

IMPROVING PRODUCTION AND CHARACTERS OF POTATO MICROTUBERS AND MINITUBERS

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ABSTRACT: *This investigation was carried out during the seasons 2012 and 2013 on potato cv. Diamant at the Tissue Culture Laboratory and the greenhouse of Potato and Vegetatively Propagated Vegetables Department, Horticulture Research Institute, Dokki, Giza, Egypt. The present study aimed to study the effect of the in vitro application of chitosan, calcium chloride and the concentration of ammonium nitrate and potassium nitrate on potato tuberization. In addition, to study the effect of foliar application of chitosan, chelated calcium and potassium silicate on growth and yield of the minituber ex vitro.*

Results revolved that 500 mg/l chitosan gave the highest values of number of shoots and node/plantlets, number of microtuber, fresh and dry weight of shoots and microtubers fresh weight after 8 weeks from culture in vitro. Furthermore, ex vitro spraying with 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicate produced the highest values of plant length, leaves number/plant, fresh and dry weight of leaves on 75 days after planting. As well as, minituber characteristics (number of minituber /plant, minituber fresh and dry weight/plant, average minituber weight and weight of minituber /plant according to its size) after 100 days from planting recorded the highest significant values with foliar application of 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicate.

It could be recommended that adding chitosan at 500 mg/l to in vitro microtuber production medium (MS + 8 mg/l BA + 80 g/l sucrose) was preferable to produce microtubers. Also, the ex vitro spraying of 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium plus 400 mg/l potassium silicate could be useful for minituber production and enhancing its yield characteristics.

Key words: *Potato, Chitosan, nitrate potassium, Potassium Silicate, Chelated calcium, microtuber and minituber*

INTRODUCTION

Potato is one of the main important vegetable crops in Egypt. It occupies the first rank in export among the different vegetable crops in 2016. The cultivated areas reached to 437386 fed. Its total productivity was 4955445 ton. Also, Egypt exported 632090 tons to the different markets especially England, West European and some Arab countries (Ministry of Agricultural, 2016).

Half a century has passed since *in vitro* tubers (microtubers) were first described in potato, but their adoption as a seed propagule has been uneven globally consensus in lacking regarding optimal production practices for microtubers and

their relative productivity in relation to the propagules for minituber production (Donnelly *et al.*, 2003). Although, many reports of the *in vitro* production of microtubers little is known about growth and yield from microtubers planted in the field (Kawakami *et al.*, 2003).

Kowalski *et al.*, (2006) added chitosan at 0.0001, 0.001, 0.01, 0.1 and 1 g/l to potato micropropagation media. Chitosan at 1 g/l showed improvement of microtuber quality, plant growth *in vitro*, helped in the acclimatization *ex vitro* and finally increased the yield of minituber. Moreover, Zakaria *et al.*, (2009) investigated the effect of chitosan at 5, 15, 50, 150, 500, 750 and 1000 mg/l on potato plantlets and found that chitosan at

500 mg/l significantly increased shoots and root fresh weight, improved acclimatization of plantlets in green house and finally increased minituber yield.

Calcium plays an important role in tuberization *in vitro*. The reducing of calcium uptake resulted in the inhibition of tuberization (Balamani *et al.*, 1986). On the other hand, Ozgen and Palta (2004) mentioned that the application of calcium decreased tuber number but increased tuber calcium content with no significant effect on tuber yield. Also, Arvin *et al.*, (2005) found that the calcium concentration at 3 mM was high enough for optimal tuberization and increased microtuber fresh weight of potato. Increasing calcium level in Murashige and Skoog medium from 3 to 5 or 15 mM improved growth of *in vitro* potato plantlets and the 15 mM concentration was the best (Habib *et al.*, 2005).

Potato plantlets growth was less with either NH_4^+ or NO_3^- as a sole nitrogen source (Avila *et al.*, 1994). Both growth and morphogenesis in tissue culture are considerably influenced by the availability and form of nitrogen (George *et al.*, 2008). Moreover Villamor (2010) indicated that nitrogen in the form of KNO_3 significantly improved the proliferation rate of ginger *in vitro*. Leaf growth and root formation was better in media devoid of NH_4NO_3 . In addition Motallebi-Azar *et al.*, (2011) reported that potato plantlets on media supplemented with 2400 mg/l of NH_4NO_3 gave the highest number of lateral shoot than the 800 and 1900 mg/l. Andreea *et al.*, (2011) studied the effect of three levels of total nitrogen (30, 45 and 60 meq/l) on tuberization. Reducing the total nitrogen supply increased number of microtubers but decreased its size. The average weight of microtuber was high with the highest concentration of nitrogen 60 meq/l.

