EFFECT OF KINETIN ON IN VITRO MICROTUBER INITIATION OF POTATO AND CRYOPRESERVATION

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ABSTRACT

The experiments were carried out in plant tissue culture labs. Date Palm Research Unit, College of Agriculture, University of Baghdad, Iraq from Jun 2012 to July 2013. Experiments included adding Kinetin at 0, 3, 5 and 7 mg/L to MS medum with 80g/L sucrose for three potato cultivars Emma, Santé, and Arnova. The study experiments were designed as factorial experiments using Completely Randomized Design (CRD) with l0 replicates for three potato cultivars for each concentration. After 8 weeks it's clear that the best concentration was 7mg/L for all traits under study (average of microtubers number/plant, average weight of the microtubers/plant (g), average diameter of the microtubers (mm), percentage of dry matter of microtubers, percentage of starch in microtubers, and percentage of protein in microtubers) but the cultivars were different, Emma was superiority in average weight of the microtubers/plant (0.566g) and average diameter of the microtubers (19.91mm) but Santé was superiority in percentage of dry matter of microtubers (19.25%) and percentage of starch in microtubers (13.15%), while Arnova was superiority in average of microtubers number/plant (4.20 microtuber/plant) and percentage of protein in microtubers (1.97%). Microtubers were harvested and preserved at 4°C for three periods 2, 4, and 6 months (after placed in glass Jars covered with cotton). All preservation periods gave 100% of success without any blighter. Experience can be concluded that increasing the concentration of kinetin (7 mg/L) led to increase the number and size of the microtubers and possible preserved for 6 months without any damage.

Key words: Gene bank, genetic preservation, tissue culture, cytokinin.

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تأثير الكياينتين على تكوين الدرينات الدقيقة للبطاطا وحفظها خارج الجسم الحي

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المستخلص

اجريت تجارب البحث في مختبرات الزراعة النسيجية لوحدة ابحاث النخيل في كلية الزراعة / جامعة بغداد للفترة من كانون الثاني 2012 ولغاية تموز 2013 . تضمنت التجارب اضافة الكاينتين بالتراكيز (0 و 3 و 5 و 7 ملغم/لتر) الى وسط MS الحاوي على 80 غرام /لتر سكروز طبقت التجربة على ثلاثة اصناف من البطاطا هي ايما وسانتي وارنوفا . صممت التجارب على اساس تجارب عاملية باستخدام تصميم القطاعات العشوائية باستخدام 10 مكررات لكل معالمة . كان واضح ان افضل المعاملات كانت باستعمال الكاينتين بتركيز 7 ملغم / لتر لجميع الصفات قيد الدراسة (عدد الدرنات ، وزن الدرنات ، قطر الدرنات ، النسبة المؤية للوزن الجاف للدرنات ، النسبة المئوية للنشا ، النسبة المئوية للبروتني) . اما الاصناف فقد اختلفت في الصفات قيد الدراسة وتفوق ايما في معدل وزن الدرنات (0.566) ومعدل قطر الدرينة (19.91 ملم) لكن تفوق سانتي في النسبة المئوية للمادة الجافة (19.25%) والنسبة المئوية للنشا (13.15 %) في حين تفوق ارنوفا في معدل عدد الدرنات / عقلة (4.20) وفي النسبة المئوية للبروتين (1.97 %) . حصدت الدرينات الدقيقة وحفظت بدرجة 4م لثلاثة مدد 2 و 4 و 6 اشهر (بعد ان وضعت الدرينات في اوعية زجاجية وغطيت بالقطن الطبي) جميع المدد اعطت نسبة نجاح 100 % بدون حدوث اي تلف للدرينات . نستنتج من الدراسة ان زيادة تركيز الكاينتين (7 ملغم/لتر) ادت الى زيادة اعداد واحجام الدرينات الدقيقة بالإضافة الى امكانية حفظها لمدة 6 اشهر بدون حصول اي تلف او اضرار .

الكلمات المفتاحية: بنك الجينات، حفظ المصادر الوراثية، الزراعة النسيجية، السايتوكاينين..

