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Research Article

CHANGES OCCURRING IN CANOLA (*BRASSICA NAPUS* L.) IN RESPONSE SILVER NANOPARTICLES TREATMENT UNDER *IN VITRO* CONDITIONS

Zahra Tabatabaee Pozveh[1], [*]Roya Razavizadeh[2] and Fatemeh Rostami[2]
[1]*Department of Biotechnology, Payamenoor University, Isfahan, Iran*
[2]*Department of Biology, Payame Noor University, PO BOX 19395 Tehran, Iran*
**Author for Correspondence*

ABSTRACT
The effect of nanosilver was investigated on protein pattern changes, silver accumulation, alterations in antioxidant capacity and reduced sugars in canola (*Brassica napus* L.) cultivar Ocapy under *in vitro* conditions. The grown seedlings on MS medium were subjected to MS medium containing 0, 0.5, 1, 1.5 and 2 ppm concentrations of silver nanoparticles for four weeks. Application of different concentrations of nanosilver had not significant effect on the total soluble protein in shoot but decreased protein content in 1.5 ppm concentration in root. The study of shoot and root protein patterns using one dimension gel electrophoresis (SDS-PAGE) and consequence analysis of bands by ImageJ program showed some remarkable changes. Relative expression of three proteins in shoot and five proteins in root were changes in response to nanosilver treatment. Silver accumulation was detected in shoot tissue only. There were some changes in antioxidant enzymes activity. The reduced sugars were increased in 1.5 ppm of nanosilver comparing with the control plants in shoot. It seems that the nanosilver at 1.5 ppm concentration is the best treatment of nanosilver as an ethylene action inhibitor under *in vitro* condition for *B. napus* L. cultivar Ocapy in this study.

INTRODUCTION
Nanosilver is a nanoparticle of the metal element silver between 1 and 100 nanometers (nm). The most common application of nanosilver is as an antimicrobial agent (Becker, 1999). Nano silver particles, also, have more surface area in contact to outer space due to their small size. Thus, the amount of adhesion to the cell surface is increasing which lead to their higher efficacy (Shah and Belozerova, 2008). Silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc (Henglein,1989).
Additionally, nano-Silver may affect the metabolism, respiration and reproduction of microorganism (Lok *et al.*, 2007). For example, the effect of nano-Silver on extend maintenance period of leaves (from 2 to 21 days) in asparagus plant is reported. Also, during this period the amount of ascorbat, chlorophyll and fiber were more in treated leaves (An *et al.*, 2008). Ethylene (C2H4), 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 development (Chi *et al.*, 1991) such as production and development of stoloniferous shoots and small leaves during short and long- term tissue culture of potato explants (Sarkar *et al.*, 2002; Sarkar *et al.*, 1999; Perl *et al.*, 1988). The negative effects of ethylene on plants under in vitro culture can be controlled using silver ions as an ethylene inhibitor action (Veen, 1987). Strader *et al.*, (2009) found that in addition to blocking ethylene signaling, Ag^+ promotes IAA efflux. Silver supplementation to the medium has been used to control and improve plant regeneration and transformation *in vitro* (Kumar *et al.*, 1998; Kumar *et al.*, 2007). Improvements were achieved despite the phytotoxicity of $AgNO_3$ (Ratte, 1999). Ag^+ reacts not only with halide ions; it also bonds to various compounds such as lipo-polysaccharides, amino acids, proteins, RNA, and DNA to form silver nanoparticles, a property frequently used for biochemical analyses (Blum *et al.*, 1987; Shevchenko *et al.*, 1996; Tsai and Frasch, 1982). It has been reported that ethylene induced 33 KD and 60 KD peroxidases during senescence of cucumber (*Cucumis sativus* L.) cultivar Poinsett 76

(Abels *et al.,* 1988). It has also been reported an increase in some proteins by exogenouse application of ethylene using SDS-PAGE. It is believed that silver ion, as an inhibitor of ethylene action, reveals its effects by interaction with the ethylene percention mechanism (Davies *et al.,* 1988). Proteins are compounds of fundamental importance for all functions in the cell (Dose, 1980). It is well known that alteration of gene expression is always involved in plants under specific culture condition. Protein variation is an essential part of plant response to stress as well as for adaptaion to environmental conditions (Vierstra, 1933; Hieng *et al.,* 2004). Proteins are final products of informational pathways in cells that produce in response to cellular needs and transfer to proper location in cells (Rostami and Ehsanpour, 2009). Higher plant cells have evolved enzymatic and non-enzymatic antioxidant defense systems in order to reduce ROSs accumulation and oxidative damages by detoxifying free radicals (Borsani *et al.,* 2001). Superoxide dismutase (SOD), Ascorbate proxidases (APX) and catalase (CAT) are some of the antioxidant enzymes which can participate in elimination of ROSs. 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 (GR) 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).

Superoxide Dismutase activities: The activity of superoxide dismutase was assayed by measuring its ability to inhibit to the photochemical reduction of nitroblue tetrazolium. Exposure to abiotic stresses such as drought, Cold, heat, and pollutants, including herbicides and heavy metals, can give rise to excess accumulation of reactive oxygen species (ROS) in plant cells (Price *et al.,* 1989). ROS are potentially harmful to the cell, as they can raise the level of oxidative damage through loss of cellular structure and function. Among all the antioxidative enzymes, superoxide dismutase (SOD) plays key roles in ROS detoxification in cells. SOD, the first enzyme in the detoxifying process, converts superoxide anion radicals (O-2) to hydrogen peroxide (H_2O_2) (Asada, 2006) which is broken down to oxygen and water.

