Changes in macroelemental composition of red clover under influence of phytopathogenic fungi

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Abstract. The article discusses the issue of metabolic changes in the leaf tissues of red clover under the influence of the spectrum of phytopathogens, determined by means of X-ray microanalysis. In the course of the research, qualitative characteristics of metabolic changes under the influence of various phytopathogens were obtained, and quantitative parameters were determined for changes in the macroelement composition of clover plant tissues. All this makes it possible to obtain data on changes in plant metabolism, as well as changes in transport processes under the influence of phytopathogens.

Introduction

The X-ray microanalysis technique is an effective tool for studying the macroelement composition of plants at various levels - from tissue to subcellular [1–4]. Data on the macroelement composition of plants provide an opportunity to solve phytopathological problems using physiological approaches (osmotic pressure, membrane permeability, transport of elements, etc.) [5–7].

Under the conditions of Western Siberia, fodder crops are annually affected by a complex of pathogens, leading to significant crop losses [8–9]. However, the etiology and pathogenesis of various diseases are poorly understood. In this regard, a promising and urgent task is the development of tools for determining plant damage at different stages of the pathological process. In this case, a comprehensive study of plant immunity is of particular importance. It will allow studying the mechanisms of resistance to phytopathogens in fodder crops, as well as improving methods for diagnosing resistant genotypes, which can be used for breeding for plant resistance to infectious diseases.

1. Objects of research

Red clover (Trifolium pratense L.); diseases: anthracnose (Kabatiella caulivora Kirchn.), brown spot (Pseudopeziza trifolii Biv.); macroelements: magnesium, phosphorus, sulfur, potassium and calcium in healthy and diseased tissues of the leaves of clover plants.

2. Methods

In the work on X-ray microanalysis, plants from field experiments and a herbarium were used [4, 5]. The studies were carried out on a JEOL JSM-6510LV scanning electron microscope under the following conditions: accelerating voltage - 20 kV, X-ray radiation registration time - 20 s, electron probe current - 15 nA. We used an Oxford X-MAX-80 energy dispersive detector.

3. Research results
The content of potassium in the tissues of the leaves of healthy plants of red clover was almost twice the amount of calcium. The content of calcium in the control is twice that of magnesium. The content of phosphorus and sulfur in the tissues of the leaves of healthy plants of red clover was noted in the range of 0.5–0.3 % (Figure 1).

![Bar chart showing the mass fraction of macroelements in leaf tissues of red clover - control](image)

**Figure 1.** Average value and standard deviation of macroelements in leaf tissues of red clover – control

The graphs of the correlation coefficients of magnesium, phosphorus and calcium in the tissues of the leaves of healthy plants of red clover showed similarity. The graphs of the correlation coefficients of sulfur and potassium in the leaf tissues of red clover had common features (Figure 2).
There was a high positive relationship between potassium and calcium in the leaves of red clover with anthracnose (causative agent – *Kabatiella caulivora* Kirchn.), with a significance level below 0.95 (Figure 3, r = 0.75).

A weak positive relationship between potassium and calcium was found (Figure 3, r = 0.2). The graphs of the correlation coefficients of magnesium, potassium, calcium changed in a similar way. The
graphs of the correlation coefficients of the levels of sulfur and phosphorus in the tissues of red clover leaves were qualitatively different from the graphs of the correlation coefficients of the levels of other macroelements (Figure 3).

Figure 4 shows the correlation coefficients between macroelements in leaf tissues of red clover affected by brown spot (causative agent – *Pseudopeziza trifolii* Biv.), obtained using X-ray microanalysis.

![Figure 4](image-url)

**Figure 4.** Coefficients of correlation between macroelements in leaf tissues of red clover – brown spot

The graphs of the correlation coefficients of potassium and magnesium changed in a similar way. However, the graphs of the correlation coefficients of phosphorus and sulfur changed in antiphase relatively to the distribution of the correlation coefficients of potassium, calcium and magnesium in the leaves of red clover affected by brown spot (Figure 4). Hence, it can be assumed that the macroelements magnesium, potassium and calcium were in an ionic form, in contrast to the macroelements phosphorus and sulfur, which were in a bound form in macromolecules. The analysis of the graphs of the correlation coefficients fairly objectively shows the nature of changes in the metabolism of macroelements and can be used to study changes in the metabolism of macroelements and in other studies on the physiology of the infectious process.

4. **Conclusions**

1. The important role of potassium and calcium in response to the damaging effect of phytopathogenic fungi in the tissues of red clover leaves was revealed. When plants were damaged by phytopathogenic fungi, the content of potassium in the leaves of plants decreased, and calcium increased.

2. Despite the differences in the nature and species of pathogens, there was a similarity both in the content of macroelements and in the correlation coefficients between macroelements in plant leaf tissues under the influence of phytopathogenic fungi.

3. The study of the macroelement composition of plants using X-ray microanalysis opens up new possibilities for studying the mechanisms of plant resistance not only to phytopathogens, but also to other negative external factors (viral, bacterial and other infectious plant diseases). The use of X-ray microanalysis makes it possible to count on a wider application of this method,
both due to the simplicity of the preparation of plant objects, and the depth and breadth of possibilities for studying the metabolism of macroelements at the cellular and tissue levels.

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