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CHANGES IN THE LIPID PEROXIDATION INTESITY IN AUXIN TREATED CHERRY ROOTSTOCKS SOFTWOOD CUTTINGS*

ĐORĐE MALENČIĆ, DEJAN PRVULOVIĆ, MILAN POPOVIĆ, VLADISLAV OGNJANOV, MIRJANA LJUBOJEVIĆ, GORAN BARAĆ, BOŠKO BORKOVIĆ, SLAVKO KEVREŠAN, BILJANA KIPROVSKI[1]

SUMMARY: In order to investigate the effect of phytohormones exogenous auxins on the level of the mechanical injury induced oxidative stress, intensity of lipid peroxidation (LP) was measured in rootstocks of cherry softwood cuttings. Basal parts of the cuttings and leaves of five rootstocks (Mahaleb 1 and 2, Gisela 5, European ground cherry (EGC) and "Oblačinska" sour cherry) were sampled 0, 2, 4 and 6 days after cutting. Cuttings were treated with 0.5% solutions of three auxins: α-naphthylacetic acid (NAA), indolebutyric acid (IBA) and combination of these two (INCIT K). Results obtained for the LP intensity varied depending on the rootstock, plant organ and auxin applied. The best LP-lowering effect of auxins occurred in all genotypes when treated with NAA. In the leaves, the LP peaked on the 2nd day while the best LP-lowering effect was recorded in European ground cherry rootstocks on the 4th day, independently of auxin applied. The highest MDA production in leaves was recorded in Mahaleb 2 and European ground cherry rootstocks treated with IBA (60-90% higher then control) two days after the cuttings were made. It has been established that in the most of the rootstocks examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant protection system, as well.

Key words: *sour cherry cuttings, oxidative stress, auxins, lipid peroxidation.*

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[1]PhD Đorđe Malenčić, Full Prof., PhD Dejan Prvulović, Assistant Prof., PhD Milan Popović, Full Prof., PhD Vladislav Ognjanov, Full Prof., PhD Mirjana Ljubojević, research assistant, MSc Goran Barać, research assistant, MSc Boško Borković, PhD student, PhD Slavko Kevrešan, Full Prof., MSc Biljana Kiprovski, research assistant; Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.

Corresponding author: Đorđe Malenčić, phone: +381214853401, e-mail: malencic@polj.uns.ac.rs

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INTRODUCTION

Abiotic stress factors, such as UV light and other forms of radiation, photooxidation, air pollution, drought, herbicides, certain injuries, hyperoxia, ozone, temperature fluctuations and other stresses are known to induce free radical formation in most aerobic organisms (Malenčić et al., 2010).

At early times after wounding, plants transiently produce active oxygen species (AOS), including the superoxide anion in damaged tissue and hydrogen peroxide, both locally and systematically (Orozco-Cardenas, Ryan, 1999). Production is maximal at several minutes after wounding for superoxide and at 4-6 h for hydrogen peroxide, and then declines (Orozco-Cardenas, Ryan, 1999). AOS can react with unsaturated fatty acids causing peroxidation of membrane lipids of plasmalemma and other cell organelles, which affect the regular metabolism by damaging the cellular components and leads to loss of membrane integrity (Smirnoff 1998).

It has been shown that wounding triggers an increase in the endogenous levels of the plant hormones, such as jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), ethylene (ET) that mediate wound-activated gene expression through unified signal transduction pathway. In contrast, phytohormones gibberellic acid (GA) and auxins/ indole-3-acetic acid (IAA) levels are affected after wounding, due to decreased biosynthesis or by oxidation during oxidative stress (Normanly, 2010; Blomster et al., 2011). The endogenous levels of auxin decline upon wounding and recovery of the initial levels of active auxins has been proposed as mechanism to limit the duration of the response to wounding (Rojo et al., 1998).

Auxin plays crucial role as a negative regulator of stress-induced morphogenic response, caused by prolonged stress exposure (Blomster et al., 2011). Following wounding, the regeneration of plant tissue is controlled by auxin produced by the young leaf directly above the wound site (Taiz and Zeiger, 2006). The phytohormone auxin plays a central role in the control of cell and plant growth. It can stimulate or inhibit cell expansion, stimulate cell division, promote differentiation of vascular tissues, inhibit shoot branching, and promote lateral root formation (Marchant et al., 2002; Aloni et al., 2003). The synthetic auxin analogue, 2,4-D, is especially active in inducing cell division, and indeed somatic embryogenesis (Pasternak et al., 2005).

