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Effects of Gelling Agents and Antioxidant Treatments on *In Vitro* Potato Micro-Tuberization and on Common Scab Development Using Virus-Indexed Plantlets

A. A. Galal, S.H. Gad El-Hak*, Y. T. Abd El-Mageed*, N. S. Youssef* and A. Z. Osman**

*Department of Plant Pathology , *Department of Horticulture, Faculty of Agriculture, El-Minia University, and **Veg. Res. Dept., Hort. Res. Inst., Agric. Res. Centre, Cairo, Egypt.*

A VIRUS- FREE (free from PVA, PVM, PVS, PVX, PVY and PLRV) plantlets of potato cv. "Alpha", "Diamant" and "Spunta" were obtained by tissue culture technique and further used throughout this study

A significant improve in the micro-tuberization of the tested potato cutivars "Alpha", "Diamant" and "Spunta" was obtained when corn starch was used instead of the gelling agent agar in the tissue culture media. Potato cv. "Spunta" gave the best growth characters *in vitro, i.e.* number of micro-tubers (1.26 micro-tubers/ plantlet),fresh weight as well as dry weight of micro-tubers(106.51 and 17.89 mg/plantlet) .

Amendment corn starch medium with ascorbic acid (AA) caused significant increase in the micro-tuberization while these characters were significantly decreased in the medium supplemented with salicylic acid (SA).

Micro-tubers variously responded to *Streptomyces scabies* the causal agent of potato common scab, infection depending on potato cultivars. The micro-tubers of potato cv. " Spunta" showed the highest susceptibility followed by "Diamant".

Complete inhibition of *S. scabies* growth (colony number and size of colony mm)was expressed when cultured on the growth medium amended with 1.0 , 5.0 or 10.0 mM SA and when SA combined with $CaCl_2$ at rate 1.0 mM $CaCl_2$ with 1.0, 5.0 or 10.0 mM SA, but AA alone or with $CaCl_2$ decreased the growth of *S. scabies*.

In Vitro production of micro-tubers is a very useful way to propagate and store nuclear potato stocks (Valicek & Nianu, 1990) and to study some factors related to tuberization (Ortiz-Montiel & Lozoya-Saldana, 1987) as well as for facilitating storage and transport of germplasm (Estrada *et al.*, 1986). For many years, tissue culture has been applied to improve potato production by means of micropropagation, pathogen elimination and germplasm conservation (Westcott, 1980 and Schilde-Rentschler *et al.*, 1982).

Wang (1977) reported that using meristem culture methods have regenerated potato plantlets free from virus x (PVX) and the potato crinkel virus. Meristem culture is frequently used to regenerate virus-free plants from infected stocks of numerous clonally propagated crops (Wang & Hu, 1982). However, several factors were found to affect success of meristem culture and micro-tubers formation such as meristem length (Kassanis & Verma, 1967), concentration of growth regulators (Obordo & Zamora, 1988 and Amezoueta *et al.*, 1989), physical characters of media (Pennazio & Redolfi, 1973) and light intensity (Lentini & Earle, 1991).

In several studies, differences in response of cultures to brands or types of agar have been reported. Differences in the performance of agars have been attributed to the limited diffusion of medium components and water (Romberger & Tabor, 1971), differences in gel strength (Debergh, 1983) and to impurities (Naira *et al.*, 1995).

Several authors have demonstrated that size of micro-tubers produced within two to three months depends on media conditions (Abbot & Belcher, 1986 and Nowak & Colborne, 1988), temperature (Nowak & Colborne, 1988) and photoperiod (Ortiz-Montiel & Lozoya-Saldana, 1987).

Estrada *et al.* (1986) reported that the use of Gelrite rather than agar as a gelling agent was found to be benefecial. Exposure to light retarded tuberization responses and stimulated shoot and root growth and the effect of media varied with the used cultivar. Dodds (1988) showed that phenolic compounds such as trans-cinnamic acid (TCA) have been shown to stimulate tuberization from stem cutting *in vitro*. Abscisic acid (ABA) is present in potato tubers and is being involved in the control of dormancy where it is used to act as a natural growth retardant.

Nowak & Asiedu (1992) found that tuberization was earlier and more uniform (a higher proportion of total tubers mass and dry matter content of potato) on Gelrite than on agar solidified medium and earlier in the darkness than in the light.

Zimmerman *et al.* (1995) showed that the cost of component for tissue culture medium is a concern for both research and production laboratories. Prices for agar vary considerably depending on brand and type and more expensive than other gelling agents. Furthermore, the starch - Gelrite mixture is easy to prepare and gelling agent costs is only 10-15% of agar, or less if starch was purchased in bulk.

When the problem of medium browning persists at each subculture, the addition of antioxidants, such as cysteine-HCl (100mg/l), ascorbic acid (50-100 mg/l) or citric acid (150mg/l), to the culture medium recommended (Gupta *et al.*, 1980). Also, polyvinyl pyrrolidon (PVP), which can absorb phenolic compounds, has been used to save tissue from the toxic effects of the oxidized phenols.

