±SD clovers	3.40	6.29	0.07	8.67	1.20	0.14	9.67	
Cocksfoot (100%)	92.91	67.36	1.38	160.27	35.53	4.51	195.80	-
Cocksfoot +	109.57	77.86	1.41	187.43	40.12	4.67	227.55	+16.2
Trs brach (50:50)	163.99	121.06	1.35	285.05	46.89	6.08	331.94	+10.6
Cocksfoot +	93.03	67.36	1.38	160.39	34.47	4.65	194.86	-0.5
Trs yanin (50:50)	135.79	99.21	1.37	235.00	40.5	5.80	275.50	-2.8
Cocksfoot +	79.4	57.07	1.39	136.47	27.95	4.88	164.42	-16.0
Trs subter (50:50)	143.74	104.47	1.38	248.21	49.08	5.06	297.29	+4.9
SE (P = 0.05) grass	6.17	4.24	0.01	10.41	2.50	0.01	12.88	
SE (P = 0.05) clover	8.39	6.58	0.01	14.89	2.57	0.30	16.43	
±SD grass	12.34	8.48	0.01	20.82	5.01	0.15	25.77	
±SD clover	14.54	11.40	0.02	25.79	4.45	0.52	28.46	
Tall fescue(100%)	101.71	72.05	1.41	173.76	38.67	4.49	212.43	-
Tall fescue+	104.19	73.84	1.41	178.03	38.44	4.63	216.47	+1.9
Trs brach (50:50)	132.42	99.7	1.33	232.12	40.81	5.69	272.93	-9.1
Tall fescue+	84.28	59.79	1.41	144.07	30	4.80	174.07	-18.1
Trs yanin (50:50)	152.25	110.4	1.38	262.65	43.5	6.04	306.15	-8.0
Tall fescue+	103.17	76.7	1.35	179.87	35.17	5.11	215.04	+1.2
Trs subter (50:50)	160.49	115.94	1.38	276.43	47.79	5.78	324.22	+14.4
SE (P = 0.05) grass	4.71	3.72	0.01	8.38	2.02	0.13	16.66	
SE (P = 0.05) clover	8.33	4.76	0.01	13.09	2.03	0.10	31.83	
±SD grass	9.42	7.45	0.03	16.77	4.04	0.27	33.32	
±SD clover	14.42	8.25	0.03	22.67	3.52	0.18	55.14	

roots due to the activity of nitrate reductase, while in subclovers nitrogen fixation process was included. Upon successful competition among the components for available nitrogen, the efficiency of use of nitrogen is higher.

By comparing the leaflet area of clovers in the mixtures with grasses, it is seen that in mixtures with cocksfoot, the leaflet area was greater than formed in mixtures with tall fescue. We assume it is due to the allelopathic effect of tall fescue found by Siegel and Bush (1997) and Renne et al. (2004).

Plastid pigments content

The content of plastid pigments as an indicator of the physiological status of the plant is changing under the influence of various factors.

In our study, the average data for the three years showed that the highest total plastid pigments content in pure grown subclovers was found in *Trifolium subterraneum ssp. brachycalicinum* (300.18 mg/100 g FW), followed by *Trifolium subterraneum ssp. subterraneum* (283.53 mg/100 g FW) and *Trifolium subterraneum ssp. yaninicum* (283.35 mg/100 g FW) (Table 1). For grasses, total plastid pigments content in tall fescue was 212.43 mg/100 g FW, and in cocksfoot – 195.80 mg/100 g FW. It is evident that the results in the two groups of crops slightly varied.

In mixtures plastid pigments content varied in a stronger degree, due to the competitive relationship between the components.

The physiological status in terms of total plastid pigments content was changed more significant in plants from the mixtures of cocksfoot with subterranean clover compared to when grass component was tall fescue. In mixtures with *Trifolium subterraneum ssp. brachycalicinum* plastid pigments content in cocksfoot increased by 16.2% and in tall fescue remains unchanged. In mixtures with *Trifolium subterraneum ssp. yaninicum* plastid pigments content in cocksfoot did not change, but decreased significantly (by more than 18.0%) in tall fescue. The trend was reversed only in mixtures with *Trifolium subterraneum ssp. subterraneum*, where the plastid pigments content did not change in tall fescue, but decreased by 16.0% in cocksfoot.

