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Effect of Modified Tissue Culture Medium by Nanomaterial on Microtuber Formation of Potato Cultivar Hermes

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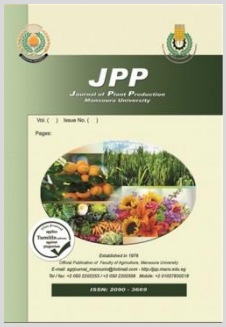

ABSTRACT

Nanoparticles (NPs)are involved in multiple areas, i.e, electronics, energy, medicine, life sciences, and agriculture.Induction and accelerating of multiplication and microtuber formation of potato plants (Hermes cultivar) by some treatments i.e. nano-nutrients, medium composition as well as explant type and cultivation orientation were examined in the current study. Datarevealed that bothtreatments of Murashige and Skoog medium (MS) +1.0mg/l benzyl adrnine (BA)+200mg/l thiamine and MS+2.0mg/l BA + 100mg/l thiamine significantly maximized the shoot proliferationcapacity. The highest shoot length was obtained from MS-free BA medium in combination with all thiamine concentrations. The shoot numbers were augmented when calcium (Ca^{2+}) were substituted with 660 or 1320 µg /l Ca^{2+} NPs compared with MS (2.77,2.77 and 2.53, respectively), also, shoot length and node numberswas not affected by areplacement which means Ca^{2+}ion could be substituted with CaNPs in MS medium without decreasing in potatoshoot proliferation. The promising results of substitution magnesium (Mg^{2+}) with Mg^{2+}NPs were recorded, where 185 or 375 µg /l Mg^{2+}NPs gave anequal or augmented number of shoots relative tothose obtained on MS medium of Hermes with no significant difference between them, node numbers and shoot length were superior as a result of replacement with Mg^{2+}NPs. Shoot tip necroses did not appear with Mg^{2+}NPs. Generally, Mg^{2+}NPs (375 µg/l) can replace the Mg^{2+}ions in MS medium and enhance growth parameters of potato cultivar Hermes.

Keywords: Potato-Nanoparticles- Multiplication- Microtuber-Thiamine-Sucrose-Orientation

INTRODUCTION

Potato (*Soalnum tuberosum* L., Family Solanaceae) considers one of the most important food crops, came after wheat, rice, and maize (Abdelaleem, 2015). Modern agriculture techniques become familiar and commercial methods of vegetative propagation (Mohapatra and Batra, 2017). There are many factors affecting plant micropropagation, the plant genetic make-up, nutrients medium composition and strength, the vitamins, the amino acids, the type and concentration of growth regulators and carbohydrates (Pierik, 1987; Rabbani *et al*., 2001). To stimulate the multiplication, cytokinin plays a great role in shoot formation by accelerating the induction and development of meristematic centers (Badoni and Chauhan, 2012). Also, the nature of explants as well as explant orientation influence *in vitro* plant growth. In this regard, Buckseth *et al.* (2018) suggested that the vertical single or double nodes orientation were successfully produced rapid healthy potato shoots during multiplication, meanwhile, horizontally cultured potato sprouts over the media maximized the shoots number per explant.

In vitro plant cells and tissues culture need several essential vitamins and amino acids to achieve the best growth and development (Yaseen *et al*., 2017). According to Mokhtarzadeh *et al.* (2018), addition of nicotinic acid, pyridoxine- HCl, and thiamine-HCl gave positive effects on shoot proliferation and maximized the number of lateral shoots in potato, additionally, benzyle adenine (BA) supplementation increased the number of lateral shoots and reduce the shoot length. Hamza (2019a), stated that multiplication and growth improvements are significantly boosted by both MS strength with BA supplementation.

Sucrose is often implicated in *in vitro* plant culture as a carbon and energy source. It is hydrolyzed and transformed into glucose and fructose. Also, it is a basic substance in metabolism that acts as a signal molecule in number of biological processes in plants (Yu *et al*., 2000; Wazir *et al*., 2015; Lembrechts *et al.* 2017). The plantlet height shoots fresh weight and chlorophyll content of potato plantlets increased as a result of increasing sucrose concentrations (Mohamed and Alsadon, 2010). Enhancement of plant growth was associated with longer shoots, deeper roots, and a greater number of leaves when the concentration of sucrose was higher than 3% (Abbas *et al*., 2020**).**

Calcium (Ca^{2+}), Magnesium (Mg^{2+}), and zinc (Zn^{2+}) are essential elements, whereas, the plant cannot complete its life cycle without them. They play an important roles in plant developmental processes i.e., motivation of many metabolic processes, building and the stability of the cell membrane, involve with chlorophyll pigments, plant maintenance, and reproduction as well as improving stress tolerance (Epstein and Bloom, 2005; White and Broadley, 2009; Marschner, 2012; El Habbasha and Ibrahim, 2015).

Microtuber formation is a method of asexual potato propagation. It can save time and space, give a great output, and free diseases (Islam *et al*., 2017). Multiple factors are affecting microtubers formation, like concentrations of sucrose, the balance between growth regulators, incubation temperature, light quality, photoperiod as well as genotypes (Emaraa *et al*., 2017; Hossain *et al*., 2017; Hamza 2019b). The effective concentrations of sucrose in microtubers induction medium-ranged between 60 to 90 g/l. The presence of abscicic acid (ABA) and low temperature gave a good microtuber and

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induced microtubers formation (Islam *et al*., 2017; Khalil *et al*., 2017).

This century is the "Nanoparticles century". Nanoparticles are involved in multiple areas, in electronics, energy, medicine, life sciences, and agriculture. The previous research on different plants confirmed that there are both positive and negative effects of nanoparticles on plants growth, depending on its size, shape, and concentration (Tripathi *et al*., 2017). Nanoparticles have multiple uses in agriculture, like, gen transformation, shoot multiplication, and microtuber formation (Naqvi *et al*., 2012; Javed et al., 2017; Hamza., 2019a and b). Javed *et al*. (2017) confirmed that the high frequency of *Stevia rebaudiana* shoot formation was obtained when nodal explants were cultured on MS medium supplemented with 1 mg/l nano-zinc particles (ZnONPs). Additionally, Hamza, (2019b)i concluded that explant types, medium composition, concentrations of sucrose, presence of ABA and cobalt-nanoparticles (CoNPs) affected microtuber formation, weight, and size. Plantlet explant produced the highest number of microtuber followed by double nodes when cultured on MS medium supplemented with 60g/l sucrose and 2.5mg/l ABA. The results may indicate the importance of physiological age, where plantlet as explant is more effective to begin microtubers formation earlier than other explants. On the contrary, slight effects of various explant types on the size of microtubers were observed. Also, Hamza. (2019b) reported that the adding of CoNPs to microtubers formation media induces the formation of the microtubers, while, negative effects were observed in the case of the size and weight of the formed microtubers. The goal of the current study is to evaluate the impact of the factors which may affect multiplication and microtuber formation of potato cultivar Hermes.

MATERIALS AND METHODS

Plant materials: Potato cultivar Hermes was *in vitro* cultured in Plant Biotechnology Department laboratory. The shoot tips and nodes, after two subcultures, were used as starting materials (Explants).

