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Breeding assessment of polycros progeny of elite genotypes of red clover (*Trifolium pratense* L.)

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Abstract. The purpose of this study is to evaluate the genetic variability between red clover half–sib families in terms of yields of dry forage mass during regrowing, as well as seasonal yield stability and years of use. Ten red clover elite plants selected in the past by one or more breeding criteria underwent a re–pollination in 2014. Their polycross progeny (HSFs) were examined by cuts from the first to the fourth vegetation (2015–2018) for the following indicators: green matter yield, dry matter content, dry matter yield, time for cuts formation. 'Nika 11' and 'Sofia' 52 cultivars were used as a control for high summer productivity and adaptability. In early spring growth, the polycross progeny of elite genotypes did not significantly differ in dry forage mass. In contrast, as in late spring and summer regrowing, the effect of the family was significant ($P<0.05$). Genotypic effects are also observed in terms of growth rate. A genetic potential for increasing the yield of dry forage mass for both late–spring and summer regrowing was found for family 2 and 3. Valuable genotypes for the breeding in relation to yield stability over seasons and age of plants, respectively years of use, can be selected in family 7, which originated from a local wild–type germplasm. The high expression in the level and stability of the observed characteristics, as well as the high estimates of general combination ability, determined 'Nika 11' as an important genetic source in the breeding for high forage productivity in regrowing. According to these results, 'NIKA 11' is used as a major component in the formation of polycross groups with the selected families.

Keywords: red clover, *Trifolium pratense*, breeding, half–sib families.

Introduction

Growth rate after mowing, the yield of dry vegetative mass from summer regrowth and persistence are the qualities that determine the profitability of red clover as a fodder crop.

Accordingly, the breeding of adapted cultivars first relates to the improvement of these characteristics.

In our previous studies it was found that the genotypic effects on the productivity during the regrowing were more pronounced than those in the initial /spring growth and that the genotypic differences in summer yield were higher in an area with insufficient moisture in the summer months, which are predominantly the conditions in the plain and hilly part of Bulgaria. In this aspect, it is necessary to establish a genetic variability concerning drought resistance, which can be used in the breeding for high summer productivity respectively.

The polycross is used successfully in the breeding for increasing fodder and seed productivity of perennial legumes [KERTIKOVA, 2008, BOLLER *et al.,* 2010, MILIĆ *et al.,* 2010, IRANI *et al.,* 2015].

The creation of complex hybrid populations combining genotypes with high general combination ability (GCA) is considered to be an effective breeding method in red clover [MONTARDO *et al.,* 2003, JALŮVKA *et al.,* 2009, ZARYANOVA & KIRYUKHIN, 2014].

The evaluation of polycross progeny of individual maternal genotypes (HSFs) is one of the most commonly used methods for determining GCA and, respectively, for the breeding of parental material according to the genotype.

The high assessment of GCA is associated with high heritability [VISSCHER *et al.,* 2008; NDUWUMUREMYI *et al.,* 2013] and can be considered sufficient to select plants of genotypic value to improve agronomically important features.

The persistence of heterosis effect in different generations in synthetic clover populations is associated with the use of source material with heterogeneous geographic origin and different degree of cultivation [NOVOSELOVA *et al.*, 2002].

In addition, the study on the combined value of wild–type forms, ecotypes and subspecies are also due to the fact that this germplasm is characterized by stress resistance and persistence [BOLLER *et al.*, 2004, NAYDENOVA *et al.*, 2015].

The purpose of this study is to evaluate the genetic variability between red clover half–sib families in terms of yields of dry forage mass during regrowing, as well as seasonal yield stability and years of use.

Material and methods

Red clover elite plants selected in the past by one or more breeding criteria in the pasture trend (high annual productivity, resistance to powdery mildew, recovery ability, persistence) underwent a re–pollination in a collection nursery in 2014.

Their progeny – half–sib families (HSF) – were sown in the spring of 2015 in a breeding nursery in rows with a length of 2.0 m, at a 0.5 m row spacing in two randomized replications. The seeding rate was 250 g.s. m^{-1}. 'Nika 11' and 'Sofia' 52 cultivars were used as a control for high summer productivity and adaptability.

Maternal genotypes of the families, with numbers from 1 to 5 in the experiment, originated from synthetic populations combining introduced breeding material. Families with numbers from 6 to 10 originated from elite plants selected in wild local populations.