The physiological status of subterranean clover was also more favorably influenced in mixtures with cocksfoot. Plastid pigments content in *Trifolium subterraneum ssp. brachycalicinum* increased by approximately 11.0% in a mixture with cocksfoot and decreased by 9.1% in mixture with tall fescue. In *Trifolium subterraneum ssp. yaninicum* the content of plastid pigments decreased slightly when grass component was cocksfoot, and by 8.0% when grass component was tall fescue. In *Trifolium subterraneum ssp. subterraneum* an increase of that plastid pigments content as well as in mixtures with cocksfoot (4.9%) and with tall fescue (14.4%) was observed. Increase the total content of photosynthetic pigments in both components was found in the mixture of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (by 16.2% and 10.6%, respectively), while proven increase the plastid pigments content in the two components in the mixtures of tall fescue was not observed.

The degree of formation of the photosynthetic apparatus was assessed by the ratio of chlorophyll a to chlorophyll b. This ratio is related to the basic activity of chlorophyll a and in its high values are indications of the greater intensity of photosynthesis (Titova, 2010).

The ratio of chlorophyll a to chlorophyll b in pure grown subterranean clovers was as follows: *Trifolium subterraneum ssp. yaninicum* (1.49), *Trifolium subterraneum ssp. subterraneum* (1.38) and *Trifolium subterraneum ssp. brachycalicinum* (1.35). In grasses, the values were in close range, i.e. – tall fescue (1.41) and cocksfoot (1.38).

In the components of mixtures a slight variation in the values of this ratio was observed, but generally it was stable.

The chlorophyll a+b/carotenoids ratio is also important as a characteristic of the photosynthetic apparatus and respond to changes in environmental factors. In our study, its values for pure grown crops, as well as mixtures were similar. Thus, in mixtures, the ratio of chlorophyll a+b/carotenoids for cocksfoot was between 4.65 and 4.88. *Trifolium subterraneum ssp. brachycalicinum* in mixtures with cocksfoot showed a higher chlorophyll a+b/carotenoids ratio as compared to that of pure grown subclovers (6.08 vs. 5.27). The same is for *Trifolium subterraneum ssp. yaninicum* (5.80 vs. 5.01).

In other grass component – tall fescue, the values of the ratio chlorophyll a+b/carotenoids were in the range of 4.63 to 5.12. In the subterranean clover the highest value of the ratio of chlorophyll a+b/carotenoids compared to pure grown subclover was recorded in *Trifolium subterraneum ssp. yaninicum* (6.04 vs. 5.01).

In mixtures the legume component through nitrogen-fixing ability is supplying grasses with nitrogen, which affects positively on the physiological status of the plants. It is known that legumes and grasses have different type of root system. Legumes have deep rooting system but grasses even when these which developed deeper root system most of the roots are concentrated in the top of the soil to a depth up to 20 cm. The ability grasses to absorb the fixed nitrogen from legumes are different for different grasses and depend on the activity of roots. According Matsunaka and Takahasi (2001) cocksfoot has the ability to quickly absorption (absorption) of nitrogen to the roots. This is a possible reason for the obtained better results in terms of data for physiological status of cocksfoot in mixtures with subterranean clover.

Conclusions

Trifolium subterraneum ssp. brachycalicinum formed the most leaflet area (2.11 cm^2), followed by *Trifolium subterraneum ssp. yaninicum* (1.98 cm^2) and *Trifolium subterraneum ssp. subterraneum* (1.42 cm^2).

Leaflet area of *Trifolium subterraneum ssp. brachycalicinum* in mixtures was by 15.2% greater as compared to pure subterranean clover, when grass component was cocksfoot and by 9.5%, when grass component was tall fescue.

In total content of plastid pigments (chlorophyll a+b and carotenoids) pure grown subclovers rank: *Trifolium subterraneum ssp. brachycalicinum* (300.18 mg/100 g FW), *Trifolium subterraneum ssp. subterraneum* (283.53 mg/100 g FW) and *Trifolium subterraneum ssp. yaninicum* (283.35 mg/100 g FW).

Plastid pigments content was found be higher in both components of the mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (by 16.2% and 10.6%, respectively).

References

Albayrak, S., M. Turk, O. Yuksel and M. Yilma, 2011. Forage yield and the quality of perennial legume-grass mixtures under rainfed conditions. *Not. Bot. Hort. Agrobot. Cluj.*, **39**: 114-118.

Chourkova, B., 2014. Productivity and Botanical Composition of a Mixed Sward of Birdsfoot Trefoil and Red Fescue Depending on the Term of Sowing and Proportion of Components. *International Journal of Agriculture Innovations and Research,* **3** (1): 276-280.

Evers, G. W., and Y. Newman., 2008. Arrowleaf, crimson, rose, and subterranean clover growth with and without defoliation in the southeastern United States. *Agron. J.,* **100**: 221-230.

