

Effect of BvAgNP on Growth, Development, and Glyoxalase Gene Expression Analysis in *Mammillaria bombycina* and *Selenicereus undatus*

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Abstract

Background: Silver nanoparticles (AgNPs) have been used in plant tissue culture as growth stimulants, promoting bud initiation, germination, and rooting. In prior studies, AgNPs were synthesized and characterized by green synthesis using extracts from *Beta vulgaris* var. Cicla (*BvAgNP*), and their functionality as seed disinfectant and antimicrobial was verified. In this study, we evaluated the effect of *BvAgNP* on the growth and development of *Mammillaria bombycina* and *Selenicereus undatus* *in vitro*, as well as the expression of glyoxalase genes.

Methods: Explants from *M. bombycina* and *S. undatus* *in vitro* were treated with 25, 50, and 100 mg/L of *BvAgNP*. After 69 days, morphological characteristics were evaluated, and the expression of glyoxalase genes was analyzed by qPCR.

Results: All treatments inhibited rooting for *M. bombycina* and no bud initiation was observed. *S. undatus*, showed a maximum response in rooting and bud generation at 25 mg/L of *BvAgNP*. Scanning electron microscopy (SEM) results exhibited a higher number of vacuoles in stem cells treated with *BvAgNP* compared to the control for both species. Expression of glyoxalase genes in *M. bombycina* increased in all treatments, whereas it decreased for *S. undatus*, however, increasing in roots.

Conclusions: This study presents the effects of *BvAgNP* on the growth and development of *M. bombycina* and *S. undatus*, with the aim of proposing treatments that promote *in vitro* rooting and budding.

Introduction

Silver nanoparticles (AgNPs) have made significant advances in various fields of science. Their synthesis can be either chemical or green. However, the green synthesis is emerging as an alternative to minimize the toxic waste produced after the reaction. Using organic materials from plants, bacteria, or fungi as reducing and stabilizing agents, this method is simple and inexpensive. The resulting NPs show an enhanced ability to interact with living organisms due to their organic coating [1, 2, 3].

Numerous studies have been conducted on AgNPs in both *in vitro* and *ex vitro* plant tissue cultures to determine their functionality in the growth and development of various plants. For example, in bean seeds, AgNPs found to enhance germination and growth [4], and in gladiolus (*Gladiolus grandiflorus* L.) seedlings, shoot growth was enhanced [5]. Garcidueñas-Piña *et al.* [2] reported synthesizing AgNPs by green synthesis with chard extracts from *Beta vulgaris* var. *Cicla*, which they named *BvAgNP*. These nanoparticles were characterized as spherical with a diameter of 78 nm and a silver content of 39.76%. They are stable in phosphate buffer. Their effectiveness was assessed in disinfecting of *Arabidopsis thaliana* and *Psidium guajava* (guava) seeds, as well as in their antimicrobial activity.

High concentrations of silver in AgNPs may cause different stress in cells, leading to significant damage or even death [6, 7]. Under stress, plants produce methylglyoxal (MG), which is a metabolic byproduct

primarily derived from glucolysis [8]. MG is a highly toxic molecule that degrades lipids and cell membrane proteins [9]. However, the glyoxalase enzymatic system biometabolizes MG, converting it into D-lactate [10]. Two glyoxalase systems exist: System I composed of glyoxalase I (Gly 1) and glyoxalase II (Gly 2), and System II, which contains only glyoxalase III (Gly DJI) [11, 12]. Therefore, glyoxalase gene expression has been used as a molecular marker to assess different stress levels in several living organisms [9, 10].

Mammillaria bombycina is a cactus native to central Mexico [13]. Its popularity as an ornamental plant has resulted in rampant poaching from its natural habitat [14]. To prevent its loss, large-scale *in vitro* propagation has been carried out and it has been chosen as a model cactus for molecular studies, such as the identification of genes related to abiotic stress and transcriptomes [8, 13]. On the other hand, *Selenicereus undatus*, also known as *Hylocereus undatus*, is a cactus mainly grown in Central America and Israel. Its fruit, "dragon fruit" or pitahaya, is widely commercialized as a functional food in many countries due to its high nutritional value, antioxidant, and antiproliferative properties [15, 16].

In this study, we examine how *BvAgNP* affects the growth and development of *M. bombycina* and *S. undatus*, as well as the morphological analysis of stem and root and the expression of glyoxalase genes in different treatments with different concentrations of *BvAgNP*. This research was conducted to propose minimally stressful *in vitro* rooting and budding treatments for these cacti.

Materials and methods

Treatments with *BvAgNP*

In vitro plantlets of *M. bombycina* and *S. undatus* were donated by the Plant Biotechnology Laboratory of the Autonomous University of Aguascalientes. These explants were cultured in MS medium [17] for six months. To assess the effect of *BvAgNP* on the plants, a range of concentrations were initially tested including 1000, 500, 200, and 100 mg/L (results not shown), choosing the concentration of 100 mg/L, that showed the least toxic response. From this, the following treatments were carried out: Three explants 5 mm thick were placed in glass jars with 30 ml of MS medium. This medium was supplemented with 25 mg/L (T1), 50 mg/L (T2), and 100 mg/L (T3) of *BvAgNP*. A control group (TC) was also treated with MS medium without *BvAgNP*. All treatments were performed in triplicate, and cultures were incubated under axenic conditions at 25°C with a 16/8 h light/dark photoperiod for ninety days.

