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EFFECT OF SILVER THIOSULPHATE AND PHOTOPERIOD ON *IN VITRO* TUBERIZATION OF THREE POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS

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ABSTRACT

The experiment was carried out to study the effect of 6 concentrations of silver thiosulphate (STS) and photoperiod on *in vitro* tuberization of three potato cultivars, namely, Herms, Spunta and Santana. The experiment was conducted in tissue culture laboratory, Vegetable Crops Dep., Fac. Agric., Cairo Univ. Tip meristems in each cultivar were excised and cultured on MS media. *In vitro* plantlets of all cultivars were multiplied as per routine by sub culturing twice. In microtuberization stage, nodal cuttings from second subculture were cultured on MS media with 0, 0.4, 0.8, 1.2, 1.6 or 2 mM of STS. The treatments were kept under dark or light conditions. STS at 0.4 and 0.8 mM recorded the tallest microplants. The highest number of leaves and branches per plantlet were obtained with 0.4mM STS. On the other hand, the STS at 2 mM recorded the lowest values of plantlet height, number of leaves and branches per plantlet. The highest percentage of microtuberization was recorded with STS at 0.4 mM under dark conditions, and at 2 mM under light one. At dark, Spunta with 1.6 mM STS produced significantly the highest number of microtubers/plantlet. Whereas at light, the maximum number of microtubers/plantlet was obtained with 0.4, 0.8 and 1.2 mM STS in Herms, Spunta and Santans, respectively. In'Spunta', application of STS at 0.4 mM under light folded the number of microtubers/plantlet 2.9 compared with dark. In Santana, number of microtubers/plantlet of 0, 0.8, 1.2, 1.6 and 2 mM STS were higher in light comparing to dark. STS applied at 0.8 and 1.2 mM produced significantly the highest value of microtuber fresh weight in Spunta and Santana, respectively under dark. In 'Spunta', the applications with 0, 1.2, 1.6 and 2 mM STS at light conditions recorded fresh weight of microtuber higher than in dark.

Keywords; Potato, *in vitro* tuberization, silver thiosulphate, dark and light conditions.

Introduction

Potato (*Solanum tuberosum* L.) is a cool season crop belonging to family solanaceae. It is cultivated for the ability to form tubers that have a contents of starch, proteins, antioxidants, vitamins (Shan *et al.,* 2013), as well as minerals, essential amino acids methionine and cysteine (Abelenda *et al.,* 2011). Potato is one of the major root and tuber crops worldwide, i.e., potato, sweet potato, taro or dasheen, cassava and yam. These crops are in the second level in importance to cereals as a global source of carbohydrates. Besides, potato represents the fourth largest food crop in terms of production after wheat (*Triticum aestivum*), maize (*Zea mays* L.) and rice (*Oryza sativa* L.). It's the world's most widely grown tuber crop, historically contributing to food and nutrition security in the world (Aksenova *et al*., 2012; Yu *et al.,* 2012 & Shan *et al.,* 2013). According to FAO STAT, 2019, the total potato production was over 368 million metric tons in the world at 2018.

Egypt is ranked the first producer of potato in Africa, with total production 4,896,476 tons produced from 176,670 ha with average yield 27.715 tons per ha However, the total exported quantity in 2017 was 671,287 tons that represent about 13.8% of the total production (FAO STAT, 2020).

The main propagation method of potato is asexually, through small tubers or divided large tubers (Li *et al.,* 2005; Zhang *et al.,* 2006). In 2017, Egypt imported 152,753 tons of seed tubers from Europe (FAOSTAT, 2020). The main problem, which faces the production of potato seed with conventional methods, is the high risk to viral, fungal and bacterial diseases with the steps of multiplication in the field, as the disease may be transferred to progeny by tubers (Dobránszki *et al.,* 2008). Moreover, the high cost of storage and transport of seed. The seed alone accounts for 40-50 % of total cost of cultivation (Struik and Wiersema, 1999).

Micropropagation technology has been successfully employed to eliminate virus infection from systemically infected potato clones, to ensure their large scale multiplication under diseases free conditions, and to maintain a constant flow of disease free plant through *in vitro* culture (Naik and Sarkar, 2000). Furthermore, plant tissue culture techniques have become powerful tool for propagation of potato to overcome the problems which facing traditional methods of propagation. Also, it is very useful for rapid multiplication, safe exchange and conservation of many vegetative propagated crops (Hussain and Tyagi, 2006) and on top of these techniques, meristem culture technique is used to produce virus free plants especially at the vegetative

propagated crops (Abo El-Nil and Zettler, 1976). Therefore, *in vitro* propagation has become the most viable alternative for ensuring efficient multiplication, and supplying the quantity and quality of disease-free plant material which is required for the establishment of large-scale plantations (Dobránszki *et al.,* 2008).

Seed production techniques of potato can be designed with *in vitro* multiplication through either plantlet regeneration or microtuber production. The main characteristics of microtubers produced through the *in vitro* culture techniques are the production of disease-free and high-quality seeds, also *in vitro* tubers are easier in the storage, handle and exchange of germplasm than the plantlets. In addition, it is stored for a long time. (Hussey and Stacy, 1981 and Seabrook *et al.,* 1993).

