Improvement of potato (*Solanum tuberosum* L.) micro-tubers formation as effected by nano-particles *in-vitro*

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Background
Applying nano-fertilizers in the agricultural sector may lead to sustainable development through lower inputs and waste generation, diminishing nutrient losses, and increasing nutrient use efficiency by releasing nutrients at a suitable rate for plant demand compared with conventional chemical fertilizers.
Objective
The current study's objective was to develop the ideal procedure for the *in vitro* creation of virus-free micro-tubers of two potato cultivars using varied doses of phosphorus and potassium nanoparticles (K-NPs) added to the Murashige and Skoog (MS) medium.
Materials and methods
The research was conducted at the Tissue Culture Laboratory, Vegetable Crops Department, Faculty of Agriculture, Cairo University, Egypt, from January 2020 to July 2021. The meristem tips of two potato cultivars were excised and cultured in solid MS medium supplemented with sucrose and agar. The multiplication stage involved re-propagation of the cultivars using nodal segments, which were cultured on MS medium.
Results and conclusion
The effects of potassium 25, 35, and 45 mM or phosphorus 2, 3, and 4 mM nanoparticles on *in-vitro* micro-tuber formation and growth of two potato cultivars (Lady Rosetta and Spunta) were cultured. Data on the number of branches, plant length, and number of leaflets were recorded after 40 days of culture. The number of micro-tubers/jar, and the fresh weight of micro-tubers were also determined. 25 mM potassium nanoparticles (K-NPs) resulted in the highest number of tubers for both cultivars, while the concentrations (35 mM and 45 mM) decreased tuber formation. For phosphorus nanoparticles (P-NPS), 4 mM resulted in the highest number of tubers and root lengths for both cultivars.

Keywords:
microtuberization, nanoparticles, phosphorus, potassium, Solanum tuberosum

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Introduction

Potato (*Solanum tuberosum* L.) is an essential food due to its better proteins, significant levels of important vitamins, minerals, and trace components, extremely low-fat content, and even therapeutic capabilities [1]. To stop viral transmission from tubers to the next generation, seed potatoes must be produced in regions with low virus transmission rates. The cultivation of seed potatoes is challenging in nations without a harsh winter since there is an annual threat of illness.

Therefore, these nations import a substantial amount of their seed tubers from areas with more accurate climatic conditions for the cultivation of seed potatoes. According to the FAO [2], the production of potatoes in Egypt in 2019 was ~6.3 million tons. According to the Food and Agriculture Organization of the United Nations (FAO) database, Egypt imported 37 336 metric tons of potato seeds in 2019. The use of nanotechnology has become a viable solution for tackling various challenges by enhancing the efficiency of resource use and subsequent production while minimizing environmental damages, as mentioned by Kashyap *et al.* [3].

According to Merghany *et al.* [4]. Nanoparticles that are smaller than 100 nm can serve as fertilizers for efficient management of nutrients. They offer the benefits of gradual release and resistance to stress. Nano-fertilizers are environmentally conscious products produced through chemical, physical, or biological means by converting standard fertilizers,

and they are believed to be comparable with, or even superior to, traditional chemical fertilizers in nutrient content and application rates [5]. Applying nano-fertilizers in the agricultural sector may lead to sustainable development through lower inputs and waste generation, diminishing nutrient losses, and increasing nutrient use efficiency by releasing nutrients at a suitable rate for plant demand compared with conventional chemical fertilizers.

There is a difference between conventional fertilizers and nano-fertilizers depending on their mechanisms in the soil and plant ecosystem, application methods, effective rates of addition, and their impact on the environment [6]. However, pathogen-tested seed tubers are often costly and could take up as much as 50% of the entire cost of cultivation [7]. Considering they do not dry out as rapidly as plantlet cultures and may survive handling better than plantlets, micro-tubers are advantageous for transportation across large distances [8].

Micro-tubers may be handled the same way as regular seed tubers and do not require the time-consuming hardening in the greenhouse that micro-propagated plants do. Since fresh tuber yields from micro-tuber plants were 82% higher than those from normal tubers, these have the potential to be used for field planting [9]. Micro-propagation is the rapid reproduction of plant material to create a high number of progeny in tissue culture and *in vitro* clonal reproduction.

Morel [10] developed this method for orchid multiplication, and it is currently used for a variety of plants. It has been demonstrated to be a very efficient method for expediting the development of pathogen-free shoots of excellent quality, both in terms of genetic and physiological uniformity. Adequate amounts of phosphorus had a positive effect on increasing the number of stems/plant. Overall, increased levels of fertilizer had a positive effect on yield until a certain point, after which it had a negative effect. However, it was observed that excessive use of P fertilizers could lead to decreased tuber weight by speeding up the maturation process and decreasing their size [11]. Phosphorus plays a crucial role in nucleic acid and is essential for seed and fruit development as well as root expansion. Moreover, it contributes significantly to enhancing early-stage leaf production in plants [12].

Phosphorus has a vital role in aiding metabolic processes in plants and is crucial for the early growth and fast tuber development of potatoes. The nutrient plays a significant role in improving plant growth and is responsible for the transfer of energy needed for metabolic processes within the plant [13]. The potato crop has a high dependency on potassium from the soil, and the tubers take in a significant amount of nitrogen and phosphate, ranging from 1 to 5 times and 4 to 5 times, respectively. Due to their high need for potassium, potatoes are used as an indicator crop for its availability [14].

