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THE EFFECT OF CHITOSAN ON ROOTING OF GRAPEVINE CUTTINGS AND ON SUBSEQUENT PLANT GROWTH UNDER DROUGHT AND TEMPERATURE STRESS

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A B S T R A C T

The effects of the treatment of grapevine cuttings with chitosan (Biochikol 020 PC) on their rooting, subsequent plant development and reaction to drought and temperature stress were investigated. 7-cm long cuttings containing a single bud were dipped in water or in Biochikol 020 PC solution at concentrations of 0.5, 1 and 2% for 24 hours at 25°C. Cutting dipped in water were served as a control. After treatment, the cuttings were rooted during 28 days at 25-28°C in the medium consisted of mixture of peat and sand (1:1, v/v). When the 1st, 3rd or 6th primary root appeared the rooted cuttings were grown in optimal watering at 20°C and 32% substrate volumetric water content (VWC), which was considered optimal, under water stress (20°C, 12, 15 and 18% VWC) or temperature stress (10 and 30°C, 32% VWC).

Biochikol 020 PC improved rooting of the cuttings, increased the number of new canes formed and their length, as well as the number of internodes and chlorophyll content in the leaves. The effectiveness of the compound depended on its concentration and a stage of plant development at a time when they were exposed to the drought or temperature stresses. Biochikol 020 PC was the most effective in alleviating adverse effects of drought stress when the rooted grapevine cuttings possessed six primary roots. The treatment remarkably stimulated the development of the root system, especially when the growing media moisture content was 18% (at 20°C) and when the compound was applied at concentration of 1 and 2%. Biochikol 020 PC applied at 1% also stimulated greatly the growth of the cuttings by increasing the number of internodes by 33%, the number of new canes by 29% and the length of canes by 71%, in comparison with the control.

Positive effect of Biochikol 020 PC application in alleviating negative impact of temperature stress also revealed when plants were exposed to 10°C or 30°C and when the biostimulator was used at 0.5 and 1% concentration.

Biochikol 020 PC increased also chlorophyll content in the leaves developed on plants kept in optimal (20°C, 32% VWC) or drought stress conditions (20°C, 12% VWC), when the biostimulator was applied at 0.5 and 1%, respectively.

Key words: *Vitis vinifera* L., biostimulator, chitosan, water deficit, temperature stress, chlorophyll

INTRODUCTION

One of the major problems in agriculture is abiotic stress which prevents plants from realizing their full genetic potential and limits food production. In grapevine (*Vitis vinifera* L.) drought and temperature stress belong to the main constraint which decrease quality of its products e.g. grape juice, jam, jelly, wine, raisins or vinegar (Flexas et al., 1999). The stress is the most dangerous at the early stages plant development field because young plantlets are very sensitive to unfavourable weather conditions.

Application of biostimulators is one of the approaches to decrease the negative effect of abiotic stress and increase yield and quality of many crops. Biochikol 020 PC (produced by GUMITEX, Sp. z o.o., Poland) became popular as it has antiviral, antibacterial and antifungal properties (Orlikowski and Skrzypczak, 2003). It contains 2% of chitosan (poly-D-glucosamine) which is one of the most common polymers found in nature (Wojdyła, 2001). Chitosan can be obtained by partial deacetylation of chitin (poly N-acetyl-D-glucosamine) from crustacean shells (Vasconsuelo et al., 2004). Naturally chitosan is formed by the action of chitin deacetylases, enzymes that have been involved either in the formation of the cell wall or in the deacetylation of chitin oligosaccharides following the action of endochitinases on cell walls during autolysis. Chitosan is structurally related to cellulose, which consists of long chain of glucose molecules linked to each other. In chitosan, the building block of the chain is a slightly modified form of glucose (Wojdyła, 2001). Chitosan is present in the shells of crustaceans – crabs, shrimps and krill; in insects and in certain other organisms including many fungi, algae, and yeast (Wojdyła, 2001). Feng et al. (2007) indicated that water-soluble chitosan is a natural antioxidant and that its antioxidant activity depends on its molecular weight. Contrary to the typical fungicidal preparations, Biochokol 020 PC applied to the soil or the leaves stimulates the resistance mechanism of plants, besides having a direct effect on pathogenic organisms. Chitosan contained in Biochikol 020 PC, as an elicitor of resistance, accelerates the activity of genes through the contact with a plant and in effect induces biosynthesis of biochemical compounds that have fungistatic or fungicidal

