
Influence of exogenous growth-regulators on physiological and growth processes of dwarf mandarin cv. ‘Miagava-Vase’

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Abstract The influence of biologically active substances of a new generation as growth regulators on the process of ovary fall, improving the quality of fruits and increasing the adaptive potential of tangerine was investigated. The tested citrus plants are dwarf mandarin cv. ‘Miagava-Vase’. The tested plants have grown since in 1986 at Plantation of Subtropical Scientific Centre, Sochi, Russia. The experimental scheme clarified the three options as exogenous growth-regulators: obstaktin (5 ml/l water); nanoelicitor (1 ml/l water) and siliplant (5 ml/l water). The content of chlorophyll and carotenoids are determined; the assessment of the functional state of plants was carried out on the slow induction of chlorophyll fluorescence. It was found that the treatment of plants with growth regulators did not affect the content of green pigments in the leaves. In the processing microbial nanoelicitor and siliplant increased the number of carotenoids, thereby enhancing the defense reactions. Treatment with growth regulators led to an improvement in the functional state of plants, which was expressed in a higher value of the viability index, especially on variants with the introduction of nanoelicitor and siliplant. The treated citrus plants with siliplant resulted to the highest number of carotenoids increased in the leaves and followed by nanoelicitor. They imply as the activation of protective reactions to the plants. The slow chlorophyll fluorescence induction expressed the higher viability index in siliplant than nanoelicitor and obstaktin. The photosynthetic activity index revealed that nanoelicitor and siliplant gave the highest activity and followed by obstaktin. The coefficient of photosynthetic activity expressed that nanoelicitor, siliplant and obstaktin were significantly higher than the control. Nanoelicitor showed significantly highest plant growth and followed by siliplant and obstaktin. Siliplant treatment confirmed significantly highest mass of fruits and followed by nanoelicitor and obstaktin. The fruits diameter was higher in siliplant than obstaktin and nanoelicitor. The treated citrus leaves with nanoelicitor encountered scoparone, a phytoalexin associated with resistance of Citrus to root rot.

Keywords: Chlorophyll, Carotenoids, Exogenous growth-regulators, Fluorescence, Mandarin

Introduction

Modern agriculture is impossible without the use of plant growth

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regulators in practice (Lebedev *et al.*, 2019; Tomar and Singh, 2006; and others). Many domestic and foreign scientists have studied the influence of certain regulators on the vital activity of agricultural plants in particular fruit trees. Significant contributions to study this problem was made by Cacco and Dell'agnola (1984), Shevelukha and Blinovskiy (1990), Rath *et al.* (2006), Gudkovskiy *et al.* (2005), Doroshenko *et al.* (2014), Tacken (2014) and others. Studies have shown that many plant regulators are physiologically active substances, increased in yield, products quality, improving fruit set and reducing the ovary falling, etc. In addition, these substances have an impact on the drought and frost resistance of plants, as well as contribute to the increase of non - specific immunity.

In fruit growing, chemicals are widely used to increase plant productivity. One of the directions is the use of phytohormones, which are physiologically active substances. According to a number of authors (Rakitin, 1963; Agafonov and Faustov, 1972; Stoyanov, 1981; Baskakov and Shapovalov, 1982; Likholat, 1983; Doroshenko *et al.*, 2014; Belous and Abilphasova, 2019), they can be used to increase the formation of a useful ovary. This is especially true in adverse weather conditions during periods of of generative bud differentiation and flowering.

In the conditions of humid subtropics of the Krasnodar region, the influence of biogenic microelements on the adaptability of the dwarf tangerine was also reported by Abilphasova (2006). The tasks facing scientists in these studies include the study of the possibility of regulating the process before harvesting ovary fall and increasing the adaptive potential of the mandarin against the background of the use of new generation biologically active substances.

Recently, a large number of new-generation fertilization have been appeared. The study of their effects on plants is currently interested. In this regard, the study of responsiveness mechanism of tangerine plants to the use of growth regulators is very relevant and is poved theoretical and practical significance. The objectives of reserch finding were proved Obstaktin (phytoregulator for plant growth), Siliplant (silicon-containing universal fertilizer) and microbial Nanoelicitor (natural product regulator for disease plant immunity induction and growth) affecting for the contents of photosynthetic pigments (chlorophyll and carotenoids), slow chlorophyll fluorescence induction and investigation of phytoalexin in citrus

Materials and methods

Tested varirties

Varieties are dwarf tangerine 'Miagava-Vase' (*Citrus reticulata* var. *unshiu* Tan.), the *Poncirus trifoliata* (L.) Raf. are served rootstock. Mandarins are grown in 1986 at plantation Subtropical Scientific Centre (Sochi).