The presence of potassium in crops facilitated their ability to withstand environmental stress and enhanced their resistance to fungal disease and insect infection. Potash fertilizer also lessened the harmful effects of frost injury and enzymatic browning. Including potassium as part of a balanced fertilizer regimen for potato crops was crucial to increasing their yield and improving the quality of their tubers. Providing potassium strengthened the stems, prevented them from bending or breaking, and promoted the growth of larger tubers by facilitating greater water retention. Studies have shown that the application of potassium boosts the size and quantity of tubers, ultimately increasing the overall yield [15]. The current study's objective was to develop the ideal procedure for the *in vitro* creation of virus-free micro-tubers of two potato cultivars using varied doses of phosphorus and potassium nanoparticles (K-NPs) added to the Murashige and Skoog (MS) medium.

Materials and methods

Plant materials

This study was carried out at the Tissue Culture Laboratory, Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period from January 2020 to July 2021. Potato tuber seed (*Solanum tuberosum* L.) cultivars Lady Rosetta and Spunta were used. The source of tubers was the Potato Research Station, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Preparation and sterilization of explants

To end their dormancy, the potato tuber seeds were sprayed with 1 ppm gibberellic acid (GA3), after which they were left in the dark for 10–15 days until sprouting took place [16]. The sprouts were detached by dipping them in 20% commercial bleaching, Clorox (5.25 NaOCl), and 2 drops of Tween 20, which make up the sterilizer solution, and they were used as explants. Thereafter, the sprouts were subjected to shaking for 20 min using an electronic shaker. The explants were properly cleaned three times with sterilized distilled water (disinfectant and detergent).

With the use of forceps and a scalpel, the tip meristem (0.5 mm) was removed under an electronic binuclear to remove the leaf primordia surrounding it.

Establishment stage

Tip meristems were cut, and they were then aseptically cultured in 250 ml culture jars with 40 ml of solid MS [17] strength basal medium with 3% sucrose and 0.8% agar. These treatments were all carried out under the hood with laminar airflow. The medium's pH was raised to 5.6–5.8, and they were autoclaved at 121°C for 20 minutes at 1.5 kg/cm^2 pressure. Tip meristems were cultured for 30 days at 24±2°C. The contamination and survival percentages were recorded weekly. The total number of excised tip meristems was 62 in Lady Rosetta cv. and 75 in Spunta cv. The establishment stage was extended from March to September 2020.

Multiplication stage

All cultivars were re-propagated with two node cuttings after 3–4 weeks using the regular micro-propagation methods. The nodal segments were separated (with 1–2 leaves), and each jar contained three explants. The nodal segments were cultured on MS-strength basal medium with 30 g (3%) sucrose and 0.8% agar. The excised jars were incubated at 24±2°C under 16 h light with a light intensity of 2000 lux for 45 days.

Synthesis of potassium and phosphorus nanoparticles

Regarding potassium and phosphorus nanoparticles, their nanoparticles were prepared and synthesized from their precursors. Phosphorus was prepared from calcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) and phosphoric acid (H_3PO_4), while potassium was prepared from potassium persulfate ($K_2S_2O_8$) and potassium chloride (KCl). All used precursors and reagents (chemicals for breaking bonds) of high purity (99.95%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphorus and K-NPs were prepared from the top-to-bottom molecular chemical approach method under high pressure according to [18,19] with some modifications by polymerizing methacrylic acid in chitosan solution as a carrier coated in buffer solution mixed with 2-ethoxy ethanol and toluene (1 : 1, v/v) for 18 h at room temperature in two-step processes. In the first step, 0.23 g of chitosan was dissolved in a methacrylic acid aqueous solution (0.5% v/v) and deionized water for 7 h under magnetic stirring at 38°C. In the second step, with continued stirring at 40°C, 0.5 mmol of $K_2S_2O_8$ and KCl (for K nanoparticles) and 0.8 mmol of $Ca(H_2PO_4)_2 \cdot H_2O$ and H_3PO_4 (for P nanoparticles) were added to the solution. 15 ml of deionized water was added until the solution became clear. Next, acetic acid, ethylene glycol, and silicic tetraethyl ester hydrolyzed (1 : 1 : 1) were slowly added under vigorous stirring, and the solution was left for 24 h. The polymerization was subsequently carried out at 75°C under magnetic stirring for 5 h, which led to the formation of a nanoparticle solution that was subsequently exposed to 1.5 psi of pressure for 6 days, discontinuous for 6 h/day, then centrifuged at 500 rpm for 45 min. After that, it was exposed to about 200°C for one day and thereafter cooled in an ice bath for 2 h. The particles were uncontrolled in shape, with a size range of (3.65–8.13 nm) for phosphorus and a size range of (2.00–9.38 nm) for potassium. The morphology and size of the nanoparticles were investigated using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Japan). One drop of the nanoparticle solution was spread onto a carbon-coated copper grid and was subsequently dried at room temperature for transmission electron microscopy (TEM) analysis. The sizes of the nanoparticles were determined directly from the figure using Image-Pro Plus 4.5 software (Fig. 1). The value is the average size of three parallels.

