

Effects of Pre-sowing Seed Treatment on the Growth Rate of Seedlings and the Activity of the Excretory System of the Wheat Root in Aquatic Culture

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ABSTRACT

The qualitative and quantitative composition of the rhizosphere is known to be the result of the metabolic activity of the root and it regulates the functional activity of the root. Therefore, it works as autoregulation in the root activity. The study of the system of autoregulation is actually not only for the physiology of plants, but also for modern biotechnologies. The contents of proteins, amino acids and total carbohydrates were determined during the formation of the rhizosphere of the wheat's roots from 1 to 3 day of growth in aquatic culture. In addition to that, the role of microorganisms in the formation of the root microenvironment with different models of pre-sowing seed treatment was investigated. It was found that, the pre-sowing seed treatment affects the qualitative and quantitative composition of root exudates. The pre-sowing treatment of seeds with potassium permanganate increased the protein content in the composition of exudates in the 1st day of seedling about 75%, but it decreased in the 2nd day about 40% if compared with the control variant. The pre-sowing treatment of seeds with ethyl alcohol and sodium hypochlorite reduced the protein content of exudates in the 2nd day of seedlings about 45% and did not affect this indicator in 1 and 3rd day of seedlings in comparison with the control variant. The pre-sowing treatment with potassium permanganate insignificantly affected the mount of microorganisms, while the pre-sowing treatment with alcohol and sodium hypochlorite reduced the amount of microorganisms in the root exudates two times if compared with the control variant.

Highlights

- The microbiota which seen on the grains did not have significant effect on the qualitative and quantitative composition of the root exudates.

Keywords: Wheat seedlings, root exudates, the microbiota, pre-sowing treatment

As it is known, from the first hours of growth of wheat sprouts, as well as other plants, the rhizosphere forms (Dennis *et al.* 2010; Mendes *et al.* 2014; Chaparro *et al.* 2014). The system of formation and functioning of the root rhizosphere has a great interest for the development of root biotechnologies. This can be explained by several important points: firstly, the root exudates are a unique natural complex of biologically active compounds that can be used in pharmacy, biotechnology and agro biotechnology; Secondly, the system of “root-rhizosphere” is a unique natural model for

studying excretory mechanisms; thirdly, the study of this system will make it possible to understand the mechanisms of allelopathy (Badri *et al.* 2009; Dmitrović *et al.* 2015; Bouhaouel *et al.* 2015; Cai *et al.* 2012).

At the same time, the stages of rhizosphere formation have been studied incompletely. In this sense, the root exudates are the products of the vital activity of specific root cells, which is called “border cells” (Hawes *et al.* 2016; Bozhkov *et al.* 2009), products of destruction of growing cells

of the root (Bozhkov *et al.* 2013), and products of vital activity of microorganisms of the rhizosphere (Ohkama-Ohtsu *et al.* 2010; Badri *et al.* 2009). The contribution of each of the elements of the excretory system in the process of rhizosphere formation is unclear.

It is well known that a large number of microorganisms (bacteria, fungi and algae) are present in the rhizosphere, which affect the intensity of root growth and alter the composition of root exudates (Zhang *et al.* 2014; Majeed *et al.* 2015; Kumar *et al.* 2014).

In natural cenoses, microorganisms are an integral component of the rhizosphere (Dennis *et al.* 2010; Badri *et al.* 2009). The composition of the rhizosphere includes both symbiotic and antagonistic relation to plants microorganisms (Rasmann *et al.* 2016; Berendsen *et al.* 2012; Bulgarelli *et al.* 2013).

It can be assumed that, when plants are growing in a hydroponic or aquatic culture with relatively sterile conditions and pre-sowing seed treatment, the microorganisms will be removed, and their contribution to the rhizosphere formation is insignificant. However, the study of different modes of pre-sowing seed treatment which aimed to remove microorganisms from the surface of the grains was not carried out. At the same time, controlling the number of microorganisms in hydroponic culture is of fundamental importance for both the development of root technologies and solving fundamental questions of plant physiology.

In this context, the present work was designed to determine the intensity of growth of plants from the 1st to the 3rd day of cultivation, the rate of rhizosphere formation by content and composition of proteins, free amino acids and total carbohydrates from the 1st to the 3rd day of growth, and the amount and intensity of growth of rhizosphere's microorganisms of 2nd day seedlings, obtained after various regimes of pre-sowing treatment and inactivation of microorganisms. These data can be used to create root biotechnologies aimed at obtaining biologically active compounds.

