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PHENOTYPIC ANALYSIS AND HERITABILITY OF SEED PRODUCTION COMPONENTS IN RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Abstract

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A comparative test of Bulgarian and Czech red clover varieties was carried out on elements, which determine the seed yield in order to evaluate the component composition of the variation and the inheritance of these indicators in relation to the high seed productivity selection. The study was conducted in the period 2013-2015 in RIMSA – Troyan, under the soil and climate conditions, representative for the foothill and mountain regions of Northern Bulgaria. The experience is set in four randomized repetitions, with size of the experimental plot of 5 m^2, as it includes the varieties Nika 11 (2n), Sofia 52 (2n), Respect (2n), Suez (2n), Vltavin (2n), Kvarta (4n) and Tempus (4n). The greatest genotypic diversity in the studied group of varieties was observed with respect to the number of flower heads per plant, the number of seeds per flower head and the absolute weight of the seeds. The diploid varieties, such as Nika 11 and Respect are characterized by the highest number of flower heads per plant. Sofia 52 and Vltavin are characterized by the highest number of seeds per flower head. These varieties are included in our selection program for increasing the seed productivity of the species.

Key words: red clover; seed yield components

Introduction

Red clover is a major alternative feed crop of alfalfa in areas with acidic or soaked soils in Bulgaria. The production of seeds of that species, in a volume demanded by the market, first requires the use of new improved varieties adapted to conditions and practices of use (Boelt et al., 2015). In cultivation of red clover in the foothill conditions of Central Northern Bulgaria, the variability of seed production and its components by variety, regrowths, age of grassland and conditions of the year is very high (Goranova, 2002). The effects of genotypic factor (including the level of ploidy, subspecies and biotype belonging) on the elements of seed productivity of the species have been examined in many studies (Malengier and Baert, 2007; Boller et al., 2010; Vleugels et al., 2015; Amdahl et al., 2016 a,b). According to our results, the late-flowering forms, as well as the varieties that are closer to the ecological and geographical origin of Bulgaria, have better setting of blossoms, and the amount of generative stems per unit area of nutrition and the fertility of the blosoms are the structural elements of seed yield, which have lower values at a tetraploid level (Goranova and Mihovsky, 2002).

The selective progress in terms of seed productivity in red clover is evaluated as weak (Vleugels et al., 2015; Boelt et al., 2015). There could be pointed out as a reason that the majority of the selection programs are aimed at the overall and seasonal productivity of the forage weight, durability, grazing sustainability, suitability for growing in mixtures. The modern European varieties are also predominantly tetraploid, and the tetraploid germplasm of the species is characterized by a significantly lower seed yield than the diploid one.

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The red clover is an entomophilic allogamous species with gametophytic self-incompatibility and the success of the selection programs depends to a large extent on the heterogeneity of the source material used (Popović et al., 2007). In most recent studies, phenotypic analysis and molecular markers identify the signs and genotypes with potential for use in the selection of red clover reproductive capacity (Herrmann, 2006; Vleugels et al., 2014). Most often in the selection of legume forage grasses, a family-group team is used, which controls seed productivity and its determining elements only for maternal genetic types. The Bulgarian red clover variety Nika 11 was also created by this method. It has very high seed productivity in different conditions than the conduction of the selection process (Nedělník et al., 2016). Vleugels et al. (2014) identify as the most effective the parallel control of maternal and paternal genotypes in the selection of diploid germplasm of the same species (2014). A comparative test of Bulgarian and Czech red clover varieties was carried out on elements that determine the seed yield in order to evaluate the component composition of the variation and the heritability of these signs in relation to the high seed productivity selection.

Materials and Methods

The present study was conducted in the period 2013-2015 in RIMSA – Troyan. The following di- and tetraploid varieties were tested in a field experiment, in four randomized repetitions in an experimental parcel of 5 m^2: Nika 11 (2n), Sofia 52 (2n), Respect (2n), Suez (2n), Vltavin (2n), Kvarta (4n), Tempus (4n). They are characterized by elements of seed productivity – number flower heads per plant; number of blossoms per flower head; seed number in flower head; fertility of the blossoms; absolute seed weight. The indicators are followed under the scheme for seed production that is suitable for Bulgarian condition – annual harvesting of the second regrowth. Biometric measurements were made according to vegetations on an average sample of 20 plants of each variety. The mean values of the observed indicators were determined, using Dunkan's multi-rate test at the least significant difference (LSD) with a significance level of 0.05. Two-factor analysis of variance model 3 was used to determine the component composition of the variance, (Annicchiarico, 2002). The components of the variation are calculated as follows:

$S^2_{GY} = (MS_{GY} – MS_E) / r$

$S^2_G = (MS_G – MS_{GY}) / ry$

$S^2_e = MS_E$

$S^2_{Ph} = (S^2_G + (S^2_{GY}/y)+(S^2_e/ry))$

The heritability coefficient in broad sense is calculated as a share of the genetic variance of the observed phenotypic variance $H^2bs = S^2_G / S^2_{Ph}$ on the studied signs under experimental conditions and for the selected group of species.

