The effect of carbon dioxide on the production of potato mini-tubers under aeroponic cultivation

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Abstract. The paper presents data on the effect of additional top dressing of potato plants with CO2 at a concentration of 2500 ppm cultivated in aeroponic plants. It was found that the increased concentration of CO2 provides better development of plant shoots (128.6%) and the root system (120.1%) relative to the control: a larger yield (123.7%) with a larger fraction of minicubes is formed, and the number of plants capable of producing 1100-1600 g of minicubes during the cultivation period increases. Additional feeding of CO2 at a concentration of 2500 ppm leads to an increase in the mass of plants, which occurs not due to increased hydration of tissues, but due to the accumulation of dry substances.

1. Introduction

Potato (Solanum tuberosum L.) is the third largest crop in the world after rice and wheat [1]. Potatoes are cultivated in conditions of wide geographical distribution and different ecological and climatic conditions [2]. High-quality seeds of new potato varieties are the key to increasing the yield of this crop. The economic and biological characteristics of potato varieties are determined by their genetic potential, which is maximally realized when using healthy high-quality seeds.

The lack of seed material in the required amount is the main reason for the low yield of potatoes, which, like most vegetatively propagated crops, has the property of gradually reducing the yield with constant use of seeds from the previous generation.

Currently, several seed production technologies are used worldwide to solve this problem: clonal micropropagation, hydroponics, and aeroponics [3, 4]. Aeroponics is one of the most effective methods due to its various advantages over other methods [5].

An important element of the technology of growing plants in aeroponics is the fertilization of plants with carbon dioxide (CO2). The low content of carbon dioxide in closed incubation systems is the main factor limiting the assimilation of carbohydrates and, consequently, the growth and development of plants. It is possible to fully cover the deficit only through the use of technical sources of carbon dioxide.

When growing plants on a soil-free basis, the lack of CO2 is a more serious problem than the lack of mineral nutrition elements. This is due to the fact that, on average, the plant synthesizes 94 % of the
dry matter mass from water and carbon dioxide, while the remaining 6% is obtained from mineral fertilizers [6].

It is known that the roots of plants in natural conditions are populated by microorganisms and emit CO2, which does not occur or occurs significantly less in more sterile conditions of aeroponic systems. Depending on the soil and plant species, the values of CO2 in the rhizosphere often reach values more than 10 times higher than the concentration of CO2 in the surrounding atmosphere [7].

According to A. Victor and M. Kramer [8], increased CO2 in the root zone can accelerate the growth of tomato plants (Lycopersicon esculentum), especially under stressful conditions, such as salinity and high temperature.

Some studies [9, 10] with lettuce grown at an ambient temperature of 20-36°C with a maximum PH of 2100 micromol m⁻² / s have shown that the increased level of CO2 in the root zone protects these plants from dynamic photoinhibition and reduces the negative impact of high air temperature on photosynthesis, nitrogen exchange, and plant growth, and thus enhances photosynthesis.

R. Tesky and M. McGuire [11] found that in the process of plant growth, CO2 released during respiration of plant tissues and soil is transported from the roots to the meristem sites through the xylem vessels. Later, it was also confirmed that in addition to atmospheric gas, soil CO2 can also be used for carbon uptake [12].

In greenhouse conditions, the combination of bright light and high temperature causes photoinhibition in plants, the same effect is observed in the cultivation of plants in tropical conditions [13]. For example, it was found that the midday Fv/Fm ratios in plants are much lower at high ambient temperature in the root zone than at 20 °C [14].

At high ambient temperature in the root zone and strong light, a low concentration of endogenous CO2 reduces photosynthesis and causes water scarcity in the shoots due to a change in the balance between water consumption by the root system and water loss in the shoots [13, 14]. The closure of stomata caused by water deficiency reduces the concentration of CO2 in the intercellular space, and at the level of chloroplasts reduces the photosynthetic assimilation of CO2 [15].

J. He and colleagues [16] investigated the effect of increased CO2 concentration in the root zone and temperature on photosynthesis, yield, nitrate content, reduced nitrogen, protein in leaves, and Rubisco protein in lettuce plants cultivated in aeroponic plants. Three weeks after transplanting, four different CO2 concentrations (360, 2000, 10000, and 50000 ppm) were applied to the plants at 20 °C and 24–38 °C in the root zone.