From the first to the fourth vegetation (2015–2018), according to regrowth's and families were reported the following: green matter yield (kg.m^{-1}) in the phenophase beginning of flowering, dry matter content (%) and dry matter yield (kg. m^{-1}), time for cuts formation (in days).

Ten cuts were harvested altogether, with two cuts being harvested in the year of sowing, in the second and third vegetation – three cuts, and in the fourth one – two.

The general combinative ability of elites (GCA), evaluated as a relative productivity for different seasons of their families compared to the average yield of all families and the standard cultivars [ZARYANOVA & KIRYUKHIN, 2014] and the daily average growth rate (g DM. day^{-1}.m^{-1}) were calculated by means of the obtained data. A comparison of the yield of dry forage mass and its stability was made according to the statistical model of Francis & Kannenberg [FRANCIS & KANNENBERG, 1978] was made.

Table 1.

Meteorological data for the vegetation period (April–October) during the experimental years

Experimental years	Months / Climate indicators	IV	V	VI	VII	VIII	IX	X
2015	Monthly precipitation, mm	39.7	66.0	86.4	20.4	72.3	46.8	37.4
	Average monthly temperatures, °C	12.2	19.8	21.0	25.6	24.8	19.9	11.6
2016	Monthly precipitation, mm	51.4	105.9	67.6	25.4	66.9	37.1	38.4
	Average monthly temperatures, °C	16.2	16.6	23.7	25.0	23.8	19.7	11.4
2017	Monthly precipitation, mm	58.5	82.4	37.8	98.9	8.0	21.5	117.5
	Average monthly temperatures, °C	11.8	16.9	22.5	23.2	24.1	19.8	12.8
2018	Monthly precipitation, mm	10.3	44.3	134.6	126.6	12.6	49.2	17.6
	Average monthly temperatures, °C	16.6	19.4	21.5	22.6	23.5	19.3	n/a

The experimental work was carried out during the period of 2015–2018 in the region of Pavlikeni (43°24'N, 25°32'E, 144 m), which is in the temperate continental climate zone with a well–established continental rainfall regime–with a maximum in May–June and a minimum in August–September.

The experimental years (Table 1) were characterized by more favourable

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conditions in the beginning of summer–with high monthly rainfall in June, which was distributed uniformly by decades.

In 2015 and 2016, the summer regrowing occurred under severe drought conditions–the rainfall sums for July were 20.2 and 25.4 mm, respectively.

In 2017 and 2018, the rainfall sum for the same period was considerably higher (98.9 and 126.6 mm).

A very strong August drought was observed for these two–vegetation period.

The soil type is a leached black earth with a 40–50 cm humus horizon, a good water–holding capacity, soil response is neutral, humus content is 3–4 %, phosphorus and nitrogen supply are medium and a good supply of potassium.

Results and discussion

The seasonal differences in climatic conditions and the age of the plants are the factors that are predominantly due to the variation in the fodder productivity of the perennial legumes used in Bulgaria [MIHOVSKY & NAYDENOVA, 2017, MARINOVA, 2017; GOLUBINOVA, 2018].

According to the results of the present experiment, the age of the plants has a more significant effect on the dry matter yield value in red clover (Table 2).

Table 1.

Dry matter yield per seasons, average over a four–year period

		Early spring production $kg.m^{-1}$	GCA %	Late spring production $kg.m^{-1}$	GCA %	Summer production $kg.m^{-1}$	GCA %
Year of utilization	1st (2015 yr)	–	–		–	0.202^{a}	–
	2nd (2016 yr)	0.437^{a}	–	0.482^{a}	–	0.104^{b}	–
	3rd (2017yr)	0.092^{b}	–	0.095^{b}	–	0.124^{b}	–
	4th (2018 yr)	0.013^{b}	–	0.033^{b}	–	–	–
Genotype	Nika 11	0.184	102.0	0.252^{a}	123.8	0.157^{a}	133.1
	Sofia 52	0.170	94.5	0.205^{ab}	100.7	0.123^{ab}	104.3
	HSF1	0.184	102.0	0.209^{a}	102.6	0.127^{a}	107.0
	HSF2	0.153	84.9	0.177^{b}	87.2	0.168^{a}	141.8
	HSF3	0.176	97.7	0.233^{a}	114.6	0.145^{a}	122.5
	HSF4	0.171	95.1	0.180^{b}	88.5	0.113^{ab}	95.6
	HSF5	0.180	99.9	0.217^{a}	106.7	0.108^{b}	91.3
	HSF6	0.158	87.6	0.137^{b}	67.6	0.117^{ab}	98.6
	HSF7	0.163	90.2	0.193^{ab}	95.0	0.107^{b}	90.4
	HSF8	0.218	120.9	0.209^{a}	102.9	0.097^{b}	81.9
	HSF9	0.214	118.8	0.226^{a}	111.2	0.090^{b}	75.7
	HSF10	0.192	106.5	0.202^{ab}	99.2	0.068^{b}	57.9
$LSD_{0.05}$		0.080		0.070		0.058	

