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The effect of silver thiosulfate (STS) on chlorophyll content and the antioxidant enzymes activity of potato (*Solanum tuberosum* L.)

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Abstract

Potato (*Solanum tuberosum* L.) auxiliary buds c.v. White Desiree were cultured in MS medium containing 0, 50, 100, 150 and 200 µM concentrations of silver thiosulfate (STS) under *in vitro* condition. After eight weeks, the effect of silver ions (Ag^+) in the form of silver thiosulfate complex (STS), as an ethylene action inhibitor, on chlorophyll contents of leaves, ascorbate peroxidase, guaiacol peroxidase and catalase activities of roots and leaves were studied. Application of silver (STS) in culture medium increased chlorophyll content comparing to the control plants significantly. After treatments of potato plants with STS, ascorbate peroxidase and guaiacol peroxidase activities in roots were higher than shoots while catalase activity was higher in leaves than roots. However, increasing of STS concentration in the culture medium resulted in higher activities of antioxidant enzymes with some variations.

***Keywords*:** Antioxidant Enzymes, Chlorophyll Content, Potato, Silver Thiosulfate

Introduction

Potato is an important tuberous crop plant worldwide (Torabi et al., 2008). Improvement of its growth and culture condition is important under *in vitro* culture for propagation and the increase of yield. Growth and development of potato under *in vitro* culture is sensitive to generation and accumulation of ethylene in closed vessels (Ehsanpour and Jones, 2001; Perl et al., 1988; Sarkar et al., 1999). Ethylene (C_2H_4), an unsaturated hydrocarbon, is a simple plant hormone that affects some of the growth and development processes in plants (Gianinetti et al., 2007). It regulates abscission, organ senescence, ripening, and plant defense (Abeles et al., 1992). Accumulation of ethylene is associated with abnormalities in *in vitro* conditions (Chi et al., 1991). The negative effects of ethylene on potato plants can be controlled using silver ions as inhibitors of ethylene biosynthesis and action (Beyer, 1976).

Higher plant cells, as aerobic cells, require oxygen for production of energy (Shcolnick and Keren, 2006). During the H_2O production from O_2, reactive oxygen species (ROS) can be formed (Fath et al., 2002; Shao et al., 2008). The most important of these ROSs are superoxide radical ($O^{\cdot}{}_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) (Dalton et al., 1986). Excessive accumulation of ROSs in cells can cause damage to cellular macromolecules such as lipids, nucleic acids and proteins (Hernandez et al., 1993). In response to the ROS generation and accumulation, plant cells can induce their antioxidant defense systems (Larson, 1988; Mizuno et al., 2005). The formation and accumulation of ROSs may be prevented by enzymatic and non-enzymatic antioxidant defense systems. Superoxide dismutase (SOD), proxidases and catalase (CAT) are some of the antioxidant enzymes which can participate in elimination of ROSs. SOD catalyzes the dismutaion of $O^{\cdot}{}_2^-$ to H_2O_2 and O_2, whereas CAT and non-specific peroxidases destroy the generated H_2O_2 in different cell compartments (Moran et al., 1994; Anderson et al., 1995). APX, dehydroascorbate reductase (DHAR) and glutathione reductase can participate in Halliwell-Asada pathway (Ascorbate-glutathion cycle) which removes H_2O_2 in cynaobacteria and plant chloroplasts (Dalton et al., 1986; May et al., 1998). Low molecular mass antioxidants as ascorbic acid, glutathione and tocopherols are non-enzymatic defense system against ROSs (Blokhina et al., 2003).

Silver (Ag) with density of 10.5 g cm^{-3} is a heavy metal (Toppi and Gabbrielli, 1999). Silver can be uptake and transport through copper-transport

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systems in many organisms (Lee et al., 2002). Silver and copper are group IB transition metals and have similar physico-chemical traits. As first reported by Beyer (1979) and subsequently noted by others (Rodrigues et al., 1999), ethylene binding site in ethylene receptors contains Cu^{+} as cofactor that is required for high-affinity ethylene binding and silver ions (Ag^{+}) can inhibit ethylene action by substituting for Cu^{+} at ethylene receptor (Beyer, 1979). It is known that several genes involved in ethylene perception in higher plants. Silver thiosulfate complex (STS [$Ag(S_2O_3)_2^{3-}$]) is an inhibitor of ethylene action which dissociates in the plant tissues and free silver ions. These ions act efficiently as anti-ethylene agents (Veen and Van De Geijn, 1978).

