ISSN NO: 2771-8840
Date of Publication: 26-12-2022

Physiological Processes of Pomegranate Seedlings Grown in Vitro at the Stages of Growth and Development

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Abstract: Murkute et al. in the direction of creating varieties of pomegranate (Punica granatum L.) in various climatic zones and zones using biotechnological methods, including seedlings free from pathogens in vitro. (2004), Singh and Khawale (2006), Chaugule et al. (2007), Sameer et al. (2009), Singh et al. (2001) conducted a study.

Boboev's studies reflected data on the dynamics of changes in some physiological parameters during the growing season and phases of pomegranate development in Central Asia.

However, on the scale of our republic, almost no scientific research has been carried out in the direction of creating pomegranate varieties that are resistant to local climatic and soil conditions, and this situation determines the relevance of theoretical and practical research in this direction

Tasks of the research:

analysis of the dynamics of adaptation in the process of transpiration and changes in the amount of photosynthetic pigments when transplanting pomegranate seedlings grown in *in vitro* conditions into the soil;

As object of the research there were selected local varieties grown in the territory of Syrdarya region, such as "Qora qayim", "Qizil anor", "Oq dona (Tuyatish)" and "Achhiq dona".

Methods of the research. In the studies were used methods, such as microcontrolling, sterilization, transpiration, chromotography, spectrophotometry.

Results and analysis are as follows:

While transplanting local pomegranate varieties into the soil of seedlings obtained by microcontrolling *in vitro* conditions, there was detected a change in the transpiration process;

Scientific novelty of the research is as follows:

in the conditions of *in vitro*, there were determined dynamics of adaptation in the transpiration process in pomegranate seedlings obtained by using the microcontroller method and transplanted into the soil;

Dynamics of transpiration process in transplanting pomegranate seedlings grown in *in vitro* conditions to soil

In our further experiment, the results of a comparative analysis of the amount of dry matter contained in the leaves of pomegranate varieties are presented. Table 1 below lists the results obtained by the proportion of the amount of dry matter in relation to control in 1 day on the leaves of seedlings grown under *in vitro* conditions in some domestic varieties of pomegranate ("Qora qayim", "Qizil Anor", "Oq dona (Tuyatish)" and "Achhiq dona") (Table 1).

Table 1
Results of a comparative analysis of the amount of dry matter in the leaves of the studied pomegranate varieties

No.	Pomegranate varieties	The amount of dry matter contained in the leaf (%)	
		Control	Grown in the condition *In vitro*
1	"Qora Qayim"	33,6±3,5	23,8±3,1**

ISSN NO: 2771-8840
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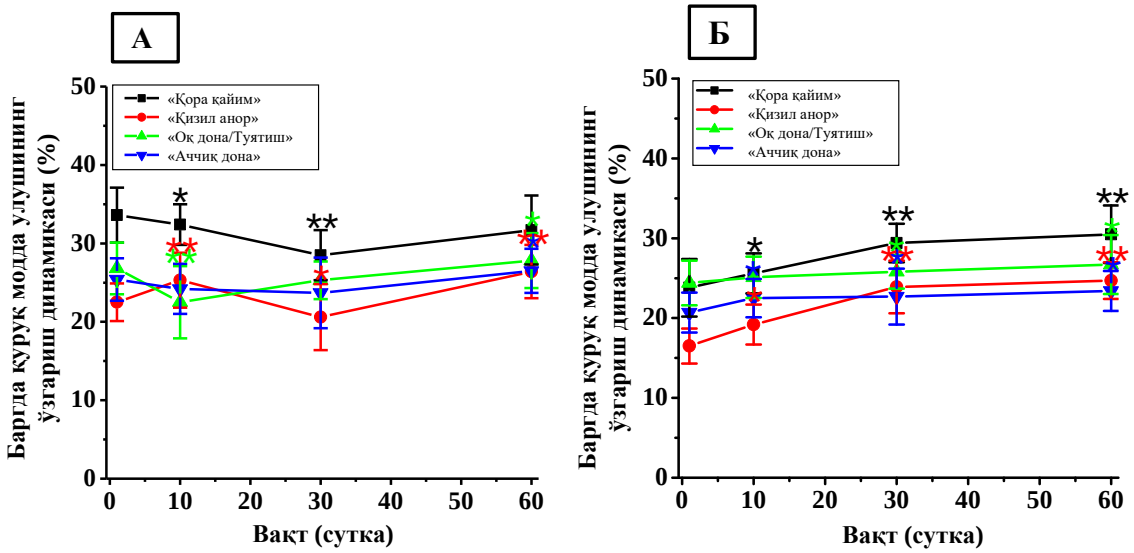
2	“Qizil Anor”	22,5±2,4	16,5±2,2*
3	“Oq Dona (Tuyatish”	26,8±3,3	24,4±2,8
4	“Acchiq Dona”	25,4±2,7	20,7±2,5

Note: * - *P<0,05 rather than controlled; ** - P<0,01 (*n*=3-4).

