ACTA AGROBOTANICA
Vol. 67 (4), 2014: 33–42
Original Research Paper
DOI: 10.5586/aa.2014.042

EFFECTS OF CYTOKININS ON ANTIOXIDANT ENZYMES IN *IN VITRO* GROWN SHOOTS OF *Pelargonium hortorum* L. H. Bayley

Agnieszka Wojtania[1,*], Edyta Skrzypek[2]

[1]Research Institute of Horticulture, Skierniewice, Poland
[2]The *F. Górski* Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland
[*]e-mail: agnieszka.wojtania@inhort.pl

Received: 09.05.2014

A b s t r a c t

The aim of this study was to determine the influence of *meta*-topolin (*m*T) and 6-benzyl-aminopurine (BAP) on the hydrogen peroxide (H_2O_2) level and antioxidant enzymes activities in relation to the shoot formation and senescence process in *Pelargonium hortorum* cultivars, which differ in their susceptibility to leaf yellowing under *in vitro* conditions.

In an early senescing cultivar 'Grand Prix', the addition of an aromatic cytokinin *m*T to abscisic acid (ABA)-enriched Murashige and Skoog (MS) basal medium more efficiently inhibited leaf yellowing than BAP. In both genotypes, *meta*-topolin was also the most effective in shoot formation. It was found that *Pelargonium* species varying in their susceptibility to senescence differ in H_2O_2 production and antioxidant enzymes activities. Generally, *meta*-topolin more effectively enhanced H_2O_2 production and POD activity than BAP and control medium, but its effect depended on genotype. The highest H_2O_2 production stimulated by *m*T was observed on day 5 of subculture in late senescing cv. 'Bergpalais'. In both geranium genotypes, superoxide dismutase (SOD) and catalase (CAT) levels were highest at the beginning of the subculture period, during the initiation of shoot formation. SOD showed the highest activity on day 5 of subculture on the medium without cytokinin and generally being higher in cv. 'Bergpalais' than in cv. 'Grand Prix'. CAT activity was positively regulated by both cytokinins. POD activity was most effectively enhanced by *m*T, but on different days of subculture - on the 2nd day of subculture in cv. 'Bergpalais' and on the 22nd day of subculture in cv. 'Grand Prix'. The enhanced activity of POD in the presence of *m*T, 4-fold higher than on control medium, at the end of subculture in *P. hortorum* 'Grand Prix' coincided with the inhibition of leaf senescence.

Key words: early culture senescence, 6-benzyl-aminopurine, hydrogen peroxide, leaf yellowing, *meta*-topolin, shoot formation

INTRODUCTION

Many *Pelargonium* cultivars cultured *in vitro* showed early culture senescence [1]. Our previous study showed that the natural cytokinin – *meta*-topolin (*m*T), inhibits early senescence in different *P. hortorum* and *P. hederaefolium* cultivars better than BAP. It was found that *m*T enhanced the regeneration capacity of recalcitrant cultivars and stimulated cyclic shoot formation. The observations of different geranium genotypes showed that they differed in their susceptibility to leaf yellowing [2, 3].

Earlier research revealed that early leaf senescence may be induced by excessive accumulation of reactive oxygen species (ROS), such as superoxide radical ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet$OH), leading to the development of oxidative stress [4, 5]. It has been found that ROS may induce the expression of many senescence genes [4]. Plants cultured *in vitro* are exposed to many extreme conditions, including wounding, high concentration of sucrose, nitrogen, hormonal imbalances, gas toxicity, and high humidity. Such conditions are very stressful and can lead to overproduction of ROS, thus promoting different physiological disorders, including early senescence [6, 7]. On the other hand, stress has been known to induce a number of acclimation responses in plants allowing them to survive the unfavorable conditions. In plants, ROS production is controlled by various non-enzymatic antioxidants and enzymatic defence system. Enzymatic protection is partly performed by superoxidase dismutases (SOD) that dismutate $\bullet O_2^-$ radicals to H_2O_2 and by catalase (CAT) and various peroxidases that degrade H_2O_2 [5, 8]. The activity of the scavenging enzymes is regulated by different factors, including hormone treatments [9,10].

The aim of this study was to determine the influence of *meta*-topolin and BAP on H_2O_2 level and antioxidant enzymes activity in relation to shoot formation and senescence process in *P. hortorum* cultivars which differ in their susceptibility to leaf yellowing under *in vitro* conditions.

MATERIALS AND METHODS

Plant material and growth conditions

The experiments were performed with two *Pelargonium hortorum* cultivars: 'Grand Prix' – susceptible to leaf yellowing, and 'Bergpalais' – not susceptible to leaf yellowing. Plant material for the experiment was derived from stock cultures initiated from shoot tips and axillary buds and multiplied for 1 year on medium containing *meta*-topolin (2.07 μM). The basal multiplication medium consisted of MS medium [11], 0.1 g L^{-1} myo-inositol, 30 g L^{-1} sucrose, and solidified with LAB-AGAR (0.6%). The pH of the medium was adjusted to 5.6 before autoclaving. The shoots were subcultured at 3 week intervals into fresh medium in an Erlenmeyer flask and kept at a temperature of 21°C, under a 16 h photoperiod provided by cool-white fluorescent lamps at 40 μmol $m^{-2}s^{-1}$ (Philips TLD 36W/95).