MATERIALS AND METHODS

In the work wheat seedlings *Triticum aestivum* were used. Wheat grains were treated in 3 different ways before soaking :

1. *Control variant*: wheat grains were washed with running water for 3 minutes and 3 times with distilled sterile water.
2. *Variant of pre-sowing treatment*: wheat grains after washing as for control processing, washed with 0.05% solution of potassium permanganate, then rinsed with sterile distilled water.
3. *Variant of pre-sowing treatment*: the grain was washed with running water, then soaked for 30 seconds in 70% ethyl alcohol, washed with sterile distilled water, soaked for 30 minutes with 5% sodium hypochlorite, and washed with sterile distilled water.

The scheme is shown in the Fig. 1.

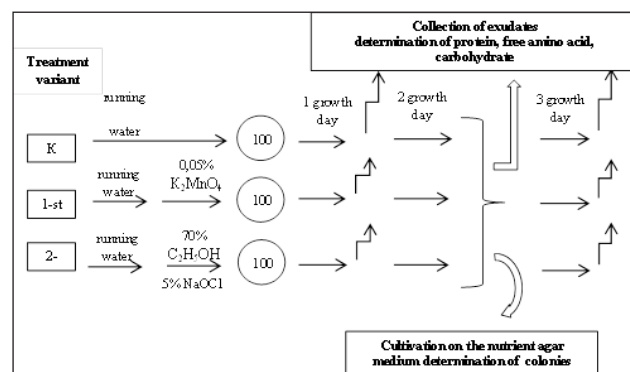


Fig. 1: Experimental scheme

After washing all variants, the grain was soaked in sterile distilled water for 24 hours at 26°C. 24 hours after soaking, the nested grains from each treatment option were spread by 100 grain in each Petri dish. 7 ml of sterile distilled water was added to each Petri dish. Petri dishes with sprouts were placed in a chamber with round-the-clock lighting and a temperature of 26°C. On the 1st, 2nd and 3rd day of growth, the number of not germinated grains and root length of wheat seedlings were measured.

Collection of root exudates of wheat germs

To characterize root exudates in the 1st, 2nd and 3rd day of growth from each petri dish, the aqueous root exudate was collected and the content of total protein, free amino acids and total carbohydrates was determined.

From the culture medium of the 2nd day seedlings, 1 ml of culture medium containing root exudates was taken, and from each Petri dish 10 sprouts

were selected. The gel cover surrounding the root apexes was removed from the roots of the selected 10 seedlings, as described in (Bozhkov *et al.* 2013). The obtained material was used to determine the presence of microorganisms after culturing the samples on meat-peptone agar.

Determination of total protein content

The content of total protein in the root exudates of wheat germs was determined by the method of Lowry which used in experiments (Bozhkov *et al.* 2013).

Determination of total carbohydrate content in root exudates

The content of carbohydrates in the composition of the root exudates was determined by the method (Kuznetsova 2008). However, 0.5 ml of a 5% aqueous phenolic reagent was added to aliquots of the soluble fraction of the root exudates. The composition of the sample was vigorously stirred, and 2.5 ml of concentrated sulfuric acid was added. After 20 min, the carbohydrate content was determined at a wavelength of 488 nm on a SF-46 spectrophotometer. The content of total carbohydrates was shown by the calibration curve for glucose and it was expressed in mg glucose/100 grains.

Determination of the content of amino acids in the composition of root exudates

The content amino acid in the root exudates was determined by the ninhydrin method which used (Bozhkov *et al.* 2013). in her experiments. Then, an equal volume of 10% trichloroacetic acid was added to the aliquot of the root exudates and the samples were incubated for 12 hours at + 40 °C to precipitate the proteins. The samples were then centrifuged at 3000g for 20 minutes. Aliquots were taken from the supernatant, diluted to 0.6 ml with distilled water, 1 ml of 1% ninhydrin and 1 ml of 10% pyridine were added. Then the samples were placed on a boiling water bath for 30-40 minutes. After cooling, the content of amino acids in the samples, was determined at a wavelength of 570 nm on a SF-46 spectrophotometer. The amino acid content was shown by the calibration curve for glycine and expressed in µg glycine/100 grains.

Cultivation of microorganisms on a universal nutrient medium

For analysis the microbiota of wheat seeds, the collected root exudates were transferred to nutrient agar medium - meat-peptone agar (MPA). The exudates of wheat seeds were applied to the nutrient medium in sterile conditions with a 3 µl micropipette. Then, the samples of microorganisms were cultured in the thermostat at 37 °C for 24 hours.

