

CREATION OF THE HAY-PASTURE TYPE BREEDING POPULATIONS OF RED CLOVER FOR THE CONDITIONS OF NORTHERN BULGARIA

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Abstract

In a field experiment conducted in the period 2019–2021, breeding populations of red clover were studied from the first to the third vegetation. They represent families – half-sib offspring of elites selected from local and breeding populations, as well as from populations representing Syn 1 and Syn 2 generation from the subspecies crossing of *T. pratense* ssp. *nivale* × *T. pratense* ssp. *pratense*. The aim is to evaluate the selection progress according to criteria significant for the combined use of the species, namely high and stable productivity over the years during secondary (summer) regrowing, as well as the related values of basic morphological traits, which are components of forage yield. According to the obtained results, the number of generative stems formed by a plant during summer regrowing, as well as a stable performance of leaf area by regrowths and years are the morphological characteristics associated with high productivity during summer regrowing, respectively suitability for combined use. The population with spreading phenotype TPNpr, Syn 2 generation of the subspecies crossing *T. pratense* ssp. *nivale* × *T. pratense* ssp. *pratense* has the highest selection value in the specified direction. It stands out with the highest summer productivity and persistence over a three-year period of use.

Key words: red clover, breeding

Introduction. In Bulgaria, sown grass stands are used in combination – the first growth is for hay or silage, and the summer regrowth is for grazing. Mixtures

of medium long-lasting legumes and grasses are recommended for their creation, as red clover is the most commonly used legume component in the semi-mountain and mountain regions of Bulgaria [1, 2]. In these areas, the productivity, quality and sustainability of grass stands depend to a large extent on the availability of adapted red clover cultivars suitable for combined use. The study by ZARZA et al. [3] identified red clover as a very important crop in sustainable pasture production systems because it has a high capacity for biological nitrogen fixation, a high crude protein content resulting in high levels of grass biomass uptake, and high levels of enzymatic polyphenols that directly improve nitrogen uptake by ruminants.

Currently, mixtures involving this legume species are created with randomly imported seeds, which are most often from tetraploid grass cultivars selected in Central or Northern Europe. However, our studies so far indicate that under the conditions of Northern Bulgaria, tetraploid cultivars are significantly inferior to the local geneplasm in terms of drought resistance, summer productivity and survival, pasture suitability and longevity [4–6]. This directed our improvement work with red clover towards selection for specific adaptation to both combined use and regional ecological conditions.

Legumes in grass mixtures are mainly assigned the function of improving forage quality in summer, so summer productivity is a particularly important genotypic characteristics of varieties for combined hay-pasture use. Thus, the creation of cultivars suitable for cultivation in grass mixtures for combined use at first requires selection for the following traits: fast rate of growth and regrowth, high productivity during summer regrowing, summer survival, forage quality ensuring high levels of uptake, etc. [7–9]. Since in Bulgaria artificial grass stands have an agro-ecological niche in areas with infertile soil, it is important that red clover cultivars are also selected for adaptive potential to unfavourable soil and climate conditions, or for specific adaptation to regional agroecological conditions.

A large number of studies have indicated morphological characteristics associated with high and uniform seasonal and annual productivity, drought and cold resistance, sustainability and longevity both in single and mixed cultivation [10–13]. This indicates that breeding for pasture suitability of red clover affects a large number of traits. In our selection programme for creating hay-pasture red clover varieties, high summer yield of dry fodder mass was the main selection criterion. The present study aimed to evaluate the selection progress according to criteria significant for the combined use of the species, namely high and stable productivity over the years during secondary (summer) regrowing, as well as the related values of basic morphological traits, which are components of forage yield.

Material and methods. The experiment was conducted in the period 2019–2021, in a hilly area in Northern Bulgaria, in the experimental field of Soybean Experimental Station of Pavlikeni. In a selection nursery, breeding populations