Microtuberization stage

During the microtuberization stage, investigate the effect of MS medium supplemented with 80 g/L sucrose and 8 g/L agar, and different concentrations of (25, 35, and 45 mM) K-NPs or (2, 3, and 4 mM) phosphorus nanoparticles (P-NPs) were examined. Nodal cuttings from the Lady Rosetta and Spunta cvs were used as explants for micro-tuber formation. The source of the nodal cuttings was from the second subculture at the multiplication stage (the best vessel jars). Five weeks of *in-vitro* micro-propagated plantlets were cultured in 250 ml glass jars each with a 40 ml micro-tuber formation medium. Forty days after culture on treatments, the number of branches/explant, plant length (cm), and number of leaflets per plantlet were recorded. Each treatment had 10 jars containing 40 ml of medium. The jars were kept in complete darkness.

Statistical analysis

Regular analyses of variance of a completely randomized design (CRD) were performed on all *in-vitro* data. Thereafter, LSD 0.05 was calculated to compare between means [20].

Results and discussions

Data in Figs 2 and 3 exhibits the effect of different concentrations of K-NPs or P-NPs on plant height and

Figure 1

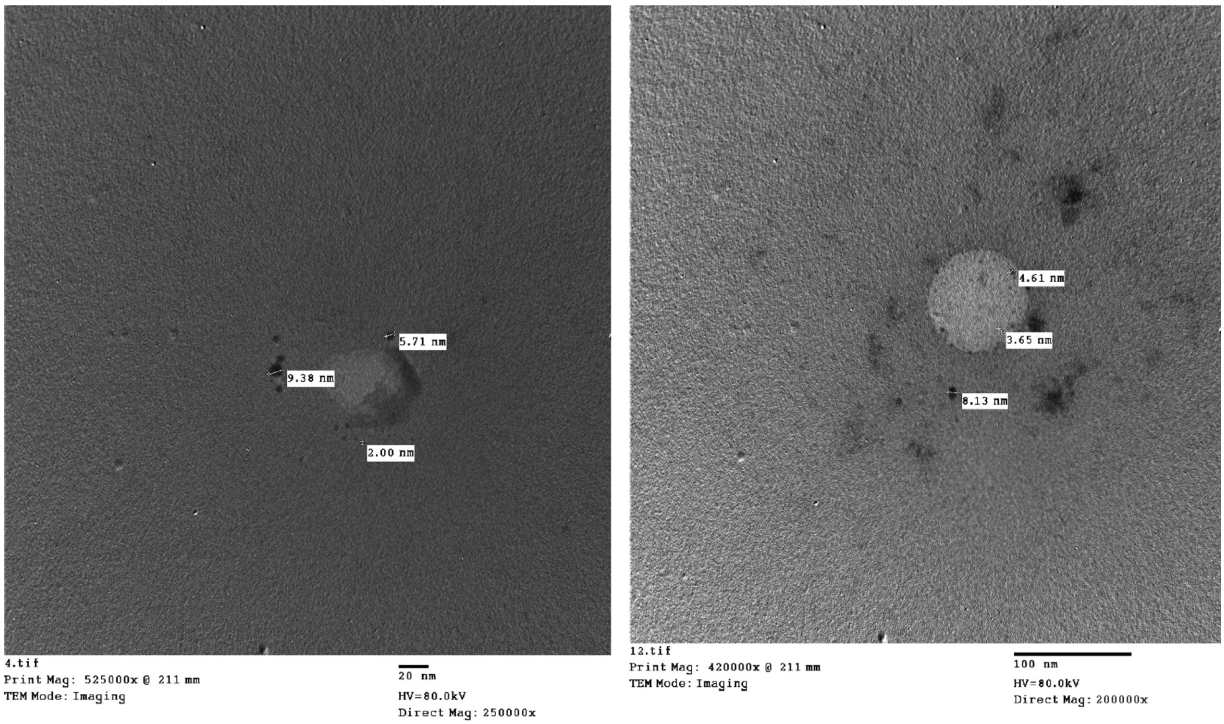
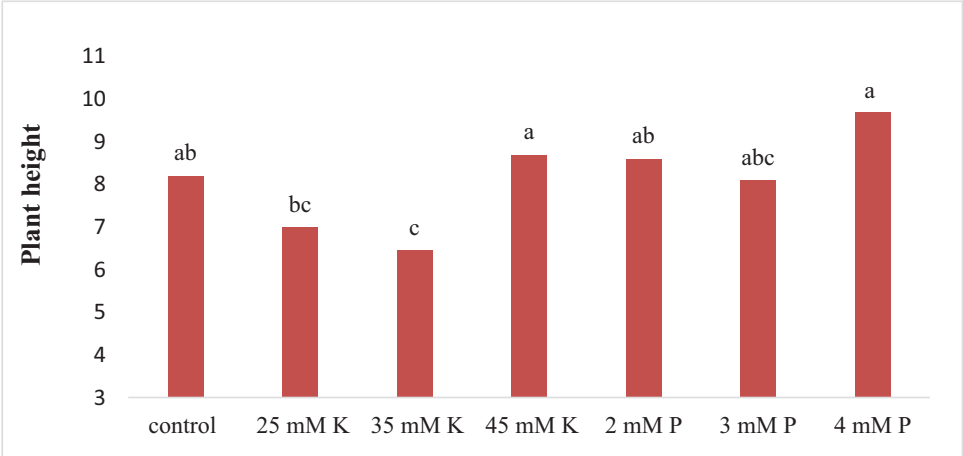


Image of potassium and phosphorus nano-particles.

