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# OBJECTIVES AND APPROACHES IN THE BREEDING OF PERENNIAL LEGUMES FOR USE IN TEMPORARY PASTURELANDS

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**Abstract:** Legumes are the major element of grassland ecosystem**,** on which the forage quality depends. Breeding of pasture varieties in perennial legumes firstly aims at achieving tolerance and persistence of the legume component in the pasture. In species having low natural grazing tolerance (lucerne and red clover) it is necessary to conduct breeding for biological, morphological and physiological characteristics, directly related to grazing tolerance. In the species having high grazing tolerance (white clover, birds foot trefoil, sainfoin), the pasture persistence is considered as a function of particular morphological characteristics, adaptive potential and stress tolerance. The indirect breeding for pasture persistence includes also breeding for competitive ability and co-adaptivity with grass pasture species adapted to the region and practices of cultivation, as well as breeding for improved nodulation and nitrogen fixation as an important element of the adaptive ability. The breeding for grazing tolerance and persistence is concentrated also on the mechanisms of self-maintenance of the legumes in the pasture – vegetative reproduction, spread and self-seeding. Many breeding programmes are concentrated also on morphology providing better intake by the animals, on main and specific quality characteristics, on anti-nutrient factors, on adaptation to systems and practices of pasture establishment and use. The breeding strategy using germplasm consolidated through the mechanisms of specific adaptation to unfavourable or specific agro-ecological conditions and influences – i.e. breeding through ecotypic selection is considered to be the most efficient for grazing purposes. The interactions in the system: pasture species – companion species in the pasture herbaceous community – grazing animals (kind, category, grazing style and behaviourial reactions) and the applied regime of use of the pasture are direct and strong. These interactions are of essential importance in genotypic differentiation of the pasture ecotypes. The adaptive variability of the pasture ecotypes is used directly, as well as in recombination breeding.

**Key words:** forage legumes, breeding for grazing use

# Introduction

Legumes are the major element of the grassland ***ecosystem***, on which the forage quality depends. Their fresh mass has high content of readily available protein, minerals, vitamins, neutral detergent fiber (*Lauriault et al., 2005; Carlier et al., 2008*). Their participation in the pasture sward is related firstly with increased intake of DM as result of higher intake rate and a longer time spent grazing (*Penning et al., 1995; Caradus et al., 1995*) and hence, with an increase of the animal productivity (*Fraser et al., 2004*). The positive nutrient effect of legumes is particularly important, consisting in increasing levels of polyunsaturated fatty acids in milk or meat (*Wu et al., 1997; Fraser et al., 2004*) and reducing methane production (*Dewhurst et al., 2009*). The inclusion of a nitrogen-fixing legume component in the sward corresponds to the principles of sustainable agriculture and extensive agricultural production (*Heichel and Henjum, 1991; Morris and Greene, 2001*). Legumes increase the nitrogen utilization in the herbaceous associations firstly through a decrease of the competition for soil nitrate ("nitrate sparing") and secondly – through N transfer (*Temperton, 2007*). They have great importance also for the fact that they contribute to even distribution of the yield from the pasture by seasons (*Sleugh et al., 2000*).

The breeding of legume varieties for grazing use can be defined as breeding for achieving a reasonable balance between persistence, quality, yield and animal safety (*Sewell et al., 2011*). The grazing use necessitates distinct plant type and selection work (*Annicchiarico et al., 2010*). The breeding process for these purposes is slow and expensive, because it requires a complete cycle of testing of perennial herbaceous species and it is conducted together with assessment of the effects of important ecological factors, such as grazing animals and companion herbaceous species in the pasture mixtures. Therefore, the test of the breeding materials requires large amounts and units of them.