Growth regulator treatments

The antisenescence activities of *meta*-topolin (*m*T, 2.07 μM) and 6-benzyl-aminopurine (BAP, 0.44 μM) in *P. hortorum* 'Grand Prix' and 'Bergpalais' were evaluated in the absence or presence of abscisic acid (ABA, 3.78 μM). The concentration of cytokinins was determined on the basis of previous experiments [3]. Explants incubated on a medium without growth regulators served as a control. The experiments were carried out with single shoots. One experimental treatment was represented by 30 explants (6×5 explants per Erlenmeyer flask). After 3 weeks of subculture, the number of shoots per explants and number of yellow leaves [%] per shoots clump were determined.

In the second experiment, hydrogen peroxide (H_2O_2) production and antioxidant enzymes activity in *P. hortorum* 'Grand Prix' and 'Bergpalais' shoots growing on media without cytokinin (control) and supplemented with *m*T (2,07 μM) or BAP (0.44 μM) were evaluated. The measurements were taken on the 2nd, 5th, 9th, 16th, 22nd day of subculture.

Determination of H_2O_2

Total H_2O_2 content was measured in fresh material by a fluorometric assay with homovanillic acid according to the method by Ishikawa et al. [12]. Samples, about 100 mg each, were homogenized in 1 ml of ice-cold 5% TCA and centrifuged at 4000 g for 5 min. The reaction mixture contained the supernatant (10 μl), 1.25 mM homovanillic acid, 1 unit of horseradish peroxidase (Sigma-Aldrich Co., Poland), and 25 mM potassium phosphate buffer (pH 7.5). The fluorescence yield was measured at an excitation wavelength of 315 nm and emission at 425 nm with a LS50B spectrofluorometer (Perkin-Elmer, USA). Knowing the exact fresh mass of the sample, the amount of H_2O_2 was calculated as μl of H_2O_2 per 1 g of FM.

Activity of antioxidant enzymes

Plant tissue was homogenized at 4°C with a phosphate buffer (pH 7.8) containing 0.01 M EDTA and 0.5% bovine serum albumin. The homogenate was centrifuged at 10 000 g for 15 min.

Peroxidases (POD, E.C. 1.11.1.11) activity was measured by the modified method of Lűck [13]. The measurement was carried out spectrophotometrically (λ=485 nm), by measuring the amount of the products in 0.1 ml 1% p-phenylenediamine (pPD) and 1 ml supernatant, in 0.05 M dm^{-3} phosphate buffer containing 0.1 mM EDTA, pH 7.0. The reaction was started in the presence of 0.05 cm^3 of 0.03 M dm^{-3} H_2O_2.

Catalase (CAT, EC 1.11.1.6) activity was measured spectrophotometrically (λ=240 nm) by the modified method of Aebi [14]. The reaction mixture consisted of 2 ml of 0.05 M dm^{-3} phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 1 ml of a solution of H_2O_2 in this buffer (0.03 mol dm^{-3}), and 50 ml supernatant.

Superoxide dismutase (SOD, EC 1.15.1.1.) activity was measured spectrophotometrically (λ=595 nm) by the modified method of McCord and Fridovich [15]. One unit was defined as the amount of enzyme necessary for 50% inhibition of cytochrome c in a coupled system with xanthine and xanthine oxidase.

The reaction kinetics for all the enzymes was examined after 60 and 120 s from the initiation of the reaction using KINLAB software. Proteins were assayed using Bradford's [16] dye-binding technique with bovine serum albumin as a protein standard.

Statistical analysis

Each experiment was repeated at least twice. The experimental data were subjected to the analysis of variance and the means were compared with Duncan's test at the α=0.05 significance level.

RESULTS

The *Pelargonium hortorum* cultivars evaluated in this study differed significantly in their susceptibility to leaf yellowing under tissue culture conditions (Fig. 1). The shoots of 'Grand Prix' growing on the control medium (without cytokinin) had 20 times more yellow leaves as compared to cv. 'Bergpalais'. In case of the early senescing cultivar, *m*T more effectively decreased leaf yellowing than BAP. In both genotypes, the highest shoot formation occurred on *m*T-medium. *Meta*-topolin showed high anti-senescence activity also in the presence of ABA, which is known as an enhancer of senescence process [17]. In the early senescing cultivar 'Grand Prix', the addition of *m*T to ABA-medium caused a 1.5-times decrease in the number of yellow leaves, whereas BAP inhibited leaf yellowing only by 9% (Fig. 1).