effect, which is termed as plants' "immunization" (Patkowska et al., 2006). Chitosan can also induce a multitude of biological processes in plant tissues, including the stimulation of chitinases, accumulation of phytoalexins, synthesis of proteinase inhibitors, and increasing lignification (Wojdyła, 2001) The literature provides mainly infor-mation about the effectiveness of Biochikol 020 PC in the protection of cereals, vegetables or ornamental plants, both in *in vitro* and *in vivo* conditions, against virus, bacterial or fungal diseases. Little is known on the effect of Biochikol 020 PC on vegetative growth of plants, especially in the production of grapevine cuttings.

The objective of the investigation reported here was to determine the effect of Biochikol 020 PC on rooting of grapevine cuttings and on the subsequent growth and development of grapevine plants in optimal and stress conditions.

MATERIAL AND METHODS

The research were performed on grapevine 'Chrupka Zlota', which is one of the most popular cultivars grown in Poland due to its tolerance to low temperature (down to -20°C). In February, grape canes were taken from vineyard. Then, the cane cuttings 7 cm long and containing one bud at the upper part were prepared. They were completely dipped for 24 hours at 25°C in water solution of Biochikol 020 PC (containing 2% of chitosan, produced by GUMITEX, Sp. z o.o., Poland), at concentrations of 0.5, 1 and 2%. Control plants were dipped in water. Afterwards, all the cuttings were rooted in the medium consisted of peat and sand mixed in a proportion 1:1 (v/v) at 25-28°C for 28 days. The rooted plants were transferred to a growth chamber at 20°C and 8/16 dark/light cycle (SON-T AGRO 400 W, 100 $\mu molm^{-2}s^{-1}$). The substrate volumetric water content (VWC) was 32%. These conditions (20°C, 32% VWC) were experimentally chosen as optimal for plant growth.

When 1st, 3rd or 6th primary root developed some of the cuttings were transferred for 156 days to drought stress conditions (VWC, 12, 15 or 18%, temp. 20°C) or temperature stress (10 or 30°C, VWC 32%)

Substrate volumetric water content was measured twice a day by the Moisture Meter (Delta-T Devices Ltd), equipped with WET sensor, and was regulated by adding the appropriate amount of water whenever necessary.

Data on the number of internodes on a plant and the number and length of canes, the visual estimation of the root system in a six-point scale as well as chlorophyll content in leaves were collected after 75 days from the beginning of rooting.

Six-point scale for estimating the root system development was based on the following criteria:

1. less than four primary roots developed,
2. four primary roots developed,
3. five primary roots developed,
4. six primary roots developed,
5. seven primary roots developed,
6. more than seven primary roots developed.

Chlorophyll content was measured spectrophotometrically according to the method described by Bruinsma (1969) in the leaves from plants kept in optimal (20°C, 32% VWC) or drought stress condition (20°C, 12% VWC). The data were expressed as µg of chlorophyll per 100 mg of leaf fresh weight.

The experiment was done in three repetitions, each including ten cuttings. The least significance differences (LSD) were calculated at $p = 0.01$ and 0.05. Standard errors of means from 40 repetitions are presented on the figures as error bars.

RESULTS AND DISCUSSION

The presented results showed that rooted grapevine cuttings exposed to drought or low temperature stress were characterized by poorly developed root system, lesser number of canes and internodes as well as lower chlorophyll content in leaves in comparison with control plants kept at 20°C (Fig. 1, 2 and 3). The extent of reaction depended on the thermal stress conditions, the moisture content of the growing media and stage of rooting during which plants were subjected to the stress. The earlier the cuttings were subjected to these conditions the less the root system and the canes were developed.