According to different authors (Hirt, 2000; Kovtun et al., 2000), auxin and AOS have antagonistic effects on cell cycle progression and gene activation and it has been proposed that a possible and emerging candidate for an intermediate function in both, stress and growth responses, seems to be the auxin (Pasternak et al., 2005). The combination of an inhibitory effect of oxidative stress on the cell cycle (Reichheld et al., 1999), and a stimulatory effect on auxin levels (Pasternak et al., 2002), point that AOS and auxin are regulators of plant development during stress (Potters et al., 2007; 2009; Tognetti et al., 2011).

In order to investigate the effect of exogenous auxins on the mechanical injury induced oxidative stress in softwood cuttings, the level of lipid peroxidation was measured in the basal parts of the cuttings and leaves of five cherry rootstocks.

MATERIAL AND METHODS

Five cherry rootstocks [Mahaleb 1 and 2, Gisela 5, European ground cherry (EGC) and "Oblačinska" sour cherry] were used for the experiment. Mother trees were grown at the nursery of Institute for Fruit science, Viticulture, Horticulture and Landscape architecture, at Rimski Šančevi, Novi Sad. Stems were cut early in the morning, placed into freezer bags and transported on ice to laboratory, in order to avoid additional stress during the sampling process. Cuttings were treated with 0.5% solutions of three auxins: α-naphthylacetic acid (NAA), indolebutyric acid (IBA) and combination of these two (INCIT K), for 60 min. Control was represented with cuttings treated with distilled water. After the cuttings have been made samples were transferred to specialised cutting substrate "Steckmedium"' (Klasmann-Deilmann, GmbH 49744 Geeste Germany) with addition of slow-released fertiliser, and placed in greenhouse with fogging system. Fogging was regulated automatically and fogging intervals lasted about 30 s, followed by 60 s pause, in order to achieve 95% humidity.

As a measure of lipid peroxidation (LP) intensity, the amount of malondialdehyde (MDA) was determined by the MDA or thiobarbituric acid-reactive-substances (TBARS) assay. MDA is formed through autooxidation and enzymatic degradation of polyunsaturated fatty acids in cells. This secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) via an acid-catalyzed nucleophilic-addition reaction yielding a pinkish-red chromagen with an absorbance maximum at 532 nm (Hodges et al., 1999). MDA or TBARS assay has been used extensively since the 1950s to estimate peroxidation of lipids in membrane and biological systems. The TBARS assay remains popular due to its simplicity, lack of expense, and rapidity with which large numbers of samples can be processed with minimal manipulation.

LP intensity was measured as TBARS production, spectrophotometrically. For this assay, plant material - basal parts of the cuttings and leaves, were first homogenized and then extracted in 10% trichloroacetic acid (TCA) in ratio 1:5 (w/v) and centrifuged at 12000 x *g* for 30 min at 4 °C. One cm^3 of supernatant was incubated with 4 cm^3 20% TCA containing 0.5% TBA for 30 min at 95 °C. The reaction was stopped by cooling on ice for 10 min and the product was centrifuged at 10000 x *g* for 15 min. The absorbance of the TBARS was measured at 532 nm and their concentration was determined using the MDA extinction coefficient of 155 mM/cm and expressed as nmol MDA/g fresh weight.

All determinations were made in triplicates, and values were expressed as the means ± standard deviation. Statistical significance was tested by ANOVA followed by comparisons of means by Duncan's multiple range test ($P < 0.05$). The results were expressed as % of control.

RESULTS AND DISCUSSION

Formation of lipid peroxides, their degradation, and the roles of these hydroperoxides in cellular metabolism has recently attracted interest. The most accurate approach to measure lipid peroxidation is to directly quantify the primary hydroperoxide products. However, these are extremely difficult to measure as a result of their lability and require lengthy procedures. Consequently, the determination of MDA, a secondary end

product of oxidative lipid degradation, has become the system of choice for estimating LP. Although there are methods available for directly quantifying MDA, such as with gas chromatography or high-performance liquid chromatography, simpler derivative-type methods, such as the TBARS assay, offer a relatively facile and rapid spectrophotometric technique with reduced sample manipulation.

Results obtained in this study varied depending on the rootstock, plant organ and auxin applied. The level of LP in the basal parts of the cuttings and leaves of different cherry rootstocks is shown in Figures 1-6.