### Breeding for survival and persistence under grazing

Persistence of the legume plants in the pasture is dynamic and determined by interactions among plant species, soil fertility, pests and pathogens, grazing stock, and seasonal weather conditions (*Widdup and Barrett, 2011*). Grazing, considered as frequent, early, selective or excessive removal of the over-ground mass, as trampling and contamination, appears to be the most important limiting factor of the persistence of species having low natural grazing tolerance (red clover and lucerne). Therefore in these species, in the first place, it is necessary to conduct breeding for biological, morphological and physiological characteristics, which provide survival of the individual plants and of the population of legumes under grazing. The traits, directly related to grazing tolerance, were studied to the greatest extent in lucerne and they were generalized by *Katepa-Mupondwa et al. (2002),* as follows: deep set crowns, broad crowns, prolific and nonsynchronous budding, extended periods of budding, subsurface budding, maintenance of stubble leaf area under grazing, early fall dormancy, and maintenance of root carbohydrates. In red clover, the plant morphology, associated with grazing tolerance is defined as follows: more profuse and finer stems, a more prostrate and lover growth habit and smaller leaves (*Rumball et al., 2003; Ford and Barrett, 2011; Boller et al., 2012*). In earlier studies the red clover persistence in pastures is considered in an aspect of the interaction of legume genotype with associate grass, season and regime of pasture use (*Pineiro and Harris, 1978; Cosgrove and Brougham, 1985*).

There are opinions that the breeding for grazing tolerance, based on individual traits, is not efficient. In the first place, because grazing tolerance must be considered as a complex trait that encompasses many morphological and physiological plant characteristics and their interactions with the environment (*Katepa-Mupondwa et al., 2002*). Furthermore, it is known that the selection for one or several characteristics, related to grazing tolerance, leads to decreased productivity and loss of other desired qualities (*Smith et al., 2000*). Morphological types, related to grazing tolerance, are different and specific, depending on the kind of the grazing animals and intensity of pasture use (*Annicchiarico et al., 2010; Ford and Barrett, 2011*), which also makes the breeding for individual traits unstable.

In white clover – the legume possessing the highest grazing tolerance – the breeding for pasture persistence is also associated with morphological characteristics - stolon diameter and branching, leaf size and root structure (*Ayres et al., 1996; Jahufer et al., 2002; Sanderson et al., 2003; Annicchiarico and Piano, 2004; Bouton et al., 2005; Jahufer et al., 2008*). The stolon and root morphology and architecture influence the white clover persistence in the pasture through the degree of utilization of water and nutrients. The stolon characteristics condition the processes of clonal persistence (*Bouton et al., 2005*). Moreover, white clover and birdsfoot trefoil form a relatively persistent seedbank. On this ground, breeding for high and ecologically stable seed productivity is conducted, which maintains a seed bank, providing the presence of these species in the pasture (*Pedersson and Brink, 1997; Ayres et al., 2008*). Birdsfoot trefoil is also mentioned in other comparative studies as a species possessing potential as a self-seeding pasture species (*Carr et al., 2005a, 2005b*). In other cases, to achieve sustainable presence, founded also on the basis of the seed ecology, the conducted breeding is for hard seed character. The hard seed character conserves the seed viability after passage through the digestive tract of animals and allows the legume spead by the animals and their natural reseeding (*Doucette et al., 2001; Abbate et al., 2003*).

Under grazing the legume persistence proves to be a more important trait than productivity. Persistence is a complex trait often negatively correlated with other important traits and its phenotyping is laborious (*Herrmann, 2007*). The plant genetic potential for persistence is connected to a great extent with primary and secondary metabolites relating to stress tolerance and defence (*Widdup and Barrett, 2011*). According to *Taylor* (2008) the persistence, considered within the framework of the life cycle, is a result of the interaction of plant adaptivity and its stress tolerance. The breeding for drought and winter stress tolerance, for tolerance and resistance to soil pathogens is considered to be a means of achieving persistence in red clover (*Taylor, 2008*); white clover (*Annicchiarico, 1997; Collins, 2002, Widdup and Barrett, 2011*), lucerne (*Smith et al., 2000; Bouton, 2012*), sainfoin (*Mowrey and Matches, 1991; Mowrey and Volesky, 1993; Morrill et al., 1998; Gray et al., 2006; Demdoum et al., 2010*). In white clover the breeding for pasture persistence is associated also with breeding for competitive ability, consisting in good growth in conditions of shading, competitive root morphology, and compatibility with adapted grass pasture species to area and practices of cultivation (*Caradus et al., 1995*). According to *Escaray et al.* (2012), rhizobial symbionts can contribute to the adaptation of legumes not only to saline and alkaline soils, but also to a wider range of adverse conditions. In this aspect, improved nodulation and nitrogen fixation also prove to be aims in the indirect breeding for persistence.