The increased CO2 content resulted in a significantly higher rate of photosynthesis, but lower stomatal conductivity. In addition, better resistance to both chronic and dynamic photoinhibition has been established. All plants accumulated better biomass at an increased CO2 concentration, with a greater increase in biomass recorded in the roots than that in the shoots, but the lowest shoot / root ratio was observed at an increased CO2 concentration and a temperature of 20 °C in the root zone.

Thus, many studies show that carbon dioxide in the system of artificial plant nutrition, as an additional factor contributing to the assimilation of carbohydrates, stimulates the development of plants, and forms additional resistance to external stressful influences. However, these studies were carried out on a limited number of plant species that are mainly used in greenhouses, such as lettuce [16], cucumber [17], tomato [18], pepper [19].

The purpose of the study is to determine the effect on the productivity of potato plants in the production of seed material of additional top dressing with CO2 during cultivation in the conditions of aeroponic installations.

2. Materials and methods

The biological object of the study was a potato of the Gulliver variety, previously propagated by the method of clonal micropropagation.

Before landing in hydroponic installations, micro-plants from one passage were tested by PCR and enzyme immunoassay methods for the presence of viral infection (PVX, PVS, PVM, PVA, PVY,
PLRV, PMTV, PSTVd). All microgrowths were rooted and had the same morphometric characteristics (microgrowth of 4-5cm, number of roots - 3-4 pcs, root length - 4-6 cm).

For plant cultivation, four UA-1500-3000 aeroponic plants (Russia) with the possibility of cultivating 32 plants in each were used. Aeroponic installations were placed in a tunnel-type greenhouse, thus ensuring a natural cycle of daylight illumination and illumination (for longitude of 52.8978N (Michurinsk)) within the period from April 10 to October 1. The air temperature was regulated automatically and did not exceed +30°C. An aqueous solution based on the Murashige-Skuga medium [20] (NH₄NO₃ – 1650 mg/l; KNO₃ – 1900 mg/l; MgSO₄·7H₂O - 370 mg/l) was used as the nutrient medium.; KH₂PO₄ - 170 mg/l; CaCl₂·2H₂O - 440 mg/l; MnSO₄·4H₂O - 22.3 mg/l; H₂BO₃ - 6.2 mg/l; ZnSO₄·7H₂O - 8.6 mg/l; Na₂MoO₄·2H₂O - 0.25 mg/l; CuSO₄·5H₂O - 0.025 mg/l; CoCl₂·6H₂O - 0.025 mg/L; KI - 0.83 mg/l; FeSO₄·7H₂O - 27.8 mg/l; Na₂EDTA·2H₂O - 37.3 mg / l).

The nutrient solution in the form of an aerosol was fed into the plant root system chamber for 1 minute every 15 minutes using 54 nozzles located in the upper part of the chamber, which ensured constant root moisture.

During the entire cultivation period, standard phytosanitary treatments were applied, which were adopted for the production of virus-free potato planting material in pot culture every 5 days.

During the experiment, the concentration of carbon dioxide (CO₂) was maintained automatically according to the principle of "deviation". The primary sensor was an infrared CO₂ analyzer MH-Z14A, the analog output of which was connected to a ten-bit analog-to-digital converter controller Atmega328. The measuring range of the sensor is 0-0.5% wt., with an error of ± 0.005%. The concentration value was controlled according to the two-position law. The value of ΔA is determined by 0.1%. The CO₂ concentration was maintained within 0.25±0.05% (2500±250 ppm) at a height of 3 cm above the root system chamber. The CO₂ feed nozzles were positioned evenly along the entire length of the aeroponic installation at a height of 35 cm.

The control was provided by aeroponic installations without an additional automatic carbon dioxide supply system.

The collection of mini-tubers was carried out 90 days after planting and then every 14 days. The criterion for collecting mini-tubers was a length of more than 15-20 mm.

To analyze the effectiveness of using CO2 as an additional source of carbon in the production of potato seed material, the number of mini-tubers, their length, width and weight on each plant were recorded using aerohydroponics methods. The raw mass of the aboveground part and the root system of the plants were taken into account, and then the dry weight was determined after drying in a thermostat (+130 °C, 180 min).

Statistical data processing was performed in the Microsoft Excel software environment.

### 3. Results

Providing plants with the necessary nutrients and selecting optimal conditions for cultivation is the fundamental basis for the development and formation of a full-fledged plant organism that can reproduce both vegetatively and sexually.

