

# COMPARATIVE EVALUATION OF DI- AND TETRAPLOID ACCESSIONS OF RED CLOVER (*TRIFOLIUM PRETENSE* L.) FOR RESISTANCE TO POWDERY MILDEW (*ERYSIPHE POLYGONI* DC)

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**Abstract:** The aim of this study was to evaluate the tolerance of twelve genetic accessions of red clover to Powdery mildew (*Erysiphe polygoni* DC). The trial was carried out during 2014-2015 at the Experimental station of soya-bean Pavlikeni at a natural infection. The plant type of reaction was evaluated by a four-grade scale depending on the % of leaves with typical symptoms. A significant effect ( $P < 0.001$ ) of the genotype, and hence the level of ploidy, on the type of reaction to the disease of powdery mildew was established. Average for the period of study, the tetraploid accessions of red clover were rated as moderately susceptible (score 3.1), and the diploid ones – as moderately resistant (score 2.7). In the selection (breeding) of red clover the diploid accessions P-3 and Sofia-52 variety can be used as genetic sources of tolerance to powdery mildew.

**Key words:** red clover, Powdery mildew

## Introduction

Red clover (*Trifolium pratense* L.) is distinguished for very good summer productivity and after grass and in sown swards it has the function to increase forage productivity and quality in summer and autumn. Powdery mildew (with causal agent *Erysiphe polygoni* DC.) attacks red clover in the mentioned seasons and is the fungal disease having the greatest economic importance to this crop in Bulgaria. The disease symptoms are increasingly larger spots of powdery covering on the stems, adaxial and abaxial leaf surfaces. The leaves become yellow and wither, which decreases the quality of the fresh forage and hay, winter survival of the plants and hence the sward productivity (Taylor, 2008). Red clover is attacked by the pathogen considerably more severely, as compared to the other cultivated species of clovers – white, alsike and crimson clover (Yarwood, 1936). The development of resistant varieties is defined as the most efficient and ecological

way of controlling the diseases in legume forage crops (Kimbeng et al., 2000; Taylor, 2008 a). The resistance or tolerance to powdery mildew is reported to be characteristic of a number of new di- and tetraploid varieties of red clover (Schubiger et al., 2004; Frick et al., 2008; Taylor, 2008; Rahjoo et al., 2012). According to Taylor and Quensenberry (1996), the resistance to the disease has a two-locus dominant-recessive model of inheritance. This resistance is defined as active inherent immunity and is manifested as necrotic reactions of the genotypes resistant to the causal agent of powdery mildew. In foreign and our studies the resistance to powdery mildew in genotypes of red clover is also associated with factors of the passive inherent immunity - micromorphological characteristics, such as synthesis and density of leaf wax covering (Mika and Bumerl, 1984) and a high degree of hairiness of the leaf surface by nonglandular trichomes (Taylor, 2008; Naydenova and Georgiev, 2013). According to Hejduk and Knot (2010), the susceptibility also depends on the age of plants (sward), the young plants being affected more severely by the disease. The ecological factors have also a significant effect on the phenotypic reaction to the disease. The susceptibility of the varieties of red clover increases considerably when grown on soils of high content of calcium (neutral reaction) and available phosphorus (Repsiene and Nekrosiene, 2006).

The objective of this study was to investigate the presence of genotypic variance of the response to powdery mildew associated with the level of ploidy in accessions of red clover and to find out genetic sources of resistance to *Erysiphe polygoni* DC.

## Material and Methods

The study was conducted in the Soybean Experimental Station of Pavlikeni (43° 24' N; 25° 32' E; 144 m above sea-level). The soil type is leached chernozem. The soil reaction is neutral. The attack by Powdery mildew with natural infection in July and August of the two experimental years 2014 and 2015 was observed in 12 accessions of red clover, including the two Bulgarian diploid varieties of the species - Nika 11 and Sofia 52, four local populations and six foreign tetraploid varieties. The accessions were sown in the spring of 2014 in randomized rows 1.5 m in length with two replications. Ten plants from each replication were observed. The plant type of reaction was evaluated by a four-grade scale depending on the % of leaves with typical symptoms: grade (1) resistant – leaves with no spots of powdery covering, (2) moderately resistant – up to 25 % of the leaves have symptoms of the disease, (3) moderately susceptible – up to 50 % of the leaves have symptoms and (4) susceptible – over 50 % of the leaves have symptoms.

A variance analysis (ANOVA model including the factors G = genotype and Y = year, cut, model 2) was used to determine the ecological and genotypic variance of type of reaction to powdery mildew in the studied group of genotypes.

