Article

Boosting Polyamines to Enhance Shoot Regeneration in Potato (*Solanum tuberosum* L.) Using AgNO₃

Walaa M. R. M. Adly¹, Yasser S. A. Mazrou^{2,3} , Mohammad E. EL-Denary^{1,*}, Mahasen A. Mohamed¹, El-Sayed T. Abd El-Salam⁴ and Ahmed S. Fouad⁴

- ¹ Horticulture Research Institute, Agriculture Research Center, Giza 12619, Egypt; DR.Walaa.Adly@gmail.com (W.M.R.M.A.); dr.mahasen.hakem@gmail.com (M.A.M.)
² Business Administration Department, Community College, King Khalid University, Guraiger, Abha 62529, Saudi Arabia
³ Department of Agriculture Economic, Faculty of Agriculture, Tanta University, Tanta 31527, Egypt; yasser.mazroua@agr.tanta.edu.eg
⁴ Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12613, Egypt; tsayed1969@hotmail.com (E.-S.T.A.E.-S.); ahmedsfouad@yahoo.com or ahmedsfouad@sci.cu.edu.eg (A.S.F.)
* Correspondence: dr.m.eldenary@gmail.com

Abstract: Advancements in shoot regeneration systems support biotechnology-based tools used in the genetic improvement of plant crops. This study aims to enhance shoot regeneration in potatoes by boosting polyamine content by adding AgNO₃ to the shoot regeneration medium (MS medium supplemented with 30 g L⁻¹ sucrose, 100 mg L⁻¹ myoinositol, and 2.25 BA mg L⁻¹). Five concentrations of AgNO₃ (2, 4, 6, 8, and 10 mg L⁻¹) were used in addition to a control. The effect of AgNO₃ on regeneration assumed a more or less concentration-dependent bell-shaped curve peaking at 4 mg L⁻¹. Enhancements in shoot regeneration were attributed to the known role of AgNO₃ as an ethylene action blocker in addition to improvements in polyamine accumulation without an increase in H₂O₂ content, lipid peroxidation, or DNA damage. The uncoupling of shoot regeneration and polyamine content recorded at high AgNO₃ concentrations can be attributed to the consumption of polyamines to counteract the synchronized oxidative stress manifested by increases in H₂O₂ content, lipid peroxidation, and DNA damage.

Keywords: potato; polyamines; callus; shoot regeneration; H₂O₂; MDA; comet

Citation: Adly, W.M.R.M.; Mazrou, Y.S.A.; EL-Denary, M.E.; Mohamed, M.A.; Abd El-Salam, E.-S.T.; Fouad, A.S. Boosting Polyamines to Enhance Shoot Regeneration in Potato (*Solanum tuberosum* L.) Using AgNO₃. *Horticulturae* **2022**, *8*, 113. <https://doi.org/10.3390/horticulturae8020113>

Academic Editor: Kin-Ying To

Received: 13 December 2021

Accepted: 17 January 2022

Published: 27 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Potato (*Solanum tuberosum*) is the most important non-cereal food crop with annual world production of 370 million tons harvested from 17.34 million hectares (FAO 2019 <https://www.fao.org/faostat/ar/#data/QCL>, accessed on 11 November 2021). Tubers are an essential origin for energy and protein. They also introduce essential nutrients to a diet, including vitamins (B₁, B₃, B₆ and C), minerals (potassium, phosphorus, magnesium, zinc, and iron) in addition to folate, pantothenic acid, riboflavin, and dietary fiber [1]. Compared to other food crops, potatoes can produce more energy, proteins, minerals, and vitamins per unit of time and land area [2]. In addition to indoor uses, potatoes are used in many food industries [3] that generate a huge amount of peel used in bioethanol production [4].

Conventional potato breeding is a difficult task due to the tetraploid nature and high heterozygosity of the potato genomes. The first is responsible for inbreeding depression and intra-species incompatibilities, while the second prevents the incorporation of new traits through conventional breeding [5]. In addition, searching for functional genetic variability in wild relatives is laborious and time-consuming [6,7].

Biotechnology can hasten potato breeding through in vitro selection [8] and transformation [9]. However, biotechnological tools require establishing a regeneration system to obtain genetically improved lines [10]. Thus, studying factors affecting regeneration in

potatoes adds to the genetic improvement of this vital crop. Generally, shoot regeneration depends on genotype, type of the explant used, the hormonal combination used in the regeneration medium, and growth conditions [11].

Different explant types were examined for their potential use in direct and indirect shoot regeneration, including tuber discs, intermodal segments, petioles, leaves, meristems, and anthers [12,13]. However, the leaf and internode are the most widely used explants.

Shoot regeneration always occurs on a medium containing the appropriate concentrations of auxins and cytokinins [14]. Most regeneration protocols for potatoes utilize a mixture of 6-benzylaminopurine (BA) and naphthalene acetic acid (NAA) that produces the highest percentage of shoot regeneration from leaf and internodal explants [13].

Polyamines are nitrogenous, aliphatic, water-soluble, low molecular weight polycationic compounds with at least three amino groups found in all living organisms [15]. They are essential for several physiological processes, including organogenesis, embryogenesis, fruit maturation, and senescence, in addition to responses to abiotic and biotic stresses [16]. However, the high cost of polyamines encourages trials to find less expensive alternatives.

Both ethylene and polyamines share the same precursor, S-adenosyl-L-methionine, for their synthesis [17,18]. In addition, ethylene inhibits methionine decarboxylase and arginine decarboxylase S-adenosyl activities necessary for polyamine synthesis [19,20]. Plant cells are sensitive to small, non-toxic concentrations of Ag^+ ions that have a profound effect on organogenesis and somatic embryogenesis. Ag^+ ions can mitigate the negative impact of ethylene accumulation on shoot regeneration by blocking the ethylene receptor through replacing the copper ion cofactor of the ethylene binding site [21]. Thus, Ag^+ incorporation in culture medium may be a cost-reasonable strategy to enhance polyamine accumulation in plant tissues and thereby alter many developmental aspects [22–24]. It is widely used to enhance regeneration in many plant species including potato [25,26].