Potato microtuberization is influenced by multiple factors, including genotype, explant type, media, and particular growth conditions (sucrose, light, temperature) (Li *et al.,* 2005). Potato nodes cultured in vessels with sealed covers or with covers screwed down too tightly develop stoloniferous shoots with small leaves, an effect that has been shown to be due to ethylene accumulation in the culture vessels (Hussey and Stacey 1981, 1984). Inhibition of ethylene action using ethylene antagonists, such as silver thiosulfate (STS), can lead to normal growth of potato tissues which consequently produce larger leaves *in vitro* and increases yield and viability of protoplasts (Perl *et al*., 1988; Chi and Pua 1989; and Chang and Chan1991).

The aim of the present study was to reach the proper protocol for *in vitro* production of virus-free microtubers of some potato cultivars, under light and dark conditions, through application of silver thiosulfate at different concentrations to MS medium.

Materials and Methods

Plant materials

This study was carried out at tissue Culture laboratory, Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period from January 2019 to July 2020. Potato tuber seeds (*Solanum tuberosom* L.) cultivars Santana, Herms and Spunta were used. The source of tubers was Potato Research Station, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt

Preparation and sterilization of explants

The potato tuber seed were sprayed with Gibberellic acid (GA_3) (1 ppm) to break the dormancy and then they were placed at dark for 10-15 days. The sprouts were sterilized by dipping in a sterilizer solution consisting of 20% Sodium hypochlorite (commercial bleaching compound, Clorox) + 2 drops of tween 20. Thereafter, the sprouts were subjected to shaking for 20 minutes using electronic shaker. The explants were washed 3 times with sterilized distilled water. The tip meristem (0.5 mm) was excised under an electronic binuclear and cultured in 250 ml culture jar, each containing 40 ml of solid media based on MS (Murashige and Skoog, 1962; Table 1) strength basal medium with 3% sucrose and 0.8 % agar. The excised explants were cultured at 24 ± 2C^{o} for 30 days.

Table 1 : Composition of Murashige and Skoog medium (MS) 1962

Constituents	Concentration (mg/l)
Macro- nutrient	
NH_4NO_3	1650
KNO_3	1900
$MgSO_4 \cdot 7H_2O$	370
$CaCl_2 \cdot 2H_2O$	440
KH_2PO_4	170
Micro- nutrient	
KI	0.83
H_3BO_3	6.20
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.60
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$Na_2EDTA \cdot 2H_2O$	37.0
$FeSO_4 \cdot 7H_2O$	27.8
Vitamins and organics	
Nicotinic acid	0.5
Pyridoxine-HCL	0.5
Thiamin-HCL	0.1
Myo-inositol	100.0
Glycin	2.0

Multiplication stage

All cultivars were repropagated with two node cuttings after 30 days. The nodal segments were separated (with 1-2 leaves) and cultured on MS strength basal medium with 3% sucrose and 0.8 % agar. *In vitro* plantlets of all cultivars were multiplied as per routine by sub culturing twice to get the required number of plantlets for experimentation. Cultures were incubated at 24±2°C under 16-hour light with a light intensity of 2000 lux for 45 days.

Microtuberization stage

Silver thiosulfate (STS) was added at 0, 0.4, 0.8, 1.2, 1.6 or 2 mM to MS medium before autoclaving. Silver thiosulfate solution preparation was as described by Roh *et al.* (2012) as following: 0.1M Sodium thiosulphate stock solution was prepared by dissolving 1.98 g of Sodium thiosulphate in 100 ml of distilled water m then 0.1 M Silver nitrate stock solution was prepared by dissolving 0.34 g of Silver nitrate in 100 ml of water distilled. The preparation of 0.02 M Silver thiosulfate solution was achieved by slowly pouring 20 ml of 0.1 M Silver nitrate stock solution in 80 ml of 0.1 M Sodium thiosulphate stock solution.

Nodal cuttings from the three cultivars (Santana, Herms and Spunta) were used as explants for microtubers formation. The source of the nodal cutting was the plantlets produced from the second subculture of the multiplication stage. Four weeks *in vitro* micropropagated plantlets were cultured on 250 ml glass jars; each having 40 ml microtuberization solid medium. The cultured vessel jars were kept under aseptic conditions in a growth room at 24±2^{0}C. Forty days after culture on treatments, number of branches per plantlet, plantlet height (cm), and leaves number per plantlet were recorded and then 20 ml of MS medium containing 80 g/l of sucrose was added to all concentrations. The jars were kept under complete darkness in boxes or under16 hrs photoperiod with a light intensity of 2000 lux at 24 ±2^{0}C for 9 weeks. At the end 9 weeks either on dark or light conditions, number of

microtubers per plantlet, fresh weight of microtuber (g) and micro tuberization percentage (%) were recorded.

Virus detection

The samples from excised tip meristems of all cultivars were tested against potato virus x (PVX), potato virus y (PVY) and potato leaf roll virus (PLRV) before using them at microtuberzation stage. In addition, at the end of microtuberzation, the micro tubers were sprouted and the small shoots were used to examined for the same three viruses: PLRV, PVX, and PVY by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) , according to Clark and Adams (1977). The detection was done at a Plant Diseases Research Center, Agricultural research center, Cairo, Giza, Egypt.

Statistical analysis

Regular analyses of variance of Completely Randomize Design (CRD) were performed on all *in vitro* data. Thereafter, $LSD_{0.05}$ was calculated for comparing between means (Snedecor and Cochran, 1980).