We were, therefore, interested in *in vitro* producing potato plantlets virus free from PVA, PVM, PVS, PVX, PVY and PLRV, improving potato productivity of micro-tubers and studying the susceptibility of potato cultivars to common scab disease. Also, reducing medium cost is a desirable objective. The major advantage of the starch-Gelrite medium is the reducing medium cost. To realize this goal, laboratory experiments were conducted.

Material and Methods

Plantlet source and micro-propagation

Potatoes (*Solanum tuberosum* L. cvs. Alpha, Diamant and Spunta) were grown in the Experimental Farm of the Faculty of Agriculture, Minia University. When the aerial stem of the mother plants was 25-35 cm tall (about 45 days after planting), the apical growing point of plants of each cultivar (about 3.0cm) were excised (Ewing, 1978). Segments were immersed for 1 min in an 80 % (v/v) ethanol solution and immediately sterilized for five minutes in 1% (v/v) NaOCl solution then rinsed thoroughly with sterilized distilled water. Segments were shecked vigorously for fifteen minutes, then rinsed three times with sterilized distilled water. Then 2 - 3 mm length of the apical meristem tips were cultured in

100 ml vessel containing 25ml of Murashige and Skoog (1962) (MS) medium. This medium was prepared by adding 30g sucrose, 100 mg myo - inositol, 0.5mg indole acetic acid (IAA), 20 mg pantothenic acid, 0.1mg gibberellic acid (GA_3) and 9 g agar/liter (Wright, 1988) to the stock solution (Table 1). These vessels were autoclaved for 20 min at 121°C. Three shoot tips per vessels were transferred to the surface of agar-solidified medium under aseptic condition in a laminar air-flow hood. Cultured vessels were sealed with Parafilm and placed in a growth chamber 16: 8 hr (day: night) photoperiod at 25 ± 2°C with a light intensity of 3000 lux (white fluorescent tubes).

TABLE 1. Detection of potato virus A, M, S, X, Y and PLRV in potato plantlets of cv Alpha, Diamant and Spunta by using ELISA test.

Cultivars and No. samples	Potato viruses					
	PVA	PVM	PVS	PVX	PVY	PLRV
Alpha						
1	- 0.139	- 0.418	- 0.075	- 0.040	- 0.418	- 0.045
2	- 0.137	- 0.406	- 0.093	- 0.010	- 0.397	- 0.036
3	- 0.036	- 0.345	+ 0.021	+ 0.285	- 0.203	+ 0.079
4	0.000	- 0.286	+ 0.045	+ 0.135	- 0.165	+ 0.084
5	- 0.047	- 0.291	+ 0.040	+ 0.102	- 0.241	+ 0.019
Diamant						
6	- 0.013	- 0.462	- 0.090	+ 0.432	- 0.178	- 0.023
7	+ 0.157	- 0.352	- 0.082	+ 0.303	- 0.307	- 0.056
8	+ 0.020	- 0.458	- 0.097	- 0.023	- 0.314	- 0.279
9	- 0.108	- 0.448	- 0.116	- 0.073	- 0.151	- 0.116
10	- 0.014	- 0.460	- 0.097	- 0.030	- 0.310	- 0.245
Spunta						
11	- 0.003	- 0.448	- 0.006	+ 0.442	- 0.128	+ 0.150
12	- 0.012	- 0.386	- 0.067	+ 0.228	- 0.315	+ 0.026
13	- 0.017	- 0.369	- 0.084	- 0.025	- 0.347	- 0.213
14	+ 0.017	- 0.428	- 0.038	+ 0.275	- 0.255	+ 0.026
15	+ 0.008	- 0.461	- 0.099	- 0.008	- 0.374	- 0.319

Absorbance at 405 nm for sample -absorbance of negative control.

Negative values (-) or 0.0 are virus-free samples.

Positive values (+) are virus content samples.

The obtained plantlets of each cultivar (Alpha, Diamant and Spunta) used were tested for virus-free using enzyme-linked immunosorbent assay (ELISA) after three times of propagation from shoot tips of plantlet. Briefly, one node from each plantlet (basal node) was taken for virus occurrence and marked while the rest of plantlet was cut for further propagation. For ELISA test, each sample (0.1 g) plantlet tissue was homogenized in three volumes of 2% polyvinyl

pyrrolydone (PVP) 40t, 0.02% diethyledithiocarbamate acid (DIECA), 10.0 mM sodium phosphate buffer (Clark & Adams, 1977).

Elisa test

A direct, double - antibody sandwich enzyme-linked immunosorbent assay (Clark & Adams, 1977) was used to detect potato virus A, M, S, X, Y and leaf roll virus (PLRV). Antibodies to potato 6 viruses were purchased from AGDIA, INC.30380 Country Road 6 ELKHART, IN 46514, USA. Alkaline phosphatase was conjugated to purified 1 gG for the third step of the assay. Block titration was performed with each batch of coating and enzyme conjugated to determine optimal concentrations for use in the assay. Coating concentration was 1.5 μg/ml, and conjugate was used at dilution of 1-500 (Flanders *et al.*, 1990).

