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SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.) AS A PROMISING FORAGE SPECIES IN BULGARIA

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Abstract

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The paper presents an overview of the possibility to use the subterranean clover (*Trifolium subterraneum* L.) (an annual leguminous self-seeding species, widespread component in the pastures of the temperate areas of Central and Northern Europe and America) for the forage production in Bulgaria. The biological and ecological characteristics of the species are given. On the basis of researches (pot and field trials), mainly performed in the Institute of Forage Crops, Pleven, Bulgaria and their results, the conclusions are drawn as for the using of subterranean clover as a forage resource under the climatic conditions of the country. 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. The subterranean clover may be included in mixtures with the following components: legumes - birdsfoot trefoil and sainfoin and grasses – cocksfoot, tall fescue, perennial ryegrass and crested wheatgrass. It decreases weed infestation, increases productivity and persistence of the pasture systems. Composition of the natural communities, in which it participates, shows that it is resistant to overgrazing, nitrification and ruderalization by domestic animals. Due to its self-seeding capacity, it may be used for under-sowing of degraded seed production stands. On the basis of its occurrence in different parts of the country and in the natural vegetation, the species is promising for establishment of forage crops. The participation of the subterranean clover in the perennial swards is also a tool to increase the biodiversity in the agrophytocoenosis. Subterranean clover as a component of sown pasture swards has practical applicability under the climatic conditions of Bulgaria.

Key words: subterranean clover, self-seeding, mixture

Introduction

The permanent changes having occurred in the last ten-year periods in the climate (increase of average yearly temperatures, long droughts in the spring and summer, increase of CO_2 concentration in the atmosphere) present a serious risk to the agricultural crops (Gornall et al., 2010; Aranjueloa et al., 2014). This requires to study new herbaceous forage species having pronounced resistance to unfavorable abiotic factors and good adaptive capacity towards the new conditions (Lelièvre and Volaire, 2009). At present the main interest in directed towards more drought resistant and drought tolerant components. Legumes species that can provide self-sowing and persist continuously in the sward become of practical importance (Carneiro, 1999).

Subterranean clover (*Trifolium subterraneum* L.) is a widespread component in the pastures and other grasslands of the temperate areas of Central and Northern Europe and America (Frame et al., 1998; Pecetti and Piano, 1998, 2002; Kyriazopoulos et al., 2008).

The species is found In Bulgaria in open, dry grasslands in the plains and lowlands. It is known from the Black Sea

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coast, Danube Plain (areas of Byala Slatina and Svishtov), 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 (Figure 1) (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 (Coombe, 1968).

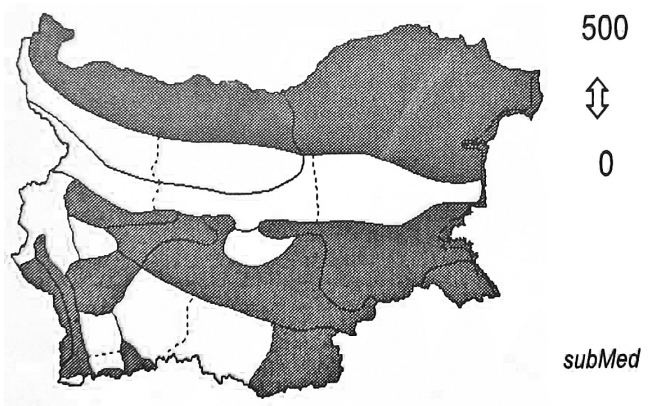

Fig. 1. Distribution of subterranean clover in Bulgaria (Assyov et al., 2012; Conspectus of the Bulgarian Vascular Flora)

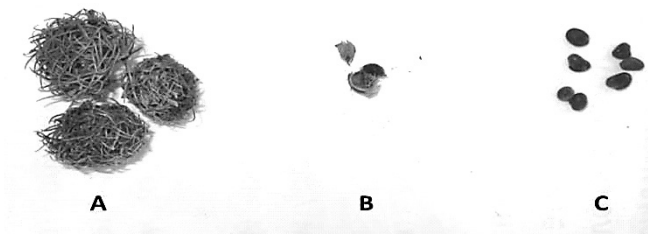

Fig. 2. Heads (A), pods (B) and seeds (C) of subterranean clover

The subterranean clover is an annual drought resistant ephemeral legume with winter-spring type of development and ability for self-sowing (Yakimova and Yancheva, 1986; Piano et al., 1996; Frame et al., 1998).