Culture medium preparation and sterilization: In all experiments, the Murashige and Skoog medium (1962) (MS) was used as a culture medium. A volume of 4.4g MS/l was added to 500ml deionized water in addition to the source of carbohydrates and growth regulators which were added and dissolved in water then the whole volume was adjusted to one litter. Then, the pH was adjusted to 5.8 ±0.2 using 0.1 N KOH and/or HCl.The medium was supported with 0.7% agar, then is distributed to glass culture jars (350 ml) with white poly-ethylene transparent closure (50 ml medium for each). In an autoclave, the prepared media were sterilized as described by Hamza (2019b).

Characterization and preparation of nanoparticles (Ca^{2+}NPs, Mg^{2+}NPs, and Zn^{2+}NPs): The nanoparticles were obtained from Nanotech, Egypt. Commonly, Ca^{2+}NPs were presented as $Ca(OH)_2$. It is a spherical-like shape with an average size of less than 100nm. While Mg^{2+}NPs were presented as MgO. Its shape is a spherical shape with an average size 50±5nm.Zn^{2+}NPs were presented as ZO. Its shape is a spherical-like shape with an average size 30 ± 5 nm. Nano-stocks were prepared by adding 100 mg of each material in 100 ml deionized water. To homogenous the nanoparticles, the sonicator **(**Ca^{2+}, Mg^{2+} and Zn^{2+}**)** was used at 100 W and 30 kHz for 45 min (Hamza, 2019a).

Experiment 1: Effect of thiamine and benzyl adenine (BA) concentrations on potato multiplication:

Different concentrations of both BA (0, 1, and 2 mg^{-1}) and thiamin-Hcl (100, 200, 300, and 400 mg/l) were added to full MS medium supplemented with 3% sucrose, 0.7% agar. Each treatment included five jars, each one contained four nodes as explant. After 21days, three homogenous jars for each treatment were selected and their data were recorded. The shoots number/explant, the length of the shoots (cm), nodes number/shoot, and shoot tip necrosis number/explant were measured.

Experiment 2: Effect of sucrose concentrations and explant types on in vitro potato multiplication

The effects of sucrose concentrations (20, 30, 40, and 50g/l)and explant types (shoot tips or nodes) were examined. Different sucrose concentrations were added to full MS medium recommended by Hamza (2019b) which contained0.5 mg/l BA, 0.2 mg/l kin ,1g/l activated charcoal (AC), 100mg/l thiamin-Hcl and 0.7% agar.The culture media was poured into a glass culture jars, each jar contains four explants: two shoot tips and two nodal cuts. After twenty-one days from culture, the shoot's number/explant, the length of the shoot (cm), nodes number/shoot, and growth vigor were determined.

Experiment 3: Evaluation of the responses of both explant types and orientations on *in vitro* potato multiplication

Explant types (shoot tips, double and single nodes) and orientation of explants on the culture medium were examined. Each jar contains four explants (two explants were vertical, and two explants were horizontal). The data were taken after one twenty days from culture; shoots number/explant, shoot length (cm), nodes number/shoot, shoot tip necrosis/explant, roots formation, and growth vigor/explant.

Experiment 4: Impact of replacement of Ca^{2+}, Mg^{2+}, or Zn^{2+} ions in MS medium by different concentrations of Nanoparticles during multiplication stage

MS medium free of Ca^{2+}, Mg^{2+}, or Zn^{2+} were employed to examine the impact of replacement these ions by different concentrations of Ca^{2+}, Mg^{2+}, or Zn^{2+} nanoparticles (220, 440, 660 and 1320µg/l Ca^{2+}NPs, 185, 375,and560 µg/l Mg^{2+}NPs, 11, 22, 33 and 44 µg/l Zn^{2+}NPs), where they were added on potato multiplication medium. The nanoparticles were prepared as recommended by Hamza (2019a). Each treatment contained three replicates (jar) each one contained three nodal segments as explants. The data were taken after 21 days; shoots number/explant; shoots length (cm); nodes number/shoot and appearance of shoot tip necrosis were observed.

Chemical analysis:

Total phenolic concentration (TPC) were determined according to Slinkard and Singleton (1977).

Chlorophyll concentration (CC): chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl.a+ Chl.b) were calorimetrically determined as described by Kamble *et al*. (2015). Where 80% Acetone was used as a blank, and the chlorophyll concentration were calculated according to the following formula:

$$\textbf{Chl a= (11.75×A662.6) – (2.35×A645.6)}$$

$$\textbf{Chl b=(18.61×A645.6) – (3.96× A662.6)}$$

Shoot elements concentration:

Shoots were collected dried in an oven at 60°C until they reach to stable weight. Then samples were digested

according to the methods of (Masson *et al*., 2010). The shoot elements contents(Ca, Mg, Zn, K, Fe, and Mn) were determined by using the Inductively Coupled Plasma (ICP) as described by Faires *et al*. (1984). While, phosphate (P) was determined by measuring the absorbance of the sample at 400 nm using a spectrophotometer as described by Pradhan and Pokhrel (2013).

Experiment 5: Impact of adding different nanoparticles concentrations to the MS medium in combination with various explant types on microtubers parameters.

In this experiment, the explants (shoot tips or single nodes) were cultured on primary micro-tuber formation medium (MS + 5% sucrose and **7** g^{-1} agar) for 15 days, then the modified liquid microtuber formation medium that contained (80g/l sucrose, 0.5 mg/l BA, 0.5 mg/l kin, 100 mg/l Thiamin-Hcl and 300 µg/l ABA)which recommended by Hamza (2019b) supplemented with different concentrations of Ca^{2+}NPs (220 and 440µg/l), Mg^{2+}NPs (90 and180µg/l) and/or Zn^{2+}NPs (4.5 and 9µg/l) individually or in combination were added to the cultures. The cultures were incubated under 1000 lux as light intensity; the photoperiod was 16/8 light/dark and the temperature was adjusted at 20 ± 2 °C. After 90 days from culture; total micro tuber number, weight (g), and volume (cm^3) were recorded. Then the microtuber were divided into two categories: large microtubers (< 0.5 cm)and small microtubers (> 0.5 cm) and the number, weight (mg), and volume (cm^3)were measured for each category in all treatments.

Statistical Analysis

Data were statistically analyzed by complete randomized design using computer software MSTAT-C (MSTAT Development Team, 1988). The least significant differences among levels of each treatment were compared using LSD. Test at 5% level according to Steel and Torrie (1997).

RESULTS AND DISCUSSION

Results

Experiment 1: Effect of thiamine and BA concentration in potato shoots multiplication.