Therefore, the aim of this study is to evaluate the impact of five different concentrations of AgNO_3 (2, 4, 6, 8, and 10 mg L^{-1}) on shoot regeneration from internode-sourced callus as well as the growth of regenerated shoots in potato (*Solanum tuberosum* L.) in relation to polyamine accumulation.

2. Materials and Methods

2.1. Explant Preparation

Virus-free tubers of potato (*Solanum tuberosum*) were kindly provided from the Agricultural Research Center, Cairo, Egypt. Tubers were stored at 10 °C till sprouting, then sprouts were detached from tubers and submerged in tap water containing a few drops of a liquid detergent, shaken for 10 min, then rinsed in running tap water for 30 min to remove the detergent. Washed sprouts were transferred to a 250 mL closed jar containing 100 mL of 20% commercial Clorox and a few drops of Tween 20, then shaken for five minutes. The solution was decanted, and sprouts were washed thoroughly with sterile distilled water in a laminar flow cabinet.

Meristems were excised aseptically under binocular microscope and placed in sterilized tubes (one shoot tip/tube), each containing 10 mL sterilized basal medium (MS medium [27] supplemented with 30 g L^{-1} sucrose and 100 mg L^{-1} myoinositol) to which 2 mg L^{-1} calcium pantothenate, 0.1 mg L^{-1} gibberellic acid (GA_3), and 0.01 mg L^{-1} NAA were added. The pH was adjusted to 5.7; the medium was solidified using 7 g L^{-1} agar and autoclaved at 121 °C for 20 min. Cultures were maintained at 25 °C under a 16/8 h light/dark cycle (light intensity 2000 lux). The same growth conditions were applied throughout the study. Six weeks later, shoots about 5–7 cm long were obtained and micropropagated by nodal cuttings with a subculture step repeated every 3 weeks. Micropropagation was carried out in 400 mL glass jars (6 cuttings/jar), each containing about 50 mL basal medium under the same growth conditions. The resulting plants were a source for internode explants for callus initiation.

2.2. Callus Induction and Plant Regeneration

Based on preliminary experiments, an approximately 1 cm segment was excised from the middle internode of each donor plant and inoculated on callus induction medium in 9 cm Petri dishes (10 explants/dish). The medium consisted of basal medium supplemented with 0.186 mg L^{-1} NAA and 2.25 mg L^{-1} BA. Explants were transferred every two weeks to the same medium, but fresh. After six weeks, callus induction % was calculated, and explants along with their resultant calli were transferred to regeneration media. Five regeneration media of basal medium fortified with 2.25 mg L^{-1} BA and filter sterilized AgNO_3 at 0, 2, 4, 6, 8, and 10 mg L^{-1} were employed. Cultures were transferred biweekly onto the same medium, but fresh.

After six weeks, shoot regeneration %, average number of shoots per explant, and the average fresh weights of the regenerated shoots were determined. Samples from each treatment were directed to polyamines, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and chlorophyll determinations, as well as a comet assay.

2.3. Quantification of Total Chlorophyll

Leaf tissues (20 mg) were ground in 2 mL pre-chilled acetone 80% (*v/v*) according to Merwad et al. [28]. The optical density of the clear extract was read at 662 and 644 nm against acetone, and chlorophyll content was determined nm using MacKinney equations [29]:

$$\text{Chlorophyll content } (\mu\text{g mL}^{-1}) = 5.13 E_{662} + 20.41 E_{644}$$

2.4. Polyamine Determinations

Polyamines were extracted by homogenizing 200 mg liquid nitrogen-powdered tissues in 1 mL of 5% perchloric acid for one hour, then centrifuged at $15,000 \times g$ at 4°C for 15 min [30]. The supernatant was neutralized using 5N KOH and stored at -20°C till use. Polyamines were colorimetrically quantified in the regenerated shoots, according to Federico et al. [31]. One mL reaction mixture containing 400 mM Na-phosphate buffer, pH 6.8, 0.1 unit of amine oxidase (Ray Biotech), and 20 μL of the sample was incubated at 37°C . After 10 min the reaction was stopped using 200 μL of 10% trichloroacetic acid (TCA), thereafter the suspension was centrifuged at $15,000 \times g$ for 15 min. The supernatant was mixed with ninhydrin reagent (2.5 g ninhydrin and 376 mg hydrindantin in 60 mL glacial acetic acid and 40 mL 6 M o-phosphoric acid) and glacial acetic acid and incubated in boiling water bath for 30 min then cooled and OD was measured at 510 nm. The polyamine content was determined based on the spermidine standard curve.

2.5. H_2O_2 Determination

H_2O_2 content was quantified following the method described by Loreto and Velikova [32]. A number of 0.2 g liquid nitrogen-powdered tissues were homogenized in 2.5 mL of 0.1% (*w/v*) TCA in an ice bath. After centrifugation for 20 min at 4°C and $15,000 \times g$, 0.5 mL of the clear supernatant was mixed with an equal volume of 10 mM potassium phosphate buffer (pH 7.0) and a double volume of 1.0 M KI. The absorbance was observed at 390 nm, and the amount of H_2O_2 (nM g^{-1} fresh weight) was calculated based on a standard curve constructed using different known concentrations of H_2O_2 .

