Optimization of the conditions of rhizogenesis and assessment of the enzymatic activity of the substrates used for rooting of cuttings of berry shrubs in Siberia

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Abstract. Meeting the needs of agricultural production in seedlings of berry crops (black currant (Ribes nigrum L.), sea buckthorn (Hippophaë rhamnoides L.), edible honeysuckle (Lonicera edulis L)) propagated by green cuttings, requires a search for ways to increase the rooting rate of cuttings. Substrates including peat, sand, sapropel with additives of ammonium nitrate were studied. Adding sapropel (pH of water 7.4) to the composition of peat (pH of water 3.0–4.1) + sand increased the rooting rate of green cuttings of berry bushes. Maximum rhizogenesis was observed in the peat + sand + sapropel variant of 20 t/ha.

Anthropogenic loads change the biological parameters of substrates and soils. The results of assessing the biological state of substrates used to obtain cuttings of berry bushes in Siberia by the level of enzymatic activity are analyzed. The quality of substrates is analyzed by studies of oxidative and hydrolytic enzymes. It was found that in the options peat + sand + sapropel 20 t/ha and peat + sand + N30, peat + sand + sapropel 10 t/ha + N30 an increase in the activity indicators of urease and invertase enzymes begins. A stable level of activity of the catalase enzyme confirms the bioecological characteristic of the substrate that is optimal for rooting cuttings in the ratio of peat + sand + sapropel 20 t/ha.

1. Introduction
In the conditions of sharply continental climate of Siberia, berry plants are of particular importance among horticultural crops, namely: blackcurrant (Ribes nigrum L.), sea buckthorn (Hippophaë rhamnoides L.), edible honeysuckle (Lonicera edulis L). In the Krasnoyarsk Territory, the share of berry-growers in the total area of garden plantings exceeds 60% [1]. The dominance of berry crops is due to their high adaptive potential, early maturity and an increased content of biologically active substances. Currently, the market for consumption of berries has grown significantly due to the increasing importance of a healthy lifestyle and proper nutrition in the world community. Accordingly, the development of industrial gardening leads to an increase in the need for planting material. To meet the needs of agricultural producers of all forms of ownership in seedlings of high quality, it is necessary to increase the efficiency of propagation of berry crops. The introduction of new varieties, as well as compliance with the rules of variety renewal, is restrained by the problem of their intensive propagation [2,3].

In berry nurseries, shrubs are mainly propagated by the method of green cuttings, the effectiveness of which is substantiated in [2, 4, 5, 6]. The rooting of the cuttings of berry shrubs to a large extent depends on the substrate used. Studies [2, 4] showed that the peat + sand substrate in a volume ratio of
1: 1 is favorable for the rhizogenes is of green cuttings of berry plants. For these purposes, use lowland peat, well decomposed, with a neutral reaction of the environment. However, analysis of the peat market shows that mainly peat, which is characterized by an acid reaction of the medium, is available to the consumer. In turn, the mismatch between the acidity level of the substrates used and the requirements for the propagation of berry crops, and especially blackcurrant, leads to poor rooting of the cuttings, therefore, we suggest using sapropel to neutralize the acidity of the base substrate (horse peat + sand).

2. Object and methods of research

The object of the research includes substrates combined according to the following scheme: 1. peat + sand (1: 1); 2. peat + sand + sapropel 10 tons/hectare; 3. peat + sand + sapropel 15 tons/hectare; 4. peat + sand + sapropel 20 tons/hectare; 5. peat + sand + N30; 6. peat + sand + sapropel 10 tons/hectare + N30; 7. peat + sand + sapropel 15 tons/hectare + N30; 8. peat + sand + sapropel 20 tons/hectare + N30. Components of the substrate: peat, water pH 3.0-4.1, fraction size 0-15 mm; sapropel of the Small Kyzykul lake, water pH 7.4. Nitrogen fertilizers - ammonium nitrate at the rate of 30 kg of active substance per hectare - were added to substrates of experimental variants from number 5 to number 8.

Green cuttings of berry bushes were carried out according to the method [7]. Test cultures included black currant, grade Sophia; sea buckthorn, Alei variety; honeysuckle, grade Blue spindle. The lower part of the green cuttings before planting in the substrate was treated with a root formation stimulator - indolylacetic acid, the exposure time of treatment was 12 hours. Prepared cuttings were planted in a substrate according to the scheme of 7 x 5 cm in the first ten days of July.

