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## The effect of live chlorella suspension on the growth and development of grafted seedlings of Cabernet Sauvignon grapes


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**Abstract.** One of the ways to grow high-quality grafted grape seedlings is to use modern, environmentally friendly biologically active preparations. Today, such preparations include live chlorella suspension. Its use in grape nursery is a new and relevant technological method of growing planting material. The aim of the study was to show the effect of live chlorella suspension on the realization of the biological potential of grapevines, with the subsequent production of high-quality grafted grape seedlings. Field (recording of plant growth and development), laboratory (determination of quantitative and qualitative parameters of plants) and statistical (confirmation of the reliability of the results) research methods were used. The obtained results showed



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that for soaking rootstock and scion components it is advisable to use the strain *Chlorella vulgaris* Beijer, dilution 1:5, soaking should be carried out for 72 hours – rootstock components, 18 hours – scion components; for irrigation of grape garden plot it is advisable to use the strain *Chlorella vulgaris* Beijer. + *Ge*, dilution 1:5, it was carried out immediately after planting scions and three times during the growing season – in June, July, and August. The use of these strains of live chlorella suspension allowed obtaining a higher yield of standard seedlings from the garden plot, the plants had a well-developed root system and annual growth. Compared to the control, which was water, the total number of roots increased. Plants in these variants were also characterized by increased growth of the aboveground part. Indicators of total and mature growth, which characterize the overall development of grafted grape seedlings, were in the range of 687.1-773.2 $cm^3$ (total growth volume), 337.9-386.6 $cm^3$ (mature growth volume), while 730.1 and 362.6 $cm^3$, respectively, in the control. The records of the output of standard seedlings from the garden plot also showed the advantage of using a suspension of live chlorella (an increase of 19.0-21.0%) compared to water. The above technological methods allow obtaining high-quality grape seedling products and can be a promising alternative for more sustainable and environmentally friendly agricultural methods

**Keywords:** cuttings; vegetative organs; annual growth; root system; microalgae *Chlorella vulgaris* Beijer.; germanium

## INTRODUCTION

Viticulture is an important economic sector of Ukraine's agro-industrial complex. This is due to the valuable consumer, energy, health, and wellness properties of fresh grapes and their products. In agricultural production, grapes are considered an "insurance fund" as they are undemanding to soils (vineyards are often planted and cultivated on land unsuitable for growing grain or other crops) and always yield a crop even under extreme conditions of drought, frost, and other negative influences. Therefore, long-term highly productive grape plantations will ensure the stable and full development of the viticulture and winemaking industry.

The analysis of scientific research on the use of live chlorella suspension in crop production showed that the work that is currently being carried out mainly concerns annual crops. In the work of T. Hajnal-Jafari *et al.* (2020), the authors studied the effect of *Chlorella vulgaris* Beijer. strain S45 suspension on the growth of *Beta vulgaris* L. subsp. *cicla*. plants and the pigment content in leaves. The best results in the development of the leaf apparatus and root system were obtained after soil treatment with 10% suspension solution.

A. Kusvuran & A. Can (2020) and A. Kusvuran & C. Kusvuran (2019) investigated the effect of a liquid fertilizer containing the microalgae *Chlorella vulgaris* Beijer. on the nutrient content of the aboveground mass of *Cyamopsis tetragonoloba (L.) Taub*. plants and their resistance to salinity and drought conditions. The authors came to the conclusion that foliar fertilization with a fertilizer based on *Chlorella vulgaris* Beijer. contributed to an increase in shoot length, wet and dry leaf weight, and their number, while an increase in the content of photosynthetic pigments, total phenolics, flavonoids, potassium, and calcium was noted in the leaves. And plants of *Cyamopsis Tetragonoloba (L.) Taub*. tolerated the negative effects of salinity better.

In the study by Y.J. Park *et al.* (2022), the positive effect of *Chlorella vulgaris* Beijer. (suspension, biomass, filtered supernatant) on the growth of *Brassica napus* var. *Pabularia* plants and the content of secondary metabolites in the leaves were shown. The authors showed that the filtered supernatant had a positive effect on the accumulation of phenols and flavonoids in the leaves, while the supernatant treatment had a positive effect on the accumulation of anthocyanins. G.E. Özer Uyar & N. Mısmıl (2022) grew *Mentha spp*. seedlings, while they introduced the microalgae *Chlorella vulgaris* Beijer into the hydroponic culture. The results of the research showed that the increase in wet weight of plants was the highest after the application of microalgae under aeration conditions. However, the height of the plants did not change significantly.

