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MITIGATING LIPID PEROXIDATION IN LEAVES OF *PRUNUS SP.* ROOTSTOCKS USING EXOGENOUSLY APPLIED AUXINS*

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Sumarry: *The effect of exogenously applied auxins on mechanical injury induced oxidative stress was studied in 6 promising rootstock selections of Prunus cerasus L., P. mahaleb L., and P. fruticosa Pall. Investigated selections were included in low-vigorous rootstock breeding programme for sweet and sour cherries. The standard rootstock PHL-A was used as a control, due to successful rooting. Leaves of investigated rootstocks were collected on: 0, 1st, 3rd and 7th day of inserting softwood cuttings into the rooting substrate under a fogging system with 95-99% average relative humidity. Exogenously applied auxin mixture consisted of 0.8% α-naphthylacetic acid (NAA) and 0.5% indolebutyric acid (IBA). Mechanical injuries during rooting period represent one of the factors that induce stress in softwood cuttings. Intensity of LP is used as a valuable biomarker of plant response to various abiotic factors. Differences in intensity of LP between auxin-treated and -untreated cuttings were examined. Almost all of investigated selections had lower LP intensity after auxin application (11.4-47.2%) between 1st and 3rd day. However, the most prominent change was in leaves of PHL-A (49.2%). The best LP-lowering effect were recorded in leaves of P. fruticosa, SV4 selection (56.9%) on 7th day and in P. mahaleb, M4 selection, on 1st and 3rd day (5.9, 5.8%), in comparison to untreated softwood cuttings. As for OV21 selection, LP intensity significantly increased in both treated and untreated cuttings on the 1st, 3rd and 7th day, but auxin-treated cuttings showed lower LP values, except on 7th day, reaching 221.3 nmol MDA equivalents g^{-1} fresh weight. Intensity of LP during vegetative propagation of selected genotypes could be used as one of the biochemical parameters in further rootstock selection for sweet and sour cherries.*

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

INTRODUCTION

Fruit rootstock breeding and selection programmes have a number of goals. Above all, rootstocks should be adapted to environmental conditions, expected to be easily propagated, to result in uniform fruit tree behavior in the orchard. Selection success in breeding of vegetative rootstocks for sweet and sour cherry is dependent not only on

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genetic variability, but also on expediting propagation processes as a precondition for investigation of special combining abilities between rootstock and scion (Bošnjaković et al., 2012; Bošnjaković et al., 2013). To expedite propagation of rootstock selections, propagation by softwood cuttings is preferable.

Oxidative metabolism of normal cells and different stress situations generate highly reactive oxygen species (ROS). The ROS, such as, superoxide radical ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), hydroxil radical (·OH), and singlet oxygen ($^1O_2$) have been implicated in a number of physiological disorders in plants (Scandalios, 1993; Blomster et al., 2011), which in turn leads to a decrease in plant productivity (Smirnoff, 1998; Pasternak et al., 2005). To prevent oxidative stress, plants have evolved a complex antioxidant system composed of non-enzymatic and enzymatic mechanisms that scavenge ROS (Casano et al., 2004; Malenčić et al., 2010). The formation of auxins conjugates may serve as a protection against oxidative degradation (Taiz and Zeiger, 2006). Antioxidant defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. A ROS signal can be generated in a group of cells in the plant in response to wounding, pathogen attack or a local abiotic stress, and be transferred to the entire plant. The integration of ROS with auxin signaling networks, triggered by environmental factors, is known as the stress-induced morphogenic response. In this response, ROS and auxin metabolism interfere and lead to morphological changes that help avoid deleterious effects of environmental stress (Mittler et al., 2011).