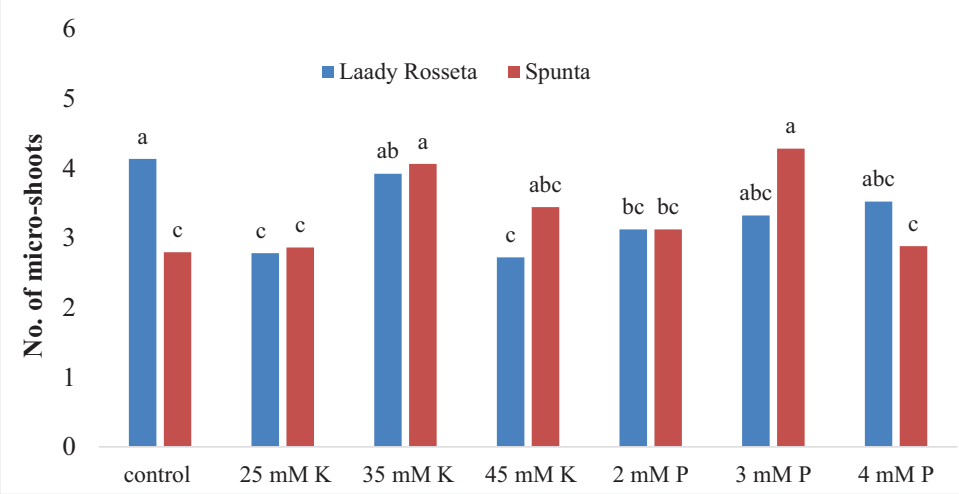
number of micro-shoots of Lady Rosetta and Spunta cvs through *in-vitro* meristem tip culture after 40 days of culturing. In terms of plant height, the control group had an average height of 8.2 cm. After treatment with 25 mM of K-NPs, the plant height decreased to 7 cm. Similarly, the plant height decreased to 6.45 cm and increased slightly to 8.7 cm when treated with 35 mM and 45 mM of K-NPs, respectively. On the other hand, treatment with 2 mM of P-NPs resulted in an average plant height of 8.6 cm. This value decreased to 8.1 cm and 9.7 cm when treated with 3 mM and 4 mM of P-NPs, respectively, with no significant difference among treatments compared with control. The highest number of micro-shoots was formed by using 35 mM K-NPs, with a significant difference compared with the control. However, treatment with P-NPs at 3 mM resulted in an increased number of branches (3.8 branches), while treatment with 4 mM of P-NPs resulted in a slightly decreased number of branches (3.2 branches). However, these results provide valuable insights into the effect of K-NPs and P-NPs on the growth and development of Lady

Figure 2



Effect of different concentrations of potassium or phosphorus nanoparticles on plant height (cm) of potato via *in vitro* meristem tip culture.

Figure 3



Effect of different concentrations of potassium or phosphorus nanoparticles on the number of micro-shoots of two potato lady Rosseta and Spunta cvs via *in vitro* meristem tip culture.

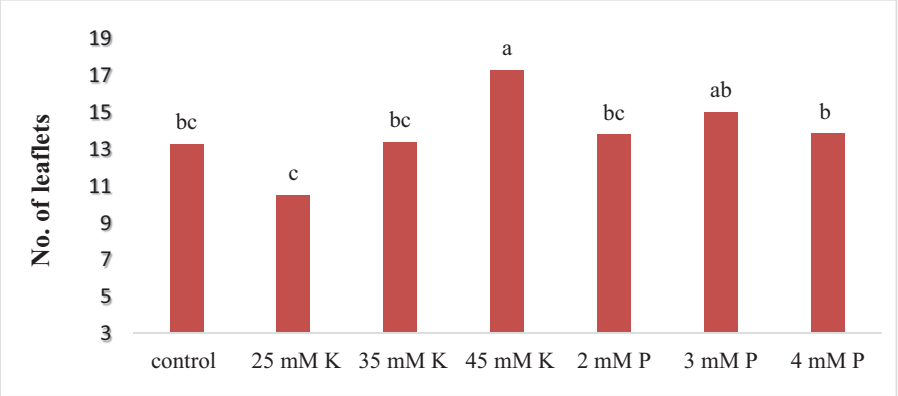
Rosetta and Spunta potato cultivars. The comparison among the cultivars under study showed that 'Spunta' recorded significantly the highest value of plant height as compared with Lady Rosetta. These findings agree with [21]. The effects of NPK nanoparticles on the *in-vitro* micropropagation of two potato varieties were evaluated. Nodal explants of potato cultivars (Spunta and Seylon) were cultured on MS basal medium supplemented with different NPK (9: 0: 6+1 silver) nanoparticles (control, 10 ml, 20 ml, 30 ml, 40 ml, and 50 ml). 30 ml exhibited the highest number of nodes, followed by 10 ml, 20 ml, and 40 ml in Seylon cv. On the other hand, the 30 ml exhibited the highest number of nodes, followed by 10 ml and 20 ml in Spunta cv. In this study, results showed that increasing the dose of both P and K-NPs increased the value of the number of branches. Also, Sarker *et al.* [22] found that the genotypes differed in their responses and that each genotype had different growth parameters. Regrinding the applied K-NPs and P-NPs concentrations, the application of P-NPs at 4 mM recorded the tallest micro-plants, but with no significant difference with the application of P-NPs at 2, 3, and 4 mM. On the other hand, the application of 35 mM K-NPs recorded significantly the lowest value of plant height. The value of plant height increased with the increment of P-NPs concentration up to 4 mM P-NPs. That result agrees with Fantaw *et al.* [23] study of the effects of nine combinations of nitrogen, phosphorous, and sulfur fertilizers. The number of stems per plant and the height of the plants did rise, though, and this may have a favorable impact on the photosynthetic area. Habtam *et al.* [24] investigated the effects of various concentrations of potassium (0, 100, 200, and 300 kg K_2O ha^{-1}) and phosphorus (0, 46, 92, 138, 184, and 230 kg P_2O_5 ha^{-1}), and they found that their main effects are significant influences on plant height, marketable tuber yields, and total tuber yields. As for the interaction between laboratories and plant varieties, the best value of the plant length was in the treatment of 4 mM in the spunta variety, while the level of 25 mM of nano-potassium was the lowest value in the plant length. There are no significant differences between the coefficients of nano-potassium and control, and there is also no significant difference between 2 and 3 mM P-NPs. Concerning to cultivars, there is no significant difference in the number of branches per plantlet between spunta and Lady Rosseta; this effect may be attributable to the genotype's performance. Maximum values were observed in both 35 mM K-NPs and 3 mM P-NPs, followed by control and 4 mM P-NPs. The previous results agree with [25], who confirmed the previous results because their results showed that increasing K in the M.S medium increased the number of shoots, which is a positive indicator of the formation of micro-tubers. They also found that too much K can prevent N from being taken in for the creation of plant organs and cell division, so raising the concentration of KNO_3 can only result in a small number of shoots. For instance, disrupting the N nutrients' absorption can be harmful to plants. N nutrients are necessary for the production of amino acids, cell division, and the creation of cells, tissues, and organs in plants Table 1.