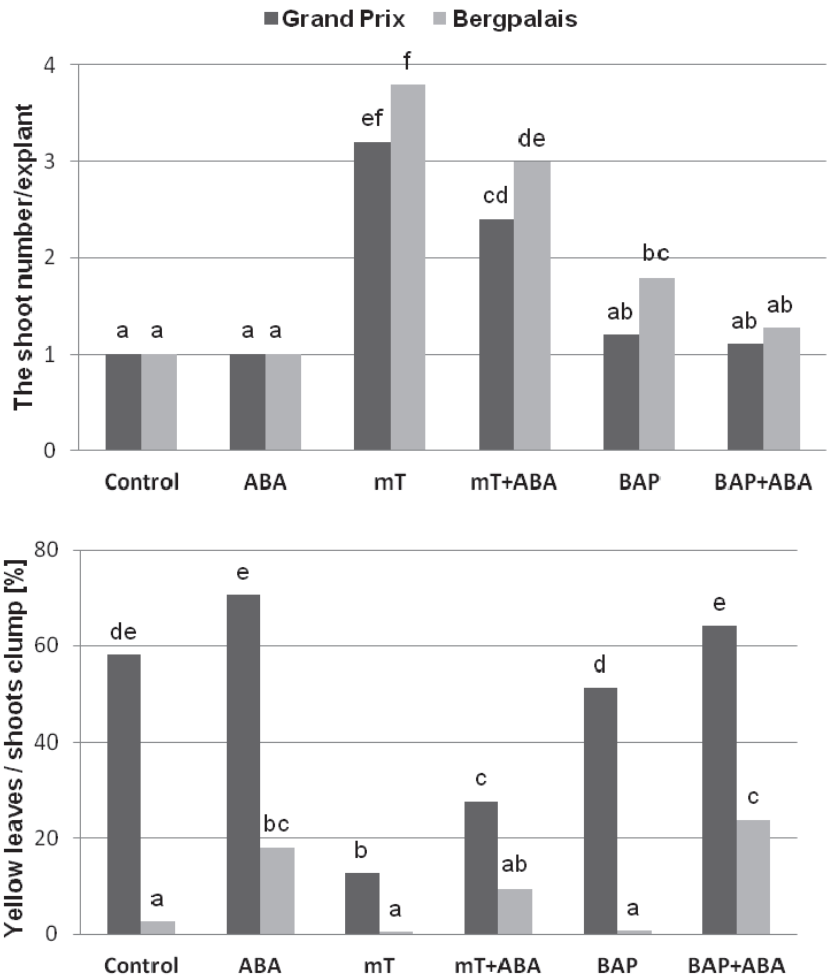

Fig. 1. The effect of different combinations of ABA (3.78 μM) and cytokinin - *m*T (2.07 μM) or BAP (0.44 μM) on the shoot formation and senescence of two *P. hortorum* cultivars after a 3-week subculture period.
Means of each growth parameter assigned the same letter do not differ significantly (α=0.05) according to Duncan's test.

The second experiment was undertaken to evaluate the production of H_2O_2 and antioxidant response in geranium shoots in the absence (control medium) and presence of different cytokinins (*m*T and BAP). In both *Pelargonium* cultivars, H_2O_2 production strongly increased between day 2 and 5 of subculture period, probably as a result of wounding (the control medium), but additionally it was stimulated by *meta*-topolin (Fig. 2). The highest H_2O_2 production was observed in late senescing cv. 'Bergpalais' in the presence of *m*T, which was the most effective in shoot formation. In all treatments, the H_2O_2 levels decreased between day 5 and 16 of subculture period and then slightly increased only in the presence of cytokinin. The highest second increase of H_2O_2 accumulation took place between day 16 and 22 in cv 'Bergpalais' in the presence of *meta*-Topolin (Fig. 2).

The study of the activity of antioxidant enzymes in *Pelargonium* shoots *in vitro* showed that CAT and POD were positively regulated by cytokinin, mainly *m*T. However, the higher activity of SOD was noted on the control medium without cytokinin (Fig. 3) and generally being higher in cv. 'Bergpalais' than in cv. 'Grand Prix'. On the control medium, SOD activity peaked on the 5th day, dropped on day 9 to increase again until the last day of measurements. In the presence of cytokinin, the activity of SOD decreased at the end of subculture.

CAT, which catalyses the decomposition of H_2O_2 into oxygen and water, showed higher activity at the earlier stage of *P. hortorum* shoot development (Fig. 4). In both cultivars, the highest CAT activity was observed on day 2 of subculture on cytokinin-medium (Fig. 4). Then, CAT activity decreased until the end of subculture period in cv. 'Bergpalais' in the presence of *m*T and in 'Grand Prix' on BAP-medium. In case of 'Grand Prix' growing in the presence of *m*T, CAT activity, after reaching the highest activity on day 2, dropped on day 5 to increase again between day 5 and 16 and further decreased until the last day of measurements (Fig. 4).