of red clover were studied from the first to the third vegetation. They represent families – half-sib offspring of elites selected from local and selective populations, as well as from populations representing Syn 1 and Syn 2 generation from the subspecies crossing of *T. pratense* ssp. *nivale* × *T. pratense* ssp. *pratense*. The Bulgarian Nika 11 cultivar was used as a reference. The populations were sown in the spring of 2019 in randomized rows 3 m in length with two replications. Manual sowing used 1000 viable seeds/m. The rows were mowed in the beginning of flowering. This phenophase is determined to be suitable for both hay and pasture use. It is a traditional practice in our country to grazing red clover at the beginning of flowering (stage 59–61 according BBCH scale). The flowers of the species contain 1.2–1.5% condensed tannins and thus reduce the risk of bloat in ruminants. The results for fresh and dry vegetative matter yield were registered (g/m) by cuts/regrowths. In the summer regrowth, at the second and third cut, six randomly selected plants from a population (family) were subjected to biometric analysis, as the following indicators were observed: number of generative stems formed by a plant, height/length of generative stems, respectively, for populations with erect or prostrate growth habit, length (*a*) and width (*b*) of the medial leaf (mm) formed at the 3rd internode, leaf area (mm²) calculated by the formula for area of an ellipse $S = 3.14(a/2)(b/2)$. One-factorial and two-factorial analysis of variance were used for static data processing (Microsoft Excel 10). Comparison of the average values of the populations and standard variety on the observed indicators was performed by Duncan's test at a significance level of 0.05, 0.01, and 0.001.

In 2019, precipitation amounts during the vegetation period were higher than the climate norm for the area ($\sum R$ Apr-Sep = 374 mm versus 330 mm). 2020 and 2021 are defined as dry, especially in the second half of summer, when monthly precipitation amounts were recorded in the range from 2.4 to 36.4 mm with a climate norm of 50.8 to 58 mm.

Results and discussion. In the year of sowing, populations TPN 13 and TPN 14, which originate from the subspecies crossing *T. pratense* ssp. *nivale* × *T. pratense* ssp. *pratense*, as well as population TPN w, which originates from a white-flowering elite genotype selected in the Syn 2 generation of this crossing, formed only leaf rosettes. The remaining breeding populations, as well as the diploid cultivar Nika 11, formed generative stems by mid-summer (20.07). The genotypic differences in dry matter yield for the only harvested regrowth in the first vegetation were significant ($P < 0.01$), and could be related to the indicated differences in the biological and morphological type of the evaluated breeding numbers. The highest yield of fresh and dry matter was obtained from the TPN pr population, which originates from a spreading phenotype, also selected in the Syn 2 generation of the studied subspecies crossing (Table 1).

In the second and third vegetation, three regrowths were harvested from all tested populations (Table 1). In the second vegetation, the highest annual yield

T a b l e 1

Dry matter yield (DMY) by regrowths and years, g/m

	First vegetation		Second vegetation		Mean	Third vegetation			Mean
	I regrowth	I regrowth	II regrowth	III regrowth		I regrowth	II regrowth	III regrowth	
1. Nika 11 St	159.7	300.0	411.2	74.9	262.0	273.0	202.8	84.4	186.7
2. TPN 13	206.0	259.4	288.1	62.9	203.4	389.0	207.2	58.4	218.2
3. TPN 14	220.0	224.2	295.7	91.1	203.7	352.0	242.0	78.0	224.0
4. TPN w	163.7	135.3	253.8	96.2	161.8*	205.0	175.8	24.7	135.2
5. E4/5	192.3	254.5	320.6	67.0	214.0	240.0	217.5	80.3	179.3
6. TPN pr	250.0*	78.1	280.6	197.0	185.2	395.2	396.6	66.8	286.2**
7. LP	167.7	183.3	204.3	226.9	204.8	257.2	129.2	39.4	141.9
I regrowth	208.5				212.4				295.4
II regrowth	–				295.1				221.4
III regrowth	–				116.4				60.4
LSD _{0.05}	85.7				77.1				50.8
LSD _{0.01}	106.5				107.0				70.7

Legend: * – indicates statistically significant positive or negative differences compared with the standard variety: $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$

of dry matter was recorded for the standard hay making cultivar Nika 11. The studied populations were inferior to it with unproven differences, except for TPN w. In the third vegetation, three of the breeding populations – TPN 13, TPN 14, and TPN pr had a higher productivity compared to the standard, and in the case of TPN pr the positive difference was significant at $P < 0.01$.

During summer regrowing (second and third regrowth) TPN 13, TPN 14, TPN w, and E4/5 populations are equal to the standard cultivar in terms of dry matter yield (Fig. 1B and C). Population LP1 created on the basis of local germplasm, was inferior in productivity to Nika 11 cv. in the second regrowth ($P < 0.01$), but significantly superior to it in the third regrowth ($P < 0.05$). The highest summer productivity was found in the population with a spreading phenotype – TPN pr, as it registered significant positive differences compared to St both in the second and third regrowth ($P < 0.05$).