Based on Figs. 4 and 5, it can be observed that both K-NPs and P-NPs have an effect on the number of leaflets and fresh weight of shoots of Lady Rosetta

Table 1 Mean squares (analyses of variance)

d.f	Plant height	No. of micro-shoots	No. of leaflets	F. W. of shoots	Root length
Treatment (T)	11.854*	1.75*	41.581*	0.103**	35.084**
Cultivars (C)	4.88	0.002	0.229	0.000	2.197
T×C	1.473	1.52*	6.695	0.005	6.998*

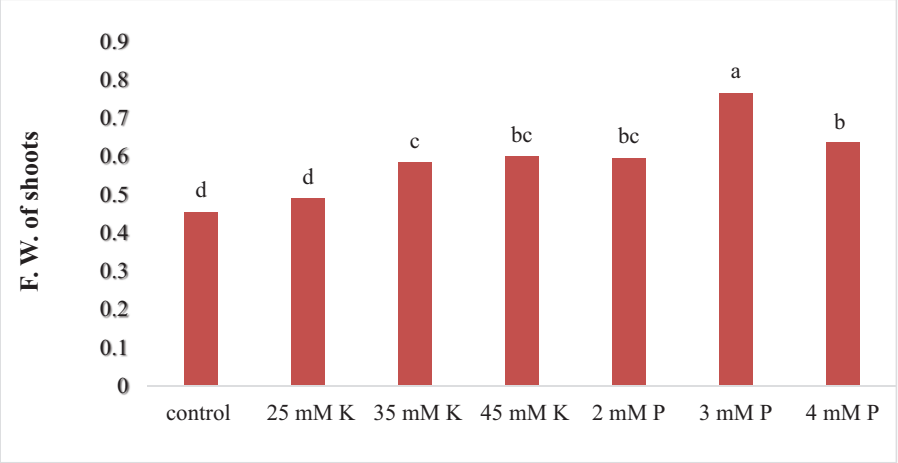
Figure 4



Effect of different concentrations of potassium or phosphorus nanoparticles on the number of leaflets of potato via *in vitro* meristem tip culture.