In each step, a volume of 100 μl was used in each well of flexible, flatbottom, polystyrene microtiter plates. The first three steps were incubated in a humid environment for at least 2 hr at 37°C, 8 hr at room temperature , or 24 hr at 4°C. The substrate step was incubated until absorbance of a standardized purified virus (positive control) exceeded 1.0. Absorbance was measured at 405 nm [Denley We II wash 4]. Plates were generally incubated until absorbance of the positive control was 2.0 and read for a second time.

Twenty-five μl of sap and 75 μl of extraction buffer were placed in each well of a microliter plate. Two replications were used per sample. Each sample from one plantlet was tested for 6 viruses duplicate. Extraction buffer was prepared by adding 0.5% Tween-20 (polyethylene sorbitan monolaurate) and 1% polyvinylepyrrolidone to 0.01 M phosphate buffer, pH 7.4. Bovine serum albumin (1%) was added to the extraction buffer when samples were tested.

Negative thresholds were determined for each plate using the healthy mean method (purchased from AGDIA). The purified virus was used to obtain antigen excess and to standardize the ELISA, by reading the plates when the absorbance of the positive control was near 1.0 and again when was near 2.0. One well, filled with extraction buffer as the antigen, was used to adjust the absorbance reader to zero (Flanders *et al.*, 1990).

The free virus-indexed plantlets were multiplied as single node explants on MS- based potato nodal cutting medium as described above, and in 3-4 weeks a plantlet with six or even more nodes became available for further propagation.

Micro-tuberization

Micro-tubers were produced by culturing nodal cuttings of virus indexed plantlets of potato cvs. "Alpha", "Diamant" and "Spunta" on MS medium with 80 g/l sucrose, 20 mg/l pantothenic acid, 5 mg/l benzyl adenine (BA) and 1.3 mg/l chlorocholine chloride (CCC) plus two levels (0.1 and 1.0 mM) from salicylic acid or ascorbic acid in two media, the first (MS medium) contained 9 g/l agar and the second (corn starch medium) contained 60 g/l corn starch and 0.6 g/l Arabic gum and prepared as mentioned above. The pH was adjusted at 5.7 before autoclaving in both media. However, the mother plantlets were 6 weeks old.

Three single nodes were used in each vessel, where 4 vessels per replicate and 4 replicates per treatment were used. Thus, 48 single nodes per treatment for each cultivar were used. The vessels were sealed with Parafilm and wrapped in aluminum foil to exclude the light and kept in the dark at 19°C (Nowak & Asiedu, 1992). The experimental treatments were arranged in randomized complete block design with four replicates.

Micro-tubers were harvested 8 weeks after culturing and average number of micro-tubers, micro-tubers mass, fresh weight and dry weight (dried at 60 °C for 24 hr) of micro-tubers were recorded. Prior to drying, micro-tubers greater than 5 mm were cut into 4 parts and smaller micro-tubers in half (Nowak & Asiedu, 1992).

Response of potato micro-tubers produced in vitro to infection by Streptomyces scabies

Potato micro-tubers of 8 weeks old plantlets, which were produced under various treatments as mentioned above, were inoculated by *S.scabies,* isolate S_2 (Galal *et al.*, 1999). Inoculation was carried out by using 3mm disk of sterilized Whatman filter paper No. 4. Sterilized paper disks were saturated by the bacterial suspension (10^6 cells/ml) and placed on the surface of micro-tuber under aseptic conditions in vessels. Inoculated vessels were capped, sealed with parafilm, wrapped in aluminum foil and kept in the dark at 19°C. Scab symptoms were examined 14 days after inoculation and surface area infected index was estimated as described by Goth *et al.* (1995).

Effects of antioxidants and calcium chloride treatments on the growth of S. scabies

Ascorbic acid and salicylic acid were dissolved in de-ionized water and ethanol for salicylic acid (Elad, 1992), then applied to bacterial growth medium, the pH of this medium was adjusted to 6.2 (King *et al.*, 1991). However, the concentrations of 0.0 (control), 0.1, 1.0, 5.0 and 10.0 mM from each antioxidant compound as well as $CaCl_2$ was used at 1.0 mM individually or combined with certain concentrations of the tested antioxidants in order to test their direct effect on the growth of potato common scab incitant bacterium, *S. scabies* isolate S_2. After the medium had been autoclaved it was cooled at 45-50°C, medium was distributed to Petri plates (15 ml per each), which contained 1.0 ml bacterial suspension and vigorously shaken for 2 min. A randomized complete block design with four replicates was used. Inoculated plates were incubated at 25°C for 10 days. After that, the number of colonies and their diameter were assayed.