The experimental scheme included three options as exogenous growth-regulators: obstaktin (5 ml/l water); nanoelicitor (5 ml/l water) and siliplant (5 ml/l water). The non-treated control was done by spraying with water.

Obstaktin is phyto regulator for plant growth which related to auxin. Active substance is a potassium salt of 1-naphthylacetic acid. It is used as a plant growth regulator, mainly for spraying the apple trees to prevent premature fruit fall and to delay flowering (when protecting from frost). Obstaktin is developed as a and registered as phyto regulator which produced by Fertico d.o.o., Serbia.

Siliplant is a silicon-containing universal fertilizer. Siliplant effectively replenishes the removal of silicon from the soil, stimulates the development of the root and aboveground parts, relieves various stresses, and activates photosynthesis. The fertilizer is developed, registered and produced by ANO "NEST M" (Russia).

Microbial nanoelicitor is constructed from active metabolites of *Chaetomium cupreum* CC3003 using electron spinning method by Dr. Kasem Soyong, Thailand which applied to increase the disease immunity. (Udompongsuk *et al.*, 2018). Nanoelicitor is registered by BioAgriTech, Vietnam namely "Nano-mocabi".

Investigation of phytoalexin in citrus

Phytoalexin in citrus was determined by thin layer chromatography (TLC). It was investigated only nanoelicitor derived *Chaetomium cupreum* applied at a concentration of 15 ppm. and the non-treated ones served as controls. The citrus leaves were inoculated with *Pythium* spp. (root rot). The leaf samples were taken at 3 days after inoculation. Each fresh leaf sample was weighed to 1.2 g and cleaned in methanol, cut into small pieces, ground, then soaked in 10 mL methanol, put in a water bath at 50 °C for 10 min, and passed through a Whatman, UK No.4 filter paper. Filtrate was evaporated with a vacuum evaporator to yield the crude extract. 3 mL of methanol and 1 mL of ethyl acetate was added. Phytoalexin was detected by TLC using a solvent ratio of toluene:ethyl acetate at 1: 1. Two mL of the TLC solvent was added to the TLC tank. The TLC plate was spotted with a crude extract sample and Scoparone as standard sample for comparison, observed under UV light at 254 nm, soaked in an anisaldehyde solvent, then dried and heated until spots appeared. The R_f value was calculated and compared with the standard one

(Udompongsuk *et al.*, 2018).

Foliar spraying during the growing season was carried out for tree data at ovary phase, dm ovary = 1.5 cm (late May - beginning June); of fruits dm = 3.0 cm (late June) and before 45-50 days at harvest in September. Experience repeatability were 5-fold randomized. Field experiments were performed in triple repetitions which were conducted in accordance by Sedov (1999). In the field experiment, growth activity was determined accounting for ovaries falling provided by selecting model shoots and counting the number of ovaries/fruits; and accounting for the productivity of trees in the garden (kg / tree) by weight methods. Weather conditions of the growing season were analyzed.

Contents of photosynthetic pigments

During the research, a spectrophotometric method was used for determining the content of chlorophyll and carotenoids with the extraction of pigments with 96 % ethanol and using the calculated formulas of Smith and Benitez (Shlyk, 1971). The optical density of extracted pigments was measured using a PE-5400VI spectrophotometer manufactured by EKROSCHEM, Russia, in cuvettes with a layer thickness of 10 mm, wavelength of 665 and 649 nm for chlorophylls a and b, and 440.5 nm for carotenoids.

Parameters of slow chlorophyll fluorescence induction

The functional state of plants was assessed using the parameters of slow induction of chlorophyll fluorescence using a portable chlorophyll fluorometer LPT-3CF/RT-Df (Russia). Before measurement, dark adaptation of objects was performed, for which the leaves were placed in low light conditions (less than 50 Lux) for 15-20 minutes (Budagovskaya *et al.*, 2006).