The diet is especially important for crops that can reproduce vegetatively, such as potatoes, as it is necessary to form a developed vegetative and root mass for further formation and growth of tubers.

A well-developed vegetative and root part of the plant indicates favorable trophic and / or external conditions of cultivation.

In this regard, the impact of any factor may lead to changes of morphometric parameters of plants, for example, the height of shoots and length of roots, as when growing in conditions of soil and conditions of the aerohydroplane system. In this regard, these indicators are considered sufficiently informative to determine the conditions of cultivation [5].

An analysis of the linear dimensions of potatoes grown under aeroponic culture conditions showed a positive effect of an additional carbon source in the form of CO₂ on plant growth (Table 1). Thus, in the experimental version, an increase in the maximum size of stolons was recorded from 126.0 cm (control) to 148.5 cm, which is 128.6%, and the root length from 128.6 cm to 159.3±8.0 (120.1%). At
the same time, the minimum values of the stolon length are almost equal, and in the case of root system growth, the minimum values are 22.0 cm higher than the experimental version.

Table 1. Effect of carbon dioxide on plant height and root length.

|                | Stolon length, cm | Root length, cm |
|----------------|-------------------|-----------------|
|                | min   | max   | χ      | min   | max   | χ      |
| Control        | 95.5  | 126.0 | 104.3±6.8 | 94.0  | 154.5 | 128.6±7.9 |
| Experience     | 92.5  | 148.5 | 124.3±8.5 | 116.0 | 185.5 | 159.3±8.0 |

Since, in the end, the root mass plays a significant role in the production of potato mini-tubers, its positively different morphometric indicators indicate a potentially high productivity.

The analysis of tuber formation showed that the increased concentration of CO2 in the zone of actively growing shoots contributes to the formation of more tubers on the roots (Fig. 1, 2). In the control variant, approximately 50% of the plants form from 20 to 60 minicubes, the remaining 50% form from 60 to 116-120 pieces. With an increased concentration of CO2, the distribution curve of the trait shifts to the right relative to the control with the formation of a peak at the level of 60 tubers/plant and decreases to 15.4% of plants that formed around 80 tubers / plant. The maximum values of the attribute in the experimental version reached 164 mini-tubers.

Figure 1. Frequency of potato miniclubber formation during the growing season during cultivation in aeroponic plants

For an objective assessment of the productivity of potato plants when growing in aeroponic conditions with a high content of CO2, it is necessary to take into account not only the quantitative characteristics of the mini-tubers, but also the qualitative ones.

Figure 3 shows the distribution of the total mass of miniclubes collected during the entire cultivation period.

Attention is drawn to the polymodal nature of the curves. This is probably due to the cyclical nature of the miniclub collection. The differences in the total mass of the miniclubbers of the control and experimental versions may also be due to the different degree of formation of the rudiments of tubers and the speed of their development at elevated concentrations of CO2.

In the control version, the main mass of plants on average forms from 400 to 800 grams of miniclubbers. In the version with CO2, this parameter has values from 400 grams to 1000 grams. At the same time, the second peak of the distribution curve of the trait lies in the region of 1100-1500 grams (25.3% of plants), which provides greater productivity of plants of the experimental variant. As a result, the average weight values of all standard mini-tubers formed during the period of active tuber formation on the plant increase from 609.4±51.9 g in the control to 753.9±54.9 g in the experiment, which is 123.7% (Table 2).
Figure 2. Formation of microtubules on potato roots in an aeroponic installation: a - at an increased concentration of CO2 2500 ppm; b - control.

Figure 3. Frequency of the total weight of minicubes during the growing season when cultivated in aeroponic plants

Table 2. Statistical indicators of potato plants grown in aeroponic conditions.

| Indicator                        | Control          | Experience       |
|----------------------------------|------------------|------------------|
| Number of mini-tubers, pcs / rast. | 53.6±5.5         | 62.8±6.6         |
| Total weight of mini-tubers, gr/rast. | 609.4±51.9      | 753.9±54.9       |
| Miniclub weight, gr              | 11.4±0.7         | 12.1±0.6         |
| Weight of the aboveground part, gr | 498.2±45.4      | 763.3±57.5       |
| Root weight without minicubes, g | 195.8±15.4       | 257.6±17.7       |
| Dry mass of shoots, g            | 31.8±5.0         | 60.4±7.4         |
| Root dry weight, g               | 7.3±1.0          | 11.9±1.9         |