MATERIALS AND METHODS

Plant Material and Culture Conditions

Canola seeds (*Brassica napus* L.) were provided by the Oil Seed Cultivation Company, Isfahan-Iran. PVP-coated Ag were purchased from the Nanopasargad Company, Tehran-Iran. The NP suspensions were analyzed with dynamic light scattering (DLS) to determine NPs size distribution. The size distributions were 54.39 nm and 288.5 nm. Initially, the seeds were planted on MS (Murashing and Skoog, 1962) solid medium (agar 1%w/v) supplemented with sucrose (3% w/v) and pH 5.8 then auxiliary buds of canola were transferred to MS medium containing concentration of 0 (control), 0.5, 1, 1.5, 2 ppm nanosilver and kept in culture room with a 16-h photoperiod at 25±2 c° for four weeks.

Protein Extraction

Approximately 0.1 gram of fresh stem-leaf and root from four-week-old plants were homogenized in liquid nitrogen, then protein extraction was carried out according to the method of (Rostami and Ehsanpour, 2009) using extraction buffer (50 mM Tris-HCL, 1m M DTT, 2 mM EDTA, 2 mM 2-Mercaptoethanol, pH 7.5). For extraction of proteins buffer modified as 1mM PMSF and pH 7.2. Total soluble protein (mg g^{1-} FW) in extracts of protein from stem-leaf and root tissues were determined according to modified Bradford (1976) method described by Olson and Markwell (2007) using bovin serum albumin as standard protein. SDS-PAGE was performed using 12% separating and 5% stacking gels (Laemmeli, 1970). After electrophoresis at 100 V, protein bands were stained using silver nitrate (Salehi and McCarthy, 2002) and finally protein bands showed remarkable changes were analyzed by ImageJ software.

Silver Measurement

Stem-leaf and root tissues from four-week-old canola oven-dried at 70 C° for 48h, silver ion content were then measured (µg g^{1-} DW) according to the method described by Reeves *et al.,* (1999).

Enzyme Extraction and Assay

Catalase (CAT, EC 1.11.1.6) activity assay was also carried out according to the method of Aebi (1984). For enzyme extraction 0.1 g of shoot and 0.03 g of root from 4 weeks 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 catalase activity measurement. The decrease in H2O2 was measured at 240 nm and activity was calculated as µM H2O2 consumed per minute (extinction coefficient 39.4 mM-1 cm-1). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to the method of Sreenvasula *et al.,* (2000). The reaction buffer for APX activity contained 50 mM sodium phosphate buffer (pH 7.8), 5 mM ascorbic acid, 5 mM EDTA, 5 mM DTT, 100 mM NaCl, 2% PVP, 44 µM H_2O_2 and 0.05 ml enzyme extract in a final volume of 1 ml. Ascorbate oxidation was measured at 290 nm for 1 min with extinction coefficient of 2.8 mM-1 cm-1. Superoxid dismutase activity was determined according to method of Cackmak and Horst (1991); Giannoplitis and Ries (1977). The reaction buffer for SOD activity contained 50 mM HEPES-KOH (pH 7.8), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH 10.2), 12 mM L-Met, 75 µM NBT (Nitroblue tetrazollium chloride), 1 µM Riboflavin and 0.1 ml enzyme extract in a final volume of 3 ml. Superoxid dismutase activity was measured at 560 nm after 10 min incubation at room temrature under continuous light. One SOD unit was definded as the amount of enzyme (volume of enzyme extract) that inhibits the rate of NBT reduction by 50% under the above assay conditions.

Measurement of Reducing Sugars

Reducing sugar content was measured by adapting Somogyi-Nelson's method (Nelson, 1944; Somogyi, 1952). Approximately 0.1 g of fresh stem-leaf and 0.03 g of root from 4 weeks old plants were extracted with 10 ml distilled water. The mixture was boiled in a boiling water bath, cooled and filtered. Then 2 ml of the extract was mixed with 2 ml of alkaline copper tartarate and the reaction mixture was heated for 20 min (Alkaline copper tartarate was prepared by dissolving 4 g anhydrous sodium carbonate, 0.75 g tartaric acid and 0.45 g hydrated cupric sulphate in 80 ml of distilled water and finally made up to 100 ml). 2 ml of phosphomolibdate solution was added and the intensity of blue color was measured at 600 nm using spectrophotometer. D-glucose was used as standard. The reducing sugar content was expressed in terms of percentage on fresh weight basis. All experiments were carried out in three replications. Data were subjected to ANOVA and the mean differences were ompared by Dunkan test at $p<0.05$.

