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Short Communication

**THE ACTIVITY OF ANTIOXIDANT ENZYMES IN *Arabidopsis thaliana*
EXPOSED TO COLCHICINE AND H₂O₂**

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Abstract: Studies on the possible interference of colchicine and H₂O₂ with the activity of some antioxidant enzymes were carried out on *Arabidopsis thaliana* v. Columbia grown in Murashige and Skooge nutrient medium. Measurements of superoxide dismutase (SOD), guaiacol peroxidase (POX), ascorbate peroxidase (APX) and catalase (CAT) activities were conducted spectrophotometrically. In the presence of colchicine, SOD activity increased, while CAT, APX and POX activities decreased. Inhibitory H₂O₂ effects on the activity of the enzymes were found. Colchicine pre-treatment resulted in an increase in CAT activity and a further increase in SOD activity in plants treated with H₂O₂.

Key Words: *Arabidopsis thaliana*, Colchicine, H₂O₂, Catalase, Peroxidases, Superoxide Dismutase

INTRODUCTION

Colchicine is known to be a drug influencing the organization of the cytoskeletal network *via* interactions with its tubular structures. For this reason, it is widely used in experiments focusing on the mechanisms of the intracellular transport of molecules such as proteins and lipids. Colchicine and its derivatives were also recently tested as cytotoxic agents against various human tumor cell lines, due to its antitubulin activity or its inhibition of topoisomerase [9]. Additionally, the

Abbreviations used: APX - ascorbate peroxidase; CAT - catalase; POX - guaiacol peroxidase; PQ - plastoquinone; PVP - polyvinyl pyrrolidone; SOD - superoxide dismutase; UQ - ubiquinone.

anti-inflammatory and anti-fibrotic properties of colchicine were found to be of interest for medication of some pathological changes, e.g. liver fibrosis [5].

In addition to its function as the electron and proton carrier in membrane (mitochondria and bacteria) electron transport coupled to ATP synthesis, ubiquinone (UQ) acts in the reduced form as an antioxidant in mammalian and plant cells [4]. A significant increase in the level of *de novo* biosynthesis of ubiquinone and plastoquinone (PQ) in plant tissue was recently observed as the result of colchicine treatment [13]. The mechanism of this stimulation is still unknown; however, the phenomenon of the elevation of the endogenous antioxidant pool could be of great interest, also in the context of eventual pharmaceutical application.

The aim of this study is to investigate the possible involvement of the enhanced UQ pool on changes in the activity of selected enzymes involved in the response to oxidative stress in plant cells.

MATERIAL AND METHODS

Arabidopsis thaliana v. Columbia was grown in Murashige and Skooge [10] growth medium (10 mg of seeds per 100 ml of the medium) in a growing chamber at 21°C, with a 16-hour photoperiod, for 3 weeks on an orbital rotating plate (100 rpm). All the cultures were subsequently transferred to continuous light conditions for the following treatment. After 3 weeks, a subset of cultures was, when indicated, supplemented with colchicine (final conc. 0.2%), and, after 16 hours, hydrogen peroxide was added to a final concentration 0.1%. Incubation was continued for 5 hours, whereupon the plants were harvested and homogenized with 50 mM phosphate buffer (pH 7.0) containing 0.1% Triton X-100 and 1% PVP-40 [8] at 4°C. The plant biomass:extraction buffer (w:v) proportion was 1:4. The homogenate was centrifuged for 15 min at 15000g. A parallel subset of *A. thaliana* cultures which were not supplemented with colchicine was directly treated with hydrogen peroxide and homogenates were prepared as described above. Crude homogenates were used for enzymatic estimations. Two independent experiments were done.

Estimation of the activity of the selected enzymes was performed as follows. Superoxide dismutase [EC 1.15.1.1.] activity was assayed according to the method of Beauchamp and Fridovich [2]. The reaction mixture consisted of 50 mM carbonic buffer (pH 10.2), 0.1 mM EDTA, 0.1 mM xanthine and 0.025 mM nitroblue tetrazolium. Xanthine oxidase ($3.3 \cdot 10^{-6}$ mM) was added at an amount at which the increase in the rate of absorbance was 0.0165/min at 560 nm. One unit of SOD was defined as the amount of enzyme that inhibited the rate of nitroblue tetrazolium reduction by 50%. Catalase [EC 1.11.1.6] activity was determined following Aebi [1]. The rate of H₂O₂ decomposition at 240 nm was measured. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0) and 10 mM H₂O₂. Guaiacol peroxidase [EC1.11.1.7] activity was determined in the reaction mixture containing 100 mM phosphate buffer (pH 6.25), 0.012%

guaiacol and 0.03% H_2O_2 . The increase in A_{470} was observed for 3 min and calculated for 1 min [8]. Measurements of ascorbate peroxidase [EC1.11.1.11] activity were based on the method of Nakano and Asada [11]. The reaction mixture contained 50 mM phosphate buffer (pH7.0), 0.5 mM ascorbate, 0.1 mM EDTA and 0.1 mM H_2O_2 . The decrease in the A_{290} was followed.