The analysis of the linear dimensions of the mini-tubers showed differences in the shape of the control and experimental versions. With approximately the same width of the tubers (control-2.7±0.1 cm; experiment-2.8±0.1 cm), their length significantly differs (control-3.7±0.2 cm; experiment-
4.2±0.2 cm), these data are confirmed by Figure 4, which shows the average size of the mini-tubers for each plant. It is obvious that the formation of more elongated tubers in the variant with CO$_2$ fertilization is associated with the cultivation conditions, and not with the genetic characteristics of the variety. With an increase in mass, the shape may increase out of proportion, but not beyond the normal response of a particular genotype, and in accordance with the type of tuber, for example, round or elongated. The fact that the shape of the tuber under the influence of an external factor does not become uncharacteristic for this genotype is also indicated by the trend line. In the case of non-normal rounding of the mini-tubers, it would tend to be horizontal.

![Figure 4. Linear dimensions of the mineclubbers.](image)

Atmospheric CO$_2$ is the main nutrient of plants. Carbon, hydrogen, and oxygen are the main elements of all plants, accounting for an average of 42%, 7%, and 45% respectively of the dry mass [21].

Photosynthesis is the main process in the synthesis of all the organic compounds that make up a plant. During the vegetative phase of the life cycle, the absorption and processing of CO$_2$ during photosynthesis is the main function of plants.

The need for daily leaf photosynthesis, and hence the response to CO$_2$ enrichment, decreases as the plant matures and approaches the end of its life cycle. However, there remains a strong positive correlation between light absorption, photosynthesis, and growth rate.

There are conditions for processing carbon from the atmosphere into the building material of plants: CO$_2$ must enter the leaves and pass to the place of carboxylation; CO$_2$ must be fixed during photosynthesis in a convenient abbreviated form, such as a sugar molecule [21]. Thus, the indicators of plant mass will allow us to give a comprehensive objective assessment of the influence of this factor when applying top dressing with CO$_2$.

It is known that in most plants, the productivity of shoots and roots is interrelated. The dispersion analysis of the masses of shoots and roots in our study showed that the ratios are significant (raw weight: control-0.73, experience-0.82; dry weight: control-0.88, experience-0.89). In general, the mass of shoots and roots was significantly higher in raw and dry form at an increased concentration of CO$_2$ (Fig. 5). The average values of these indicators are presented in Table 2.

Thus, it can be unequivocally stated that additional feeding of CO2 at a concentration of 2500 ppm leads to an increase in the mass of plants. Moreover, the weight gain is not due to increased hydration of the tissues, but due to the accumulation of dry substances. This is evidenced by an increase in the dry weight of the experimental plants in comparison with the control.
Figure 5. Indicators of the mass of potato plants when cultivated on aeroponic installations: a - raw weight; b - dry weight.

To analyze the possible redistribution of nutrients during plant development between the shoots and the root system when feeding with CO$_2$, we used the mass ratio Cm (mass of shoots / mass of roots + mass of mini-tubers) (Fig. 6).

Figure 6. The mass ratio of the aboveground and root parts of potato plants.
The closer this indicator is to one, the more equal the mass of shoots and roots with the miniclubbers formed on them, and vice versa. In our case, an increase in the concentration of CO$_2$ leads to a greater alignment of plant parts due to the development of the aboveground photosynthetic part, which in turn provides a better trophic function and, accordingly, productivity in the form of tuber formation.

4. Conclusion

For the cultivation of potato plants in aeroponic plants for the production of seed material, the conditions of cultivation and nutrition are of great importance.

Additional fertilization of potato plants with CO$_2$ at a concentration of 2500 ppm, cultivated in aeroponic plants, provides better development of plant shoots (128.6%) and root system (120.1%) relative to the control. In addition, a larger crop (123.7%) is formed with a larger fraction of minitubers. The number of plants capable of producing 1100-1600 grams of the minitubers during the period of cultivation is increasing.

Thus, additional feeding of CO$_2$ at a concentration of 2500 ppm leads to an increase in plant mass, which occurs not due to increased hydration of tissues, but due to the accumulation of dry substances. To a greater extent, the above-ground photosynthetic part of the plant develops, which in turn provides better nutrition due to the processing of photon energy and contributes to the further increase in plant productivity in the form of tuber formation.

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