Results and Discussion

Effect of silver thiosulphate (STS) concentrations on plant height (cm) and number of leaves/plantlet of three potato cultivars after 40 days from *in vitro* culture

Data in Table 2 show the effect of silver thiosulphate (STS) concentrations on plant height (cm) of three potato cultivars after 40 days from *in vitro* culture. The comparison among the cultivars under study showed that 'Spunta' recorded significantly the highest value of plant height as compared to all cultivars; however, no significant difference was recorded between 'Herms' and 'Santana'. These findings agree with Mollers *et al.* (1992), who reported that the cultivars differed in their responses during *in vitro* propagation; this difference may be attributing to the genotypes performance. Also Sarkar *et al*. 1999, they found that the genotypes differed in their responses and each genotype have a different growth parameters.

Regrinding the applied STS concentrations, the application of STS at 0.8 mM recorded the tallest microplants but with no significant difference with the application of STS at 0.4 mM. On the other hand, the application of 2 mM STS recorded significantly the lowest value of plant height. The value of plant height increased by the increment of STS concentration up to 0.8 mM STS and then the values decreased with the increment of the concentration. It was previously reported that the presence of STS in culture media enhanced the *in vitro* growth of many crops, such as lettuce (Huang and Khan, 1988), potato (Chang and Chan, 1991; Sarkar *et al.*, 1999; Rostami and Ehsanpour, 2010), *Solanum nigrum* (Sridhar *et al*, 2011) and *Brassica napus* (Roh *et al*., 2012). This enhancement refers to the inhibition affect of STS to ethylene accumulation in vessels (Sarkar *et al.*, 1999). In addition to Ehsanpour and Jones (2001), they tested the comparisons between control (MS medium, no STS) and concentrations of 50 µM, 100 µM and 200 µM STS. They reported that the presence of STS (50 or 100 µM) in the culture medium increased significantly leaf area and internode length of nodule shoots culture of potato cv. Delaware and they referred this effects to that STS decreased the ethylene accumulation in the culture vessels, therefore, inhibition of ethylene action using ethylene antagonist. On the other hand, the high concentrations of STS (200 µM) caused inhibition for *in vitro* growth of plantlets. This may be referring to that the high concentrations of STS led to toxicity of high concentrations of ion and this agree with Ibrahim *et al.* (2016), who mentioned that the increase of STS at medium to 1.5ml/l decreased the potato growth.

With respect to an interaction between cultivars and concentrations, in Herms, the application of 0.8 mM of STS to MS medium recorded the highest value of plant height without significant difference as compared to STS at 0.4 mM, while both of them were significantly the tallest plantlets. On the contrary, the highest concentration of STS (2mM) led to obtaining the shortest plantlet but without significant difference as compared to application of 1.6 mM of STS. In Spunta, there were no significant differences between 0, 0.4 and 0.8 mM of STS. In addition, the highest concentration of STS (2mM) obtained significantly the shortest plantlet as compared to all concentrations. Santan obtained the same results; tallest plantlets were recorded with 0.8 mM of STS in compared with the other concentrations. However, there were significant differences between 0, 0.4, 0.8, 1.2, 1.6 mM STS. On the other hand, the high concentration of STS (2 mM) showed significantly the lowest value of plant height in 'Santana'. The results in 'Spunta' and 'Santana' are in agreement with Rakosy *et al.* (2011) who reported that the application of STS (62.5 or 250mg/l) to MS medium of *in vitro* cultures of *Solanum Chacoense* , stimulated only the growth of leaf, root and stem length , being not significantly different as compared with control explants. The pervious findings are on the contrary of most of reports which found that the presence of STS in the culture media improved the growth as compared with control (Ibrahim *et al*., 2016). This enhancement refers to the inhibition affect of STS to ethylene accumulation in vessels. Also Sarkar *et al*., 1999, who tested the responses of 7 genotypes of potato and their interactions with 7 concentrations of STS. They suggested that the genotypes differed in their response to STS concentrations. And There were significance between genotypes and STS interactions for some recorded characters such as; microshoot height, number of nodes per microplant, number of green leaves permicroplant and leaf senescence.

Data in Table 2 show the effect of silver thiosulphate (STS) concentrations number of leaves/plantlet of three potato cultivars after 40 days from *in vitro* culture. Regrinding the tested cultivars, no significant differences were recorded among all cultivars in number of leaves/plantlet.

By comparing the applied concentrations, the application of STS at 0.4 mM recorded significantly the highest value of number of leaves. However, the application with 0.8 mM STS ranked the second but with no significant difference with control (0 mM STS). On the other hand, the lowest values of number of leaves were obtained with the highest concentrations of STS (1.2, 1.6 and 2 mM STS) with no significant differences to each other. This may be referring to the high toxicity of high concentrations of ion that caused the inhibition of *in vitro growth* of plantlets. These results agree with those of Ibrahim *et al*. (2016), who mentioned that the increase of STS at medium to 1.5ml/l decreased the potato growth. In addition Diab (2017), who observed that the increasing STS concentrations from 0.5 to 8

ml/l decreased gradually root number and their length of date palm.