Determination of the growth dynamics of microorganisms on different media

By cultivating microorganisms on a nutrient agar medium, the growth dynamics of the culture was assessed by the growth of colonies. To do this, every 3 hours the colony was photographed and the area of the colonies was measured using the computer program Axio vision, which was expressed in conventional units.

Statistical processing of results

The experiments were performed in 3 biological replicates with 3 analytical repeats. The mean values of the obtained data were determined and the error of the mean was calculated. The reliability of the differences between the samples was indicated using a nonparametric method using the Mann-Whitney U test (Thomas *et al.* 2016).

RESULTS AND DISCUSSION

The influence of different methods of pre-sowing seed treatment on germination and root growth intensity

The Pre-sowing treatment of seeds ensures the removal of the accompanying microorganisms on the surface of seeds or inactivate them, while it should not affect the germination of seeds and the intensity of root growth.

It was found that, in the control variant, 26% of the seeds did not germinate at the end of the 1st day, but to the 3rd day only 6% of seeds are not germinated (Fig. 2A). Consequently, in the control variant there were seeds which are sprouted in 1st day, and the others sprouted only in the 3rd day. Therefore 74% of seeds germinated in the 1st day, 12% sprouted in the next day, 8% (Fig. 2B) sprouted for the 3rd day,

and only 6% of seeds in the control variant are not germinated at all (Fig. 2A). Results are shown in the Fig. 2.

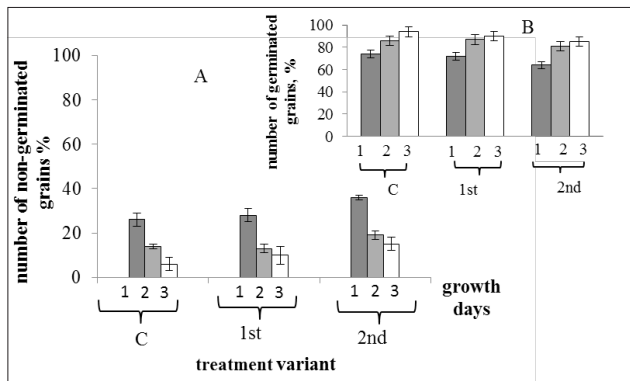


Fig. 2: Number of non-germinated (A), germinated grains (B) for 1st – 2nd – 3rd day of wheat seeds after different pre-sowing treatment: 1-Control variant; 2-Treatment variant with 0.05% solution of potassium permanganate; 3 - Treatment variant with alcohol 70% and sodium hypochlorite 5%. B: 1st day of growth - percentage of PP from total sample; 2nd - and 3rd days of growth - increase in PP in % compared with 1st day of growth

*- significant differences in comparison with the control

In this case, when the wheat seeds were treated with 0.05% solution of potassium permanganate (first method of treatment), the dynamics of seed germination was slightly different from the control variant case. Thus, 72% of the seeds are germinated during the 1st day, 15% of seeds in the following day, while 3% of them are germinated in the 3rd day, and 10% of seeds are not germinated, which is 4% more than control (Fig. 2B).

In the other case, when the seeds were treated with 70% alcohol and 5% sodium hypochlorite (second method of treatment), seed germination was decreased 10% if compared with the control variant case. So, in the 1st day, 64% of seeds are germinated, and up to the 3rd day the percentage of non-germinated seeds was 9%, which is more than the percentage of non-germinated seeds in the control variant case (Fig. 2A, B).

Such decline of seed germination can be caused by various reasons: death of the embryo, destruction of the water absorption system in the seeds, inhibition of the rate of the embryo development.

The length of the roots of wheat germs in the control variant case reached to 0.76 cm in the 1st day of growth, and 1.89 cm to the 2nd day, and the roots had a length of 2.78 cm in the 3rd day (Fig. 3A). If we illustrate the growth rate of the roots in the control

variant case, the greatest increase can be seen in the 2nd day, but in the 3rd day it has somewhat decreased (Fig. 3B). Results are shown in the Fig. 3.