RESULTS AND DISCUSSION

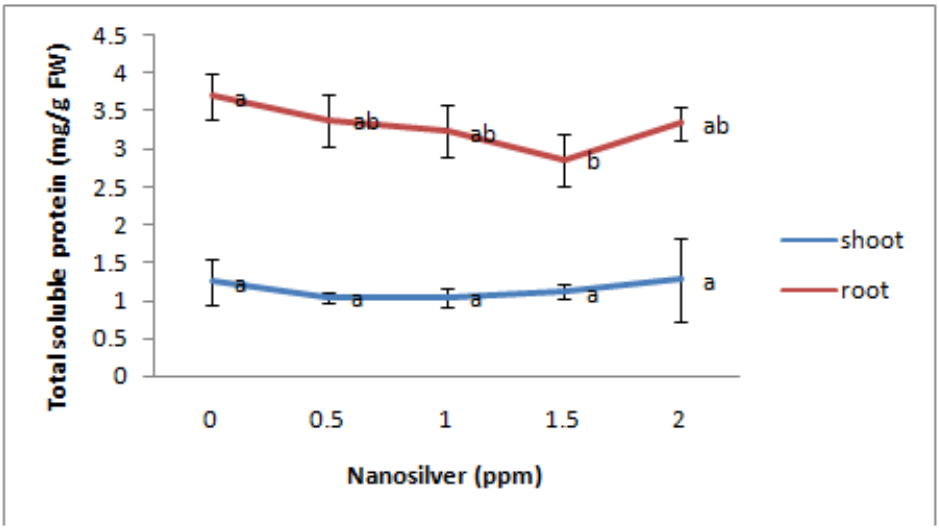

Figure 1: Total soluble protein of stem-leaf (a) and root (b) of canola cultivar Ocapy. Common letters are not significant (P<0.05) based on Duncan test

When protein patterns of stem-leaf and roots were analyzed by SDS-PAGE on four-week-old canola, expression of some specific protein bands were up regulated or down regulated in response to Nanosilver. SDS-PAGE pattern (Figure 2) showed less that 175 KD and around 14 KD and around 10.5 KD protein bands were up-regulated in root while around 42 KD and less than 29 KD and around 22 KD protein bands were up-regulated in stem-leaf at concentration of 2 ppm Nanosilver. Moreover, more than 175 KD and around 10.5 KD protein bands were up-regulated in root at concentration of 0.5 ppm Nanosilver. However, increasing of Nanosilver concentration caused down regulation of between 42 and 51 KD and more than 14 KD protein band in root at concentration of 1 and 1.5 ppm. In 2 ppm of nanosilver there was no protein band to detect. In other hand, increasing of Nanosilver concentration caused up-regulated of around 42 KD and 29 KD protein bands in stem-leaf at 1.5 ppm.

Analysis of relative intensitv of protein bands in stem-leaf showed significant difference in protein band at different concentration of a nosilver treatment (Figure 3).

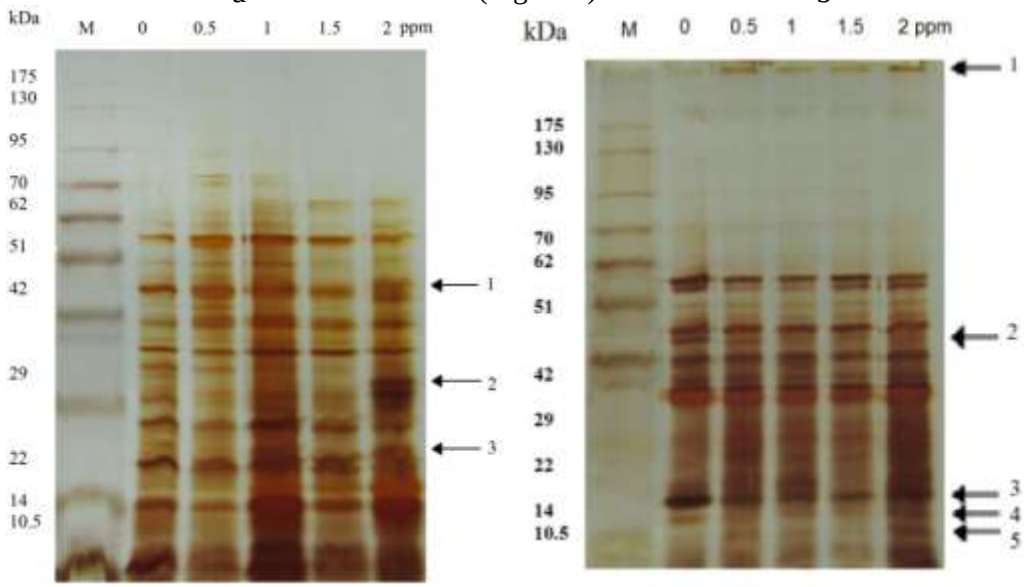

Figure 2: SDS-PAGE pattern of soluble proteins from stem-leaf (a) and root (b) of canola cultivar Ocapy. The arrows indicate the protein bands with changes of expression levels. M – Protein marker

The protein level SDS-PAGE analysis revealed remarkable differences in Nanosilver treated in comparison to untreated plants. Three proteins in stem-leaf and five proteins in root showed some changes for example most of protein bands up regulated due to Nanosilver treatments. The expression of some proteins were increased (more than 175, around 14, around 10.5 in roots and more than 42, around 29 and around 22 KD in stem-leaf) by Nanosilver. There were some increase in expression of protein bands in another researchs in shoot of potato in 20, 116 and 120 kD by use of nanosilver (Nejati, 2011) and in 17, 40, 50 and 100 KD by use of STS (Rostami, 2009). Increase in bands of 40 and 50 KD in potato ,by use of STS, is similar to increase in 42-51 KD protein band in this research. These proteins may have a role in response of canola to Nanosilver or have a critical role in adaptation to nanosilver and development of canola (*Brassica napus* L.) in mediums containing nanosilver.