Its reproductive organs are formed in early May, and the seeds ripen before the end of the spring in hedgehog-shaped heads that remain on the soil surface (Figure 2). The precipitations during the late summer contribute to emergence of new self-sown plants (Frame et al., 1998).

An important element of the biology of this species is that a big part of the seeds germinated during the autumn. Other part germinated during the spring of the next year but because of the winter-spring type of the development the plants took part in the sward during most of the vegetation season, some of them wintering and on the next spring formed seeds (Frame et al., 1998; Vasilev, 2006). Substantial part of the formed seeds is solid and germinates after two-three years. This biological specificity turns the superficial soil layer into an original seed bank which makes the species even more flexible one (Pecetti and 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; Porqueddu et al., 2003).

As a legume species, the subterranean clover is nitrogen fixing (Ferreira and 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^{-1} (Sanford et al., 1994; Bolger et al., 1995).

Some biological and ecological characteristics of the subterranean clover are listed bellow.

Biological characteristics

Subterranean clover is an annual plant, with several to numerous stems. Stems are 5.00–30.0 (45.0) cm long, branched, creeping, semi-appressed hairy. Stipules are ovate (egg-shaped), short sharp-pointed, often with auricles, with whole edges or slightly indented, membranous, with clear veins, ⅓–½ of their length accrete, much shorter than the leaf petioles, glabrous. Leaf petioles are 5.0–25.0 mm long, a little longer to much longer than leaflets, often filiform (thread-like), more or less hairy. Leaflets are 1.0–1.3 mm long and 6.00-9.00 mm wide, cordate (heart-shaped), broadly obovate (inversely egg-shaped), more rarely broadly triangular cuneate (wedge-shaped), with 8–12 lateral, protuberant veins, on the edge in the upper third part short indented, on the two sides or mostly on the underside semi-appressed rust hairy, on 0.5 mm long petioles, equal between them. Inflorescence peduncles are curved, shorter, more rarely longer than the bracts (leaves associated with inflorescences), axillary, sparsely long rust white or light rust hairy. Heads are 10.0–15.0 mm spherical, loose, appressed on the soil, self-burying. Flowers are 8.0–14.0 mm long, 2–5 (7) fertile, outer and numerous inner sterile, developing after fall of the fertile ones, almost sessile or on pedicles shorter than 0.5 mm

without bracts (leaves associated with flowers). Calyx is 5.0–6.0 mm long, glabrous; tube is pale, often red purple, membranous, at its upper end often violet, cylindrical bell-shaped, with 5–10 unclear veins, teeth are slightly unequal between them, filiform (thread-like), longer than the tube, outspread hairy. Corolla is 2 times longer than the calyx, pale pink, after fall yellow brown. Standard is fused to the wings and keel in a long tube and is a little longer than them, blade is narrowly obovate (inversely egg-shaped), smooth. Wings are with an obovate (inversely egg-shaped), shorter than the filiform (thread-like) claw, slightly veined blade, ligule is unclear. Keel is blunted with an elliptically cordate (heart-shaped) blade and a claw longer than it, with a poorly developed ligule. Barren flowers are of calyxes only, with branched teeth, during fruiting of the fertile calyx teeth they accrete, spread out radially and surround the pod forming a 10.0–15.0 mm head that at the end of the flowering is completely buried in the soil. Pod is elliptical, one-seeded, skin-like, protruding above the calyx. It flowers in March-April, and fruits in April-May (Kozhuharov, 1996).

Ecological characteristics

Two associations have been described by the dominant method where subterranean clover is a co-dominant in the literature in Bulgaria.