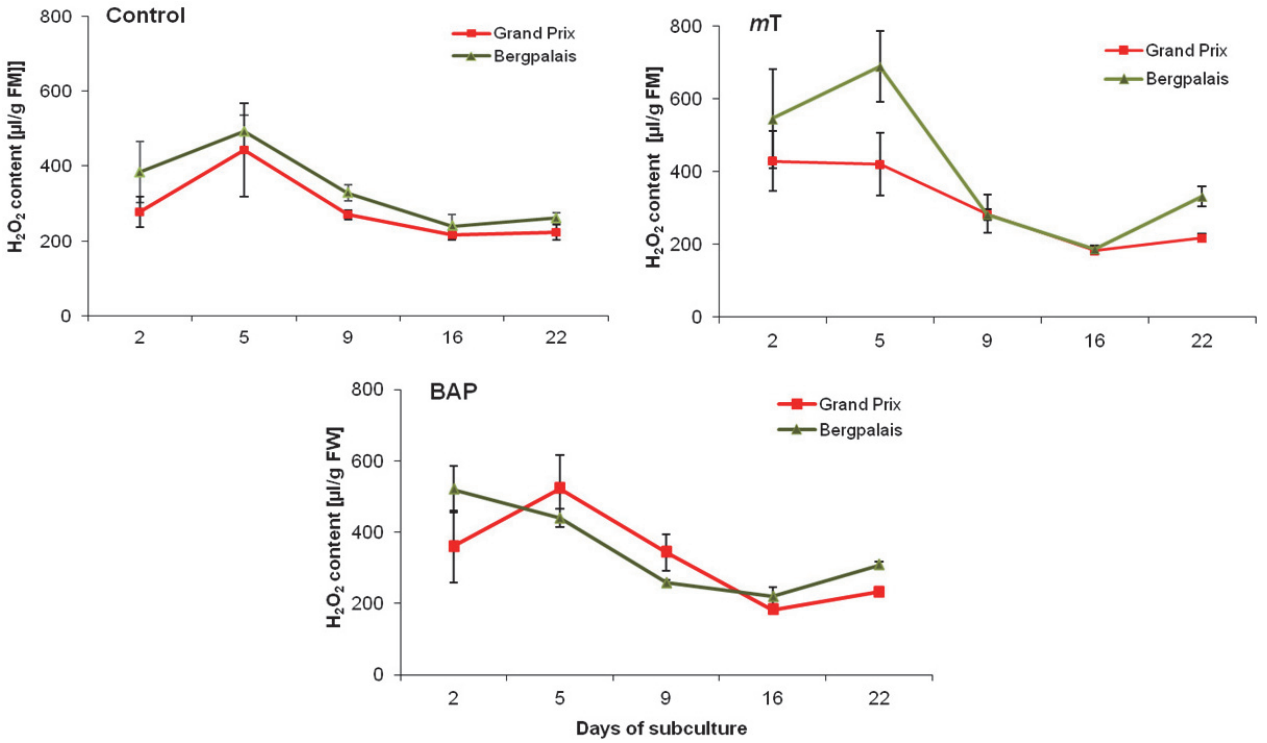

Fig. 2. The content of H_2O_2 in the 2nd, 5th, 9th, and 22nd days of subculture in shoots of *P. hortorum* 'Grand Prix' and 'Bergpalais' growing on medium containing 30 g L^{-1} sucrose, without growth regulators (Control) and treated with *m*T (2.07 µM) or BAP (0.44 µM).

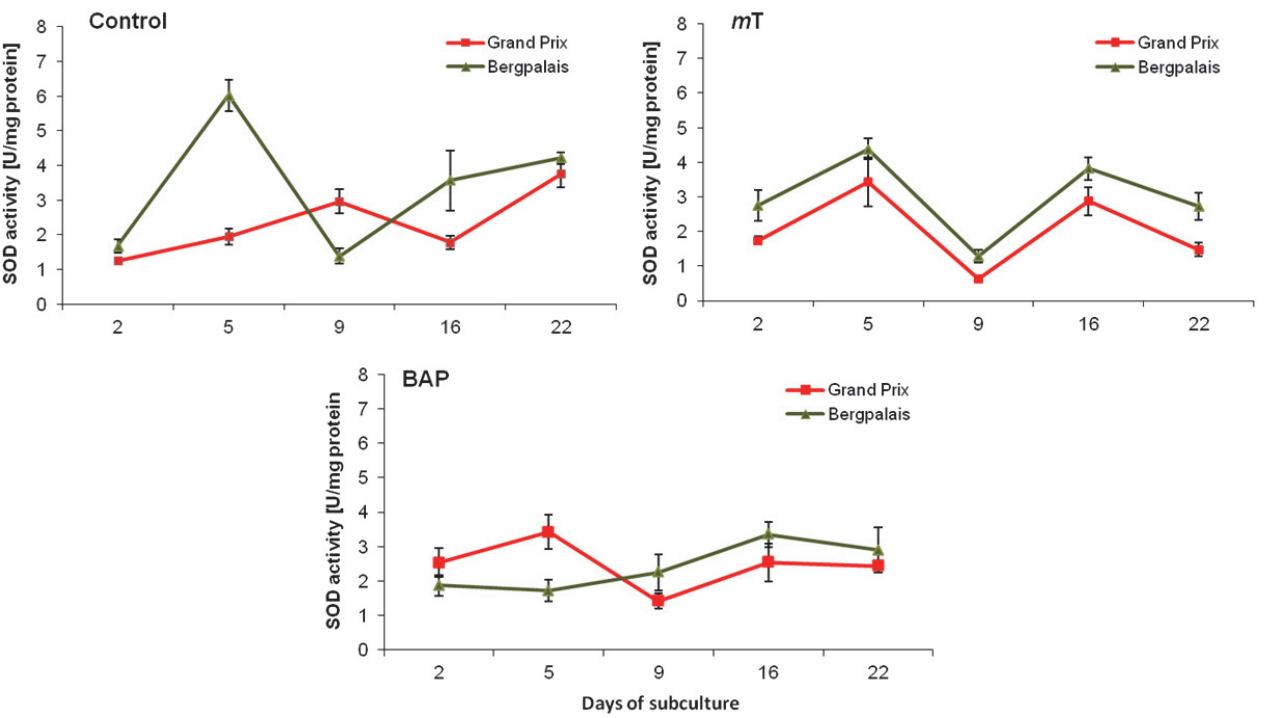

Fig. 3. The activity of superoxide dismutase (SOD) in the 2nd, 5th, 9th, and 22nd days of subculture in in shoots of *P. hortorum* 'Grand Prix' and 'Bergpalais' growing on medium containing 30 g L^{-1} sucrose, without growth regulators (Control) and treated with *m*T (2.07 µM) or BAP (0.44 µM).

