

RESEARCH ARTICLE

Polymorphism in populations of white clover (the City of Gorno-Altaysk, Western Siberia)

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The polymorphism of the populations of *Trifolium repens* L. was studied on the basis of the presence and diversity of the «gray spot» on the leaves. In 2016–2017, we studied polymorphism in the populations of white clover growing in different areas of Gorno-Altaysk. For the account and identification of phenotypes 10 trial randomized platforms in parks, on waste grounds, in the yards, along highways, at gas stations, about boiler rooms and plant of reinforced concrete products were put. The natural population of white clover growing outside the city limits is taken as a control. The collection of material for the detection of genetic polymorphism was carried out in the summer during the abundant flowering of clover (July–August). In total, we analyzed 1500 plants. The analysis of the samples was carried out in accordance with the method of Ia. Schwartzman (1986). During the observation, the shape of the pattern, the frequency of occurrence of the identified hair dryer was recorded, the presence of plants with any unique hairdryers (a picture of a different color, leaves with 4 or more leaves) was noted. A phenotypic diagnosis of the growing conditions of white clover was conducted at various points in the city of Gorno-Altaysk. A high degree of polymorphism was detected in urban clover populations, a significant decrease in the proportion of hair dryer 1 (without picture), and the appearance of new hair dryers not characteristic of control plots.

Keywords: white clover; hairdryers; phenotype; «gray» picture; genetic polymorphism; the index of frequency of occurrence of hair dryers

Introduction

Environmental monitoring of the environment can be carried out using phenotypic bioindicators. The hair dryer is a variation of any sign which reflects genetic features of individuals. When clearly identified alternative changes in the manifestation of morphological characteristics of biological objects can be quantified heterogeneity of populations, especially their organization and structure, the level of anthropogenic impac (Caradus et al., 1993; David et al., 2001; Welham et all, 2002). The degree of implementation of specific genofonda serves as an indicator of the level of favored conditions (Sokolova, 2010, 2016) as part of the hairdryers any symptom occur with high frequency and constant (conventional "normal") and the other with low frequency and rarely (deviations from normal conditions – anomalies).

As an indicator of the degree of anthropogenic load is often used white clover. It is characterized by a wide range of tolerance to a number of abiotic factors (Hirano, 2005; Helgadottir et al., 2007). It is more resistant to mechanical damage and trampling. White clover is often found in disturbed areas, in plant communities under the influence of intensive anthropogenic factors. Clover leaves are threefold, on the surface of the leaves there are spots or whitish strips in the form of a diamond – shaped pattern – "gray spot". As a result of histological studies (Cern, Harper, 1976) it was found that the spots are associated with a group of palisade cells in which the number of chloroplasts is small or they are completely absent.

One of the characteristic features of clover is polymorphism on the basis of "gray spot". Figure can be characterized by size, shape, location, color, intensity of manifestation. The severity of the "gray spot" and its shape is influenced by the age of the plant, its shape, the relative size of the leaves, as well as anthropogenic factors (Vasiliev et al., 2007; Korona, Knyazev, 2014). The presence of a "gray" spot on the leaves is a dominant sign (V), its absence is recessive (v). Various forms and levels of spots are formed during ontogenesis as a result of the action of the corresponding alleles of the V gene, which violate the normal development of chlorophyll. The effect of gene V in the leaves of the clover is manifested in the decrease in the content of chlorophyll in the cells of the "gray spots", changing their shape and earlier death (Nakhaeva et al., 2015). Alleles (dominant or co-dominant) often have a fuzzy phenotypic manifestation, only about 10% of plants can definitely be attributed to a particular genetic type (Inge-Vechtomov, 1989). But the analysis on this basis can be carried out using the frequency of certain phenotypes (Valiev, Yakovleva, 2008). The degree of realization of the species gene pool can serve as an indicator of environmental conditions (Gorshkova, 2012; Levitsky, 2013). The shape of the gray pattern on the plates of the white clover leaf and the frequency of its occurrence are good indicators of environmental pollution in urban ecosystems (Kupriyanova, Semenova, 2014; Aleksandrova, Nakhaeva, 2015).

Materials and methods

White clover (*Trifolium repens* L.) is a perennial plant with a highly branched root system, shortened main aboveground shoots and lateral creeping rooted shoots up to 10–50 cm long. White clover grows well on different soils with a sufficient amount of nutrients and water. This light-loving plant, which under favorable conditions grows rapidly, forming a closed cover. White clover is represented by individuals of seed origin (genets) and individuals of vegetative reproduction. Genes are more common in areas with anthropogenic load and disturbed vegetation. In stable and stable communities, white clover is mainly represented by elongated creeping shoots, growing tops and forming axillary lateral shoots (Denisova, 1995).

In 2016–2017, we studied polymorphism in the populations of white clover growing in different areas of Gorno-Altaysk. For the account and identification of phenotypes 10 trial randomized platforms in parks, on waste grounds, in the yards, along highways, at gas stations, about boiler rooms and plant of reinforced concrete products were put. The natural population of white clover growing outside the city limits is taken as a control. The collection of material for the detection of genetic polymorphism was carried out in the summer during the abundant flowering of clover (July–August). In total, we analyzed 1500 plants. The analysis of the samples was carried out in accordance with the method of Ia. Schwartzman (1986). During the observation, the shape of the pattern, the frequency of occurrence of the identified hair dryer was recorded, the presence of plants with any unique hairdryers (a picture of a different color, leaves with 4 or more leaves) was noted. For populations of white clover at each test location was calculated the frequency of occurrence of individual hairdryers according to the formula:

$$P_i = \frac{n_i}{N} * 100\%$$

where p_i is the frequency of the i -th hair dryer; n_i is the number of recorded plants with this pattern on the leaf blade; N is the total number of recorded plants.