The silicon (Si) is effective on nutrients balance, increased tubers and increased absorption of nitrogen and phosphorus in plants (Khan *et al.*, 1994). After silicon

absorption, the element transportation in plants is done through vessel element and transpiration flow from roots to shoots (Ma and Takahashi, 2002). Also, Silicon plays role in growth improvement, photosynthesis increase, efficiency of transpiration and evaporation, increasing the strength of leaves, chlorophyll concentration per leaf area and product quality (Hwang *et al.*, 2005). Best effect of silicon treatment was recorded with 5 mM silicate acid on the growth of potato. The ratio of bound water to free water was increased, the chlorophyll content was enhanced, the respiration rate was decreased significantly, the cell wall extraction rate was increased significantly, the cellulose content was increased by the silicon treatment (Fang and Long 2006). The tomato plants treated with 50 or 100 mg/l Si produced heavier weights and larger fruit size (Darshani and Dilshan 2015).

The aim of this work was to investigate the effect of the *in vitro* application chitosan, calcium chloride and the ratio of ammonium nitrate (NH_4NO_3) to potassium nitrate (KNO_3) on potato microtuberization. Besides studying the effect of foliar application of chitosan, chelated calcium and potassium silicate on the production of minituber *ex vitro*.

MATERIAL AND METHODS

Two experiments were conducted during the two successive seasons 2012 and 2013 at the Dept. of Potato and Vegetatively Propagated Vegetables, Horticulture research Institute, Dokki, Giza, Egypt. Potato (*Solanum tuberosum* L.) Diamant cultivar was used. Clean sprouts from tubers were surface sterilized in 10% v/v Clorox solution (5.75% of sodium hypochlorite) plus two drops of tween-20, then rinsed three times with sterile distilled water. Shoot tip explants were used as a source of explants. Shoot tip explants established on Murashige and Skoog (1962) (MS) medium +3% sucrose +0.01 mg/l NAA + 0.25 mg/l GA3 + 2.0 mg/l calcium panthothenic acid after one month the obtained plantlets were used as a

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source of explants for the first experiment (microtuberization). Single node explants (cuttings) were transferred onto jars containing 40 ml of microtuberization media (Table 1) i.e., MS medium +8 mg/l BA+80 g/l Sucrose (control) Wang and Hu (1982) (T1), the same medium with chitosan (T2, T3, T4), modified CaCl_2 concentrations (T5, T6, T7, T8) or modified potassium nitrate (KNO_3) to ammonium nitrate (NH_4NO_3) ratio (T9, T10, T11). Each treatment was replicated five times with five jars per replicate. Each jar contained 3 nodal cutting. Jars were distributed randomly in incubation room for month. The statistical design was complete randomize design.

Data on number of shoots/plantlet, node number/plantlet, plantlet height, number of microtubers/ plantlet, shoot fresh weight, shoot dry weight, root fresh weight, microtuber weight/ plantlet, microtuber dry weight were recorded after 8 weeks from culture.

The *in vitro* produced microtubers were cleaned with tape water, then immersed for 10 minutes in 1g/l Rizolex after that microtubers were dried for one night then in incubated at 25°C for 4 weeks to be used in the minituber production experiment.

The second experiment was conducted

in the greenhouse using the microtubers produced in the first experiment. Planting dates were September 17th and 22th in 2012 and 2013 seasons, respectively. Microtubers were transferred into pots (40 cm diameter) filed with mixture of peat moss and sand soil (2:1 v/v). Three microtubers were cultured in each pot. Each treatment contained 4 pots per replicate. After full germination the plants were sprayed twice at 45 and 60 days after planting by foliar treatments i.e., Control (water), chitosan, chelated calcium, potassium silicate or mixture of chelated calcium and potassium silicate (Table 2).

