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AGRONOMIC CHARACTERIZATION AND THE POSSIBILITY FOR POTENTIAL USE OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.) IN THE FORAGE PRODUCTION IN BULGARIA

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Abstract

The permanent climate changes having occurred in the last decade present a serious risk to the agricultural crops. This requires to study new herbaceous forage species having pronounced resistance to unfavorable abiotic factors and good adaptive capacity towards the new conditions. Legumes species that can provide self-sowing and persist continuously in the sward become of practical importance. Subterranean clover (*Trifolium subterraneum* L.) is an annual drought resistant legume with winter-spring type of development and ability for self-sowing. The studies with subterranean clover during the last years showed that it has practical applicability under the climatic conditions of Bulgaria. When sown at an appropriate time in the autumn, it establishes a uniform stand before the beginning of the permanent cold spell and grows up early in the spring and forms a dense sward. Subterranean clover was found as a suitable component for mixtures with widely used perennial grass and legume forage crops and contributed to weed infestation decreasing, higher productivity and persistence of the pasture systems. In addition, due to the prostrate habit it is strongly tolerant to grazing. The subterranean clover is adaptable to the changing climatic conditions and its use as a natural bio-recourse in the pastures could be a contribution to finding a solution in the field of forage production to mitigate the adverse effects of climatic change.

Key words: *Trifolium subterraneum*, Forage production, Self-sowing

Introduction

The permanent climate changes having occurred in the last decade present a serious risk to the agricultural crops, including forage crops. They have a negative impact on the species composition, productivity and quality of dry matter, as well as their durability. This requires to study new plant species having pronounced resistance to unfavorable abiotic factors and good adaptive capacity towards the new conditions (Gornall *et al*., 2010). Having in a mind the need of adaptation of forage crops to the changed conditions, the species that can provide self-sowing and persist continuously in the sward become of practical importance (Nichols *et al*., 2012).

Subterranean clover (*Trifolium subterraneum* L.) belongs to Fabaceae family. Common names are subterranean clover and subclover. Native to southern Europe, north Africa and southern England. It is a widespread component in the pastures and other grasslands of the Mediterranean zones and Australia. The species is found in Bulgaria in open, dry grasslands in the plains and lowlands. It is known from the Black Sea coast, Danube Plain, Northeastern Bulgaria, Sofia Region, Western Border Mountains, Struma Valley, Mesta River Valley, Thracian Lowland, Tundzha Plain, Eastern Rhodopes, Rila, Strandzha to 500 m above the sea level (Assyov *et al*., 2012). The area of the species also includes Western and Southern Europe (in the north to Transylvania, in the east to the Crimea), the Mediterranean countries, Southwestern Asia, Northern Africa, Macaronesia. It is an annual drought resistant plant with winter-spring type of development (Nichols *et al*., 2012).

Subclover does best on well drained, slightly acidic soils. It is strongly self-fertile; diploid with 2n=2x=l6 chromosomes. Adapted to regions with a Mediterranean climate, viz. hot dry summers, moist, mild winters, and annual rainfall, 350-1200 mm, also to warm, temperate areas. It flowers in March-April. Its reproductive organs are formed in early May and the seeds ripen before the end of the spring in hedgehog-shaped heads (10.0-15.0 mm spherical shape) that remain on the soil surface (Porqueddu *et al*., 2003). The precipitations during the late summer contribute to emergence of new self-sown plants. Life cycle of subclover is adapted to escape summer drought. It germinates in autumn with main growth during autumn to spring, produces seeds prolifically and remains dormant during summer with the seed burrs embedded or buried in the upper layers of the soil.

Subclover swards die back during summer drought but regenerate from the soil seed bank following autumn rainfall. Provided the seed bank is adequate, and there is sufficient time and suitable conditions for further hard seed breakdown, the sward will re-establish from hard seed reserves. In these environments it is advantageous to use cultivars with higher levels of hard seed. Small seeds may also be more advantageous than large seeds since they need less water for germination because of a higher ratio of surface to volume (Pecetti & Piano, 1994).

Subterranean clover is distinguished for very good winter resistance, and in the spring it grows up quickly after the forced winter dormancy forming a dense sward (Porqueddu *et al*., 2003). The effective utilization of autumn-winter soil moisture, successful seed formation and self-sowing at the end of spring allow to the subterranean clover to avoid summer droughts (Piano *et al*., 1996).

As a legume species, the subterranean clover is nitrogen fixing (Ferreira & Castro, 2005). It develops symbiotrophic relations with bacteria from the species *Rhizobium leguminosarum biobar trifolii*. The amount of fixed nitrogen per year varied from 50 to 188 kg/ha

(Bolger *et al*., 1995). Nowadays, this crop is of interest and we tried to study the potentiality of its growing as a forage resource under the climatic conditions of Bulgaria.