Exposure of plants to heavy metals induces many responses at cellular and molecular levels. To understand the moleculalar basis of canola response to nanosilver, as an inhibitor of ethylene action, variation of protein pattern was detected using SDS-PAGE. Some studies have demonstrated that, heavy metals such as Cd, Pb, Ni and Ag changed the total protein amount in plants (Ewais, 1997; Rostami and Ehsanpour, 2009). In our study the total proteins did not change and no significant differences were observed after application of nanosilver in stem-leaf but decreased in roots in 1.5 ppm of nanosilver

treatment. Silver ions inhibit ethylene action by substituting for Cu^{+} at ethylene receptor site (Beyer, 1979) and probably nanosilver induces protein alternations of stem-leaf and roots of canola. Addition of nanosilver to the culture medium changed growth and activity of antioxidant enzymes in canola under *in vitro* culture. These results showed that application of nanosilver in canola (*Brassica napus* L.) regulates the expression of some specific proteins. In another study on *Arabidopsis thaliana* many genes differentially expressed by AgNPs and Ag^{+} were found to be involved in the response of plants to various stresses: upregulated genes were primarily associated with the response to metals and oxidative stress (e.g., vacuolar cation/proton exchanger, superoxide dismutase, cytochrome P450-dependent oxidase, and peroxidase), while downregulated genes were more associated with response to pathogens and hormonal stimuli [e.g., auxin-regulated gene involved in organ size (ARGOS), ethylene signaling pathway, and systemic acquired resistance (SAR) against fungi and bacteria] (Kaveh *et al.*, 2013). So in this study increase of expression in some protein bands may shoes upregulated genes to response to metals and oxidative stress and decrease of expression in some protein bands may shoes downregulated genes involved in ethylene signaling pathway, and systemic acquired resistance (SAR) against fungi and bacteria.

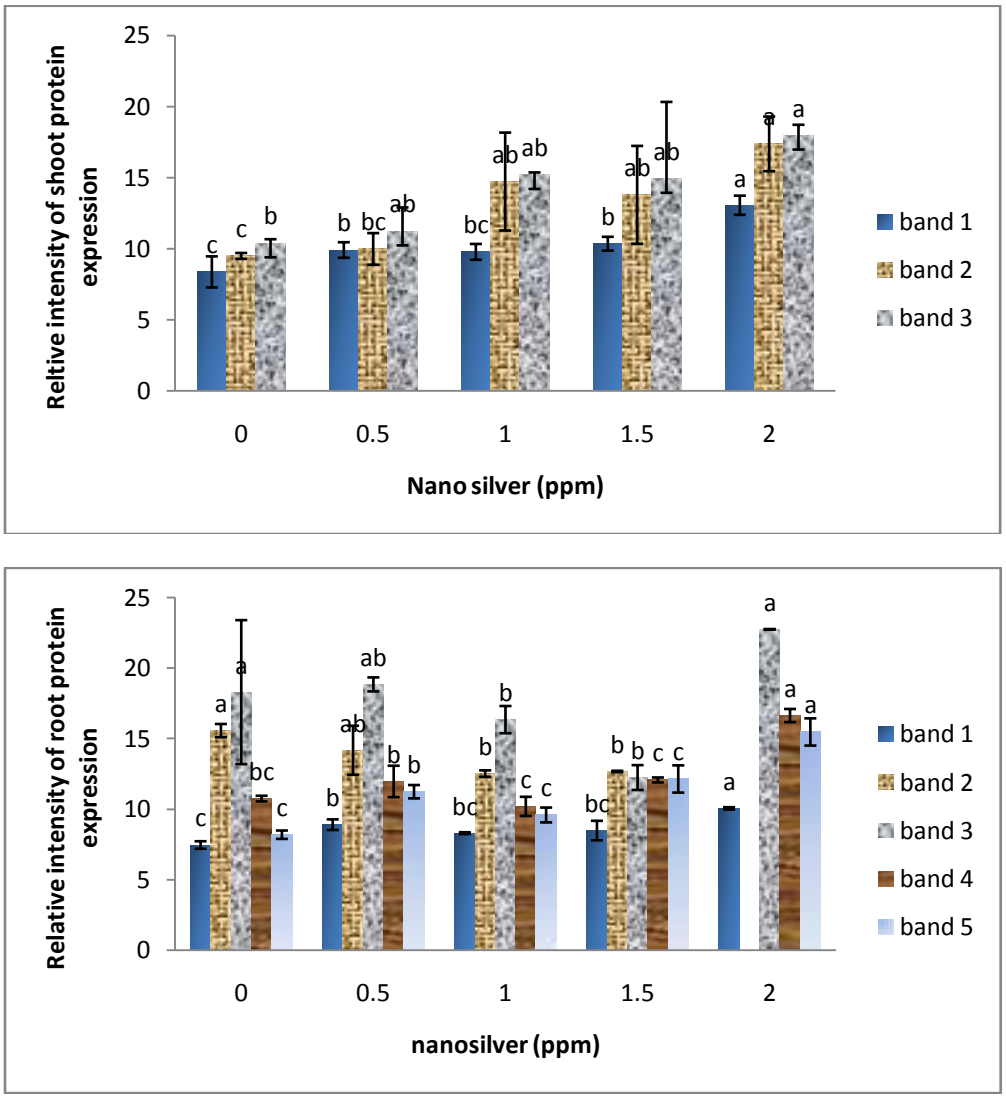

Figure 3: Relative levels of protein expression in stem-leaf (a) and root (b) of canola in response to different concentration of Nanosilver (0, 0.5, 1, 1.5, 2 ppm). Uncommon letters are significant ($p<0.05$) based on Duncan test