Laboratory studies were conducted for photosynthetic pigments and chlorophyll fluorescence induction at Physiology and Plant Biochemistry, Federal Research Centre in the Subtropical Scientific Centre, The Russian Academy of Sciences, Sochi, Russia. Phytoalexin production was tested at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Statistical analysis was done. All chemical analyses were performed in triple repetitions. Statistical processing of experimental data were performed using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint Technologies) and MS Excel 2007. Statistical analysis included one-dimensional variance analysis (a method of comparing averages using variance analysis, t-test). The significance of the difference between the mean values at $p < 0.05$ is considered statistically significant. All experiments

were performed in six-fold repetitions. Differences between treatments were evaluated using an unpaired t-test. The results of the study are expressed as an arithmetic mean with a standard deviation.

Results

Contents of photosynthetic pigments

Studies showed that the treatment of plants with growth regulators did not affect the content of green pigments in the leaves (Figure 1). The nanoelicitor resulted the content ranged from 1.67 mg. g⁻¹ in nanoelicitor treatment up to 1.83 mg. g⁻¹ in siliplant treatment.

Result showed that the treated plants with siliplant yielded the highest number of carotenoids increased in the leaves which was 3.11 mg. g⁻¹ and followed by nanoelicitor (2.69 mg. g⁻¹) and 2.57 mg. g⁻¹ in the control). It acts as the activation of protective reactions to the tested plants.

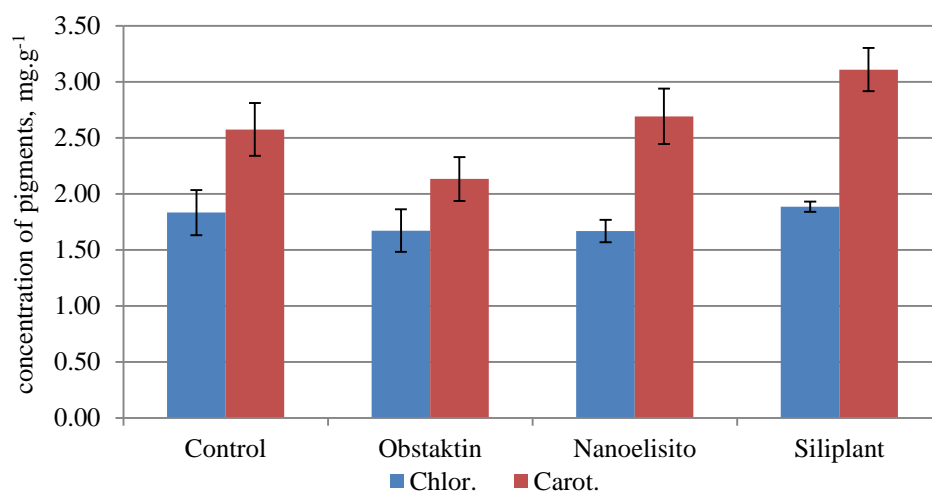


Figure 1. Content of photosynthetic pigments in the leaves of experimental plants during treatment by growth regulators

Parameters of slow chlorophyll fluorescence induction

The parameters of slow chlorophyll fluorescence induction showed that viability index in siliplant was 5.33 Fm/F_T, and followed by nanoelicitor (5.19 Fm/F_T), and Obstaktin (4.61 Fm/F_T) as compared to the control (4.37 Fm/F_T). The index of photosynthetic activity revealed that nanoelicitor and siliplant expressed the highest activity (80 and 79 Kf_T), and followed by

obstaktin (77 Kf_T) and the lowest activity foundv in conteol (Kf_T). Moreover,

The coefficient of photosynthetic activity yielded that nanoelicitor, siliplant and obstaktin were 0.57, 0.56 and 0.54 Kf_n, respectively which significantly differed in higher than the control (0.46 Kf_n) as seen in Table 1.