The exogenous supply of auxins to leaf cuttings readily induces the new root formation, which ensures vegetative propagation. Adventitious root formation in some plant species initiates without any specific treatment, while in others, a medium supplied with growth regulators is required. In horticulture, the stimulatory effect of auxin on the formation of adventitious roots has been very useful for the vegetative propagation of plants by cuttings (Taiz and Zeiger, 2006). Lipid peroxidation (LP) is a natural metabolic process occurring in aerobic conditions and presents the most investigated effect of ROS on structure and function of cell membrane. Autocatalytic peroxidation of membrane lipids by ROS leads to loss of membrane semipermeability (Xu et al., 2006). ROS can cause cellular and molecular damage, protein modification and LP (Ali et al., 2005). Thus, the aim of this study was to measure the intensity of LP in the leaves of six cherry rootstocks selections in order to investigate the effect of exogenously applied auxins on the mechanical injury induced oxidative stress during rooting of softwood cuttings.

MATERIAL AND METHODS

Intensity of LP was determined in leaves of six promising rootstock selections of *Prunus cerasus* L., (OV21, OV22), *P. mahaleb* L., (M4, M6) and *P. fruticosa* Pall. (SV2st, SV4). Standard vegetative rootstock-PHL-A was used as a control, due to successful rooting. Softwood cuttings of investigated selections were collected from *ex situ* mother trees from the experimental field of the Faculty of Agriculture at Rimski Šančevi, near Novi Sad. The experiment was carried out in a plastic house under a fogging system with 95-98% relative humidity in average, where intervals lasted 90 s with 720 s pause. Fogging wasn't carried out during the night. The rooting substrate was a blend of white sphagnum and perlite. The 15-20 cm long terminal cuttings were treated with exogenously applied auxin mixture consisted of 0.8% α-naphthylacetic acid (NAA) and 0.5% indolebutyric acid (IBA). Leaves of investigated rootstocks were collected on 0, 1st, 3rd and 7th day after cutting and inserting softwood cuttings into the rooting substrate. Experiment was carried out with total 60 softwood cuttings per selection (30 auxin-treated and 30 auxin-untreated cuttings).

As a measure of LP intensity, the amount of malondialdehyde (MDA) was determined spectrophotometrically 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, 1999). Leaves of softwood cuttings 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 600 nm and their concentration was determined using the MDA extinction coefficient of 155 mM cm^{-1} and expressed as nmol MDA g^{-1} 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 presented as a % of control.

RESULTS AND DISCUSSION

Relatively little is known about how ROS regulate plant growth and development under stress conditions, and how they interact with other signaling molecules, including phytohormones (Pasternak et al., 2005). Measurement of MDA levels is routinely used as an index of LP under stress conditions. The present investigation showed that mechanical injury induced oxidative stress in the leaves of rootstock selection for sweet and sour cherry, characterized by an accumulation of MDA, may be attributed to the genotypic differences in wounding tolerance. According to Ljubojević et al. (2011), differences in rooting capability among investigated rootstock selections were presented. Satisfying percentage of rooting was achived in *P. cerasus* selections, while for *P. fruticosa*, method for vegetative propagation should be improved. Propagation protocol within and between species could not be uniformed, because it is strongly influenced by genotype (Bošnjaković et al., 2012). Results of Malenčić et al. (2012) showed a positive effect of exogenously applied NAA on LP-lowering effect in standard vegetative rootstock Gisela 5, as well as in four investigated sweet and sour cherry rootstocks selections. Similar was recorded for standard rootstock PHL-A in our experiment where a positive auxin effect on lowering LP intensity was noticed between 1st and 3rd collecting day, but on 7th day, an increase of LP was 65% compared to control (Fig. 1). In *P. mahaleb*, selection M4, MDA production was significantly lower in auxin-treated cuttings on 1st and 3rd collecting day. On 7th day LP intensity was similar in auxin-treated cuttings of both *P. mahaleb* selections, M4 and M6 (24.2% and 2.5%, respectively), comparing to untreated cuttings (23.9%, 5.5%) (Fig. 2 and Fig. 3). The enhancement of MDA accumulation, a cytotoxic product of lipid peroxidation, was recorded in *P. fruticosa* selection SV2st. on 3rd collecting day in both treated and untreated cuttings, contrary to 7th collecting day where auxin application showed lowering effect on LP intensity (Fig. 4). Lowering effect on LP intensity was noticed on 1st and 7th day in SV4 selection, also. Higher degree of membrane damage was noticed in untreated cuttings on 7th collecting day (154.5% of control) (Fig. 5).

