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RESEARCH ARTICLE

Effect of 6-Benzylaminopurine on Morphogenesis and Production Process of Sweet Pepper (*Capsicum annuum* L.)

V.G. Kuryata, O.V. Kushnir, O.O. Kravets

Vinnytsia Mykhailo Kotsiubynskyi State Pedagogical University, Ostrozhskogo Str., 32, Vinnytsia, 21000, Ukraine

Author E-mail: kravets07041992@gmail.com, vgk2006@ukr.net, olenakushnir766@gmail.com

Kuryata V.G: https://orcid.org/0000-0002-7801-933X
Kushnir O.V: https://orcid.org/0000-0001-9343-6636
Kravets O.O: https://orcid.org/0000-0003-1977-1617

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We were determined the effect of 6-benzylaminopurine (6-BAP) on morphogenesis, formation of leaf mesostructure and leaf surface area of sweet pepper plant (*Capsicum annuum* L.) and phytocenosis. The application of 0,005% aqueous solution of 6-BAP at the budding phase accelerated the growth and thickening of plants, increased in the total number and weight of leaves, and improved the leaf surface area. At the coenotic level, drug interaction caused changes in leaf surface area of treated planted that increased in an important indicator of the production process — leaf index (LI). The relative proportion of leaf weight among the organs of 6-BAP-treated plant grew compared to control. Drug application led to a thickening and optimization of leaf mesostructure, intesificated a development of assimilative parenchyma due to an increase in the volume and linear dimensions of assimilative cells. The chlorophyll content of experimental trials increased. Consequently, the photosynthetic activity of a unit leaf surface area increased – net photosynthetic productivity (NPF) under 6-benzylaminopurine was higher. These changes created the prerequisites to increase gross photosynthetic crop production and accumulation of assimilates in the leaves. Accumulation of nitrogen, phosphorus and potassium by the vegetative organs of 6-BAP treated plant improved, and supply of the elements to fruits increased. The obtained results indicate significant accumulative capacity of stems and root of sweet pepper plants. A significant part of nitrogen-containing compounds, phosphorus and potassium contained in those organs and gradually decreased due to intensive nitrogen outflow to fruits during the whole vegetation period. The yield of sweet pepper plants increased under 6-BAP application due to the optimization of leaf mesostructural organization, an increase in the leaf surface area and phytocenosis, an improvement in the supply of phosphorus, potassium and nitrogen compounds to the fruits.

Keywords: Sweet pepper; 6-benzylaminopurine; Morphogenesis; Mesostructure; Mineral nutrients; Productivity

Introduction

Knowledge of physiological and biochemical principles of phytohormones action and their synthetic analogues is a significant theoretical and practical importance for understanding the laws of plant growth and development, regulation of the plant organism integrity and the potential capabilities of assimilative apparatus. Application of growth regulators for the redistribution of plastic and mineral substances to the economically valuable plant parts will allow to develop ways to optimize the crops production process (Greene, 2010; Kuryata & Polyvanyi, 2018a), establish the procedure of effective drugs application in order to increase their productivity and reduce pesticide load on the environment (Rademacher, 2016; Vishal et al., 2017; Kuryata et al., 2017).