All the measurements were carried out in triplicate at 25°C using a Shimadzu UV-160A spectrophotometer (Kyoto, Japan) equipped with a CPS 240A thermostated chamber (Shimadzu).

RESULTS AND DISCUSSION

Colchicine interfered not only with the biosynthesis of PQ and UQ [13] known as antioxidants [4, 13], but also with the enzymatic antioxidant system in *A. thaliana* (Fig. 1), while the total cellular protein content was not changed significantly (data not shown). Interestingly, the response of individual antioxidant enzymes to the treatment was different. The activities of enzymes decomposing H_2O_2 , such as catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (POX) were decreased (51%, 46% and 65% of control, respectively) as the result of colchicine treatment; by contrast, there was an increase in superoxide dismutase (SOD) activity, which reached 157% of control (Fig. 1).

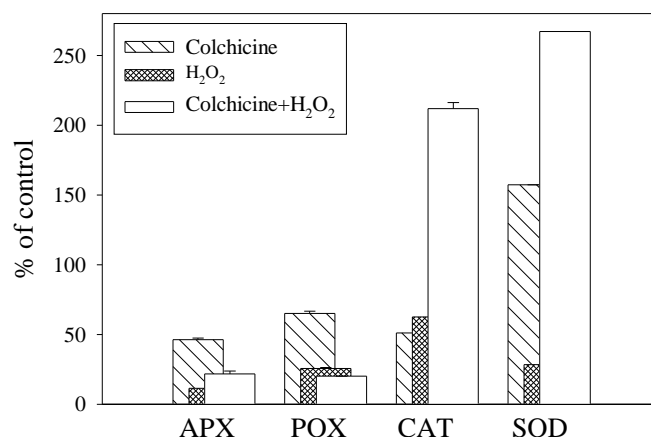


Fig. 1. The activity of antioxidant enzymes in *Arabidopsis thaliana* treated with colchicine or H_2O_2 , or pre-treated with colchicine prior to exposure to H_2O_2 . (means \pm SE).

The cultivation of *A. thaliana* in the presence of H_2O_2 resulted in diminished activities of all the studied enzymes (Fig. 1). Under these conditions, APX appeared to be the most sensitive enzyme, while CAT was the most resistant. Their activities were decreased to 11% and 63% of control, respectively. Similar results were obtained by Gueta-Dahan *et al.* [6]. They showed the inhibiting

effect of H₂O₂ on APX and SOD activities, especially on Cu/Zn-SOD, as well as a greater sensitivity of APX than SOD to H₂O₂. The different SOD isoforms show a wide range of responses with respect to H₂O₂ concentration. For example, the activity of FeSOD was inhibited by 0.075 mM H₂O₂, while CuZnSOD was stimulated by 0.1 mM H₂O₂ and inhibited by 0.25 mM H₂O₂ [3]. At a 0.1% H₂O₂ concentration, SOD activity in *A. thaliana* decreased to 28% of control (Fig. 1). Further studies are necessary to determine which SOD isoforms are inhibited in these conditions. However, in the plants pre-treated with colchicine before the addition of H₂O₂, APX activity was diminished to 22% of control, POX activity reached 20% of control, but both CAT and SOD activities increased up to 212% and 267% of control, respectively (Fig. 1). These results indicate that pre-treatment of *A. thaliana* plants with colchicine prior to exposure to H₂O₂ not only eliminated the inhibitory effect of both colchicine and H₂O₂ on CAT activity but increased this activity and enhanced the stimulatory effect of colchicine on SOD activity. In *A. thaliana*, MnSOD was unresponsive to environmental stress in contrast to FeSOD and CuZnSOD [7]. This suggests that the synergistic effect of colchicine and H₂O₂ on SOD activity (Fig. 1) could be related to the induction of CuZnSOD and/or FeSOD isoform activity. In young *A. thaliana*, oxidative stress induced CAT3 isozyme activity [12]. A similar induction could occur in those plants pre-treated with colchicine prior to H₂O₂ treatment (Fig. 1), where the extremely high SOD activity was favourable for H₂O₂ accumulation as a result of superoxide radical conversion to H₂O₂. Such an accumulation, in combination with H₂O₂ added to the growth medium of *A. thaliana*, could ensure a substrate concentration suitable for CAT activity, while being insufficient when colchicine and H₂O₂ were applied alone. Enzymes scavenging reactive oxygen species show a highly varying affinity for their substrate, and this further confirms the existence of different thresholds in the different cell compartments [3]. The beneficial effect of the observed increase of plastoquinone and ubiquinone biosynthesis under plant treatment with colchicine on the redox status of the cells [13] could also be taken into consideration.

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