The data in Table 1, discuss the effects of both BA and thiamine concentrations on shoots number of Hermes cultivar. The data confirmed that the presence of BA on the culture medium enhanced shoot proliferation, MS supplemented with 1.0 mg/l BA gave the highest shoots number (1.87 shoot/explant), but there are no significant differences between the effects of BA concentrations on shoots number. On the other hand, results show that there is a significant negative relationship between thiamine concentrations and shoots number, where the lowest concentrations of thiamine (MS+100 or MS+200mg/l thiamine) resulted in the highest shoot number (1.97 or 1.83 shoot/explant). Regarding the interaction between BA and thiamine, data revealed that both MS+1.0mg/l BA+200mg/l thiamine and MS+2.0mg/l BA + 100mg/l thiamine significantly maximized the shoot proliferation (2.03 and 2.07 shoot/explant, respectively). Shoots length was significantly inhibited with an increase of BA concentrations, the highest shoot length was obtained from MS-free BA (10.29cm). Also, the results indicated that there were negative effects of thiamine concentrations on shoot length but the differences between the effects were not significant. The interaction between the BA and thiamine cleared that the highest shoot length was obtained from MS-free BA media in combination with all thiamine concentrations (100, 200, 300, or 400 mg/l thiamine) (10.60, 10.07, 10.23 or 10.27 cm, respectively) with no significant difference between them.

Table 1. Effect of thiamine and BA concentration in potato shoots multiplication.

BA concentration /mg/l (A)	Thiamine concentration/mg/l (B) 100	200	300	400	Mean
Shoots number /explant					
0.0	1.87	1.63	1.33	1.80	1.66
1.0	1.97	2.03	1.67	1.80	1.87
2.0	2.07	1.83	1.70	1.40	1.75
Mean	1.97	1.83	1.57	1.67	
LSD at 5%	A		B		AxB
	NS		0.34		0.60
Shoots length(cm)					
0.0	10.60	10.07	10.23	10.27	10.29
1.0	5.60	5.23	4.73	5.23	5.20
2.0	4.90	5.17	5.23	4.20	4.88
Mean	7.03	6.82	6.73	6.57	
LSD at 5%	A		B		AxB
	0.84		NS		1.68
Nodes number/shoot					
0.0	7.00	5.33	5.33	5.67	5.83
1.0	4.67	4.67	2.67	3.67	3.92
2.0	3.00	2.67	3.33	2.67	2.92
Mean	4.89	4.22	3.7	4.00	
LSD at 5%	A		B		AxB
	0.80		0.92		1.60
Roots number /explant					
0.0	0.80	0.90	1.00	1.00	0.93
1.0	0.80	1.00	0.57	0.80	0.79
2.0	1.17	0.33	0.47	0.00	0.49
Mean	0.92	0.74	0.68	0.60	
LSD at 5%	A		B		AxB
	0.24		0.28		0.49

Concerning the nodes number, the date concluded that the node number significantly decreased with increasing BA concentrations. While, thiamine concentrations slightly affected nodes number, where MS+ 100, 200, or 400 mg/l thiamine gave the highest nodes number with no significant differences between them (4.89, 4.22, and 4.00 nodes/shoot, respectively). The interactions between BA and thiamine concentrations show that the MS media-free BA amendment with 100 or 400 mg/l thiamine significantly maximized nodes number (7.00 or 5.67 nodes/shoot, respectively). The results revealed that BA has negative effects on root number (formation), the free BA media produce the highest root number followedby1.0 and 2.0 mg L^{-1} (0.93, 0.79, 0.49 roots/plantlet, respectively).

Concerning the effects of thiamine concentrations, the MS medium contained 100 mg/l resulted in the highest roots number (0.92 root /plantlet). The interaction between the BA and thiamine concentrations shows that MS substituted with 2.0 mg/l BA+ 100 mg/l thiamine gave the most effective results of roots number (1.17 roots/plantlet).

Experiment 2: Effect of sucrose concentrations and type of explant on *in vitro* potato multiplication

The data in Table 2, discuss the effects of both sucrose concentrations and type of explant on shoot number of Hermes cultivar. Results indicate that there are significant effects of sucrose concentrations on potato shoot number, where, the highest shoots number were obtained from 50gm/sucrose, followed by 20 and 40 gm L^{-1} sucrose (1.92, 1.58, and 1.42 shoots/explant, respectively). Regarding the effects of explant types, the nodal explants significantly augmented shoot proliferation than shoot tip explants (1.75 and 1.29 shoot/explant, respectively). Concerning the effects of

interaction between sucrose concentrations and explant types on shoot proliferation, the nodal explants with 50, 40, or 30 g/l sucrose as well as shoot tip explant with 50g/l sucrose significantly enhanced shoot number/ explant (2.17, 1.83, 1.67 and 1.67 shoot/ explant, respectively). There is a positive relationship between shoot length and sucrose concentrations. The nodal explant enhanced shoot length more than shoot tip explants. The nodal explants with 50g/l sucrose significantly maximized shoot length (10.40cm). The same trend was observed with nodes number/explant, the nodal explants with 50, 40, or 30 g/l sucrose as well as shoot tip explant with 50g/l sucrose significantly induced nodes number (7.67, 6.83, 6.17 and 6.17nodes/shoot, respectively). Growth vigor were significantly affected by increasing sucrose concentrations,50g/l sucrose maximized the growth vigor (4.78). The explant types did not significantly affect growth vigor. The two types of explant (Nodal and shoot tip explants) gave the significant growth vigor at 50g/l sucrose (5.00 and 4.57 respectively).

Table 2. Effect of sucrose concentration and the type of explant on *in vitro* potato multiplication

Sugar concentration (g/l) (A)	Type of explant (B)		Mean (A)
	Node	Shoot tip	
	Shoots number /explant		
20 g / L	1.67	1.50	1.58
30 g / L	1.33	1.00	1.17
40 g / L	1.83	1.00	1.42
50 g / L	2.17	1.67	1.92
Mean (B)	1.75	1.29	
LSD 5%	A	B	AxB
	0.47	0.33	0.66
	shoots length (cm)		
20 g / L	6.08	5.38	5.73
30 g / L	3.93	3.13	5.53
40 g / L	3.58	3.83	3.71
50 g / L	10.40	8.70	9.55
Mean	6.00	5.26	
LSD 5%	A	B	AxB
	1.08	NS	1.52
	Nodes number /shoot		
20 g / L	6.83	4.33	5.58
30 g / L	6.17	4.00	5.08
40 g / L	3.67	3.50	3.58
50 g / L	7.67	5.67	6.67
Mean	6.08	4.38	
LSD 5%	A	B	AxB
	1.34	0.95	1.99
	Growth vigor/jar		
20 g / L	3.90	3.77	3.83
30 g / L	2.50	2.73	2.62
40 g / L	2.77	3.13	2.95
50 g / L	5.00	4.57	4.78
Mean	3.54	3.55	
LSD 5%	A	B	AxB
	0.61	NS	0.97

Experiment 3: Evaluation of the responses of both explant types and orientations on *in vitro* potato multiplication.

The effects of explant types and orientation on *in vitro* potato multiplication of Hermes cultivar are presented in Table 3. The results indicated that the shoot's number was significantly affected by the types of explant. The shoot tip gave the highest shoots number followed by double node and node (2.67, 2.08 and 1.50shoots/explant, respectively).

The data showed that there was no significant relationship between cultural orientation on shoot number. The interaction between cultural orientation and type of explants revealed that the shoot tip in a vertical or a horizontal position and double nodes in vertical position produced the highest shoots number (3.00, 2.33, and 2.33 shoots/explant, respectively) with no significant difference between them.