One of the central issues in modern phytophysiology is a problem of artificial redistribution of assimilates and mineral nutrition elements between plant organs (Kiriziy et al., 2014; Yu et al., 2015; Kuryata et al., 2017). In the growth processes, the meristematic zones are themselves acceptors of assimilates and compete with the reserve organs – storage root, tubers, and seeds (Poprotska & Kuryata, 2017). Excessive development of the leaf surface does not lead to increased crop production, since leaves use significant reserves of assimilates for morphogenesis. Therefore, it is necessary to establishe the physiological and biochemical mechanisms of assimilates and mineral nutrition elements flows regulation between plant organs in order to direct them to yield formation (Rogach et al., 2018; Kuryata & Polyvanyi, 2018a; Khodanitska et al., 2019). The processes of accumulation and redistribution of photoassimilates and mineral nutrition elements between plant organs under the action of growth regulators should be analyzed by the concept of donor-acceptor plant relations ("source-sink" - system). According to this concept, photosynthesis is considered as a donor, and processes of growth, accumulation of reserve substances and an active metabolism zone during autotrophic nutrition are considered as an acceptor (Bonelli et al., 2016; Poprotska et al., 2019), or interaction between stock organs and growth processes of seedling development at the heterotrophic stage (Poprotska and Kuryata, 2017). The donor and acceptor spheres of the plant are connected by the system of direct and feedback (hormonal, trophic) relations, which provides mutual correction of the growth processes and photosynthesis. Application of growth regulators can artificially change the morphogenesis (Macedo et al., 2017; Sugiura et al., 2019), activity of growth (Rogach et al., 2016) and photosynthetic processes (Zhang et al., 2013; Yan et al., 2015), induce flowering (Sousa Lima et al., 2016), regulate plant loading with fruits and seeds (Carvalho et al., 2016; Koutroubas and Damalas, 2016), increase the plants resistance to adverse environmental conditions (Fahad et al., 2016, Zhao et al., 2017; Peng et al., 2014). In essence, the utilization of such drugs makes it possible to artificially simulate a different degree of stress "source-sink" relation system in the plant and find out through which morphological, anatomical and physiological changes occur the flows of assimilates and mineral nutrition elements redistribution between plant organs.

Native hormone stimulators are used in crop production to intensify histo- and morphogenesis, accelerate cell proliferation and differentiation, that increase formation of root branching and caused changes of anatomo-morphological, mesostructural and physiologo-biochemical organization of the leave (Hedden, & Thomas, 2016; Sugiura et al., 2019). Such changes determine the formation of a more powerful assimilative apparatus, which is able to provide active synthesis of plastic compounds that will be directed to generative organs and reserve organs in greater quantities (Rademacher, 2016; Rogach et al., 2018; (Vedenicheva & Kosakivska, 2017). However, the effectiveness of the native hormones action during their exogenous application is usually significantly inferior to the action of synthetic analogues. This is due to the lack of splitting enzymes of synthetic growth regulators in plants, which determines the prolongation of their action.

Application of cytokinins is common for the ontogenesis regulation and enhancement of plant resistance to the action of extreme environmental factors and crop yields (Fahad et al., 2016 Fagherassi et al., 2017). However, it was not carried out the study of cytokinin compounds application on the functioning of donor-acceptor system in connection with optimization of crop production process. In this case, the issue of this study was to establish the formation of donor – acceptor relations in connection with changes in morphogenesis, establish the features of redistribution of assimilates and nutrients stocks synthesized and deposited in the vegetative organs to the fruits formation and growth needs under synthetic cytokinin - 6-benzylaminopurine treatement.

Materials and Methods

The experimental part of work was carried out at the plant physiology and biochemistry laboratory of department of biology, Vinnitsya Mykhailo Kotsiubynskyi State Pedagogical University and a field-based micro-trial setup was established at a specialized agricultural farm " Berzhan P." in Gorbanovka, Vinnitsa region from 2013 to 2015 vegetation periods. It was studied the effect of 6-benzylaminopurine (6-BAP) on the morphogenesis and productivity of sweet pepper Antey. 6-benzylaminopurine (benzyladenine) is a yellowish-white powdery substance with a molecular weight - 225,25 D and a molecular formula - $C_{12}H_{11}N_5$. Melting point is 229,51°C. It decomposes at a boiling point of 245°C. The technical name of compound is 6-BAP. The compound is poorly soluble in water and soluble in organic solvents. 6-BAP is an analogue of natural phytohormones - cytokinins It is used as a growth regulator with a stimulating effect that gets into the plant through root and leaves.

Seeds of pepper were sown for seedlings in greenhouses on February 20, 2013, February 18, 2014 and February 22, 2015. Seedlings were planted on May 22, 2013, May 8, 2014 and May 15, 2015 by a tape method with the planting formula 80+50+50×25. The treatment was applied via foliar spraying OP-2 with aqueous solution of 0,005% 6-benzylaminopurine to complete wetting of leaves at the beginning of budding phase on July 17, 2013, July 10, 2014 and July 9, 2015 respectively. The experiment followed a randomized block design (10 m^2) with five replication.