Material and Methods

The studies were mainly performed in the Institute of Forage Crops, Pleven (43º 23'N, 24º 34'E, 230 m altitude) during the last 15 years. The experiments, both, pot and field were carried out on haplustoll and podzolized soil subtype. Three subspecies of subclover, i.e. *Trifolium subterraneum* ssp. *brachycalicinum* (cv. Antas), *Trifolium subterraneum subterraneum* ssp. *yaninicum* (cv. Trikkala) and *Trifolium subterraneum 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 for the filed trials 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 sowing for pot experiments was done on the depth 1-1.5 cm for subclover, white clover and birdsfoot trefoil; 0.5-1 cm for cocksfoot, and 3 cm for sainfoin.

Results and Discussion

When subterranean clover was sown at an appropriate time in the autumn, it establishes a uniform stand before the beginning of the permanent cold spell and grows up early in the spring and forms a dense sward. Rainfall has a major influence and within an annual dry mass yield range of 360 to 430 kg/da (Fig. 1).

Dry mass yield depends on the leaf ness of subclovers. Leaflet area is also essential for the competitiveness of subterranean clover (Pecetti & Piano, 1998; Evers & Newman, 2008). However, subspecies differ in this regard. Leaflet area of different subclovers was assessed measuring length and breadth of the middle leaflet of clovers on three representative leaves. Formulaes of Pecetti & Piano (1998) were used: (length x breadth) x 0.769 for *Trifolium subterraneum* ssp. *brachycalicinum*, (length x breadth) x 0.645 for *Trifolium subterraneum* ssp. *yaninicum* and (length x breadth) x 0.730 for *Trifolium subterraneum* ssp. *subterraneum*. Most leaflet area formed *Trifolium subterraneum* ssp. *brachycalicinum* (2.11 cm^2), followed by ssp. *yaninicum* (1.98 cm^2) and ssp. *subterraneum* (1.42 cm^2) (Fig. 2) (Vasileva & Ilieva, 2017).

Mixed crops have an essential role in building a system of sustainable and ecologically friendly farming (Luscher *et al*., 2014). They are more effective than pure grown in using environmental resources, better withstand adverse conditions and are more productive and long-term (Elessesser, 2004). In addition, mixed cropping are more effective over pure with regard to the nitrogen transfer from legume to grasses (Frame, 2005; Frame & Laidlaw, 2005; Vasilev *et al*., 2005; Nyfeler *et al.*, 2006; Pozdisek *et al*., 2011) (for grass-legume mixtures).

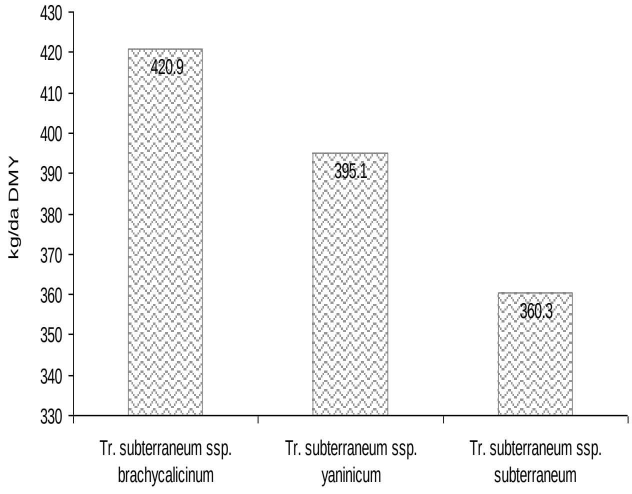

Fig. 1. Dry mass yield of subterranean clover [SE (P=0.05), 0.17 kg/da].

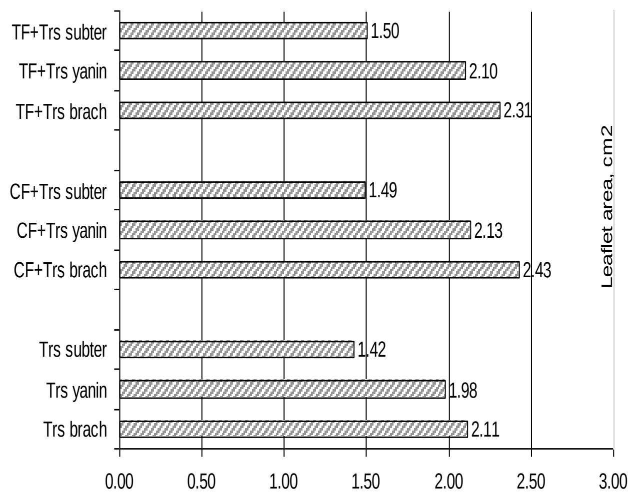

Fig. 2. Values of leaflet area of subterranean clover (pure and mixtures). (for subclovers pure grown, ± SD=0.37, SE (P=0.05)=0.21; for subclovers in mixture with cocksfoot, ± SD=0.48, SE (P=0.05)=0.27; for subclovers in mixture with tall fescue, ± SD=0.42, SE (P=0.05)=0.24.