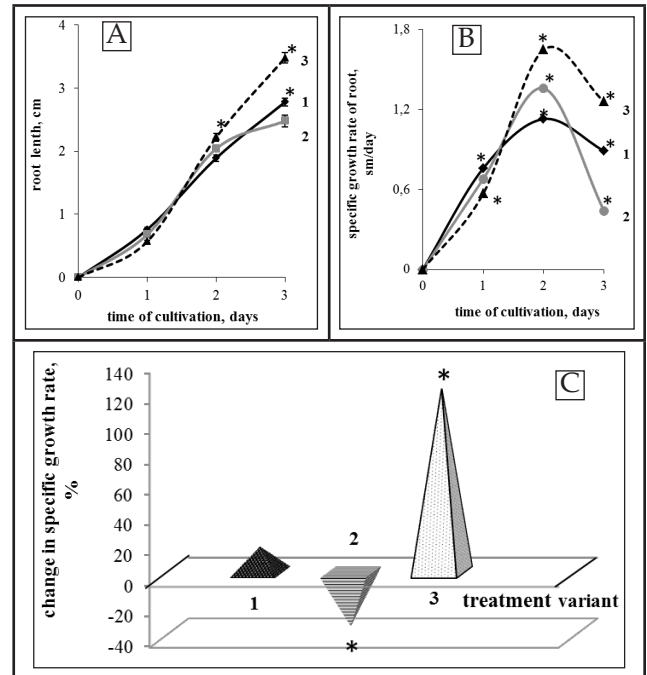


Fig. 3: Root length (A), growth rate (B), change in specific growth rate, expressed in the 3rd day of growth compared with the 1st day, which is taken as 100% (C). For 1st, 2nd and 3rd days sprouts of wheat after different pre-sowing treatments: 1- control variant; 2- treatment variant with 0.05 % solution of potassium permanganate; 3- treatment option 70% alcohol and 5% sodium hypochlorite; 1- Control variant; 2- Treatment variant with solution of potassium permanganate; 3- Treatment variant with alcohol and sodium hypochlorite

*- significant differences compared with control

If the seeds were treated within the second method (treatment variant with K_2MnO_4), the intensity of root growth would be affected. Roots are increased intensively in the 2nd day, but later if compared with the control variant case, they were grow slowly and this can be clearly seen in the growth rate (Fig. 3A, B).

There are unexpected results which appeared in the second method of seed treatment, the length of the roots of wheat seeds in this method reached to 0.57 cm in the 1st day of growth (which means that the length of the roots is 25% less than roots length in the control variant case) (Fig. 3A). In the 2nd day, the growth of roots was obviously increased (Fig. 3B). While in the 3rd day the root growth was 25% longer than its length in the control variant case.

So, in the 1st day the growth rate for the control variant case was 0.76 cm / 24 hours, for the 1st treatment method 0.68 cm / 24 hours, and for the 2nd method 0.57 cm / 24 hours, while in the 3rd day the growth rate of seedlings was 0.89; 0.44 and 1.26 cm / 24 hours (Fig. 2B). If we compare these three experimental variants of pre-sowing seed treatments, it should be noted that the growth rate of the root in the control in 3rd day increased about 17% in comparison with 1st day, in the case of the first treatment method, it decreased about 36%, and for the second treatment the growth rate increased about 121% (Fig. 3C). Consequently, in the case of the second method of pre-sowing treatment of wheat seeds, there was an effect of growth inhibition followed by growth stimulation.

Such stimulation of root growth by pre-sowing seed treatment with 70% alcohol and 5% sodium hypochlorite, which comes after the inhibition of seed germination, can be caused by several reasons: 1) the effect of synchronization of cell cycle; 2) effect of hormesis; 3) changes in the ratio between the formed microbiota of the root and its influence on the growth rate.

When talking about the effect of hormesis, it should be noted that small “disturbances” have stimulatory effects (Jia *et al.* 2015) and in this formula can occur. Finally, it is known, that the intensity of growth largely depends on the quantitative and qualitative composition of the microorganisms which are formed in the root rhizosphere and provided root nutrition for plants. The main components of the microenvironment of the root are carbohydrates, proteins and free amino acids (Badri *et al.* 2009). So, the root exudates exert direct and indirect (through the regulation of the qualitative and quantitative composition of the microbiota), the influence on the growth rate at the next stage of the work, is that the qualitative and quantitative composition of the root exudates were determined in the case of different methods of pre-sowing treatment of wheat seeds.

The qualitative and quantitative composition of root exudates after different methods of pre-sowing treatment of wheat seeds

Excretory activity of the roots of the control variant repeatedly increased from 1st to 3rd days of growth in aquatic culture. So, in the 1st day 1418 µg of root exudates were detected in the aquatic environment

per 100 seeds, at the end of 2nd day 2739 µg/100 grains, after 3 days there were 5880 µg / 100 grains (Fig. 4). Therefore, during the 2nd day the amount of exudates increased about 1321 µg /100 grains if compared with 1st day, while at the 3rd day in comparison with the 2nd day about 3141 µg/100 grains, there was almost an exponential increase in the number of root exudates in the case of the control variant/ Results are shown in the Fig. 4.