Previous investigations have demonstrated that heavy metals such as cadmium can induce oxidative stress and change of antioxidant enzymes (Schuzendubell and Polle, 2002), but to our knowledge, so far no report has been published on the effect of silver (Ag), as a heavy metal, in silver thiosulfate complex (STS [$Ag(S_2O_3)_2^{3-}$]) on changes of chlorophyll content and activity of the antioxidant enzymes in potato (*Solanum tuberosum* L.) c.v. White Desiree under *in vitro* condition.

In this study, we aimed to understand the effect of silver thiosulfate (STS) as an ethylene action inhibitor on chlorophyll content and the activity of antioxidant enzymes in potato plant.

Materials and Methods

Plant material and culture conditions

Potato plants, cultivar White Desiree, were propagated on MS (Murashig and Skoog, 1962) medium supplemented with agar (1% w/v) and sucrose (3% w/v), pH 5.8. Then, auxiliary buds were transferred to MS medium containing concentrations of 0 (control), 50, 100, 150 and 200 µM STS. All cultures were then kept in the culture room with a 16/8-h light/dark photoperiod with 2000 Lux intensity at 25 ± 2 ˚C for eight weeks. STS solutions were prepared by mixing 800 µM $AgNO_3$ and 3200 µM $Na_2S_2O_3.5H_2O$ in 1:1 ratio based on the method of Ehsanpour and Jones (2001).

Chlorophyll measurement

Total chlorophyll content from 0.1 g fresh leaves from eight-week-old potato plants was extracted. According to the method of Arnon (1949) using 80% acetone in darkness and measured at 645 and 663 nm by spectrophotometer.

Protein extraction and enzyme assay

For protein and enzyme extraction 0.1 g of fresh leaf and root from eight-week-old plants were homogenized using a mortar and pestle with 1 ml of 100 mM sodium phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The whole extraction procedure was carried out on ice. The homogenates were then centrifuged for 30 min at 14000 rpm at 4˚C and supernatants were used for protein and enzyme activity measurement.

Ascorbate peroxidase (APX, EC 1.11.1.11) and guaiacol peroxidase (GP, EC 1.11.1.7) activities were determined according to the method of Nakano and Asada (1981). The reaction buffer for APX activity contained 50 mM sodium phosphate buffer (pH 7), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.25 mM H_2O_2 and 0.02 ml enzyme extract in a final volume of 1ml. Ascorbate oxidation was measured at 290 nm at extinction coefficient of 2.8 mM^{-1} cm^{-1}. For GP activity mixture (1 ml) contained 50 mM sodium phosphate buffer (pH 7), 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.1 ml enzyme extract. Enzyme activity was assayed by monitoring formation of tetraguaiacol from guaiacol at 470 nm at extinction coefficient of 26.6 mM^{-1} cm^{-1} in the presence H_2O_2.

Catalase (CAT, EC 1.11.1.6) activity assay was also carried out according to the method of Aebi (1984). The decrease in H_2O_2 was measured at 240 nm and activity was calculated as µM H_2O_2 consumed per minute (extinction coefficient 39.4 mM^{-1} cm^{-1}).

Total soluble protein was determined using modified Bradford (1976) method described by Olson and Markwell (2007). Bovine serum albumin as the standard protein was used.

Statistical analysis

All experiments were carried out in three replications and mean values ± standard deviation were presented. Data were subjected to ANOVA and the mean differences were compared by Dunkan test at $p < 0.05$.

Results

Presence of silver thiosulfate (STS) in culture medium increased the total chlorophyll content of plants in comparison with the control. The highest amount of chlorophyll was observed at 100, 150 and 200 µM STS while, the lowest amount of chlorophyll was observed in the medium without STS (figure 1).