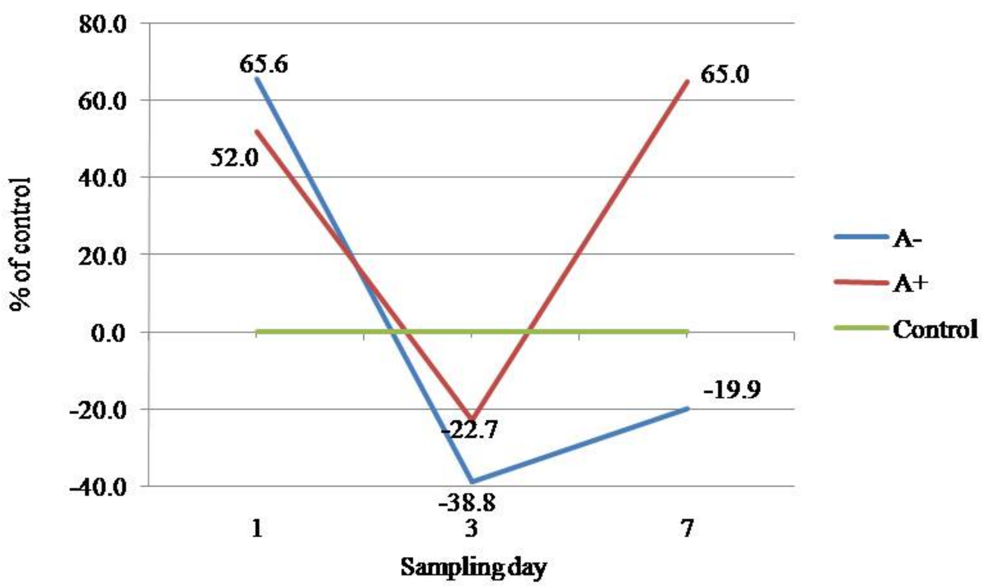

Figure 1. LP intensity in auxin treated and untreated standard vegetative rootstock PHL-A