Tuberization medium (Dodds, 1988)

MS Bassal salts		
Sucrose	80.00	g/l
Pantothenic acid	0.020	g/l
Benzyl adenine (BA)	0.005	g/l
Chlorocholine chloride (CCC)	0.0013	g/l
Agar	9.000	g/l

(The costs/Liter range from 2.7 to 7.2 Egyptian pounds)

pH 5.7

Modified medium for tuberization

MS Bassal salts		
Sucrose	80.00	g/l
Pantothenic acid	0.020	g/l
Benzyl adenine (BA)	0.005	g/l
Chlorocholine chloride (CCC)	0.0013	gl
Corn starch	60.0	g/l
Arabic gum	0.6	g/l

(The costs/Liter range from 0.35 to 0.45 Egyptian pounds)

pH 5.7

Ascorbic acid or salicylic acid was added singly to either tuberization medium or modified medium at 0.1 or 1.0 mM

Solid modified glucose medium (King et al., 1991)

Yeast extract (powder) Oxoid	4.0 g/l
An-Hydrous D- glucose	5.0 g/l
K_2HPO_4	0.250 g/l
KH_2PO_4	0.250 g/l
$MgSO_4$	0.10 g/l
$NaCl_2$	0.05 g/l
$FeSO_4 . 7H_2O$	0.005 g/l
Agar	20.0 g/l
Distilled water	1000 ml
pH 6.8	

Statistical analyses

All recorded data were subjected to the analysis of variance procedures and treatment means were compared using the LSD, and Standard Deviation (SD) as described by Gomez & Gomez (1984).

Results

Data presented in Table 1 showed that 5 potato plantlets were virus-free from A, M, S, X, Y and PLRV viruses out of the 15 tested plantlets of the three potato cultivars (Alpha, Diamant and Spunta). Two plantlets (coded 1 and 2) belonged to cv. Alpha, two plantlets (coded 9 and 10) belonged to cv. Diamant and one plantlet (coded 13) belonged to cv. Spunta was free from all the tested viruses. Thus, the indexed virus-free plantlets; code one from each potato cutivar codes 1, 9 and 13 from Alpha, Diamant and Spunta cvs., respectively was then micro-propagated to use it for the subsequent investigation throughout this study.

Number of micro- tubers/ plantlet

In vitro tuberization of potatoes was differed with cultivars, gelling agents and antioxidant treatments (Table 2). Potato cv. "Spunta" gave the highest number of micro-tubers (1.26 micro tubers/plantlet) followed by "Alpha" (1.16) and "Diamant" (1.08) but insignificant differences were found between cvs "Alpha" and "Diamant".

Corn starch significantly increased number of micro- tubers / plantlet (1.23) than the agar (1.10) (Fig. 1 a and c) .

Adding SA to the growth medium caused a substantial reduction in average number of micro tubers even at 0.1mM (0.84) while, AA caused significant increase and the highest increase was obtained with 1.0mM (1.49) compared to the control (1.31).

Both cvs. "Spunta" and "Alpha" had the highest number of micro tubers (1.34 and 1.25 respectively), when cultured in media contained corn starch, but insignificant differences between them were found. Also, when the medium supplemented with AA at 1.0 mM micro-tubers were 1.63 for "Spunta" cv. and 1.51 for "Alpha" cv. However, the untreated control gave 1.31 and 1.44 but insignificant differences were found between them.

SA caused a reduction in number of micro tubers with both gelling agents and concentrations, where the highly decrease was recorded with agar medium with 1.0mM SA (0.69 micro tubers/ plantlet) compared to the control treatment (1.22) and the highest number of micro-tubers was recorded with the medium containing corn starch supplemented with AA at 1.0mM (1.58). However, insignificant differences were found between corn starch + 0.1mM AA treatment and the corn starch control medium.

TABLE 2. Average number of micro-tubers / plantlet as affected by potato cultivars gelling agents and antioxidant treatments.

A: Cultivar (A)	Medium (B)		C: Treatment and Concentration (mM) Control (0.0)	SA 0.1	SA 1.0	AA 0.1	AA 1.0	Mean of AxB	Mean of A	Mean of B
Alpha a_1	AB C	b_1	1.34	0.71	0.60	1.29	1.41	1.07	1.16	
		b_2	1.54	0.85	0.71	1.52	1.63	1.25		
	Mean of AxC		1.44	0.78	0.65	1.40	1.51			
Diamant a_2	AB C	b_1	1.16	0.82	0.70	1.29	1.31	1.06	1.08	
		b_2	1.23	0.84	0.77	1.28	1.37	1.10		
	Mean of AxC		1.19	0.83	0.73	1.28	1.34			
Spunta a_3	AB C	b_1	1.16	0.90	0.77	1.59	1.52	1.19	1.26	
		b_2	1.46	0.97	0.85	1.64	1.75	1.34		
	Mean of AxC		1.31	0.93	0.81	1.61	1.63			
Mean of C			1.31	0.84	0.73	1.43	1.49			b_1= 1.10
Mean of BxC			1.22	0.81	0.69	1.39	1.41			b_2=
			1.41	0.88	0.77	1.48	1.58			1.23