Low temperature (10°C) was the most unfavourable among all examined stress conditions. The growth of the rooted control cuttings (soaked in water), with one or three primary roots developed, subjected to this temperature was almost completely inhibited (Fig. 1 and 2). It was especially visible in the case of root development, the number of internodes and the length of canes. This is in agreement with the results obtained by Hendrickson et al. (2004), who reported 34-63% reduction in growth rates due to temperature decreased by 2°C. Chilling temperatures, similarly to drought stress, apart from limitation of growth (Buttrose, 1969) decrease also leaf water potential (Báló et al., 1991). Low soil water temperatures decreases root function and water transport because hydraulic resistance, stomatal conductance and leaf transpiration are decreased (Flexas et al., 1999). Reduced plant growth due to chilling temperature is probably indirectly caused by photosynthesis inhibition, which is more pronounced in the light condition because it may impair activation of the carbon reduction cycle and lead to photoinhibition. However, long-term exposure to combined high and low temperature is needed to photoinhibit grapevines (Chaumont et al., 1997). Less than 6 h exposure does not affect photochemical yields. In our experiments, grapevine plants were stored in these conditions for 4 months. Therefore, photoinhibition phenomenon may have occurred.

On the contrary to the effect of 10°C, the grapevine plants exposed to 30°C were better developed in comparison to the control. The earlier the plants were transferred to these conditions the better the root system was developed and the more accelerated was the growth of plants (Fig. 1 and 2).

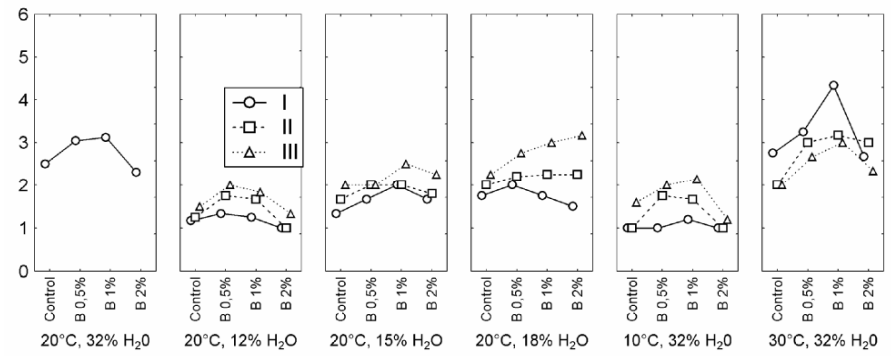

Figure 1. The visual estimation of the grapevines root system development in six-point scale after 75 days from appearance of the first root. The cuttings were soaked at 25°C for 24 h in Biochikol 020 PC (B) at concentrations of 0 (control); 0.5; 1 and 2% and then transferred from optimal condition (20°C; 32% VWC in the media) to the drought (20°C; 12, 15, 18% VWC) or temperature stress (10, 30°C; 32% VWC) when first (-O-), second (-□-) or sixth (-Δ-) root appeared

Drought stress at 20°C also remarkably slowed down the development of roots, growth of plants as well as chlorophyll contents in leaves (Fig. 1, 2 and 3). The lower was water content of growing media and the earlier were the cuttings subjected to these conditions the less developed was the root system and the slower plant growth. The most adverse effect of drought conditions was observed when rooted cuttings with one developed primary roots were subjected to the lowest water content in the growing media (12%). After 75 days of such treatment development of the root system was inhibited by 52% as compared with the control (non stressed plants). Plants obtained from stressed cuttings were also characterized by poorer growth. It was expressed as reduced number of internodes and canes and reduced length of canes by 73%, 33% and 90%, respectively, in comparison with the control (Fig. 2). Drought stress also remarkably reduced chlorophyll content in the leaves (Fig. 3).

It is commonly known that drought stress affects virtually every aspect of plant physiology and metabolism. Numerous changes that occur under this stress have been well documented. The effect of drought stress, similarly like chilling stress, is usually perceived as a decrease in photosynthesis and growth, and it is associated with alterations in C and N metabolism. Drought-related physiological changes, such as decrease in leaf water content and stomatal closure, result in limited CO_2 availability and the channelling of the reducing equivalents to the production of active oxygen species rather than to CO_2 fixation (Osmond, 1981). The oxidative damage of important molecules is a result of the imbalance