In the study by Sh.L. Tian *et al.* (2022), the effect of *Chlorella vulgaris* Beijer extract on the growth of Capsicum annuum plants of the "Chao Tian Jiao" variety was investigated. Two weeks after transplanting into pots, the seedlings of the experimental groups were sprayed with Chlorella extracts. The results showed that the use of the extract significantly increased the height of the seedlings, stem diameter and leaf area, increased the pigment content and enzyme activity in the leaf tissues, and the root system was more powerful and branched. Similar results in terms of biometric indicators of growth and development of vegetative organs were obtained when growing *Eruca vesicaria* ssp. *sativa* Mill plants in greenhouse conditions with a closed root system (Turhan *et al.,* 2022).

Few studies have been devoted to the use of live chlorella suspension in viticulture, which are mainly aimed at increasing berry yields. For example, S. Tangolar *et al.* (2019) studied the effect of root and foliar treatments with *Chlorella vulgaris* Beijer algae extract on the yield and quality of grape varieties "Trakya ilkeren", "Yalova incisi" and "Prima". The results of the experiments showed that the highest grape yield, weight of one bunch, 100 berries were obtained after the complex application of a suspension of live chlorella: root + foliar treatment. After such application, a high

content of K, Ca, Mg and Fe, P, and Zn was also noted in the leaves of plants.

To date, there are no studies related to the use of a suspension of live chlorella — *Chlorella vulgaris* Beijer. in a grape nursery. Separate works on the use of dry extract of *Chlorella vulgaris* Beijer. in the cultivation of vegetative rootstocks of grape variety "Palieri" affected by root ectoparasite *Xiphinema index* were conducted at the Agricultural University of Plovdiv, Bulgaria (2010-2013). *Chlorella vulgaris* Beijer. suspension was not generally used in the technology of growing grafted grape seedlings. Thus, at present, there is no data on its influence on the development of vegetative organs of grape planting material and the production of standard seedlings.

Given the above, the study aimed to determine the effect of live chlorella suspension on the development of vegetative organs of grafted grape seedlings. To achieve this goal, the following research objectives were set: to determine the effect of live chlorella suspension on the development of annual growth and root system of grafted grape seedlings; to determine the effect of live chlorella suspension on the yield of grafted grape seedlings from the garden plot and their compliance with DSTU 4390:2005 (2006); to assess the influence of certain technological factors on the formation of quality indicators of grafted grape seedlings.

## MATERIALS AND METHODS

The work was carried out during 2018-2022 at the Department of Nursery, Propagation, and Biotechnology of Grapes of the National Research Centre "Tairov Institute of Viticulture and Winemaking" of the National Academy of Agrarian Sciences of Ukraine. The material for the research was graft components and grafted seedlings of the Cabernet Sauvignon technical grape variety. Cabernet Sauvignon is a late-ripening dark berry grape variety. The growth of the bushes is strong, the ripening of the shoots is satisfactory, and it is relatively winter-hardy. It is characterized by high resistance to mildew and grey rot. Productivity is 6.0-9.0 t/ha, average bunch is weight 73 g. Tasting score: 7.9-8.0 points.

The suspension of live chlorella was used at the following stages of the technology for the production of grafted grape planting material — soaking of scion components and during the cultivation of seedlings in the open ground plot. The strain of *Chlorella vulgaris* Beijer. pure and enriched with germanium (aqueous working solutions) were used in the study. *Chlorella vulgaris* Beijer is a unicellular microalgae belonging to the domain *Eukaryota* → kingdom *Protista* → division *Chlorophyta* → order *Chlorellales* → family *Chlorellaceae* → genus *Chlorella* → species *Chlorella vulgaris* Beijer.

Cultivation of microalgae *Chlorella vulgaris* Beijer. IFR No. C-111 (grade A, TU U 03.0-37613791- 001:2017) and *Chlorella vulgaris* Beijer BIN are grown at the farm "At Samvel's" (Odesa region). The product is used as a plant growth stimulant to increase crop yields (grade B). In terms of organoleptic, physicochemical parameters and toxicity, the chlorella suspension must meet the technical specifications of TU U 03.0-37613791-001:2017.

Scientists of the I.I. Mechnikov Odesa National University together with the International Research and Production Company "Living Chlorella" have developed a method for growing a suspension culture of live cells of the microalgae *Chlorella vulgaris* Beijer with the addition of germanium. The preparation contains vitamins ($B_1$, $B_2$, $B_5$, $B_6$, $B_9$, $B_{12}$, A, C, D, E, PP, K), macro- and microelements (N, P, K, Ca, Cu, Mg, Fe, S, Zn, Mn, Zr, Rb, I, Co), amino acids (alanine, valine, glycine, leucine, threonine), vegetable protein, growth regulators (gibberellins, cytokinins, auxins, natural steroids and phenols), and a natural antibiotic – chlorelin.