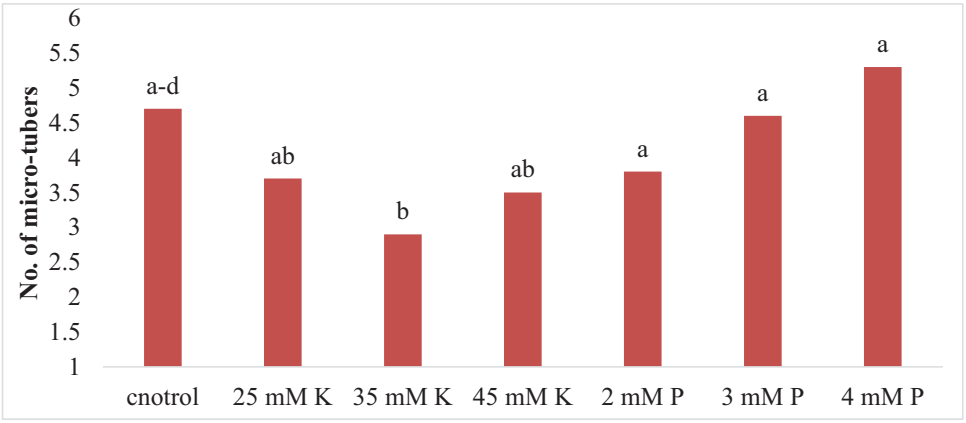
and Spunta potato. From Figs. 3 and 4, it can be observed that both K-NPs and P-NPs have an effect on the number of leaflets and fresh weight of shoots of Lady Rosetta and Spunta potato cvs after 40 days using *in vitro* meristem tip culture. For K-NPs, a concentration of 45 mM resulted in the highest number of leaflets and fresh weight of shoots for both cultivars, while a lower concentration of 25 mM resulted in a decrease in both parameters. For P-NPs, the highest concentration of 3 mM resulted in the highest number of leaflets and fresh weight of shoots for both cultivars, while the lowest concentration of 2 mM resulted in a slight decrease in both parameters. Overall, it can be concluded that the optimal concentration of K-NPs and P-NPs for the number of leaflets and fresh weight of shoots (growth parameters) of Lady Rosetta and Spunta potato cultivars is 45 mM and 3 mM, respectively. These findings are in line with previous studies that have identified the importance of potassium and phosphorus in plant growth and development. The comparison between the cultivars under study showed that 'Spunta'

Figure 5



Effect of different concentrations of potassium or phosphorus nanoparticles on the fresh weight of shoots of potato via *in vitro* meristem tip culture.

Figure 6

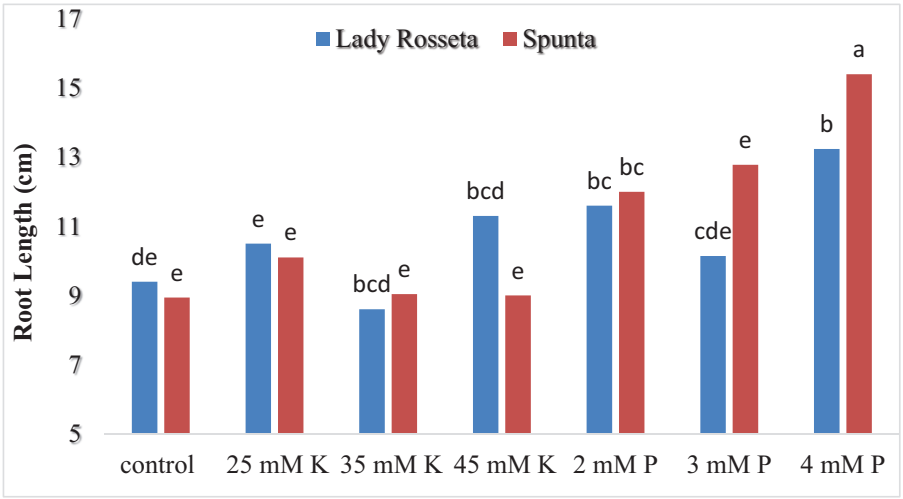


Effect of different concentrations of potassium or phosphorus nanoparticles on the number of micro-tuber of potato via *in-vitro* meristem tip culture

recorded significantly the highest number of leaflets as compared with Lady Rosetta. These findings agree with [21] who reported that raising doses of potassium and phosphorus for potatoes leads to an increase in both the number of leaflets and the fresh weight of shoots per plant. This result agrees with [26], who found that increasing concentrations of K led to an increase in the number of leaflets. [27] studied the effect of different concentrations of potassium K (0, 1.88, 2.5, 5, 10, 20, and 40 mM) and phosphorus P (0, 0.30, 0.60, 1.25, 2.5, 5, and 10 mM) on *Nidularium minutum* via modified media *in vitro*. They find out that increasing K increases the number of leaflets, but they mention that the level of 40 mM causes a toxic effect and decreases the number of leaflets. P increased the number of leaflets, but there was no significant difference among treatments. They found that the fresh weight of plants grown in media containing 0.60–10 mM showed no changes in the analysis of shoot mass, and increasing the concentration of K led to an increase in the fresh weight of shoots. This result disagrees with Abd El Wadod *et al.* [28], who studied the effect of different concentrations of KNO_3 on potatoes *in vitro*. They reported that increasing KNO_3 decreased shoots' fresh and dry weights.

Figures 6 and 7 show that the different concentrations of K-NPs and P-NPs also have an affect on the root length after 40 days and the number of tubers after 100 days of initiation treatments *in vitro* under full darkness of Lady Rosetta and Spunta potato cvs. For K-NPs, a concentration of 25 mM resulted in the highest

Figure 7



Effect of different concentrations of potassium or phosphorus nanoparticles on the root length of two potato lady Rosseta and Spunta cvs via *in vitro* meristem tip culture.