2.6. Lipid Peroxidation

Lipid peroxidation was measured by the quantification of MDA formation following Stewart and Bewley [33]. Samples of regenerated shoots of all treatments were homogenized in Tris-HCl buffer (100 mM, pH 7.4) containing PVP (1.5%). The homogenates were filtered, and then the filtrates were centrifuged for 15 min at $12,000 \times g$. The supernatant was mixed with 4 mL 2-thiobarbituric acid (0.5%) dissolved in 20% trichloroacetic acid in a 1:4 ratio and kept at 90°C for 30 min and then cooled rapidly. The cooled samples were centrifuged at $10,000 \times g$ for 20 min and the OD of the supernatants was recorded at 532 nm.

After subtraction of the nonspecific absorbance at 600 nm, MDA content was calculated as nM g^{-1} fresh weight using its extinction coefficient ($155 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.7. Comet Assay

DNA damage was estimated in regenerated shoots using an alkaline comet assay outlined by Tice et al. [34] with some modifications. Tissues were homogenized in a cold mincing solution to release the nuclei. The mincing solution consists of Hanks' Balanced Salt Solution (HBSS), lacking calcium and magnesium cations and supplemented with 20 mM EDTA and 10% dimethylsulphoxide (DMSO). A 10 μL aliquot of cell suspension containing approximately 10,000 cells was blended with 75 μL (0.5%) low melting point agarose and spread on an ice-cooled glass slide pre-immersed in 1% normal melting agarose. Thereafter, the solidified slides were kept in chilled lysis buffer (10 mM Tris, pH 10, 2.5 M NaCl, and 100 mM EDTA) freshly supplemented with 1% Triton X-100 and 10% DMSO at 4 °C in darkness. After 24 h, the slides were kept for 20 min in fresh alkaline buffer (pH 13): 300 mM NaOH and 1 mM EDTA. The uncoiled DNA was electrophoresed for 20 min at 25 V (0.90 V/cm) and 300 mA. The products were neutralized in 0.4 M Trizma base and finally fixed in chilled absolute ethanol.

After air-drying at room temperature, the extent of DNA fragments' departure from the nucleoid was recorded by capturing and scoring images of 50 ethidium bromide-stained cells using Komet 5 image analysis software established by Kinetic Imaging, Ltd. (Liverpool, UK). The amplitude of DNA damage was evaluated by calculating % tail DNA (tDNA). tDNA calculated in shoots regenerated on AgNO_3 -free medium was considered as the unit for DNA damage.

2.8. Statistical Analysis

Results of each treatment were represented as the mean of 3 replicates $\pm$ standard deviation (SD). The least significant difference (LSD) between treatments was evaluated at $p \leq 0.05$ using Statistica software version 7.

3. Results

3.1. Callus Induction and Shoot Regeneration

In vitro potato plants (Figure 1) were used as a source for the internode explants. On the callus induction medium, the explants swelled, and nodular green calli started to appear at the cut margins of all internode explants after 2 weeks (Figure 2). Following transfer to shoot regeneration media, calli extended to cover the whole surface of explant, and regenerated shoots started to appear at the third week on all regeneration media (Figure 3).

In the absence of AgNO_3 , about 37% of calli produced shoots on the regeneration medium (Figure 4a). Shoot regeneration % increased gradually following the increase in AgNO_3 concentration, reaching about 1.33 folds of control at 4 mg L^{-1} . The stimulatory effect of AgNO_3 decreased at 6 mg L^{-1} , disappeared at 8 mg L^{-1} , and finally reversed at 10 mg L^{-1} , where shoot regeneration % reached about 75% of control. The average number of shoots per explant increased gradually with an increase in AgNO_3 concentration reaching about 2.3 folds of control at 4 mg L^{-1} , then fell to 1.6 folds of control at 6 mg L^{-1} (Figures 3 and 4b). Exceeding 6 mg L^{-1} , the number of shoots dropped below the control level reaching about 46% of control at 10 mg L^{-1} .

Figure 1. Four week old potato in vitro plants propagated by nodal cuttings, grown on basal medium and maintained at 25 °C under 16/8 h light/dark cycle (light intensity 2000 lux).

Figure 2. Six week old calli induced on internode explants grown on basal medium supplemented with 0.186 mg L⁻¹ NAA and 2.25 mg L⁻¹ BA and maintained at 25 °C under 16/8 h light/dark cycle (light intensity 2000 lux).

Figure 3. Six week old shoots regenerated from calli grown on basal medium supplemented with 2.25 mg L^{-1} BA and different concentrations of AgNO_3 and maintained at 25°C under 16/8 h light/dark cycle (light intensity 2000 lux).

AgNO_3 gradually improved the average fresh weight of regenerated shoots, reaching about 1.5 folds of control at 4 mg L^{-1} (Figure 4c). Further increases in AgNO_3 concentration were accompanied by a gradual decrease in fresh weight to reach about 65% of control at 10 mg L^{-1} , remaining significantly higher than control at 6 mg L^{-1} . The same behavior was reflected by the chlorophyll content of the regenerated shoot in response to an increasing AgNO_3 concentration, except for the absence of significant change at 6 mg L^{-1} (Figure 4d).

3.2. Polyamine Content

In the absence of AgNO_3 , polyamine determination reflected about $450 \mu\text{g}$ in each gram of regenerated shoots on a fresh weight basis (Figure 5). Polyamine accumulation increased in response to introducing AgNO_3 into the regeneration medium, in a concentration-dependent manner, reaching about 3.6 folds of control at 8 mg L^{-1} that remained insignificantly changed with further increases in AgNO_3 concentration to 10 mg L^{-1} .

Figure 4. Effect of AgNO₃ at concentrations of 2, 4, 6, 8, and 10 mg L⁻¹ on % of shoot regeneration (a), average number of regenerated shoots per explant (b), average weight of regenerated shoot (c), and chlorophyll content of regenerated shoots (d) of potato (*Solanum tuberosum* L.). Values are represented as mean $\pm$ SD of triplicates; bars with different letters are significantly different, according to the LSD test, at $p < 0.05$.