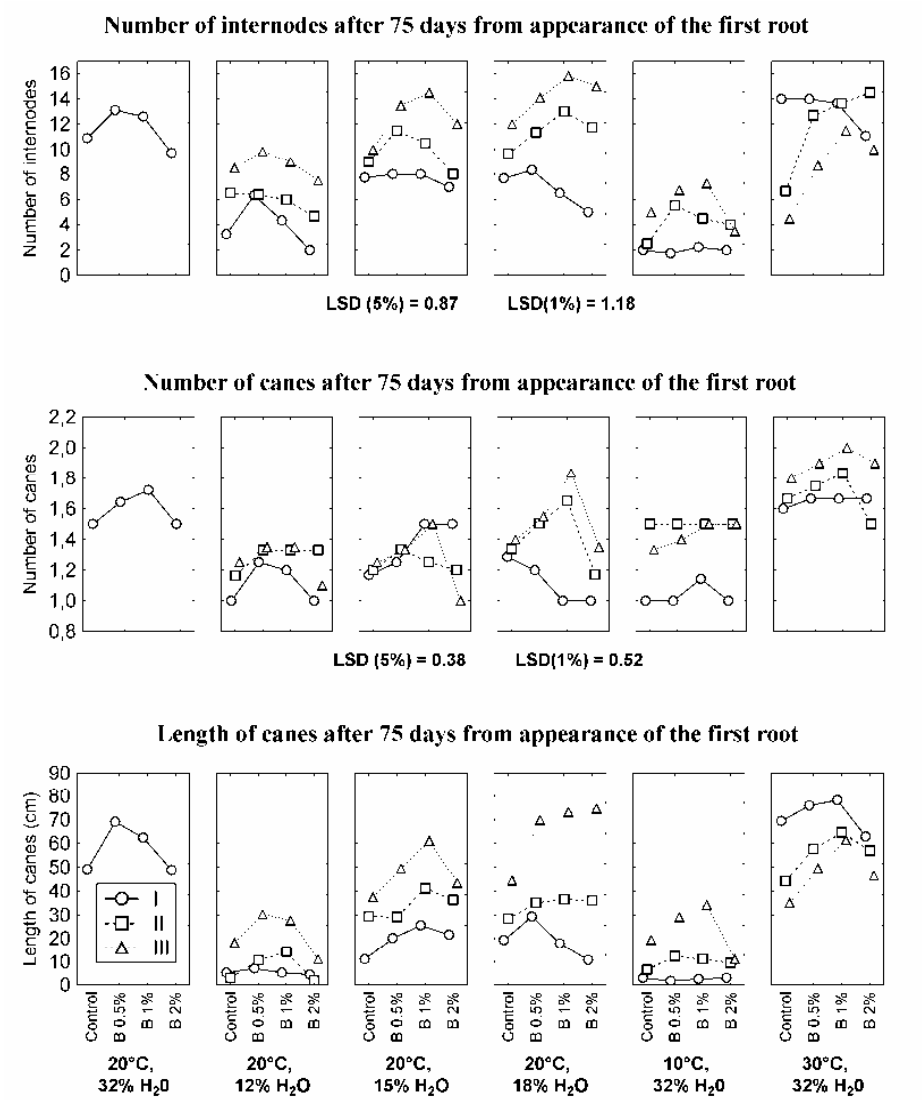

Figure 2. The number of internodes, canes and the length of canes after 75 days from appearance of the first root. The cuttings were soaked at 25°C for 24 h in Biochikol 020 PC (B) and then transferred from optimal condition (20°C; 32% VWC in the media) to the drought (20°C; 12, 15, 18% VWC) or temperature stress (10, 30°C; 32% VWC) when first (-O-), second (-□-) or sixth (-Δ-) root appeared