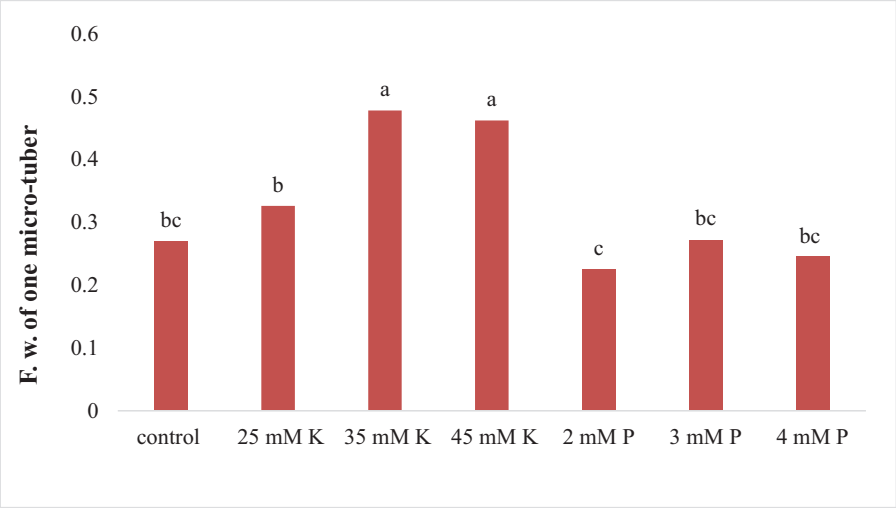
Table 2 MeanOVA

d.f	No. of micro-tubers	F. W. of one micro-Tuber	F. W. of micro-tubers	F. W. of large micro-Tuber	D. of large micro-tuber
Treatment (T)	5.148*	0.107**	0.267*	3.859**	0.054**
Cultivars (C)	0.000	0.053	0.720	13.394	0.148
T×C	2.300	0.008	0.059	1.505**	0.011**

number of tubers, while 35 mM and 45 mM resulted in a decrease in tuber formation. Meanwhile, the root length was not significantly affected by different concentrations of K-NPs. For P-NPs, a concentration of 4 mM resulted in the highest number of tubers and root length for both cvs, while 2 mM and 3 mM resulted in a decrease in both parameters. It can be observed that the optimal concentrations of K-NPs and P-NPs for shoot growth and leaflet formation (45 mM and 3 mM, respectively) were not the same as the optimal concentrations for tuber formation (25 mM and 4 mM, respectively). The results agree with [22,29]; they found that Kufri Sindhuri and Diamant cvs micro-tuber numbers decreased when potassium content increased. Zakaria [30] found that more micro-tuber/jar emerged at lower concentrations of both N and K. Islam *et al.* [31] show that adding N and K had a significant impact on these factors when they were added in various amounts in the medium of cultivation. The correlation to NxK levels is also not significant. Iranbakhsh *et al.* [32] suggested that potassium has a positive effect on the formation of cells, cell division, and growth of seeds in doses up to 1.5 times that of the normal range. However, when potassium levels increased, the number of microtubers decreased. Nistor *et al.* [33] showed that when used in the Christian variety, the micro-tuber number reduced as the potassium level rose. De Andrade and Tamaki [27] studied the effect of different concentrations of potassium K (0, 1.88, 2.5, 5, 10, 20, and 40 mM) and phosphorus P (0, 0.30, 0.60, 1.25, 2.5, 5, and 10 mM) on *Nidularium minutum* Mez via modified media *in vitro*. Root length increased with increasing concentrations of potassium and phosphorus. This result agrees with Ashraf *et al.* [21] who reported that increasing doses of K and P increase root length Table 2.

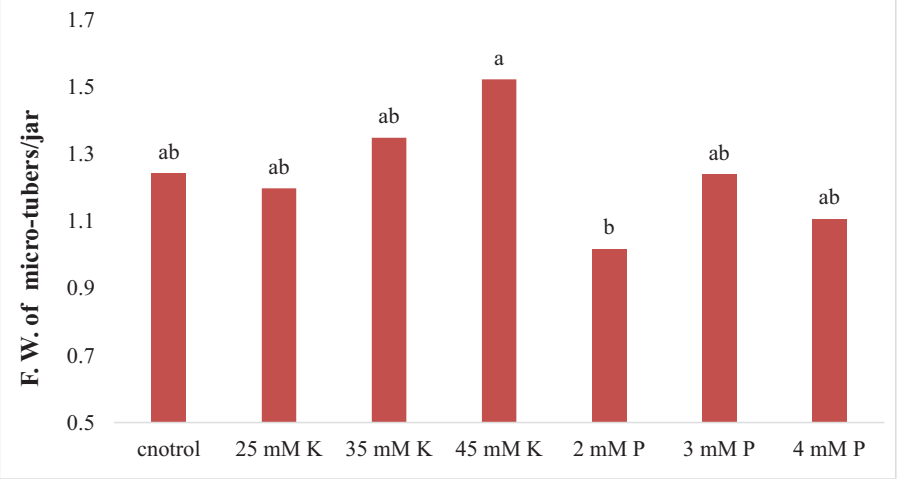
Figures 8 and 9 show the effect of different concentrations of K-NPs or P-NPs on the fresh weight of micro-tubers per jar and the average fresh weight of two potato cvs, 'Lady Rosetta' and 'Spunta', after 100 days of initiation treatments *in vitro* under fully dark conditions. The control group, which received no treatment (0 mM), had a fresh weight of 0.27 g for the mean of both potato cultivars. Comparatively, the introduction of K-NPs at different concentrations resulted in increased fresh weight. The highest concentration of K-NPs at 35 mM produced the highest fresh weight of micro-tubers, with a mean value of 0.478 compared with the

Figure 8



Effect of different concentrations of potassium or phosphorus nanoparticles on the fresh weight of one micro-tuber (g) of potato via *in vitro* meristem tip culture.