b_1 = Agar
b_2 = Corn starch
L.S.D. at $_{0.05}$ for A = 0.09
B = 0.07
C = 0.11

SA = Salicylic acid
AA = Ascorbic acid
AB = 0.12
AC = 0.19
BC = 0.16
ABC = 0.27

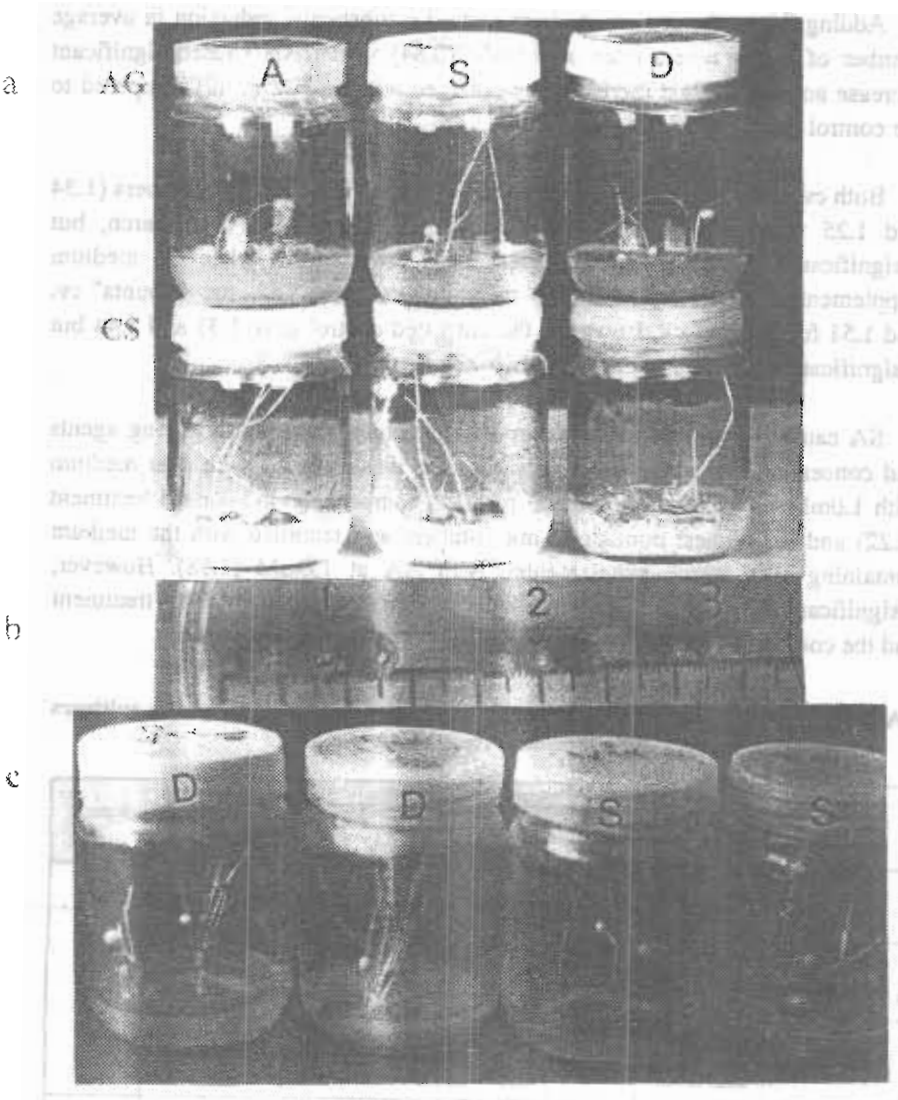

Fig. 1. a and c. Micro-tuber formation of potato cvs. "Alpha", "Diamant" and "Spunta" grown on MS medium with the gelling agent agar (AG) or corn starch (CS)

b) Size of micro-tubers formed by cv."Spunta" grown on MS medium amended with corn starch and ascorbic acid (1), corn starch alone (2) or agar alone (3).

Results in Table 2 showed that the combination effects of cultivar, gelling agent and antioxidant treatments on this character were significant. Spunta cv. with corn starch medium containing ascorbic acid at either 1.0 or 0.1 mM and Alpha cv. with corn starch and AA at 1.0 mM only gave the highest values of micro-tubers/plantlet (1.75, 1.64 and 1.63, respectively) without significant difference among them.

Fresh weight of micro -tubers (mg)/plantlet

Data in Table 3 showed that the highest significant increase in average fresh weight (mg) of micro-tubers (106.51 mg) was obtained by cv. "Spunta" compared to "Alpha" cv. (87.63 mg) and "Diamant" cv. (64.14 mg) Corn starch gave higher values (102.18 mg) than agar (70.01 mg)with significant difference between them.

TABLE 3. Average fresh weight of micro-tubers (mg) / plantlet as affected by potato cultivars, gelling agents and antioxidant treatments .

Cultivar (A)		Medium (B)	(C) Treatment and Concentration (mM) Control	SA 0.1	SA 1.0	AA 0.1	AA 1.0		Mean of A	
Alpha a_1	ABC	b_1	92.76	30.77	23.38	99.75	101.70	69.66	87.63	
		b_2	133.40	47.97	30.51	149.40	166.70	105.60		
	of Mean AxC		113.10	39.37	26.94	124.60	134.20			
Diamant a_2	ABC	b_1	71.47	26.33	25.50	66.26	82.20	54.35	64.14	
		b_2	104.00	30.96	26.42	107.50	100.90	73.94		
	Mean of AxC		87.72	28.64	25.96	86.87	91.54			
Spunta a_3	ABC	b_1	66.13	36.51	25.76	148.00	153.80	86.03	106.51	
		b_2	136.60	46.51	30.88	203.00	217.80	127.00		
	Mean of AxC		101.30	41.51	28.32	175.50	185.80			
Mean of C			100.70	36.51	27.07	129.00	137.20			b_1= 70.01
Mean of BxC			76.78	31.20	24.88	104.67	112.56			b_2= 102.18
			124.66	41.81	29.27	153.30	161.80			

b_1 = Agar
b_2 = Corn starch
L.S.D. at $_{0.05}$ for A = 4.89
B = 3.99
C = 6.31

SA = Salicylic acid
AA = Ascorbic acid
AB = 6.91
AC = 10.93
BC = 8.92
ABC = 15.45