The interaction between cultivars and concentrations on the number of leaves/plantlet was significant. In this respect, the highest number of leaves was recorded in the three used cultivars, i.e., 'Herms', Santana and 'Spunta' with the application of STS at 0.4 mM. However, the application with 0.8 mM STS was without significant difference as compared to at 0.4 mM in Herms and Santana. On the contrary, the lowest value of number of leaves was obtained with the concentration of 1.6 mM in'Herms' and 2 mM STS in Santana and 'Spunta'. The number of leaves values at 0.4 STS were doubled 2.5 and 2.4 times in 'Spunta' and 'Santana', respectively, as compared with the values at 2 mM STS. The previous results are disagree with findings of Rakosy *et al.* (2011) who reported that the application of STS with 250mg/l to MS medium of *in vitro* cultures of *Solanum Chacoense* , recorded no significant differences in number of leaves but there were an inverse ration between leaf fresh weight and stem and root length. On the other hand, our results agree with EL-Shobaky and Ibrahim (1997). They tested the effect of STS at levels 0, 0.25, 0.50 and 1.0 ml of 4mM on potato cvs. Cara and Spunta. And they reported that the potato cv. Cara showed the highest number of leaflets/plantlet while, MS media with 0.25ml STS/l produced leaflets number higher than the medium with 0.50ml STS /l. in addition Chang and Chan (1991), they found that the STS treatment (2µM) enhanced the fresh weight of plantlet 6 times greater than that without STS. In addition, Roh *et al*., 2012 who found that the optimal *Brassica napus* shoots regeneration response was observed in medium supplemented with 50 µM STS. The frequency of shoot regeneration ranged from 52 to 60 % on STS medium. Moreover, shoot length and fresh weight was also enhanced in medium supplemented with STS. In addition to the above, Sarkar *et al*., 1999 concluded that the number of leaves increased with increasing concentrations of STS in medium. A linear relationship between STS and number of green leaves per microplant was significant in all genotypes. It could be concluded from the previous reports and our findings that the each cultivar has a specific concentration of STS and this is genotype dependent.

Effect of silver thiosulphate (STS) concentrations on number of branches/plantlet of three potato cultivars after 40 days from *in vitro* culture:

Data in Table 3 show the effect of silver thiosulphate (STS) concentrations on number of branches/plantlet of three potato cultivars after 40 days from *in vitro* culture. With respect to cultivars, Spunta recorded the highest value of number branches/plantlet. Meanwhile, no significant difference was obtained between 'Spunta' and 'Santana. The same trend was obtained by Mollers *et al* (1992), who reported that the cultivars differed in their responses to the addition of STS to *in vitro* potato; this effect may be attributing to the genotypes performance. In addition to Sarkar *et al*. 1999, they found that the genotypes differed in their responses and each genotype have a different growth parameters.

Concerning the effect of STS concentrations, the application of 0.4 mM STS recorded the highest number of branches. The value was significantly higher than the control (0 mM STS). The concentration of 0.8 mM STS came at the second rank in this respect, but with no significant difference comparing with 0.4 mM of STS. The number of branches was negatively affected by the increment of STS concentration, where the lowest value of number of branches was noticed at the concentration of 2 mM STS. The pervious results are in contradiction with Sarkar *et al*. (1999), who studied the effect of different concentrations of STS silver thiosulfate (STS) in reducing ethylene-induced culture during *in vitro* growth of seven genotypes of potato. They reported that the addition of STS significantly improved the microplant growth of potato shoot cultures *in vitro*. The number of leaves per microplant increased and leaf senescence decreased linearly as the levels of STS in the medium increased except at the high concentrations (200 µM). They explained their findings by that the inhibition affects by the accumulation of ethylene during potato shoot culture can be suppressed by the addition of STS to the medium. Also, Sridhar (2011) reported that supplementing MS medium with STS led to highest frequency of regeneration (95%) and increasing number of shoots of *Solanum nigram*. However, the low concentrations of STS gave maximum number of shoots.

With respect to an interaction between cultivars and concentrations, the application of 0.4 mM STS recorded the highest number of branches in 'Herms' and 'Spunta' without significant difference as compared to STS at 0.8 mM. The concentration of 0.4 mM STS doubled the values of number of branches 1.8 and 1.3 times in Herms and Spunta, respectively, as compared with the values of control. On the other contrary, the lowest number of branches was obtained in 'Herms' by using STS at 1.6 mM. The highest concentration of STS (2 mM STS) recorded significantly the lowest values of number of branches in 'Spunta' and 'Santana'. This may be refer to that the high concentrations of STS cause the inhibition of *in vitro growth* of plantlets due to the high toxicity of high concentrations of ion (Ibrahim *et al*., 2016). Also, the responses of cultivars were differed under tested concentrations. This agree with EL-Shobaky and Ibrahim (1997), they reported that there are differences between potato cvs; Cara and Spunta to their responses to STS at levels 0, 0.25, 0.50 and 1.0 ml of 4mM. Cara cultivar showed the highest number of leaflets/plantlet with 0.25ml STS/l. Also Sarkar *et al*., 1999, who suggested that the genotypes differed in their response to STS concentrations. The values of number branches at 0.4 mM STS were folded 3.6, 4.3 and 6.3 in Herms, Spunta and Santana, respectively, as compared with the values of 2 mM STS. It is noticeable that the high concentrations of STS at our study recorded the lowest values of plant height, number of leaves and number of branches/ plantlet in all cultivars. Within the same findings, Mollers *et al., (*1992) found that the addition of silver thiosulphate (STS) to *in vitro* potato propagation medium at higher STS concentrations caused toxic symptoms in the plants of most of the tested genotypes. And these effects were differed among genotypes.