The amount of silver content in shoots of canola (*Brassica napus* L.) grown at 0, 0.5, 1.5 and 2 ppm nanosilver is plotted in (Figure 4). Application of nanosilver caused significant difference of silver ion accumulation at 0.5, 1, 1.5 and 2 ppm nanosilver compared to the non treated plants in shoot tissues. Maximum silver content was observed at 2 ppm concentration of nanosilver. When silver content from root was measured, no silver ion was detected.

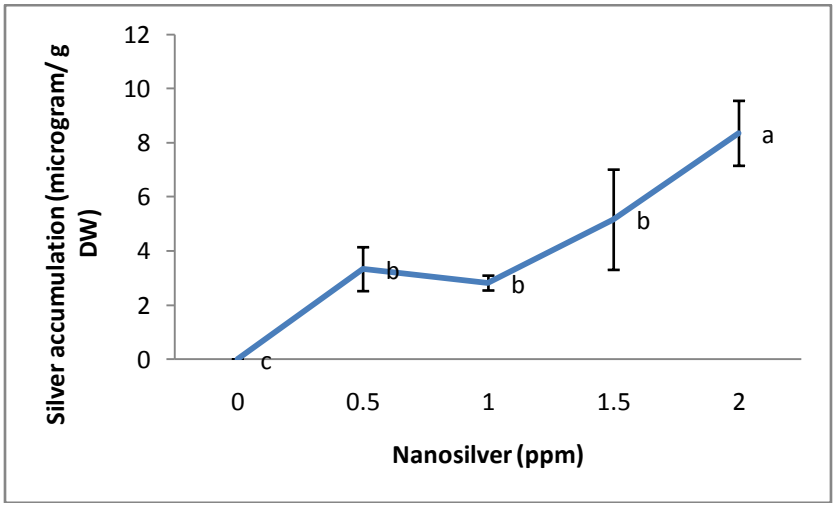

Figure 4: Silver content in canola shoot. Data are means of three replications. Uncommon letters are significant (P<0.05) based on Duncan test

In another study There was no significant difference between 100 ppm of silver nitrate and 60 ppm treatment of nano silver on the shoot silver concentration in *Borago officinalis*. Therefore, permeability of nanosilver is far greater than silver nitrate. The reason of this matter is the small size of nano particle, which causes more adhesion of nano particles to plant tissues (Seif, 2011). In another study, treatment of silver thiosulfate (STS) in medium of potato showed presence of silver ion in root tissues only that indicate less mobility of silver ions compare with nanosilver in plants. Variations in the antioxidant levels can serve as a signal for the modulation of ROSs scavenging mechanisms and ROSs signal transduction (Mittler, 2002). The activity of APX increased significantly in 1.5 ppm of nanosilver in shoots. Catalas activity had no significant changes comparing with control in shoots but it decreased in 2ppm comparing with 0.5 ppm of nanosilver. Catalas activity in roots increased in 0.5 ppm of nanosilver. The activity of superoxide dismutase in shoots decreased in all treatments of nanosilver but it increased in 2 ppm of nanosilver in roots (Figure 5).

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 and Sresty, 2000). The high level of APX activities in shoots indicated efficient conversion of H_2O_2 to H_2O. CAT activity in shoot 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 cannot 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 shoot of canola plant under STS treatment and other enzymatic (APX and SOD) and non-enzymatic pathways could also cooperate to detoxify ROSs in the shoot tissues. SOD enzymes are involved in antioxidation during stress which dismutates superoxide into hydrogen peroxide and molecular oxygen.

Hydrogen peroxide, produced by superoxide dismutase, is scavenged by catalase and a variety of peroxidase with concomitant oxidation of co-substrates such as phenolic compounds and/ or antioxidant molecules (Usha, 2008; Jyothsna *et al.*, 2009).