Table 1. Functional state of the photosynthetic apparatus of plants during treatment by growth regulators

Option	Parameters of slow chlorophyll fluorescence induction					
	Fm/F_T ¹		Kf_T ²		Kf_n ³	
	unit	V, %	unit	V, %	unit	V, %
Control	4.37±0.41	64.40	0.67±0.08	24.80	0.46±0.07	29.60
Obstaktin	4.61±0.41	18.00	0.77±0.03	6.60	0.54±0.02	8.50
Nanoelicitor	5.19±0.58	22.40	0.80±0.02	4.90	0.57±0.02	6.20
Siliplant	5.33±0.95	35.80	0.79±0.04	9.40	0.56±0.03	10.60

¹/Fm/F_T - viability index

²/Kf_T - calculated index of photosynthetic activity

³/Kf_n - coefficient of photosynthetic activity

Table 2. Growth processes and productivity of plants during treatment by growth regulators

Option	Growth, cm	Mass of fruit, g	Diameter of fruit, mm	Crop, pieces/tree
Control	21.39±9.64	56.5±5.7	48.6±3.9	64.0±22.2
Obstaktin	22.55±9.63	61.6±2.1	52.8±1.6	125.0±13.8
Nanoelicitor	31.38±5.32	61.7±1.3	50.6±0.9	Undefined
Siliplant	23.08±9.15	64.7±2.5	55.4±2.9	94.0±16.5

Nanoelicitor showed significantly highest growth of plant (31.38 cm), and followed by siliplant, obstaktin which were 31.38 and 22.55 cm, respectively as compared to the control (21.39 cm). Siliplant treatment showed significantly highest mass of fruits (64.7 g), and followed by nanoelicitor and obstaktin which were 61.7 and 61.6 g., respectively when compared the the control (56.5 g). The highest dimeter of fruits was shown in siliplant treatment (55.4 mm), and followed by obstaktin and nanoelicitor when were 52.8 and 50.6 nn, respectively as compared to the control (48.6 mm) as seen in Table 2.

Investigation of phytoalexin in citrus

Result showed that the treated citrus leaves treated with nanoelicitor

derived from a potent fungus *Chaetomium cupreum* applied at a concentration of 15 ppm found to produce Scoparone, a phytoalexin associated with resistance of Citrus to root rot which the R_f value of 0.6 under UV light at 254 nm (Figure 2).

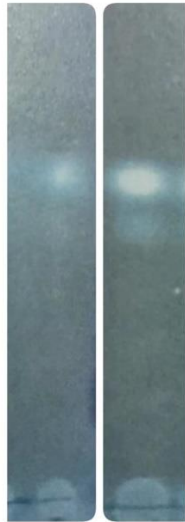


Figure 2. The presence of possible phytoalexins of Scoparone, at an R_f value of 0.6 in the TLC plates when using a combination toluene: ethyl acetate (1: 1.) under UV light at 254 nm.

Discussion

Result showed that the treatment of plants with growth regulators; Obstaktin (phytoregulator for plant growth), Siliplant (silicon-containing universal fertilizer) and microbial nanoelicitor (natural product regulator for plant immunity induction and growth) did not affect the content of green pigments in the leaves. The nanoelicitor resulted the content ranged from 1.67 mg. g⁻¹ and 1.83 mg. g⁻¹ in siliplant treatment. It revealed that the treated plants with siliplant gave the highest number of carotenoids increased in the leaves and followed by nanoelicitor. It acts as the activation of protective reactions to the citrus plants. However, there are reported on other factors involved. The soil and climate conditions of the Krasnodar region are unique, favorably distinguishing this region from other territories of Russia. However, even in the southern territories, there is a systematic negative impact of various climatic stress factors on fruit plants (Doroshenko *et al.*, 2014; Ryndin *et al.*, 2019; Abilphasova and Belous, 2019). For mandarin/tangerine plants, limiting

abiotic stressors are frosts in the absence of snow cover, droughts and elevated air temperatures in the summer. At a temperature of +35 °C, the mandarin reduces photosynthesis; there is an inhibition of vital processes and active ovary fall.

The research finding on slowed the chlorophyll fluorescence induction expressed higher viability index in siliplant than nanoelicitor and Obstatkin as comparison with the non-treated control. Photosynthetic activity index showed that nanoelicitor and siliplant gave significantly highest activity than obstatkin. Photosynthetic activity coefficient showed significantly higher in nanoelicitor, siliplant and obstatkin than the control.