Table 2: Effect of silver thiosulphate (STS) concentrations on plantlet or height (cm) and number of leaves/plantlet of three potato cultivars after 40 days from *in vitro* culture.

Treatment	mM	Plant height (cm)				Number of leaves/plantlet			
		Herms	Spunta	Santana	Mean	Herms	Spunta	Santana	Mean
Silver Thiosulphate (STS)	**0**	9.75	17.625	11.75	10.04	24.8	29.8	28.12	27.54
	0.4	16.75	15.625	11.37	14.58	35.8	43.37	34.63	37.92
	0.8	18.50	16.75	13.62	16.29	34.9	23.37	32.00	30.00
	1.2	12.00	10.86	11.37	11.42	22.50	19.37	23.25	21.1
	1.6	6.38	13.50	9.87	9.92	14.5	29.00	16.9	20.1
	2	6.75	7.6	4.125	6.17	25.5	16.87	14.4	18.91
	Mean	**11.69**	**13.67**	**10.35**	**11.90**	**26.31**	**26.96**	**24.88**	**26.0**
LSD $_{0.05}$	**Cultivars**	1.76				4.849			
	Concentrations	2.49				6.858			
	Conc. × Cultivars	4.32				11.88			

Table 3: Effect of silver thiosulphate (STS) concentrations on number of branches/plantlet of three potato cultivars after 40 days from *in vitro* culture.

Treatment	mM	Number of Branches/plantlet			
		Herms	Spunta	Santana	Mean
Silver Thiosulphate (STS)	**0**	8.8	8.1	8.2	8.4
	0.4	16.6	11.2	8.6	12.2
	0.8	14.8	9.4	9.5	11.2
	1.2	7.8	5.4	8.1	7.0
	1.6	3.2	6.5	4.7	4.8
	2	4.6	2.6	1.5	2.9
	Mean	**9.3**	**7.2**	**6.8**	**7.7**
LSD $_{0.05}$	**Cultivars**	2.0			
	Concentrations	2.8			
	Conc. × Cultivars	4.9			

Effect of Silver Thiosulphate (STS) concentrations on microtuberazation percentage (%) for three potato cultivars after 9 weeks of culture under dark or light conditions

Data in Table 4 show the effect of silver thiosulphate (STS) concentrations on percentage (%) of microtuberazation for three potato cultivars after 9 weeks of culture under dark or light conditions. Regrinding the tested photoperiods, microtuberazation percentage (%) under dark conditions (76.9%) was higher than under light conditions (74.5 %). This agree with the Hussain *et al. (*2006), who observed that complete obscurity was an essential factor in tuber induction. Cultures kept under 16 hrs., photoperiod were not able to produce microtubers. During incubation under light, GA3 is synthesized which inhibits tuber induction while darkness enhanced tuberonic acid synthesis, which plays important role in tuber formation. Dobranszki *et al.* (1999).

With respect to an interaction between photoperiods and cultivars, under dark conditions, 'Herms' showed the highest value of microtuberazation (%), while Santana cultivar recorded the best performance under light conditions. It was previously reported that the genotypes were differed in their responses to photoperiod. And each genotype has a specific photoperiod requirement (Seabrook *et al.,* 1993 and Dobranszki *et al.,* 1999). Also Gopal *et al.* (1998), who stated that it is important to develop genotype specific protocols for optimum microtuberization.

The interaction between photoperiods and the applied concentrations showed that, under dark condition, STS at 0.4 mM concentrations recorded the highest microtuberazation (%), while the control treatment (without STS) gave the lowest value of microtuberazation (%). Under light conditions, the application with 2 mM STS recorded the highest microtuberzation %. Reversely, STS at 0.8 mM recorded the lowest microtuberazation (%).

Regrinding the interaction among photoperiods, the applied concentrations, and cultivars. The data at dark showed that the highest microtuberazation (%) were recorded in 'Herms' and 'Spunta' with the application of 0.4 mM STS. On the other hand, 'Santana' recorded the highest value at 0.8 mM STS. The microtuberazation (%) at 0mlSTS/l in 'Herms' was lower than the other concentrations except the application with 1.6 mM STS. In 'Spunta' the application with 0 mM STS showed the lowest value of microtuberazation (%). The pervious findings are in agreement with most of reports which found that the presence of STS in the culture media improved the growth as compared with control (Sarkar *et al*., 1999; Ibrahim *et al*., 2016). On the other hand, this disagree with Chang and Chan (1991), they found that the STS treatments (2µM) did not showed any improvement of tuberization of *in vitro* cultured potato.

On the contrary, the data at light conditions showed that the highest values of microtuberazation (%) were recorded at the concentration of 2 mM STS in Herms and Spunta cultivars. While, the application with 1.6 mM STS showed the highest percentage of microtuberzation in 'Santana'. The lowest values of microtuberazation (%) were recorded with 0.4 mM STS in 'Herms' and 'Spunta'. On the other hand, the lowest value of microtuberazation (%) was recorded with 0.8 mM STS in 'Santana'.

