Effect of Auxins on Mechanical Injury Induced Oxidative Stress in *Prunus sp.* Rootstocks

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Abstract. The effect of exogenously applied auxins on oxidative stress was studied in six rootstock selections of *Prunus cerasus*, *P. mahaleb* and *P. fruticosa* aiming at the effect of phytohormones on the level of the lipid peroxidation (LP). Standard rootstock PHL-A was used as a control. Leaves were collected on 0, 1st, 3rd and 7th day of inserting cuttings into the rooting substrate. Auxin mixture consisted of 0.8% α-naphthylacetic acid and 0.5% indolebutyric acid. Differences in intensity of the 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%). The most prominent change was in leaves of PHL-A (49.2%). LP-lowering effect were recorded in leaves of *P. fruticosa* (56.9%) on 7th day and in *P. mahaleb* on 1st and 3rd day (5.9, 5.8%). Intensity of LP could be used as one of the biochemical parameters in further rootstock selection and production.

Keywords: *Prunus* sp. rootstocks, softwood cuttings, auxins, oxidative stress, lipid peroxidation

1 Introduction

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 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), hydroxyl radical (·OH), and singlet oxygen ($^1O_2$) have been implicated in a number of physiological disorders in plants (Malenčić et al., 2012; Blomster et al., 2011), which in turn leads to a decrease in plant productivity. 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

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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).

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. 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 oxidative stress induced by mechanical injury during rooting of softwood cuttings.

2 Material and Methods

The effect of exogenously applied auxins on mechanical injury induced oxidative stress was studied in six rootstock selections: *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. 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 a total of 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 thiobarbituric acid-reactive-substances (TBARS) assay (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.

3 Results and Discussion

The present investigation showed that the oxidative stress induced by mechanical injury in the leaves of rootstock selection of sweet and sour cherry, characterized by an accumulation of MDA, may be attributed to the genotypic differences in wounding tolerance. 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 SV2 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).

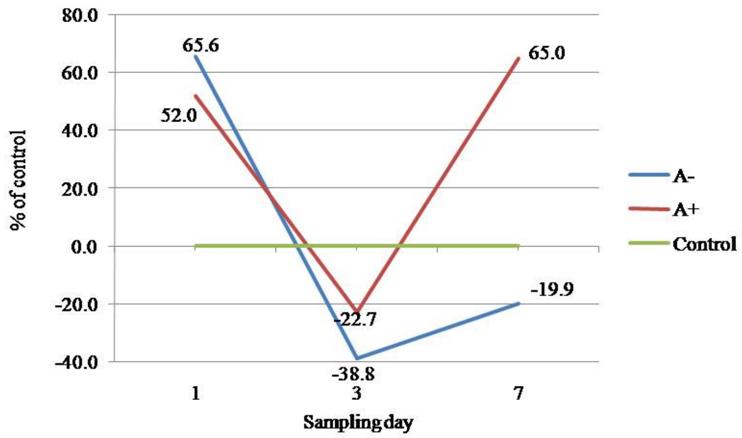

Fig. 1. LP intensity in auxin treated and untreated standard vegetative rootstock PHL-A

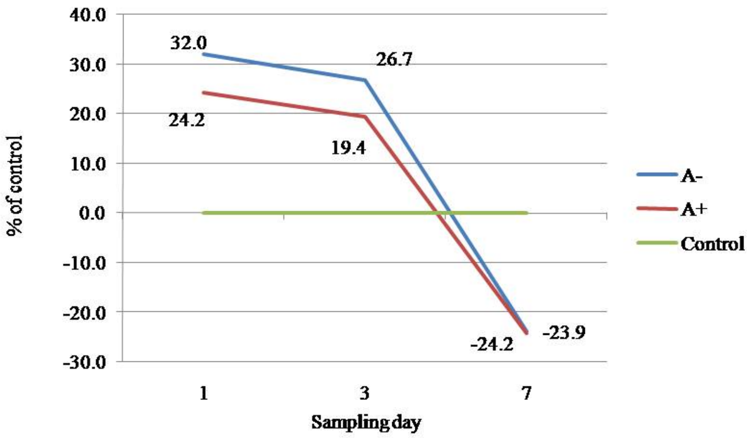

Fig. 2. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb* - M4

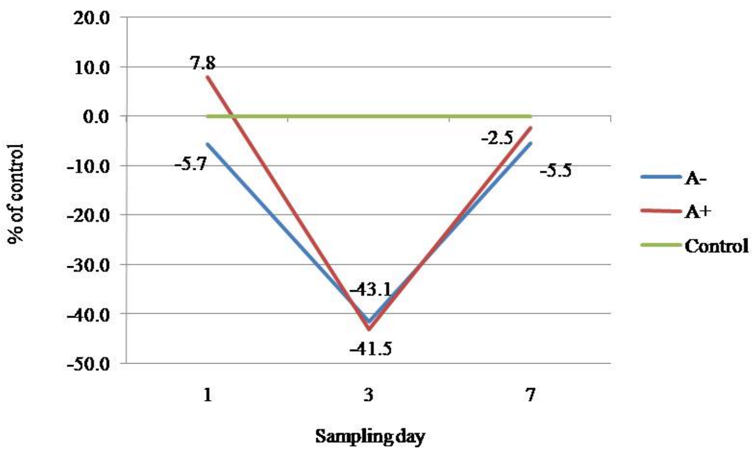

Fig. 3. LP intensity in auxin treated and untreated rootstock selection *P. mahaleb* - M6