Morphological measurements (plant height, stem thickness, weight of dry and fresh matter of plants, leaf area) were determined in the middle of each vegetation stage. Mesostructural organization of leaves and anatomical structure of stem were studied at the fruit formation phase on a fixed material. It was used a mixture of equal parts of ethanol, glycerol and water with addition of 1% formalin for its preservation. Measurement of cells sizes, individual tissues and organs was performed by using a microscope "Mikmed-1" and ocular micrometer MOB-1-15x. It was used a partial maceration of leaf tissue Determination of individual cells size was carried out after the maceration of leaf tissues with a 5% solution of acetic acid in 2 mol/l hydrochloric acid. It was analyzed the middle-layer leaves and stems of the shoots on the same stage of plant growth development for anatomical structure. The frequency of microscopic studies is twenty times. The phosphorus content was determined by the formation of phosphorus - molybdenum complex, the potassium content by the flame photometric method and the total nitrogen content by the Kjeldahl method. The chlorophyll content (per leaf fresh matter weight) was determined by spectrophotometric method on the spectrophotometer SF-16 (AOAC, 2010). It was determined the coenotic indicator – leaf index (LI), as the green leaf area per unit ground surface area. Statistical analysis of experimental data was performed by computer program «STATISTICA-6» StatSoft Inc. The reliability of obtained results between control and experiment varient was assessed with the use of Student's t-test.

Results and Discussion

Anatomical and morphological changes of the plant under the growth regulators application can have a significant impact on crop production. This interaction is manifested in changes of the ratio activity of donor and acceptor of the plant (Rogach et al., 2016). An essence of leaf donor function is processes of photosynthesis that depend on the physiological and mesostructural features of leave and on the total leaf surface area at the cenosis level (Kiriziy et al., 2014). Therefore, it is important to analyze the effect of growth regulators on the growth processes, number, weight and area of plant leaves. The results of the study indicate a significant effect of 6-BAP on the rate of growth processes and morphogenesis of sweet pepper plants (Figure 1). The drug treatment led to increase in the height of sweet pepper plants compared to control. Moreover, the application of 6-BAP led to a stem thickening. In particular, the thickness of drug-treated plants was 1,3 ± 0,07 cm compared to 1,1 ± 0,05 cm in control.

It is known that growth regulators affect on the formation of leaf apparatus (Kuryata & Polyvanyi, 2018a), and therefore influenced on the photosynthetic productivity of crops. However, the analysis of literature data indicate that there is no results of the comparative effect of synthetic growth regulators of various chemical nature on the morphological measurements and anatomical structure of sweet pepper leaves. It was found that application of 6-BAP led to increase in the number of leaves, formation of leaf fresh and dry weight matter and total leaf surface area. The total leaf surface area is one of the most important indicators of potential photosynthetic productivity of plants. Thus, the obtained results indicate that 6-BAP interaction stimulates the formation of leaf apparatus of sweet pepper. Leaf index (LI) is an important ceonotic indicator for the total crop yield. It has been established that the changes of leaf area are increased in leaf index under drug application. Consequently, the analysis has shown that leaf index of drug-treated plants was 1,65 m^2/m^2 compared to control – 1,43 m^2/m^2.

It is known that a donor-acceptor system of plants has a significant role in the formation of general productivity and crop yields. One of the perspective areas of production process regulation is the assimilates redistribution in a plant. In particular, it was found that a partial restriction of growth rate of vegetative organs promotes the formation of assimilates excess in plants that contribute to the formation of economically valuable parts of plant - fruits, seeds and storage root (Khodanitska et al., 2019). Plant productivity is determined by the power of donor sphere and the power of acceptor zones. At the fruitification stage, it was analysed the ratio of vegetative organs weights. The results indicate that application of 6-BAP increased the relative proportion of leaf weight compared to control (Figure 2). This indicates an increase in formation of a more powerful donor sphere and better support of assimilates for the processes of growth, development and yield formation.