Figure 9



Effect of different concentrations of potassium or phosphorus nanoparticles on the fresh weight of micro-tuber/jar (g) of two potato cultivars via *in-vitro* meristem tip culture.

control. Similarly, the use of P-NPs also showed an increase in fresh weight, with the highest concentration of 3 mM resulting in a mean fresh weight of 0.276 g and no significant difference between comparisons. The average weight of two cultivars was increased by increasing the concentration of K-NPs, but increasing the concentration of P-NPs reduced the weight of micro-tuber in each cultivar. The highest value was in treatments at 45 mM and then 34 mM compared with control. This previous result is similar to [33], who examined how three potassium concentrations (10, 25, and 40 mM/L) affected *in vitro* tuberization. The size emerged, but the total number of micro-tubers decreased when the potassium supply was raised. Sarker *et al.*[22] Reported that at a potassium concentration of 40 mM, the weight of micro-tubers in the Kufri Ashoka cultivar massively increased. The amount of potassium in potatoes is significantly larger than that of other elements like Ca, which is known to exist in the form of K+ and move at an accelerated rate [34]. The lack of degree of tuberization may be caused by the detrimental impact of high nitrogen and low potassium levels on *in vitro* tuber initiation; this could be considered a conclusion. The vital elements needed for the generation and growth of potato micro-tubers by cytokinin-induced *in vitro* tuberization are inorganic nitrogen and potassium. 40 mM K-NPs was the maximum dose of potassium that gave a high weight of micro-tuber in the previous treatments, and the weight of micro-tuber decreased in treatment 45 mM. This is in agreement with those of [22], who reported that 60 mM nitrogen mixed with 40 mM potassium resulted in the highest standard micro-tuber weight.

As shown in Table 3, different concentrations of K-NPs and P-NPs were tested on Lady Rosetta and Spunta cultivars after 100 days of initiation treatments *in vitro* under full darkness. The control group showed a mean fresh weight of 0.319 g for Lady Rosetta and 0.278 g for Spunta, and a mean diameter of

Table 3 Effect of different concentrations of potassium or phosphorus nanoparticles on the fresh weight of large micro-tuber and Diameter of large micro-tuber of two potato Lady Rosetta and Spunta cvs via *in vitro* meristem tip culture

		F. W. of large micro-tubers			Diameter of large micro-tuber		
Treatment	mM	Leady R	Spunta	Mean	Leady R	Spunta	Mean
Control	0	0.36f	0.278 h	0.32 e	7.54 def	7.8 bc	7.67 c
K-NPs	25	0.46 c	0.326 fg	0.39 c	8.28 bc	8.28 bc	7.88 bc
	35	0.51 b	0.416 d	0.47 b	8.61 ab	7.15 fg	8.28 ab
	45	0.61 a	0.414 d	0.51 a	8.99 a	7.82 cde	8.40 a
P-NPs	2	0.33 f	0.356 f	0.34 de	7.24 e-i	6.63 gh	6.93 d
	3	0.41de	0.334 f	0.37 cd	7.57 def	6.23 h	6.90 d
	4	0.36 ef	0.284 gh	0.32 e	8.06 bcd	6.26 h	7.16 d
	Mean	0.436**	0.344	0.39	8.041**	7.167	7.60

small micro-tubers of 7.671 mm for Lady Rosetta and 7.8 mm for Spunta. Increasing the concentration of K-NPs to 25 mM resulted in an improved fresh weight of large micro-tubers, while the mean diameter of small micro-tubers remained the same. Further increases in the concentration of K-NPs to 35 mM and 45 mM showed higher fresh weights and mean diameters of small micro-tubers for both cultivars, with a significant difference compared with the control. The concentration of 2 mM of P-NPs resulted in lower fresh weights and mean diameters of small micro-tubers for both cultivars, while increasing the concentration to 3 mM and 4 mM showed improvements with a significant difference compared with the control. The fresh weight of large micro-tubers remained higher for Spunta compared with Lady Rosetta, while the mean diameter of small micro-tubers ranged from 6.9 mm to 7.162 mm for Lady Rosetta and from 6.23 mm to 7.162 mm for Spunta.

Conclusion

In conclusion, the application of K-NPs and P-NPs at various concentrations had a positive effect on the growth and development of Lady Rosetta and Spunta potato cultivars through *in-vitro* meristem tip culture. Different concentrations of K-NPs and P-NPs led to varying degrees of improvement in shoot growth, leaflet formation, tuber formation, and root length. The optimal concentrations of K-NPs and P-NPs for different growth parameters were not the same, indicating that different nutrients may play different roles in various aspects of plant growth and development. Overall, these findings provide important insights for optimizing potato production through the use of nutrients and *in-vitro* meristem tip culture techniques. Increasing the phosphorous element in the MS environment led to an increase in plant height, number of leaflets, fresh weight of shoots, root length and number of micro-tubers. Increasing the potassium element led to a significant increase in the number of branches, number of leaflets, fresh weight of tubers micro-tubers, the fresh weight of one tuber and the diameter of each micro-tuber. The best treatments were 35 mM K-NPs and 3 mM P-NPs.

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Conflicts of interest

There are no conflicts of interest.

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