In many cases it is assumed that pasture persistence of the species can be increased through a change in their system of reproduction from closed (such one with seeds) to open (with the opportunity also for vegetative reproduction in the sward) (*Taylor, 2008*). In this case, the ideotype of pasture legume is considered to be the white clover, which has excellent grazing tolerance, as well as due to its open system of reproduction through stolons. In wild ecotypes of the seed-propagating species, ability of vegetative reproduction was also found. Creeping forms (spreading by rhizomes or stolons) were found in birdsfoot trefoil (*Beuselinck, 1989; 2004*), lucerne (*Heinrichs, 1973; Piano et al., 1996*) and red clover (*Smith and Bishop, 1993; Brock et al., 2003; Rumball et al., 2003*). However, it should be mentioned that there is a substantial microenvironmental influence on the expression of this ability of vegetative spread in the pasture sward and it proves to be strongly linked to specific ecological conditions, this applying also to the varieties developed on the basis of these ecotypes (*Hyslop et al., 1998; Kallenbach et al., 2001; Beuselinck et al., 2005*).

Interspecific hybridization has been also used as a means of introgression of different types of systems of vegetative reproduction. In red clover the work was done with interspecific hybrids, obtained from crosses with the rhizomatous species *Trifolium medium* (*Merker, 1991; Sawai et al., 1995*). Similarly, through the hybridization with *Trifolium ambiguum* in white clover it was achieved to combine a stoloniferous system with a rhizomatous system of reproduction (*Anderson, 1991*) and the drought tolerance and persistence was increased in large leaved white clover varieties under grazing (*Meredith et al., 1995; Marshall et al., 2001*).

Interspecific hybridization has been also used for introgression of other traits, providing survival and persistence under grazing. Yellow (sickle) lucerne (*Medicago sativa ssp. falcate*), obtained from crosses of *Medicago sativa X Medicago falcate* possesses tolerance to summer drought, winter tolerance and deeper position of root crown. In contrast to subspecies sativa, the plant survival does not depend on duration of the period of carbohydrate recovery in the roots after removal of the over-ground mass (*Berdahl et al., 1989; Bittman et al., 1991*). Through hybridization with *Trifolium uniflorum*, in white clover the depth of development of the root system was increased and hence the drought tolerance was increased (*Pandey, 1987*). The hybrids of *Trifolium repens X Trifolium occidentale* were intended to be used as a source of viral resistance (*Pederson and McLaughlin, 1989*). Nevertheless it should be mentioned that in legumes there are no practically spread varieties, developed through interspecific hybridization (*Abberton, 2007*), as it is for grasses, in this case for Festulolium.

**Breeding for traits conditioning grazing suitability**

Breeding criteria connected with grazing suitability, are numerous and heterogeneous. Selection for morphological traits, which provide better intake of legumes by animals, should be considered as a kind of breeding for productivity. The examples of such traits in red clover are great leafiness of stems, more profuse and thin stems, lower and flatter growth habit, smaller leaves (*Boller et al., 2012*), in birdsfoot trefoil - fine stem, prostrate growth (*Radović et al., 2008*), in white clover - upright habit with long petioles, large-leaved character (*Widdup and Barrett, 2011; Mihovski and Goranova, 2006*). It should be also considered that the intake of legumes depends very much on species, style of grazing and behavioural reactions of the grazing animals (*Taylor et al., 1987; Marten et al., 1990; Popp et al., 1999*). That is an important reason to include animals in the breeding process.