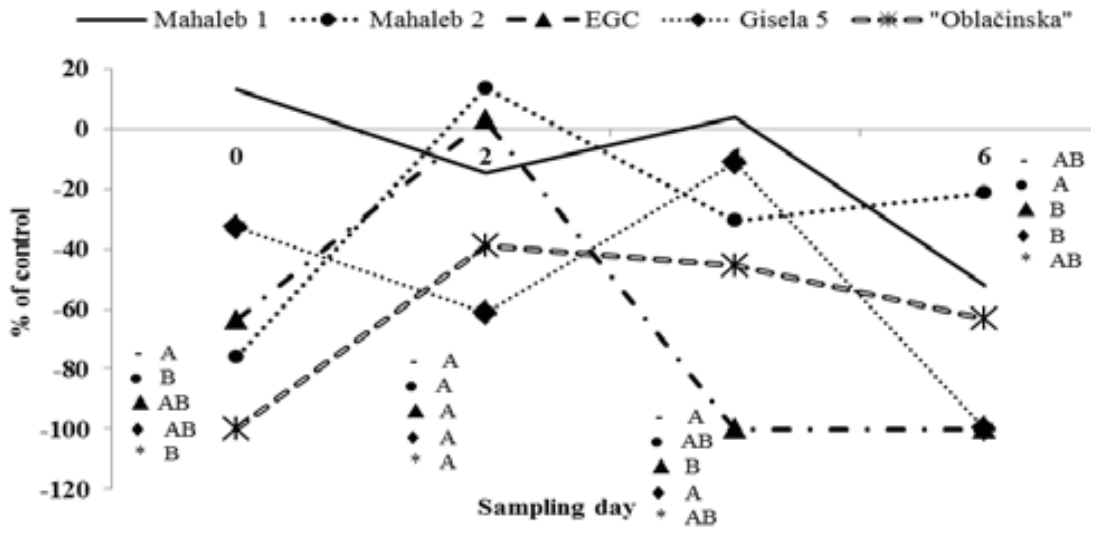

Fig. 1. Changes in LP levels in leaves of different cherry rootstocks treated with NAA.

The values marked with the same letter do not differ significantly at $P<0.05$.

All cherry rootstocks reacted in the same manner after mechanical injury was made, but some differences concerning auxin applied are visible. The accumulation of TBARS occurred in the leaves of all genotypes between 0 and 2nd day, but it seems that auxin application had positive lowering effect on LP intensity in the following days. This effect was noticeable in leaves of all rootstocks investigated when NAA was applied, during the whole experiment. The positive effect of NAA was especially pronounced between days 2 to 6, in the most of the genotypes (Fig. 1). Similar happened in Mahaleb 1 and 2, and EGC between days 2 to 4 when treated with IBA (Fig. 2). Same as for NAA, Gisela 5 and "Oblačinska" rootstocks showed no increase in LP in their leaves compared to control when IBA was applied, as well. Application of INCIT K seems also to help plants and their antioxidant protection systems to cope with the stress because in all rootstocks, 6 days after the cutting, LP intensity was significantly lower compared to control (Fig. 3).

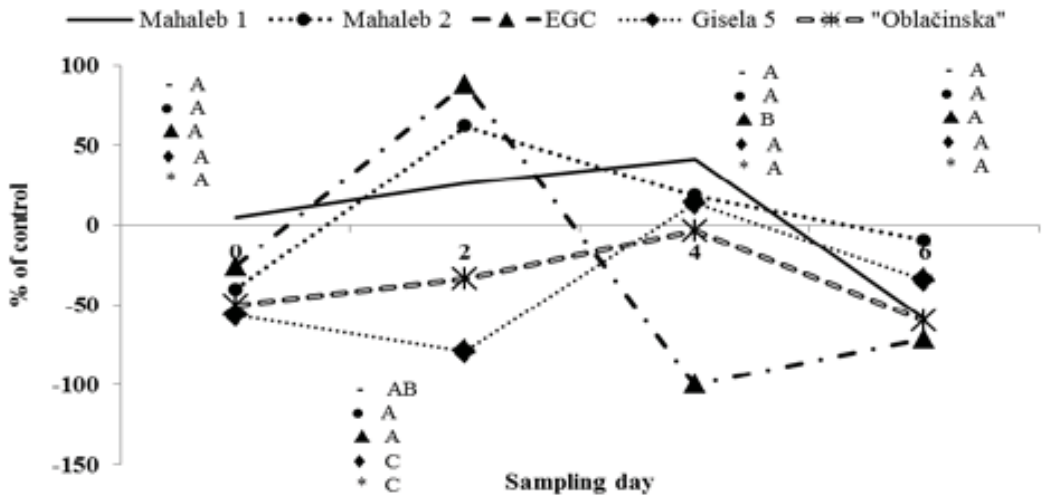

Fig. 2. Changes in LP levels in leaves of different cherry rootstocks treated with IBA.
The values marked with the same letter do not differ significantly at P<0.05.