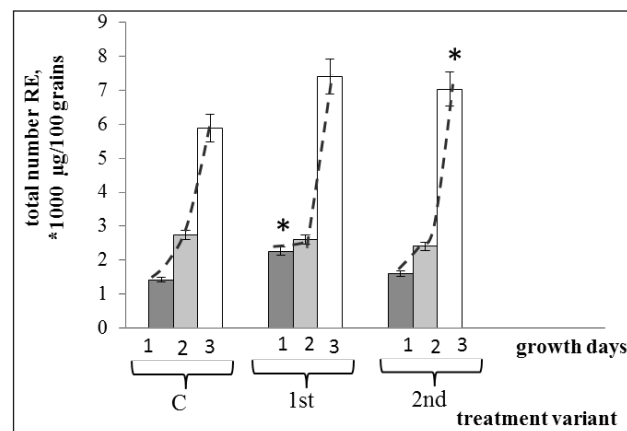


Fig. 4: Content of the total number of root exudates at the 1st, 2nd and 3rd days of wheat sprouts after different pre-sowing treatments

* - significant differences compared with control

In this case when the seeds were treated with potassium permanganate, the intensity of the root excretion in the 1st day of growth was 60% higher if compared with the control variant case (Fig. 4). But, at the 2nd day of growth, the rate of root excretion did not change if compared with control variant (Fig. 4). While in the 3rd day of growth, the amount of excreted substances increased about 4790 µg/100 roots if compared with 2nd day and exceeded about 25% more than the excretion of the control variant case (Fig. 4). Consequently, the first method of pre-sowing seed treatment increased the rate of root exudates and influenced the dynamics of this process. A similar nature of excretory activity took place in the case of the 2nd method of pre-sowing seed treatment (Fig. 4). The difference is only, that the intensity in the 1st and 2nd days does not differ practically from the control variant, but in the 3rd day it is exceeded the control variant about 20% and came close to the first method of treatment at the same time (Fig. 4).

The obtained results allow us to conclude that pre-sowing treatment of wheat seeds can be, firstly, used

to induce excretory activity in root biotechnologies because it allows firstly, obtaining up to 7.5-8 mg of root exudates/100 grains. Secondly, influenced the intensity growth and development of plants with regulating the composition and number of components formed microenvironment of the root.

At the same time, the most important characteristics of the influence of seed treatment methods on excretory activity and understanding of the biological role of root exudates is their composition, specially the relationship between the main components of exudates.

Despite the wide range of substances excreted by the root system, more than 90% of them located on 3 main components: carbohydrate, proteins and free amino acids, at least for wheat (Bozhkov *et al.* 2013).

At the next stage of work, the content of total sugar, proteins and amino acids in the root exudates of wheat seeds was determined after different methods of pre-sowing seed treatment.

The content of carbohydrates in the 1st day of growth of the roots of the control variant was 950 g of glucose/100 grains, which was 67% of the total amount of root exudates (Table 1). At the next day it increased about 41% when compared with 1st day, and in 3rd day the amount of carbohydrates was 3311 g of glucose/100 grains, which was 146% higher if compared with 2nd day in the control variant case (Table 1).

The dynamics of the excretory activity of carbohydrates in roots in the first and second methods of pre-sowing seed treatment generally

was similar to that of common exudates (Fig. 4). Thus, in the 1st day of growth, especially after the first treatment method, the roots excreted up to 1527.3 µg of glucose/100 grains into the aqueous medium, which was 1.6 times greater than control variant at same time. Later, in the 2nd day, the sugar content remained the same as the 1st day, while in the 3rd day the excretion increased and the carbohydrate content was 4929.3 µg glucose/100 grains, which was 211% more if compared with the 2nd day of growth, and 30% more than the control variant at that time (Tab 1). The content of sugar in the composition of root exudates after the second method of pre-sowing wheat seed treatment did not differ from the control variant case in the 1st and 2nd day of seedlings but it was increased by 37% in 3rd day of seedlings (Tab 1).

Consequently, pre-sowing seed treatment with potassium permanganate increased the rate of carbohydrate excretion in the medium on the 1st and 3rd day of seedlings.

The protein content of root exudates in wheat seeds in the control variant case increased from the 1st to the 3rd day of cultivation (Table 1). So, its number in the control variant increased from 1st to 3rd day about 3, 5 times (Table 1).

Pre-sowing treatment of seeds with potassium permanganate increased the protein content in the composition of root exudates on the 1st day of seedlings about 75%, but on the 2nd day the protein content was 40% less than its content in the control variant.