From the point of view of grazing use, the breeding for main nutritional forage characteristics (crude protein, acid detergent fibre, neutral detergent fibre, ash, lignin, lipid, metabolizable energy and organic matter digestibility), as well as the breeding for specific qualitative characteristics are of importance. The following examples of the latter can be mentioned: the breeding for high levels of the polyphenol oxidase enzyme, protecting proteins and glycerol-based lipid in the rumen (*Winters and Minchin, 2001; Lee et al., 2009; Weiher et al., 2010*); or the breeding for optimal content (2-4%) of condensed tannins, which in a grazing situation have the important functions, such as: to reduce protein fermentation to ammonia in the rumen and methane gas emissions, control of rumen bloat and internal parasite infections (*Arrigo et al., 2009; Escaray et al., 2012*).

In connection with the intake, as well as due to the direct and quick effect of the grazed herbage on the biochemical processes in animal organism, the breeding against antinutrient factors is taken into consideration. Such ones are: content of cyanogenic glucosides in white clover (*Collinge and Hughes, 1982;*

*Paplauskienë and Sprainaitis, 2003; Annicchiarico, 2012*), content of phytoestrogens, formononetin, biochanin-A and coumestrol in red clover (*Francis and Quinlivan, 1974; Gosden et al., 1979*) and lucerne (*Cantero, 1993*), high levels of condensed tannins (above 6%) in birdsfoot trefoil (*Aerts et al., 1999; Wen et al., 2003*). As a particularly important antinutrient factor is considered to be the bloating potential of legumes, which have very low natural content of condensed tannins – lucerne, red and white clover. In order to overcome this limitation the work is done mostly with lucerne, because its bloating potential is higher than that of clovers, on the one hand, and due the necessity (search) for high and sustainable productivity and reduced cost of pastured forage, on the other hand. The selection for lower initial rate of dry matter disappearance (low initial rates of digestion) has been proposed to develop alfalfa cultivars with reduced bloating potential (*Goplen, 1972; Goplen and Howarth, 1976; Basigalup et al., 2003*). This new strain reduces the incidence and severity of frothy bloat on pasture. Their effectiveness in controlling bloat was related to feeding or grazing management practices, the maturity of the plants and the season of use (*Berg et al., 2000*). The methods of genetic transformation prove to be very successful in overcoming the bloating potential. The newest studies show that in the leaves of genetically modified lucerne and white clover plants condensed tannins can be synthesized and accumulated up to levels preventing the bloat (1.8%) (*Hancock et al., 2012*). There are programmes for breeding of sainfoin varieties suitable for cultivation in mixtures with alfalfa, thus reducing also the bloating problems (*Acharya, 2006; Wang et al., 2007*).

In their turn, the different systems and practices of establishment and use of pastures also necessitate specific breeding objectives. For instance, often legumes become a component of existing pasture swards through underseeding (*Guretzky et al., 2004*). The best result of this method was observed in red clover and birdsfoot trefoil (*George, 1984*). That justifies in red clover to conduct breeding for increased frost-seeded seedling establishment (*Riday 2006, 2008*). Furthermore, the legumes in the pasture are assigned the function to prolong the grazing season, to increase the yield and quality of grazing in the second half of summer and autumn. In this aspect, major breeding criteria prove to be productivity in secondary growth and rate of regrowth – in birdsfoot trefoil (*Blumenthal and McGraw, 1999; Radović et al., 2008; Scheffer-Basso et al., 2011*) and sainfoin (*Martiniello, 2005*), drought tolerance – in white clover (*Caradus et al., 1995*) and sainfoin (*Demdoum et al., 201*0), late beginning of fall dormancy and aftergrass character in combination with winter survival – in red clover and lucerne (*Bouton, 2012*).