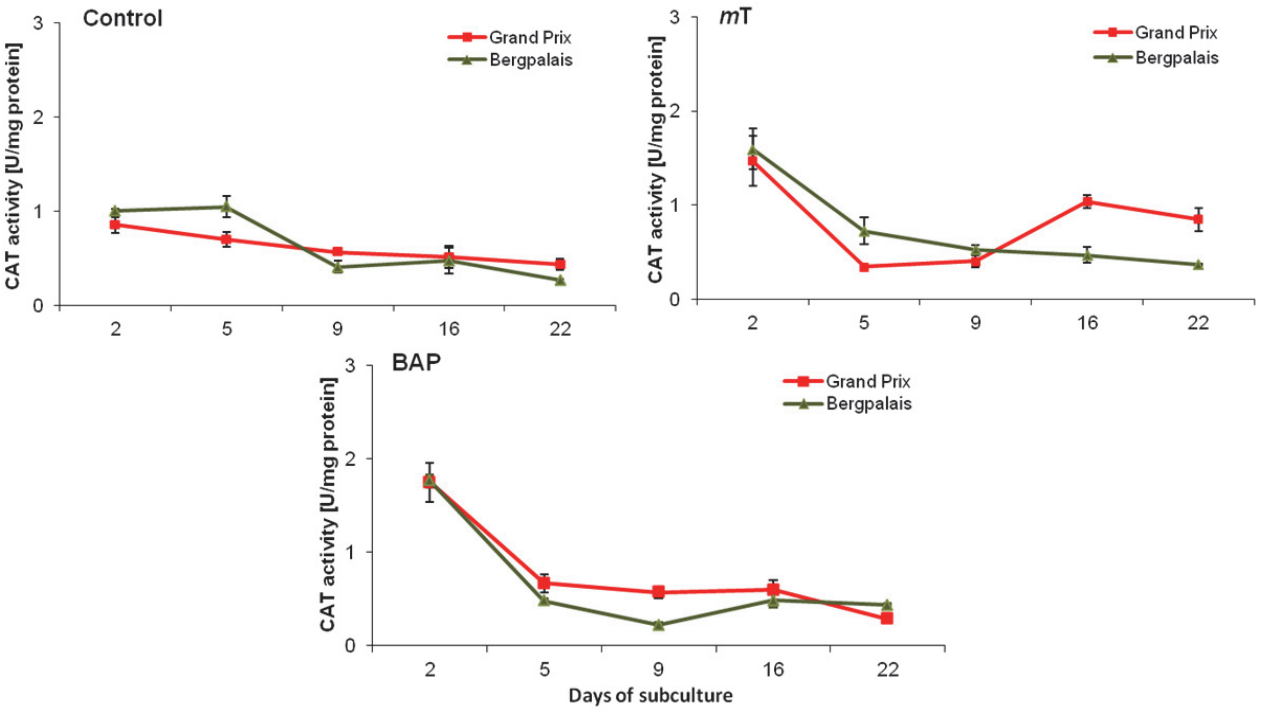

Fig. 4. The activity of catalase (CAT) in the 2nd, 5th, 9th, and 22nd days of subculture in in shoots of *P. hortorum* 'Grand Prix' and 'Bergpalais' growing on medium containing 30 g L^{-1} sucrose, without growth regulators (Control) and treated with *m*T (2.07 μM) or BAP (0.44 μM).

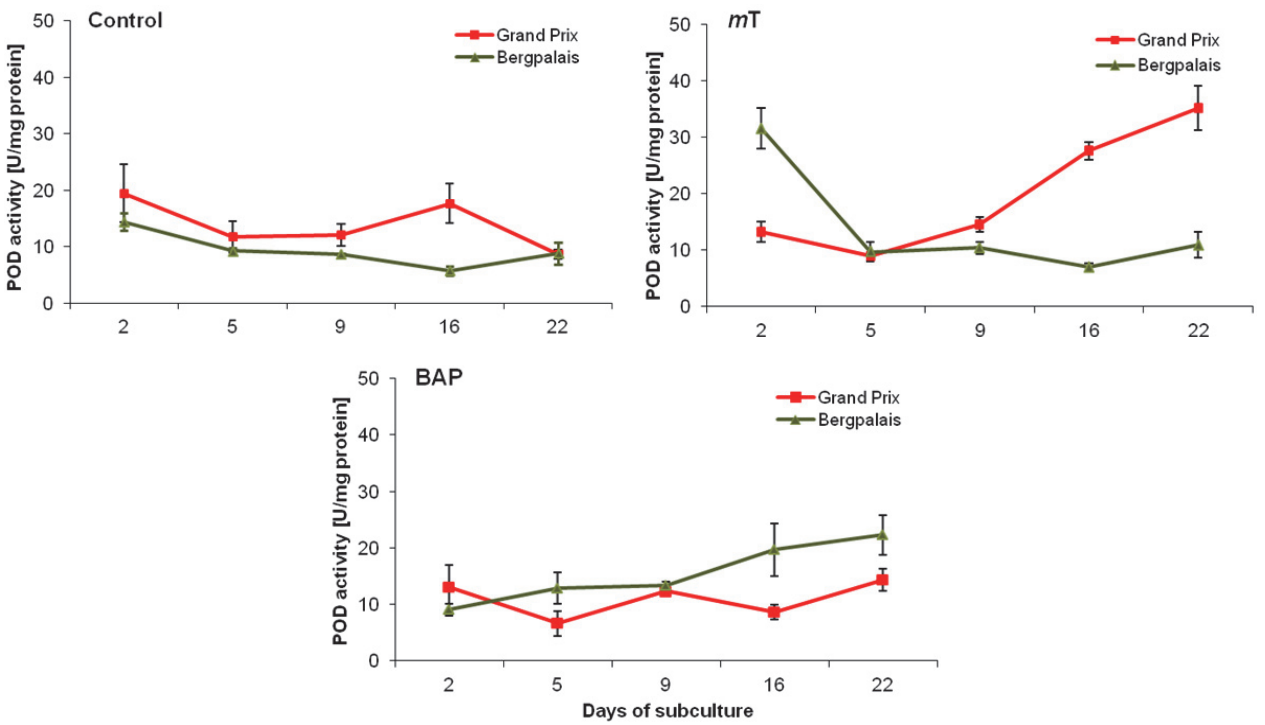

Fig. 5. The activity of peroxidases (POD) in the 2nd, 5th, 9th, and 22nd days of subculture in shoots of *P. hortorum* 'Grand Prix' and 'Bergpalais' growing on medium containing 30 g L^{-1} sucrose, without growth regulators (Control) and treated with *m*T (2.07 μM) or BAP (0.44 μM).

