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Improving Growth and Tuberization of Potato During *In vitro* Multiplication and Rooting Stages via Nano Chitosan and Magnetized Water

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Abstract: The experiment was carried out to study the effect of magnetized water (MW) and nano-chitosan on *in vitro* tuberization of potato cultivar, namely, Diamant. The experiment was conducted in tissue culture laboratory, of Strawberry and non-traditional crops center, faculty of Agriculture, Ain shams University. In multiplication and microtuberization stages, nodal cuttings from the second subculture were cultured on MS media with magnetized water (MW) and or o.4g/l of nano-chitosan. The results indicated that, magnetized water recorded the tallest plantlet and branches. Also it increased nods number per plantlet. On the other hand, control plants recorded the lowest values of plant height, number of branches and nods plantlet. The highest percentage of microtuberization was recorded with magnetized water. Medium treated with magnetized water produced significantly the highest number of microtubers/plantlet. Using magnetized water produced significantly the highest values of microtuber fresh weight.

Key words: Potato・*In vitro*・Tuberization・Nano-chitosan・Magnetic water

INTRODUCTION

Potato is one of the most important vegetable crops grown in Egypt for local consumption, export and processing. The area cultivated with potatoes was about 475, 000 Fed., producing about 58 million tons, with an average of 12.3 tons per fed. and the exportation reached 678, 000 tons of potatoes. Egypt imported 152, 753 tons of seed tubers from Europe [1]. In order to optimize potato productivity and quality, various aspects of production chain should be considered in an integrated way. These aspects include potato seed quality. The lack of sufficient quality of quality seed is a major constrain to improve potato productivity and quality. The main propagation method of potato is asexually, through small tubers or divided large tubers [2, 3]. Seed potato is usually the most expensive input in potato cultivation, accounting from 40 to 50 percent of production costs [4]. SIn developing countries whereas no formal seed supply system exists, farmers have devised their own method for selecting seed tubers: they sell the largest potatoes for cash, eat the medium sized ones at home and keep the smallest as future planting material [5].

Tissue culture techniques have become a powerful tool for propagation of potato to overcome the problems faced by traditional propagation methods. It is also very useful for rapid multiplication, safe exchange and conservation of many vegetative propagated crops [6]. In these techniques, meristem culture used to produce virus free plants [7]. Furthermore, potato micropropagation technology has been successfully employed to eliminate virus infection from systemically infected 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 [8]. 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 [9]. Potato microtuberization is influenced by multiple factors, including genotype, explant type, media and particular growth conditions [2].

Recently, nano materials have been involved in multiplication of the plant biotechnology; such as antiseptic, eliminate the harmful components of agricultural ecosystems as a safe, high potential for

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identify and eliminate pathogens, elicitor which enhance production of secondary metabolites, developing fruit packaging to elongate product shelf-life. Nanomaterials have positive morphological effects for example 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 the most crop plants Agrawal and Rathore [10] and Doklega [11] reported that spraying potato with nano material the best treatment of beneficial effects for minimizing the environmental pollution, consumer health and production costs, moreover increasing yield quantity and quality.

Nanostructured chitosans can be used as bioactive ingredients carriers. They have the potential to be encapsulation or immobilization carriers. Due to their favorable biological properties such as nontoxicity, biocompatibility, biodegradability and antibacterial ability, they are also interesting options as drug delivery carriers and as cell proliferation enhancers Divya and Jisha [12] and Zayed *et al.* [13] indicated that, nano chitosan in all concentrations significantly promoted seed germination and radical length under salt stress. The best treatment on ermination was 0.3 % of nano chitosan. Growth variables (plant height, leaf area, fresh and dry weights of the shoot and root) were increased significantly.

Magnetized water can improve the growth and development of plants both quantitatively and qualitatively. It can improve the germination of seeds, increase in the pH of treated water. Moreover, physicochemical characteristics of water were improved leading to better yield of potato crop. Magnetic water technology shown promising potential in various fields especially agriculture. It safety compatibility, simplicity, environmental friendliness, low cost operating and non-adverse effects. Magnetized water has unique physical and chemical properties that make it a multi-purpose compound with potential benefits in medical treatment industrial as well as, environmental applications [14]. It can be stimulate the growth and influence the activity of respiratory enzymes in plants [15].