The association of *Cynodon dactylon-Lolium perenne-Trifolium subterraneum* is from the valley of Studena River near village of Batak (Pavlikeni area) (Ganchev and Kochev, 1962). It is a slightly sloping (4–5°) ground with southwesterly exposure. Soils are chernozemic, trampled. The ground is a pasture. Characteristic species are *Trifolium repens, Trifolium campestre, Plantago lanceolata, Erodium cicutarium*. The characteristic for the associations is that they have moderate ruderalization mainly due to the effect of overgrazing by domestic animals. This shows that the species is influenced rather favourably by the effects of overgrazing and trampling.

Association of *Poa bulbosa-Trifolium subterraneum* – it was described by Ganchev (1958). It is a pasture on some alluvial-meadow soils.

By the method of Braun-Blanquet (Braun-Blanquet, 1964) it is mentioned among the species composition of sparse forests of pubescent oak in Southwestern Bulgaria (association of *Cisto incani-Quercuetum pubescentis* and *Teucrium polium-Quercus pubescens*) (Gogushev, 2009).

This is an indicator for the high ecological flexibility of the species and its presence in habitats in sparse oak forests, but also in pastures differing in slope, soil cover, degree of grazing and nitrification.

Materials and Methods

Studies with subterranean clover in Bulgaria

During the last 15 years studies with subterranean clover were conducted in Bulgaria. They were mainly performed in the Institute of Forage Crops, Pleven, but some of analyses and pot experiments were performed in the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia; University of Forestry, Sofia; The Institute of Soil Science, Agro-Technology and Plant Protection "Nikola Pushkarov", Sofia; the Sofia University "St. Kliment Ohridski"; the Institute of Plant Physiology and Genetics National Academy of Sciences of Ukraine, Kiev (Vasilev, 2006, 2009; Ilieva and Vasileva, 2011; Vasileva et al., 2011; Vasileva and Vasilev, 2012; Vasileva and Vasilev, 2012; Vasileva, 2014, 2015; Ilieva et al., 2015).

The subterranean clover was tested pure and in mixture with the traditional grasses (cocksfoot, tall fescue, ryegrass) and new one as crested wheatgrass, and with the legumes (birdsfoot trefoil, sainfoin, alfalfa, white clover). The experiments were carried out on haplustoll and podzolized soil subtype.

Studies with the subterranean clover are directed to its growth and development, relations with the crops tested, botanical composition (percentage of participation in the swards) and productivity of dry matter; as legume plant – on the productivity of dry root mass, nodulating ability, nitrogen fixing activity, accumulation of nitrogen in the soil.

From the pot experiments carried out

Plant and development, productivity of dry shoot and root mass, nodulating ability and some other parameters of subterranean clover pure and in two component (50:50%) and three component mixtures (33:33:33%) were studied. Birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis viciifolia* Scop.) were used for legume components. Different grasses were included in the studies – cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) as traditional for the practice and new – wheatgrass (*Agropyron cristatum* (L.) Gaertn.) (highly productive, winter and dry resistant with the possibility for pasture and hay-pasture direction of use) and ryegrass (*Lolium perenne* L.) (winter resistant, tolerant to drought, suitable for hay-pasture direction of use) (Katova, 2011, 2012).

No inhibition in the growth and development of the plants as well significantly higher dry shoot and root mass productivity in the mixtures with grasses were observed (Vasileva and Vasilev, 2012; Vasileva, 2014; Vasileva, 2015). Better growth of subterranean clover in mixtures was determined from the accumulation of greater amount of total nitrogen,

reducing in the water-soluble sugars content and amino acids in the leaves. One possible explanation for the higher nitrogen-use efficiency of subterranean clover under inter cropped conditions could be sought in the successful competition between the two species for available inorganic nitrogen in the solution which favored nitrogen fixation (Vasileva et al., 2015a). Fresh weight leaves/fresh weight stems in grass components was higher in mixtures as compared to subterranean clover pure.

Subterranean clover formed the highest number of nodules in the mixtures. One of the reasons for this could be enhanced competition between the two species for available nitrogen in the media.