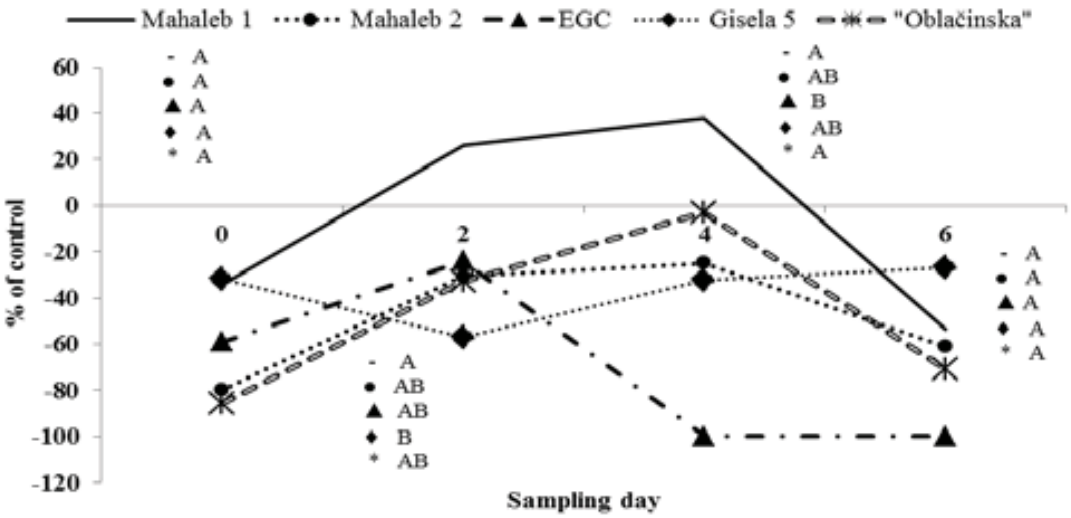

Fig. 3. Changes in LP levels in leaves of different cherry rootstocks treated with INCIT K.
The values marked with the same letter do not differ significantly at P<0.05.

Contrary to leaves, LP intensity in the basal parts of the cuttings was significantly higher. As expected, the LP occurred predominantly in the tissue where cuttings have been made – in their basal parts (Figs. 4-6). Still, rootstocks reacted differently – Mahaleb 1 was at least affected by the injury and its LP level was the lowest on the beginning of the experiment (day 0). The best LP-lowering effect was detected once again with the phytohormone NAA (Fig. 4).

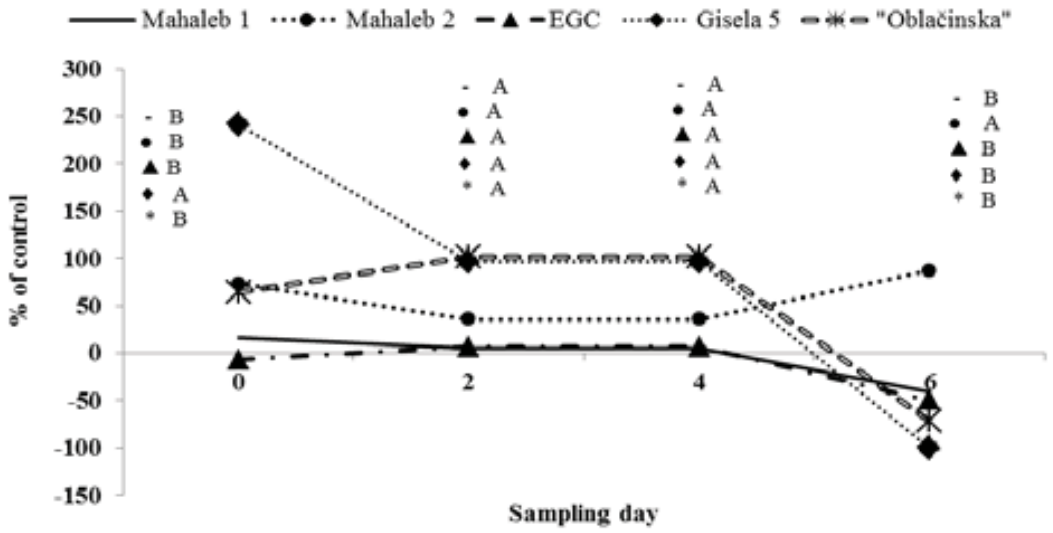

Fig. 4. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with NAA.