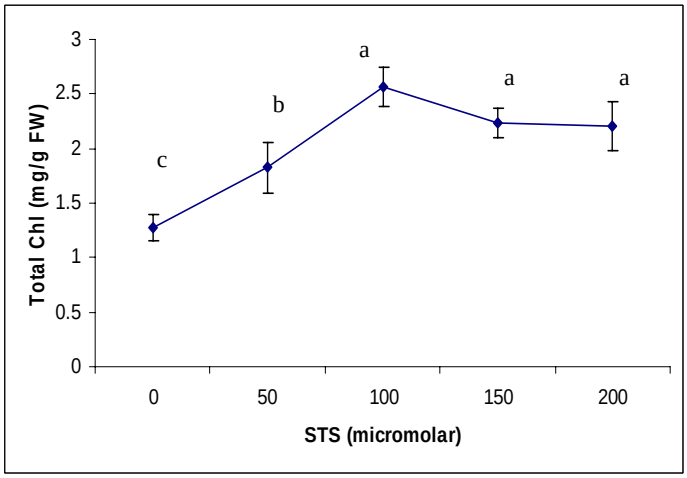

Figure 1. Effect of STS on chlorophyll content of potato leaf c.v. White Desiree. Values are means of three replications ± Std. Uncommon letters are significant ($p < 0.05$) based on the Duncan test.

By increasing the STS concentration in the culture media, the activity of the assayed enzyme was also increased in roots. So that the APX activity was increased significantly at 200 µM STS whereas, the activity of GP and CAT increased at 100 µM STS. The activity of APX and GP in roots was much higher than that in leaves. In contrast, CAT activity in leaves was higher than roots and did not show a significant difference in leaves of potato (figure 2).