In the experimental variants, the scion components were soaked in *Chlorella vulgaris* Beijer working solutions for 72 (rootstock scions) and 18 (grafting scions) hours, in the control variants they were soaked in water for similar time intervals. Watering of grape planting material in the open field (plot) with aqueous solutions of live chlorella suspension was carried out immediately after planting and three times during the growing season – in June, July, and August. Control grafts and seedlings were watered at the indicated times.

*Scheme of the experiment:*

| | |
|---|---|
| | **Soaking components of grafts:** |
| Option 1 | Water (control 1). |
| Option 2 | *Chlorella vulgaris* Beijer. (dilution of the suspension solution: water 1:5). |
| Option 3 | *Chlorella vulgaris* Beijer. + *Ge* (dilution of the suspension solution: water 1:5). |
| | **Watering the scion in the nursery:** |
| Option 4 | Water (control 2). |
| Option 5 | *Chlorella vulgaris* Beijer. (dilution of the suspension solution: water 1:5). |
| Option 6 | *Chlorella vulgaris* Beijer. + *Ge* (dilution of the suspension solution: water 1:5). |
| Option 7 | *Chlorella vulgaris* Beijer. (dilution of the suspension solution: water 1:1). |
| Option 8 | *Chlorella vulgaris* Beijer. + *Ge* (dilution of the suspension solution: water 1:1). |

***Source:*** *developed by the authors*

The produced grape scions were stratified in a closed way on coconut substrate for 20 days, hardening – for 7 days openlyon water in the greenhouse complex of the Institute. After the end of the growing season and digging of the grafted grape seedlings, the following indicators were determined: total number of roots (including those with a diameter of more than 2 mm) (pcs.); root length (including gradations) (cm); shoot height and part of the matured vine (cm); shoot maturation (%); shoot diameter (mm); volume of total, matured growth ($cm^3$); yield of standard seedlings (%). Determinations and calculations were made according to the methods described in the textbook "Features of the grape plant and methods for assessing organ and tissue indices" (Scherer & Zelenyanskaya, 2011). The quality of the grafted grape seedlings was determined by determining whether the development of vegetative organs – annual shoots and roots – corresponded to the parameters and characteristics specified in DSTU 4390:2005 (2006).

To determine the effect of live chlorella suspension on the biometric parameters of growth and development of grafted grape planting material, the value of stimulating efficiency (R, %) was calculated: $R=(\sum K_1/N_1) - (\sum K_2/N_2)$, where R is the value of stimulating efficiency (%); $\sum K_1$ is the number of plants characterized by the studied trait after exposure to the suspension (%); $N_1$, $N_2$ is the number of replications during the study period; $\sum K_2$ is the number of plants characterized by the studied trait without the influence of the suspension (%). Depending on the value of R, the results were evaluated as follows: R>0 – the suspension showed stimulating properties, R=0 – the suspension did not show stimulating properties; R<0 – the suspension had an inhibitory effect. The study was conducted in accordance with the requirements of the Convention on Biological Diversity (1992).

## RESULTS AND DISCUSSION

The development of the root system of plants in general and grapes in particular determines the degree of development of aboveground organs, fruiting, and yield of bushes. The development of the root system of a grape plant and the placement of roots in soil horizons depends on many agronomic practices, including plant nutrition. Therefore, the issue of determining the effect of live chlorella suspension on the development of the root system of grafted grape seedlings is relevant and timely.

In the process of growing grafted grape seedlings, it is very important to obtain plants with well-developed roots with a diameter of more than 2.0 mm. Since these roots are characterized by a perfect conductive system and thick parenchyma, the latter performs protective, reserve and mechanical functions. Therefore, during the autumn and winter storage of seedlings, these roots counteract the negative effects of environmental factors to the maximum extent possible, are better preserved, and in the spring, when laying out a vineyard or planting individual bushes, they ensure high plant survival. Roots with a smaller diameter are more often exposed to abiotic and biotic factors during storage, are damaged and die.

The development of the root system of the grafted grape seedlings was recorded after excavation from the garden plot. The results showed the following. In the control variants, the grafted grape seedlings, on average, had 10-11 roots, of which 31.0% were roots with a diameter of more than 2.0 mm and 69.0% were roots with a diameter of less than 2.0 mm (Table 1).