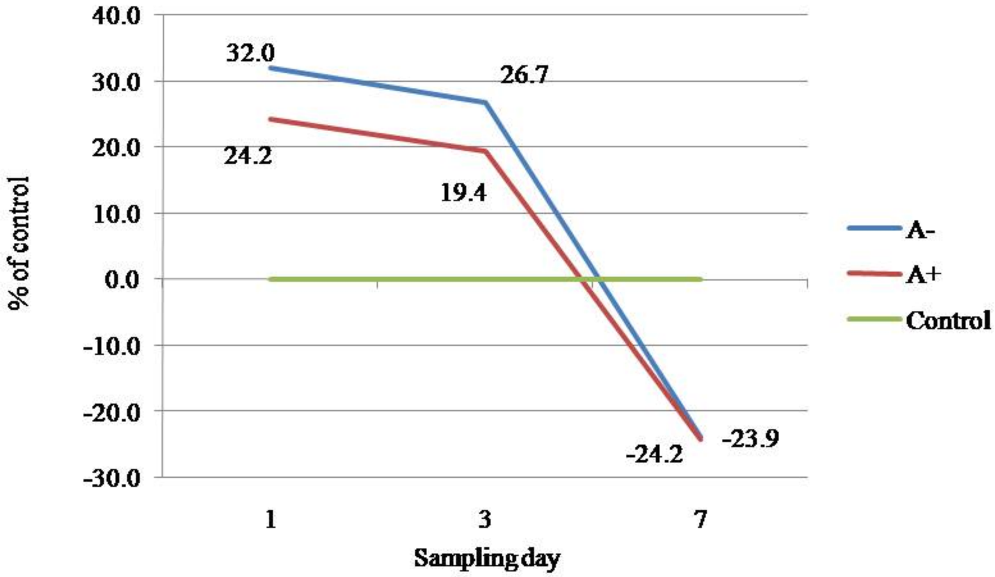

Figure 2. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb*-M4

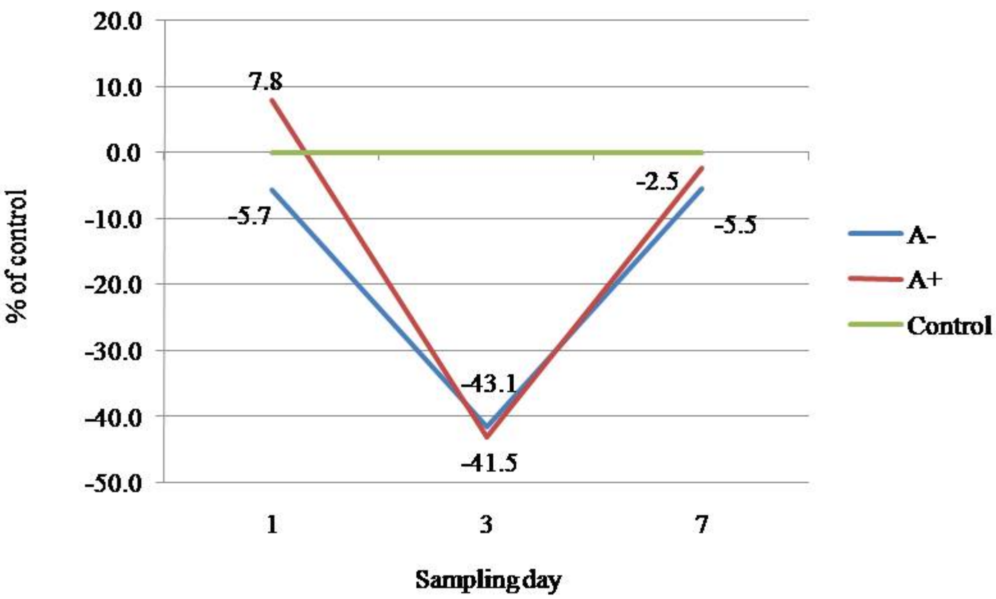

Figure 3. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb*- M6

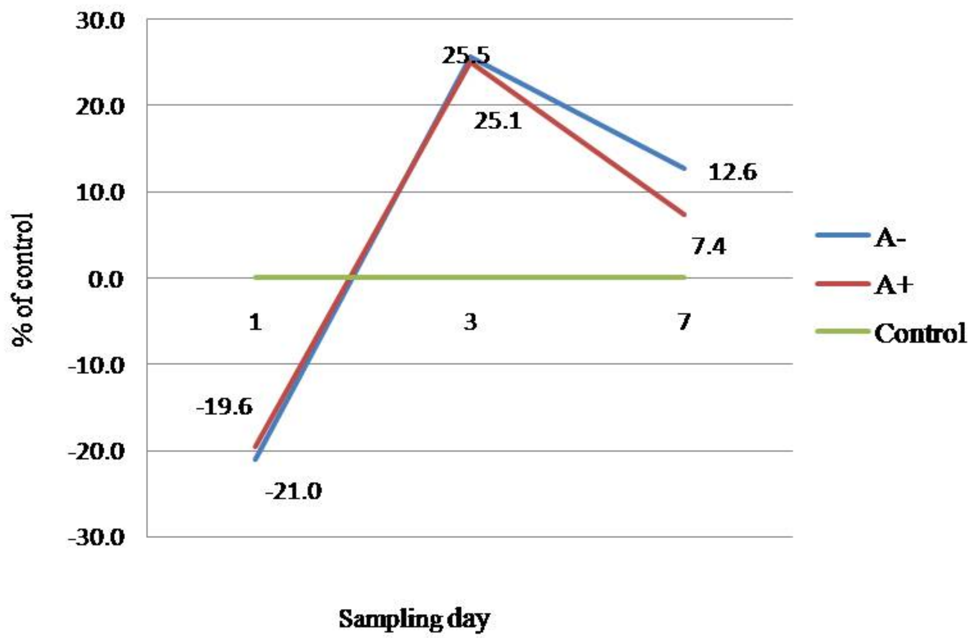

Figure 4. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV2st.

Figure 5. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV4

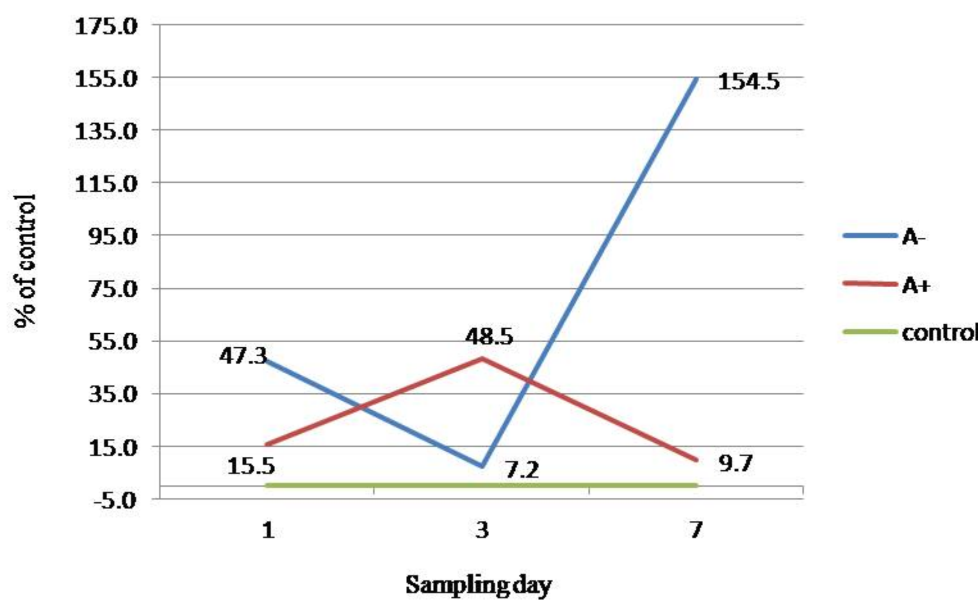

Figure 5. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa*-SV4

On 1st and 3rd collecting day auxin-treated cuttings of OV21 selection had lower LP intensity, but damage in membrane structure after auxin application was presented on 7th collecting day, reaching 221.3 nmol MDA equivalents g^{-1} fresh weight (Fig. 6). As for OV22 selection (Fig. 7), higher LP intensity resulted from enhanced ROS production, indicate that this selection was under higher oxidative stress conditions. Positive effect of auxin application was recorded on 3rd collecting day, where LP intensity was significantly lower compared to untreated cuttings and control. Plants respond to wounding by activating self-defense systems to restore damaged tissue (Castro-Mercado et al., 2009). According to Cheong et al. (2002), wounding negatively regulates IAA responsive genes, revealing a new level of crosstalk between wounding and auxin response in plants. Studies of expression patterns of genes regulated by wounding provided new information on the interactions between wounding and other signals, such as pathogen attack, abiotic stress factors, and plant hormones (Cheong et al., 2002). Different abiotic factors induce oxidative stress in cell, which increase MDA production in different plant species (Shalata and Tal, 1998; Sudhakar et al., 2001; Bor et al., 2003; Wu et al., 2003). Possibly, due to the fact that wounding inhibits auxin level in plants, exogenously applied auxin had a positive effect on rooting in our experiment, however, LP-lowering effect of applied hormone, had no uniform effect on all investigated selections.