***البحث مستل من أطروحة دكتوراه للباحث الاول.**

INTRUDUCTION

Since it seemed that human knowledge of the art of agriculture and economic importance of the plant, carried out some of germplasm conservation, and that's when he started the selection of seeds or vegetative reproductive parts from season to another. The Inscription on the walls of ancient tombs indicate that the ancients practiced methods of preservation and selection for the most important crops like sesame, wheat, onion,,,,etc. (3). The first cultivars and the wild species are the main source for genetic diversity and they are important to provide farmers and plant breeding with options to develop their crops, through selection and breeding programs (33). Potato is one of the most common cultivated species on earth and ranks on place four in produced crops after maize, rice and wheat (l4); (40); (8); (l1); (43). Potato genetic resources *(Solarium tuberosum* L. ssp. *tuberosum)* and related cultivated species are preserved through storage of tubers, *in vitro* plants and in cryopreservation (21). Potato plants are highly heterozygous, and the seeds are not true to type. Thus, maintenance of cultivated potato accessions by seeds is not possible (13). In gene banks, the plant material is maintained vegetatively. Potato belongs to the botanical family *Solanaceae* and the genus *Solanum*, which consists of more than 2,000 species (17). Today, there are more than 4,500 varieties of *S. tuberosum ssp*. *tuberosum* (19)/Potato is known to have the highest genetic diversity of any cultivated plant because of its high number of varieties and related species (14)/The origin of cultivated potatoes lies in the high Andes of South America and the coastal strip of central to southern Chile (17) After the first potatoes were brought to Europe in the late 16th century, and entered to Iraq in the end of 19th century (2) this crop plant was distributed and utilized all over the world in *in vitro and in vivo* (l and 46) mostly in temperate regions (18). Because of the high number of varieties and related species, potato is known to have the richest genetic diversity of any cultivated plant (14). To prevent the loss of potato genetic resources, long term conservation of plant material is accomplished in gene banks and private collections worldwide. Since a half of century, *in vitro* micro tubers were first described in potato, but the adoption as a seed propagule has been unequally globally. Growth regulator used has led to change effects in other plant system (l5); (7). The literature depicts the utility of cytokinins and growth retardants for induction are contradictory, partially because different cultivars, propagules, and incubation conditions were applied. Plantlets 1 month old with many nodes generally produced from one to four microtubers, usually at the basal nodes. Exogenous cytokinins may catalyze or enhance this process (26), stimulate both microtuber initiation and growth (27) although this is cultivar- dependent and they may only promoting growth not induction (16). Since apical dominance is completely eliminated in single-node cuttings, exogenous cytokinins were not necessary for microtuberization and 50 to 75% microtuberization occurred in the presence of ancymidol (5 mg/L).

Materials and Methods

All experiments were carried out in plant tissue culture labs., Date Palm Research Unit, College of Agriculture, University of Baghdad, Iraq. The experiments were implemented from June 2012 to July 2013. Three commercial potato (*Solarium tuberosum*) cultivars were used. Cultivars were obtained from the General Company for Horticulture and Forestry, Ministry of Agriculture, Iraq .These tuber seeds were imported at Elite stage from IPM company for Emma and Agico company for Santé and Arnovas. Three selected cultivars (Emma, Santé and Arnova) were tested in the General Authority for Examination and Certification of Seeds, Ministry of Agriculture, Iraq, for free of viral etiology and pathogenesis. Tubers seeds were washed with tab water to remove dust and then left to dry, incubated in dark at 15-20°C for 10-15 day to break dormancy and stimulate the growth of buds (45). After the arrival of the buds to 1.5-2.5cm, they were isolated to conduct sterilization. Isolated grown sprouts were cut in nodal segments and immersed in molten Paraffin wax (30°C).Then rinsed in 70% ethanol. Segments were placed into an Erlenmeyer flask and shaken in 50% bleach's solution (sodium hypochlorite with 6% active chlorine) for 15 min. Then segments