Diagnostics of the biological activity of the investigated substrates was carried out using enzyme activity indicators. The selection and preparation of substrate samples was carried out according to GOST [8, 9]. Determination of the activity of the catalase enzyme was carried out according to the method of Johnson and Temple (1964) by titration with a 0.1 N potassium permanganate solution, the activity was expressed in ml 0.1 N. KMnO₄ g⁻¹.20 min⁻¹. The determination of urease activity was carried out according to the method of Shcherbakova (1983) by colorimetry at a wavelength of 400 nm and was expressed in mg N-NH₃ g⁻¹.4h⁻¹. The activity of the protease enzyme was determined by the method of Hoffmann and Teicher (1957) at a wavelength of 650 nm and was expressed in mg N-NH₃ g⁻¹.20 h⁻¹. The activity of the invertase enzyme was studied by the method of colorimetry according to Hoffmann and Pallafu (1965) at a wavelength of 578 nm and was expressed in mg glucose g⁻¹.24 h⁻¹ [10]. To evaluate the data obtained, they were guided by the comparison scale proposed by Gaponyuk and Malakhov [11].

3. Research results and discussion

The ranking of berry crops according to the response to the acidic environment of the soil solution according to the increasing vector is as follows: honeysuckle < sea buckthorn < black currant (figure 1). The most severe reaction was observed in blackcurrant; on a peat substrate (water pH 3.0-4.1) + sand, only 28% of the planted cuttings were rooted, which is the minimum value in the experiment. The root-forming ability of honeysuckle cuttings in the control variant is higher than in other cultures and amounted to 72%.

The addition of sapropel, having a pH of 7.4, created more favorable conditions for the rooting of green cuttings. A tendency toward an increase in the rhizogenic activity of cuttings of all test cultures with an increase in the dose of sapropel in the substrate was noted. The maximum regenerative ability of the cuttings was obtained using the option peat + sand + sapropel 20 tons/hectare, where 96% of sea buckthorn cuttings and 100% of blackcurrant and honeysuckle cuttings were rooted. The addition of ammonium nitrate to the peat-sand substrate provided better rooting of the stem cuttings relative to the control plots, but in honeysuckle and sea buckthorn, the survival rates were 6 and 18% respectively lower than in the peat + sand + sapropel 20 tons/hectare variant. Comparing the block of options peat + sand + sapropel and the block of options peat + sand + sapropel + N30, we note that only in sea buckthorn the addition of ammonium nitrate to the substrate increases the efficiency of rhizogenes is (figure 1). This trend was not recorded in the areas of currant and honeysuckle.
The use of agrochemicals significantly degrades the quality of the habitat for biota, as a result, its biological activity decreases. Biological methods for assessing the state of soil, substrates, and enzymatic activity, including, have proved their effectiveness and are confirmed by many years of research [12, 13, 14].

The low biological activity of the substrates recommended for obtaining cuttings of berry crops does not allow them to take root and develop normally. Monitoring the level of biological activity of the substrate can be performed by studying the activity of different groups of enzymes. The activity of the catalase enzyme helps to control the overall biogenicity of the substrates and their environmental safety, the activity of the urease and protease enzymes determines the level of nitrogen metabolism, and invertase shows the availability of the substrate with organic matter [14].

The analysis of the activity level of the investigated substrates for planting cuttings of berry shrubs according to the rating scale [11] showed that they are characterized by an average enrichment, therefore, an optimal selection of composite components. A substrate with high activity indices for all studied enzymes in comparison with the initial peat + sand variant (1: 1) has not been established, but variants have been identified in which an increase in the level of activity of urease and invertase enzymes is noted (table 1).