Ilieva, A., V. Vasileva and A. Katova, 2015: The effect of mixed planting of birdsfoot trefoil, sainfoin, subterranean clover, and tall fescue on nodulation, and nitrate reductase activity in shoots. *Journal of Global Agriculture and Ecology,* **3** (4): 222-228.

Kusvuran, A., Y. Ralice and T. Saglamtimur, 2014. Determining the biomass production capacities of certain forage grasses and legumes and their mixtures under Mediterranean regional conditions. *Acta Adv. Agric. Sci.*, **2**: 13-24.

Luscher, A., I. Mueller-Harvey, J. F. Soussana, R. M. Rees and J. L. Peyraud, 2014. Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science,* **69**: 206-228.

Matsunaka, T. and H. Takahasi, 2001. Root weight as a principal factor responsible for difference in nitrogen absorption among cocksfoot (*Dactylis glomerata* L.), Meadow Fescue (*Festuca elatior* L.) and Timothy *(Phleum pratense* L.) during first growing period. *Plant Nutrition, Developments in Plant and Soil Sciences*, **92**: 592-593.

Nichiporovich, A., 1971. Some principles to optimize photosyntethetic activity of plants in crops. *L. Nauka*, pp. 104-108.

Nichols, P. G. H., C. K Revell, A. W. Humphries, J. H. Howie, E. J. Hall, G. A. Sandral, K. Ghamkhar and C. A. Harris, 2012. Temperate pasture legumes in Australia – their history, current use and future prospects. *Crop and Pasture Science,* **63**: 691-725.

Nurmakova, J., 2013. Photosynthetic characteristics of sorghum, soybeans and mixed crops in agro-ecosystems. *Natural Science*, **2**: 196-201.

Østrem, L., B. Volden and A. Larsen, 2013. Morphology, dry matter yield and phenological characters at different maturity stages of ×Festulolium compared with other grass species. *Acta Agriculturae Scandinavica*, Section *B-Soil & Plant Science*, **63** (6): 531-542.

Pecetti, L. and E. Piano, 1998. Leaf size variation in subterranean clover *(Trifolium subterraneum* L. sensu lato). *Genetic Resources and Crop Evolution*, **45** (2): 161-165.

Porqueddu, C., G. Parente and M. Elsaesser, 2003. Potential of grasslands. In: A. Kirilov, N. Todorov and I. Katerov (Eds.). *Grassland Science in Europe,* **8**: 11-20.

Renne, I. J., B. G. Rios, F. S. Jeffrey and B. F. Benjamin, 2004. Low allelopathic potential of an invasive forage grass on native grassland plants: a cause for encouragement. *Basic and Applied Ecology*, **5** (3): 261-269.

Siegel, M. R. and L. P. Bush, 1997. Toxin production in grass/endophyte associations. *Plant Relationships*, **5**: 185-207.

Smart, A. J., W. H. Schacht, J. F. Pedersen, D. J. Undesander and L. E. Moser, 1998. Prediction of leaf:stem ratio in grasses using near infrared reflectance spectroscopy. *Journal of range Management*, **51** (4): 447-449.

Smirnova, E., V. Reshetnikova, T. Makarova and G. Karavaeva, 2013. Features of genotic relations in the one-specy and mixed crops of *Mellilotus officinalis* L. *Proceedings of the Samara scientific center of RAS*, **15** (3): 793-795.

SPSS, 2012. SPSS Version 20.0. SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois.

Titova, M., 2010. Content of photosynthetic pigments in needles of *Picea Abies* и *Picea koraiensis*. *Vestnik of taiga station of DVO RAS,* **12** (118): 9-12.

Vasilev, E., 2006.Productivity of subterranean clover (*Tr. subterraneum* L.) in pasture mixtures with some perennial grasses for the conditions of Central North Bulgaria. *Plant Science S.*, 4 (2): 149-152 (Bg).

Vasileva, V. and E. Vasilev, 2012. Dry mass yield from some pasture mixtures with subterranean clover (*Trifolium subterraneum* L.). *Journal of Mountain Agriculture on the Balkans,* **15**: 1024-1033.

Vučković, S. M., 2004. Travnjaci. Poljoprivredni fakultet Univerzitet, ISBN868073375X, 506 p.

Zelenskii, M. and G. Mogileva, 1980. Comparative Evaluation of Photosynthetic Ability of Agricultural Crops by Photochemical Activity of Chloroplasts. *VIR*, Leningrad, pp. 36 (Ru).

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