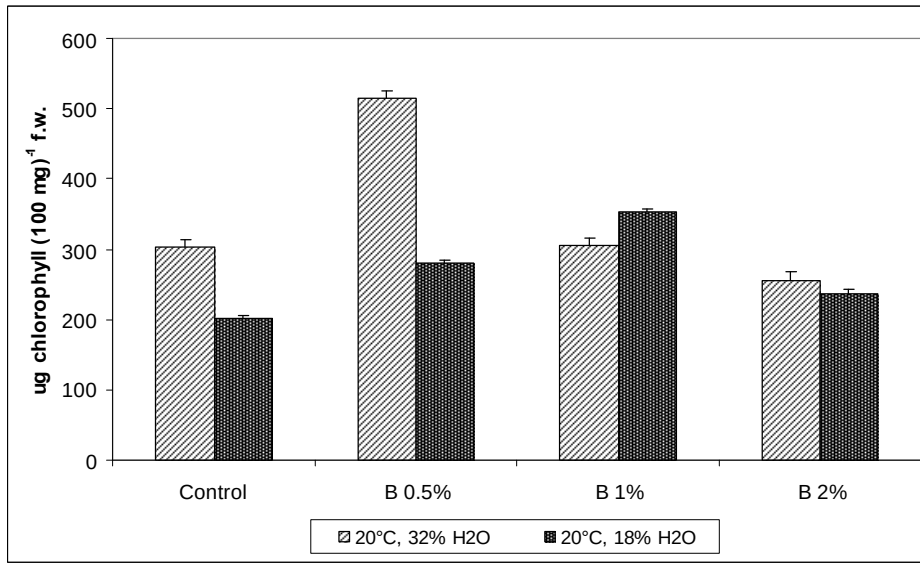

Figure 3. Chlorophyll content in the leaves of grapevine cuttings after 75 days from appearance of the first root. The cuttings were soaked at 25°C for 24 h in Biochikol 020 PC (B) and then kept under optimal (20°C; 32% VWC in the media) or drought stress conditions (20°C; 18% H_2O VWC) when sixth root appeared. Data are the mean ± standard error of forty replicates

between production of reactive oxygen species (ROS) from reduced O_2, i.e. superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) and the antioxidant defences (Iturbe-Ormaetxe et al., 1998).

The results obtained indicate that Biochikol 020 PC applied to the cutting prior to rooting, during their subsequent growth in optimal conditions (20°C, 32% VWC) or under drought stress (20°C, 12, 15 and 18% VWC) positively affected the length and number of canes, number of internodes, root establishment and the chlorophyll content in leaves (Fig. 1, 2 and 3). The effectiveness of its application depended on concentration of the compound, substrate water content, temperature, stage of rooting and plant development during exposure to drought stress. The research carried out by Borkowski et al. (1999 and 2006) indicated that chitosan, applied as Biochikol 020 PC, increased fruit yield of tomato and cabbage.

With regard to drought stress, the most profitable effect of Biochikol 020 PC application was observed when grapevine cuttings with six primary roots developed were transferred to these conditions. After that treatment the compound stimulated to the largest extent the development of the root system. It was especially visible when the substrate water content was 18% and temperature 20°C and when the compound was applied at concentration of 1 and 2% (Fig. 1). This

is in agreement with the results of Żółtańska (2006), who indicated that this biostimulation stimulates the increase of root mass of wheat at soil water potential of 20 kPa. Biochikol 020 PC applied at 1% also greatly stimulated other growth parameters of the cuttings. Its application increased the number of internodes by 33%, the number of canes by 29% and the length of canes by 71% in comparison with the control (Fig. 2). This treatment was especially beneficial because it entirely eliminated adverse effect of drought stress by increasing the examined plant growth parameters to the level obtained in nonstressed, control grapevine cuttings. These results suggest also that due to Biochikol 020 PC application the plants conferred a tolerance to drought stress conditions. However, under the most severe drought stress (20°C, 12% VWC in the growing media, i.e. moisture content under which plants untreated with Biochikol 020 PC die) the improvement of grapevine rooting and plant growth were not as significant as under moderate drought stress conditions (20°C, 15 and 18% H_2O). Hwang et al. (1998) suggested that chitosan can decrease negative effects of drought stress by its specific properties similar to waxes. These properties prevent water loss from fruits and improve their storability.

Biochikol 020 PC significantly increased chlorophyll content in the leaves developed on plants kept in optimal (20°C, 32% VWC.) or drought stress conditions (20°C, 12% VWC) (Fig. 3). The highest increase of chlorophyll content in leaves was observed after Biochikol 020 PC application at concentration of 0.5% for plants grown in optimal condition and 1%, for cuttings kept in drought stress condition (Fig. 3). The results of Barka et al. (2004) obtained from experiments carried out on grapevines showed enhanced both O_2 production and CO_2 fixation. Their findings also indicated a beneficial effect of chitogel (a formulated chitosan solution) on plantlet photosynthesis.

Similar effect of Biochikol 020 PC application on grapevine growth was observed when drought stress was initiated earlier i.e. when cutting with three primary roots developed were exposed to drought stress (20°C, 15 and 12% VWC). After such treatment the cuttings had 30% more internodes and 14% more canes in comparison with untreated plants (Fig. 2). Application of Biochikol 020 PC in these conditions also positively affected the development of the root system (Fig. 1).