The activity of the catalase enzyme is reduced in the options peat + sand + sapropel 20 tons/hectare + N30 and peat + sand + sapropel 15 tons/hectare + N30 to 0.14 and 0.16 ml 0.1 N. KMnO₄ g⁻¹·20 min⁻¹, respectively. A high dose of ammonium nitrate could suppress the activity of microflora, which was reflected in the level of catalase enzyme. The optimal ratio of components in the substrate is the option peat + sand + sapropel 20 tons/hectare, the activity of the enzyme catalase remained stable and amounted to 0.22 ml 0.1 N. KMnO₄ g⁻¹·20 min⁻¹ (table 1). Thus, for the initial diagnosis of the condition and suitability of the substrate for rooting cuttings, one can use the activity of this enzyme, and to characterize the level of nutrition with hydrolytic enzymes, urease, protease, and invertase.

The activity of the urease enzyme in comparison with the initial version in the combination of peat + sand (1: 1) components is significantly higher in the following variants: peat + sand + sapropel 20 tons/hectare and peat + sand + N30 - 0.48 and 0.50 mg N-NH₃ g⁻¹·4h⁻¹, respectively. The maximum activity of the invertase enzyme - 32.4 mg glucose 1g⁻¹·24 h⁻¹, was established in the variant with the

![Figure 1](image-url)

**Figure 1.** The effect of substrate components on the rooting rate of green cuttings of berry bushes (numbering options was fulfilled in accordance with the scheme of experience).
ratio of pea + sand + sapropel components 10 tons/hectare + N30, which determines a high level of the substrate with organic matter.

**Table 1.** Indicators of enzymatic activity of substrates.

| Experiment variants | Catalase, ml 0.1 N. KMnO₄ g⁻¹20 min⁻¹ | Urease, mg N-NH₃ g⁻¹4h⁻¹ | Protease, mg N-NH₃ g⁻¹20 h⁻¹ | Invertase, mg glucose 1g⁻¹24 h⁻¹ |
|---------------------|--------------------------------------|--------------------------|-------------------------------|-------------------------------|
| 1. peat + sand (1:1)| 0.22±0.003                           | 0.25±0.005               | 0.73±0.009                    | 28.25±0.63                    |
| 2. peat + sand + sapropel 10 t/ha | 0.18±0.009                           | 0.28±0.011               | 0.64±0.003                    | 21.56±0.38                    |
| 3. peat + sand + sapropel 15 t/ha | 0.20±0.009                           | 0.30±0.007               | 0.62±0.026                    | 25.54±0.54                    |
| 4. peat + sand + sapropel 20 t/ha | 0.22±0.015                           | 0.48±0.009               | 0.73±0.014                    | 26.10±0.62                    |
| 5. peat + sand + N30 | 0.22±0.014                           | 0.50±0.012               | 0.72±0.006                    | 25.13±0.77                    |
| 6. peat + sand + sapropel 10 t/ha + N30 | 0.18±0.003                           | 0.30±0.007               | 0.62±0.011                    | 32.40±0.47                    |
| 7. peat + sand + sapropel 15 t/ha + N30 | 0.16±0.009                           | 0.29±0.005               | 0.75±0.023                    | 24.1±0.87                     |
| 8. peat + sand + sapropel 20 t/ha + N30 | 0.14±0.003                           | 0.28±0.005               | 0.76±0.020                    | 21.25±0.30                    |

The activity of the protease enzyme, showing the availability of the substrate with nitrogen-containing organic matter, shows a slight increase in the values compared to the initial value only to 0.75-0.76 mg N-NH₃ g⁻¹20 h⁻¹ in the options peat + sand + sapropel 15 tons/hectare + N30 and peat + sand + sapropel 20 tons/hectare + N30, respectively (table 1). Based on the data presented in the table it is clear that the use of sapropel increases the biological activity of the substrate.

4. Conclusion

Thus, the addition of sapropel (pH of water 7.4) to the substrate peat (pH of water 3.0-4.1) + sand helps to increase the rooting of green cuttings. The maximum efficiency of the rhizogenes is of test cultures of sea buckthorn, blackcurrant and honeysuckle was obtained on a substrate of peat + sand + sapropel 20 tons/hectare. A stable level of activity of the catalase enzyme confirms the best bioecological characteristics of the substrate in this ratio. The options were identified - peat + sand + sapropel 20 tons/hectare, peat + sand + N30, peat + sand + sapropel 10 tons/hectare + N30, in which there is an increase in the activity of urease and invertase enzymes. The main task of using the developed soil mixtures, adjusting the ratio of their components is the need to reduce the number of days of rooting of plants and increase their yield.

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