Morphological characteristics evaluation

Fifteen explants per treatment of *M. bombycina* and *S. undatus* were randomly analyzed. Wet weight, elongation, number and length of shoots, number and length of primary, secondary, and aerial roots, callus formation and contamination were evaluated. The measurements were taken with the ImageJ® 1.43u software [18, 19], scaling with 1 cm.

Micromorphological characteristics evaluation

From each *BvAgNP* treatment, explants were fixed in triplicate using FAA solution (formaldehyde-ethyl alcohol-glacial acetic acid-distilled water, 1:5:0.5:3.5) for 72 h. The middle area of the explant and the main root were assessed through cross-section for *M. bombycina*, while for *S. undatus* was evaluated for the middle area of the shoot. The tissue was dehydrated in immersion processes in distilled water with 70, 80, and 96% alcohol, then cleared with xylene and infiltrated with paraffin, all within 12 hours. Samples were embedded in paraffin blocks and cut transversely to a thickness of 16 μm using a rotary microtome. The slides were fixed by dehydration at 65°C for 48 hours, dewaxed with 100% xylene for 5 min and washed three times for 1 min each. After rehydration with 100% alcohol twice for 1 min each, followed by 96% alcohol for 1 min three times, and 50% alcohol for 5 min, they were differentially stained with safranin and alcian blue. Finally, they were mounted with synthetic resin and left to dry overnight. The sections were analyzed using a light microscope.

Scanning Electron Microscopy (SEM)

Samples of stem and root from *M. bombycina* and *S. undatus* from treatments T1, T2, T3, and TC were processed using the histological technique for plant tissue, were transversely cut, and analyzed by Scanning Electron Microscopy (SEM). An energy-dispersive X-ray spectroscopy (EDS) analysis of elements was carried out in at least three different areas of both plants stem and root.

Glyoxalase expression analysis by qPCR

Stem and root samples from all treatments of *M. bombycina* and *S. undatus* were used for RNA extraction following the protocol described by Hernández-Camacho *et al.* [13]. The RNA samples were treated with RNase-Free DNase Promega, and then cDNA was synthesized using the Radiant™ cDNA Synthesis Kit – 1 Step. The concentration and purity of the cDNA were analyzed by spectrophotometry in NanoDrop 2000 (Thermo scientific) to adjust the cDNA concentration to 100 ng/ μL .

For the qPCR analysis of gene expression, we utilized *M. bombycina* glyoxalase oligonucleotides proposed by Enríquez- González *et al.* [8]. In the case of *S. undatus*, we designed oligonucleotides *in silico* from the *S. undatus* genome available in the Sequence Read Archive of the NCBI with the Bioproject ID PRJNA691451 (<https://www.ncbi.nlm.nih.gov/genome/99797>) [15]. The 25S ribosomal gene was used as the reference gene selected based on the oligonucleotides and [13], whose sequences are presented in Table 1. The analysis was performed in triplicate and the results were normalized to the control and reference gene using the $2^{-\Delta\Delta\text{Ct}}$ method [20].

Table 1
Oligonucleotide sequences for expressing Glyoxalases in *M. bombycina* (MBGly) and *S. undatus* (SuGly).

MBGly1.1	F5' ctatttcttcgttttaggtgc 3'	R5' gagaacaactgaaaccgat 3'
<i>MBGly 2 - 1</i>	F5' cgtccttaacagatgagaca 3'	R5' tactgcgtaaatagagctacg 3'
<i>MBGly 2-3</i>	F5' agccgactagagtttattgt3'	R5' caatggtgaaggaacagtt 3'
<i>MBGlyDJ-1</i>	F5' ggagccggagccaatagacc 3'	R5' tcactcagaatgttacacggatgc 3'
<i>SuGly1</i>	F5' agtgacccggtggatcgta 3'	R5' gaagccacgaggggtctgtg 3'
<i>SuGly2-1</i>	F5' agtgtgtattgcgggcatga 3'	R5' cgtaaatagagctacgcggga 3'
<i>SuGly2-3</i>	F5' agccgactagagtttattgt 3'	R5' caatggtgaaggaatggt 3'
<i>SuGlyDJ-I</i>	F5'tacacggataccgaggagcg3'	R5'ggagcctgagccaatagaccg3'
25Sr	F5'cgtaaggcgtaaggaagctg3'	R5' tcggagggaaccagctacta 3'

The experimental design was completely randomized. We analyzed all quantitative results in the R statistical program (R Foundation for Statistical Computing, Vienna, Austria) using a one-way ANOVA and multiple comparison test by the Tukey method ($\alpha = 0.05$). The mean of the repetitions with their standard deviation was plotted using GraphPad Prism version 8.4.2 for Windows. (GraphPad Software, San Diego, California, USA, www.graphpad.com).

Results

Morphological characteristic evaluation

For *M. bombycina*, in all the treatments analyzed, the explants did not show any increase in weight, length or budding compared to the control (Table 2, Fig. 1A). The average number of roots per explant in the control was 7.33 ± 2.10 (Fig. 1A), whereas it was lower in all the treatments, with the lowest being in T1 with 1.73 ± 1.58 roots (Fig. 1A). The average length of the roots in TC was 2.09 ± 0.70 cm, but decreased in all treatments specially T3 with an average length of 1.26 ± 0.60 cm. Some explants even failed to develop roots (Fig. 1A). Callus formed in all treatments except the control, with highest yield in T1 representing 20% of the explants (Table 2). Contamination was absent in all the treatments, whereas the control had 20% contamination.