Table 1: The content of carbohydrates, total protein and amino acids in the root exudates in 1st, 2nd and 3rd days of wheat sprouts after different pre-sowing treatment

Content of RE	Growth days	Control	Treatment variant	
			1 st variant	2 nd variant
Carbohydrate, µg glucose/100 grains	1	949,7±106,2	1527,3±458,4*	1136,4±93,5
	2	1342,8±98,2	1582,3±169,4	1227,3±123,8
	3	3311,5±318,3	4929,3±507,9*	4546,2±432,3*
Total protein, µg/100 grains	1	338,5±40,8	593,1±108,4*	311,0±46,6
	2	693,8±101,4	410,2±103,8*	382,5±46,2*
	3	1456,3±158,4	1344,9±156,9	1359,7±184,7
Free amino acid, µg/100 grains	1	130,0±14,7	141,3±11,2	143,7±11,4
	2	702,4±55,2	614,7±29,1	799,8±36,4
	3	1112,2±64,4	1122,9±66,5	1132,3±113,4

The seed treatment with ethyl alcohol and sodium hypochlorite reduced the protein content on the 2nd day about 45% if compared with control case (Table 1).

Pre-sowing seed treatment did not affect the content of free amino acids in the composition of root exudates (Table 1). At the same time, the content of amino acids in the composition of root exudate increased in 7.9-8 times from 1st to 3rd day of growth regardless of the methods of pre-sowing seed treatment.

It should be noted that the intensity of amino acid excretion increased linearly from the 1st to the 3rd day of cultivation, but the content of proteins and sugars increased in nonlinear way during cultivation. Results are shown in the Fig. 5.

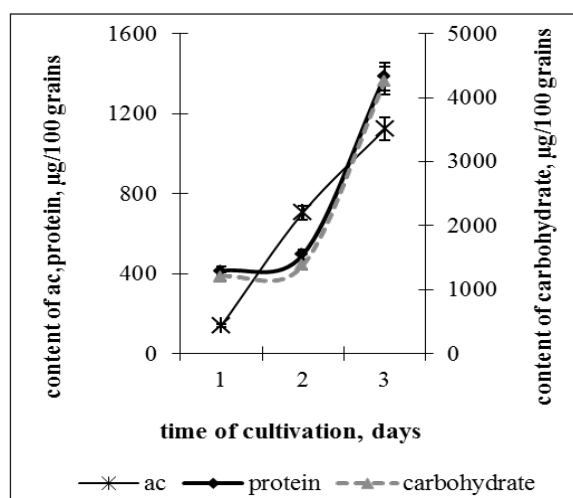


Fig. 5: The content of free amino acids, protein and carbohydrates in the composition of root exudates in 1st, 2nd and 3rd days under different pre-sowing treatments

Such differences in the dynamics of excretion of different components led to a change in the ratio between its components in the 1st, 2nd, and 3rd day-old seedlings, which must be taken into account when obtaining root exudates. So, different methods of pre-sowing seed treatment lead to a change in the composition of carbohydrates and proteins, especially expressed on 1 day of growth. Results are shown in the Fig. 6.

The most important components of the microenvironment of the root, along with proteins, amino acids and carbohydrates is a microbiota. As is known, microorganisms of the root rhizosphere are not only utilize the root exudates, but also excrete their exudates and as a consequence, they change

the microenvironment of the root and can affect the intensity of root growth and the composition of the obtained root exudates.

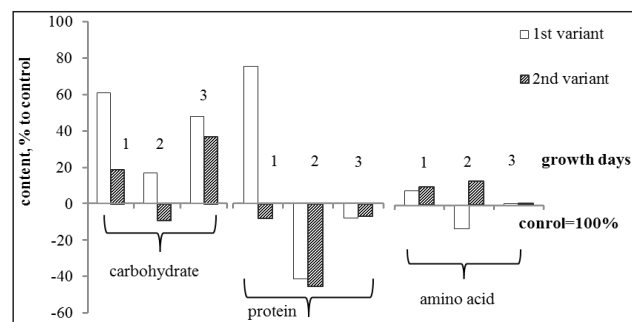


Fig. 6: Change in the content of carbohydrate, total protein and free amino acids (in relation to control, which is taken as 100%) in 1st, 2nd and 3rd days of root exudates with different pre-sowing treatments: first method - 0.5% solution of potassium permanganate; the second method is 70% alcohol and 5% sodium hypochlorite

At the next stage of the work, the content of the total number of microorganisms was determined in the 2nd day of wheat sprouts after different methods of pre-sowing seed treatment and in the cultivation of seedlings in the same culture with the observance of aseptic conditions.