Figure 5. Effect of AgNO₃ at concentrations of 2, 4, 6, 8, and 10 mg L⁻¹ on polyamine content in regenerated shoots of potato (*Solanum tuberosum* L.). Values are represented as mean $\pm$ SD of triplicates; bars with different letters are significantly different, according to the LSD test, at $p < 0.05$.

3.3. H_2O_2 and MDA

Compared with control, H_2O_2 content in regenerated shoots remained unaffected on media containing $AgNO_3$ up to 6 mg L^{-1} (Figure 6a). Further increases in $AgNO_3$ concentration were associated with a gradual increase in H_2O_2 content, reaching about 1.65 folds at 10 mg L^{-1} . Similarly, lipid peroxidation, indicated with MDA formation, remained comparable with control up to 6 mg L^{-1} (Figure 6b). However, MDA content reached about 1.4 folds of control at 8 mg L^{-1} and remained without significant change following an increase in $AgNO_3$ concentration.

Figure 6. Effect of $AgNO_3$ at concentrations of 2, 4, 6, 8, and 10 mg L^{-1} on H_2O_2 content (a) and MDA content (b) in regenerated shoots of potato (*Solanum tuberosum* L.). Values are represented as mean $\pm$ SD of triplicates; bars with different letters are significantly different, according to the LSD test, at $p < 0.05$.

3.4. DNA Damage

Estimation of DNA damage in regenerated shoots, represented as tDNA in comet assay, showed no harmful effect related to the utilization of $2\text{--}6\text{ mg L}^{-1}$ $AgNO_3$ on genetic material (Figure 7). The application of higher concentrations of $AgNO_3$ was associated with

a gradual increase in DNA damage as indicated with the doubling of tDNA at 10 mg L^{-1} , compared with control.

Figure 7. Effect of AgNO_3 at concentrations of 2, 4, 6, 8, and 10 mg L^{-1} on tail DNA in regenerated shoots of potato (*Solanum tuberosum* L.). Values are represented as mean $\pm$ SD of triplicates; bars with different letters are significantly different, according to the LSD test, at $p < 0.05$.

4. Discussion

In the present work, callus induction was induced on all explants on medium containing 0.186 mg L^{-1} NAA and 2.25 mg L^{-1} BA and shoot regeneration was carried out following auxin elimination. Similarly, Kumlay, and Ercisli [13] used medium containing both auxin and cytokinin to initiate callus on internodes and leaf explants of four potato cultivars while shoot regeneration started following auxin withdrawal. The same strategy was applied by Ghosh et al. [35] starting with leaf explants of three potato cultivars. Auxins play an important role in the callus induction; they are believed to be related to a change in cell fate during which some somatic cells acquire pluripotency [36]. However, auxins provide limited flexibility of cell fate transition [37] that necessitates their elimination or at least decreasing their concentration in regeneration media. On the other hand, cytokinins affected callus induction through decreasing the cell wall lignification, facilitating callus initiation and in vitro growth [13]. They are the major players in regeneration media, where the shoot regeneration process is the result of interconnections among cytokinin receptors, cell cycles, and the development of shoot meristem [38].

Results of the current investigation emphasize the positive impact of small concentrations of AgNO_3 on shoot regeneration and the growth of regenerated shoots. The response of the related parameters to AgNO_3 concentration assumed a more or less concentration-dependent bell-shaped curve peaked at 4 mg L^{-1} . With a genotype-dependent peak, the same trend was recorded for shoot regeneration in response to the increasing concentrations of AgNO_3 in the potato cultivar 'Kufri Chipsona 1' [25,26,39], tomato [40], primrose [41], oat [42], and wheat [43]. It was also observed dealing with shoot growth and chlorophyll content in potato [26] and robusta coffee [44]. The dependence of the optimum concentration of the genotype may reflect a different tolerance to Ag^+ ion toxicity. In a related context, Kaur et al. [45] recorded the dependence of direct somatic embryogenesis in potato on the presence of AgNO_3 in a culture medium. Moreover, the positive effect of AgNO_3 on in vitro growth of potato shoots was recorded by Turhan [46].

The AgNO₃-related improvements in shoot regeneration, recorded at low concentrations, can be explained by interference with ethylene perception that ameliorates the inhibitory impact of the accumulation of the gas hormone on shoot regeneration [21,43] and shoot growth [47]. The current results reflected only 33% improvement in shoot regeneration % at 4 mg L⁻¹, while the average number of regenerated shoots per explant reached 2.5 folds of control. Similar results were recorded in potato by Kaur and Kumar [39] reflecting the advantageous role of AgNO₃ in the enhancement of the number of regenerated shoots per explant rather than the percentage of explants carrying regenerated shoots. These observations may highlight the unequal competence of explants for shoot regeneration and the appearance of the role of AgNO₃ with competent explants only.

Our results afford another advantageous role for the addition of AgNO₃ to the regeneration medium where improvements in shoot regeneration were associated with significant increases in polyamine content. The role of polyamines in the enhancement of morphogenesis and growth was documented early in potato shoot cultures [48]. A similar role for polyamines concerning shoot regeneration was documented in *Picea abies* [49], *Cedrela fissilis* [50], and *Capparis decidua* [51]. In addition, the role of polyamines in the enhancement of growth and chlorophyll content was also observed in *Bakraii citrus* [52] and *Rosa hybrida* [53].

Both ethylene and polyamines share the same precursor, S-adenosyl-L-methionine, for their synthesis [17,18]. In addition, ethylene inhibits methionine decarboxylase and arginine decarboxylase S-adenosyl activities necessary for polyamine synthesis in pea seedlings [19,20]. Thus, the promotive effect of ethylene inhibitors, e.g., AgNO₃, on organogenesis was attributed to enhancements in polyamine biosynthesis rather than a reduction in ethylene production [22]. Interestingly, Kim et al. [54] reported improvements in the shoot regeneration in *Polygonum tinctorium* in response to introducing AgNO₃ and the polyamine putrescine to the regeneration medium.