No changes in weight or elongation were observed for of *S. undatus* explants in all analyzed treatments, compared to the control (Table 2). T1 produced the highest number of buds with 2.06 ± 0.36 , while TC had 1.33 ± 0.33 (Fig. 1B and Table 2). The average bud elongation remained consistent across was all treatments and the control (Table 2). For the number of main roots, there were no significant differences in all the analyzed treatments including the control (Fig. 1B, Table 2). Differences in root length were observed in all treatments, with the longest average root length measuring at 10.96 ± 6.64 in T1 (Fig. 1D,

Table 2). While no differences in quantity were found for aerial roots in all treatments, there were variations in their length (Table 2).

All treatments showed no contamination or callous formation, but necrosis was observed in 20% of the explants in T3. For all treatments, it was noted that every root displayed reddish pigmentation and thickening in the elongation zone and apical zone (Fig. 1D).

Table 2
Morphological characteristics in cacti *in vitro* treated with BvAgNP

<i>M. bombycina</i>				
	Control	25 mg/L BvAgNP	50 mg/L BvAgNP	100 mg/L BvAgNP
Increased weight (Proportion)	1.5476 ± 0.2036 a	1.5698 ± 0.1428 a	1.3160 ± 0.06951 a	1.3609 ± 0.4433 a
Explant lenght (Proportion)	1.4076 ± 0.1105 ab	1.3016 ± 0.3540 ab	1.6935 ± 0.2571 a	1.2352 ± 0.0730 b
Number of shoots (Unit)	0.6667 ± 0.7817 a	0.0667 ± 0.1491 a	0.2667 ± 0.3651 a	0.0 ± 0.0 a
Shoot lenght (cm)	0.2200 ± 0.1304 a	0.1 ± 0.0 a	0.15 ± 0.05773 a	0.0 ± 0.0 a
Number of roots (Unit)	7.3333 ± 2.1082 a	1.7333 ± 1.5882 b	3.2000 ± 0.9888 b	1.8000 ± 0.1826 b
Root length (cm)	2.0997 ± 0.7029 a	1.7469 ± 0.9340 ac	2.4080 ± 1.5110 ab	1.2621 ± 0.6009 c
Callus (Percentage frecuency)	0.0 ± 0.0	20 ± 44.7213	13.3333 ± 18.2574	6.6666 ± 14.9071
Plants with dead tissue (%)	0.0	0.0	0.0	0.0
Contaminated plants (%)	20	0.0	0.0	0.0
<i>S. undatus</i>				
	Control	25 mg/L BvAgNP	50 mg/L BvAgNP	100 mg/L BvAgNP
Increased weight (Proportion)	3.5742 ± 1.0440 a	4.2416 ± 1.6809 a	4.3735 ± 1.0559 a	2.7136 ± 0.7209 a
Explant lenght (Proportion)	1.1329 ± 0.1383 a	1.1929 ± 0.0614 a	1.1741 ± 0.0806 a	1.2400 ± 0.1113 a
Number of shoots (Unit)	1.3333 ± 0.3333 a	2.0667 ± 0.3651 b	1.9333 ± 0.6412 ab	1.3333 ± 0.3333 a
Shoot lenght (cm)	1.5158 ± 0.7078 a	2.0084 ± 0.9303 a	1.7605 ± 0.8860 a	1.6217 ± 0.9941 a

<i>M. bombycina</i>				
Number of main roots (Unit)	1.1333 ± 0.1826 a	1.4000 ± 0.4346 a	1.5333 ± 0.6055 a	1.0000 ± 0.4714 a
Main root length (cm)	5.4548 ± 2.9907 a	10.9660 ± 6.6400 b	7.6932 ± 5.0147 ab	8.9611 ± 4.1161 ab
Number of aerial roots (Unit)	1.4667 ± 0.8367 a	2.2000 ± 1.2156 a	1.1333 ± 0.3801 a	0.6667 ± 0.6667 a
Aerial root length (cm)	1.6127 ± 1.3254 a	4.3748 ± 3.8936 b	4.2391 ± 1.6390 ab	5.4385 ± 3.4960 b
Callus (Percentage frequency)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Plants with dead tissue (%)	0.0	0.0	0.0	20 ± 18.2541
Contaminated plants (%)	0.0	0.0	0.0	0.0

Values (mean ± SD, n = 15) different letters indicate significant differences according to the mean comparison test with the Tuckey method ($\alpha = 0.05$).

Micromorphological characteristic evaluation

The analysis showed the accumulation of vacuoles for both *M. bombycina* and *S. undatus* (Fig. 2), with the number increasing depending on the concentration of BvAgNP. For *M. bombycina* in all treatments including the control, the number of vacuoles per cell was 8.67 ± 0.81^a (TC), 11.87 ± 0.81^{ab} (T1), 13.73 ± 1.68^b (T2), 14.87 ± 2.23^b (T3), and for *S. undatus* it was 2.20 ± 0.40^a (TC), 4.60 ± 0.72^{ab} (T1), 6.53 ± 1.17^b (T2), 10.33 ± 1.72^c (T3) vacuoles per cell in the same order of treatments.