The statistical design was complete randomize design with three replicates. The samples were taken at the plant age of 75 days after planting. Data on plant length (cm), leaves number/plant, leaves fresh and dry weight (g.), leaf area (cm^2), stem fresh and dry weight (g.). Minitubers were harvested after 100 days from planting. At harvest time the minituber characteristics (number of minituber/plant, minituber fresh weight/plant (g), minituber average weight (g), minituber weight of three sizes ($\leq 1.49\text{cm}$, $1.5 - 2.99\text{cm}$ and $\geq 3\text{cm}$) and the minituber dry weight/plant (g) were recorded.

Table 1. Additives and modifications to microtuberization media (MS+8 mg/l BA+80 g/l Sucrose).

Treatments	Treatments
T1	Control
T2	500mg/l chitosan
T3	700mg/l chitosan
T4	900mg/l chitosan
T5	5mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
T6	10mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
T7	15 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
T8	20 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
T9	50 mM KNO_3 : 10 mM NH_4NO_3
T10	55 mM KNO_3 : 5 mM NH_4NO_3
T11	60 mM KNO_3 : 0 mM NH_4NO_3

Table (2): Foliar application treatments for enhance minitubers production.

Treatment	Treatment
T 1	Control (water)
T 2	Chitosan 500 mg/l
T 3	Chitosan 700 mg/l
T 4	Chitosan 900 mg/l
T 5	Chelated Ca 350 mg/l
T 6	Chelated Ca 500 mg/l
T 7	K ₂ SiO ₃ 200 mg/l
T 8	K ₂ SiO ₃ 400 mg/l
T 9	Chelated Ca 350 mg/l + K ₂ SiO ₃ 400 mg/l

The obtained data of both seasons were subjected to the analysis of variance method and the means of the treatments were compared by using the least significant difference (L.S.D) at the level of 0.05 probability according to Snedecor and Cochran (1981).

RESULTS AND DISCUSSIONS

First experiment:

Microtuberization (*in vitro*)

Data in Table 3 showed the tallest plantlets were recorded with the application of NO₃:NH₄ =55:5mM followed by the treatments 5mM CaCl₂.2H₂O and 10mM CaCl₂.2H₂O without significant difference between the application of NO₃:NH₄ =55:5mM.

It was clear from results that chitosan at 500 mg/l gave the highest number of shoots/plantlet (6.4) followed by chitosan at 700 mg/l (5.8) and the application of NO₃:NH₄ =55:5mM (5.0) compared with control treatment (4.4). Due to calcium chloride concentration, it was obvious that number of shoot/plantlet was significantly decreased with increasing calcium concentrations (3.2, 2.6, 2.2. and 1.6). The nitrogen treatments (NO₃:NH₄ =55:5mM and 50 mM KNO₃: 10 mM NH₄NO₃) followed chitosan treatments were preferable after chitosan treatments for number of node. Moreover, this result was parallel with Avila

et al., (1994) who found that potato plantlets growth were less with NH₄ or NO₃ as a sole source of nitrogen and Villamor (2010) who indicated that nitrogen in the form of KNO₃ significantly improved proliferated rate of ginger *in vitro*. Also, Avila *et al.*, (1994) found that decreasing proportion of NH₄ from 50% to 30 % increased shoot growth. The application of NO₃:NH₄ =55:5mM was the best treatment (5.0). There were no significant effect between all the three chitosan treatments and the treatments of nitrogen of 50 mM KNO₃: 10 mM NH₄NO₃ and NO₃:NH₄ = 50:10 or 55:5mM followed by the control.

Number of microtubers/planlet and microtubers fresh weight took the same trend which were significantly high with chitosan at 500 or 700 mg/l followed by nitrogen treatments the application of NO₃:NH₄ =55:5mM and 50 mM KNO₃: 10 mM NH₄NO₃. In addition, calcium chloride treatments always produced the lowest number and lightest weight of microtuber. These results parallel with Ozgen and Palta (2004). They found that Ca application decreased tuber number. These results disagree with Habib *et al.*, (2005) who found that 15 Mm of Ca application was best for potato plantlets. Moreover, chitosan at 500 mg/l produced the heaviest fresh and dry weight followed with significant effect by the application of 50 mM KNO₃: 10 mM NH₄NO₃ or NO₃:NH₄ =55:5mM.