*mean values according to productivity, followed by same letters does not differ significantly at P<0.05.

The average values of the indicator are significantly higher in the first and the second year of utilization.

The greatest difference between the spring and the summer productions was observed in the second vegetation–dry matter yield in summer cut average for all genotypes was only 23 % of that in the first and second cuts.

In the third and fourth vegetation, respectively, the seasonal fluctuations were small, despite the significantly lower moisture content in the summer months.

Reliable genotypic effects on the average seasonal yield of dry forage mass were also found (Table 2).

In early spring growth, the polycross progeny of elite genotypes did not significantly differ in dry forage mass. In contrast, as in late spring and summer regrowing, the effect of the family was significant (P<0.05).

In late spring regrowing, abiotic environmental factors had optimal values (Table 1).

Under these conditions, the highest average yield was recorded for 'Nika 11', as families 1, 3, 5, 8 and 9 fell into a homogeneous group with it.

The summer regrowths in the first and second vegetation were formed in periods of severe drought.

In these conditions, there were vivid differences in the genotypic productivity response.

The highest summer yields of dry forage mass were found in families 2 and 3, as well as 'Nika 11'.

According to the average performance of the families, a high general combination ability in dry forage mass yield in regrowing under the optimal conditions was found in family 3 (with a relative productivity compared to the average yield of all families and standard cultivars 114.6 %), 9 (111.2 %) and 5 (106.7 %).

The highest GCA for yield in summer drought were found in family 2 (141.8 %) and 3 (122.5 %).

The high productive assessment for GCA of 'Nika 11', both in late spring (123.8 %) and in summer regrowing (133.1 %), determined this cultivar as a valuable genetic source in the breeding for high forage productivity during regrowing.

'Nika 11' was created through a recurring family–group team according to persistence and a high summer productivity in foothill conditions, and according to current results it performs these characteristics under conditions of significantly lower moisture than that during the conducting the breeding process.

Genotypic effects are also observed in terms of growth rate (Figure 1).

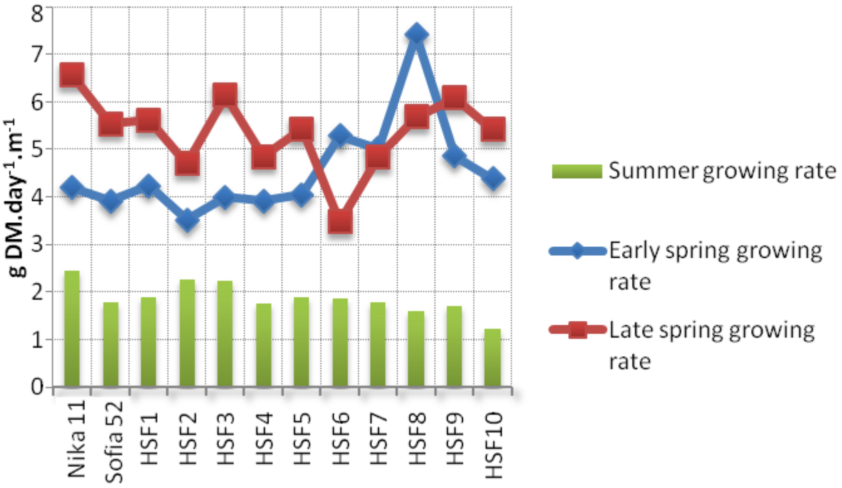

Figure 1. Daily growth rate over seasons, average over a four–year period

Family 6, 7 and 8, originating from elites selected in local populations, showed a distinctly higher growth rate in the initial spring growth.