Scanning electron microscopy (SEM) analysis

The SEM analysis of the stem of *M. bombycina* showed tissue damage in all treatments, with greater damage observed in T3 for both root and stem (Fig. 3). In both stem and root samples for *S. undatus*, no tissue damage was detected in all analyzed treatments (Fig. 3). No accumulation of BvAgNP was found in the analyzed samples of both cactus species. EDS element analysis detected silver in both species in all treatments, except for the control. The quantity of silver relied on the amount of BvAgNP added per treatment (Fig. 4).

Expression analysis of glyoxalases by qPCR

The expression analysis of the glyoxalase genes in *M. bombycina* showed an increase in all analyzed treatments compared to the control. In the stem, Gly 2 – 1 expression increased compared to the control at 25 mg/L (T1) of *BvAgNP*. However, in the case of Gly 1–1, Gly 2–3, and Gly DJI, the increase started from T3. In the root, the expression increased for all genes except Gly 2 – 1, which peak at 50 mg/L of *BvAgNP* (Fig. 5A).

Regarding *S. undatus*, the glyoxalase genes expression in the stem decreased when treated with *BvAgNP* between 50 to 100 mg/L compared to the control. Meanwhile, in root samples, the expression was similar to stem, increasing with 25 mg/L compared to the control, remaining the same with 50 mg/L and increasing again with 100 mg/L (Fig. 5B).

Discussion

Cacti are plants that thrive in extreme environments such as extreme temperatures, drought, or salinity. Furthermore, most of them are used as ornamental plants, making them prone to illegal harvesting. Due to the aforementioned natural factors, their growth and development are slow. As a result, numerous studies have been conducted for their *in vitro* propagation and conservation [13, 14].

Many propagation studies have been done on different cacti using various hormones to accelerate their development. In some cases, the results have been less than satisfactory because rapid growth was not achieved [21]. Therefore, our study focuses on analyzing the growth and development of *M. bombycina* and *S. undatus*, under the influence of *BvAgNP* at different concentrations.

Several studies have used AgNPs synthesized chemically or through green synthesis, as means of promoting growth and development in different *in vitro* plants. For example, in the regeneration and multiplication of *V. planifolia* seedlings [22], and in the growth of *Stevia rebaudiana* [23]. However, most studies have focused on agricultural plants [6, 24], and to date, there are no reports regarding the effect of AgNPs on cacti, there for it is necessary to studier it, as every plant species reacts differently to AgNPs [4].

Regarding the impact of *BvAgNP* on *M. bombycina*, all treatments showed a decrease in root numbers compared to the control. There was no root formation even at T3 concentrations. Garcidueñas-Piña *et al.* [2] observed an increase in plant length in *A. thaliana* germinated in MS medium supplemented with 0.01 mg/mL of *BvAgNP*, however, they reported a reduction in plant length at 10 mg/mL. High concentrations of Ag have been demonstrated to cause oxidative stress and negatively impact the growth and development of plants, as noted in previous research [25]. AgNPs reduces the plants' ability to absorb macro and microelements, as well as altering their ionic homeostasis, ultimately impacting growth [26]. Furthermore, alterations to the plant's root system from AgNPs exposure can limit water and nutrient absorption from the medium, resulting in direct effects on plant development [27]. Studies conducted by Mirmoeini *et al.* [28] demonstrated changes in plant height when working with *Camelina sativa* L. at concentrations of 2 g/L of AgNPs, but higher concentrations resulted in decreased growth.

On the other hand, for *S. undatus*, there was sprouting with T1 and T2. These results are similar to reported by Chávez-García *et al.* [5], who used concentrations of 50 mg/L of AgNPs on gladiolus apices, resulting in a shoot length 35.5% longer than the control. In 2020, Pastelín-Solano *et al.* [22] observed increased plant multiplication, elongation, and leaf formation using 25 and 50 mg/L of AgNPs in solid MS medium. This finding is consistent with our study on *in vitro* *S. undatus*, where 25 mg/L of BvAgNP stimulated sprouting and rooting.

Notably, the roots of *S. undatus* treated with BvAgNP *in vitro* displayed thickening and a reddish coloration from the elongation zone to the apical zone. These results are consistent with the findings of Vannini *et al.* [7], who observed a greater effect on roots than shoots in *Eruca sativa* comparing the effects of AgNPs and Ag⁺ as AgNO₃, due to the low translocation of silver from roots to shoots. This coloration is partially caused by the production of secondary metabolites, such as betalains, which are red pigments specific to cacti like *S. undatus*. Their main significance is acting as antioxidants for the plant [16].

Another effect of BvAgNP on the stems of both *M. bombycina* and *S. undatus* was the formation of vacuoles in all treatments, which increased with higher BvAgNP quantities. The presence of vacuoles suggests the accumulation of toxic compounds and metallic ions within the cells [29]. This finding is consistent with the discoveries of Mirmoeini *et al.* [28], who noted the buildup of vacuoles in the stem and root of *C. sativa* L. that underwent treatment with AgNPs, as well as the similar outcome observed by Vannini *et al.* [7] in *E. sativa* and by Cvjetko *et al.* [29] in tobacco plants (*Nicotiana tabacum*) exposed to AgNPs.