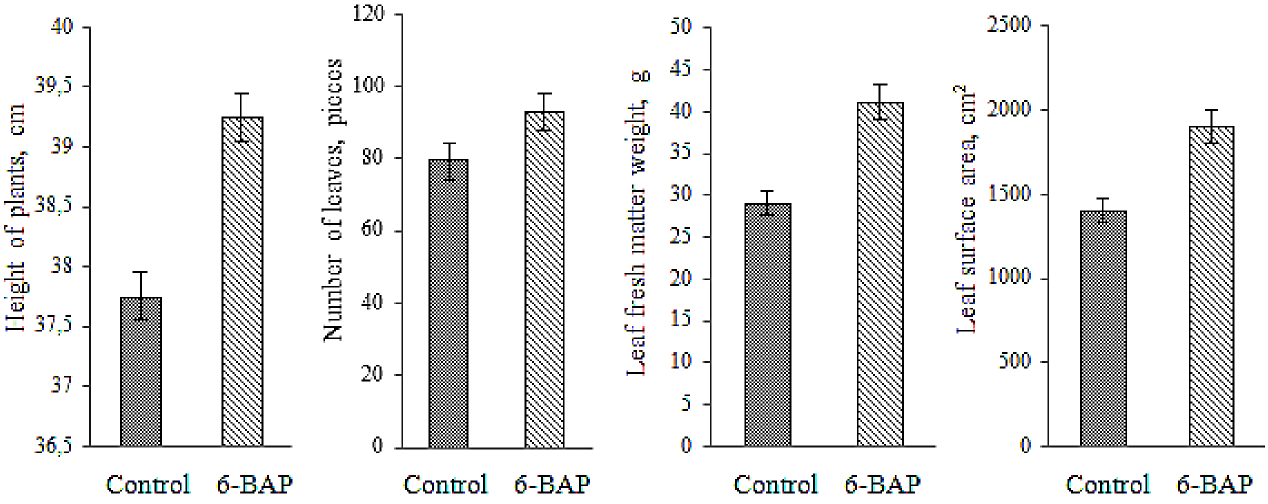

Figure 1. Growth rate and formation of leaf apparatus of sweet pepper sv. Antey under 6-BAP treatment (full ripeness fruitification stage).

Regulation of the plant's donor – acceptor system under synthetic growth regulators makes it possible to artificially redistribute the products of photosynthesis to economically valuable organs and therefore has a significant role in increasing the crop yields.

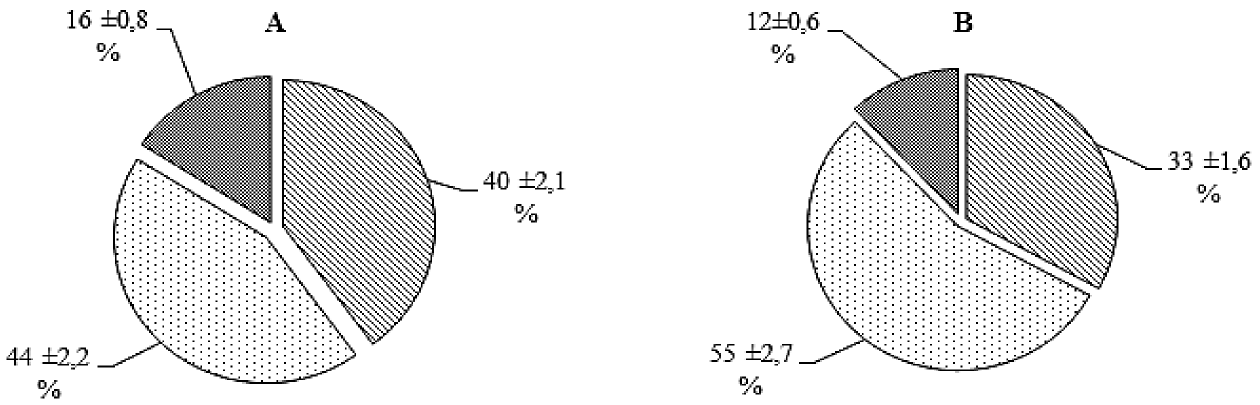

Figure 2. Application of 6-BAP on vegetative organs dry matter weight of sweet pepper at the full ripeness fruitification stage.