The effect of pre-sowing seed treatment on the number of microorganisms in the 2nd day of wheat sprouts in the aquatic culture

Cultivation of inoculants, obtained from the rhizosphere of wheat seedlings on nutrient agar medium, showed that:

1. In the composition of root exudates there are microorganisms which can actively proliferate on nutrient agar medium.
2. In the case of the control variant, the stationary phase of the culture growth arrived at 24 hours with the attainment of the relative area of the colonies of about 4 thousand usl unit.
3. In the case of the first method of treatment, the yield to the stationary phase of microorganism also occurred at 24 hours, but the relative area of the colonies was less about 20-25% compared with the control variant.
4. In the case, when an inoculant was prepared in the 2nd day of seedling after second method of pre-sowing seed treatment, the output to

the stationary phase was carried out at 6 hours, and the relative area of the colonies was 2 times less than the relative area in the control variant case.

Results are shown in the Fig. 7.

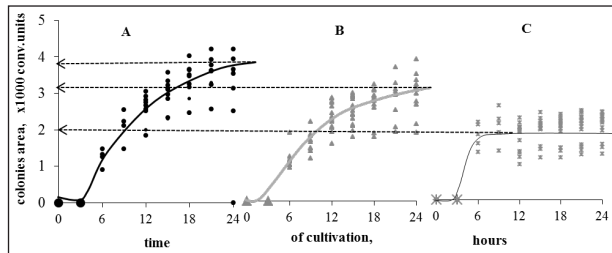


Fig. 7: Relative colonies area of microorganisms (conventional units) isolated from 2nd day of wheat sprouts after different pre-sowing treatments when cultivated on the nutrient agar medium; A - Control variant; B- treatment variant with 0.5% solution of potassium permanganate; C- Treatment variant with 70% alcohol and 5% sodium hypochlorite

Consequently, pre-sowing treatment of wheat seeds with 0.05% of potassium permanganate insignificantly inactivated located microorganisms on the seed's surface. The seed treatment with 70% ethyl alcohol and 5% sodium hypochlorite inactivated most of the microorganisms which were on the seed's surface and delayed the germination of seeds.

The results of the work showed that; in the soft short-term pre-sowing treatment (treat with potassium permanganate) of wheat seeds there was a slight delay in the growth rate of seedlings, an increase in the intensity of excretion of root exudates, a change in the composition of root exudates, and a decrease in the number of microorganisms in the rhizosphere of the wheat's root in aquatic culture. The change in these out comes depended on the method of pre-sowing seed treatment. The results indicate the existence of a relationship between growth characteristics and excretory system function. It is possible to manage the process of obtaining root exudates by using different methods of pre-sowing seed treatment.

So, in particular, the pre-sowing seed treatment slightly delayed the growth rate of seedlings in the 1st day of germination, and this delay was accompanied by a pronounced increase in the specific growth rate of seedlings in the 2nd day. Then it should be noted that in the 3rd day the specific growth rate again decreased (Fig. 3B).

Consequently, the specific growth rate had rhythmic feature. The pre-sowing treatment of seeds increased the amplitude of fluctuations in the rhythmic nature of the specific growth rate from 1st to 3rd day of germination.

We believe that, the effect of delayed growth rate with the stimulation of seedling growth can be considered as a symptoms of the hormesis effect on the seedling model. As it is known, the hormesis effect is known as the stimulation of biological processes by small doses of toxic substances or physical factors and the effect is described for various types of radiation, that was shown in the model of action of heavy metal ions and other chemical compounds (Jia *et al.* 2015; Tang *et al.* 2015; Bozhkov *et al.* 2010).

It should be noted that in this paper there is a direct relationship between the degree of "delay" of root length and the subsequent increase in specific growth rate of seedlings when using these methods of pre-sowing seed treatment. So, the soft way method (with potassium permanganate) showed less effect than the hard way (70% ethyl alcohol and 5% sodium hypochlorite) of seed treatment (Fig. 3B). So, the hormone's effect can be realized by various mechanisms. It can be realized by the synchronizing passage of the cell cycle of cells of an intensively growing root.

As it is known that, the first mitoses of cells of sprouts occur relatively synchronously, but in the future there is an asynchronous occurrence of the cell cycle (Moubayidin *et al.* 2010; Marshall *et al.* 2012; Hayashi *et al.* 2013). The delay in root growth after pre-sowing seed treatment may be associated with a short-term delay in forming rate of the cell cycle. The subsequent "removal" of the inhibitory effect of the components of pre-sowing seed treatment is accompanied by the synchronous entry of cells into the cycle, which is manifested with increase in the growth rate in the 2nd day of growth. Further, in the 3rd day of growth there is again an asynchrony occurrence of the cell cycle, and as a consequence a decrease in the specific growth rate of the seedlings.