Regarding the main effect of the antioxidant treatments, treatment with AA at 1.0 mM gave the highest value (137.2 mg), while SA particularly at 1.0 mM

significantly reduced the average weight of micro tubers (27.07 mg) compared to the control treatment (100.7 mg).

Significant increase in average fresh weight of the micro- tubers (127.0 mg) was recorded with "Spunta" cv when cultured on the medium that contained corn starch and also when supplemented with AA at 1.0 mM (185.80 mg) or 0.1 mM (175.50 mg) compared to the other tested cutivars, SA and control treatments.

Corn starch medium containing AA at 1.0 mM and 0.1 mM concentrations produced higher micro tuber weight (161.80 and 153.30 mg, respectively), with insignificant differences between both levels, compared to agar which had 112.56 and 104.67 mg for 1.0 and 0.1 mM AA, respectively. The highest reduction was found with SA at 1.0 mM with the agar medium (24.88 mg) compared the control treatment (76.78 mg). Also, the results in Table 3 showed that culturing "Spunta" cv. on corn starch medium supplemented with AA at 1.0 mM produced the heaviest micro tubers (217.8 mg) and the highest depressing was recorded with "Alpha" cv when cultured on agar medium supplemented with SA at 1.0 mM (23.38 mg).

Dry weight of micro-tubers (mg)

Data in Table 4 showed that the highest significant increase in the average dry weight (mg) of micro-tubers was obtained for "Spunta" cv. (17.89 mg) compared with the other tested cultivars (14.44 mg for "Alpha" and 10.48 mg for "Diamant").

Corn starch showed the highest value (17.72 mg) when compared to agar (10.83 mg).

SA particularly at 1.0 mM significantly decreased the average dry weight of micro tubers (3.18 mg), while AA at 1.0 mM significantly increased this character (24.14 mg) compared to the control (17.01 mg).

With regard to the interaction between cultivar and medium treatments, cultivar "Spunta" with corn starch produced the highest dry weight of micro tubers (22.83 mg). Regarding cultivar x antioxidant interaction, the medium supplemented with AA at 1.0 mM when using cv. "Spunta" gave the highest value (32.52 mg).

TABLE 4. Average Dry weight of micro-tubers (mg) / plantlet as affected by potato cultivars, gelling agents and antioxidant treatments.

Cultivar (A)	Medium (B)		(C) Treatment and Concentration (mM) Control (0.0)	SA 0.1	SA 1.0	AA 0.1	AA 1.0	Mean of AxB	Mean of A	Mean of B
Alpha a_1	ABC	b_1	15.50	4.00	2.61	16.67	16.94	11.14	14.44	
		b_2	22.89	6.54	4.03	25.58	29.81	17.75		
	Mean of AxC		19.19	5.23	3.32	21.12	23.37			
Diamant a_2	ABC	b_1	11.44	3.74	2.62	10.93	13.21	8.3911	10.48	
		b_2	17.35	4.49	3.24	18.04	19.82	2.58		
	Mean of AxC		14.40	4.12	2.93	14.47	16.52			
Spunta a_3	ABC	b_1	10.66	4.08	3.02	23.50	23.52	12.96	17.89	
		b_2	24.23	5.66	3.59	39.16	41.52	22.83		
	Mean of AxC		17.45	4.87	3.30	31.33	32.52			
	Mean of C		17.01	4.74	3.18	22.31	24.14			b_1= 10.83
	Mean of BxC		12.53	3.94	2.75	17.13	17.89			b_2= 17.72
			21.45	5.53	3.62	27.58	30.38			

b_1 = Agar
b_2 = Corn starch
L.S.D. at $_{0.05}$ for A = 0.58
B = 0.47
C = 0.75

SA = Salicylic acid
AA = Ascorbic acid
AB = 0.82
AC = 1.30
BC = 1.06
ABC = 1.84

Meanwhile, the medium containing corn starch that supplemented with AA at 1.0 mM gave the heaviest weight (30.38 mg) while S A at 0.1 or 1.0 mM with both gelling agents caused inhibitory effect (2.75 mg and 3.62 mg) compared to the control treatment with either agar (12.53 mg) or corn starch (21.45 mg).