The data obtained were processed by conventional statistical methods using the program Statistica.

Results and description

Table 1. Average frequency of phenotypic classes, %

hair dryer	The symbol of the phenotype	Genotype	Frequency of hair dryers, %
1 – without spot	O	vv	40,41
2 – high wide triangle	A ^H	V ^H V ^B	15,66
3 – low and wide triangle	A ^H	V ^H V ^H	10,86
4 – high narrow triangle	A	VV	3,91
5 – low narrow triangle	A	Vv	1,44
6 – triangle with a notch at the top	B ^H C	V ^{Bh} V ^p	8,32
7 – low triangle with a spot at the top	A ^H C	V ^H V ^p	8,74
8 – ^- shaped spot	C	V ^p V ^p	5,02
9 – high triangle with a spot at the top	A (C)	V ^p V	5,44
Leaves with 4 leaves			0,23

Table 2. The diversity and frequency of occurrence of clover hair dryers creeping in different places of growth

Place of growth	Types of hair dryers and frequency of their occurrence, %									
	Hd1	Hd 2	Hd 3	Hd 4	Hd 5	Hd 6	Hd 7	Hd 8	Hd 9	new forms
Control	71.7	15.1	7.5	5.7	–	–	–	–	–	–
Parks	62.0	19.0	2.0	9.0	–	8.0	–	–	–	–
Yards	47.9	14.6	12.5	–	–	7.3	15.6	2.1	–	–
Lawns	27.0	14.0	27.0	–	–	8.0	11.0	12.0	1.0	–
Wastelands	39.0	21.0	7.0	–	3.0	8.0	15.0	7.0	–	–
Highways	44.0	23.0	5.0	–	3.0	13.0	5.0	3.0	4.0	–
Autorefuellings	24.2	15.1	20.2	–	–	13.1	4.0	5.0	17.2	1.2
Boiler	26.9	5.8	9.7	19.2	2.9	7.7	8.6	6.7	12.5	–
Reinforced Concrete products plant	35.9	22.9	6.5	2.1	1.1	13.0	10.9	2.2	5.4	–
Circuit	25.5	6.1	11.2	3.1	4.1	5.1	17.3	12.2	14.3	1.1

The results of the study showed that there are 10 types of hair dryers in clover populations on the territory of the city (Table 1). Assessment of the frequency of occurrence of fen revealed a high prevalence in all populations of the dryer 1 without pattern

(40%). The average frequency of occurrence is characterized by the following hair dryers: hair dryer 2 (16%), hair dryer 3 (11%), hair dryer 6 (8%), hair dryer 7 (9%). Low frequency of occurrence was noted for 4, 5, 8, 9 hair dryers, as well as for anomalies of complex leaves (leaf with 4 leaves).

For all the studied populations of white clover is characterized by the presence of the dryer 1, dryer 2 and a dryer 3 (Table 2). In the control and in the parks is not met, the dryer 5, the dryer 7. The smallest phenotypic diversity and dominance of the hair dryer 1 is typical for control clover populations (3 types of hair dryers), as well as for populations growing in parks (5 types of hair dryers). A high level of polymorphism (8–10 types of hair dryers) was observed in clover populations associated with roads and gas stations, boilers, motor racing and reinforced concrete products plan.

For the white clover population at each trial site the total frequency of occurrence of all forms with a pattern (the index of correlation of hairdryers – ICH) was calculated by the formula:

$$ICH = (n_1 + n_2 + n_3 + n_i) / N \cdot 100 \%,$$

where $(n_1+n_2+n_3+n_i)$ – the number of hair dryers on the test site; N –the total number of recorded plants.

Phenotypic diagnosis of growth conditions white clover, in the city of Gorno-Altaysk showed that the index of frequency of occurrence of fen clover was in control of the population 4%. The increase in the intensity of anthropogenic load leads to an increase in the frequency index of occurrence of phenes by 1–6% (Table 3).

Table 3. Results of phenotypic diagnosis of creeping clover in the city of Gorno-Altaysk

No	Region	The index of correlation of hairdryers (ICH), %
1	Control	3.77
2	Parks	5.00
3	Yards	6.25
4	Lawns	7.00
5	Wastelands	7.00
6	Highways	8.00
7	Autorefuellings	8.01
8	Boiler	8.65
9	Reinforced Concrete products plant	9.78
10	Circuit	10.20

Conclusions

Thus, within the city of Gorno-Altaysk in the populations of white clover 10 phenotypes were identified, four of which were noted in all habitats. The clover populations in the control areas are more homogeneous, characterized by a smaller variety of phenotypes and a high frequency of hair dryer 1 (without figure), which is most likely due to more stable conditions and the lack of anthropogenic load.

A high degree of polymorphism has been established for urban populations of white clover exposed to various anthropogenic loads (mowing, trampling, atmospheric pollution, soil salinization, etc.). For urban populations of clover characterized by a significant decrease in the proportion of hair dryer 1 (without picture) and the emergence of new backgrounds with different shapes and colors of "gray spots" on the leaves.

In urban populations, polymorphism is most likely due to adaptive superdominance effects and stabilizing natural selection.

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