Concerning the effects of explant types on shoot length, results cleared that the best shoot lengths were produced from double node and shoot tips explants (10.05 and 9.90cm, respectively). Also, there was no significant difference between the effect of both culture positions in the plant's shoot length. Interaction showed that the highest shoots length was obtained from double nodes and shoot tips in both horizontal and vertical culture positions with no significant differences between them (11.07, 9.03, 10.23, and 9.57cm, respectively).

Regarding the effects of the explant types on nodes number, the data showed that there were no significant differences between either the types of explant or the culture position as well as the interaction between them. Anyway, the highest nodes number resulted from double nodes in a horizontal position (7.83 node/ explant).

On the other hand, explant types significantly affected growth vigor, shoot tips, and double nodes gave the highest growth vigor (5.00). The explant position on the medium did not significantly affect the growth vigor. The interaction showed that shoot tips and double nodes in both culture positions significantly enhanced growth vigor (5.00).

Table 3. Evaluation the responses of both explant types and orientations on *in vitro* potato multiplication

Type of explant(A)	Orientation (B)		Mean (A)
	Vertical	Horizontal	
	Shoots number/explant		
Shoot tip	3.00	2.33	2.67
Node	1.17	1.83	1.50
Double nodes	2.33	1.83	2.08
Mean(B)	2.17	2.00	
LSD at 5%	A	B	AxB
	0.73	NS	1.04
	shoots length(cm)		
Shoot tip	9.57	10.23	9.90
Node	4.97	5.40	5.18
Double nodes	9.03	11.07	10.05
Mean	7.86	8.90	
LSD at 5%	A	B	AxB
	2.10	NS	2.96
	Nodes number /shoot		
Shoot tip	7.33	6.83	7.08
Node	5.83	5.33	5.58
Double Node	7.17	7.83	7.50
Mean	6.78	6.67	
LSD at 5%	A	B	AxB
	NS	NS	NS
	Growth vigor/jar		
Shoot tip	5.00	5.00	5.00
Node	2.67	3.00	2.83
Double nodes	5.00	5.00	5.00
Mean	4.22	4.33	
LSD at 5%	A	B	AxB
	0.56	NS	0.79

Experiment 4: Impact of replacement of Ca^{2+}, Mg^{2+}, or Zn^{2+} ions in MS medium by different concentrations of Nanoparticlesduring multiplication stage

The data in Figure (1a) and Photo 1, examine the multiplication responses of potato cultivar, Hermes, when Ca^{2+} ions were substituted by different concentrations of Ca^{2+}NPs in MS medium. The shoots number were augmented when Ca^{++}were substituted with 660 or1320 µg /l Ca^{2+}NPs compared with MS (2.77, 2.77 and 2.53, respectively), while, the substitution with 220 or 440 µg/l Ca^{2+}NPs decreased the Hermes shoots number (1.53 and 1.73 shoot/explant, respectively) with no significant difference among all concentrations, which mean that Ca^{2+} ions could be substituted with Ca^{2+}NPs in MS medium without decreasing in shoot

proliferation in potato cultivar Hermes. On the other hand, data showed clear significant reserve of shoot length and nodes number as a result of the replacement of Ca^{2+} by different Ca^{2+}NPs concentrations, with one exception where shoot length and nodes number were near to moderate(6.67 cm and 4.67 node/shoot, respectively) at 1320 µg/l Ca^{2+}NPs with no significant difference between this substitution and MS (9.60 cm and 5.0 node/shoot, respectively). The shoot tip necrosis observation cleared an insignificant increase of necrosis with the most substitution concentrations of Ca^{2+}NPs (220, 440 and 660 µg/l Ca^{2+}NPs) compared with MS (3.7, 3.3, 2.0, and 2.7, respectively), but shoot tip necrosis was significantly decreased (disappeared) when Ca^{2+} ions was substituted with 1320 µg/l Ca^{2+}NPs (0.0 necrosis). The increase of shoot tip necrosis may reflect the lake of Ca^{2+} in low concentrations of nanoparticle substitution which stimulated the same deficient of Ca^{2+} in MS that is an essential element in building cell walls and when the concentrations of Ca^{2+}NPs were increased the phenomenon disappeared.

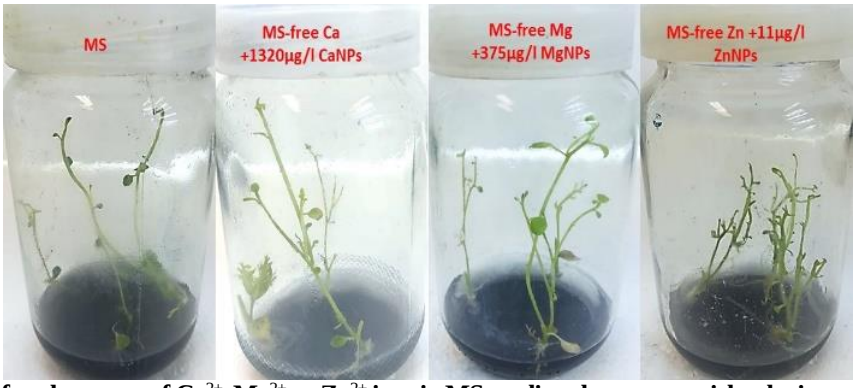


Photo 1. Impact of replacement of Ca^{2+}, Mg^{2+} or Zn^{2+} ions in MS medium by nanoparticles during multiplication stage

The best growth vigor was recorded in MS followed by Ca^{2+} substituted with 1320 µg/l Ca^{2+}NPs (4.9 and 3.7, respectively). Figure (1b) concerns with the replacement of Mg^{2+} ions in MS medium by different concentrations of Mg^{2+}NPs. Data revealed that Mg^{2+}NPs significantly enhanced Hermes shoot proliferation, substitution Mg^{2+} with 185 or 375 µg/l Mg^{2+}NPs gave an equal or augmented shoot number of Hermes when compared with MS with no significant difference between them (2.53, 2.63, or 2.53 shoot/explant, respectively), while an excess of Mg^{2+}NPs (555 µg/l) leads to a significant reduction of the shoot number (1.40 shoot/ explant). Likewise, shoot length, and nodes number/shoot of Hermes were positively affected by replacement of Mg^{2+} ions by 375 µg/l Mg^{2+}NPs (8.40cm, and 5.7 node/shoot, respectively) when compared with MS (9.60 cm, and 5.0 node/shoot, respectively), but the differences were not significant. It is important to refer that all Mg^{2+}NPs concentrations (185, 375, or 555 µg/l) decreased shoot tip necroses in Hermes compared with MS containing Mg^{2+} ions (1.0, 0.0,0.0, and 2.7, respectively). The growth vigor was significantly maximized with Mg^{2+}NPs at 375 µg/l and MS (4.8 and 4.9, respectively). Generally, Mg^{2+}NPs (375 µg/l) can replace the Mg^{2+} ions in MS medium and enhance the growth parameters of potato cultivar Hermes. Figure (1, c) deals with the observation of the response of potato cultivar Hermes when Zn^{2+}NPs replaced Zn^{2+} ions in MS medium during multiplication. Shoot number/explant was slightly affected by replacement Zn^{2+} ions with Zn^{2+}NPs, 11 or 44 µg/l Zn^{2+}NPs gave the maximum shoot number after MS with no significant difference between all treatments (2.17, 2.07, and 2.53 shoot/explant, respectively). Shoot length is negatively affected by replacement Zn^{2+} ions with Zn^{2+}NPs. While nodes number/Shoot was positively affected by replacement Zn^{2+} ions with 11, 22, or 33 µg/l Zn^{2+}NPs compared with MS (6.0, 5.7, 5.7 and 5.0 nodes/shoot, respectively). All concentrations of Zn^{2+}NPs greatly decreased the shoot tip necroses of Hermes. Also, growth vigor was slightly affected by Zn^{2+} ions replacement by Zn^{2+}NPs, but the differences between Zn^{2+}NPs concentrations and MS was not significant, which means that Zn^{2+}NPs can replace Zn^{2+} ions in MS without decreasing in growth proliferation and parameters of potato cultivar Hermes.