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Table 3

Finally, chitosan at 500 mg/l showed the best results of number of shoot, node, microtuber/planlets, microtuber fresh weight (mg), and shoot fresh or dry weight. This results matched with Zakaria *et al.*, (2009) who reported that 500 mg/l significantly increased shoot number followed by nitrogen treatments at $\text{NO}_3:\text{NH}_4 = 50:10$ or $55:5\text{mM}$, respectively. increasing calcium concentration caused dramatic decline in characteristic.

Second experiment: Minituber production (greenhouse experiment).

Data in Table (4) indicate that, severe changes in plant growth, manifested by vegetative parameters were recorded at 75 days after planting as affected by the foliar application of chitosan, chelated calcium, calcium silicate and the mixture between chelated calcium and potassium silicate. In this respect, all the presented results of plant growth characters significantly increased due to the application of the experiment treatments. The largest increase in plant length was observed with the application 400 mg/l of potassium silicate followed by the mixture of 350 mg/l chelated calcium +400 mg/l potassium silicate or 200 mg/l the potassium silicate without significant differences between the three treatments. These results agree with Hwang *et al.*, (2005) who declared that Silicon plays very important role in growth improvement, photosynthesis increase, efficiency of transpiration and evaporation, increasing the strength of leaves, chlorophyll concentration per leaf area and product quality. While, there was a clear reduction in the plant length with the foliar application of chitosan as a result of the decrease of internodal distance as mentioned by Zakaria *et al.*, (2009)

The highest significant values of leaves number arranged in descending with the 500 mg/l chitosan, mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicate and

500 mg/l. chelated calcium. The results also cleared that the leaves fresh and dry weight have the same trend, with concern that the heaviest significant values recoded with the 500mg/l chitosan and the mixture of chelated calcium 350mg/l+ 400 mg/l potassium silicates. Otherwise, the highest leaf area per plant were obtained with the mixture of 350 mg/l chelated calcium +400 mg/l potassium silicates followed by the first and second chitosan levels respectively. These findings agree with Zakaria *et al.*, (2009) who cleared that the chitosan level of 500 mg/l increased either the leaves or stems fresh and dry weight.

Data referred to the response of stems fresh and dry weight to the foliar application of the chitosan, chelated calcium, potassium silicate and the mixture of chelated calcium + potassium silicate at the plant age of 75 days after planting. The highest value of stem fresh weight was recorded with the 500 mg/l chitosan. These results agree with Zakaria *et al.*, (2009) who found that the application of chitosan at 500 mg/l significantly increased the stems fresh and dry weight. On the other side, the stem dry weight was recorded with the mixture of chelated calcium 350 mg/l+400 mg/l potassium silicates followed with 350 mg/l chelated calcium or 400 mg/l potassium silicate. Such results confirm those of Salim *et al.*, 2014 who mentioned that the higher rate of potassium silicate had a significant effect on stems fresh and dry weight on the open field.

Data in Table (5) showed that the minituber characteristics at the harvest time after 100 days from planting were significantly affected by the foliar spray of chitosan, chelated calcium, calcium silicate and the mixture between chelated calcium and potassium silicate. The acquired data indicated that the 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium +400 mg/l potassium silicates gave the highest significant minituber number. While the control, 900 mg/l chitosan and 200 mg/l

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Table 4

Table 5

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potassium silicate gave the lowest significant minituber number. Moreover, the heaviest minituber fresh weight was mentioned with the 500 mg/l chitosan with concern that there were no significant differences between this level of chitosan and the mixture of 350mg/l chelated calcium +400 mg/l potassium silicates or 400 mg/l potassium silicate . Furthermore, the highest minituber weight was obtained from the foliar application of chitosan 500 mg/l and the mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicates but this result has no significant differences with 500 mg/l chelated calcium or 400 mg/l potassium silicate.

The recorded data cleared that the highest values of small minitubers (≤ 1.49 cm) were obtained in control followed by 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium +400 mg/l potassium silicates. In contrary, the lowest significant weight was found with 350 mg/l chelated calcium or 200 mg/l potassium silicate.

Data in Table (5) cleared that the highest significant weights of medium size minituber (1.5 -2.99 cm) were arranged in descending order as the mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicates, 500 mg/l chitosan, 350 mg/l chelated calcium or 400 mg/l potassium silicate. Regarding the results in the same table, data clearly showed that 500 mg/l chitosan and the mixture of 350mg/l chelated calcium +400 mg/l potassium silicates recorded the highest significant values of the weight of tuber size equal or more than 3cm. Also, the data showed that the mixture of 350 mg/l chelated calcium + 400 mg/l potassium silicate gave the heaviest significant minituber dry weight with concern that there were no significant differences between this treatment and 500 mg/l of chitosan. The results on minituber yield and its components agree with those obtained with Arvin *et al.*, (2005) who mentioned that the minituber number and weight were increased with the application of calcium.