The values marked with the same letter do not differ significantly at P<0.05.

Rootstock EGC reacted similarly to NAA, having LP level same as in control cuttings. Mahaleb 2 rootstock was the only one that showed significant damage in membrane structure after NAA was applied, having LP level 30-80% higher then control. IBA also helped rootstocks to lower their TBARS accumulation and all rootstocks had their LP levels on the same or lower level then control (Fig. 5). Application of INCIT K affected LP intensity in the basal parts of rootstocks investigated quite differently; in Mahaleb 2 and "Oblačinska" oxidative damage could not be prevented and LP level was about 100% higher compared to control (Fig. 6). On the other hand, in the rest of the rootstocks this auxin helped in lowering the peroxidation of cellular components including cell membranes.

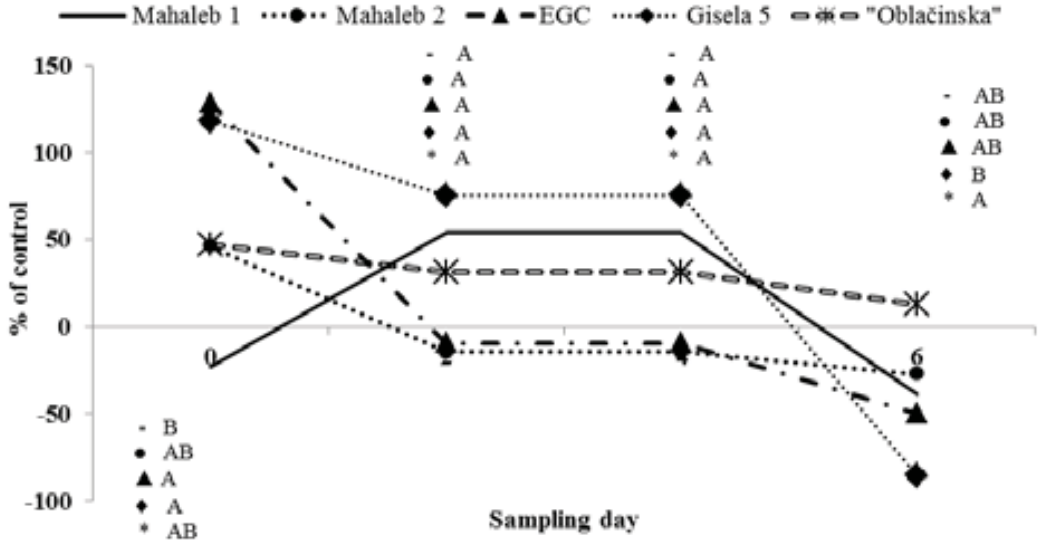

Fig. 5. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with IBA.

The values marked with the same letter do not differ significantly at P<0.05.

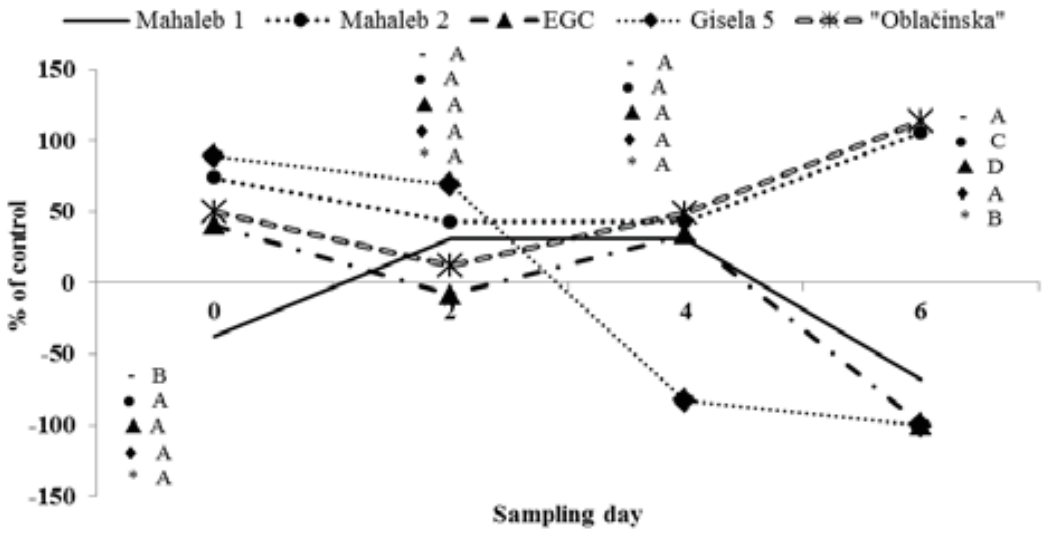

Fig. 6. Changes in LP levels in softwood cuttings of different cherry rootstocks treated with INCIT K.