The beneficial effect of this compound was also recorded when cuttings were exposed to drought stress very early, i.e. when one primary roots appeared and when the moisture content in the growing media was 15%.

Positive effect of Biochikol 020 PC application was revealed when the plants were exposed to 30°C and when the biostimulator was used at 0.5 and 1% (Fig. 1 and 2). Under these conditions the compound stimulated development of the root system, increased the number of canes and their length as well as the

number of internodes. When plants were subjected to this temperature earlier, i.e. when one root appeared, Biochikol 020 PC stimulated the development of root system only but did not increase the number of internodes and canes.

Application of Biochikol 020 PC was also advantageous when the grapevine plants were exposed to 10°C (Fig. 1 and 2). Generally, the most beneficial effect was observed when Biochikol 020 PC was applied at concentration of 0.5-1% and when plants with three or six primary roots were transferred to 10°C. Under such conditions Biochikol 020 PC improved the development of the root system, increased the number of internodes and length of canes.

Beneficial effects of Biochikol 020 PC application on grapevine grown under optimal or drought and temperature stresses showed that this biostimulator can be widely used in order to improve cuttings rooting and their further growth.

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REFERENCES

Báló B., Lajkó F., Garab G. 1991. Effects of chilling on photosynthesis of grapevines. PHOTOSYNTHETICA. 25: 227-230.

Barka E. A., Eullaffroy P, Clément C., Vernet G. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. PLANT CELL REP. 22: 608-614.

Borkowski J., Felczyńska A., Stępowski J., Niekraszewicz A. 2006. Effect of different compounds Biochikol 020 PC, calcium nitrate, tytanit and pomonit on the healthiness and the yield of Chinese cabbage. POLISH CHITIN SOCIETY, MONOGRAPH XI: 201-207.

Borkowski J., Kowalczyk W. 1999. Influence of tytanit and chitosan sprays and other treatments on the tomato plant growth and the development of powdery mildew (*Oidium lycopersicum*). BULL. POLISH ACAD. SCI. BIOLOG. SCI. 47(2-4): 129-132.

Bruinsma J. 1969. The quantitative analysis of chlorophylls a and b in plant extracts. PHYTOCHEM. PHYTOBIOL. 2: 241-249.

Buttrose M.S. 1969. Vegetative growth of grapevine varieties under controlled temperature and light intensity. VITIS 8: 280-285.

Chaumont M., Osório M.L., Chaves M.M., Vanacker H., Morot-Gaudry J.F., Foyer C. 1997. The absence of photoinhibition during the mid-morning depression of photosynthesis in *Vitis vinifera* grown in semi-arid and temperature climates. J. PLANT PHYSIOL. 150: 743-751.

Feng T., Du Y., Li J., Wei Y., Yao P. 2007. Antioxidant activity of half N-acetylated water-soluble chitosan *in vitro*. EUR. FOOD RES. TECHNOL. 225: 133-138.

Flexas J., Badger M., Chow W.S., Medrano H., Osmond B. 1999. Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low temperature and/or water stress. PLANT PHYSIOL. 121: 675-684.

Hendrickson L., Ball1 M.C., Wood J.T., Chow W.S., Furbank R.T. 2004. Low temperature effects on photosynthesis and growth of grapevine. PLANT CELL ENVIRON. 27: 795-805.

Hwang Y.S., Kim Y.A., Lee J.C. 1998. Effect of postharvest application of chitosan and wax and ethylene scrubbing on the quality changes in stored 'Tsugaru' apples. J. KOREAN SOCIETY HORT. SCI. 39 (5): 579-582.

Iturbe-Ormaetxe I., Escudero P.R., Arrese-Igor C., Becana M. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. PLANT PHYSIOL. 116: 173-181.

Orlikowski L.B., Skrzypczak Cz. 2003. Biocides in the control of soil-borne and leaf pathogens. HORTIC. VEGET. GROW. 22(3): 426-433.

Osmond C.B. 1981. Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. BIOCHIM. BIOPHYS. ACTA 639: 77-98.

Patkowska E., Pięta D., Pastucha A. 2006. The effect of Biochikol 020 PC on microorganism communities in the rhisosphere of *Fabaceae* plants. POLISH CHITIN SOCIETY, MONOGRAPH XI: pp. 171-178.