The negative impact of introducing AgNO₃ to a regeneration medium starts at 8 mg L⁻¹, where the average number of shoots per explant, the average weight of regenerated shoot, and the chlorophyll content decreased significantly without a significant decrease in the percentage of regeneration, reflecting that high concentrations impair shoot regeneration qualitatively then quantitatively. Both kinds of negative impacts of AgNO₃ at high concentrations can be attributed to the accompanying significant increases in H₂O₂ content and the subsequent lipid peroxidation indicated by an increase in MDA content as well as the increase in DNA damage expressed as tDNA%. The AgNO₃-induced oxidative stress manifested in our study by an increase in H₂O₂ content coupled with an increase in MDA content was also documented in *Cucumis sativus* [55] and tomato [56]. Heavy-metal-induced oxidative stress accompanied by elevated levels of H₂O₂ and MDA was also recorded in potato shoot cultures exposed to cadmium [8].

H₂O₂ and other ROS generated during oxidative stress is associated with damage to cellular macromolecules, including DNA [57,58] and chlorophyll [59,60]. The injurious impact of H₂O₂ on DNA supports its utilization as a positive control in plant comet assays [61]. In a similar context, Szpunar-Krok et al. [62] documented the hazardous effect on chlorophyll content of spraying potato plants with H₂O₂. Similarly, the synchronized increase in both H₂O₂ content and DNA damage manifested by tDNA% was documented in potato plants experiencing salinity stress [63]. Additionally, Vishwakarma et al. [64] recorded the deterioration of DNA and photosynthetic pigments associated with growth retardation in response to AgNO₃ at 1–3 mM in *Brassica* sp. The increase in polyamine content recorded at high AgNO₃ concentrations synchronized with a decline in shoot regeneration reflects the depletion of polyamines in alleviating the ROS load [65].

5. Conclusions

In conclusion, in a specific concentration range, the potent ethylene action inhibitor (AgNO_3) enhances shoot regeneration from potato callus through an increase in polyamine content. At higher concentrations, polyamines are exhausted by the accompanying oxidative stress with subsequent inhibition of shoot regeneration.

Author Contributions: Conceptualization, E.-S.T.A.E.-S.; A.S.F.; M.E.E.-D., Y.S.A.M. and M.A.M.; methodology, W.M.R.M.A. and Y.S.A.M.; software, Y.S.A.M.; W.M.R.M.A.; validation, Y.S.A.M.; E.-S.T.A.E.-S., A.S.F., M.E.E.-D. and M.A.M.; formal analysis, W.M.R.M.A.; investigation, W.M.R.M.A.; resources, M.E.E.-D. and M.A.M.; data curation, W.M.R.M.A.; writing—original draft preparation, Y.S.A.M. and W.M.R.M.A.; writing—review and editing E.-S.T.A.E.-S., A.S.F., Y.S.A.M., M.E.E.-D. and M.A.M.; visualization, Y.S.A.M. and W.M.R.M.A.; supervision, E.-S.T.A.E.-S., Y.S.A.M., A.S.F., M.E.E.-D. and M.A.M.; project administration, Y.S.A.M., M.E.E.-D. and M.A.M.; funding acquisition, M.E.E.-D., Y.S.A.M., M.A.M. and W.M.R.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the Deanship of Scientific Research at King Khalid University for funding this work through the Program of Research Groups under grant number (RGP 2/67/43).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are represented in the text and figures.

Acknowledgments: The authors acknowledge the Deanship of Scientific Research at King Khalid University for funding this work through the Program of Research Groups under grant number (RGP 2/67/43). They also acknowledge Salah El-Din Ahmed Mohamedien, the director of the main vegetable crops and hybrid production project, the Egyptian Ministry of Agriculture and Land reclamation, for the facilities he provided for the completion of this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Devaux, A.; Goffart, J.-P.; Kromann, P.; Andrade-Piedra, J.; Polar, V.; Hareau, G. The Potato of the Future: Opportunities and Challenges in Sustainable Agri-Food Systems. *Potato Res.* **2021**, *64*, 681–720. [[CrossRef](#)] [[PubMed](#)]
2. Zaheer, K.; Akhtar, M.H. Potato Production, Usage, and Nutrition—a Review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 711–721. [[CrossRef](#)] [[PubMed](#)]
3. Zhang, H.; Fen, X.; Yu, W.; Hu, H.; Dai, X. Progress of Potato Staple Food Research and Industry Development in China. *J. Integr. Agric.* **2017**, *16*, 2924–2932. [[CrossRef](#)]
4. Maroufpour, B.; Rad, F.A.; Yazdanseta, S. Bioethanol Production as Biofuel from Potato Peel Using *Saccharomyces Cerevisiae* PTCC 5052 and *Zymomonas Mobilis* PTCC 1718. *Bioagro* **2019**, *31*, 177–184.
5. Diambra, L.A. Genome Sequence and Analysis of the Tuber Crop Potato. *Nature* **2011**, *475*, 189–195.
6. Hameed, A.; Zaidi, S.S.-A.; Shakir, S.; Mansoor, S. Applications of New Breeding Technologies for Potato Improvement. *Front. Plant Sci.* **2018**, *9*, 925. [[CrossRef](#)]
7. Fouad, A.; Hafez, R.; Hosni, H. Authentication of Three Endemic Species of the Caryophyllaceae from Sinai Peninsula Using DNA Barcoding. *Egypt. J. Bot.* **2019**, *59*, 483–491. [[CrossRef](#)]
8. Ashrafzadeh, S.; Leung, D.W. Novel Potato Plants with Enhanced Cadmium Resistance and Antioxidative Defence Generated after in Vitro Cell Line Selection. *PLoS ONE* **2017**, *12*, e0185621. [[CrossRef](#)]
9. Ricoch, A.E.; Hénard-Damave, M.-C. Next Biotech Plants: New Traits, Crops, Developers and Technologies for Addressing Global Challenges. *Crit. Rev. Biotechnol.* **2016**, *36*, 675–690. [[CrossRef](#)]
10. Ohnuma, M.; Teramura, H.; Shimada, H. A Simple Method to Establish an Efficient Medium Suitable for Potato Regeneration. *Plant Biotechnol. J.* **2020**, *37*, 25–30. [[CrossRef](#)]
11. Mukhtadir, M.A.; Habib, M.A.; Mian, M.A.K.; Akhond, M.A.Y. Regeneration Efficiency Based on Genotype, Culture Condition and Growth Regulators of Eggplant (*Solanum melongena* L.). *Agric. Nat. Resour.* **2016**, *50*, 38–42. [[CrossRef](#)]
12. Bordallo, P.N.; Silva, D.H.; Maria, J.; Cruz, C.D.; Fontes, E.P. Somaclonal Variation on in Vitro Callus Culture Potato Cultivars. *Hortic. Bras.* **2004**, *22*, 300–304. [[CrossRef](#)]
13. Kumlay, A.M.; Ercisli, S. Callus Induction, Shoot Proliferation and Root Regeneration of Potato (*Solanum tuberosum* L.) Stem Node and Leaf Explants under Long-Day Conditions. *Biotechnol. Biotechnol. Equip.* **2015**, *29*, 1075–1084. [[CrossRef](#)]
14. Skoog, F.; Miller, C. Chemical Regulation of Growth and Organ Formation in Plant Tissues Cultured In Vitro. *Symp. Soc. Exp. Biol.* **1957**, *11*, 118–130. [[PubMed](#)]