These results agree also with Zakaria *et al.*, (2009) who stated that 500 mg/l chitosan significantly increased the minituber yield. Also, Salim *et al.*, (2014) found that foliar application of potassium silicate significantly increased the tubers number/plant and the tubers weight/plant.

Conclusion:

The treatment of chitosan enhanced the microtuber growth characters followed by NO_3/NH_4 ratio 50: 10 or 55: 5 mM. Also 500 mg/l chitosan or the mixture of 350 mg/l chelated calcium +400 mg/l potassium silicate enhanced the minituber production and yield characteristics.

REFERENCES

- Andreea, N., C. Nicoleta and B. Carmen (2011). Effect of inorganic nitrogen on *in vitro* production of potato microtubers. Proceeding of the 4th International Symposium. New research in biotechnology "USAMV" Bucharest, Romania. 51-55.
- Arvin, M. J., A. Habib and D. J. Donnelly (2005). Effect of calcium concentrations in medium on microtuberization of potato (*Solanum tuberosum* L.) . Iranian J. Biotech. Vol. 3, No. 3., 152-156.
- Avila, A. L., S. M. Pereyra, D. J. Collino and J. A. Arguello (1994). Effects of nitrogen source on growth morphogenesis of three micropropagated potato cultivars. Potato Res. 37: 161-168.
- Balamani, V., K. Veluyhambi and B. W. Poovaiah (1986). Effect of calcium on tuberization in potato. Plant Physiol. 80 : 856-858.
- Darshani W. and D. Dilshan (2015). Effect of silicon and potassium on tomato anthracnose and on the postharvest quality of tomato fruit (*Lycopersicon esculentum* Mill.). J. Natn. Sci. Foundation Sri Lanka (3):273-280.
- Donnelly, D. J., W. K. Coleman, and S. E. Coleman (2003). Potato microtuber and performance; A review. Am. J. potato Res. 80: 103-115.

- Fang J. Y. and M. X. Long (2006). Effect of silicon on the growth of test- tube potato plantlets and the cell wall formation. *Acta Agronomica Sinica.* 32 (1) : 152-154.
- George, E. F, M. A. Hall and G. Dekerk (2008). Plant propagation by tissue culture 3rd edition Vol. 1. The background. published by springer 2008.pp 305.
- Habib, A., J. Abdulnour and D. J. Donnelly (2005). Increased calcium availability improves potato micropropagation and microtuberization. *Potato Res.* 47 :139 - 150.
- Hwang, S.J., H. M. Park and B. R. Jeong (2005). Effect of potassium silicate on the growth of miniature rose 'Pinocchio' grown on rock wool and its cut flower quality. *Journal of the Japanese Society for Horticultural Science.* 74: 242-247.
- Kawakami, J., K. Iwama, T. Hasegawa and Y. Jitsuyama (2003). Growth and yield potato plants grown from microtubers in fields. *Amer J. of Potato Res.* 80:371-378.
- Khan, H.R., S. Elahi, M. S. Hussain and T. Adachi (1994). Soil characteristics and behavior of potassium under various moisture regimes. *Soil Science and Plant Nutrition.* 40(2): 234-254.
- Kowalski, B., F. J. Terry, L. Herrera and D. A. Penalver (2006). Application of soluble chitosan *in vitro* and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Res.*, 49: 167-176.
- Ma, J.F. and E. Takahashi (2002). Soil, Fertilizer and Plant Silicon Research in Japan. Elsevier Science, Amsterdam, The Netherlands. P 294.
- Motallebi-Azar, A., S. Kazemiani, F. Kiumarsie and N. Mohades (2011). Shoot proliferation from node explants of potato (*Solanum tuberosum* L.) cv. Agrida. II. Effect of different concentrations of NH₄ NO₃ hydrolyzed casein and BAP. *Romanian Biotechnology Letters* 16 (3): 6181-6186.
- Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Plant Physiology*, 15: 473-497.
- Ozgen, S. and J. P. Palta (2004). Supplement al calcium application influences potato tuber number and size. *HortScience.* 40: 102-105.
- Salim, B. B. M., H. G. Abd El-Gawad and A. Abou El-Yazied, (2014). Effect of Foliar Spray of Different Potassium Sources on Growth, Yield and Mineral Composition of Potato (*Solanum tuberosum* L.). *Middle East Journal of Applied Sciences*, 4(4): 1197-1204.
- Snedecor, D. M. and W. G. Cochran (1981). *Statistical Methods.* 7th Ed., Iowa state Univ. press., Ames, USA, p.305.
- Villamor, C. C. (2010). Influence of media strength and sources of nitrogen on micropropagation of ginger, *Zingiber officinale* rosc. *E-International scientific Res. Jour.* 2: 1749-2094.
- Zakaria, R. A., B. M. Zanjani and E. Sedghi (2009). Effect of *in vitro* chitosan application on growth and minituber yield of *Solanum tuberosum* L. *Plant Soil Environ.*, 55, (6) : 252-256.
- Wang, p. j. and C. Y. Hu (1982). Effect of medium composition on *In vitro* tuberization. *Am. Potato J.* 64: 462.