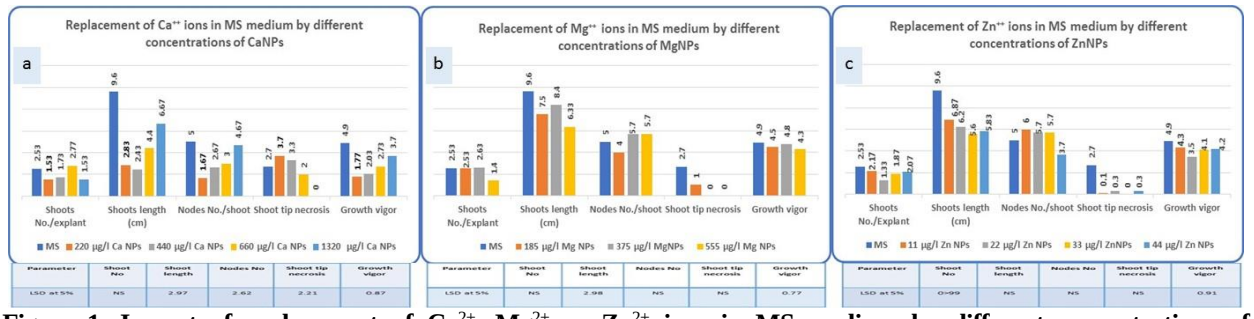


Figure 1. Impact of replacement of Ca^{2+}, Mg^{2+} or Zn^{2+} ions in MS medium by different concentrations of nanoparticlesduring multiplication stage

Chemical analysis:

Total phenolic concentration:

As shown in Fig. 2a, there is a significant difference between the effects of replacement Ca^{2+}, Mg^{2+}, and Zn^{2+} ions in MS medium with different concentrations of NPs on TPC, the results cleared that all replacement treatments (Ca^{2+}NPs, Mg^{2+}NPs, and Zn^{2+}NPs) maximized the TPC, the highest TPC was obtained in Zn^{2+}-free MS that contain 11µg/l Zn^{2+}NPs (50.271mg GA/g) followed by Ca^{2+}-free MS that contains 1320 µg/l Ca^{2+}NPs (43.141 mg GA/ g) and Mg^{2+}-free MS that contains

375 µg/l Mg^{2+}NPs (38.302 mg GA/g). The results indicated that all nanoelements replacement induced the TPC in potato cultivar Hermes more than plants which were grown on MS medium.

Chlorophyll concentration (CC):

Data in Fig. 2b, indicated that slightly impact were observed of the replacement of Ca^{2+}, $Mg^{2+,}$ or Zn^{2+} ions in MS medium by different concentrations of NPs during multiplication stage on chlorophyll concentration (Chl.a, Chl. b and total chlorophyll a+b). Although MS medium gave the highest values of CC when compared with replacement treatments, all growth parameters did not affect, this result may be due to the low photosynthesis activity of *in vitro* plants which means the low need for CC *in vitro*, as well as the high delivery and special characters of the nanoparticles which may give it the ability to do the same jobs of ions in low concentrations without the appearance of ions deficiency.

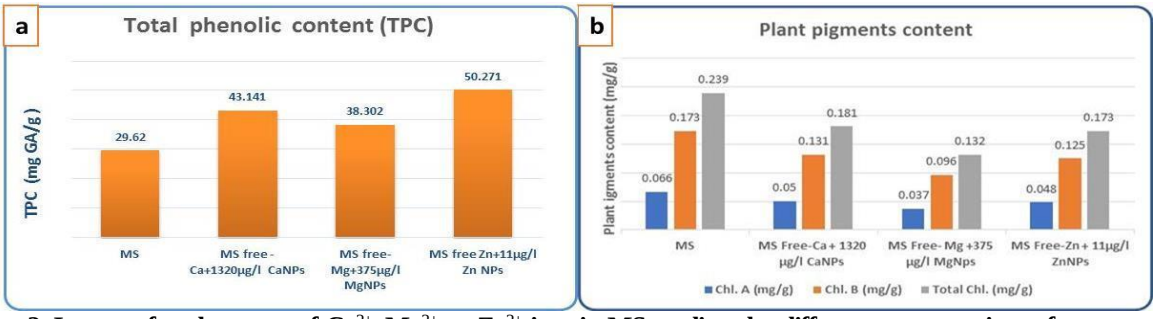


Figure 2. Impact of replacement of Ca^{2+}, Mg^{2+} or Zn^{2+} ions in MS medium by different concentrations of nanoparticles during multiplication stage on: (a): Total phenolic content (TPC) and (b): Chlorophyll concentration (CC)

Shoot elements concentration:

The replaced ions content (Ca^{2+}, Mg^{2+} and Zn^{2+}) in the multiplied shoots were significantly affected compared with the ions content of the shoots which grew on MS medium. Ca^{2+} content was maximized (478.9ppm) when shoots were grown on MS-free Mg^{2+}+ 375 µg/l Mg^{2+}, while, Mg^{2+} and Zn^{2+} concentration of multiplied shoots was maximized on MS medium free of nanoparticles. On the contrary, replaceMg^{++}ions in MS medium with 375 µg/l Mg^{2+}NPs increased the K, Fe, and Mn shoot concentration (68.39, 0.21, and 2.11 ppm, respectively). Also, replace Ca^{2+}, Mg^{2+} or Zn^{2+} ions in MS medium by nanoparticles led to significantly maximize the P shoot concentration (45.39, 42.92 or 61.42 ppm, respectively) compared with MS (10.85ppm).