- leaf dry matter weight; - stems dry matter weight; - roots dry matter weight
A- control; B- 6-BAP

The evidence from this study suggests that analysis of morphological features of 6-BAP-treated plants may be supplemented with the data of anatomical features of leaf (mesostructures). This indicator clearly shows a synthetic activity of the plant. The results of the study indicate that interaction of drug influenced on the thickness of leaf blade due to the growth of photosynthetic tissue - chlorenchyme and thickness of upper and lower epidermis (Table 1). It was found that the main tissue of leave in photosynthesis is a palisade assimilative parenchyma. The data suggest that 6-BAP application increased the cell volume of palisade assimilative parenchyma.

Leaf area density value (LADV) characterizes the ratio of leaf dry matter weight to unit leaf surface. The importance of this indicator is determined by the concentration of structural elements, which are directly involved in the photosynthetic processes. The obtained results indicate that changes of leaf mesostructural organization led to increase in this indicator. The analysis of the data has shown that application of treptolem (Kuryata & Polyvanyi, 2018a) and various types of retardants (Kuryata & Kravets, 2018; Kuryata & Polyvanyi, 2018b; Rogach et al., 2018) on the agricultural plants has similar interaction on the formation of mesostructure and leaf area density value. Increase of the total chlorophyll content contributed to the intensification of photosynthetic activity in leaves.

Net photosynthetic productivity (NPP) is characterized by photosynthetic productivity of unit leaf surface, which with the increase in total leaf area of an individual plant and phytocenosis under drug action created the prerequisites to enhance a gross photosynthetic crop production and cenosis in general. Consequently, optimization of leaf mesostructure and increase of the pigments content under 6-benzylaminopurine treatment intensificate the photosynthetic activity of a unit of leaf area and phytocenosis that created the prerequisites to enhance the crop yield.

It is known that the total productivity and crop production are determined by the features of nitrogen exchange in plant. It was analyzed the dynamic of nitrogen content in vegetative organs under the action of 6-BAP during vegetation stages. It was established that the application of growth regulator made a influence on the dynamic of total nitrogen in ontogenesis (Figure 3).

Table 1. Influence of 6-BAP on the leaf mesostructural organization, chlorophyll content and net photosynthetic productivity of sweet pepper sv. Antey.

Measurements	**Control**	**6-BAP**
Thickness of leave, μm	263,72 ± 13,18	298,63 ± 14,93
Thickness of upper epidermis, μm	23,32 ± 0,62	*28,71 ± 0,73
Thickness of chlorenchyma, μm	216,48 ± 1,68	*244,85 ± 4,13
Thickness of lower epidermis, μm	23,92 ± 0,49	25,07 ± 0,85
Volume of palisade parenchyma, μm^3	19857,02 ± 896,32	*23058,58 ± 1147,19
Length of spongy cells, μm	33,28 ± 0,95	34,06 ± 1,30
Width of spongy cells, μm	24,95 ± 0,75	26,92 ± 1,04
Leaf area density value, mg/cm^2	7,94 ± 0,39	*9,01 ± 0,45
Total chlorophyll content (a+b), % per leaf fresh matter weight	0,62 ± 0,03	*0,69 ± 0,03
Net photosynthetic productivity, $g/(m^2 \cdot day)$	1,73 ± 0,08	*2,07 ± 0,10

Note. * - Difference is significant at P<0,05.