## Results and Discussion

According to the analysis of variance, the genotypic factorial influence had a significant effect ( $P < 0.001$ ) on the phenotypic variation in type of reaction to Powdery mildew – Table 1. The differences in the climatic conditions by years and cuts were also a significant source ( $P < 0.001$ ) of variability in the response of the studied genotypes to the pathogen. The disease develops in moderately dry conditions and according to the recorded average scores (Table 2) a more severe attack was observed in the periods with droughts – in 2014 it was in August, and in 2015 – in July (Figure 1).

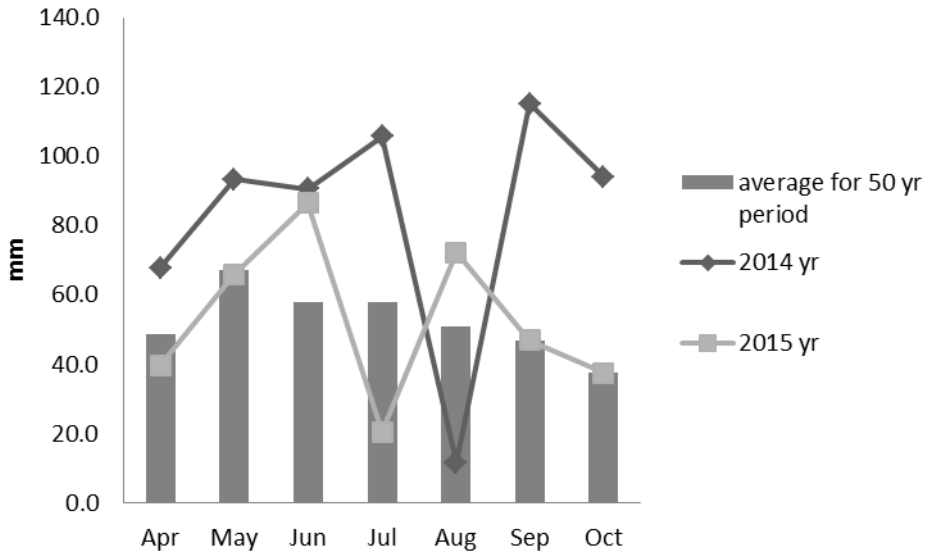
**Table 1. Analysis of variance by reaction to powdery mildew**

Source of variance	SS	df	MS	F	P-value	F crit
Genotypes	17.19	11	1.56	10.93	0.000000	2.09
Year, cut	4.94	3	1.65	11.52	0.000025	2.89
Residual	4.72	33	0.14			
Total	26.84	47				

**Table 2. Type of reaction to powdery mildew of red clover genotypes**

Genotypes	Ploidy	Type of reaction to <i>Erysiphe polygoni</i> DC., Mean score from 20 plants			
		Jul 2014	Aug 2014	Jul 2015	Aug 2015
1. Nika 11(2x)	2x	3.00	2.55	2.75	2.50
2.Sofia 52 (2x)	2x	2.25	2.60	2.50	2.50
3.Kvarta (4x)	4x	3.15	3.35	4.00	3.60
4.Astur (4x)	4x	2.00	2.00	3.40	3.00
5.Carbo (4x)	4x	2.25	2.70	4.00	3.25
6.Elanus (4x)	4x	2.00	2.45	3.25	2.50
7.Fregata (4x)	4x	3.00	3.55	4.00	3.75
8.Larus (4x)	4x	2.60	3.25	4.00	3.50
9.P1 (2x)	2x	1.90	3.15	3.00	2.80
10.P2 (2x)	2x	1.55	3.40	2.75	2.00
11.P3 (2x)	2x	1.25	2.00	2.15	1.60
12.P4 (2x)	2x	4.00	4.00	4.00	4.00
Mean for diploids		2.3	2.9	2.9	2.6
Mean for tetraploids		2.5	2.9	3.8	3.1

\* type of reaction – score: (1) - resistant, (2) - moderately resistant, (3) - moderately sensitive, (4) - sensitive