***Table 1.*** *The effect of live chlorella suspension on the formation of the root system of grafted grape seedlings (average for 2018-2022)*

| Research options | Total number of roots, pcs. | Number of roots, d≥2 mm, pcs. | Number of roots, d≤2 mm, pcs. | The length of one root d≥2 mm, cm | The total length of the roots, d≥2 mm, cm | The length of one root d≤2 mm, cm | Total length of roots d≤2 mm, cm |
|---|---|---|---|---|---|---|---|
| 1 | 11.3±0.5 | 3.0±0.2 | 7.8±0.4 | 27.0±1.5 | 94.8±5.5 | 9.3±0.7 | 73.1±3.8 |
| 2 | 16.0±0.3 | 5.8±0.3 | 10.1±0.5 | 43.3±2.7 | 253.3±7.2 | 16.3±0.9 | 166.2±5.6 |
| 3 | 13.6±0.6 | 4.6±0.3 | 9.0±0.5 | 34.6±2.1 | 162.0±5.8 | 9.3±0.8 | 84.3±4.0 |
| 4 | 10.6±0.5 | 3.0±0.2 | 6.9±0.3 | 29.0±1.4 | 108.0±5.0 | 9.6±0.7 | 67.1±4.2 |
| 5 | 14.4±0.5 | 3.8±0.2 | 10.6±0.6 | 30.4±1.6 | 116.5±4.6 | 10.0±0.6 | 106.3±5.0 |
| 6 | 19.2±0.8 | 5.7±0.4 | 13.4±0.7 | 43.8±1.8 | 251.5±6.6 | 19.0±0.8 | 255.8±5.9 |
| 7 | 14.9±0.4 | 4.4±0.3 | 10.5±0.5 | 37.5±2.3 | 165.7±6.0 | 11.3±0.6 | 119.5±3.5 |
| 8 | 16.4±0.3 | 4.8±0.3 | 11.6±0.4 | 35.3±2.2 | 170.8±6.5 | 14.6±0.6 | 170.7±3.3 |

***Source:*** *developed by the authors*

After applying the method of soaking the grafted components in aqueous solutions of live chlorella suspension with subsequent grafting and growing seedlings in a garden plot, the number of roots increased to 14.0 (*Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5)) – 16.0 (*Chlorella vulgaris* Beijer. (dilution 1:5)), of which 33.8-36.2% were roots with a diameter of more than 2.0 mm and 63.1-66.1% were roots with a diameter of less than 2.0 mm. In general, after soaking the scion components in the live chlorella suspension, a significant difference was observed in the total length of roots of different gradations. Compared to control 1 (water application), the total length of roots d≥2 mm increased by 1.7-2.6 times, and the total length of roots d≤2 mm – by 1.5-2.2

times, respectively. At the same time, the length of one root differed significantly from the control only after soaking the scion components in a solution of *Chlorella vulgaris* Beijer (dilution 1:5).

After applying the suspension of live chlorella for watering grape scions and seedlings during the growing season in the garden plot, the total number of roots increased by 33.3 and 54.7% compared to the control after using aqueous solutions of *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5, 1:1) and by 35.8 and 40.5% after using aqueous solutions of *Chlorella vulgaris* Beijer. of the corresponding dilutions. The number of roots with a diameter of more than 2.0 mm, on average, increased by 29.0% in variants and in relation to control 2. After the application of *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5, 1:1), a significant increase in the length of roots of both gradations was observed. Compared to the control, such an increase for roots of the first order (d⩾2 mm) was 1.5 (*Chlorella vulgaris* Beijer. + *Ge*, *Chlorella vulgaris* Beijer. (dilution 1:1)) – 2.3 (*Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5)) times, for roots of the second order (d⩽2 mm), respectively, 2.5-3.8 times after application of *Chlorella vulgaris* Beijer. + *Ge* and 1.5-1.7 times after application of *Chlorella vulgaris* Beijer.

In grapes, a certain relationship has been established between the root system and the aerial part of the bushes: the leaf apparatus provides organic matter to both the aerial parts and the root system, the latter provides water and minerals to the aerial part. Therefore, by regulating the growth of the grape root system through the use of appropriate agricultural products, it is possible to stimulate the growth of shoots and leaf surface. Enhanced growth of the aerial part, on the contrary, promotes the development of the root system. In the process of growing grafted grape seedlings, it is very important to stimulate the growth of shoots, as the development of the assimilation (leaf) apparatus will depend on their development. According to the results of the work, it was found that the longest annual shoots were formed in seedlings after the use of *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5, 1:1) (sixth, eighth variants, watering of scions) and *Chlorella vulgaris* Beijer. (second variant, soaking of components) (dilution 1:5) (Table 2).