Table 4. Impact of replacement of Ca^{2+}, Mg^{2+}, or Zn^{2+} ions in MS medium by different concentrations of nanoparticles during multiplication stage on shoot elements content

Treatment	Elements concentration (ppm)						
	Ca	Mg	Zn	P	K	Fe	Mn
MS	313.2	70.96	29.00	10.85	51.57	0.16	1.91
MS-free Ca^{++}+ 1.32ppm/l CaNPs	239.3	55.17	20.93	45.39	35.41	0.09	1.95
MS-free Mg^{++}+ 0.375ppm/l MgNPs	478.9	51.66	28.05	42.92	68.39	0.21	2.11
MS-free Zn^{++}+ 0.011 ppm/l ZnNPs	367.6	68.98	23.05	61.42	54.56	0.13	1.54
LSD 5%	0.62	0.48	0.60	0.52	0.92	0.11	0.26

Experiment 5: Impact of adding different nanoparticles concentrations to the MS medium in combination with various explant types on microtubers parameters

I-Impact of adding different nanoparticles concentrations to the MS medium in combination with various explant types on the total, large and small microtubers number

Data in Table(5) interested with the total number of formed microtuber as well as the number of large, and small size microtubers. The total number were enhanced with adding the NPs to MS medium in the most NPs concentrations, the significant maximum number obtained from MS+220µ g/l Ca^{2+}NPs +90µg/l Mg^{2+}NPs +4.5 µg/l Zn^{2+}NPs/l (11.33 microtuber/ jar) followed by 90 µg/l Mg^{2+}NPs, both concentrations of Ca^{2+}NPs and 90 µg/l Mg^{2+}NPs+4.5 µg/l Zn^{2+}NPs (10.83, 9.83, 9.67 and 10.00 microtuber/jar, respectively). The nodes explant significantly increased the total number of microtuber than shoot tip explants. The interaction between NPs concentrations and explant type showed the excellence of 90µg/l Mg^{2+}NPs and shoot tips as explants and 90µg/l Mg^{2+}NPs+4.5 µg/l Zn^{2+}NPs in combination with nodes explants (13.33 and 12.33 microtuber/jar, respectively). Also, the number of large microtubers was significantly affected by the most NPs concentrations despite Zn^{2+}NPs concentration individually or in combination with Ca^{2+}NPs which gave the lowest numbers of large microtubers. On contrary, the explant types cleared no significant differences between shoot tips and nodes in several large microtubers. The interaction between NPs concentrations and explant types showed that the high number of largely formed microtubers resulting from MS medium contained either 440µg/l Ca^{2+}NPs or 220µg/l Ca^{2+}NPs+4.5µg/l Zn^{2+}NPs (2.67 or 2.33 large microtuber/ jar, respectively). The small size microtubers were significantly affected by NPs concentrations, MS+ 220 Ca^{2+}NPs + 90Mg^{2+}NPs +4.5 Zn^{2+}NPs/l produced the highest number of small microtubers followed by MS+ 90 µg/l Mg^{2+}NPs and MS+220 µg/l Ca^{2+}NPs+90µg/l Mg^{2+}NPs, with no significant difference between them (10.00, 9.17 and 8.67 small microtuber/jar, respectively). There were no significant differences between explant types in the number of small microtuber. Interaction cleared a role of adding Mg^{2+}NPs in increasing small microtuber formation where MS+ 90µg/l Mg^{2+}NPs in combination with shoot tip explants or MS+90µg/l Mg^{2+}NPs+4.5µg/l Zn^{2+}NPs in combination with nodes explants or MS+220µg/l Ca^{2+}NPs+90µg/l Mg^{2+}NPs+4.5µg/l Zn^{2+}NPs in combination with shoot tip explants significant maximized the number of small microtubers (12.00, 10.67 and 10.33 small microtuber/jar, respectively) (Photo, 2).

II- Impact of adding different Ca, Mg, and Zn nanoparticles concentrations to the MS medium in combination with various explant types on the total, large and small microtubers weight

Results in (Fig3.a,b, and c) revealed that NPs concentrations significantly affected the total weight of microtubers, MS culture medium free of NPs significantly enhanced total weight of formed microtubers (2.57g) followed by MS supplemented with either 9µg/l Zn^{2+}NPs or 180µg/l Mg^{2+}NPs or 440µg/l Ca^{2+}NPs (2.39g, 2.37 and 2.31g, respectively) with no significant difference between them. Observation of total microtuber weight formed on both types of explant revealed that nodes explant significantly maximized the total microtuber weight (2.25g). The interaction between NPs concentrations and types of explant showed that MS media produced the highest total weight (3.48g) with the node explant followed by MS medium+220µg/l Ca^{2+}NPs+90µg/l Mg^{2+}NPs+4.5µg/l Zn^{2+}NPs (2.99gm) on shoot tip without significant differences between them, while there were significant differences among all combinations additions of nanoparticles concentrations and MS medium free of NPs. Similarly, the weight of large formed microtube was significantly affected by NPs concentration and both types of explant. The large weight significantly increased on the MS media that contain MS+220µg/l Ca^{2+}NPswith the node explants. Also, the small microtubers weight was significantly varied according to NPs concentrations, but the explant types showed non-significant differences in the small microtuber weight. The maximum enhancement of small microtubers weight was observed with the MS medium+220µg/l Ca^{2+}NPs +90µg/l Mg^{2+}NPs when the nodes were the explant (1.45gm).

Photo 2. The best impact of adding nanoparticles to MS medium and explant types on microtubers formation as compared with microtuber medium free of nanoparticles

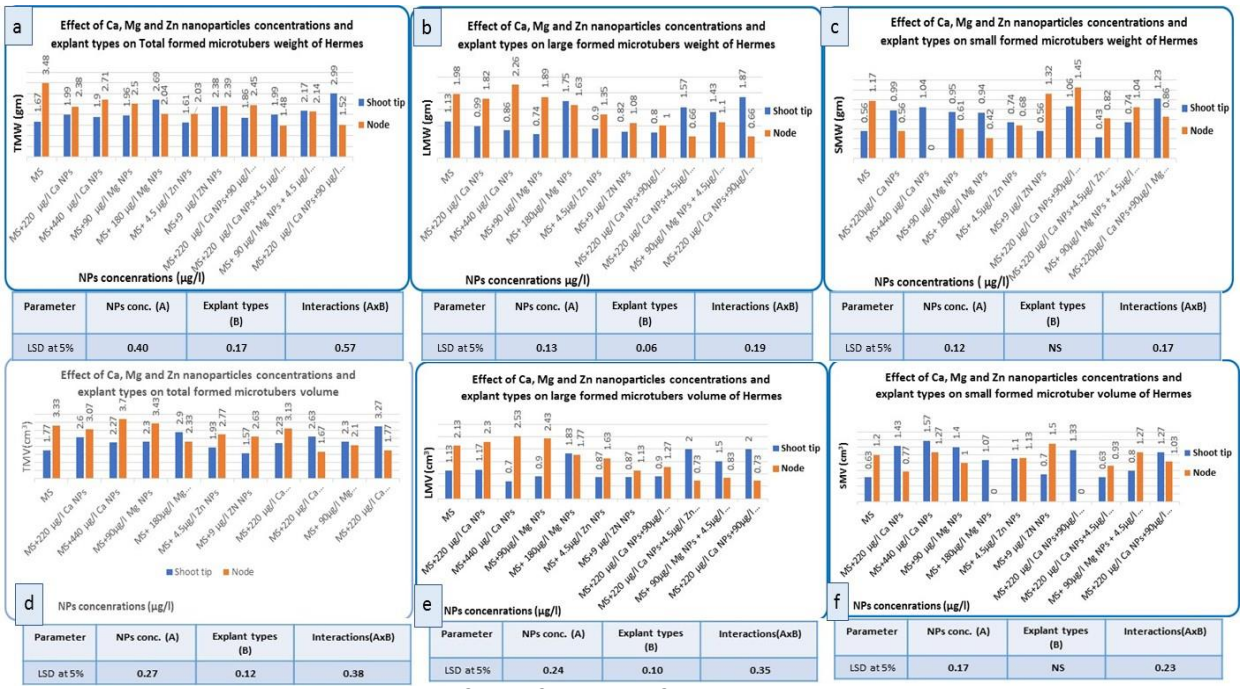
Table 5. Impact of adding different concentrations of Ca^{2+}, Mg^{2+}, and Zn^{2+} nanoparticles to MS medium and explant types on total, large, and small microtubers number