Therefore, the aim of the present study was to reach the proper protocol for *in vitro* production of virus-free microtubers of Diamant potato cultivar, through application of magnetic water (MW) and nano-chitosan to MS medium.

MATERIALS AND METHODS

This study was carried out at the tissue Culture laboratory, of Strawberry and non-traditional crops center, faculty of Agriculture, Ain shams University, Cairo, Egypt, during the period from 2018 to 2019. Potato explants from cultivar Diamant were used. The source of explant was Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt. Four treatments, magnetize water, nano-chitosan at 0.4 g/l and magnetized water plus nano-chitosan in addition to control were conducted to improve the growth and tuberization of potato *in vitro*.

Magnetized Water Treatment: Magnetic device (1.45 Tesla) was obtained from Delta Water Company, Alexandria, Egypt. When water passes through the magnetic field it becomes magnetized, which causes some physical changes to the composition and shape of water molecules.

Chitosan Nanoparticles:

Synonyms: nanopoly (D-glucosmine): deacetylated chitin nanoparticles

Chemical Formula: (C6H11NO4) n Chemical Family: Polymer

Table 1: Constituents of murashige and skoog basal nutrient

Macro elements				
$NH4NO_3$	KNO_3	$CaCl_2 .2H_2O$	$MgSO_4.7H_2O$	KH_2PO
1650 mg/1	1900 mg/1	440 mg/1	370 mg/1	170 mg/1
Micro elements				
H_3BO_3	$MnSO_4.4H_2O$	KI	$N8MO_4.2H_2O$	$CuSO_4.H_2O$
6.2 mg/1	8.6 mg/1	0.83 mg/1	0.25 mg/1	0.025 mg/1
$CoCl_2.6H_2O$	N8-Fe-EDTA	----	----	----
0.025 mg/1	36.7 mg/1	----	----	----
Vitamins				
Myo-Inositol	Glysine	Nicotinic acid	Pyridcxin-HCl (B6)	Thiamine-HCl (B1)
80 mg/1	2 mg/1	0.5 mg/1	0.5 mg/1	0.1 mg/1

The experimental included two stages:

1.Multiplication stage of potato *in vitro* culture.

2.Microtuberization stage of potato *in vitro* production

Preparation and Sterilization of Explants: Certified virus-free potato tubers of "Diamant" cultivar were kindly supplied from Agricultural Research Center in Giza. Explants of 2-3 mm length from potato tubers was excised under an electronic binuclear and cultured in 250 ml culture jar, each containing 40 ml of solid media based on MS [16]; Table (1) strength basal medium with 3% sucrose and 0.8 % agar. The excised explants were incubated at 16/8 hr. Light of 4000 Lux and 25 ±2°C for 4 weeks.

Multiplication Stage: Magnetized water and or nano chitosan at 0.4 g/l were added to MS medium before autoclaving. Plantlets obtained from establishment stage with (6-9 cm length) were repropagated with two node cuttings after 30 days. The nodal segments were separated (1-2 leaves) with five nodes per jar and cultured on MS medium with 30g/l sucrose and 7 g/l agar. plantlets of all cultivars were multiplied by sub culturing twice to get the required number of plantlets. Then, cultures were incubated at 24±2°C under 16-hours light with a light intensity of 4000 lux and 8.hours dark for three weeks. At the end of the multiplication stage, plantlet length (cm), number of branches, number of nodes and plantlet fresh and dry weight (g) were recorded.

Rooting Stage: The source of the nodal cutting was the plantlets produced from the second subculture of the multiplication stage. Four weeks *in vitro* micro propagated plantlets were cultured on 250 ml glass jars; each having 50 ml microtuberization solid medium.(MS) 3, 3 g that was supplemented with either 80g/l sucrose , 3mg/1 kinetin, 2mg/1 benzyl adenine BA and 7g/l agar adjusting the PH at 5, 7. The cultured vessel jars were kept under aseptic conditions in a growth room at 24±2°C. Thereafter, the jars were kept under complete darkness at 24-±2°C for two weeks. Then, the jars were kept at 16 hours light and 8 hours dark with a light intensity of 2000 lux at 24-±2°C for two weeks number of branches per plantlet, plantlet height (cm) and leaves number per plantlet were recorded and then, the jars were kept with a light intensity of 3000 lux at 24-±2°C for two weeks. After that, the jars were kept with a light intensity of 4000 lux at 24-±2°C for two week. At the end 8 weeks either on dark or light conditions, plantlet length (cm), number of branches, number of nodes, number of microtubers per plantlet, fresh and dry weight of microtuber (g) were recorded.