تحسين انتاج و مواصفات الميكروتيوبور والميني تيوبور في البطاطس

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الملخص العربي

اجريت هذه الدراسة خلال موسمي النمو 2012 و 2013 علي البطاطس صنف دايمونت بمعمل زراعة الانسجه وقسم البطاطس والخضر خضرية النكاثر - معهد بحوث البساتين بالدقي الجيزة. تهدف هذه الدراسة لمعرفة تاثير اضافة الشيتوسان وكوريد الكالسيوم ونسب مختلفة من تركيز نترات الامونيوم الي نترات البوتاسيوم علي انتاج الميكروتيوبور في البطاطس . بالاضافة الي دراسة تاثير الرش الورقي بالشيتوسان والكالسيوم المخلي وسيليكات البوتاسيوم علي نمو ومحصول الميني تيوبور في البطاطس خارج المعمل

اظهرت النتائج ان اضافة الشيتوسان الي بيئة النمو بتركيز 500 ملجم/لتر في مرحلة تكوين الميكروتيوبور معمليا ان عدد الافرع وعدد العقل الساقية / جزء نباتي وعدد الميكروتيوبور والوزن الطازج والجاف /للافرع والوزن الطازج للميكروتيوبور الناتجة بعد 8 اسابيع من الزراعة كانت الافضل. و بالاضافة الي ذلك ادي الرش بالشيتوسان بتركيز 500 ملجم/لتر وكذلك الرش بخليط من الكالسيوم المخلي بمعدل 350 ملجم/لتر + سيليكات البوتاسيوم بمعدل 400 ملجم/لتر الي الحصول علي اعلى طول نباتات وعدد الاوراق والوزن الطازج والجاف للاوراق بعد 75 يوم من زراعة الميكروتيوبور. وكذلك ادي الرش بالمعاملات السابقة لزيادة متوسط وزن الميني تيوبور المحصودة بعد 100 يوم من الزراعه.

وتوصي الدراسة باستخدام الشيتوسان بتركيز 500 ملجم/لتر داخل المعمل لانتاج افضل صفات نمو ومحصول للميكروتيوبور. وكذلك الرش بالشيتوسان بتركيز 500 ملجم/لتر او الرش بخليط من الكالسيوم المخلي بمعدل 350 ملجم/لتر + سيليكات البوتاسيوم بمعدل 400 ملجم/لتر خارج المعمل لتحسين نمو وانتاج الميني تيوبور.

Table (3): Effect of some additives and modifications of MS medium on growth characteristics and microtuber production of potato after 8 weeks from culturing (average of 2012 and 2013 seasons).