Also, data recorded in Table 4 showed that, all tested cultivars when cultured on the medium containing agar or corn starch and each medium supplemented with SA at the two tested concentrations depressed average dry weight of micro-tubers (mg). On the other hand, AA at 1.0 or 0.1 mM with both gelling agents increased dry weight of micro- tubers and the heaviest weight was recorded when corn starch medium at 1.0 mM AA was used on "Spunts" cv. (41.52 mg)

Effects of gelling agents and antioxidants on common scab development in vitro

Data presented in Table 5 showed that the tested potato cultivars are variously responded to *S. scabies* infection. Micro-tubers of "Spunta" cv. were

more infected than the other cultivars. Both gelling agents, agar or corn starch had no effect on common scab infection. SA significantly reduced the severity of common scab, but AA had an insignificant effect in this respect.

TABLE 5. Response of potato micro-tubers produced *in vitro* to the infection by *Streptomyces scabies*.

Cultivars	Media	Antioxidant treatments				
		Control	SA		AA	
			0.1 mM	1.0 mM	0.1 mM	1.0 mM
Alpha	Agar	2.50±0.09	1.7±0.11	1.00±0.02	2.40±0.10	2.28±0.04
	Corn starch	2.38±0.06	2.09±0.03	1.32±0.06	2.36±0.04	2.27±0.05
Diamant	Agar	2.53±0.02	1.78±0.02	1.40±0.02	2.45±0.05	2.30±0.02
	Corn starch	2.70±0.04	2.3±0.02	2.00±0.02	2.50±0.04	2.34±0.01
Spunta	Agar	2.76±0.01	2.48±0.01	2.06±0.12	2.78±0.01	2.70±0.02
	Corn starch	3.00±0.06	2.60±0.02	2.33±0.11	2.80±0.02	2.71±0.01

[1]-Surface area infected index (SAI) (SA) = Salicylic acid (AA) = Ascorbic acid

Data are mean of 3 replicates ± SD.

Effect of antioxidant and $CaCl_2$ on the growth of Streptomyces scabies

Data in Table 6 showed a substantial inhibition of *Streptomyces scabies* on the growth medium supplemented with SA and AA individually. SA was more suppressor for *S. scabies* growth than AA. Even at 1.0 mM, SA caused a complete inhibition while, AA did not cause a complete inhibition even at 10.0 mM. Also, using $CaCl_2$ alone at 1.0 mM caused a substantial reduction (more than 50% inhibition) in the number of CFU of *S. scabies*. Meanwhile, adding $CaCl_2$ with SA, increased the inhibitory activity but with AA, it decreased the inhibitory potential.

TABLE 6. Effects of the antioxidants, *i.e.*, ascorbic acid or salicylic acid and / or calcium chloride on *Streptomyces scabies* growth.

Growth of *Streptomyces scabies*	Control	Calcium chloride (1mM)	Antioxidants and / or calcium chloride treatments													
			Ascorbic acid (mM)				Salicylic acid (mM)				Ascorbic acid + calcium chloride (mM)				Salicylic acid + calcium chloride (mM)			
			0.1	1.0	5.0	10.0	0.1	1.0	5.0	10.0	0.1 + 1.0	1.0 + 1.0	5.0 + 1.0	10.0 + 1.0	0.1 + 1.0	1.0 + 1.0		10.0 + 1.0
No colonies Per plate	78.66±1.00	34.33±1.52	218.33±0.08	163.66±2.08	142.66±1.15	79.00±1.00	4.66±1.52	0.00	0.00	0.00	255.66±3.21	196.33±1.52	137.00±2.64	35.00±2.64	108.33±2.51	0.00	0.00	0.00
Colony Size (mm)	68.0±2.00	37.7±1.52	43.0±2.88	33.6±3.05	20.0	10.0	13.3±3.05	0.00	0.00	0.00	38.66±2.30	32.6±0.57	18.0±1.00	7.33±0.57	43.44±3.05	0.00	0.00	0.00

Data are mean of 3 replicates ± standard deviations (SD).

Discussion and Conclusion

Tissue culture technique became an essential procedure for micro-propagation, micro-tuberization and germplasm potato conservation. Otherwise it is possible to use these techniques for producing a virus-free plantlets, pathogens - or environment stress - resistant once. For these purposes, tissue culture technique is used world wide in a large scale. Thus, large amount of agar is relatively used per liter of medium. This amount is expensive and the agar is price increasing year by year. Thus, this study tried to use another gelling agent, *i.e.* corn starch, which is available and cheaper than agar (Sovari, 1986a and Zimmerman *et al.,* 1995).

A substantial increase in the micro-tubers productivity, *e.g.* number, and fresh or dry weights of micro-tubers, was pronounced when potato explants were cultured on cornstarch gelled medium than agar gelled one. Data suggested that the stimulatory effect of corn starch on the micro-tuberization may be due to the improvement of the nutritional status and that agar may contain some elements which reacted as inhibitor factors (Scholten & Pierik, 1998).

A significant increase was also found with corn starch medium supplemented with ascorbic acid and the best micro-tuberization was expressed at 1.0mM with corn starch medium. This increase may be attributed to the role of ascorbic acid in the absorbance of phenolic compounds to save tissue from the toxic effects (Gupta *et al.*, 1980). Ascorbic acid can inhibit also the ethylene production (Elad, 1992) where these factors are reflected on the absorption and nutritional status in the growing media.