The dry matter yield of the third regrowth can also be determined as a function of the drought resistance of the breeding materials, since in both experimental years strong droughts were observed in the second half of the summer. The indicated populations LP1 and TPN pr exhibited significantly better regrowth potential under dry conditions. In summer conditions, they are distinguished by a significantly greater number of generative stems formed by a plant ($P < 0.05$), as well as by the lowest variance for this characteristics (Table 2). And the study of LOUCKS et al. [14] indicated genotypic differences in terms of maintaining the

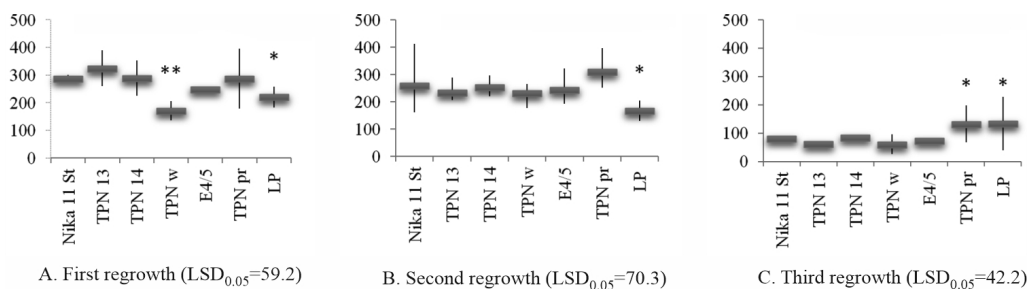


Fig. 1. Average seasonal dry matter productivity over the experimental period, g/m

viability of the meristem tissue in the root neck and, accordingly, the ability to form generative shoots in dry conditions, as the characteristic number of generative stems formed by a plant is determined by as the main criterion in breeding for persistence.

Number of generative stems and plant height are the two traits directly correlated with dry mass productivity in red clover [15]. According to the present study, the first trait is of greater importance in explaining the variation between breeding populations in productivity during summer growth.

A spreading growing habitus and shorter plant height are also associated with higher persistence [16]. And according to the results obtained by us (Table 2), the population with the laying phenotype TPN pr, created by subspecific crossing, is inferior to the standard hay variety in height/length of generative stems ($P < 0.01$), but stands out with a significantly higher yield in third undergrowth of third vegetation, which is indicative of the highest persistence. On the other hand, the greater stem height and upright growing habitus give the species an advantage in conditions of interspecies competition in mixed breeding [12]. In this regard, the high-growing population TPN 14, also originating from the subspecies cross *T. pratense* ssp. *nivale* $\times$ *T. pratense* ssp. *pratense* has breeding potential given the direction of our improvement work – suitability for combined use in mixed cultivation.

Leaf characteristics, such as length and width of the leaf blade and leaf area were studied during secondary regrowth as indicative of active growth and good productivity in the summer season. Larger leaf size is associated with higher forage quality and higher uptake rates in grazing [17]. Particularly important is that the leaves contain high levels of the enzyme polyphenol oxidase, which is specific to this species and like the condensed tannins protects proteins and glycerol-based lipid in the rumen [18,19]. Therefore, high leafiness should be viewed as a main morphological feature determining the high nutritive value of red clover forage. In the present experiment, significant genotypic differences in the leaf area were found for the different regrowths and vegetations. They are most distinct in the second regrowth of the third vegetation, and the slightest in the third one.

T a b l e 2

Values of the observed morphological indicators in the summer regrowths
of the second and third vegetation