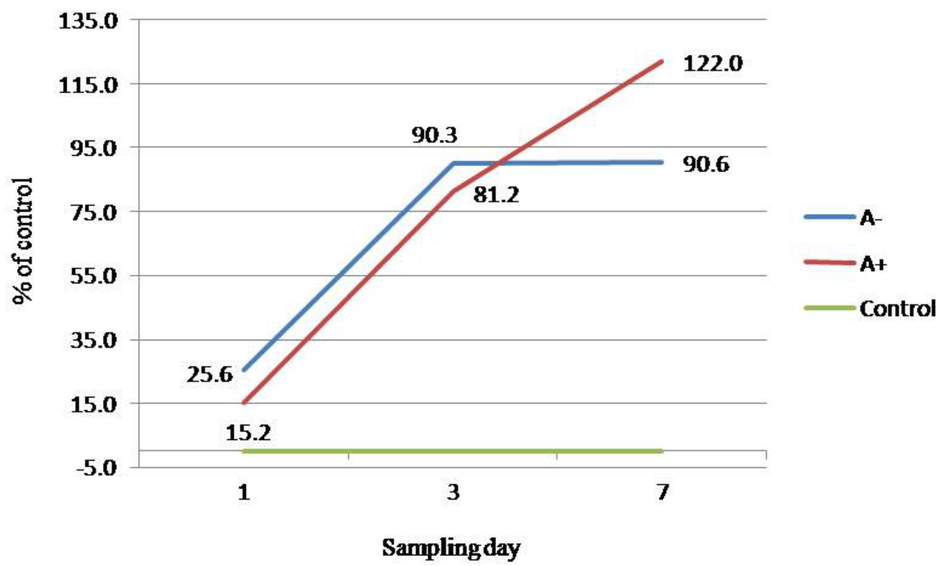

Figure 6. LP intensity in auxin treated and untreated rootstock selection *P. cerasus*-OV21

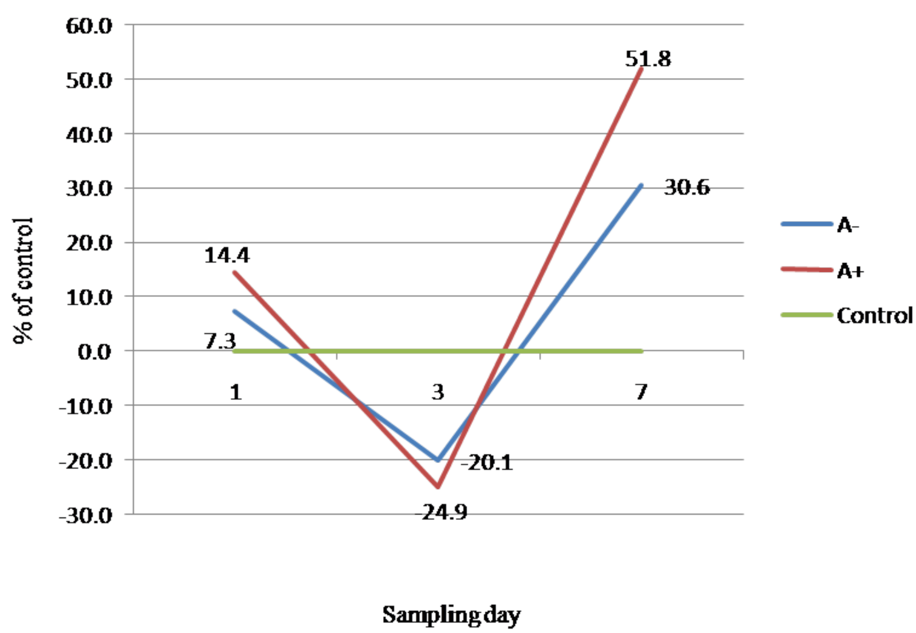

Figure 7. LP intensity in auxin treated and untreated rootstock selection *P. cerasus*-OV22