***Table 2.*** *The influence of live chlorella suspension on the development of the vegetative mass of grafted grape seedlings (average for 2018-2022)*

| Research options | The total length of the shoot, cm | Shoot diameter, mm | The length of the mature part of the shoot, cm | Ripening of shoots, % | Total growth volume, $cm^3$ | Volume of mature growth, $cm^3$ |
|---|---|---|---|---|---|---|
| 1 | 49.5±3.1 | 4.2±0.10 | 24.3±1.6 | 49.1 | 687.1±22.5 | 337.9±12.5 |
| 2 | 60.3±3.8 | 5.0±0.31 | 34.6±1.8 | 57.5 | 1216.6±23.3 | 698.4±14.5 |
| 3 | 55.0±3.4 | 4.6±0.24 | 25.6±1.5 | 46.6 | 929.8±23.4 | 434.5±13.0 |
| 4 | 50.1±3.0 | 4.4±0.22 | 25.0±1.5 | 49.9 | 773.2±21.0 | 386.6±12.4 |
| 5 | 53.7±3.5 | 4.5±0.32 | 27.6±1.7 | 51.5 | 885.2±20.5 | 450.3±12.5 |
| 6 | 71.2±4.5 | 6.0±0.51 | 45.6±2.0 | 63.9 | 2017.7±23.1 | 1295.1±14.1 |
| 7 | 60.2±3.7 | 5.0±0.20 | 31.6±2.1 | 52.5 | 1200.4±22.2 | 625.0±13.8 |
| 8 | 65.9±4.1 | 5.4±0.50 | 39.0±2.4 | 58.9 | 1526.0±23.0 | 899.6±13.2 |

***Source:*** *developed by the authors*

In comparison with the controls, the shoot height in plants of the second variant was 10.8 cm or 21.8% higher, in plants of the sixth and eighth variants, respectively, by 21.1, 15.8 cm or 42.1 and 31.5%. The plants of these variants also had a larger shoot diameter. And this is an important feature, since this indicator affects the formation of the volume of total and mature growth. The latter determines the degree of development of grape plants in general, the content of reserve and plastic substances in lignified and perennial parts of the bushes, which indicate the quality of the planting material and its stability during storage.

The use of a suspension of live *Chlorella vulgaris* Beijer. (dilution 1:5) for soaking the components of grape scions contributed to an increase in the volume of total and mature growth, compared to the control, by 1.8 (total growth), 2.0 (mature growth) times. The use of the strain *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5) also contributed to different results from the control, but these indicators were lower and increased by 1.3 times compared to the control. After applying a suspension of live chlorella for watering grape scions and seedlings in the garden plot, the best results were obtained after using the strain *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5 (sixth variant) and 1:1 (eighth variant)). In plants of these variants, the volume of total growth was 2.6 times (sixth variant) and 1.9 times (eighth variant) higher than the control value, the volume of mature growth was 3.3 times (sixth variant) and 2.3 times (eighth variant). After the use of the strain *Chlorella vulgaris* Beijer. dilution 1:5, these indicators were at the level of control values, and after the use of the solution dilution 1:1 increased by 1.5 times.

A similar dependence was found for the maturation of annual shoots. In the technological process of growing grafted grape seedlings, it is very important to achieve maximum ripening of shoots (vines). It is of great practical importance – it is associated with the

winter hardiness of shoots, buds, and the quality of rootstock and scion in the production of planting material. Therefore, the issue of shoot ripening is of great interest, especially after the phylloxera invasion. The best shoots ripened in the variants where the plants were watered in the garden plot with a suspension of live *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5) – 63.9%, which is 14.0% more than in the control. After the use of *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:1) for watering plants and *Chlorella vulgaris* Beijer. (dilution 1:5) for soaking the components of scions – shoots maturation was in the range of 57.5-58.9%, which was 8.4-9.0% higher than in the control. In all other variants, this increase was not significant.Obtaining grafted grape seedlings with the specified parameters of vegetative organs development allows increasing the survival rate of plants in a permanent place. Comparison of plant development indicators obtained after the use of a suspension of live chlorella and water in the technology of growing grafted grape seedlings showed that the root system development of seedlings of the experimental variants, where the strain *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5 and 1:1) for irrigation and *Chlorella vulgaris* Beijer. (dilution 1:5) for soaking the scion components fully met the parameters of DSTU 4390:2005 (2006).

Plants of control variants and after application of the strain *Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5, soaking), *Chlorella vulgaris* Beijer. (dilution 1:1, 1:5, watering) the number of roots corresponded to the minimum value, but the roots were placed asymmetrically (on the one hand) on the "heels" of plants, which does not correspond to DSTU 4390:2005 (2006). According to the development of one-year growth, namely the thickness of the shoot, only plants in the second group (*Chlorella vulgaris* Beijer. (dilution 1:5, soaking)), sixth (*Chlorella vulgaris* Beijer. + *Ge* (dilution 1:5, watering)), seventh (*Chlorella vulgaris* Beijer. (dilution 1:1, watering)) and eighth (*Chlorella vulgaris* Beijer. + *Ge* (dilution 1:1, watering)) variants.

Thus, summarizing the above, it should be noted that the suspension of live chlorella promotes the powerful development of both the root system and the aboveground vegetative mass of grafted grape seedlings. Its use at the stage of soaking the scion components involves the use of the strain *Chlorella vulgaris* Beijer. dilution 1:5, at the stage of watering plants in the garden plot during the growing season — the use of the strain *Chlorella vulgaris* Beijer. + *Ge* dilution 1:5 or 1:1.