As shown in Fig. 5, POD activity was significantly influenced by *meta*-topolin. However, the *Pelargonium* genotypes showed enhanced POD activity in different days of subculture. The shoots of the late senescing cultivar 'Bergpalais' showed the highest POD activity on the 2nd day of subculture, over 2-fold higher as compared to cv. 'Grand Prix'. At the end of subculture, however, POD activity in early senescing *P. hortorum* 'Grand Prix' was over 3-fold higher than in 'Bergpalais'. Moreover, POD activity in 'Grand Prix' growing in the presence of *m*T was 4-fold higher in comparison with the control medium and 2.5-fold higher than on BAP-medium. The high activity of POD in the presence of *m*T at the end of subculture in *P. hortorum* 'Grand Prix' coincided with the inhibition of leaf senescence.

DISCUSSION

Early senescence of plants cultured *in vitro* is a physiological disorder leading to leaf yellowing, decreased regeneration ability, reduced shoot formation capacity, and subsequently to the death of cultures [7]. Susceptibility to early senescence greatly depends on plant species. Our previous [2, 3] and this study showed significant differences among *Pelargonium* genotypes in their susceptibility to leaf yellowing. Early senescence is widely influenced by stress conditions [18]. Plantlets cultivated *in vitro* are exposed to very stressful conditions due to wounding, abnormal mineral nutrition, unusual hormonal treatment, and high osmoticity [7, 18]. For example, MS medium provides more than 60 mM of nitrogen, which exceeds about 80 times the level normally found in the soil [19].

It is believed that enhanced stress tolerance and inhibition of senescence processes correlate with higher activity of antioxidant enzymes [20]. It has been demonstrated that a low H_2O_2 content as well as an increased activity of antioxidant enzymes or application of antioxidants enhanced regeneration potential and shoot formation capacity in different plant species, including *Lycium barbarum* [21], *Gladiolus hybridus* [22], strawberry [23], *Vicia faba* [24], and *Pinus strobus* [25]. It has been reported that cytokinins play an important role in the functioning of the antioxidant system in plants [9].

Cytokinins are plant hormones involved in the regulation of plant growth and development as well as stress response. Moreover, they are the major positive regulator of senescence [25]. Our previous [3] and this study showed that *meta*-topolin can delay senescence in *Pelargonium* shoot cultures more efficiently than BAP. This study demonstrated that *m*T partially overcame senescence induced by ABA. The inhibition of senescence by *m*T was previously observed in the wheat chlorophyll retention bioassay [26, 27], radish cotyledons [28] and *Solanum tuberosum* 'Kennebec' cultures as well as in *Zantedeschia aethiopica* fruit [29]. In wheat, *m*T retarded senescence of leaf segments by decreasing protease activity and increasing endogenous polyamines and total nitrogen content [27].

In this study, it was found that *P. hortorum* 'Bergpalais' growing on *m*T-medium produced the highest amount of H_2O_2 on 5 day of subculture. More recently, H_2O_2, in addition to being a toxicant, has been regarded as a signaling molecule that mediates responses to various stimuli [30]. As compared to early senescing cv. 'Grand Prix', higher H_2O_2 production was observed in late senescing cv. 'Bergpalais'. The enhanced H_2O_2 production in this genotype coincided with higher shoot formation. Tian *et al.* [22] reported that in strawberry callus a continuous production of low-level H_2O_2 coincided with the emergence of meristemoid and vascular tissue, and with bud formation. There are suggestions that cytokinin can act as a "switch" of defense response [31]. It has been shown that TDZ-induced morphogenesis in *Pelargonium* roots was preceded by the accumulation of various minerals and stress markers, including proline, ABA, and 4-aminobutyrate [32].

The study on antioxidant activity of *Pelargonium* shoots *in vitro* showed that the activities of CAT and POD were positively regulated by cytokinin, mainly *m*T. However, the highest activity of SOD was found on the medium without cytokinin. In both geranium genotypes, SOD and CAT levels were highest at the beginning of the subculture period, during the initiation of shoot formation. Our studies are in agreement with previous findings that SOD and CAT activities increased in young tissue [9, 22, 33]. Among the antioxidant enzymes studied, POD showed the highest activity in *Pelargonium* shoot cultures and was greatly induced by *m*T. Similarly to our study, an increase in POD activity and chlorophyll content in wheat leaf segments followed the *m*T treatment [27]. Peroxidases have been shown to be involved in response to stress [34]. Several studies have shown that POD changes its activity during maturity and senescence. However, some researchers have observed increased POD activity with advancing senescence, others when senescence was delayed [35, 36]. Our study showed that *Pelargonium* genotypes, differing in their susceptibility to leaf yellowing *in vitro,* showed enhanced POD activity in different days of subculture. It is known that peroxidases exist as multiple isoforms [34] and this might also be a reason for different POD activity in *Pelargonium* genotypes.

Geranium is one of the most economically important bedding and pot plant in the world. Solving any problems related to its micropropagation can facilitate the application of *in vitro* technique to produce disease-free elite material in breeding programs.