This biological feature of the local germplasm can be described as adaptive as the early red clover flowering is positively associated with persistence in dry areas [FORD & BARRETT, 2011, GEORGIEVA *et al.*, 2018], as well as the persistence of the population in the grassland [PYŠEK & RICHARDSON, 2008].

These families have a selective value in the pasture as they can provide productivity and quality of grazing grassland in the early–spring period.

Families that originate from material already subjected to a phenotypic breeding according to total/annual productivity (numbers 1 to 5) had a higher average daily growth rate in late–spring conditions during the maximum rainfall for the area.

Higher productivity under optimal conditions of the germplasm with a high degree of cultivation is also recorded in other studies on that crop [ANICCHIARICO & PAGNOTTA, 2012; NAYDENOVA *et al.*, 2015].

Summer growth rates for all genotypes were significantly lower compared to the spring season.

A relatively quicker summer regrowing was recorded only for family 2, 3 and 'Nika 11'.

The severe delay of vegetative growth during drought, which was characteristic for some of the local families (10 and 8), was also a mechanism of draught resistance observed in many species and genotypes of forage grasses [SANDERSON *et al.*, 1997; SALIS et al. 2006; ZWICKE *et al.*, 2013].

The stability in yields over seasons and years, which according to a number of studies is subject to genetic control [ROSSO & PAGANO, 2005; DUNEA, 2008, NAYDENOVA et al. 2015; NAYDENOVA, 2016] can also be seen as indicative of high productivity during summer regrowing as well as high persistence.

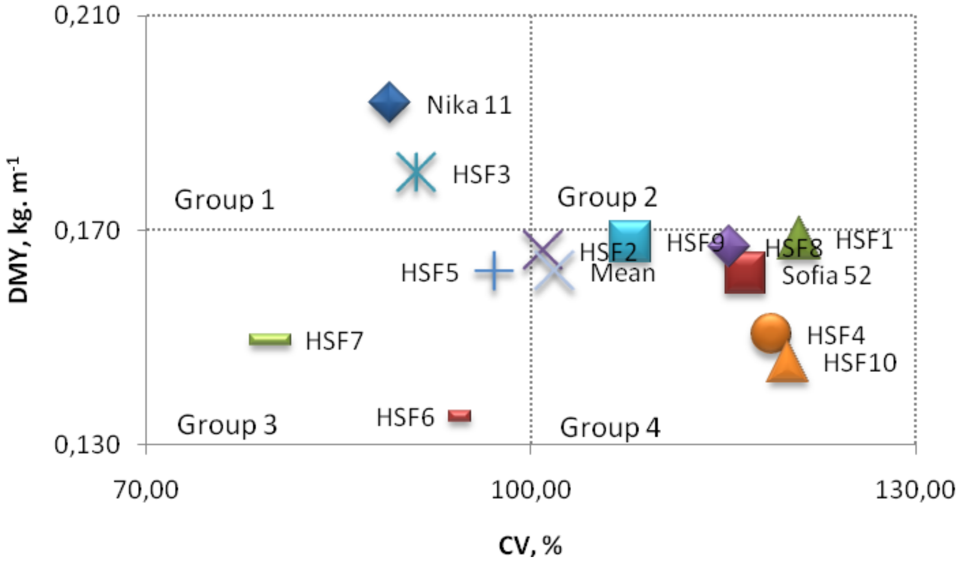

Figure 2. Comparison of the average yield of dry forage mass with its variation over seasons, years and age of plants.

According to the data presented in Figure 2, only one of the studied families – HSF3, together with 'Nika 11' – showed a high and relatively constant yield of dry forage mass per seasons, years and age of the plants.

Family 7 showed the most stable performance of that indication during a four–year period, although the average productivity of this local genotype was low.

Most of the studied material, together with 'Sofia 52', was a homogeneous in the low–level ratio of value and stability of the forage mass yield.

Valuable genotypes for the breeding in relation to yield stability over seasons and age of plants, respectively years of use, can be selected in family 7, which originated from a local wild–type germplasm.

The high expression in the level and stability of the observed characteristics, as well as the high estimates of general combination ability, determined 'Nika 11' as an important genetic source in the breeding for high forage productivity in regrowing. According to these results, 'Nika 11' is used as a major component in the formation of polycross groups with the selected families.

Conclusions

A genetic potential for increasing the yield of dry forage mass for both late–spring and summer regrowing was found for family 2 and 3.

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