A notable finding in the micromorphological analysis revealed a reduction in the diameter of the root epidermis in both species. The effect of AgNPs on plant cells is contingent upon the AgNPs characteristics, the applied concentration, the duration of exposure, the species of plant, and the age of the plant being treated [7, 30]. Furthermore, the SEM analysis conducted on *M. bombycina* and *S. undatus* revealed cellular membrane damage in both stem and root samples, with a higher incidence in treatment T3. Lin and Xing [31] obtained similar findings, confirming the translocation of zinc oxide nanoparticles in the endodermis of *Lolium perenne* L. (ryegrass) cells, ultimately impairing root growth and causing damage.

The EDS analysis of stem and root areas of both species revealed the presence of silver from BvAgNP. The base MS medium formulation does not contain silver. Treatment T3 for *M. bombycina* and treatment T1 for *S. undatus* showed silver presence. SEM photographs indicated no BvAgNP within plant cells. Therefore, the silver comes from Ag⁺, which was released from the BvAgNP in solution [30]. This is in accordance with Vannini *et al.* [7], who reported that the root elongation results of *E. sativa* were influenced by the release of Ag⁺ from the applied AgNPs.

The analysis of glyoxalase gene expression indicates distinct expression profiles between the stem and root of both species. The expression of glyoxalase genes in the stem and root of *M. bombycina* increased

from T1, while in *S. undatus*, the expression in the stem decreased but increased in the root for all treatments. Our findings align with those of Mirmoeini *et al.* [28], who reported an increase in the enzymatic activity of Gly 1 and Gly 2 at 1 g/L of AgNPs. However, the activity decreased in both lower and higher concentrations tested, suggesting a possible insufficient biometabolization of MG at high concentrations of AgNPs.

The expression profiles of Gly genes in *M. bombycina* differ from those reported by Enríquez-González *et al.* [8], however, both studies confirm that these genes are stress inducible. The glyoxalase genes are involved in a complex response network to biotic and abiotic stress stimuli. Therefore, their expression profiles may be affected by the oxidative stress caused by the contact between Ag + and the root. The glyoxalase DJ-1 gene's expression might help keep MG levels and reduce oxidative stress, as per *Yadu et al.* [32]. Thus, the increase in rooting is how *S. undatus* combats stress caused by Ag + and nutrient deficiency.

In conclusion, the growth and development of *M. bombycina* and *S. undatus* are affected differently by *BvAgNP* at 25, 50, and 100 mg/L. These treatments cause adverse effects on rooting for *M. bombycina*, and it is not recommended to apply them. However, for *S. undatus*, they enhance root elongation at an optimal concentration of 25 mg/L of *BvAgNP*, perhaps as a plant defense mechanism against stress.

Abbreviations

NP	Nanoparticles
AgNPs	Silver nanoparticles
<i>BvAgNP</i>	AgNP by green synthesis with chard extracts from <i>Beta vulgaris</i> var. <i>Cicla</i>
Gly DJ1	Glyoxalase III gen
MS	Murashige & Skoog culture medium
ROS	Reactive oxygen species
MEB	Scanning Electron Microscopy
EDS	Energy-dispersive X-ray spectroscopy

Declarations

AUTHOR CONTRIBUTIONS

Author Contributions: M.D.L Methodology, formal analysis, investigation, writing—original draft preparation; P.M.B.E. and L.M.A. Methodology, supervision; G.P.C. Methodology, supervision; M.D.J.F

Conceptualization, supervision, funding acquisition, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no relevant financial or non-financial interests to disclose.

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Data availability Statement: No data associated in the manuscript

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Figures



Figure 1

Morphological effects in *M. bombycina* and *S. undatus* in vitro after 90 days of treatment with BvAgNP

A) Apical area of *M. bombycina* root; B) Apical area of *S. undatus* root; C) *M. bombycina* explants D) *S. undatus* explants. The photos (A and B) show damage to the rooting of *M. bombycina* in vitro from 25 mg/L of BvAgNP (C), and the induction of rooting in *S. undatus* in vitro with 25 mg/L of BvAgNP (D). They also (C and D) show thickening and reddish coloring from the elongation zone to the apical root zone of *S. undatus* in vitro treated with BvAgNP.