Vasconsuelo A., Giulietti A.M., Boland R. 2004. Signal transduction events mediating chitosan stimulation of anthraquinone synthesis in *Rubia tinctorum*. PLANT SCI. 166: 405-413.

Wojdyła A.T. 2001. Chitosan in the control of rose diseases – 6 year-trials. BULL. POLISH ACADEMY SCI. BIOLOG. SCI. 49(3): 233-252.

Żółtańska E. 2006. The effect of soil moisture and temperature on efficacy of seed dressing preparations Biochikol 020 PC and Baytan universal 19,5 WS in control of *rhizoctonia* fungi on wheat. J. PLANT PROTEC. RES. 46. 3: 261-274.

WPŁYW PREPARATU BIOCHIKOL 020 PC NA UKORZENIANIE I WZROST ROŚLIN WINOROŚLI W WARUNKACH SUSZY I STRESU TERMICZNEGO

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S T R E S Z C Z E N I E

Celem badań było określenie wpływu preparatu Biochikol 020 PC (chitozan) na ukorzenianie sadzonek winorośli, ich rozwój oraz zawartość chlorofilu w liściach roślin uprawianych w warunkach optymalnych oraz stres suszy i temperatury. Sadzonki o długości 7 cm moczono w roztworze preparatu Biochikol 020 PC w stężeniach 0; 0,5; 1,0 i 2,0% w 25°C przez 24 godziny, a następnie ukorzeniano

w wilgotnym uniwersalnym substracie (torf:piasek – 1:1) przez 28 dni w 25°C -28°C. Ukorzeniane sadzonki przetrzymywano w warunkach optymalnych (20°C, 32% H_2O) oraz poddano działaniu stresu suszy (20°C, 12, 15 i 18% H_2O w podłożu) lub temperatury (10 °C i 30°C, 32% H_2O w podłożu).

Zarówno stres suszy, jak i temperatury (10°C) negatywnie wpłynęły na formowanie się korzeni oraz rozwój liści oraz na zawartość chlorofilu w liściach. Spośród badanych warunków stresowych wpływ 10°C był najbardziej niekorzystny.

Uzyskane wyniki wykazały, że 24-godzinne moczenie sadzonek w preparacie Biochikol 020 PC w okresie poprzedzającym ukorzenianie korzystnie wpływało na formowanie się korzeni, liczbę wytworzonych międzywęźli, pędów oraz ich długość oraz zawartość chlorofilu w liściach. Skala oddziaływań i łagodzenia niekorzystnego wpływu stresu zależała od stężenia preparatu, zawartości wody w podłożu, temperatury oraz stadium ukorzeniania sadzonek, podczas którego były one poddawane działaniu stresu. Najkorzystniejszy wpływ preparatu w łagodzeniu skutków suszy zaobserwowano, gdy rośliny przeniesiono do tych warunków w chwili uformowania się sześciu korzeni podstawowych. Preparat stymulował wówczas w największym stopniu rozwój systemu korzeniowego. Szczególnie było to widoczne, gdy wilgotność podłoża utrzymywano na poziomie 18% i gdy preparat zastosowano w stężeniu 1 lub 2%. Biochikol 020 PC zastosowany w stężeniu 1% stymulował również rozwój pędów, zwiększając liczbę międzywęźli o 33%, liczbę pędów o 29% oraz ich długość o 71% w porównaniu z kontrolą.

Pozytywny wpływ preparatu stwierdzono również w przypadku ukorzeniania sadzonek oraz wzrostu roślin w 10°C i 30°C oraz gdy jego stężenie wynosiło 0,5-1%. W tych warunkach preparat stymulował rozwój systemu korzeniowego, zwiększał liczbę międzywęźli i pędów oraz ich długość. Korzystny wpływ preparatu na rozwój systemu korzeniowego i wzrost roślin był również wyraźny, gdy siewki przetrzymywano w 30°C od chwili pojawienia się pierwszego korzenia podstawowego.

Biochikol 020 PC zwiększył również zawartość chlorofilu w liściach u roślin przetrzymywanych w warunkach optymalnych (20°C, 32% H_20) i stresu suszy (20°C, 12% H_2O), gdy był zastosowany odpowiednio w stężeniu 0,5 i 1,0%.

Słowa kluczowe: *Vitis vinifera* L., biostymulator, chitozan, stress suszy, stress temperatury, chlorofil