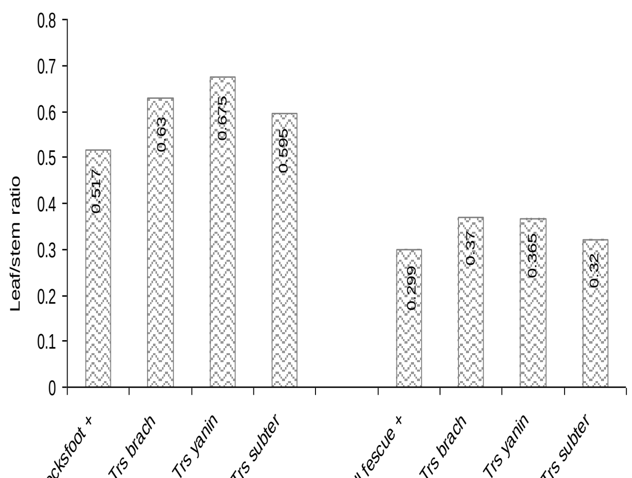

Fig. 3. Leaf/stem ratio of cocksfoot and tall fescue pure and in mixture with subterranean clover.

The subterranean clover was tested pure and in mixtures. Traditional grasses were used for components of subclover in mixtures, i.e. cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), and legumes - birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis viciifolia* Scop.), alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) in different ratios. Their use in the pastures of temperate countries is known practice (Vučković, 2004).

Subterranean clover was found as a suitable component for mixtures with above-mentioned perennial grass and legume forage crops and contributed to weed infestation decreasing, higher productivity and persistence of the pasture systems. In subclover-grass mixtures subclover yields are best with companion grasses which have a complementary growth rhythm together with a grazing management which favours the legume component. It is known competition between weeds and crops in agro-ecosystems is important factor which leads to decreasing crop yield (Nakova & Christova, 2012).

Subterranean clover and white clover, both alone and in mixtures with perennial ryegrass (50:50%) were compared in four-year field trial (Vasileva & Vasilev, 2012). Dry mass yield, botanical composition and other characteristics as the main chemical composition of the forage biomass obtained were assessed. It was found that dry mass productivity of pure sward of subterranean clover (401.1 kg/da) was higher as compared to white clover (296.0 kg/da) by 26.2%. Botanical composition showed that the proportion of subterranean clover was significantly higher as compared to that of white clover. Comparing white clover with subterranean clover, the subterranean clover forms a dense sward with insignificant part of weeds (the weed infestation was significantly less than that of white clover) and after the occurrence of late summer and early autumn precipitations the subclover grows up faster than white clover.

Subterranean clover as a legume component contributed to increasing the leaf/stem ratio of cocksfoot and tall fescue mixtures (Vasileva, 2016) (Fig. 3). This ratio is an important factor affecting quality and forage intake and it was calculated on the base of fresh weight. More leaf biomass formation from grasses in mixtures with legume component was associated to better nitrogen assimilation (Ledgard & Steel, 1992).

Due to the prostrate growth habit subterranean clover is adapted to intensive grazing systems and has high tolerance to grazing (Evers & Newman, 2008). It can flower and set seed under close grazing. In *In vivo* trials the palatability or the preference of sheep to the subterranean clover and to other commonly used perennial forage crops was studied (Kirilov & Vasileva, 2016). Thirty sheep number of Pleven Blackface sheep were used to graze the plots in three consecutive days, one hour a day. Cells were placed on the swards before grazing and through the difference between the amount before and after grazing, the accepted amounts from each grasses was established. According to data for grazing amount as share - percentage of the quantity available before grazing, the types of grasses were classified. Highest palatability had this crops from which were grazed the most compared to the initial amount available.

Results obtained showed that subterranean clover was grazed at 100% and from the legumes sheep prefer most this crop followed by birdsfoot trefoil and sainfoin, and from the grasses - cocksfoot (Table 1). Subterranean clover consist higher crude protein and lower crude fiber content, thus is a palatable and preferred for grazing by sheep forage crop compared to sainfoin, cocksfoot and tall fescue.

Table 1. Comparative data for the palatability during the grazing of some forage crops by sheep.

Crops	Dry matter before grazing	Dry matter after grazing	Before grazing	Grazed grass	Grazed grass	
	%		kg/da		%	
Subclover	21.63	-	55.4	55.4	100.00	(1)
B. trefoil	30.70	40.20	265.6	209.6	78.92	(3)
Sainfoin	29.46	41.18	196.8	136.7	69.46	(4)
Cocksfoot	29.02	38.78	193.9	165.4	85.30	(2)
Tall fescue	31.42	35.85	154.9	103.4	66.75	(5)
SE (P=0.05)	1.7	1.16	34.4	26.2	5.98	

Conclusions

Subterranean clover could be grown both, alone and in mixtures. It is a suitable component for mixtures with widely used perennial grass (cocksfoot, tall fescue, perennial ryegrass, and legume (alfalfa, birdsfoot trefoil, sainfoin) forage crops. Subterranean clover has practical applicability under the climatic conditions of the country. It is adaptable to the changing climatic conditions and responsible to the challenges of agriculture nowadays for bigger resource use efficiency.

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