It was found that the share of the amount of dry matter in the composition of the leaves of the varieties “Qora Qayim” and “Qizil Anor” is reliably reduced by 29.2% and 26.7%, respectively, in relation to controlled. Obtained as an object of research, it was found that the proportion of dry matter in seedling leaves grown in in vitro conditions of the pomegranate varieties “Oq Dona (Tuyatish” and “Acchiq Dona” (Table 1) in relation to controlled was reduced by 8.96% and 18.5% unreliable, respectively (Table 1). From the experimental results obtained, it can be seen that the total dry matter content in seedling leaves grown in *in vitro* conditions was noted to be statistically convincing in relation to controlled. This situation is due to the mechanism of adaptation to the intensity of nutrient absorption by the root in the initial period planted in natural soil conditions, when the dry matter in the leaves of pomegranate seedlings grown in vitro conditions is relatively low in control, which can be explained by the low content of inorganic substances in the leaves, which determines the value of this analyzed indicator was determined by the presence of mutual differentiation in the varieties. This situation can be explained by the fact that the genetic physiological characteristics of the varieties, the degree of adaptation to soil and climatic conditions differ among themselves.

At the next stage of research, the dynamics of changes in the time interval range of the share of the amount of dry matter in relation to controlled in the leaves of seedlings grown in *in vitro* conditions in local pomegranate varieties “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona” were analyzed (Picture 1, A and B).



Picture 1. Dynamics of change in the time interval range of the share of the amount of dry matter in relation to controlled in the leaves of seedlings (A) of local pomegranate varieties “Qora Qayim”, “Qizil Anor”, Oq Dona (Tuyatish)” and “Acchiq Dona” and *in vitro* conditions.

On the ordinate axis, the percentage of dry matter content in the leaf is expressed in percent (%). Time (day) is expressed in the abscissa axis. * - relative to controllked r<0.05, * * - r<0.01 (n=3-5).

In this case, it was found that in the local varieties of pomegranate “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona”, no general changes in the dynamics of change in the proportion (%) of the amount of dry matter in the leaf composition in 1-60 days are observed (Picture 4.1.) The proportion of the amount of dry matter (%) in leaf composition in 1-60 days in seedling leaves grown in *in vitro* conditions in the dynamics of change, the minimum and maximum values are 23.8/30.5; 16.5/24.7,

ISSN NO: 2771-8840
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respectively, in local varieties of pomegranate “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona”; It was found that it is equal to 24.4 / 26.7 and 20.7 / 23.4, that is, it increases in the form of a law, having a value close to control in 30-60 days (Picture 1, B). The results obtained indicate that the mechanism of adaptation of pomegranate seedlings grown in *in vitro* conditions when planted in natural soil conditions is carried out at a high intensity in 1-30 days.

The picture below shows an overview of the grown seedlings of selected pomegranate varieties from cuttings and the growth - development of pomegranate seedlings grown in *in vitro* conditions (Pic.2) The structure of the leaves of pomegranate seedlings, bred from cuttings of selected pomegranate varieties and grown in in vitro conditions, occupies a special place in the process of photosynthesis in them. In this case, water storage indicators in the process of transpiration on the leaves have an important importance in plants.

Picture 2. An overview of the growth and development of pomegranate seedlings grown from cuttings of pomegranate varieties and *in vitro* conditions.

At this point, at the next stage of experiments, a change in the amount of water in the leaf composition was analyzed when planting pomegranate seedlings grown in *in vitro* conditions in natural soil (Table 2). In this control group, it was found that the water content of the leaf (%), in the “Qora Qayim”, is at the lowest value and and in the variety “Qizil Anor”, is at a higher value. It was noted that the water content of the leaf of local pomegranate varieties “Qora Qayim”, “Qzili Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona” is on average 72.7%. The lowest value indicator of the amount of water contained in the leaf of pomegranate seedlings grown in *in vitro* conditions is indicated in this “Oq Dona (Tuyatish)”, while the highest indicator was again observed in the “Qizil Anor” variety. In pomegranate seedlings grown under *in vitro* conditions, it was found that the amount of water contained in the leaf is 78.7% compared to control seedlings (Table 2).