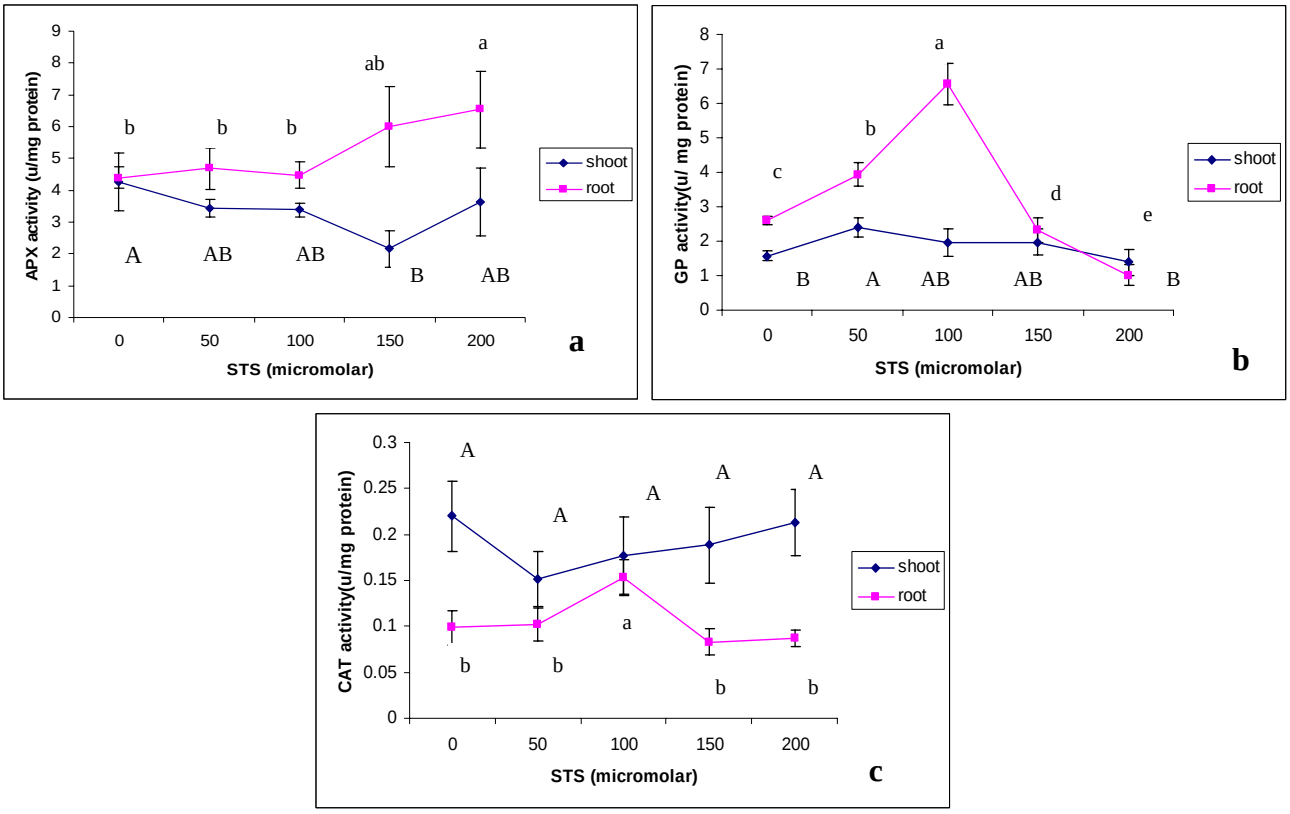

Figure 2. The effect of STS on (a) Ascorbate peroxidase (APX), (b) guaiacol peroxidase (GP) and (c) Catalase (CAT) activities in roots and leaves of potato cultivar White Desiree. Values are means of three replications ± Std. Uncommon letters are significant ($p < 0.05$) based on the Duncan test.

Discussion

Chlorophyll has a unique and essential role in higher plants (Eckhardt et al., 2004). Biosynthesis and breakdown of chlorophyll in plants are complex pathways that are regulated by different factors. It has been documented that ethylene has a negative effect on chlorophyll content of plants (Jona et al., 1997). For example, Jakob-Will et al., (1999) reported that ethylene induced expression of chlorophyllase genes (Chlase) in Citrus fruits, but inhibition of ethylene action by STS increases the chlorophyll content. The STS also increases the leaf area (Ehsanpour and Jones, 2001; Perl et al., 1988) as well as, chlorophyll content, viability and the number of protoplasts in potato cultivar Delaware (Ehsanpour and Jones, 2001). In the present study, we found similar results in chlorophyll content by increaseing the STS concentration. Increasing of the chlorophyll content and leaf area may be due to the inhibition of ethylene action by STS treatment. It has been reported that accumulation of ethylene and depletion of oxygen in thigthly closed vessel is associated with various morphological abnormalities during *in vitro* plant tissue culture (Ehsanpour and Jones, 2001; Perl et al., 1988; Sarkar et al., 1999; Sarkar et al., 2002). Therefore, the potato growth can be improved by suppression of ethylene action using the STS treatment.

Heavy metal-induced changes in CAT, APX and GP activities has already been reported (Gallego et al., 1996; Chaoui et al., 1997; Gallego et al., 1999; Roa & Sresty, 2000). In our study, APX and GP activities were much higher in roots while CAT showed lower activity in roots. The high level of APX and GP activities in roots indicated efficient conversion of H_2O_2 to H_2O. In contrast, the CAT activity in roots was lower than shoots, it is possibly due to some compensation mechanism between APX and CAT enzymes. However, the details of this hypothesis will need to be studied in the future. CAT activity remained without significant changes in comparison to the control plants. Although catalase may be present in all plant cells, it tends to be restricted largely to peroxisomes. The Catalase has a high *K*m for H_2O_2, as substrate, and this enzyme alone can not be sufficient for omitting and degrading all the generated H_2O_2 (Halliwell, 1974). Thereby, according to our study, the catalase seems poorly suited scavenger for H_2O_2 in root of potato plant c.v. White Desiree under STS treatment and other enzymatic (APX and GP) and non-enzymatic pathways could also cooperate to detoxify ROSs in the root tissues.

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