CONCLUSIONS

1. *Pelargonium* species varying in their susceptibility to senescence differ in H_2O_2 production and antioxidant enzymes activities.
2. The results indicated that the enhanced shoot formation capacity and inhibition of leaf yellowing in *Pelargonium hortorum* shoots growing on MS medium supplemented with *meta*-topolin coincided with higher levels of H_2O_2 and POD activity, as compared to BAP and the control medium.
3. Generally, the late senescing cv. 'Bergpalais' produced more H_2O_2 than the early senescing cv. 'Grand Prix'. The highest H_2O_2 production in cv. 'Bergpalais' was observed on *m*T-medium on day 5 of subculture.
4. In both *Pelargonium* species, superoxide SOD and CAT levels were highest at the beginning of the subculture period, as following on control and cytokinin-medium (both *m*T and BAP).
5. In both genotypes, the activity of POD was most effectively enhanced by *m*T, but on different days of subculture, at the beginning of subculture in cv. 'Bergpalais', and at the end of subculture period in case of cv. 'Grand Prix'.

Acknowledgements

This work was financially supported by grant no. N N310 726740 from the National Science Centre, Poland.

Authors' contributions

The following declarations about authors' contributions to the research have been made: concept of the study – AW, morphological observations – AW, biochemical analysis – ES, writing the paper – AW.

REFERENCES

1. Wojtania A, Węgrzynowicz-Lesiak E. Ethylene and cytokinin interaction in the morphogenesis of *Pelargonium* × *hortorum* L.H. Bailey in vitro. Acta Physiol Plant. 2012; 34(6): 2407–2412. http://dx.doi.org/10.1007/s11738-012-1005-z
2. Wojtania A, Gabryszewska E. Effect of cytokinins and amino acids on multiplication of *Pelargonium* cultivars. Acta Soc Bot Pol. 2001; 70(3): 203–207. http://dx.doi.org/10.5586/asbp.2001.026
3. Wojtania A. Effect of Meta-topolin on in vitro propagation of *Pelargonium* × *hortorum* and *Pelargonium* × *hederaefolium* cultivars. Acta Soc Bot Pol. 2010; 79(2): 101–106. http://dx.doi.org/10.5586/asbp.2010.013
4. Lim PO, Kim HJ, Gil Nam H. Leaf senescence. Ann Rev Plant Biol. 2007; 58(1): 115–136. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105316
5. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010; 48(12): 909–930. http://dx.doi.org/10.1016/j.plaphy.2010.08.016
6. Cassells AC, Curry RF. Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. Plant Cell Tiss Organ Cult. 2001; 64(2-3): 145–157. http://dx.doi.org/10.1023/A:1010692104861
7. Joyce SM, Cassells AC, Jain SM. Stress and aberrant phenotypes in vitro culture. Plant Cell Tiss Organ Cult. 2003; 74(2): 103–121. http://dx.doi.org/10.1023/A:1023911927116
8. Alscher RG, Donahue JL, Cramer CL. Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant. 1997; 100(2): 224–233. http://dx.doi.org/10.1111/j.1399-3054.1997.tb04778.x
9. Synkova H, Semoradova S, Schnablova R, Witters E, Husak M, Valcke R. 09 - Cytokinin--induced activity of antioxidant enzymes in transgenic Pssu--ipt tobacco during plant ontogeny. Biol Plant. 2006; 50(1): 31–41. http://dx.doi.org/10.1007/s10535-005-0071-0
10. Mýtinová Z, Motyka V, Haisel D, Lubovská Z, Trávníčková A, Dobrev P, et al. Antioxidant enzymatic protection during tobacco leaf ageing is affected by cytokinin depletion. Plant Growth Regul. 2011; 65(1): 23–34. http://dx.doi.org/10.1007/s10725-011-9571-4
11. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 1962; 15(3): 473–497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x
12. Ishikawa T, Takeda T, Shigeoka S, Hirayama O, Mitsunaga T. Hydrogen peroxide generation in organelles of *Euglena gracilis*. Phytochemistry. 1993; 33(6): 1297–1299. http://dx.doi.org/10.1016/0031-9422(93)85078-6
13. Lück H. Methoden der enzymatischen Analyse. In: Bergmeyer HU, editor. . Weinheim: Verlag Chemie; 1962. p. 885–894.
14. Aebi H. Catalase in vitro. Meth Enzym. 1984; 105: 121–126.
15. McCord JM, Fridovich I. Superoxide dismutase an enzimic function for erytrocuperein (hemocuperein). J Biol Chem. 1969; 244: 6049–6055.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72(1-2): 248–254. http://dx.doi.org/10.1016/0003-2697(76)90527-3
17. Yang J, Zhang J, Wang Z, Zhu Q, Liu L. Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling. Planta. 2002; 215(4): 645–652. http://dx.doi.org/10.1007/s00425-002-0789-2
18. Gaspar T, Franck T, Bisbis B, Kevers C, Jouve L, Hausman JF, et al. Concepts in plant

stress physiology. Application to plant tissue cultures. Plant Growth Regul. 2002; 37(3): 263–285. http://dx.doi.org/10.1023/A:1020835304842