The reliability of the data obtained on the development of vegetative organs of grafted grape seedlings was proved by the results of analysis of variance. The main factors of influence are: technological method (factor 1), strain of live chlorella suspension (factor 2) and dilution of live chlorella suspension solution (factor 3). The identified differences between the experimental and control variants are reliable, since the actual values of Fisher's criterion (for most of the main factors of influence) at the level of significance P – 0.05 were higher than their tabulated values. $F_{fact.} = 4.35$, $F_{theor.} = 6.58\text{-}55.7$ (limits for different indicators). However, the results of the interaction of factors 2 and 3 were not always reliable in the development of annual growth and root system of plants.

Determination of the share of influence of the main factors on the development of annual growth and root system showed that the factor "technological method"(i.e. how the live chlorella suspension was used: soaking or for irrigation) was of the greatest importance – 27.0-52.0%. The influence of other factors was as follows: factor "dilution of the live chlorella suspension solution" – 11.2-33.0% (vegetative mass), 14.0-29.6% (root system), factor "strain of live chlorella suspension" – 12.1-25.0% (vegetative mass), 10.0-17.3% (root system), interaction of factors "technological method" and "strain of live chlorella suspension" – 11.1-37.2% (vegetative mass), 13.1-22.2% (root system).

In nursery farming, the effectiveness of new or improved technological methods is assessed by the yield of planting material, in this work it is the number of grafted grape seedlings that must meet the parameters of DSTU 4390:2005 (2006). The largest number of such plants was obtained in the variants after the use of live chlorella suspension for irrigation of grape garden plot – 48.0-55.5% and these are variants with the strain *Chlorella vulgaris* Beijer. + *Ge* and after the use of live chlorella suspension for soaking the grafted components – these are variants with the strain *Chlorella vulgaris* Beijer.

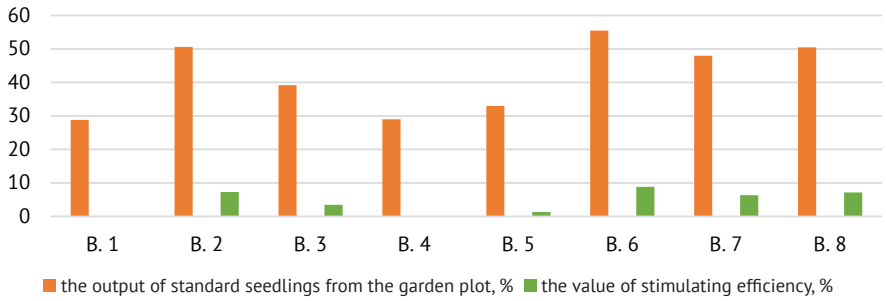


***Figure 1.*** *The yield of grafted grape seedlings from the nursery and the assessment of the effect of live chlorella suspension on this indicator (average for 2018-2022)*

***Source****: developed by the authors*

The effectiveness of the influence of live chlorella suspension on the yield of standard grape seedlings from the garden plot is confirmed by the value of stimulating efficiency. It had the highest values in the variants identified as the best (sixth, eighth, second, and seventh). Thus, the results obtained indicate the positive effect and expediency of using a suspension of live chlorella *Chlorella vulgaris* Beijer. in the technology of production of grafted grape planting material, in particular at the stages of soaking the scion components and watering the seedlings in the garden plot. Its use allowed increasing the yield and improving the quality of planting material.

Microalgae produce a wide range of metabolites, which, due to their biological activity, can be used in agriculture as biofertilizers, biostimulants or biopesticides. They play an important role in: soil decontamination and fertilization, protection of plants from biotic and abiotic factors, and promoting better plant development (Gonçalves, 2021). In the current study, the authors concluded that the live chlorella suspension has a stimulating effect and promotes the growth and development of the vegetative organs of grafted grape seedlings, in particular the development of shoots and root systems. According to the authors, these results are associated with the influence of biologically active substances contained in the live chlorella suspension. These are: vitamins ($B_1$, $B_2$, $B_5$, $B_6$, $B_9$, $B_{12}$, A, C, D, E, PP, K), macro- and microelements (N, P, K, Ca, Cu, Mg, Fe, S, Zn, Mn, Zr, Rb, I, Co), amino acids (alanine, valine, glycine, leucine, threonine), plant protein, plant growth and development regulators (auxins, gibberellins, cytokinins, natural steroids, phenolic compounds). The conclusions about the stimulating effect of algal fertilizers were also reached by C. Schreiber *et al.* (2018), who grew *Triticum aestivum* L. on two poor substrates: "null Erde" and sand, and liquid algal fertilizer was used for irrigation. As a result, it was shown that plants grown in sand were smaller in terms of biometric parameters, but fertilization with algal fertilizer led to increased growth processes, which, according to the authors, was similar to the use of chemical fertilizers. These results showed that algal biomass and its nutrients can be a natural alternative to support agriculture.