From the field experiments carried out

Chemical composition of the forage and crude protein yield of subterranean clover and white clover (pure) and in binary mixtures (1:1) with some perennial grasses (wheatgrass and ryegrass) in the four-year field experiment were studied (Vasilev, 2009). Chemical composition (crude protein content, calcium, phosphorus) of the forage of subterranean clover (pure and in mixtures) was closed to this of white clover (pure and in mixtures). Crude protein yield was significantly higher (more than 20%) in the mixtures of subterranean clover with wheatgrass as compared to ryegrass. 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 it was found that subterranean clover forms a dense sward with insignificant part of weeds and grow up faster that white clover after the occurence of late summer and early autumn precipitations.

The productivity of pasture mixtures with components subterranean clover, white clover and wheatgrass in the four-year field experiment was studied (Vasileva et al., 2011; Vasileva and Vasilev, 2012; Vasileva, 2015). The crops were tested pure and in mixtures with different rations between them (subterranean clover + wheatgrass 50%:50%; white clover + wheatgrass 50%:50%; subterranean clover + white clover 50%:50%; subterranean clover + white clover + wheatgrass 25%:25%:50%).

It was also found that the subterranean clover in mixture with wheatgrass formed significantly higher number of nodules and bigger amount of dry root mass. Subterranean clover showed better nodulating ability as compared to white clover.

Regarding the botanical composition, subterranean clover predominated in the mixtures with wheatgrass and white clover decreasing the weed infestation in the stands. During the fourth year the proportion of subterranean clover was significantly higher as compared to that of white clover.

The possibility for under sowing of degraded alfalfa seed production stands with three subterranean clover subspecies – *Tr. subterraneum ssp. brachycalicinum* (cv. "Antas"), *Tr. subterraneum ssp. yaninicum* (cv. "Trikkala") and *Tr. subterraneum ssp. subterraneum* (cv. "Denmark") was studied (Vasileva et al., 2015b). The under sowing with clovers was performed during the autumn of the fourth year of using of alfalfa stands. It was found that subterranean clover contributed to the improvement of botanical composition, two- and three fold reduced the weed infestation and increased dry matter yield in the four cuts obtained during the first and second year after under sowing, and three cuts during the last. *Trifolium subterraneum ssp. brachycalicinum* showed the best self-seeding ability. Under sowing with subterranean clover of degraded alfalfa stands used for seed production is possible agro technical measurement.

The results from the studies showed that subterranean clover has practical applicability as a component of pasture stands under the conditions of the Central part of the Danube plain region.

Results and Conclusions

The subterranean clover sown at an appropriate time in the autumn establishes a uniform stand before the beginning of the permanent cold spell grows up early in the spring and forms a dense sward.

As legume species an annually formation of root and nodule biomass and their decomposition contributed to soil fertility improvement.

Subterranean clover may be included in mixtures with the following components: legumes – birdsfoot trefoil and sainfoin and grasses – cocksfoot, tall fescue, perennial ryegrass and crested wheatgrass. The composition of the natural communities, in which it participates, shows that it is resistant to overgrazing, nitrification and ruderalization by domestic animals.

An annual leguminous species, but because of the biological ability for self-seeding with the presence in the sward at the beginning and end of vegetation, subterranean clover decreases weed infestation and increases the persistence of the pasture systems.

The chemical composition of subterranean clover (pure and in mixture) was closed to that of white clover (pure and in mixture).

Some units of the technology of artificial and natural perennial swards as weed control and nitrogen fertiliza-

tion could be replaced by alternative, ecologically friendly ones including subterranean clover as a component. Subterranean clover may be used for under-sowing of degraded seed production stands from alfalfa.

The subterranean clover is adaptable to the changing climatic conditions and its use as a natural bio-recourses in the establishment of artificial perennial swards could be a contribution to finding a solution in the field of agriculture to mitigate the adverse effects of climatic change.

On the basis of its occurrence in different parts of the country and in the composition of the natural vegetation, the species is promising for establishment of forage crops.

The participation of subterranean clover in the perennial swards is also a tool to increase the biodiversity in the agrophytocoenosis.

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