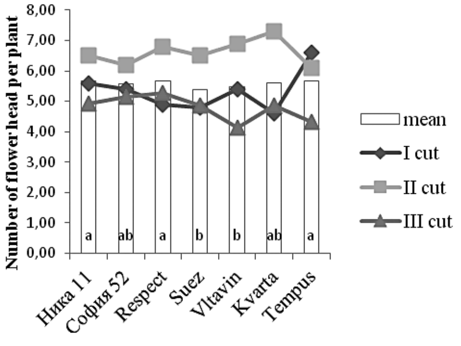
The study was conducted in soil and climate conditions, representative for the mountain regions of Northern Bulgaria. The soils are planosols, unsaturated, with low to moderate content of mobile forms of nitrogen, very low content of assimilating phosphorus and medium reserve of assimilating potassium. The average monthly precipitation for the vegetation period was 74.7 mm and the average monthly temperature was 15.9°C. August and September were very dry in the year of sowing. In the second and third experimental years, the monthly rainfall during vegetation was higher than the usual for the region.

Results and Discussion

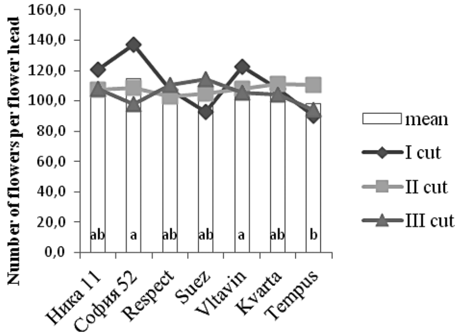
Higher mean values for the number of flower head per plant were established for Nika 11 and Respect diploid varieties as well as for the tetraploid variety of Tempus (Figure 1a). Suez and Vltavin varieties yielded with proven difficulties. The values of the indicator for all genotypes were the highest in the second vegetation of the species, and the lowest in the third, with the exception of Respect variety. Less blossom setting with the increase of plant age was observed in our other research. It is believed that for tetraploid varieties, the lower seed productivity is associated with less formation of flower heads (Vleugels et al., 2014; Amdahl, 2016a), but such dependence in the present study has not been observed. A lower variation was observed for that attribute by years for the Bulgarian varieties Nika 11 and Sofia 52.

The number of blossoms in flower head has the highest average values for the diploid varieties Sofia 52 and Vltavin, and the lowest for the tetraploid Tempus – Figure 1b. The high variation between the studied genotypes in the first vegetation is of no practical and selective significance, as grasslands of that species are rarely harvested for seed in the year of creation. The studied varieties fall into a homogeneous group according to the values of the indicator in the second and third vegetation. According to Vleugels et al. (2014) the tetraploid varieties do not yield to the diploid in number of blossoms per flower head. In a study by Muntean (2006), the amount of blossoms per flower head depends on the subspecies belonging of varieties. Those from *subvar. intermedium*, originating from Central Europe, as the species in the present study also belong to them, form significantly more flower heads than *var. serotinum.*

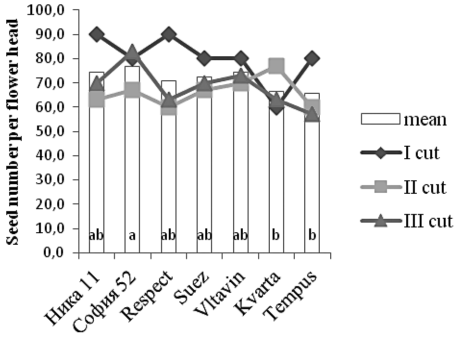
The number of seeds per flower head and the fertility of the flowers are seed production components strongly correlated with the yield of plant seeds (Herrmann et al., 2005). In the genotype study group, the highest number of seeds per flower head was observed for the variety that formed the most numerous