Statistical Analysis: The experimental design of this trial was factorial distributed in full randomized design with three replicates each replicate consisted of five Jars with five stem cuttings (each 5 explants) per jar. The experiment was repeated twice. Data were tested by analysis of variances [17].

RESULTS AND DISCUSSIONS

Plant Length and Number of Nods and Branches During Multiplication Stage: Data in Table (2) show the effect of magnetic water and nano chitosan on plant length (cm) and number of leaves/plantlet of "Diamant" potato cultivar after muliplication stage *in vitro*. Results indicate that the application of magnetized water recorded the tallest plantlets, highest values of number of branches and nods per plantlet but without significant difference with the other treatments in the two tested seasons. On the other hand, control plants recorded the lowest values of plant height, number of branches and nods per plantlet. These findings agree with Amor *et al.* [18] who reported that the magnetized water has a stimulating effect on the number of the plant growth. In addition, Doklega [11] who found that magnetized water produced the highest significant values of plant growth. Plant height significantly increased when broad plants were irrigated with magnetically treated water compared to plants irrigated with untreated water [19]. These increases may be attributed to increasing ions mobility and ions uptake improved under magnetic field, which leads to a better stimulation in plants.

Fresh and Dry Weight During Multiplication Stage: Applied magnetized water and / or nano chitosan significantly influenced the plantlet fresh weight as well as dry weight. Magnetized water recorded significantly maximum plantlet fresh weight (0.73 and 0.94 g) as well as dry weight (0.07 and 0.11 g) in the first and second seasons respectively. Whereas, the control /plantlet gave the lowest values (Table 3). These results may be attributed to the role of magnetized water in increasing absorption and assimilation of nutrients consequently increasing plantlet growth and hence the shoot fresh and dry weight Abd Ellateef and Mutwali [19] who found that the shoot fresh and dry weight of broad bean showed a significant increase in treatment treated with magnetized water against control.

Plant Length and Number of Nods and Branches During Microtuberazation Stage: Table (4) shows the effect of magnetized water and or nano chitosan on plant length, number of nods and branches of potato plant. Magnetized water recorded significantly the highest value of plant

Table 2: Effect of magnetized water (mw) and nano chitosan on plant length (cm), number of nods and branches /plantlet of "Diamant" potato cultivar during multiplication stage *in vitro* in 2018 and 2019 seasons

	Plantlet length (cm)		Number of nods per plantlet		Number of branches / plantlet	
Treatments	2018	2019	2018	2019	2018	2019
Magnetized water	2.44a	2.33a	4.11ab	3.55a	4.54a	5.44a
Nano chitosan	1.66ab	1.77ab	3.66ab	2.99a	4.17a	5.36ab
Magnetized water +Nano chitosan	1.44ab	1.10ab	2.99ab	2.99a	4.21a	4.38ab
Control	1.99ab	1.77ab	2.99ab	2.88a	3.78a	4.05ab

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level.

Table 3: Effect of magnetized water (mw) and nano chitosan on fresh and dry weight (g) plant of "Diamant" potato cultivar during multiplication stage *in vitro* in 2018 and 2019 seasons. of"Diamant" potato cultivar during multiplication stage *in vitro* in 2018 and 2019 seasons

	Fresh weight (g)/plant		Dry weight (g)/plant	
Treatments	2018	2019	2018	2019
Magnetized water	0.73a	0.94a	0.07a	0.11a
Nano chitosan	0.24c	0.73c	0.03b	0.05b
Magnetized water + Nano chitosan	0.52b	0.83b	0.06a	0.07b
Control	0.12d	0.45d	0.02b	0.04b