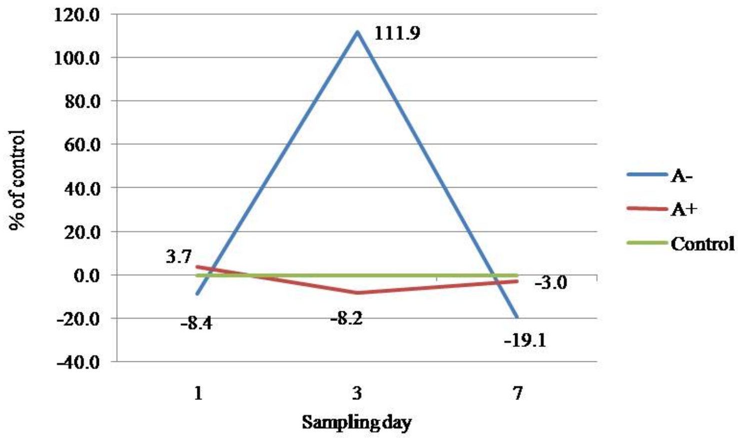

Fig. 4. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa* - SV2

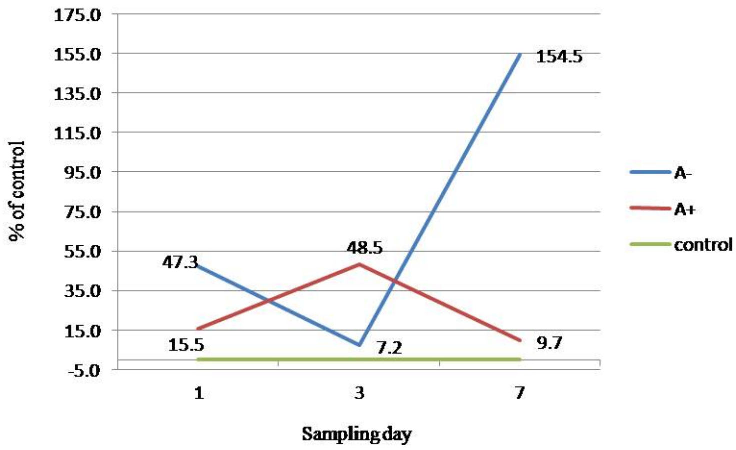

Fig. 5. LP intensity in auxin treated and untreated rootstock selection *P. fruticosa* - SV4

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.

A great genetic variability in response of rootstocks of *Prunus cerasus* L., *P. mahaleb* L. and *P. fruticosa* Pall. toward wounding and oxidative stress was established. 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 the oxidative stress induced by mechanical injury and impact of propagation in sweet and sour rootstock selection.

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References

1. Blomster, T., Salojärvi, J., Sipari, N., Broche, M., Ahlvors, R., Keinänen, M., Overmyer, K., Kangasärvi, J. (2011) Apoplastic reactive oxygen species transiently decrease auxin signaling and cause stress-induced morphogenic response in Arabidopsis. Plant physiology, 157, p.1866-1883.
2. Bošnjaković, D., Ognjanov, V., Pranjić, A., Ljubojević, M., Barać, G., Mladenović, E., Čukanović, J. (2012) Vegetative reproduction of the autochtonous sour cherry germplasm. 14th Serbian Congress of fruit and grapevine producers, Vrnjačka banja, Serbia, 9-12.10.2012., Book of abstracts, p.93.
3. Bošnjaković, D., Ognjanov, V., Barać, G., Ljubojević, M., Pranjić, A., Dugalić, K. (2013) Micropropagation selection of dwarf rootstocks for cherry and sour cherry. Voćarstvo (Fruit growing), 47. p.121-128.
4. Casano, L.M., Martin, M., Sabater, B. (1994) Sensitivity of superoxide dismutase transcript levels and activities to oxidative stress is lower in mature senescent than in young barley leaves. Plant physiology, 106, p.1033-1039.
5. Cheong, Y.H., Chang, H-S., Gupta, R., Wang, X., Zhu, T., Luan, S. (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant physiology, 129, p.661-677.
6. Hodges, D.M., Delong, J.M., Forney, C.F., Prange, R.K. (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanins and other interfering compounds. Planta, 207, p.604-611.
7. Malenčić, Dj., Kiprovski, B., Popović, M., Prvulović, D., Miladinović, J., Djordjević, V. (2010) Changes in antioxidant system in soybean as affected by

Sclerotinia sclerotiorum (Lib.) de Bary. Plant physiology and biochemistry, 48, p.903-908.

8. Malenčić, Dj., Prvulović, D., Popović, M., Ognjanov, V., Ljubojević, M., Barać, G., Borković, B., Kevrešan, S., Kiprovski, B. (2012) Changes in the lipid peroxidation intensity in auxin treated cherry rootstocks softwood cuttings. Savremena poljoprivreda/Contemporary agriculture, 61, p.221-229.
9. Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, T.B., Vandepoele, K., Gollery, M., Shulae, V.V., Breusegem, F.V. (2011) ROS signaling: the new wave? Trends in Plant Science, 16, p.300-309.
10. Taiz, L., Zeiger, E. (2006) Plant physiology. Sinauer Associated Inc., p.478-499.