**Ecotypic selection as efficient breeding approach**

Breeding of varieties for grazing use should be considered in the first place as breeding for specific adaptation (*Annicchiarico et al., 2010*). From this point of view, in the breeding for grazing purposes, the breeding strategy using germplasm consolidated through the mechanisms of specific adaptation – i.e. breeding through ecotypic selection is considered to be the most efficient.

The use of specific adaptations to soil, climatic and topographic conditions is important to legumes, because they are considerably more susceptible to unfavourable conditions than grasses. On the other hand, most often the pastures have an agro-ecological niche in areas with low-fertile lands and in mountainous areas. A great deal of tolerance to constrained environments has been lost in commercial cultivars of legume grass, because breeding emphasis has been centered on dry matter production and persistence under non-restrictive conditions (*Papadopoulos et al., 1999; Vasiljević et al., 2008*). Many studies mention the advantages of the naturally formed populations of legumes, when searching for preadaptive and acclimatization potential to a stress abiotic influence. The genotypic characteristics achieved through ecotypic selection are: cold tolerance (*Finne, 2002*), tolerance to soils poor in phosphorus (*Spencer, 1980*), to soils of high concentrations of aluminium and manganese (*Mitev and Goranova, 2008*), drought tolerance (*Ayres et al., 1996*), hard seed character, providing self-reseeding (*Asci et al., 2011*). The ecotypes are also used as a source of other characteristics of importance to grazing use, such as: persistence, combined with productivity (*Piano and Annicchiarico, 1995;Goranova et al., 2005; Radović et al., 2008*), nutritional quality (*Goranova and Mihovsky, 2005*; *Vučković et al., 2007*), persistence (*Lugic et al., 2004*), good yield in the later stages of growth cycle (*Naydenova, 2008*). As a base of these facts, it could be mentioned that the high degree of intra-population variability and allogamy of perennial legumes allow a rapid genetic change in the genotype of the populations under natural or artificial breeding pressure (*Collins, 2002*). This adaptive variability of the ecotypes, being important to the breeding for grazing purposes, can be used directly, as well as it can be transferred into a cultural germplasm, using conventional crossing methods (*Garcia De Los Santos et al., 2001; Boller et al., 2012*). Up to now, the recombination breeding has been used most successively in white clover, to combine the high productivity of large leaved forms and the stoloniferous characteristics of wild-growing medium and small leaved ecotypes, which provide persistence and drought tolerance (*Jahufer et al., 1999*). In red clover there are also many contemporary varieties directly developed from ecotypes or after hybridization of ecotypes (*Lehmann et al., 1998; Boller, 2000*).

The genotypic result of the interaction between ecological conditions and mode of use are the cultivated ecotypes that have a long history of cultivation in a definite area, under a specific agronomic practice. The interactions in the system: pasture species – companion species in the pasture herbaceous community – grazing animals (kind, category, grazing style and behaviourial reactions) and the applied regime of use of the pasture are direct and strong. These interactions are of essential importance in the consolidation of the so-called pasture ecotypes. The pasture ecotypes can also originate from varieties with a broad area of spread. *Bouton and Gates* (2003), *Moutray* (2000), as well as *Smith et al.* (1989) described procedures for breeding of pasture lucerne varieties through submitting populations of adapted varieties to continuous grazing. In this case, pasture ecotypes are developed through recurrent phenotypic selection. With regard to duration of use and yield the varieties bred in this way, due to good ecological adaptation, are equal or exceed the widespread varieties also in other regimes of use.

The interspecific interactions in the pasture mixture have a significant influence on the morphology (*Pineiro and Harris, 1978*), productivity, quality (*Wen et al., 2003*) and survival (*Guretzky et al., 2004*) of legume components. In this respect, the breeding strategy, based on the ecotypic selection, is considered to be very suitable, because the ecotypes develop in natural plant communities and their genotype is also a result of coevolution. The choice of components from jointly existing populations, with high "general ecological combination ability" is of crucial importance to establishment of productive pasture mixtures, in which the dynamic stability of the sward is maintained (*Hill, 1990*). *Collins and Rhodes* (1989), *Turkington* (1996) reported such results for white clover and perennial ryegrass. Similar data in breeding of pasture lucerne varieties were presented by *Berdahl et al.* (1986). According to them, the populations with natural adaptation to grazing, as well as to species and genotype of grass components in the mixture have the highest value.