19. Desjardins Y, Dubuc JF, Badr A. In vitro culture of plants: a stressful activity. Acta Hort. 2009; 812: 29–50.
20. Kukavica B, Jovanovic SV. Senescence-related changes in the antioxidant status of ginkgo and birch leaves during autumn yellowing. Physiol Plant. 2004; 122(3): 321–327. http://dx.doi.org/10.1111/j.1399-3054.2004.00410.x
21. Gupta SD, Datta S. Antioxidant enzyme activities during in vitro morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. Biol Plant. 2004; 47(2): 179–183. http://dx.doi.org/10.1023/B:BIOP.0000022248.62869.c7
22. Tian M, Gu Q, Zhu M. The involvement of hydrogen peroxide and antioxidant enzymes in the process of shoot organogenesis of strawberry callus. Plant Sci. 2003; 165(4): 701–707. http://dx.doi.org/10.1016/S0168-9452(03)00224-3
23. Skrzypek E, Szechyńska-Hebda M, Dąbrowska G. Zmiany aktywności enzymów antyoksydacyjnych podczas regeneracji kalusa bobiku (*Vicia faba* L. minor). Zesz Probl Post Nauk Rol. 2007; 53: 213–221.
24. Tang W, Newton RJ. Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos. Plant Physiol Biochem. 2005; 43(8): 760–769. http://dx.doi.org/10.1016/j.plaphy.2005.05.008
25. Jordi W, Schapendonk A, Davelaar E, Stoopen GM, Pot CS, de Visser R, et al. Increased cytokinin levels in transgenic PSAG12-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. Plant Cell Env. 2000; 23(3): 279–289. http://dx.doi.org/10.1046/j.1365-3040.2000.00544.x
26. Holub J, Hanuš J, Hanke DE, Strnad M. Biological activity of cytokinins derived from Ortho- and Meta-Hydroxybenzyladenine. Plant Growth Regul. 1998; 26(2): 109–115. http://dx.doi.org/10.1023/A:1006192619432
27. Palavan-Ünsal N, Çağ S, Çetin E. The role of meta-topolin in senescence of wheat leaf segments. J. Cell Mol. Biol. 2004; 3: 23–31.
28. Cağ S, Palavan-Ünsal N. The effect of meta-topolin on protein profile in radish cotyledons. J Cell Mol Biol. 2003; 2: 31–34.
29. Baroja-Fernández E, Aguirreolea J, Martínková H, Hanuš J, Strnad M. Aromatic cytokinins in micropropagated potato plants. Plant Physiol Biochem. 2002; 40(3): 217–224. http://dx.doi.org/10.1016/S0981-9428(02)01362-1
30. Hunk PD, Yu CW, Lin CH. Hydrogen peroxide functions as a stress signal in plants. Bot Bull Acad Sin. 2005; 45: 1–10.
31. Hare PD, Cress WA, van Staden J. The involvement of cytokinins in plant responses to environmental stress. Plant Growth Regul. 1997; 23(1-2): 79–103. http://dx.doi.org/10.1023/A:1005954525087
32. Murch SJ, KrishnaRaj S, Saxena PK. Thidiazuron-induced morphogenesis of Regal geranium (*Pelargonium domesticum*): a potential stress response. Physiol Plant. 1997; 101(1): 183–191. http://dx.doi.org/10.1111/j.1399-3054.1997.tb01835.x
33. Casano LM, Martin M, Sabater B. Sensitivity of superoxide dismutase transcript levels and activities to oxidative stress is lower in mature-senescent than in young barley leaves. Plant Physiol. 1994; 106(3): 1033–1039. http://dx.doi.org/10.1104/pp.106.3.1033
34. Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep. 2005; 24(5): 255–265. http://dx.doi.org/10.1007/s00299-005-0972-6
35. Kukavica B, Jovanovic SV. Senescence-related changes in the antioxidant status of ginkgo and birch leaves during autumn yellowing. Physiol Plant. 2004; 122(3): 321–327. http://dx.doi.org/10.1111/j.1399-3054.2004.00410.x
36. Mitrović A, Janošević D, Budimir S, Bogdanović Pristov J. Changes in antioxidative enzymes activities during *Tacitus bellus* direct shoot organogenesis. Biol Plant. 2012; 56(2): 357–361. http://dx.doi.org/10.1007/s10535-012-0098-y

Wpływ cytokinin na aktywność enzymów antyoksydacyjnych u *Pelargonium hortorum* L. H. Bayley w warunkach *in vitro*

Streszczenie

Badano wpływ cytokinin (*m*T i BAP) na tworzenie H_2O_2 i aktywność enzymów antyoksydacyjnych – dysmutazy ponadtlenkowej (SOD), katalazy (CAT) i peroksydaz (POD) w procesie tworzenia i starzenia pędów *in vitro* u odmiany pelargonii rabatowej wolno ('Bergpalais') i szybko starzejącej się ('Grand Prix').

U obydwu odmian najwyższy współczynnik mnożenia pędów i ich jakość uzyskano w obecności *m*T. Przy łącznym podaniu z ABA, *m*T znacząco obniżała żółknięcie liści u odmiany 'Grand Prix'. Odmiany pelargonii różniły się zdolnością do akumulacji H_2O_2 i aktywnością enzymów antyoksydacyjnych, a ważnym czynnikiem wpływającym na ich poziom była cytokinina, głównie *m*T. W obecności *meta*-topoliny obserwowano najwyższą produkcję H_2O_2 w 5. dniu pasażu u odmiany 'Bergpalais' i najwyższą aktywność POD u obydwu odmian, przy czym u odmiany ‚Bergpalais' na początku pasażu a u odmiany 'Grand Prix' w 22. dniu pasażu. U odmiany szybko starzejącej się, wzrost aktywności POD w obecności *meta*-topoliny, czterokrotnie wyższy w porównaniu z pożywką bez cytokininy był zbieżny z opóźnionym starzeniem pędów. U obydwu odmian, aktywność SOD i CAT była najwyższa na początku pasażu, w czasie indukcji

tworzenia pędów. Najwyższy poziom SOD obserwowano na pożywce bez cytokininy, natomiast CAT pod wpływem cytokininy, zarówno *m*T jak i BAP. Sugeruje się, iż indukowana w obecności *meta*-topoliny produkcja H_2O_2 i aktywność POD mogą odgrywać istotną rolę w procesie tworzenia i starzenia pędów *Pelargonium in vitro.*

Handling Editor: Elżbieta Weryszko-Chmielewska