The microtuberzation % of treated plantlets with high concentrations of STS was higher at light than under dark conditions. This may be refer to the treated plantlets with STS under light took all the period of culture (9 weeks) to enhance the growth and stimulated the growth of leaf, root and stem length which reflected on the microtuberazation %. On the contrary, this effect under dark was ended early. This may be explained by the previous studies which reported that the application of STS in culture media delayed leaf senescence (Sarkar *et al*., 1999). They reported that maximum number of green leaves per microplant and minimum leaf senescence were observed when the microplants were conserved in medium supplemented with 9.0 mg ml^{-1} STS (The highest concentration). This agrees with Rostami and Ehsanpour, 2010, they reported that total chlorophyll content of plants increased at medium with STS in comparison with the control. In addition, the highest amount of chlorophyll was recorded by the highest concentration of STS.

In conclusion, the highest microtuberazation percentage (%) in Herms cultivar was recorded under light condition and in presence of STS at a concentration of 2 mM. Meanwhile, the highest microtuberazation percentage (%) in 'Spunta' and 'Sansta' was achieved in presence of STS at a concentration of 0.4 and 0.8 mM, respectively and under dark condition. These results clearly indicated that each potato cultivar of studied cultivars in the present investigation has its specific conditions concerning the concentration of STS and light or dark requirement to achieve the highest microtuberization. These findings agree with Gopal *et al*. (1998), who stated that it is important to develop genotype specific protocols for optimum microtuberization. In addition to Seabrook *et al.,* 1993, who reported that the day length influenced the tuberization responses under the four tested cultivars. And these influences refer to genotype dependent and their interactions with media components (Nistor *et al*., 2010 and Refaie *et al*., 2020).

Table 4: Effect of silver thiosulphate (STS) concentrations on percentage (%) of potato microtuberazation for three potato cultivars after 9 weeks of culture under dark or light conditions.

Treatment	mM	Microtuberazation (%)							
		Dark				Light			
		Herms	Spunta	Santana	Mean	Herms	Spunta	Santana	Mean
Silver Thiosulphate	**0**	72.2.	50.0	61.2	61.1	83.3	74.1	85.7	**81.0**
	0.4	91.7	91.7	80	87.8	66.6	41.7	85.7	**64.6**
	0.8	88.9	61.1	95.2	81.7	76.2	61.9	61.9	**66.6**
	1.2	88.9	88.9	80	85.9	66.7	56.0	62.5	**61.7**
	1.6	72.2	80.1	73.3	75.2	70.0	83.3	91.7	**81.6**
	2	73.3	75.0	61.1	69.8	96.7	90.5	86.7	**91.3**
	Mean	**81.2**	**74.5**	**75.1**	**76.9**	**76.6**	**67.9**	**79.0**	**74.5**

Effect of Silver Thiosulphate (STS) concentrations on number of potato microtubers/plantlet for three potato cultivars after 9 weeks culture under dark or light conditions

Data in Table 5 show the effect of silver thiosulphate (STS) concentrations on number of microtubers/plantlet for three potato cultivars after 9 weeks of culture under dark or light conditions. As comparing the means of interaction between photoperiod and cultivars, firstly, there were no significant differences among the means of three cultivars under dark. Secondly, at light condition, Santana recorded significantly the highest number of microtubers/plantlet. The cultivars were differed in their responses to photoperiod. Each genotype has a specific photoperiod requirement (Seabrook *et al.,* 1993 and Dobranszki *et al.,* 1999)

Regrinding the means of interaction between photoperiod and concentrations, there were no significant differences between means of concentrations either in dark or light condition.

With respect to an interaction among photoperiods, cultivars and concentrations, the data at dark showed that the untreated microplants (0 mM STS) obtained the lowest number of microtubers/plantlet among all concentrations. Although, these differences were no significant either in Herms or Santana. In agreement with Chang and Chan (1991), they reported that 2µM STS enhanced the fresh weight of plantlet 6 times greater than without STS whereas, it did not exhibit any improvement of tuberization of *in vitro* cultured potato. On the other hand, Spunta obtained different trend, that the treated planlets with 1.6 mM STS produced significantly the highest number of microtubers/ plantlet as compared to control. However, no significant differences were obtained among other concentrations.

In 'Herms' under light, the maximum number of microtubers/plantlet was significantly obtained with 0.8 mM STS as compared with the all concentrations except with 1.6 mM STS (Fig. 1). In 'Spunta', the application with 0.4 mM STS exceed significantly all other concentrations. The obtained value with 0.4 mM STS was folded 1.7, 1.8, 1.6 and 2.0 times as compared with the values with 0.8, 1.2, 1.6 and 2 mM STS, respectively. This may be referring to that the increment of STS concentrations negatively affected the number of microtubers/plantlet due to the toxicity of silver ion in high concentrations and this agree with Ibrahim *et al*., (2016) and Diab (2017). No significant differences were detected among all concentrations in Santana. In 'Herms', no significant differences were detected among all values under light comparing with dark conditions. An exception was with 0.8 mM STS, it obtained under light significantly number of microtubers/plantlet higher than under dark. In 'Spunta', no significant differences were detected among all values in light comparing with dark except for 0.4 mM STS. The application with 0.4 mM STS at light folded the number of microtubers/ plantlet 2.9 times higher than in dark. On the other hand, in 'Santana', the number of microtubers/plantlet of 0, 0.8, 1.2, 1.6 and 2 mM STS were higher in light as compared with values in dark. (Fig. 2). This is agree with Seabrook *et al.,* 1993, who reported that the number of

microtubers per vessel varied with cultivar, photoperiodic treatment and their interaction. . In addition Gopal *et al.* (1998), who stated that it is important to develop genotype specific protocols for optimum microtuberization. Their findings and ours suggested employing to adapt light regimes for microtuberization to specific requirements for each cultivar and the interaction with STS concentrations

Table 5: Effect of Silver Thiosulphate (STS) concentrations on number of microtubers/plantlet for three potato cultivars after 9 weeks of culture under dark or light conditions.