The positive effect of minerals and vitamins in their works is pointed out by R. Rahman *et al.* (2022). The authors note that, in addition to vitamins and minerals, the intensification of the growth processes of grape seedlings is also explained by the presence of a number of active amino acids in the live chlorella suspension, which are central to metabolic processes and building blocks of cell walls. H. Baqer *et al.* (2019) also associate active growth processes with the presence of active amino acids. The authors believe that a group of natural regulators of plant growth and development contained in the suspension – auxins, cytokinins, gibberellins – also contributes to the activation of the metabolism of the plant organism in general, and grape seedlings in particular. They ensure the architecture and development of vegetative organs and the vascular system. Y. Durán-Medina *et al.* (2017) also reported a similar effect on the development of *Arabidopsis thaliana* (L.) Heynh. under the influence of these plant growth regulators.

After applying a suspension of live chlorella *Chlorella vulgaris* Beijer. + *Ge.*, dilution 1:5, grafted grape seedlings with a well-developed root system were obtained. During the growing season, it was noted that immediately after planting the grafts in the open ground plot, they took root faster, rooted and moved to the vegetation stage. The positive effect of microalgae on plant rooting is emphasized in the works of N. Pereira *et al.* (2018). They showed that the use of chlorella stimulated the rooting of the orchid *Schomburgkia crispa in vitro*. Other authors explain this effect through the influence of microalgae directly on soil microbial populations (changes in biomass, activity, species composition and diversity), improvement of soil physical properties (structure, water holding capacity), enrichment of soil with phytohormones and other biologically active substances (Marks *et al.,* 2019; Nisha *et al.,* 2018; Chanda *et al.,* 2019).

The use of live chlorella suspension *Chlorella vulgaris* Beijer., dilution 1:1, 1:5 at the stage of soaking the scion components was also aimed at intensifying callusogenesis in the current study. And, according to the results obtained, it intensified the formation of callus tissue on rootstock and scion sections, the formation of the vascular-conductive system (xylem and phloem), and the formation of "fusion rods". This was facilitated by auxins, cytokinins, amino acids and vitamins that activate callus growth and vascular tissue formation. These data are consistent with the results of A.K. Nanda and C.W. Melnyk (2018), who showed the overall effect of biologically active substances on the formation of a whole organism after vaccination and A. Aydin *et al.* (2022), who proved that the treatment of cucumber (*Cucumis sativus* L.) cuttings during the grafting process with auxin substances contributed to better plant formation (increased moisture, dry weight of roots and stems). The use of a suspension of live chlorella with germanium at this technological stage did not give the desired results. In previous laboratory studies by N.M. Zelenianska and O.M. Mandych (2022), the authors showed that after soaking the inoculated components in aqueous solutions of *Chlorella vulgaris* Beijer. suspension with germanium, the mass of callus was lower, and callus tissue was formed unevenly around the circumference of the copulation cut. Most likely, germanium, in this case, had an inhibitory effect, and the strain *Chlorella vulgaris* Beijer. gave positive results.

When comparing the parameters of the development of vegetative organs of grafted grape seedlings after application of live chlorella suspension, it should be noted that they were higher than the standard ones after application of *Chlorella vulgaris* Beijer. + *Ge* for

watering plants in the garden plot. According to the authors, this is due to the introduction of germanium into the suspension. In plant tissues, it promotes the decomposition of water into oxygen and hydrogen, which is then used in physiological processes, thus stimulating plant growth. K.V. Chernega *et al.* (2019), in their work on *Cucumis sativum* L., *Cucurbita pepo* subsp., *Solanum lycopersicum* L. and *Lepidium sativum* L. plants, also showed the stimulating effect of germanium obtained from the culture fluid of the microalgae *Chlorella vulgaris* Beijer. on seed germination and further morphometric development of plants.

Thus, taking into account the theoretically substantiated effect of physiological and biochemical substances that are components of the suspension of live chlorella, it becomes clear that the formation of better parameters of the development of the aboveground mass and root system of grafted grape seedlings after its application is possible. Therefore, based on the theoretical and practical results obtained, it is advisable to use the suspension of live chlorella – *Chlorella vulgaris* Beijer. at the technological stages of growing grafted grape seedlings – pre-stratification soaking of grafted components and for irrigation of grape garden plot.