15. Hasanuzzaman, M.; Alhaithloul, H.A.S.; Parvin, K.; Bhuyan, M.; Tanveer, M.; Mohsin, S.M.; Nahar, K.; Soliman, M.H.; Mahmud, J.A.; Fujita, M. Polyamine Action under Metal/Metalloid Stress: Regulation of Biosynthesis, Metabolism, and Molecular Interactions. *Int. J. Mol. Sci.* **2019**, *20*, 3215. [\[CrossRef\]](#)
16. Rakesh, B.; Sudheer, W.; Nagella, P. Role of Polyamines in Plant Tissue Culture: An Overview. *Plant Cell Tissue Organ. Cult.* **2021**, *145*, 487–506. [\[CrossRef\]](#)
17. Evans, P.T.; Malmberg, R.L. Do Polyamines Have Roles in Plant Development? *Annu. Rev. Plant Biol.* **1989**, *40*, 235–269. [\[CrossRef\]](#)
18. Bais, H.P.; Ravishankar, G. Role of Polyamines in the Ontogeny of Plants and Their Biotechnological Applications. *Plant Cell Tissue Organ. Cult.* **2002**, *69*, 1–34. [\[CrossRef\]](#)
19. Apelbaum, A.; Goldlust, A.; Icekson, I. Control by Ethylene of Arginine Decarboxylase Activity in Pea Seedlings and Its Implication for Hormonal Regulation of Plant Growth. *Plant Physiol.* **1985**, *79*, 635–640. [\[CrossRef\]](#)
20. Smith, T. Plant Polyamines Metabolism And Function Polyamine Synthesis. In *Polyamines And Ethylene: Biochemistry, Physiology And Interaction*; American Society of Plant Physiologists: Rockville, MD, USA, 1985; pp. 1–23.
21. Zhao, X.-C.; Qu, X.; Mathews, D.E.; Schaller, G.E. Effect of Ethylene Pathway Mutations upon Expression of the Ethylene Receptor ETR1 from *Arabidopsis*. *Plant Physiol.* **2002**, *130*, 1983–1991. [\[CrossRef\]](#)
22. Kumar, V.; Parvatam, G.; Ravishankar, G.A. AgNO₃: A Potential Regulator of Ethylene Activity and Plant Growth Modulator. *Electron. J. Biotechnol.* **2009**, *12*, 8–9. [\[CrossRef\]](#)
23. Salem, J. Effects of Anti-Ethylene Compounds on Vitrication and Genome Fidelity of *Stevia rebaudiana* Bertoni. *Egypt. J. Bot.* **2020**, *60*, 519–535. [\[CrossRef\]](#)
24. Strader, L.C.; Beisner, E.R.; Bartel, B. Silver Ions Increase Auxin Efflux Independently of Effects on Ethylene Response. *Plant Cell* **2009**, *21*, 3585–3590. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Kaur, A.; Reddy, M.S.; Kumar, A. Efficient, One Step and Cultivar Independent Shoot Organogenesis of Potato. *Physiol. Mol. Biol. Plants* **2017**, *23*, 461–469. [\[CrossRef\]](#)
26. Alva Ticona, S.; Oropeza, M. Effect of Culture Medium Consistence and Silver Nitrate on Micropropagation of Two Potato (*Solanum tuberosum*) Cultivars. *Rev. Colomb. Biotechnol.* **2013**, *15*, 55–62. [\[CrossRef\]](#)
27. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant* **1962**, *15*, 473–497. [\[CrossRef\]](#)
28. Merwad, A.-R.M.; Desoky, E.-S.M.; Rady, M.M. Response of Water Deficit-Stressed *Vigna unguiculata* Performances to Silicon, Proline or Methionine Foliar Application. *Sci. Hortic.* **2018**, *228*, 132–144. [\[CrossRef\]](#)
29. Sestak, Z.; Catsky, J.; Jarvis, P. Determination of Chlorophylls a and b. *Plant Photosynth. Prod.* **1971**, 672–701.
30. Minocha, R.; Shortle, W.C.; Long, S.L.; Minocha, S.C. A Rapid and Reliable Procedure for Extraction of Cellular Polyamines and Inorganic Ions from Plant Tissues. *J. Plant Growth Regul.* **1994**, *13*, 187–193. [\[CrossRef\]](#)