**Figure 1. Monthly distribution of rainfall (mm) for investigation period**

In some studies conducted with a large number of origins of red clover it was found that the tetraploid genotypes had lower susceptibility to fungal diseases (Vleugels *et al.*, 2012; Bukauskaite *et al.*, 2014), and in other studies they had higher susceptibility (Jacob *et al.*, 2010; Schubiger *et al.*, 2003). In this study the diploid Bulgarian varieties and populations, except for population 4, showed higher resistance to Powdery mildew, as compared to the foreign tetraploid germplasm – Figure 2. The lowest attack to the leaves was observed in the local population with number 3, for which the scores of the four records were within the range of 1.25 to 2.15 and therefore showed a resistant to moderately resistant type of reaction (Table 2). The reaction of the Bulgarian varieties Sofia 52 and Nika 11 to the disease remained constant when the conditions of regrowth and plant age changed. This characteristic can be associated with presence of genetic resistance to the local strains of the pathogen and therefore Sofia 52 variety, which is moderately resistant, can be used in plant breeding for tolerance to powdery mildew. In the group of tetraploid genotypes, variety Elanus, for which a predominately moderately resistant type of reaction was recorded, had lower susceptibility to the disease. This genotype also showed the best resistance to anthracnose, as compared to all tetraploid varieties of red clover registered in the European variety list (Jacob *et al.*, 2010).

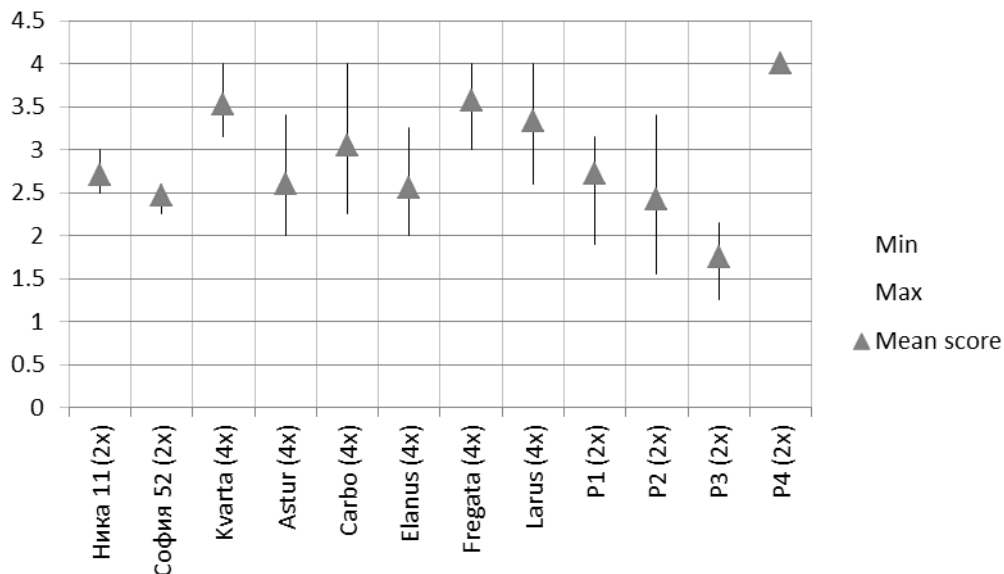


Figure 2. Marginal and mean scores by type of reaction to Powdery mildew at red clover accessions

## Conclusion

A significant effect ( $P < 0.001$ ) of the genotype, and hence the level of ploidy, on the type of reaction to the disease of powdery mildew was established. Average for the period of study, the tetraploid accessions of red clover were rated as moderately susceptible (score 3.1), and the diploid ones – as moderately resistant (score 2.7). In the plant breeding of red clover the diploid accessions P-3 and Sofia-52 variety can be used as genetic sources of tolerance to powdery mildew.

## Komparativna ocena di- i tetraploidnih formi crvene deteline (*Trifolium pratense* L.) na otpornost na pepelnicu (*Erysiphe polygoni* DC)

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Cilj ovog istraživanja je bio da se proceni tolerancija dvanaest genetskih formi crvene deteline na pepelnicu (*Erysiphe polygoni* DC). Oglad je sproveden

tokom 2014-2015 godine, u eksperimentalnu stanicu Pavlikeni u uslovima prirodne infekcije. Tip reakcije biljke je procenjivan na skali četiri razreda zavisno od % lišća sa tipičnim simptomima. Utvrđen je značajan efekat ( $P < 0,001$ ) genotipa, a time i nivoa ploidnosti, na tip reakcije na bolest pepelnicu. Prosek za period istraživanja, tetraploidne forme crvene deteline su ocenjene kao umereno osetljive (rezultat 3.1), a diploidne forme - kao umereno otporne (rezultat 2.7). U selekciju (uzgoj) crvene deteline, diploidne forme P-3 i sorta Sofija-52 se mogu koristiti kao genetski izvori tolerancije na pepelnicu.

**Ključne reči:** crvena detelina, pepelnica

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