## CONCLUSIONS

The suspension of live chlorella *Chlorella vulgaris* Beijer. has a stimulating effect on the development of vegetative mass, root system of grafted grape seedlings and their exit from the garden plot. It should be recommended for use at the following stages: soaking of grape scion components with subsequent production of scions and when growing grafted seedlings in an open ground plot for irrigation through a drip irrigation system. For soaking grape scion components, the strain *Chlorella vulgaris* Beijer. should be used at a dilution of 1:5. In this case, the scion components should be soaked for 18 hours, and the rootstock components – for 72 hours. For irrigation of grape scions and seedlings in the garden plot – use the strain *Chlorella vulgaris* Beijer. + *Ge* dilution of the working solution 1:5 or 1:1. Watering should be carried out with aqueous solutions in time – immediately after planting the scions in the garden plot and three times during the growing season – in June, July, and August.

The use of such strains at different technological stages contributed to the formation of more roots, including roots with a diameter of more than 2 mm, an increase in their length and better branching of the root system. Compared to the controls, the total number of roots increased by an average of 41.5-81.1%, the number of roots with a diameter of more than 2 mm – by 29.3-36.2%, and the length of roots – by 1.5-2.6 times. After the application of live chlorella suspension of these strains, the plants were also characterized by better development of annual growth: the volume of total growth was in the range of 1216.6-2017.7 $cm^3$, mature growth – 698.4-1295.1 $cm^3$, while 730.1 $cm^3$ and 362.2 $cm^3$ in the control. The use of such strains and dilutions allows producing grafted grape seedlings that fully meet the parameters of DSTU 4390:2005. The yield of standard seedlings from the garden plot exceeded the control values by an average of 21.5-26.5%.

The reliability of the obtained results was confirmed by the results of the analysis of variance. The identified differences between the experimental and control variants are reliable, since the actual values of Fisher's criterion (at the significance level $P \leqslant 0.05$) were higher than their tabulated values. Determination of the share of influence of the main factors (technological method, strain of live chlorella suspension, dilution of live chlorella suspension solution) on the development of annual growth and root system of grafted grape seedlings showed that the factor "technological method" was the most important – 27.0-52.0%. The influence of other factors was in the range of 10.0-33.0%. Prospects for further work in this area are to determine the effect of live chlorella suspension on the course of physiological and biochemical processes in the tissues of grape leaves and shoots.

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## CONFLICT OF INTEREST

There is none.

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# Вплив суспензії живої хлорели на ріст і розвиток щеплених саджанців винограду сорту «Каберне Совіньйон»

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**Анотація.** Одним із шляхів вирощування високоякісних щеплених саджанців винограду є використання сучасних, екологічно безпечних біологічно активних препаратів. До таких препаратів сьогодні відносять суспензію живої хлорели. Її застосування у виноградному розсадництві є новим та актуальним технологічним прийомом вирощування садивного матеріалу. Метою роботи було показати вплив суспензії живої хлорели на реалізацію біологічного потенціалу виноградної лози з подальшим одержанням високоякісних щеплених саджанців винограду. У роботі використовували польові (обліки росту та розвитку рослин), лабораторні (визначення кількісних та якісних параметрів рослин) і статистичні (підтвердження достовірності результатів) методи досліджень. Отримані результати показали, що для вимочування підщепних і прищепних компонентів доцільно використовувати штам *Chlorella vulgaris* Beijer., розведення 1:5, вимочування проводити протягом 72 годин – підщепні компоненти, 18 годин – прищепні компоненти; для поливу виноградної шкілки доцільно використовувати штам *Chlorella vulgaris* Beijer + *Ge*, розведення 1:5, його здійснювали одразу після висаджування щеп та тричі протягом вегетації – у червні, липні та серпні. Застосування вказаних штамів суспензії живої хлорели дозволило отримати більший вихід стандартних саджанців зі шкілки, рослини мали добре розвинену кореневу систему та однорічний приріст. Порівняно з контролем, яким слугувала вода, у рослин збільшувалась загальна кількість коренів. Рослини у цих варіантах характеризувалися і посиленим ростом надземної частини. Показники загального та визрілого приросту, які характеризують загальний розвиток щеплених саджанців винограду, знаходилися у межах 687,1-773,2 см$^3$ (об'єм загального приросту), 337,9-386,6 см$^3$ (об'єм визрілого приросту), при 730,1 та 362,6 см$^3$ відповідно у контролі. Обліки виходу стандартних саджанців із шкілки також показали перевагу застосування суспензії живої хлорели (збільшення на 19,0-21,0 %) у порівняні з водою. Наведені технологічні прийоми дозволяють отримувати високоякісну продукцію виноградного розсадництва та можуть бути перспективною альтернативою для більш стійких і екологічно чистих методів ведення сільського господарства

**Ключові слова:** щепи; вегетативні органи; однорічний приріст; коренева система; мікроводорість *Chlorella vulgaris* Beijer.; германій