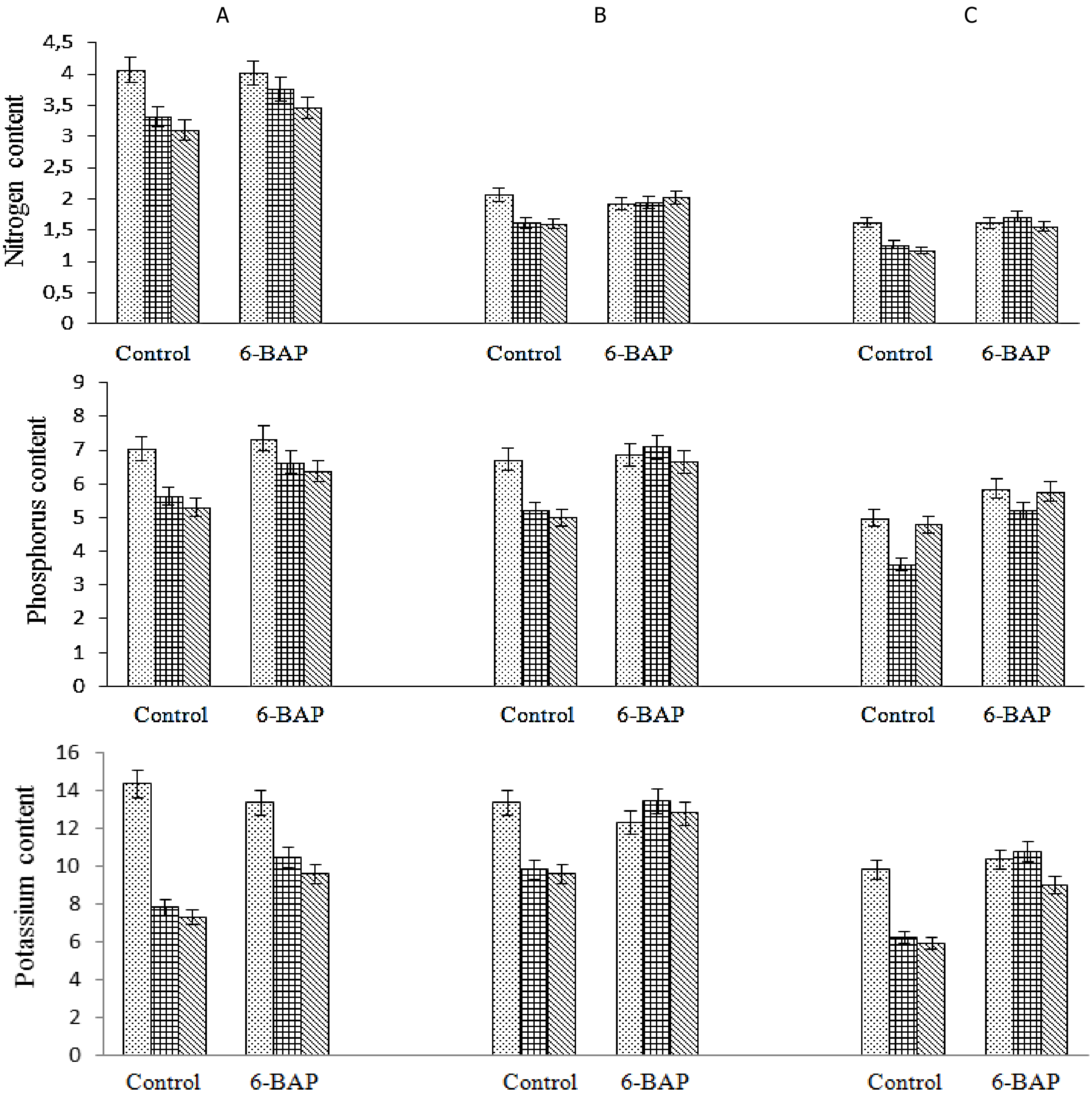

Figure 3. Nitrogen, phosphorus and potassium content (% per dry matter weight) in organs of sweet pepper plants sv. Antey under 6-BAP treatment during vegetation.

A - leaves; B - stems; C – roots.

- fruit formation stage; - fruitification stage; - full ripeness fruitification stage.

In most cases, it was noted a decrease in the nitrogen content in the leaves of sweet pepper plants. The growth rate of leaves reduced in ontogenesis, therefore it is impossible to explain that with biodilution processes. The data suggest that accumulated nitrogen in leaves is used for the processes of fruits growth and formation. The obtained results indicate that the stems and root of sweet pepper plants have a significant accumulative capacity. A significant part of nitrogen-containing compounds contained in those organs and gradually decreased due to intensive nitrogen outflow during the whole vegetation period on the fruits formation. It was established that the 6-benzylaminopurine application contributed to a more intensive nitrogen supply of the vegetative organs of pepper plants. This correlates with the obtained data of protein-synthesizing systems stimulation under cytokinins interaction (Vedenicheva & Kosakivska, 2017).