Treatment	mM	Number of microtubers/plantlet							
		Dark				Light			
		Herms	Spunta	Santana	Mean	Herms	Spunta	Santana	Mean
Silver Thiosulphate	**0**	1.29	1.25	1.16	**1.23**	1.46	1.74	3.27	**2.16**
	0.4	2.29	1.54	1.95	**1.92**	1.87	4.41	3.14	**3.14**
	0.8	2.00	2.20	1.66	**1.96**	3.83	2.55	3.88	**3.42**
	1.2	1.97	1.87	2.25	**2.03**	2.25	2.42	3.92	**2.86**
	1.6	2.33	2.95	2.17	**2.49**	2.50	2.60	3.04	**2.71**
	2	2.41	1.87	2.00	**2.09**	1.92	2.16	3.75	**2.61**
	Mean	**2.04**	**1.95**	**1.87**	**1.95**	**2.31**	**2.65**	**3.50**	**2.82**
LSD $_{0.05}$	**Photoperiod × cultivars**	0.64							
	Photoperiod ×conc.	1.67							
	Photoperiod × cultivars × conc.	1.57							

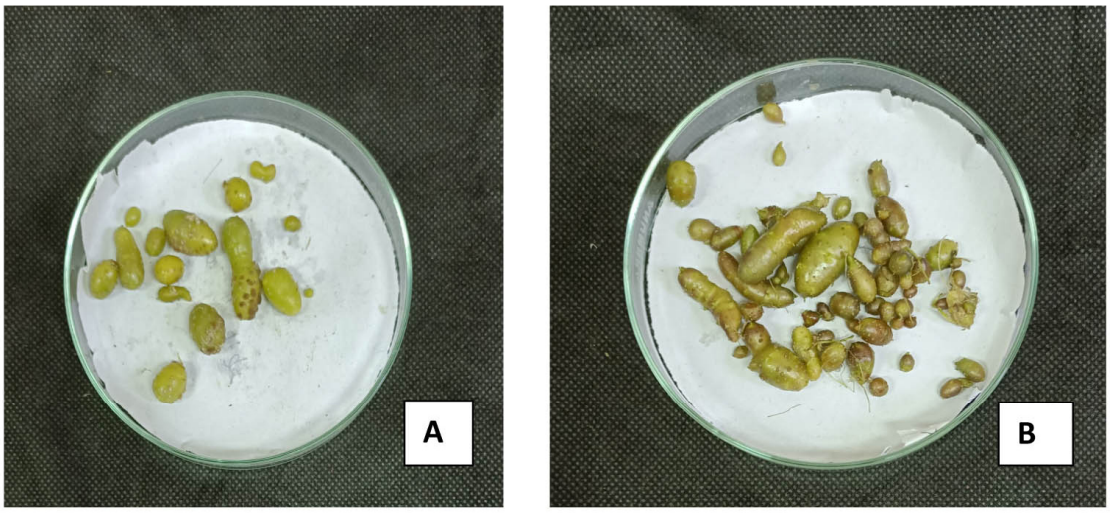

Fig. 1. Microtubers of Herms after culture at lightning for 9 weeks a: MS media + 0 mM STS and b: MS media + 0.8 mM STS

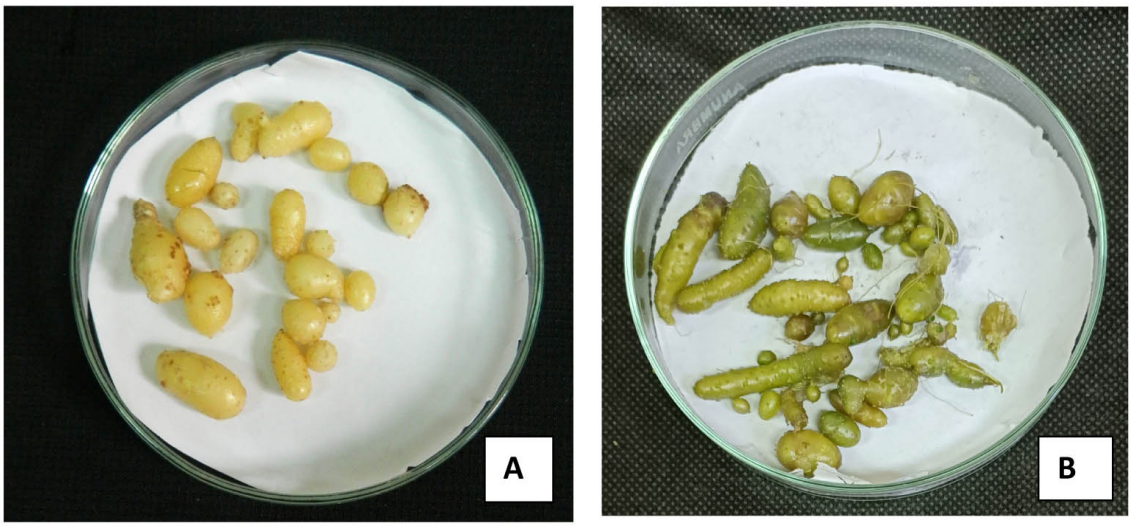

Fig. 2. Microtubers of Santana after culture in MS media with 1.2 mM STS under culture for 9 weeks a: Dark and b:Light