The values marked with the same letter do not differ significantly at $P<0.05$.

It has been established that in the most of the sour cherries examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant system, as well. LP-lowering effect of the auxins may be due to their interaction with other hormones involved in the process of LP that is tightly connected with cell death responses, such as auxin antagonist JA, which regulates cell death responses (Blomster et al., 2011).

Comparison of the rootstocks investigated showed that Mahaleb 1, EGC and Gisela 5 reacted positively to application of all auxins, having the lowest production of end-products of the LP. This good regenerative characteristic should be acknowledged and used in the fruit softwood cutting production. In the same time it should be mentioned that phytohormone NAA showed the best antioxidant and regenerative effect and affected beneficially most of the rootstocks which recommends it for rooting of softwood cuttings.

CONCLUSION

Results obtained for the LP intensity varied depending on the rootstock, plant organ and auxin applied. The best LP-lowering effect of auxins occurred in all genotypes when treated with NAA. In the leaves, the LP peaked on the 2nd day while the best LP-lowering effect was recorded in European ground cherry rootstocks on the 4th day, independently of auxin applied. The highest MDA production in leaves was recorded in Mahaleb 2 and European ground cherry (EGC) rootstocks treated with IBA (60-90% higher then control) two days after the cuttings were made. It has been established that in the most of the rootstocks examined auxins showed lowering effect on LP which points to their positive effect, not only on rooting of softwood cuttings, but on their antioxidant protection system, as well.

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PROMENE INTENZITETA LIPIDNE PEROKSIDACIJE U REZNICAMA PODLOGA ZA VIŠNJU I TREŠNJU TRETIRANIM AUKSINIMA

ĐORĐE MALENČIĆ, DEJAN PRVULOVIĆ, MILAN POPOVIĆ, VLADISLAV OGNJANOV, MIRJANA LJUBOJEVIĆ, GORAN BARAĆ, BOŠKO BORKOVIĆ, SLAVKO KEVREŠAN, BILJANA KIPROVSKI

Izvod

U radu je ispitan nivo oksidativnog stresa izazvanog mehaničkom povredom biljke prilikom proizvodnje reznica, u bazalnom delu i listovima pet podloga za višnju i trešnju (Magriva 1 i 2, Gizela 5, Stepska (EGC) i "Oblačinska" višnja). Istovremeno, reznice su tretirane 0.5% rastvorima tri auksina (α-naftilsirćetna kiselina, NAA, indolbuterna kiselina, IBA i kombinacija ovih hormona, INCIT K), u periodu od 60 min, kako bi se utvrdio efekat auksina na antioksidantni status reznica višnje. Uzorci su uzimani nakon 0, 2, 4 i 6 dana. Kontrolu su činile reznice držane u vodi bez hormona. Intenzitet lipidne peroksidacije (LP) je meren kao produkcija malondialdehida (MDA), spektrofotometrijski na 532 nm, i izražen je u nmol MDA g^{-1} sveže mase.

Dobijeni rezultati su varirali u zavisnosti od podloge, organa biljke i primenjenih auksina. Utvrđeno je da je intenzitet LP u bazalnom delu bio najveći prvog dana, nakon ozleđivanja, a da su svi ispitivani auksini snižavali produkciju MDA u narednim danima. Najbolji efekat pokazala je Magriva 1 tretirana sa NAA. U listovima, LP je bila najveća 2. dana, a najbolji efekat na smanjenje pokazali su primenjeni auksini u Stepskoj višnji (4. dana). Najveća produkcija MDA u listovima zabeležena je u Magrivoj 2 i Stepskoj višnji tretiranih sa IBA, 2. dana nakon odsecanja reznica (60-90% više od kontrole). Utvrđeno je da u većini podloga pod dejstvom ispitivanih hormona dolazi do smanjenja intenziteta LP što ukazuje na pozitivan efekat primenjenih auksina, ne samo na ožiljavanje reznica nego i na njihov antioksidantni sistem.

Ključne reči: reznice, oksidativni stres, auksini, lipidna peroksidacija.

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