It is known that phosphorus and potassium play an important role at the period of fruit formation. The analysis of those elements content in the vegetative organs of sweet pepper ontogenesis indicates a significant effect of 6-BAP on their accumulation and redistribution between plant organs. The obtained data indicate that during ontogenesis, it was noted a decrease of the phosphorus content in leaves and stems of the plant from fruit formation to full ripeness fruitification stage. The phosphorus content was higher in the 6-BAP experimental trial. We argue that decrease in the phosphorus content in vegetative organs indicates an intensive reutilization of this element on the needs of fruit formation and growth. It has been established the essential accumulative value of the roots of sweet pepper plants in the process of using phosphorus. It was noted that the content of this element decreased from fruit formation to fruitification stage with a subsequent increase in the phosphorus content at the end of vegetation period. In our opinion, that indicates about the cessation of phosphorus transport from the roots and the gradual accumulation of the element in them. It should be noted that the phosphorus content in all vegetative organs of 6-benzylaminopurine treated pepper plants was higher.

It was found a gradual decrease in the potassium content during ontogenesis in the leaves of control and experimental trials of sweet pepper plants from fruit formation to full ripeness fruitification stage. Obviously, this is due to the reutilization of element primarily from plant leaves to the needs of fruit formation and growth. It was not observed such decrease in potassium content in the roots and stems of plants during ontogenesis due to outflow of the element to the fruit during the ripening stage and full ripeness fruitification stage. In contrast, the element content in those phases increased. It can be concluded about earlier cessation of potassium transport from these organs to the fruits. In most cases, it was noted a significant potassium accumulation capacity of stems and roots of 6-BAP -treated sweet pepper plants during the vegetation period.

Application of 6-BAP increased the yield of sweet pepper plants due to the optimization of leaf mesostructural organization, an increase in the leaf surface area and phytocenosis, an improvement in the supply of phosphorus, potassium and nitrogen compounds to the fruits (Figure 4).

Thus, the application of growth stimulator 6-BAP is an effective methods for product process optimization of sweet pepper plants.

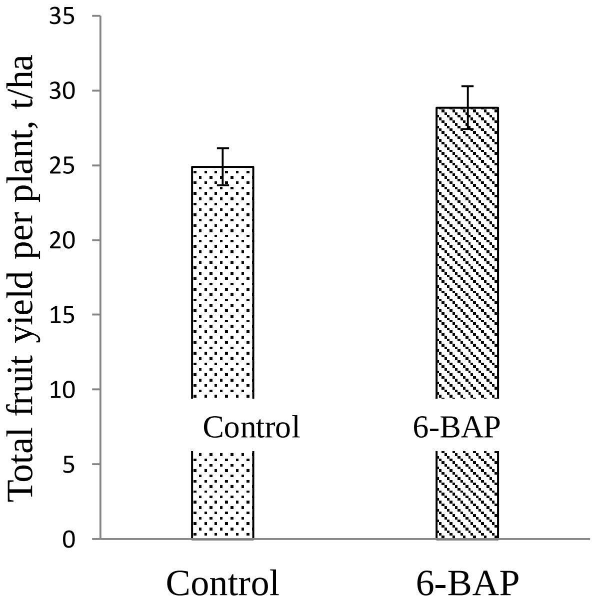

Figure 4. Effect of 6-BAP on yield of sweet pepper.

Conclusion

The application of 0,005% 6-benzylaminopurine on sweet pepper plants by spraying method during the budding phase led to the optimization of leaf mesostructure, better development of assimilative parenchyma and increase in the chlorophyll content. As a result obtained effect improved the photosynthetic activity per unit leaf area. Leaf surface area of the plant and an important coenotic indicator leaf index (LI) are increased under the drug treatment that created the prerequisites to increase gross photosynthetic crop production and accumulation of assimilates in the leaves. The accumulation of nitrogen, phosphorus and potassium content in the vegetative organs of the plant improved and their assimilation in the fruits augmented under the influence of 6-BAP. Consequently, such anatomical and morphological and physiological-biochemical changes increased crop yields.

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