Table 2
Results of a comparative analysis of the amount of water in the leaf of the studied pomegranate varieties

No.	Pomegranate varieties	The amount of dry matter contained in the leaf (%)	
		Control	Grown in the condition *In vitro*
1	“Qora Qayim”	66,4±4,3	76,2±2,5
2	“Qizil Anor”	76,5±3,2	83,5±4,4
3	“Oq Dona (Tuyatish)”	73,4±4,7	75,6±3,4
4	“Acchiq Dona”	74,6±2,4	79,5±2,1*

Note: * - P<0,05 rather than controlled; ** - P<0,01 (*n*=3-4).

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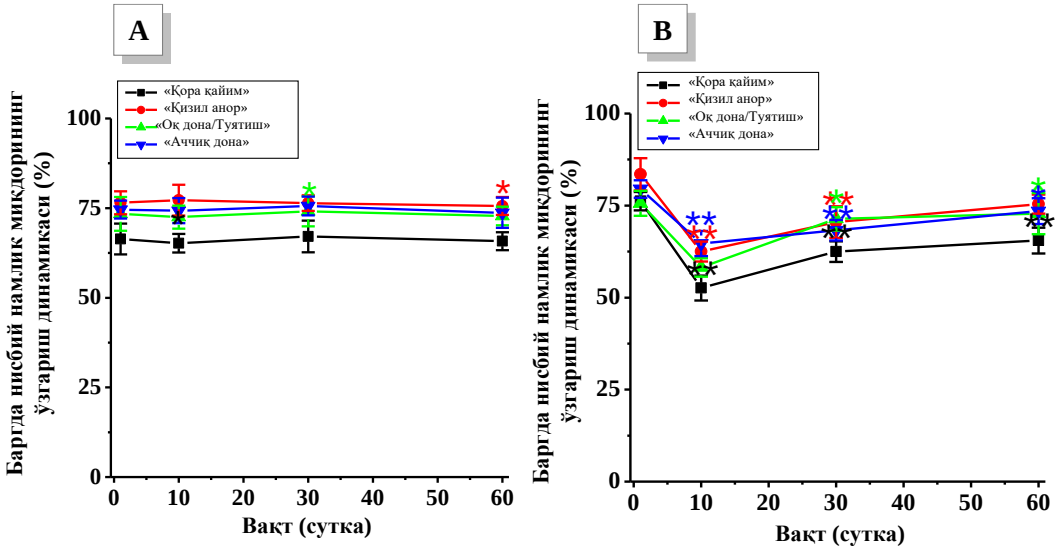
At the next stage of research, the dynamics of changes in the time interval range of the amount of water relative to control in seedling leaves grown in *in vitro* conditions in local pomegranate varieties “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona” were analyzed (Pic. 3 A and B). From the results of the experiment, it can be seen that in the control group, a significant change in the amount of water during 1-60 days in the leaf of selected pomegranate varieties was not recorded (Pic. 3, A). The amount of water in the leaves of seedlings grown under *in vitro* can be sharply reduced in the first days, and in the varieties “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)” and “Acchiq Dona” the value is from 76.2±2.5% to 52.6±3.4% in 10 days, respectively; from 83.5±4.4% to 62.5±a decrease of 2.4% to 64.7±3.4% was found. In the next 30-60 days, the amount of water in the leaves was to approach the control, and in the selected pomegranate varieties, the value in 10 days, respectively 65,5±3,5; 75,4±2,6; 72,8±5,6 and an increase of 73.5±3.5% was found (Pic 3, B).



Picture 3. Dynamics of changes in the time interval range of the amount of water in seedling leaves (Control A) and seedling leaves (B) of local pomegranate varieties “Qora Qayim”, “Qizil Anor”, “Oq Dona (Tuyatish)”, “Acchiq Dona” and grown under *in vitro* conditions *P<0,05; ** - P<0,01; (*n*=3-4).