	Second vegetation/ II regrowth	Second vegetation/ III regrowth	Third vegetation/ II regrowth	Third vegetation/ III regrowth	Mean	Variance
Generative stems formed by a plant during summer regrowing, number						
1. Nika 11 St	26.1	14.0	17.5	6.8	16.1	8.0
2. TPN 13	22.4	18.0	16.7	9.0	16.5	5.6
3. TPN 14	26.0	11.9	15.2	7.7	15.2	7.8
4. TPN w	19.5	12.8	14.5	5.5	13.1	5.8
5. E4/5	20.2	13.0	21.4	8.2	15.7	6.2
6. TPN pr	19.7	19.0	27.7	16.0	20.6*	5.0
7. LP	20.0	18.0	24.0	18.7	20.2*	2.7
<i>LSD</i> _{0.05}				3.8		
Height/length of generative stems during summer growth, cm						
1. Nika 11 St	76.0	36.0	53.0	32.0	49.3	20.0
2. TPN 13	58.0	30.0	39.0	30.0	39.3*	13.2
3. TPN 14	62.0	42.0	51.0	35.0	47.5	11.7
4. TPN w	43.0	32.0	40.0	31.0	36.5**	5.9
5. E4/5	57.0	37.0	45.0	34.0	43.3	10.3
6. TPN pr	48.0	36.0	34.0	30.0	37.0**	7.7
7. LP	47.0	43.0	27.0	26.0	35.8**	10.8
<i>LSD</i> _{0.05}				8.1		
<i>LSD</i> _{0.01}				10.9		
Leaf surface, mm ²						
1. Nika 11 St	9.5	9.8	8.4	3.9	7.9	2.7
2. TPN 13	9.2	7.3	9.4	3.1	7.2*	2.9
3. TPN 14	7.6	6.0	4.4	3.9	5.5***	1.7
4. TPN w	9.5	6.8	9.3	5.9	7.9	1.8
5. E4/5	10.7	11.4	9.4	5.0	9.1***	2.9
6. TPN pr	8.3	5.4	9.0	4.5	6.8***	2.2
7. LP	6.2	6.9	6.7	6.6	6.6**	0.3
<i>LSD</i> _{0.05}				0.7		
<i>LSD</i> _{0.01}				0.9		
<i>LSD</i> _{0.001}				1.14		

Legend: * – indicates statistically significant positive or negative differences compared with the standard variety: $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$

Population E4/5 is distinguished by significantly higher mean values for the length and width of the leaf blade (data not shown) and, accordingly, by the leaf area (Table 2) compared to the standard. Populations with high summer productivity, such as TPN pr and LP1 had leaves of significantly ($P < 0.01$; $P < 0.001$) smaller size. The interconnection between small leaf area as a genotypic characteristics and drought resistance was also determined by Loucks et al. [14], as well as by HOEKSTRA et al. [20]. A predominant tendency to decrease leaf size was observed

from the second to the third vegetation, as well as from the second to the third regrowth. An exception was observed for LP1, which was created on the basis of a local germplasm adapted to low summer soil moisture reserve. During summer regrowing, regardless of the sequence of the regrowth and the age of the plants, this population had a stable performance of its leaf area and its transpiration area. The strongest reduction of the leaf area in the third regrowth of the third vegetation was in the large leaf genotypes, such as Nika 11, E4/5 and TPN13.

The two-factor variance analysis of the morphological traits (Table 3) showed that the genotypic effect was significant for the regrowths formed in summer conditions. The regrowing conditions/season (regrowth succession) and plant age (vegetation succession) are a stronger source of variability in the traits, such as number of generative stems formed per plant and leaf area according to ratio values factorial/total variance (η^2 , %). For all three traits, specific genotypic expression was observed in relation to regrowing conditions/season and plant age.

T a b l e 3

Significance of factorial variances (MS) and degree of factorial influence (η^2 , %)

Traits	Factorial effect	Genotype		Regrowth and age of plants		Interaction	
		MS	η^2 , %	MS	η^2 , %	MS	η^2 , %
Generative stems formed by a plant during summer regrowing		49.1**	24.2	160.8***	52.8	11.7**	5.8
Height of plants in summer regrowing		219.2**	38.8	500.5***	29.5	29.3***	10.4
Leaf surface		45.8***	20.1	145.2***	31.8	17.8***	23.4

Conclusion. The presented results are indicative of a good potential of researched material in the breeding of cultivars for combined use. According to the obtained results, the number of generative stems formed by a plant during summer regrowing, as well as a stable performance of leaf area by regrowths and years are the morphological characteristics associated with high productivity during summer regrowing, respectively, suitability for combined use. The population with spreading phenotype TPN pr, Syn 2 generation of the subspecies crossing of *T. pratense* ssp. *nivale* $\times$ *T. pratense* ssp. *pratense* has the highest breeding value in the specified direction. It stands out with the highest summer productivity and persistence over a three-year period of use.

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