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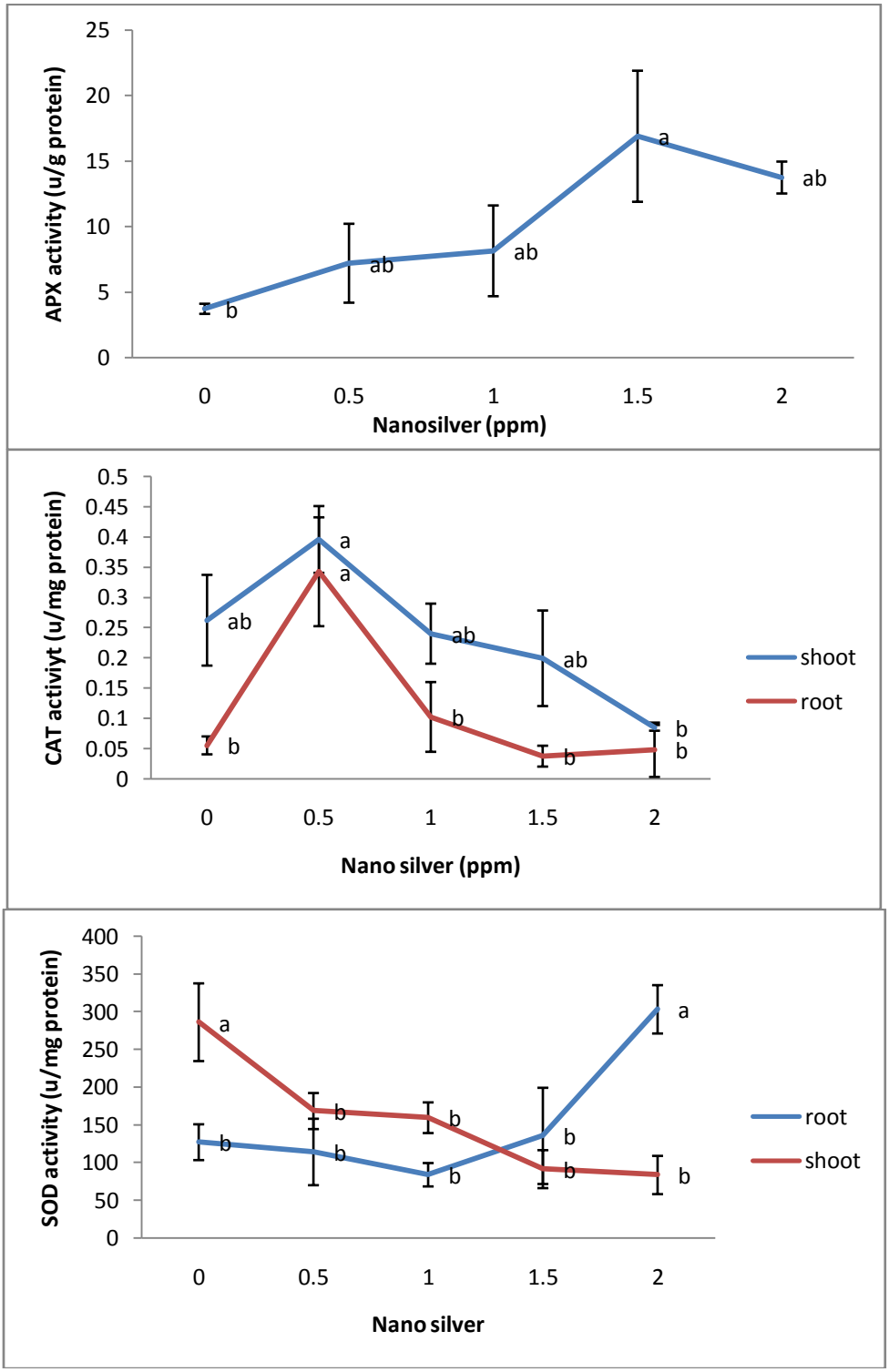

Figure 5: The effect of Nano silver on (a) Ascorbate peroxidase (APX), (b) Catalase (CAT), in roots and shoots and (c) Superoxid dismutase (SOD) activities in shoots of canola cultivar Ocapy. Uncommon letters are significant (P<0.05) based on Duncan test

Purvis (1980) reported that ethylene caused an increase in enzyme activity of chlorophyllase and destruction of internal membrane of chloroplast, while that 100 ppm of silver nitrate caused decreasing the production of ethylene and destruction of chlorophyll in calamondin fruit. It seems that in this study the presence of silver in shoots is effective to reducing oxidative stress and decrease of SOD activity by prevention at ethylene action.

Accumulation of reducing sugar could be acting as activators of carbohydrates synthesis (Kodandaramaiah, 1983). The present results demonstrated that nanosilver was significantly improved the reducing sugars production or/and accumulation in shoot of canola in 1.5 ppm. It was similar to finding of Jiang (2012) on Spirodela polyrhiza with 1 mg/lit gum arabic–coated AgNPs (1.7±6 nm) in hydroponic culture. In addition to playing a central role in metabolism, soluble sugars such as Glc and Suc help regulate many developmental and physiological processes in plants (for review, see Koch, 1996; Smeekens, 1998; Sheen *et al.*, 1999; Yu, 1999).

Many jasmonate, ABA, and stress-inducible genes are coregulated by sugars (Reinbothe *et al.*, 1994; Sadka *et al.*, 1994). The delayed senescence and increased stress resistance observed in Arabidopsis *HXK* antisense plants (Xiao *et al.*, 2000) similarly connect plant sugar metabolism and sensing with the control of stress resistance and aging.

Abiotic and biotic stress stimuli, such as drought, salinity, wounding, and infection by viruses, bacteria, and fungi, can modulate source-sink activities. Because extracellular invertase, a key enzyme for hydrolyzing Suc (Sturm, 1999), is regulated by stress stimuli and hormones, it has been proposed to be a central modulator of assimilate partitioning, integrating sugar, stress, and hormone signals (Roitsch, 1999).

REFERENCES

Beauchamp and Fridrovich I (1971). Superoxide dismutase improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44** 276-286.

Foyer, Descourvierse and Kunert (1994). Protection against oxygen radicals. An important defense mechanism studied in transgenic plants. *Plant, Cell & Environment* **17** 507–23.

Asada (1999). The water-water cycle in chloroplasts scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50** 601–39.

Price AH, Atherton NM and Hendry GAF (1989). Plants under drought stress generate activated oxygen. *Free Radical Research Communications* **8** 61-66.