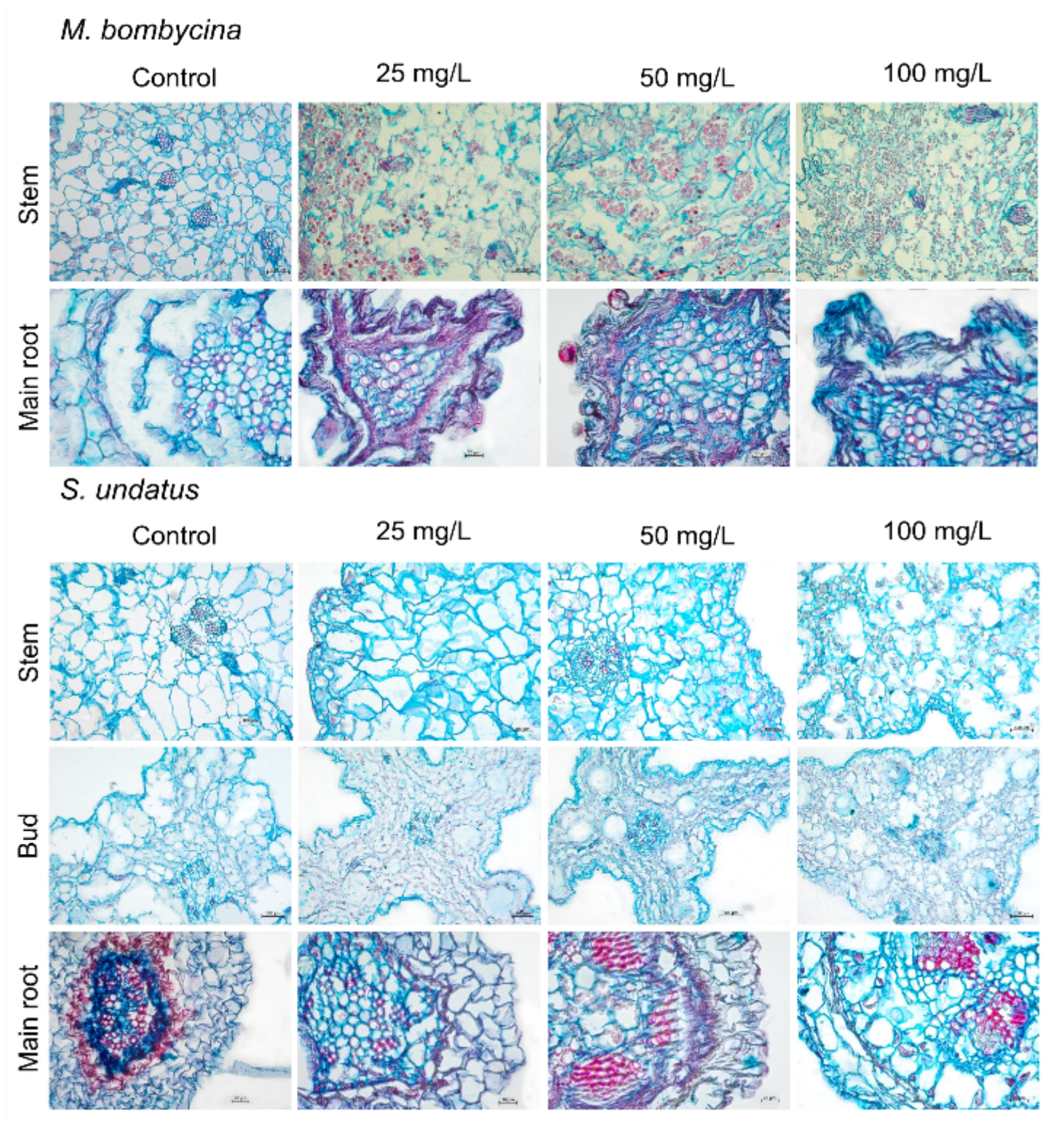


Figure 2

Micromorphology of the stem and root of *M. bombycina* and *S. undatus* Photographs in an optical microscope of transverse sections of the explant stem (10X) and the main root (40X) stained with safranin and alcian blue. The photos show an accumulation of safranin-stained vacuoles inside the stem cells of *M. bombycina* and *S. undatus* in treatments with BvAgNP.