Studies revealed that the treatment of plants with these growth regulators did not affect the content of green pigments in the leaves. It can be explained that the participation of the pigment apparatus in the stability and adaptation of plants is directly related to the protective effect of carotenoids, for example, the suppression of excess energy of triplet states of chlorophyll and singlet oxygen (Rakitin, 1963; Baskakov and Shapovalov, 1982; Khan *et al.*, 2009). Treating plants with nanoelicitor and siliplant increased in the number of carotenoids in the leaves which contributes to the activation of protective reactions.

With this, the activity of photosynthetic processes determined the productivity, resistance to stress factors, adaptability and viability of plants. The constant companion of photosynthesis is chlorophyll fluorescence and its level depends on the intensity of photochemical processes. Weak fluorescence is characteristic of a viable photosystem and strong fluorescence is characteristic of a weakened or inhibited photosystem (Gaevsky and Morgun, 1993; Krause and Weis, 1991; Budagovsky *et al.*, 2002). For higher plants, at a temperature of + 20 - 25 °C, the duration of reaching the stationary level is within the range of 60 - 180 seconds. As the temperature increases, the decline accelerates, and as the temperature decreases, it slows down (Sorokina *et al.*, 1985; Georgieva, 2000; Budagovskaya *et al.*, 2006).

The authors of this article were defined the main parameters of chlorophyll fluorescence. These parameters allow us to assess the functional state of the plants photosynthetic apparatus (FA), since changes in the fluorescence directly or indirectly relate to all stages of the light phase of the photosynthesis process (Doroshenko *et al.*, 2017). It is shown that treatment with growth regulators led to an improvement in the state of FA and, in general, the functional state of the whole plant (Table 1). As it is known, in plants, the efficiency of light utilization during photosynthesis (the so - called coefficient of photosynthetic activity-Kf_n) is 0.6 or higher, and in pathologies of various origins it decreases in proportion to the weakening of photosynthetic function (Norikane and Kurata, 2001; Budagovsky *et al.*, 2002; Lootens *et al.*, 2004).

Treatment with regulators led to a smaller weakening of photosynthetic function, which was expressed in a higher value of the viability index, especially in the variants with the introduction of nanoelicitor and siliplant.

Nanoelicitor showed significantly highest growth of plant and followed by siliplant, obstaktin. Siliplant treatment showed significantly highest mass of fruits and followed by nanoelicitor and obstaktin. The highest diameter of fruits was shown in siliplant treatment and followed by obstaktin and nanoelicitor as results. The calculation of growth parameters and the number of fruits left on the tree during treatment with growth regulators was made. The active growth of shoots on the variant with all growth regulators was determined, for example, during applying nanoelicitor, the growth of shoots was 1.5 % times higher compared to the control. It is increased by 1.5 – 1.9 times in the number of remaining fruits on the tree when treated with growth regulators was shown.

Treatment with regulators led to a smaller weakening of photosynthetic function, which was expressed in a higher value of the viability index, especially in the variants with the introduction of nanoelicitor and siliplant. The calculation of growth parameters and the number of fruits left on the tree during treatment with growth regulators was recorded. The active growth of shoots on the variant with all growth regulators was determined, for example, during applying nanoelicitor, the growth of shoots was 1.5 times higher than the non-treated control.

Result also showed that the treated citrus leaves with nanoelicitor derived from *C. cupreum* at a concentration of 15 ppm expressed Scoparone, a phytoalexin associated with resistance of Citrus to root rot which the RF value of 0.6. With this, Udompongsuk *et al* (2018) found that root rot is one of the most serious disease in Tangerine which caused by *Pythium* spp. and consequently reported that active metabolites of *C. cupreum* actively against *Pythium* sp. A research finding of Afek and Szejnberg (1988) stated that accumulation of Scoparone which is a phytoalexin associated with resistance of Citrus rot of *Phytophthora citrophthora*. Recently, Song *et al.* (2020a,b) reported that the natural products nano-CBH constructed to be nanoparticles derived from a fungus *Chaetomium brasiliense* significantly inhibited *Magnaporthe oryzae* and reduced the rice blast disease, and the rice leaves treated with nano-CBH produced Sakuranertin and Oryzalexin B as phytoalexin in rice. It is noted that the processing plant nanoelicitor and siliplant increased the number of carotenoids, thereby enhancing the defense reactions.

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