Effect of Silver Thiosulphate (STS) concentrations on fresh weight of microtuber (F.W) (g) for three cultivars after 9 weeks of culture under dark or light conditions

The effect of silver thiosulphate (STS) concentrations on fresh weight of microtuber (F.w) (g) for three cultivars after 9 weeks of culture under dark or light conditions is shown in Table 6. As comparing the means of interaction between photoperiod and cultivars, firstly, there were no significant differences among the cultivars under dark. Secondly, under light, Spunta recorded significantly the highest value of F.w. The previous results can be explained by Gopal *et al*. (1998), who stated that it is important to develop each genotype a specific protocol for optimum microtuberization. In addition to Seabrook *et al.,* 1993, there are a different photoperiod requirements for each genotype.

Regrinding the interaction between photoperiod and concentrations, in dark, the application with 0.8 mM STS obtained higher value of F.W as comparing with 0.4 mM STS only. However, there were no significant differences between the concentrations under light.

With respect to an interaction among photoperiods, cultivars and concentrations, in 'Herms' under dark, no significant differences were recorded among concentrations. On the other side 'Spunta' showed a different response, the application with 0.8 mM STS recorded significantly the highest value of F.W as compared with 1.2 and 2mM STS. The STS at 0.8 mM exceeded the other concentrations to fold the values to 2.8 and 3.1 with 1.2 and 2 mM STS, respectively. This may be referring to that the increment of STS concentrations negatively affected the fresh weight of microtuber due to the toxicity of silver ion in high concentrations and this agree with Ibrahim *et al*. (2016) and Diab (2017). In 'Santana', highest value of F.W. was recorded with 1.2 mM STS comparing with 0.4 mM STS. It is noticeable from the results, that there were differences between the cultivars on their response to concentrations of STS. The same trend was reported by Mollers *et al.* (1992), who reported that response to STS depended on the genotype performance. In addition, EL-Shobaky and Ibrahim (1997), they reported that there were differences between potato cvs; Cara and Spunta to their responses to STS at levels 0, 0.25, 0.50 and 1.0 ml of 4mM.

In 'Herms' under light, the highest value of F.W. was recorded with 1.6 mM STS while, the lowest value was obtained with 0.4 mM STS. But no significances were detected among all concentrations. In Spunt, STS at 0.8 mM STS obtained significantly the highest value of F.W of microtuber as compared with 0.4 mM STS but with no significances among all concentrations. The performance of Santana was differed than Spunta'. There were no significant differences were detected among all concentrations. These results agree with Chang and Chan (1991), they found that no great impact on potato *in vitro* tuberization when grown in MS medium either with (2µM) or without STS. In Herms and Santana, no significant differences were detected among all values in light comparing with dark at all concentrations. In'Spunta', no significant differences were detected among all values in light comparing with dark at 0.4 and 0.8 mM STS. Nevertheless, the applications with 0, 1.2, 1.6 and 2 mM STS under light recorded significantly values of F.W higher than under dark. These values were folded 2.8, 2.5, 2.2 and 3.7 in applications with 0, 1.2, 1.6 and 2 mM STS, respectively. This may be refer to the treated plantlets with STS under light took all the period of culture (9 weeks) to enhanced the growth and stimulated the growth of leaf, root and stem length which reflected on fresh weight of microtuber. On the contrary, this effect under dark was ended early. However, this trend was not obvious at all cultivars because of the different performance of cultivars to the application of STS. The same finding was reported by Mollers *et al.* (1992), who reported that the response of addition STS to *in vitro* potato shoot propagation was differed depends on the genotype performance. Adding to El-Shobaky and Ibrahim (1997), they reported that potato cvs; Cara and Spunta had different responses to different levels of STS.

Table 6: Effect of Silver Thiosulphate (STS) concentrations on fresh weight of microtuber (g) for three cultivars after 9 weeks of culture under dark or light conditions.

Treatment	mM	Fresh weight of microtuber (g) (F.W)							
		Dark				Light			
		Herms	Spunta	Santana	Mean	Herms	Spunta	Santana	Mean
Silver Thiosulphate	**0**	0.42	0.32	0.43	**0.39**	0.61	0.92	0.38	**0.64**
	0.4	0.28	0.32	0.16	**0.25**	0.41	0.38	0.21	**0.53**
	0.8	0.34	0.62	0.39	**0.45**	0.49	0.94	0.18	**0.54**
	1.2	0.48	0.22	0.49	**0.39**	0.52	0.55	0.43	**0.50**
	1.6	0.46	0.34	0.47	**0.42**	0.68	0.77	0.47	**0.64**
	2	0.48	0.20	0.34	**0.34**	0.49	0.75	0.35	**0.53**
	Mean	**0.41**	**0.34**	**0.38**	**0.38**	**0.53**	**0.71**	**0.33**	**0.52**
LSD $_{0.05}$	**Photoperiod × cultivars**	0.14							
	Photoperiod ×conc.	0.19							
	Photoperiod × cultivars × conc.	0.33							

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