were washed three times with sterile distil water under aseptic conditions. Nodal segments were dried on filter paper and bleached ends were cut off. At last segments were transferred to culture medium All these steps were done inside the laminar air flow cabinet. Pre-mixed (30). (MS) medium (HiMedia Laboratories Pvt. Limited, India) was used as culture medium. Sucrose was added at 3%then pH was adjusted to 5.7+ 0.1 using IN HC1 or NaOH . Media were solified by adding 6g/L Agar-Agar and heated until boiling using hot plate magnetic stirrer. Media were dispensed in 150x25mm test tubes at 15 ml /tube using media automated pump dispenser and capped with polypropylene caps before autoclaved at 121°C, 1.04 kg/cm2 for 20 min. Segments were cultured onto MS medium prepared previously. They were maintained under a 16h photoperiod at 22°C with 1000 lux light intensity (23) for four weeks. Shoots were then cut to hold onto single nodes and cultured on the same media. Two subcultures were done before preparing the explants for preservation. These explants were shoot tips and single nodes (1cm length each), shoots (for three cultivars) consisted of 3 nodes were cultured onto MS media supplemented with 80g/L sucrose and Kinetin at 0, 3, 5 and 7 mg/L. Media were dispensed in jars at 40ml/jar and 2 explants were cultured per jar. Cultures were incubated at 18°C with 8h light and 16h dark for 2 weeks, then full darkness was applied for 6 weeks at the same temperature. Microtubers were harvested and preserved at 4°C for 2, 4 and 6 months. This experiment was applied with 10 replicate for all preservation periods and cultivars, then the following measurements were calculated:

1- The average of microtubers number/ plant:

The number of microtubers/plant for each replicate was calculated by dividing the number of microtubers produced form each jars by 2.

2- The weight of the microtubers/plant (g): The weight of microtubers was determined by using a sensitive balance. The average weight of the microtubers/plant for each replicate was calculated by dividing the total microtubers wight produced from jar by 2.

3-The percentage of dry matter of microtubers: Potato microtubers were dried using an electric oven (60°C) until weight constancy and used the following equation: The percentage of dry matter = (Dry weight of microtubers/ Wet weight of microtubers) ×l00 (5).

4- The percentage of starch in microtubers: It was calculated using the following equation: The percentage of starch= 17.55+0.891 (The percentage of dry matter- 24.18).

5- The percentage of protein in microtubers: It was measured by estimating the total nitrogen according to Kieldahl method using the Micro Kieldahl device (5)'and calculated the percentage of protein on the basis of dry weight, as in the following equation: The percentage of protein on the basis of dry weight = The percentage of the total nitrogen 6.25 (6).

Results and Discussion

1- Number of microtubers

Results in table (1) showed that Emma predomination in the average of microtuber number (3.00microtuber) which wasn't significant different with Arnova that gave (2.80 microtuber) While Santa cultivar gave lowest average of microtuber number which is significant different with previous two cultivars. There were significant different between the kinetin concentrations, 7mg/L kinetin gave the highest average of microtuber number (3.90 microtuber) and it increased with increasing kinetin concentration. For the interaction between the cultivars and the concentration, it was noticed outweigh Arnova that cultured on MS media contained 7mg/L kinetin in the average of microtuber number (4.20 microtuber), which was not significant different with Emma and Sante cultivars that gave 3.90 microtuber and 3.60 microtuber respectively, and Emmas cultured on MS medium modified with 5mg/L of kinetin gave (3.60 microtuber), and it was significant different with the other interactions. While Arnova cultured on MS free medium gave lowest average of microtuber number (1.30 microtuber). The differences between cultivars can be attributed to the difference in the genotype so these cultivars differ in the number of microtuber.

Table 1. Effect of kinetin concentrations on the no. microtubers /plant for three potato cultivars and their interaction.

Cultivars	Kinetin conc. (mg/L)				Average
	0	3	5	7	
Emma	2.10	2.40	3.60	3.90	3.00
Sante	1.40	1.90	2.30	3.60	2.30
Arnova	1.30	2.30	3.40	4.20	2.80
L.S.D 5%	0.71				0.35
Average	1.60	2.20	3.10	3.90	
L.S.D. 5%	0.41				