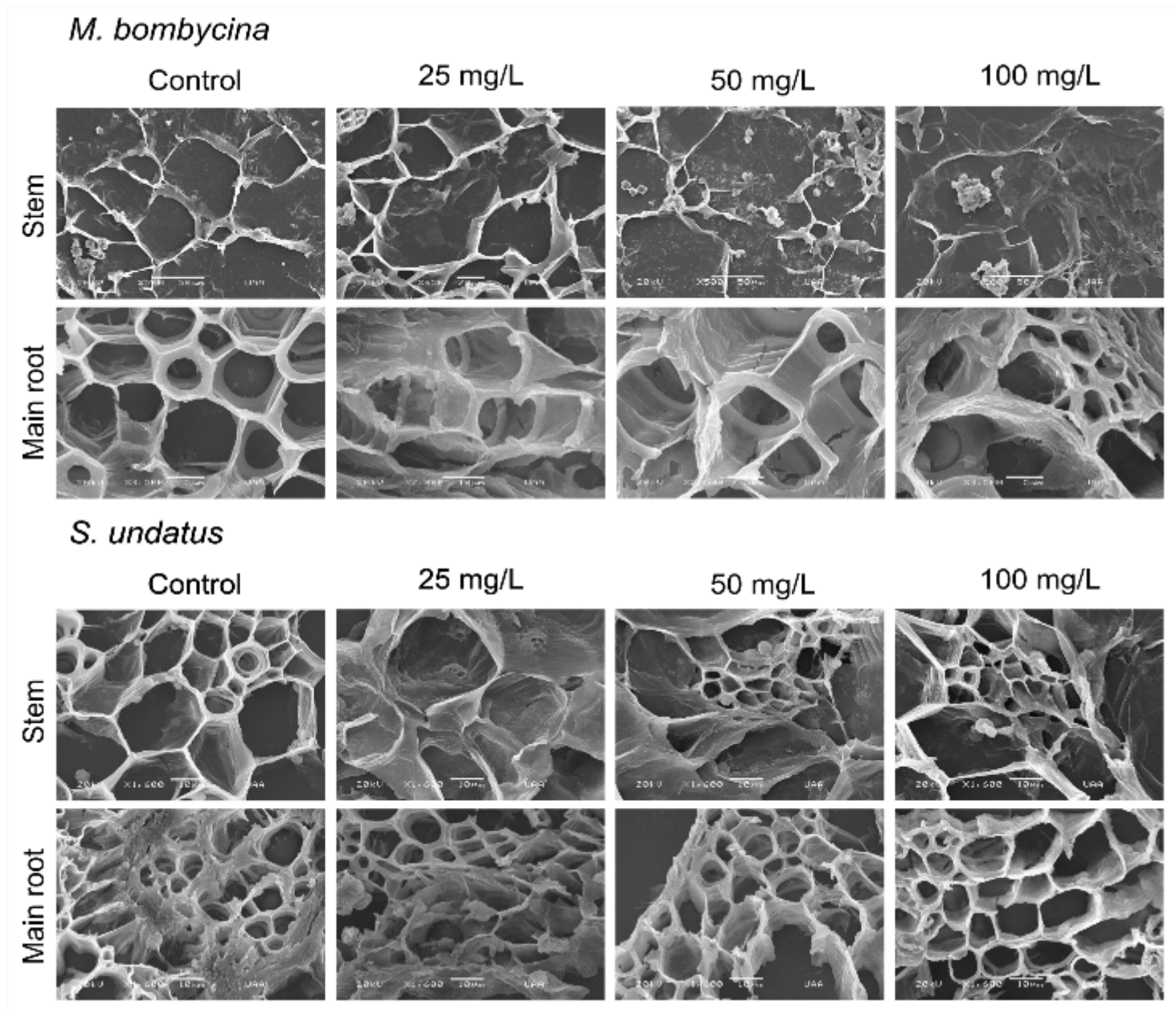


Figure 3

SEM photographs of stem and root of *M. bombycina* and *S. undatus* Control treatments without *BvAgNP*, 25 mg/L, 50 mg/L, and 100 mg/L of *BvAgNP*. The photographs show the accumulation of crystals inside the stem cells in treatments with *BvAgNP* of *M. bombycina* and *S. undatus*.

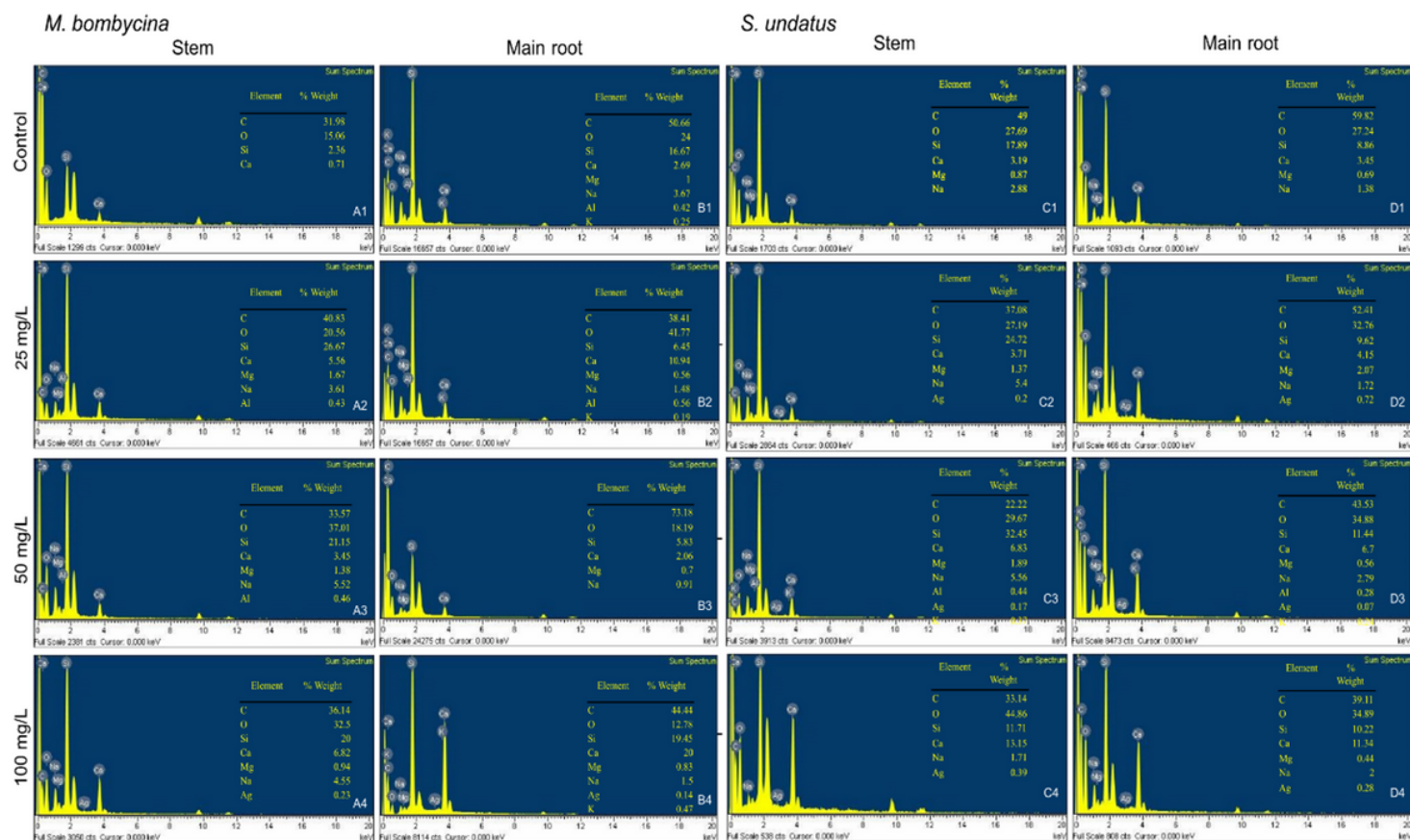


Figure 4

EDS element analysis on stem and root of *M. bombycina* and *S. undatus* A) *M. bombycina* stem: control (1), 25 (2), 50 (3), and 100 (4) mg/L of BvAgNP; B) *M. bombycina* root: control (1), 25 (2), 50 (3), and 100 (4) mg/L of BvAgNP; C) *S. undatus* stem: control (1), 25 (2), 50 (3), and 100 (4) mg/L of BvAgNP; D) *S. undatus* root: control (1), 25 (2), 50 (3), and 100 (4) mg/L of BvAgNP;; The graphs show the presence of silver in *M. bombycina* stem and root of from 100 mg/L and in *S. undatus* stem and root of from 25 mg/L of BvAgNP.