Becker RO (1999). Silver ions in the treatment of local infections. *Metal-Based Drugs* **6** 297–300.

Jyothsna Y, Kapil M and Usha Rani P (2009). Effects of herbivore feeding on biochemical and nutrient profile of castor bean, *Ricinus communis* L. plants. *Allelopathy Journal* **24**(1) 131–142.

Usha Rani P and Rajasekharreddy P (2011). Green synthesis of silver-protein (core-shell) nanoparticles using *Piper betle* L. leaf extract and its ecotoxicological studies on *Daphnia magna*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **389**(1–3) 188–194.

An J, Zhang M, Wang SH and Tang J (2008). Physical, chemical and microbiological change in stored green asparagus spears as affected by coating of silver nanoparticle-pvp. *LWT- Food Science and Technology Journal* **41** 1100-1107.

Shah V and Belozerova I (2008). Influence of metal nanoparticles on the soil microbial community and germination of Lettuce seeds. *Water, Air, & Soil Pollution*.

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PKH, Chiu JF and Che CM (2007). Silver nanoparticles: partial oxidation and antibacterial activities. *Journal of Biological Inorganic Chemistry* **12** 527-534.

Purvis AC (1980). Sequence of Chloroplast Degreening in Calamondin Fruit as Influenced by Ethylene and AgNO3. *Plant Physiology* **66** 624-627.

Seif Sahandi M, Sorooshzadeh A, Rezazadeh HS and Naghdibadi HA (2011). Effect of nano silver and silver nitrate on seed yield of borage. *Journal of Medicinal Plants Research* **5**(5) 706-710.

Henglein A (1989). Small-particle research: physicochemical properties of extremely small colloidal metal and semiconductor particles, *Chemical Reviews* **89** 1861-1864.
Jiang HSH, Li M, Yichang F, Li W and Yin L (2012). Physiological analysis of silver nanoparticles and $AgNo_3$ toxicity to Spirodela polyrhiza. *Environmental Toxicology*.
Reinbothe S, Mollenhauer B and Reinbothe C (1994). JIPs and RIPs: The regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell* **6** 1197–1209.
Roitsch T (1999). Source-sink regulation by sugar and stress. *Current Opinion in Plant Biology* **2** 198–206.
Sadka A, DeWald DB, May GD, Park WD and Mullet JE (1994). Phosphate modulates transcription of soybean *VspB* and other sugar-inducible genes. *Plant Cell* **6** 737–749.
Sturm A (1999). Invertases: Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiology* **121** 1–8.
Xiao W, Sheen J and Jang JC (2000). The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Molecular Biology* **44** 451–461.
Koch KE (1996). Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47** 509–540.
Smeekens S (1998). Sugar regulation of gene expression in plants. *Current Opinion in Plant Biology* **1** 230–234.
Sheen J, Zhou L and Jang JC (1999). Sugars as signaling molecules. *Current Opinion in Plant Biology* **2** 410–418.
Yu SM (1999). Cellular and genetic responses of plants to sugar starvation. *Plant Physiology* **121** 687–693.
Gianinetti A, Laarhoven LJJ, Persijn ST, Harren FJM and Petruzzelli L (2007). Ethylene production is associated with germination but not seed dormancy in red rice. *Annals of Botany* **99** 735-745.
Abeles FB, Morgan PW and Saltiveit ME (1992). *Ethylene in Plant Biology,* 2nd edition (Academic Press) San Diego.
Chi GL, Pua EC and Goh CJ (1991). Role of ethylene on *de novo* shoot regeneration from cotyledonary explants of *Brassica campestris ssp. Pekinesis* (lour) Olsson *in vitro*. *Plant Physiology* **96** 178-183.
Sarkar D, Sud KC, Chakrabarti SK and Naik PS (2002). Growing of potato microplants in the presence of alginate-silver thiosulfate capsules reduces ethylene-induced culture abnormalities during minimal growth conservation *in vitro*. *Plant Cell, Tissue and Organ Culture* **68** 79-89.
Sarkar D, Kaushik SK and Naik PS (1999). Minimal growth conservation of potato microplants: silver thiosulfate reduces ethylene-induced growth abnormalities during prolonged storage *in vitro*. *Plant Cell Reports* **18** 897-903.
Perl A, Aviv D and Galun E (1988). Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. *Plant Cell Reports* **7**(6) 403-406.
Kumar RP, Lakshmana P and Thorpe TA (1998). Regulation of morphogenesis in plant tissue culture by ethylene. *In Vitro Cellular and Developmental Biology-Plant* **34** 94-103.
Kumar V, Ramakrishna A and Ravishankar GA (2007). Influence of different ethylene inhibitors on somatic embryogenesis and secondary embryogenesis from Coffea canephora P ex Fr. *In Vitro Cellular and Developmental Biology-Plant* **43** 602-607.
Beyer EM (1979). Effect of Silver Ion, Carbon Dioxide, and Oxygen on Ethylene Action and Metabolism. *Plant Physiology* **63** 169-173.
Bradford M (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* **72** 248-254.
Cakmak I and Horst JH (1991). Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). *Physiologia Plantarum* **83**(3) 463-468.