31. Federico, R.; Angelini, R.; Cogoni, A.; Floris, V. Enzymatic Methods for the Quantification of Polyamines Using Plant Amine Oxidases. *Biochem. Physiol. Pflanz.* **1991**, *187*, 113–119. [\[CrossRef\]](#)
32. Loreto, F.; Velikova, V. Isoprene Produced by Leaves Protects the Photosynthetic Apparatus against Ozone Damage, Quenches Ozone Products, and Reduces Lipid Peroxidation of Cellular Membranes. *Plant Physiol.* **2001**, *127*, 1781–1787. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Stewart, R.R.; Bewley, J.D. Lipid Peroxidation Associated with Accelerated Aging of Soybean Axes. *Plant Physiol.* **1980**, *65*, 245–248. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Tice, R.R.; Agurell, E.; Anderson, D.; Burlinson, B.; Hartmann, A.; Kobayashi, H.; Miyamae, Y.; Rojas, E.; Ryu, J.-C.; Sasaki, Y. Single Cell Gel/Comet Assay: Guidelines for in Vitro and in Vivo Genetic Toxicology Testing. *Environ. Mol. Mutagen.* **2000**, *35*, 206–221. [\[CrossRef\]](#)
35. Ghosh, S.; Majumdar, S.; Sarkar, D.; Datta, K. An Efficient Adventitious Shoot Regeneration System for Potato (*Solanum tuberosum* L.) Using Leaf Discs. *J. Plant Biochem. Biotechnol.* **2015**, *24*, 298–304. [\[CrossRef\]](#)
36. Xu, C.; Cao, H.; Zhang, Q.; Wang, H.; Xin, W.; Xu, E.; Zhang, S.; Yu, R.; Yu, D.; Hu, Y. Control of Auxin-Induced Callus Formation by BZIP59–LBD Complex in *Arabidopsis* Regeneration. *Nat. Plants* **2018**, *4*, 108–115. [\[CrossRef\]](#)
37. Shin, J.; Seo, P.J. Varying Auxin Levels Induce Distinct Pluripotent States in Callus Cells. *Front. Plant Sci.* **2018**, *9*, 1653. [\[CrossRef\]](#)
38. Bidabadi, S.S.; Jain, S.M. Cellular, Molecular, and Physiological Aspects of in Vitro Plant Regeneration. *Plants* **2020**, *9*, 702. [\[CrossRef\]](#)
39. Kaur, A.; Kumar, A. The Effect of Gelling Agent, Medium PH and Silver Nitrate on Adventitious Shoot Regeneration in *Solanum Tuberosum*. *bioRxiv* **2020**. [\[CrossRef\]](#)
40. Shah, S.H.; Ali, S.; Jan, S.A.; Din, J.; Ali, G.M. Assessment of Silver Nitrate on Callus Induction and in Vitro Shoot Regeneration in Tomato (*Solanum lycopersicum* Mill.). *Pak. J. Bot.* **2014**, *46*, 2163–2172.
41. Hayta, S.; Smedley, M.A.; Li, J.; Harwood, W.A.; Gilmartin, P.M. Plant Regeneration from Leaf-Derived Callus Cultures of Primrose (*Primula vulgaris*). *HortScience* **2016**, *51*, 558–562. [\[CrossRef\]](#)
42. Warchol, M.; Juzoń, K.; Dziurka, K.; Czyczyło-Mysza, I.; Kaploniak, K.; Marcińska, I.; Skrzypek, E. The Effect of Zinc, Copper, and Silver Ions on Oat (*Avena sativa* L.) Androgenesis. *Plants* **2021**, *10*, 248. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Malik, W.A.; Mahmood, I.; Razzaq, A.; Afzal, M.; Shah, G.A.; Iqbal, A.; Zain, M.; Ditta, A.; Asad, S.A.; Ahmad, I.; et al. Exploring Potential of Copper and Silver Nano Particles to Establish Efficient Callogenesis and Regeneration System for Wheat (*Triticum aestivum* L.). *GM Crops Food* **2021**, *12*, 564–585. [\[CrossRef\]](#) [\[PubMed\]](#)