Microtubers have become an important method for rapid multiplication for pre-basic stock in seed tuber multiplication as well as gerrnplasrn exchange. The number and size of microtubers produced *in vitro* depend on several factors, like optimum concentration of sugar, growth regulators and anti-gibberellin compounds in the culture medium (41); (l2) Cytokinins are thinked to have strong enhance effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (31 and 32). Cytokinins were supplemented "in the media for *in vitro* tuberization of potatoes (44). Cytokinins promote in vitro tuberization of potato by altering GA balance in non-induced stems (25) inhibiting root formation and transferring the upright leafy shoots into horizontal stolons (37). Cytokinin failed to exert stimulating effect of tuherization at any concentration when media supplemented with 2% sucrose (24). They noticed that sucrose concentrations at above 4%, cytokinins exhibited a promoting effect on tuberization. Request of high concentration of sucrose by cytokinins for *in vitro* tuberization was also reported by (31 and 44). For many reasons, cytokinin has often been considered to be an important factor for tuberization process. First, cytokinin is known to stimulate cell division (39); second, there are indications that it inhibits/cell elongation (42), and promotes cell expansion (36). These phenomena are desired for tuber formation and development. Many workers have, therefore, suggested that the unknown tuberization stimulus could be a cytokinin like substance (29 and 9). Although cytokinin is not directly responsible for tuberization as reported by many workers, without doubt, it plays role in cell division and thus creating sink activity of the developing microtuber. Results showed that these phytohormones can produce big effects on tuberisation parameters, but these effects depend on sucrose content and potato genotype. For example, the effect of phytohormones on tuber yield in some cultivars is inductive at low, stimulatory at intermediate and even repressive at high sucrose in the medium (34). The stimulatory effect of phytohormones on microtuber yield is directed to different tuberisation stages, kinetin was shown to act mainly on tuber initiation thereby increasing microtuber number. Many of researchers have shown that cultivars have a different potential in production of microtuber (4) and (16). lt seems that in the same condition, genotypic potential of cultivars has the greatest effect and will result in different yields because of instinctive capacity of the genotypes in production of endogenous levels of growth regulators. The main factor of microtuber initiation and induction are different, since increase in cytokinen level, the number of genotypes responding to microtuberization decreases (l6) whether some cultivars of the potato haven't potential for microtuber production (34). Effect of Kin on microtuberization may be referred to its relationship with the ethylene biosynthesis (38). Due to Kin mainly influenced the microtuber initiation, therefore microtuber number increased; besides, effect of phytohormones on microtuberization parameters depends on plant genotype and the amount of sucrose in media (32and 22).

2- The weight of the microtuber (g): Table (2) indicated that Emma predomination in the average weight of microtuber (0.216g) which significant different with sante cultivar that gave 0.172g. While Arnova gave lowest average of microtuber weight (0.131g) which is significant different with previous two cultivars. As for the kinetin concentrations shown in table (2) there were no significant differences between the kinetin concentration 3mg/L kinetin (0.08g) and the control treatment (0.07g), while 7mg/L gave the highest average of microtuber weight (0.38g) which is significant different with the others kinetin concentrations. For the interaction between the cultivars and the concentrations, it

was noticed outweigh the Emma that cultured on MS medium supplemented with 7mg/L kinetin in the microtuber weight (0.56 g) which was significant differences with the other interactions. While santé cultured on MS free medium gave lowest average of microtuber weight (0.06g). Because cytokinins interfere with cell division, induction and production of potato were increased (22) and among the various concentrations, with an increase in concentration, size and weight of microtuber increased since there is linear relation between them (28) but the mean number of microtubers were decreased because the external using of hormone disturbs the balance of endogenous levels of growth regulators (16)There is a linear relation between size and weight of microtuber (28), (i.e. each factor that influence microtuber weight, directly influence microtuber size). In many cases, Kin usually induced no significant change in microtuber size, cytokinins, has more potential for microtuberization and have promoted effect on reduction of total sugar and subsequently have increased starch content (35) Although cytokinins such as Kinetin have antagonistic effect on microtuber yield, has smaller size (35).

Table 2. Effect of kinetin concentrations of on microtubers weight (g) for three potato cultivars and their interaction

Cultivars	Kinetin conc. (mg/L)				Average
	0	3	5	7	
Emma	17.800	17.930	17.490	19.190	18.102
Santé	13.210	17.060	18.770	19.250	17.072
Arnova	17.110	18.320	18.200	19.130	18.190
L.S.D 5%	1.685				0.842
Average	16.040	17.770	18.153	19.190	
L.S.D. 5%	0.973				

3- The percentage of dry matter of microtubers:

Table (3) showed that Arnova predomination in the percent of microtuber dry matter and gave the highest percentage (18.19%) which was not significant different with Emma that gave(18.10%). While Santé gave lowest percent of microtuber dry matter(17.07%). As for the kinetin concentrations shown in table (3) It was noticed that the consentration 7mg/L of kinetin gave the highest percentage of microtuber dry matter which is significant different with the other kinetin concentrations (19.19%). While there was no significant different between concentration 5mg/L and 3mg/L which gave (18.15%) and (17.77%) respectively. The control treatment gave lowest percentage of microtuber dry matter (16.04%). For the interaction between the cultivars and the concentration, it was noticed outweigh Santé that cultured on MS media contained 7mg/L kinetin in the percentage of microtuber dry matter (19.25%) which wasn't significant different with Emma and Arnovas cultured on the same medium (19.19%) and (19.13%) respectively. While santé cultured on MS free medium gave lowest percent of microtuber dry matter (13.21%). The beneficial effects of high sucrose concentrations on microtuberization as observed in this study, are in agreement with earlier reports (44); (20) and (16). The present study, showed that high sucrose concentrations also improved biomass production and microtuber dry matter content. We suggest that, for germplasm conservation, microtubers should be induced on media supplemented with cytokinine and a high concentration of sucrose (60-80g/L), as this enhances tuberization (31); (44); (20); and (16).

Table 3. Effect kinetin concentrations on the dry weight percentage of microtubers for three potato cultivars and their interaction.

Cultivars	Kinetin conc. (mg/L)				Average
	0	3	5	7	
Emma	0.077	0.096	0.125	0.566	0.216
Santé	0.062	0.070	0.213	0.345	0.172
Arnova	0.073	0.093	0.111	0.249	0.131
L.S.D 5%	0.054				0.027
Average	0.070	0.086	0.149	0.386	
L.S.D. 5%	0.031				

4- The percentage of starch in microtubers:

Results in table (4) indicated that Arnova predomination in the percent of microtuber starch and gave the highest percent (12.21%) which was not significant different with Emma that gave (12.13%). While Santé gave lowest percent of microtuber starch (11.34%). As for the kinetin concentrations, It was noticed that 7mg/L of kinetin gave the highest percentage of microtuber starch which is significant different with the others kinetin concentrations (13.09%).while there were no significant

different between concentration 5mg/L and 3mg/L which gave 12.17 and 11.83% respectively. And the control treatment gave lowest percent of microtuber starch (10.48%). For the interaction between the cultivars and the concentrations, it was noticed outweigh Santé cultivar that cultured on MS medium contained 7mg/L of kinetin in the percentage of microtuber starch (13.15%) which wasn't significant different with Emma and Arnovas cultured on the same media (13.09%) and(13.04%) respectively. While santé cultured on MS free medium gave lowest percent of microtuber dry matter (8.30%). The increase of starch percentage of microtubers may be due to the high percentage of dry matter caused by high concentrations of kin in culture medium (10).

Table 4. Effect of of kinetin concentrations on the starch percentage of microtubers for three potato cultivars and their interaction.

Cultivars	Kinetin conc. (mg/L)				Average
	0	3	5	7	
Emma	11.86	11.97	11.59	13.09	12.13
Santé	8.30	11.19	12.72	13.15	11.34
Arnova	11.27	12.32	12.21	13.04	12.21
L.S.D 5%	1.29				0.64
Average	10.48	11.83	12.17	13.09	
L.S.D. *5%*	0.74				

5 -The percentage of protein in microtubers:

Table (5) showed that there were no significant different between the cultivars in the percentage of protein in microtubers. As for the kinetin concentrations, It was noticed that 7mg/L of kinetin gave the highest percentage of microtuber protein which was significant different with the other kinetin concentrations (1.95%).While there were no significant different between 5mg/L and 3mg/L of Kinetin which gave 1.84 and 1.78% respectively. The control treatment gave lowest percent of microtuber protein (1.70%). For the interaction between the cultivars and the concentration, it was noticed outweigh Arnova that cultured on MS medium contained 7mg/L of kinetin in the percent of microtuber protein (1.97%) which wasn't significant different with Emma and Santé cultivars cultured on the same medium 1.95 and 1.91% respectively.While santé cultured on MS free medium gave lowest.

Table 5. Effect of kinetin concentrations on protein percentage in microtubers for three potato cultivars and their interaction.

Cultivars	Kinetin conc. (mg/L)				Average
	0	3	5	7	
Emma	1.773	1.819	1.844	1.956	1.848
Santé	1.584	1.773	1.839	1.918	1.778
Arnova	1.764	1.766	1.837	1.976	1.836
L.S.D 5%	0.147				N.S.
Average	1.707	1.786	1.840	1.950	
L.S.D. 5%	0.085				

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