The intensity of the process of photosynthesis in a plant is calculated based on the value of the specific density of the leaf surface. Also, the value of this indicator is an indicator that allows to assess the intensity of transpiration on the plant [1; p. 38-42; 2; p. 141-143.]. In the studies, it was noted that the specific density of the surface of the pomegranate leaf depends on the growing conditions, the phases of development during the growing season [2; p. 141-143.]. In experiments, the value of the specific density of the surface of the pomegranate leaf was determined using the standard method, based on the calculation of the ratio of the weight of the leaf in a dry state to the surface of the leaf [2; p. 141-143; 5; p. 15-19.]. At the next stage of research, a comparative density of pomegranate leaf was analyzed (Table 3).

Table 3.
Analysis results of the value of the comparative density of the leaf surface of the studied pomegranate varieties

No.	Pomegranate varieties	Compared density of the leaf surface (g/dm^2)	
		Control	Grown in the condition *In vitro*
1	“Qora Qayim”	0,75±0,04	0,54±0,02**
2	“Qizil Anor”	0,83±0,03	0,66±0,03**
3	“Oq Dona (Tuyatish)”	0,74±0,05	0,62±0,04*
4	“Acchiq Dona”	0,76±0,04	0,53±0,05**

Note: * - P<0,05 rather than controlled; ** - P<0,01 (*n*=3-4).

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Thus, it was found that the specific density of the leaf surface of pomegranate seedlings grown in *in vitro* (g/dm^2) was statistically believable lower than the control in the initial day (1 day). In particular, in the control group, the value of this indicator averaged 0.77±0.04 g/dm^2; in the experimental group, it was determined that it was equal to 0.59±0.03 g/dm^2 (Table 3). The results obtained correspond to the results of the existing literature. Typically, at the stage of plant development, an increase in the specific density value of the leaf surface is noted, a decrease in humidity with an increase [2; p. 141-143.].

The results obtained are explained by the fact that the specific density of the leaf surface of pomegranate seedlings grown in in vitro conditions (g/dm^2) is lower than the control adaptation to natural soil conditions with a high water content in the leaf during the initial period. To clarify the physiological mechanism of this, the intensity of transpiration on the leaves was analyzed when transferring seedlings grown in *In vitro* conditions of local pomegranate varieties Qora Qayim", "Qizil Anor", "Oq Dona (Tuyatish)" and "Acchiq Dona" to natural soil.

Transpiration is an integral indicator of the water regime in a plant [3; p. 11-12]. It is considered important to study the effect of stress associated with water exchange when transplanting a plant bred *in vitro* conditions into non-sterilized unsterilized soil environment [10; p. 148-150.]. Therefore, when transplanting pomegranate sprouts bred *in vitro* conditions to natural soil conditions, the study of water exchange, the dynamics of changes in the amount of water in the leaves and the intensity of transpiration was considered appropriately.

In the studies, it is recommended that the intensity of transpiration at the initial stage of the adaptation period in the transplantation of a plant bred *in vitro* conditions to the non-sterilized soil environment is relatively high, the amount of water in the leaves decreases, the increase in the humidity level in the atmosphere by 90-95% with gradual decrease to 50% in order to ensure optimal adaptation mechanisms [5; p. 148–150].

When transplanting pomegranate sprouts propagated *in vitro* conditions into non-sterilized soil, it is recommended to ensure that the humidity level of the environment is relatively high in the initial period. The results obtained above can be used to develop a system of comprehensive measures in optimizing the cultivation of pomegranate sprouts in the natural environment, which are propagated in in vitro conditions.

Conclusions. Thus, the intensity of the process of photosynthesis in a plant is calculated based on the value of the specific density of the leaf surface, the value of this indicator is an indicator that allows you to assess the intensity of transpiration in a plant. In doing so, it was found that the specific density of the leaf surface of pomegranate seedlings grown in in vitro conditions (g/dm^2) was statistically believable lower than the control in the initial day (1 day).

The results obtained can be explained by the fact that the specific density of the leaf surface of pomegranate seedlings grown in *in vitro* (g/dm^2) is lower than the control due to the high water content in the leaf during the initial period of adaptation to natural soil conditions.

It was noted that the intensity of transpiration at the initial stage of the adaptation period in the transplantation of a plant bred *in vitro* conditions to the non-sterilized soil environment is relatively high, as well as a decrease in the amount of water in the leaves, that is, a high water deficit value, and in turn, in order to ensure optimal adaptation mechanisms in the atmospheric environment around the plant, it is recommended to increase the humidity level to ~90-95% and gradually lower it to 50%.

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