Significant decrease was noticed when salicylic acid was supplemented in the medium of either agar or corn starch gel medium and also with the 3 tested cultivars. These decreases may be due to the inhibition of phosphate uptake and reducing potassium absorbtion, which play the main role in the cell division and cell elongation of micro-tubers production. Simialar results in barely and oat roots in the reduced absorbtion of potassium and phosphorus uptake were reported by Glass (1973 and 1974) and Harber & Balke (1981).

Micro-tubers were variously responded to *S. scabies,* the casual agent of potato common scab, infection depending on potato cultivars. The microtubers of potato cv. Spunta showed the highest susceptibility followed by Diamant cv.,

while partial resistance was expressed by cv. Alpha. Data are consistent with the reaction of potato cvs when they are grown in the greenhouse under artificial inoculation. Thus this procedure could be used to screen potato cultivars for common scab resistance *in vitro* and to test the pathogenicity of *S. scabies* rapidly. However, till now no potato cultivar was immune to *S. scabies* infection but different degrees of resistance were existent (Goth *et al.*, 1995).

Complete inhibition of *S.scabies* growth was expressed when it was cultured on the growth medium amended with 1.0, 5.0 or10.0mM salicylic acid (even at pH 6.2) and when SA combined with $CaCl_2$ at the rate of 1.0mM (SA + $CaCl_2$), 5.0 mM SA + 1.0mM $CaCl_2$ and 10.0mM SA+ 1.0mM $CaCl_2$ but ascorbic acid alone or with $CaCl_2$ decreased the growth of *S. scabies,* where the rate of this decrease was enhanced with increasing the SA concentration compared to the control treatment. Data indicated that SA and AA had a direct inhibitory effect to the growth of *S. sacbies*. The present data are consistent with those reported by Elad (1992), Prusky *et al.* (1995), Galal & Abdou (1996) and Galal *et al.* (2000) who found direct inhibitory effects for these compounds on some plant pathogenic fungi.

It may be concluded from the results of this investigation the following points

Potato plantlets free from six viruses, PVA, PVM, PVS, PVX, PVY and PLRV were obtained from the tested cvs. Alpha, Diamant, and Spunta *in vitro,* Medium containing the salts of MS medium plus corn starch at 60 g/l plus Arabic gum at 0.6 g/l could be used for producing micro-tubers, the corn starch medium can produce more and heavier micro-tubers by using ascorbic acid at 1.0 or 0.1 mM and the virulence of common scab *S. scabies* isolates could be simply and accurately detected by using micro-tuberization.

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تأثيرات مواد الصلابة الجيلاتينية ومضادات الأكسدة على تكوين درينات البطاطس وتطور مرض الجرب العادى معمليا باستخدام نبتات مختبرة فيروسيا

أنور عبدالعزيز جلال ، سيف النصر حسين جاد الحق * ، يسرى تمام عبدالمجيد * ، ناصر سيد يوسف * و عباس زكى عثمان **

قسم أمراض النبات ، *قسم البساتين (خضر) - كلية الزراعة - جامعة المنيا و ** قسم بحوث الخضر - معهد بحوث البساتين - مركز البحوث الزراعية - القاهرة - مصر .

تم الحصول على نبيتات بطاطس خالية من الفيروسات (A, M, S, X, Y and LRV) للأصناف المختبرة الفا - دايمونت - وسبونتا باستخدام تكنيك زراعة الأنسجة . وجد تحسين معنوى فى عملية تكوين الدرينات للأصناف المختبرة السابقة عند استخدام نشا الاذرة بديلا عن الآجار كمادة جيلاتينية فى بيئة زراعة الأنسجة . صنف البطاطس سبونتا أعطى أحسن صفات نمو معمليا وعلى سبيل المثال (عدد الدرينات ١ر٢٦ درينة / نبيتة) ، الوزن الطازج والوزن الجاف للدرينة (٥١ ، ١.٦ مجم ، ٨٩ ، ١٧ مجم / نبيتية على الترتيب) . وجد أن اضافة حمض الأسكوربيك مع نشا الاذرة أحدث زيادة معنوية فى عملية تكوين الدرينات بينما حمض السالسيليك أحدث انخفاض معنوى .

اختلفت استجابة الدرينات المتكونة لبكتريا ستربتومايسيس سكابيس المسببة لمرض الجرب العادى حيث أظهرت درينات صنف البطاطس سبونتا أعلى حساسية للبكتريا ويليه صنف البطاطس دايمونت .

وجد تثبيط كامل فى النمو للبكتريا استربتومايسيس سكابيس (عدد المستعمرات - حجم المستعمرة ملليمتر) عند

تنميتها على بيئة مغذية مضافا اليها ١٠ ، ٥ ، ١ ملليمولر حمض سالسيليك وعند اضافته مشتركا مع كلوريد الكالسيوم (١ ملليمولر) بالتركيزات السابقة ولكن حمض الأسكوربيك منفرداً أو مضافاً مع كلوريد الكالسيوم أحدث انخفاض فى نمو بكتريا استربتومايسيس سكابيس.