Treatments	plantlet height cm	No. of Shoots /plantlet	No. of Nodes /plantlet	No. of Micro tubers /plantlet	Microtuber fresh weight (mg)	Shoot fresh weight (mg)	Shoot dry weight (mg)
Control	9.4	4.4	7.6	4.40	178.0	953.20	90.72
500mg/l chitosan	8.88	6.4	9.6	7.00	249.8	1496.6	153.07
700mg/l chitosan	7.72	5.8	9.6	6.80	245.4	1029.2	104.82
900mg/l chitosan	7.36	4.0	9.6	4.60	193.2	1017.2	98.70
5mM CaCl ₂ .2H ₂ O	11.32	3.2	6.4	3.40	172.8	942.40	89.34
10mM CaCl ₂ .2H ₂ O	11.4	2.6	5.6	2.80	159.0	933.40	87.09
15mM CaCl ₂ .2H ₂ O	11	2.2	5.6	3.00	158.6	818.80	75.08
20mM CaCl ₂ .2H ₂ O	8.28	1.6	4.8	1.60	139.4	754.20	66.34
50 mM KNO ₃ : 10 mM NH ₄ NO ₃	11	4.0	9.6	4.80	194.6	1137.8	115.20
55 mM KNO ₃ : 5 mM NH ₄ NO ₃	12.6	5.0	10.2	5.00	201.2	1132.0	121.00
60 mM KNO ₃ : 0 mM NH ₄ NO ₃	10.28	2.8	6.4	4.20	176.2	1047.2	104.80
L.S.D. 50.0	1.81	1.3	1.82	0.98	15.5	166.61	17.47

Table 4: The effect of chitosan, chelated calcium, potassium silicate and the mixture between chelated Calcium and potassium silicate on potato growth characteristics after 75 days from *ex vitro* (average of 2012 and 2013 seasons).

Treatment	Plant length (cm)	Leaves number /plant	Leaf fresh weight/ (g)	Leaf dry weight/ (g)	Leaf area/ plant (Cm ²)	Stem fresh weight/ (g)	Stem dry weight/ (g)
Control	22.20	11.60	22.82	3.07	335.25	15.84	1.59
Chitosan 500 mg/l	21.33	14.45	26.3	3.76	356.21	16.19	1.62
Chitosan 700 mg/l	21.07	13.60	23.06	3.01	341.68	15.13	1.19
Chitosan 900 mg/l	20.27	12.45	22.69	2.80	312.22	11.48	1.16
Chelated Ca 350 mg/l	22.47	13.45	21.24	2.80	318.51	8.95	1.65
Chelated Ca 500 mg/l	22.37	13.75	21.90	3.10	301.30	12.44	1.28
K ₂ SiO ₃ 200 mg/l	24.63	11.60	23.50	3.11	327.55	9.37	1.23
K ₂ SiO ₃ 400 mg/l	25.37	12.45	24.11	3.43	272.78	14.80	1.64
Chelated Ca 350 mg/l + K ₂ SiO ₃ 400 mg/l	25.07	14.10	25.64	3.65	383.60	11.88	1.83
L.S.D. 50.0	1.43	1.18	1.82	0.64	66.03	3.68	0.35

Table 5: The effect of chitosan, chelated calcium, potassium silicate and the mixture between chelated calcium and potassium silicate on the minituber characteristics of potato after 100 days from *ex vitro* (average of 2012 and 2013 seasons).

Treatment	Minituber number/ plant	Minituber fresh weight/ plant (g)	Minituber average weight (g)	Minituber fresh weight / plant (g)			Minituber dry weight/ plant(g)
				size ≤ 1.49 cm	size 1.5-2.99 cm	size ≥ 3 cm	
Control	3.00	10.70	3.57	5.37	3.17	2.17	1.41
Chitosan 500 mg/l	4.67	20.77	4.50	5.27	7.03	8.47	2.24
Chitosan 700 mg/l	4.00	12.30	3.10	3.40	3.77	5.13	1.98
Chitosan 900 mg/l	3.33	10.67	3.20	5.03	3.13	2.50	1.78
Chelated Ca 350 mg/l	3.67	12.93	3.50	2.27	6.90	3.77	1.47
Chelated Ca 500 mg/l	4.00	14.77	3.73	4.47	4.73	5.57	1.80
K ₂ SiO ₃ 200 mg/l	2.67	9.33	3.50	2.03	3.90	3.40	2.08
K ₂ SiO ₃ 400 mg/l	4.00	16.00	4.00	4.43	5.77	5.80	1.98
Chelated Ca 350 mg/l + K ₂ SiO ₃ 400 mg/l	4.67	16.17	4.43	5.13	7.17	8.20	2.50
L.S.D. 50.0	1.09	5.66	0.84	2.65	1.72	1.81	0.38