Of course, the synchronization of the cell cycle is not only the factor in the nonlinear growth rate of seedlings, but also an appreciable contribution in this process which can also be made by the process



of “stretching” in root cells, and an increase in their size. However, the basis of this process, of course, is the rate and the direction of the metabolism of the cells of the growing root.

One of the indicators of metabolic activity is the characteristic of the excretory system of the root.

Earlier, it was shown that an increase in the secretion density of the root exudates on a root inhibition model by phytotoxicant - sodium fluoride (Bozhkov *et al.* 2009, Bozhkov *et al.* 2013). The slow growth rate of wheat sprouts, which comes from the natural storage of the seeds, was also accompanied by an increase in the excretory activity of the root. Consequently, when the root grows more slowly, at least from 1st to 3rd day, it excites more active exudates into the culture (Bozhkov *et al.* 2009; Bozhkov *et al.* 2013).

The results of this study confirm what we have explained above, and they also show the relation between the period of growth and excretion is not strong enough, but it is a passageway or the range of wide interconnections, which must be taken into consideration in the technology of obtaining exudates.

Thus, a small delay in root growth after pre-sowing seed treatment in a “soft” way (potassium permanganate) was accompanied by an increase in excretory activity in the 1st day of growth. So, in 1st day of seedlings, the rate of excretion increased to 60%, and at the same time the ratio between root exudates is changed. Acceleration of the growth rate in the 2nd day was accompanied by a decrease in the rate of excretion of the root exudates. But the decrease in the specific growth rate in the 3rd day was accompanied by a 25% increase in the rate of excretion of the root exudates.

At the same time, in the “hard” treatment of seeds with alcohol and sodium hypochlorite, the process did not exert a pronounced effect of stimulation of excretory activity.

Consequently, the process of excretion is regulated by a various factors and probably there are specific mechanisms of excretion, as well as general mechanisms which reflect the overall metabolic level in the root cells.

As we know, the growing root, from the first hours of growth, forms its own specific microenvironment. The study of the highly specific

“root-microenvironment” system can be carried out on the model of the water culture of seedlings. The knowledge of the specifics formation of the “root-microenvironment” system is important for the fundamental principles of plant physiology, for obtaining biologically active compounds and for the development of root technologies.

It should be noted that, the components of the microenvironment of the root are formed from several sources: 1 - specific excretory system of the root represented by the border cells of the root (Hawes *et al.* 2016; Bozhkov *et al.* 2013); 2 - products of degradation of cells of a growing root; 3 – by inhabiting microorganisms for the rhizosphere of the root, as well as by the products of their vital activity (Badri *et al.* 2009; Berendsen *et al.* 2012). There are enzymes and microorganisms in the microenvironment, its composition dynamically changes. Furthermore, the “contribution” of these constituent components in the microenvironment during the growth of the root will vary greatly.

If (in the 1st -3rd day) the main contribution to the microenvironment is made by exudates, then in the future, during the growth process, the contribution of the components of cell degradation and metabolites of microorganisms will increase.

Consequently, from 1st to 3rd day of growth of seeds in aquatic culture under maintaining sterile growth conditions, the main components of the microenvironment of the root are sugars, proteins and amino acids that are actively excreted into the aquatic environment.

The intensity and composition of these root exudates, during this period, depend on the conditions of cultivation, and species composition of the culture, and they can be obtained in industrial quantities. The pre-sowing treatment of wheat seeds allows to increase the yield of dry biomass of root exudates from 5.8 mg / 100 roots in the control to 7.5-8.0 mg/100 roots. As shown earlier, such root exudates have biological effect and can be used in biotechnology (Bozhkov *et al.* 2013).

It should be noted that, a small amount of microorganisms was exist in the composition of wheat exudates in the water culture, which grew only on a universal nutrient medium, but they did not grow in the composition of root exudates. The pre-sowing seed treatment reduced the growth rate



of these microorganisms, which suggests that they exist on the surface of grain, and they did not have any significant contribution in the excretory root system in aquatic culture.

CONCLUSION

However, wheat sprouts, in the 1st-3rd day in aquatic culture, have excretory activity, but the amount of excreted substances in the medium and the composition of the components are affected by pre-sowing seed treatment. By using different methods of pre-sowing seed treatment and collecting exudates in the 1st, 2nd, and 3rd day of wheat sprouts, it is possible to obtain different amounts of exudates with different compositions.

Concomitant microorganisms do not make any significant contribution to the quantitative and qualitative composition of the exudates in the aquatic culture from 1st to 3rd day of germination.

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