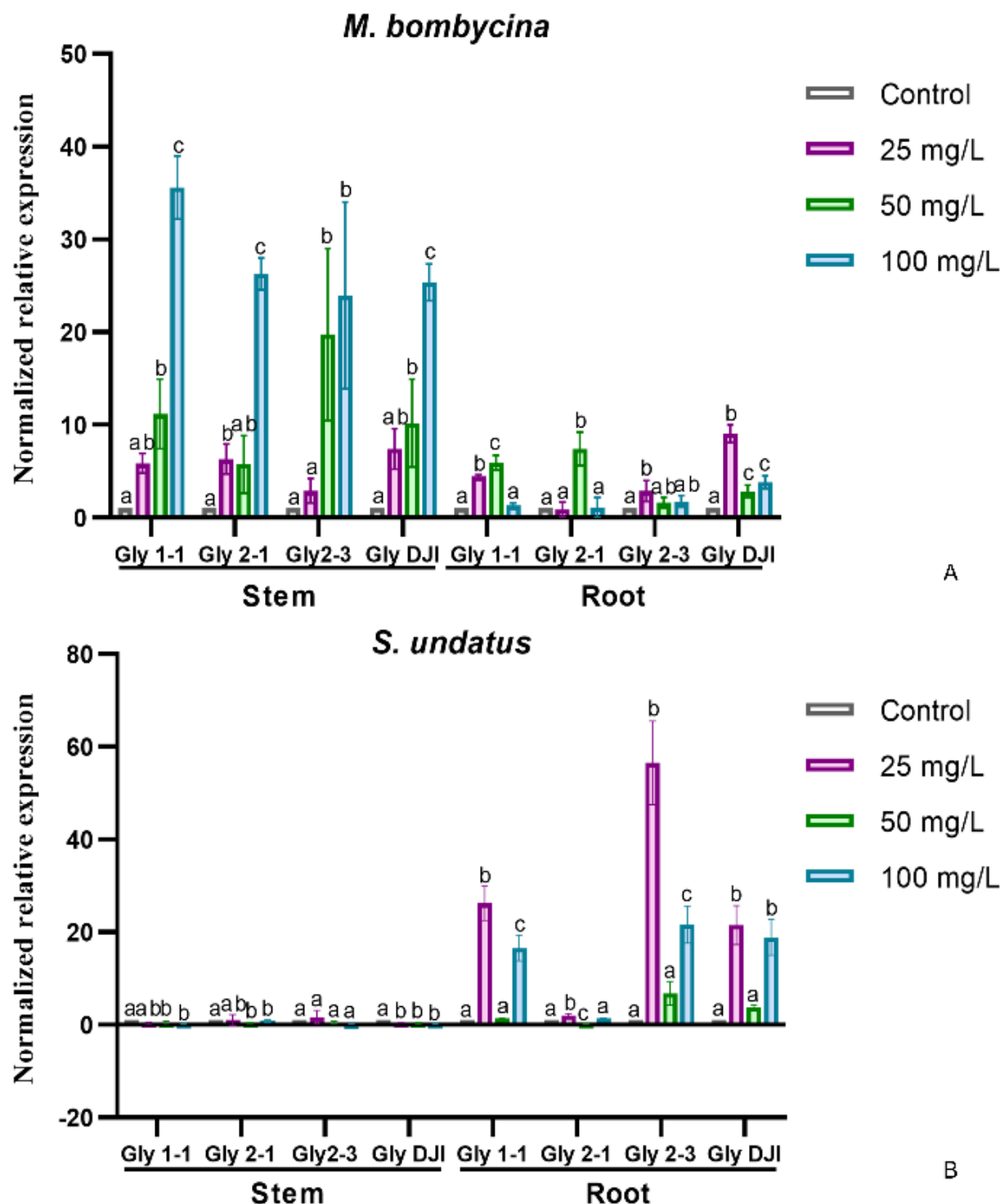


Figure 5

Expression analysis of glyoxalase genes in *M. bombycina* (A) and *S. undatus* (B) with *BvAgNP* treatments Different letters indicate significant differences between treatments according to the Tukey method ($\alpha=0.05$). The values were previously normalized using the $2^{-\Delta\Delta C_t}$ method, the bars represent the standard deviation. The graph shows an increase in the expression of glyoxalase genes in the stem and root of *M. bombycina* *in vitro* from 25 mg/L of *BvAgNP*; in addition to a decrease in the expression of

glyoxalase genes in the stem of *S. undatus in vitro* from 25 mg/L of BvAgNP and an increase in the expression of glyoxalase genes in the root with 25 and 100 mg/L of BvAgNP.