44. Giridhar, P.; Indu, E.; Vijaya Ramu, D.; Ravishankar, G. Effect of Silver Nitrate on in Vitro Shoot Growth of Coffee. *Trop. Sci.* **2003**, *43*, 144–146. [\[CrossRef\]](#)
45. Kaur, A.; Reddy, M.S.; Kumar, A. Direct Somatic Embryogenesis of Potato [*Solanum tuberosum* (L.)] Cultivar ‘Kufri Chipsona 2’. *Plant Cell Tissue Organ. Cult.* **2018**, *134*, 457–466. [\[CrossRef\]](#)
46. Turhan, H. The Effect of Silver Nitrate (Ethylene Inhibitor) on in Vitro Shoot Development in Potato (*Solanum tuberosum* L.). *Biotechnology* **2004**, *3*, 72–74.
47. Vaseva, I.I.; Qudeimat, E.; Potuschak, T.; Du, Y.; Genschik, P.; Vandenbussche, F.; Van Der Straeten, D. The Plant Hormone Ethylene Restricts *Arabidopsis* Growth via the Epidermis. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E4130–E4139. [\[CrossRef\]](#)
48. Feray, A.; Hourmant, A.; Brun, A.; Penot, M. Effect of Polyamines on Morphogenesis of in Vitro Potato Plants (*Solanum tuberosum* Cv. Bintje). *C. R. Acad. Sci. Sér. 3 Sci. Vie* **1993**, *316*, 1446–1451.
49. Vondráková, Z.; Eliášová, K.; Vágner, M.; Martincová, O.; Cvikrová, M. Exogenous Putrescine Affects Endogenous Polyamine Levels and the Development of *Picea abies* Somatic Embryos. *Plant Growth Regul.* **2015**, *75*, 405–414. [\[CrossRef\]](#)
50. Aragão, V.P.M.; Reis, R.S.; Silveira, V.; Santa-Catarina, C. Putrescine Promotes Changes in the Endogenous Polyamine Levels and Proteomic Profiles to Regulate Organogenesis in *Cedrela fissilis* Vellozo (Meliaceae). *Plant Cell Tissue Organ. Cult.* **2017**, *130*, 495–505. [\[CrossRef\]](#)
51. Ahlawat, J.; Sehrawat, A.R.; Choudhary, R.; Samarina, L.; Bandaralage, J.-H.; Chaudhary, R. Quantifying Synergy of Plant Growth Hormones, Anti-Oxidants, Polyamines and Silver Nitrate for Optimizing the Micro Propagation of *Capparis decidua*: An Underutilised Medicinal Shrub. *Nucleus* **2020**, *63*, 313–325. [\[CrossRef\]](#)
52. Khoshbakht, D.; Asghari, M.; Haghighi, M. Influence of Foliar Application of Polyamines on Growth, Gas-Exchange Characteristics, and Chlorophyll Fluorescence in *Bakrati citrus* under Saline Conditions. *Photosynthetica* **2018**, *56*, 731–742. [\[CrossRef\]](#)
53. Yousefi, F.; Jabbarzadeh, Z.; Amiri, J.; Rasouli-Sadaghiani, M.H. Response of Roses (*Rosa hybrida* L. ‘Herbert Stevens’) to Foliar Application of Polyamines on Root Development, Flowering, Photosynthetic Pigments, Antioxidant Enzymes Activity and NPK. *Sci. Rep.* **2019**, *9*, 1–11.
54. Kim, J.K.; Baskar, T.B.; Park, S.U. Silver Nitrate and Putrescine Enhance In vitro Shoot Organogenesis in *Polygonum tinctorium*. *Biosci. Biotechnol. Res. Asia* **2016**, *13*, 53–58. [\[CrossRef\]](#)
55. Tripathi, A.; Liu, S.; Singh, P.K.; Kumar, N.; Pandey, A.C.; Tripathi, D.K.; Chauhan, D.K.; Sahi, S. Differential Phytotoxic Responses of Silver Nitrate (AgNO₃) and Silver Nanoparticle (AgNps) in *Cucumis sativus* L. *Plant Gene* **2017**, *11*, 255–264. [\[CrossRef\]](#)
56. Noori, A.; Donnelly, T.; Colbert, J.; Cai, W.; Newman, L.A.; White, J.C. Exposure of Tomato (*Lycopersicon esculentum*) to Silver Nanoparticles and Silver Nitrate: Physiological and Molecular Response. *Int. J. Phytoremediation* **2020**, *22*, 40–51. [\[CrossRef\]](#) [\[PubMed\]](#)
57. De Veylder, L.; Beeckman, T.; Inzé, D. The Ins and Outs of the Plant Cell Cycle. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 655–665. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Hafez, R.; Fouad, A. Mitigation of Genotoxic and Cytotoxic Effects of Silver Nanoparticles on Onion Root Tips Using Some Antioxidant Scavengers. *Egypt. J. Bot.* **2020**, *60*, 133–145. [\[CrossRef\]](#)
59. Chen, X.-B.; Zhao, X.-H.; Zhu, Y.; Gong, Y.-D.; Li, L.-B.; Zhang, J.-P.; Kuang, T.-Y. Hydrogen Peroxide-Induced Chlorophyll a Bleaching in the Cytochrome b 6 f Complex: A Simple and Effective Assay for Stability of the Complex in Detergent Solutions. *Photosynth. Res.* **2006**, *90*, 205–214. [\[CrossRef\]](#)
60. Dai, H.; Shan, C. Others Effects of Lanthanum on the Antioxidant Capacity of Chloroplasts and Chlorophyll Fluorescence Parameters of Maize Seedlings under Chromium Stress. *Photosynthetica* **2019**, *57*, 27–31. [\[CrossRef\]](#)
61. Santos, C.L.; Pourrut, B.; Oliveira, J.M.P. The Use of Comet Assay in Plant Toxicology: Recent Advances. *Front. Genet.* **2015**, *6*, 216. [\[CrossRef\]](#)
62. Szpunar-Krok, E.; Jańczak-Pieniążek, M.; Skrobacz, K.; Bobrecka-Jamro, D.; Balawejder, M. Response of Potato (*Solanum tuberosum* L.) Plants to Spraying by Hydrogen Peroxide. *Sustainability* **2020**, *12*, 2469. [\[CrossRef\]](#)
63. Dikilitas, M.; Collins, A.R.; Kocyigit, A.; El Yamani, N.; Karakas, S. DNA Damage in Potato Plants Exposed to High Level of NaCl Stress. In Proceedings of the Frontiers in Genetics, Conference Abstract: ICAW, Antwerpen, Belgium, 1–4 September 2015.
64. Vishwakarma, K.; Upadhyay, N.; Singh, J.; Liu, S.; Singh, V.P.; Prasad, S.M.; Chauhan, D.K.; Tripathi, D.K.; Sharma, S. Differential Phytotoxic Impact of Plant Mediated Silver Nanoparticles (AgNPs) and Silver Nitrate (AgNO₃) on *Brassica* Sp. *Front. Plant Sci.* **2017**, *8*, 1501. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Seo, S.Y.; Kim, Y.J.; Park, K.Y. Increasing Polyamine Contents Enhances the Stress Tolerance via Reinforcement of Antioxidative Properties. *Front. Plant Sci.* **2019**, *10*, 1331. [\[CrossRef\]](#) [\[PubMed\]](#)