CONCLUSION

There is a noticeable difference in the alternation of MDA content among 7 investigated genotypes of *Prunus cerasus* L., *P. mahaleb* L. and *P. fruticosa* Pall., which implies to a great genetic variability in response of these rootstocks toward wounding and oxidative stress induced by investigated abiotic factor. Despite the fact that auxin has a positive effect on rooting and LP-lowering effect in most of treated selections, its application seems to has no universal LP-mitigating effect for treated rootstocks. Among all investigated selections, the best auxin LP-lowering effect was in *P. mahaleb*, M6 selection during the entire experiment. Also, the results gained from LP assay proved to be invaluable marker for resistance to mechanical injury induced oxidative stress and impact of propagation in sweet and sour rootstock selection.

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SMANJENJE INTENZITETA LIPIDNE PEROKSIDACIJE U LISTOVIMA SELEKCIJA VEGETATIVNIH PODLOGA ZA VIŠNJU I TREŠNJU UPOTREBOM EGZOGENO PRIMENJENIH AUKSINA

Dušica DORIĆ, Biljana KIPROVSKI, Đorđe MALENČIĆ, Vladislav OGNJANOV, Mirjana LJUBOJEVIĆ, Goran BARAĆ

Izvod: Uticaj egzogeno primenjenih auksina na oksidativni stres izazvan mehaničkom povredom izučavan je kod 6 selekcija vegetativnih podloga vrsta- *Prunus cerasus L*., *P. mahaleb L*., i *P. fruticosa Pall*. koje su uključene u oplemenjivački program na stvaranju slabobujnih podloga za višnju i trešnju. Kao kontrola korišćena je standardna vegetativna podloga za višnju i trešnju- PHL-A. Listovi ispitivanih selekcija sakupljani su 0, 1, 3 i 7 dana od termina tretiranja reznica u supstratu za ožiljavanje, u kontrolisanim uslovima vlaženja, sa 95-99 % relativne vlažnosti. Reznice su tretirane egzogeno primenjenom kombinacijom auksina- 0,8 % α- naftilsirćetna kiselina (NAA) i 0,5 % indolbuterna kiselina (IBA). Mehanička povreda nastala odvajanjem letorasta od matične biljke predstavlja jedan od faktora koji utiču na stvaranje oksidativnog stresa u biljkama. Intenzitet lipidne peroksidacije je korišćen kao pokazatelj reakcije biljaka na različite abiotičke faktore. U radu su praćene razlike intenziteta lipidne peroksidacije između reznica tretiranih auksinima i reznica bez primene egzogenih auksina. Skoro sve ispitivane selekcije su imale smanjen intenzitet LP nakon primene auksina (11,4- 47,2 % manje od kontrole) između prvog i trećeg dana. Najbolje smanjenje intenziteta LP je uočeno kod standardne podloge PHL-A (49,2 %). Smanjenje intenziteta LP je zabeleženo kod selekcije *P. fruticosa*, SV4 (56,9 %), sedmog dana, i kod selekcije *P. mahaleb*, M4, prvog i trećeg dana (5,9 i 5,8 %), u poređenju sa netretiranim reznicama. Kod selekcije *P. cerasus*- OV21 uočen je porast intenziteta LP, kako kod netretiranih, tako i kod tretiranih reznica, prvog, trećeg i sedmog dana. Vrednosti LP su bile manje kod tretiranih reznica, osim sedmog dana, dostižući 221,3 nmol MDA ekvivalenata g^{-1}svm. Pozitivan efekat primenjenih auksina na smanjenje intenziteta LP tokom postupka vegetativnog ožiljavanja može biti jedan od biohemijskih parametara u budućem selekcionom radu koji se odnosi na oplemenjivanje podloga za trešnju i višnju.

Ključne reči: zelene reznice, auksini, lipidna peroksidacija, oksidativni stress.

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