NPs		Type of explant		Mean	Type of explant		Mean	Type of explant		Mean
Element	Conc. (µg/l)	Shoot tip	Node	(A)	Shoot tip	Node	(A)	Shoot tip	Node	(A)
MS free of NPs	0.0	5.67	9.33	7.50	1.67	1.67	1.67	4.00	7.67	5.83
Ca^{2+}	220	9.67	10.00	9.83	1.33	2.00	1.67	8.33	8.00	8.17
	440	9.00	10.33	9.67	1.00	2.67	1.83	8.00	7.67	7.83
Mg^{2+}	90	13.33	8.33	10.83	1.33	2.00	1.67	12.00	6.33	9.17
	180	7.33	6.33	6.83	1.67	2.00	1.83	5.67	4.33	5.00
Zn^{2+}	4.5	7.00	7.33	7.17	1.00	1.33	1.17	6.00	6.00	6.00
	9	4.00	10.33	7.17	1.00	1.00	1.00	3.00	9.33	6.17
Ca^{2+} x Mg^{2+}	220 Ca +90Mg	10.00	9.33	9.67	1.00	1.00	1.00	9.00	8.33	8.67
Ca^{2+} x Zn^{2+}	220 Ca +4.5Zn	4.67	9.33	7.00	2.33	0.67	1.50	2.33d	8.67	5.50
Mg x Zn	90Mg + 4.5Zn	7.67	12.33	10.00	1.33	1.67	1.50	6.33	10.67	8.50
Ca^{2+} x Mg^{2+} x Zn^{2+}	220 Ca +90Mg +4.5Zn	12.00	10.67	11.33	1.67	1.00	1.33	10.33	9.67	10.00
Mean(B)		8.21	9.42		1.39	1.55		6.81	7.88	
LSD at 5%		A:1.40	B:0.60	AxB:1.98	A:0.66	B:NS	AxB:0.94	A:1.49	B: 0.64	AxB:2.10

III- **Impact of adding different Ca^{2+}, Mg^{2+}, and Zn^{2+} nanoparticles concentrations to the MS medium in combination with various explant types on the total, large and small microtubers volume**

Data in Fig 3(d, e, and f) discuss the total volume of microtubers as well as the volume of large and small microtubers as affected by NPS concentrations and type of explants. Commonly, NPs concentrations significantly enhanced the total volume of microtubers, the MS fortified with 440 or 220µg/l Ca^{2+}NPs or 90µg/l Mg^{2+}NPs significantly increased the total volume of produced microtubers (2.98, 2.83, 2.87, and 2.68cm^3, respectively) with no significant difference between them. Also, nodes explants significantly augmented the total volume of microtuber compared with those produced from shoot tip explants (2.27 and 2.34cm^3, respectively). The data of interaction revealed that MS medium contained 440µg/l Ca^{2+}NPs and nodes explant significantly ascended the total volume of microtubers (3.70cm3). The volume of large microtubers was significantly enhanced as a result of adding 180 or 90µg/l Mg^{2+}NPs or 220or 440µg/l Ca^{2+}NPs to the MS medium with no significant differences between them and MS medium free of NPs (1.80, 1.67, 1.62,1.73 and 1.63cm^3, respectively). The nodes explant significantly induced the volume of large microtubers (1.59cm^3). Finally, the volume of large microtubers significantly increased when MS contained 440µg/l Ca^{2+}NPs or 90 µg/l Mg^{2+}NPs with node explants in both (2.53 ad 2.43cm^3, respectively). The volume

of small microtubers was significantly improved by adding NPs to the medium, MS supplemented with 220 µg/l Ca^{2+}NPs +90 µg/l Mg^{2+}NPs gave the highest volume of small microtubers (1.60cm^3). While, the explant types affected the volume of small microtubers, but the difference was not significant. Data of interaction proved that 220 µg/l Ca^{2+}NPs +90µg/l Mg^{2+}NPs and node explant significantly increased the volume of small microtubers (1.87cm^3).



Parameter	NPs conc. (A)	Explant types (B)	Interactions (AxB)
LSD at 5%	0.40	0.17	0.57

Parameter	NPs conc. (A)	Explant types (B)	Interactions (AxB)
LSD at 5%	0.13	0.06	0.19

Parameter	NPs conc. (A)	Explant types (B)	Interactions (AxB)
LSD at 5%	0.12	NS	0.17

Parameter	NPs conc. (A)	Explant types (B)	Interactions(AxB)
LSD at 5%	0.27	0.12	0.38

Parameter	NPs conc. (A)	Explant types (B)	Interactions(AxB)
LSD at 5%	0.24	0.10	0.35

Parameter	NPs conc. (A)	Explant types (B)	Interactions(AxB)
LSD at 5%	0.17	NS	0.23

Figure 3. Impact of adding different Ca^{2+}, Mg^{2+}, and Zn^{2+} nanoparticles concentrations to the MS medium incombination with various explant types on weight and volume of microtubers.

Where: a, b, and c: the total, large and small microtubers weight of Hermes, d, e, and f: the total, large and small microtubers volume of Hermes

Discussion

Results showed that the *in vitro* potato multiplication was significantly affected by thiamine concentrations; MS substituted with 100mg/l thiamine-HCl gave the highest shoots number, shoots length, nodes and roots number (1.97 shoots, 7.03cm, 4.89 nodes, and 0.92 roots, respectively). While, the different concentrations of the BA significantly affected all growth parameters except for the number of shoot multiplication. The interaction revealed that MS+2mg/l BA+100mg/l thiamine-HCl and MS+1.0mg/l BA+200mg/l thiamine-HCl maximized potato proliferation and growth parameters. Results supported with those published by Kazemiani *et al*. (2018), who ensured the positive effect of thiamine-HCl and confirmed that the maximum node number, the longest shoots in potato was produced in MS medium which supported with thiamine-HCl. On contrary, results disagree with the finding of Emaraa *et al.* (2017) and Hamza (2019a), who stated that multiplication and growth parameters are significantly induced by both MS strength and concentrations of BA. The difference may be due to the difference in the number of subculture or may be related to the genotype. Similarly, explant types and sucrose concentration promoted the *in vitro* potato multiplication, the nodal explant that cultured in MS media supplement with 50g/l sucrose gave the highest shoots number, shoots length, node number (1.75, 6.00, and 6.08, respectively), while, the double node gave the highest value of growth vigor (3.55) at the highest sucrose concentration (50g/l). Results agree with the finding of Chen *et al*. (2020); Abbas *et al*. (2020) who confirmed that enhancement of plant growth was associated with longer shoots, deeper roots, and a greater number of leaves when concentration of sucrose was higher than 3%

Concerning the effect of explant orientation in *in vitro* potato multiplication, the results indicated that the shoot tip produced the highest shoot number (2.67 shoot) with no significant difference between both explant orientation. On another side, the double node produced longer plantlets (10.05cm) with no significant difference between the effect of both culture orientations, there were no significant difference between the explant's types and its orientations on node number.The results disagree with the studies of Rani *et al. (*2013); Jainol and Gansau (2016); Morais *et al. (*2018) who reported that culture sprouts on horizontally position over the media produced a significantly higher number of shoots per explant in potato.