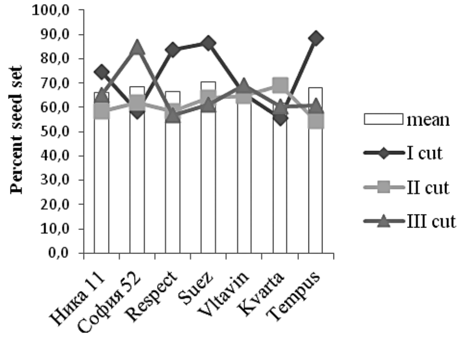
a) Number of flower heads per plant



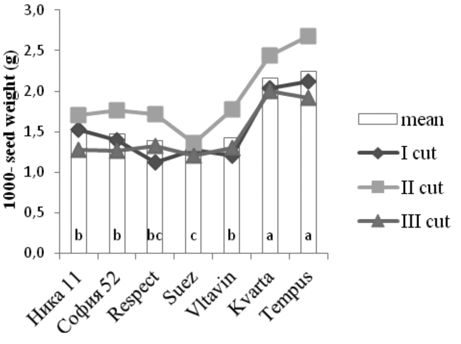
b) Number of flowers per a flower head



c) Seed number per a flower head



d) Fertility of flowers (%)



e) Absolute weight of seeds (g)

Figure 1 a-e. Seed yield components of red clover cultivars by years and mean for period. Mean values, denoted by the same letters, do not differ significantly, P = 0.05

flower heads – Sofia 52 (Figure 1c). Both tetraploid varieties Tempus and Kvarta yielded to it with significant differences. The lowest phenotypic variability of this indicator was observed for Vltavin diploid variety by years. According to the results of another our study, the main reason for the lower yield of seeds in tetraploid varieties in the region is the inferior fertility of flowers compared to that of the diploid varieties (Goranova and Michovski, 2002). This was observed only in individual vegetations in the present study – in the Kvarta variety in the first vegetation and in Tempus variety in the second (Figure 1d). According to data, the factors that were observed in the experiment – genotype/ level of ploidy, age of grassland and conditions of the year do not reliably influence the fertility of flowers. The predominant random variance of the indicator (Table 2) can be explained by the entomophilic pollination of the species – with the presence and activity of the pollinators and with the nectar secretion, which is related to climate conditions during flowering. These results support the conclusion of Amdahl et al. (2016) that a discrepancy between the flower morphology of tetraploids (characterized by longer flower tubes) and pollinators is not the main or only reason for the inferior fertility of their flowers.

The absolute weight of seeds is determined by the level of ploidy of the studied varieties – Figure 1e. In the case of diploid varieties, higher average values were observed for varieties Nika 11, Sofia 52 and Vltavin. Suez variety yielded to them with a proven difference. The differences between both tetraploid varieties are unreliable, and for Tempus variety the variability in the values of the indicator by years/vegetations is greater.

The analysis of the variant shows that all observed elements of the seed yield in red clover, with the exception of the absolute weight of seeds, are under the prevailing influence of external factors (Table 1), including also the age of the grassland because of the experimental setting. A higher effect of the genotypic-mediated interactions was reported on the phenotypic value of the indicator seeds per flower and absolute weight of seeds. According to the values of the heritability coefficient (H^2_{bs}), the genotype variant is relatively higher in terms of number of flower heads per plant and seed number per flower head (H^2_{bs} = 0.33; 0.27). According to Herrmann et al. (2005) the number of flower heads per a plant is a particularly useful indicator of increasing seed productivity of red clover since it can easily be determined before seed maturation. Amdahl et al. (2016a, b) determine the yield of seeds per flower head as the component in the strongest positive dependence with the yield of seeds per unit area of red clover, both at di- and tetraplod levels. And, according to the results of our study, these two seed yield components should be used as a selection criteria in our selection program. The established high heritability by indicator absolute seed weight (H^2_{bs} = 0.82) in the experiment is related to the different level of ploidy of the studied material. Also, given the small-sized seed of red clover, it is not efficient to use the indicator in the seed selection to increase the seed yield.

Table 1

Component composition of the variance and heritability coefficient in a broad sense (H^2bs) according to seed yield of red clover

Traits	Estimates of variance components				Heritability
	S^2_G	S^2_{GxY}	S^2e	S^2_{Ph}	H^2_{bs}
Number of flower head per plant	0.18	2.9	0.4	0.6	0.32
Number of flowers per flower head	27.9	22.0	125.5	153.3	0.08
Seed number per flower head	28.6	151.2	78.7	107.3	0.27
Percent seed set	5.5	54.3	138.8	144.3	0.04
1000-seed weight (g)	0.07	0.20	0.02	0.09	0.82

S^2_G – genotypic variance; S^2_{GxY}– variance for interaction of genotype X year; S^2e – random variance; S^2_{Ph} – phenotypic variance; H^2_{bs} – coefficient of heritability broad-sense heritability

Conclusion

The seed components with the highest genotypic diversity in the studied group of varieties are the number of flower heads per plant, the number of seeds per flower head and the absolute weight of seeds. The diploid varieties, such as Nika 11 and Respect are characterized by the highest number of flower heads per plant. Sofia 52 and Vltavin are characterized by the highest number of seeds per flower head. These varieties are included in our selection program for increasing the seed productivity of the species.

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