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level

Table 4: Effect of magnetized water (mw) and nano chitosan on plant length (cm), number of nods and number of branches /plant of "Diamant" potato cultivar during microtuberazation stage *in vitro* culture in 2018 and 2019 seasons

	Plant length (cm)		Number of nods/plant		Number of branches/plant	
Treatments	2018	2019	2018	2019	2018	2019
Magnetized water	7.21a	5.49a	2.55a	2.55a	2.44a	2.33a
Nano chitosan	5.72b	5.16a	1.44b	1.44b	2.11ab	1.33bc
Magnetized water + Nano chitosan	5.46b	5.47a	1,55b	2.44a	1.55ab	1.77bc
Control	3.27c	3.44b	1.44b	1.55b	1.11bc	1.44bc

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level

Table 5: Effect of magnetized water (mw) and nano chitosan on number of microtubers/plant, microtubers fresh and dry weight of "Diamant" potato cultivar during microtuberzation stage *in vitro* in 2018 and 2019 seasons

	Number of microtubers/plant		Microtubers fresh weight (g)		Microtubers dry weight (g)	
Treatments	2018	2019	2018	2019	2018	2019
Magnetized water	4.66a	4.44a	2.62a	2.19a	0.45ab	0,37a
Nano chitosan	1.99b	2.10b	1.16b	1.78a	0.13ab	0.15b
Magnetized water + Nano chitosan	3.99a	4.00a	1.99a	2.17a	0.16ab	0.29a
Control	1.77b	1.88b	1.25b	1.16 b	0.15ab	0.12b

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level

height as compared to other used treatments. However, no significant difference was recorded between nano chitosan and magnetic water plus nano chitosan. On the other side, the lowest values of plant length were obtained from the control treatment in the two tested seasons.

Concerning number of nods, the application of magnetized water recorded the highest number of nods with no significant difference comparing with nano chitosan and magnetic water plus nano chitosan in the second season.

Regarding the number of branches per plant, the highest number of branches was recorded in the Daimont cultivar with the application of magnetized water. However, the application with nano chitosan was in significant as compared to magnetic plus nano chitosan in the two seasons. This result agrees with Shahin *et al.* [20] who revealed that all growth parameter were improved significantly by using irrigation water and magnetized seeds as compared to the control treatment (un-treated plants *in vitro* and untreated water through the growing season). Similar conclusions were also obtained by Cheikh *et al.* [21] and Abd Ellateef and Mutwali [19].

Fresh and Dry Weight During Microtuberazation Stage: It is clear from results presented in Table (5) that magnetize water and nano chitosan treatments had positive effects on number of microtubers/plant as well as microtuber fresh and dry weight in two tested seasons. The application of magnetized water significantly increased the number of microtubers/plant and fresh and dry weight. On the other side, the control treatment gave the lowest values. Similar conclusions were obtained by Hozaya and Abdul Qados [22] who associated the mechanism of magnetic field with the activation of phytohormone such as gibberellic acid-equivalents, indole-3-acetic acid and trans-zeatin as well as activation of the bio-enzyme systems which leads to the growth improvement and increased the crop yield Ahmed and Abd El-Kader [23] concluded that the irrigation of potato plants by magnetic water at 55% or 60% of the field capacity could be recommended to enhance vegetative growth, yield and its components of potato.

These studies provide a holistic approach of an agricultural cultivation that can lead to the comprehension of the exact mechanism of magnetize water effect on plant tissues and lead to the appropriate application of the magnetize water. The determination of the optimal duration of exposure, the type of magnetic field used and the effect of magnetic field on quality characteristics are some major factors that deserve further investigation. The magnetic treatment had a positive effect on number and weight of microtubers/plant *in vitro*. This means that this technology can be recommended for agriculture to improve their crop yield 1` particularly when we are facing poor water quality.

CONCLUSION

Our results indicate that the application of magnetized water in tissue culture can be an ecofriendly practice that improves plant characteristics in all stages, from multiplication medium to micro-tuberazation. Magnetized water treatment, in certain times of exposure, improved plantlet length (cm) and number of leaves/plantlet, fresh and dry weight per plantlet number of nods and branches of potato plant.

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