# Conclusion

Genetic providing of the functions, which are assigned to legumes in the pasture, requires from the breeding for grazing purposes to search for or to develop germplasm, in which persistence, forage quality, productivity and animal safety are positively related. The pasture persistence is also examined in the relation with grazing tolerance, plant morphology, adaptive potential and stress tolerance. The indirect breeding for pasture persistence includes also breeding for competitive ability and coadaptivity towards other pasture species and genotypes, as well as breeding for improved nitrogen fixation as a particularly important element of the adaptive ability. The breeding for grazing tolerance and persistence is concentrated also on the mechanisms of self-maintenance of the legumes in the pasture – vegetative reproduction, spread and self-seeding. Many breeding programmes are directed also to morphology providing better intake by the animals, to main and specific quality characteristics, to anti-nutrient factors, to adaptation to systems and practices of pasture establishment and use. The adaptive variability of the pasture ecotypes of perennial legumes are of prime importance in the breeding of pasture varieties.

# Ciljevi i pristupi u proizvodnji višegodišnjih leguminoza za upotrebu na privremenim pasnjacima

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## Rezime

Leguminoze su glavni element ekosistema travnjaka od čega zavisi kvalitet krmnih biljaka. Oplemenjivanje pašnjačkih sorti višegodišnjih leguminoza prvo ima za cilj postizanje tolerancije i upornosti komponente leguminoze u u pašnjaku. Kod vrsta koje imaju nisku prirodnu toleranciju ispaše (lucerka i crvena detelina), neophodno je sprovesti uzgoj za biološke, morfološke i fiziološke karakteristike, u direktnoj vezi sa tolerancijom ispaše. U vrstama koje imaju visoku toleranciju ispaše (bela detelina, žuti zvezdan, esparzeta), perzistencija se smatra funkcijom pojedinih morfoloških karakteristika, adaptivni potencijalom i tolerancijom na stres. Indirektno gajenje na perzistenciju pašnjaka uključuje i oplemenjivanje na konkurentsku sposobnost i koadaptivnost vrsta trave sa pašnjacima prilagođeno regionu i praksi kultivacije, kao i uzgoja na poboljšanju nodulaciju i fiksaciju azota kao važan element adaptivne sposobnosti. Oplemenjivanje na toleranciju ispaše i istrajnost je takođe koncentrisano na mehanizmima samostalnog održavanja mahunarki na pašnjacima - vegetativnog razmnožavanja, širi i samo-setve. Mnogi programi za uzgoj su takođe usmerena na morfologiju kojom se obezbeđuje bolji unos od strane životinja, na magistralne i specifične karakteristike kvaliteta, na faktor anti-nutrijenata, na prilagođavanje sistemu i praksi osnivanja i korišćenja pašnjaka. Strategije oplemenjivanja korišćenjem germplazme konsolidovana kroz specifične mehanizme adaptacije na nepovoljne ili određene agroekološke uslove i uticaje - tj kultivacije kroz ekotipičnu selekcije se smatra da je najefikasnija za svrhu ispaše. Interakcije u sistemu: pašnjačke vrste - prateće vrste u pašnjačkoj zajednici zeljastih biljaka - ispaša životinja (vrsta, kategorija, stil ispaše i bihaviurlne reakcije) i primenjeni režim korišćenja pašnjaka je direktan i jak. Ove interakcije su od suštinskog značaja u genotipskim diferencijacijama ekotipova pašnjaka. Adaptivna varijabilnost ekotipova pašnjaka se direktno koristi, kao i u rekombinantnom oplemenjivanju.

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