The results showed that *in vitro* potato multiplication was affected with the nanoparticles, the MS media free of $CaCl_2$+660µg/l Ca^{2+}NPs produced the superior rate of shoots number (2.97 shoot/explant), Also, increase the Ca^{2+}NPs to 1320µg/l prevent shoot tip necrosis. The increase of shoot tip necrosis in low concentrations may reflect the lake of Ca^{2+} in low concentrations of nanoparticle substitution which stimulated the same deficient of Ca^{2+} in MS which is an essential element in building cell walls and when the concentrations of Ca^{2+}NPs were increased the disorder phenomenon disappeared. Results came in harmony with Agrawal and Rathore (2014) who stated that in general nanomaterials have positive morphological effects like improvement germination rate and percentage; shoot and root length through enhancement the physiological parameters such as enhancing the photosynthetic activity and the metabolism of nitrogen in most plants. Also, the result is

compatible with Busse *et al*. (2008) who reported that shoot tip necrosis was observed in cultures of potato 'Dark Red Norland' when $CaCl_2$ was insufficient in the medium and when the media supplement with 1360 µg Ca^{2+} the shoot tip necrosis was reduced or prevent. Results indicated that Mg^{2+}NPs (375 µg /l) can replace the Mg^{2+} ions in MS medium free of Mg^{2+} ions, and enhance growth parameters of potato cultivar Hermes. Also, Zn^{2+}NPs can replace Zn^{2+} ions in MS medium free of Zn^{2+} ions without decreasing in growth proliferation and parameters of potato cultivar Hermes. Results came in line with the finding of Javed *et al*. (2017) who recorded that the highest shoot formation (89.6%) was obtained when nodal explants of *Stevia rebaudiana* were cultured on MS medium supplemented with 1mg/l Zn^{2+}ONPs.Similarly, the nodes number are affected with different NPs. Also, microtuber formation was enhanced by adding nanoparticles (Ca^{2+}, Mg^{2+} and Zn^{2+}) to MS medium as well as the type of explant. The highest total microtuber number was obtained when MS was fortified with 90mg/l Mg^{2+}NPs and shoot tips as explants and 90mg/l Mg^{2+}NPs+4.5mg/l Zn^{2+}NPs in combination with nodes explants (13.33 and 12.33 microtuber/jar, respectively). Also, large microtuber numbers and small microtuber numbers were significantly affected by NPs concentrations and explant types. Weight and volume of total, large and small microtubers were differed according to NPs concentrations and explant types. Results came to agree with Hamza (2019b), who stated that explant types and NPs concentrations affected the number of induced microtubers, as well as weight and volume of microtubers.

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اضافه عناصر النانو فى بيئه زراعه الانسجه النباتيه وتاثيرها على تكوين الدرينات فى البطاطس صنف هيرماس

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تشارك الجسيمات النانوية في مجالات متعددة ، في الإلكترونيات والطاقة والطب وعلوم الحياة والزراعة ، والهدف من الدراسة الحالية هو تقييم تأثير العوامل المؤثره علي إكثار وتكوين درينات لبطاطس معمليا و خاصة جزيئات النانو في صنف بطاطس هيرمس . تم استبدال بعض العناصر الأساسية بتركيزات مختلفة منها في صورة النانو في مراحلة التضاعف كذلك تم تقيم اضافة تلك العناصر في صورة النانو متداخلة مع بعضها أو كلا علي حده في مرحلة تكوين درينات لبطاطس في المعمل .تم دراسة بعض العوامل التي تؤثر إكثار وتكوين درينات لبطاطس معمليا ومنها (تركيزات مختلفة من الثيامين والبنزايل آدينين - تركيزات مختلفة من السكر مع إختلاف الجزء النباتي – نوع المنفصل النباتي وطبيعة زراعته علي البيئة –إستبدال كلا من الكالسيوم والماغنسيوم والزنك بتركيزات مختلفة منها في صورة النانو في مرحلة التضاعف كذلك اضافة تلك العناصر في صورة النانو متداخلة مع بعضها أو كلا علي حده في مرحلة تكوين درينات لبطاطس معمليا .آشارت النتائج الي أن كلا من البيئة المحتوية علي 0.1 مجم/ لتر بنزايل آدينين+200 مجم/ لتر ثيامين والبيئة التي تحتوي علي 0.2 مجم/ لتر بنزايل آدينين +200 مجم/ لتر ثيامين إدت الي زيادة معنوية في عدد الفروع المتكونة, أما بالنسبة لطول الفروع آكدت النتائج أن أعلي طول للفروع كان مع البيئة الخالية من والبنزايل آدينين مع تركيزات الثيامين المختلفة .إماعن تأثيرتركيزات السكر مع إختلاف المنفصل النباتي أوضحت النتائج أن زراعة الجزء النباتي "العقد" علي البيئة التي تحتوي علي 50 جم/ لتر سكر سجلت أعلي عدد فروع (2.17 فرع/ المنفصل النباتي) وأطول فروع (10.40سم) وأكبر عدد عقد (7.67عقد/الفرع) وأقوي قوة نمو (5.00/ البرطمان) .بالنسبة لتأثيرنوع الجزء النباتي وكذلك إتجاه زراعته أكدت النتائج أن كلا من القمم النامية في كلاً وضعي الزراعة "العمودي والأفقي " كذلك العقد المزدوجة عند زراعتها عموديا علي البيئة سجلت أعلي عدد من الفروع مع وجود فروق معنوية بين باقي القياسات (عدد العقد – طول الفروع – قوة النمو) .أدي إستبدال أيونات الكالسيوم في بيئة موراشيج وسكوج بـ (660 أو 1320 ميكروجرام/ لتر) من نانو الكالسيوم الي زيادة عدد الفروع(2.77) بالمقارنة بالكنترول (2.53) . في الوقت نفسه لوحظ من النتائج أن إحلال أيونات الماغنسيوم بـ (185 أو375ميكروجرام/ لتر)من نانو الماغنسيوم أدي إلي زيادة ملحوظة في عدد الفروع بالمقارنة بالكنترول مع عدم وجود إختلافات معنوية بينهم, وزيادة معنوية في طول الفروع وعدد العقد مع عدم ظهور إحتراق للقمم النامية في معاملات الماغنسيوم وبصفة عامة و وفقاً للنتائج يمكن إستبدال أيونات الماغنسيوم بـ (375 ميكروجرام/ لتر) من نانو الماغنسيوم في بيئة MS مع تحسين نمو البطاطس صنف هيرمس.في الوقت نفسه أوضحت النتائج إلي إمكانية إحلال أيونات الزنك بإخري في صورة النانو مع عدم التأثير علي طبيعة وقوة نمو النباتات في المعمل مقارنة بالكنترول

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