Blum H, Beier H and Gross HJ (1987). Improved silver staining of plant-proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* **8** 93–99.
Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV and Aparicio-Tejo P (1994). Drought induces oxidative stress in pea plants. *Planta* **194** 346-352.
Olson BJSC and Markwell J (2007). Current protocols in protein science. *Detection and Assay Method* **48** (3.4) 1-3.4 29.
Salehi Z and McCarthy JEG (2002). Structure and function of cap-associated proteins in yeast. PhD thesis. Department of Biomolecular Sciences, University of Manchester, Institute of Sciences and Technology (UMIST), Manchester, England.
Giannopolitis CN and Ries SK (1977). Superoxide dismutase. Occurrence in higher plants. *Plant Physiology* **59** 309-314.
Kaveh R, Shengli Y, Ranjbar S, Tehrani RL, Bruek C and Vanaken B (2013). Changes in Arabidopsis thaliana Gene Expression in Response to Silver Nanoparticles and Silver Ions. *Environmental Science & Technology* **47**(18) 10637-10644.
Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**(5259) 680-5.
Davies KM, Hobson GE and Grierson D (1988). Silver ion inhibit the ethylene-stimulated production of ripening-related mRNA in tomato. *Plant, Cell and Environment* **11**(8) 729-738.
Dose K (1980). *Biochemie* (Springer Berlin) Heidelberg, New York.
Aebi H (1984). Catalase in vitro. *Methods in Enzymology* **105** 121–126.
Abeles FB, Dunn LJ, Morgens P, Callahan A, Dinterman RE and Schmidt J (1988). Induction of 33-KD and 60-KD peroxidases during ethylene-induced senescence of cucumber cotyledons. *Plant Physiology* **87** 609-615.
Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7 405–410.
Gallego SM, Benavides MP and Tomaro ML (1996). Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science* **121** 151-156.
Gallego SM, Benavides MP and Tomaro ML (1999). Effect of cadmium ions on antioxidative defenses system in sunflower cotyledons. *Biologia Plantarum* **42** 49-55.
Chaoui A, Mazhoudi S, Gorbal MH and Ferjani EEL (1997). Cudmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Sciences* **127** 139-147.
Roa KVM and Sresty TVS (2000). Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L.) Millspaugh in response to Zn and Ni stresses. *Plant Sciences* **157** 113-128.
Halliwell B (1974). Superoxide dismutase, catalase and glutathione peroxidase: solutions to the problems of living with O2. *New Phytologist* **73** 1075-1086.
Vierstra RD (1993). Prptein degradation in plants. *Annual Review of Plant physiology and Plant Molecular Biology* **44** 385-410.
Hieng B, Ugrinovich K, Sustar-Vozlich J and Kidric M (2004). Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* cultivars differing in sensivity. *Journal of Plant Physiology* **161** 519-530.
Anderson MC, Prasad TK and Stewart CR (1995). Changes in isozyme profiles of catalase, peroxidase, and glutathione redutase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiology* **109** 1247–1257.
Dalton DA, Russell SA, Hanus FJ, Pascoe GA and Evans HJ (1986). Enzymatic reaction of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proceedings of the National Academy of Sciences USA* **38** 3811–3815.
May MJ, Vernoux T, Leavar C, Van Montagu M and Inze D (1998). Glutathione homeostasis in plants: implications for environmental sensing and plant development. *Journal of Experimental Botany* **49** 649–667.

Asada K (2006). Production and scavenging of reactive oxygen species in chloroplasts and their function. *Plant Physiology* **141** 391-396.
Reeves RD, Baker AJM, Brhidi A and Berazain R (1999). Nickel hyperaccumulation in the serpentin flora of Cuba. *Annals of Botany* **83** 29-38.
Nelson N (1944). Photometric adaptation of the somogyi method for determination of glucose. *Journal of Biological Chemistry* **153** 375–380.
Somogyi MJ (1952). Notes on sugar determination. *Journal of Biological Chemistry* **195** 19–23.
Ewais EA (1997). Effect of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. *Biologia Plantarum* **39**(3) 403-410.
Veen H and Van De Geijn SC (1978). Mobility and ionic form of silver as related to longevity of cut carnations. *Planta* **140** 93-96.
Strader LC, Beisner ER and Bartel B (2009). Silver ions increase auxin efflux independently of effects on ethylene response. *Plant Cell* **21** 3585–3590.
Ratte HT (1999). Bioaccumulation and toxicity of silver compounds: a review. *Environmental Toxicology and Chemistry* **18** 89-108.
Razavizadeh R and Rostami F (2013). Changes in growth and antioxidant capacity of canola by salinity and salicylic acid under in vitro. *International Research Journal of Applied and Basic Sciences*, ISSN 2251-838X **4**(12) 4093-4101.
Roya Razavizadeh and Fatemeh Rostam (No Date). Changes in growth and antioxidant capacity of canola by salinity and salicylic acid under in vitro, *International Research Journal of Applied and Basic Sciences* © 2013 Available: www.irjabs.com ISSN 2251-838X **4**(12) 4093-4101 (Science Explorer Publications).
Fatemeh Rostami and Ali Akbar Ehsanpour (2010). The effect of silver thiosulfate (STS) on chlorophyll content and the antioxidant enzymes activity of potato (*Solanum tuberosum* L.) *Journal of Cell and Molecular Research* **2**(1) 29-34.
Rostami F and Ehsanpour AA (2009). Application of silver thiosulfate (STS) on silver accumulation and protein pattern of potato (Solanum tuberosum L.) *under in* vitro culture, Malays. *Annals of Applied Biology* **38**(2) 46-54.