EVALUATE THE PERFORMANCE OF FIELD AND PRODUCTIVE FOR THE SEEDLINGS POTATOES RESULTING FROM *IN VITRO* PRESERVATION*

Shaimaa M. I. Al-Ahmar Assist. Instructor
Iman J. Abdul Rasool Prof.
Hussam S.M. Kheirallah Assist. Prof.
shaimaaalahmar@mnail.com
Dept. of Horticulture – Coll. of Agric. – Univ. of Baghdad

ABSTRACT

Research's 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 Cytokinen represented by kinetin (0, 3, 5 and 7 mg/L) to MS media with 80g/L sucrose for microtuber initiation and mannitol (0, 10, 20 and 40 g/1) and ABA (0, 2.0, 4.0 mg/l) to MS media with 30g/L sucrose for three potato cultivars Emma, Sante, and Arnova. For microtuber initiation, after 8 weeks it's clear that the best concentration was 7mg/L for all recipes 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, microtubers were harvest and preserved at 4°C for three periods 2, 4, and 6 months (after placed in glass Jars covered with cotton) after those periods microtubers cultured in plastic pot to growth. For the slow growth conservation using mannitol and ABA the explant also preserved for 2, 4, and 6 months and then transferred to regeneration media (MS free hormones) and maintain for four weeks then transferred to acclimation stage for four weeks also, 40g/I mannitol was the best treatment for acclimation (data not shown) then those plant transferred to greenhouse which supplemented with cooling (fan and pad) system, until plant reaches the production stage. Before the shoots dry and yellowing, vegetative growth parameters were measured. Tuber harvesting has been done in 1-6-2013 and yield parameters were calculated. The best method for conservation to production tubers was slow growth preservation compared to microtuber preservation for all traits under study.

Key Word: Genetic Preservation, Tissue culture, conservation
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تقييم الاداء الحقلي والانتاجي لشتلات البطاطا الناتجة من الحفظ خارج الجسم الحي

شيماء محمد سعيد الاحمر مدرس مساعد
ايمان جابر عبد الرسول أستاذ
حسام سعد الدين محمد خير الله أستاذ مساعد
shaimaaalahmar@mnail.com
قسم البستنة وهندسة الحدائق – كلية الزراعة – جامعة بغداد

المستخلص

اجريت تجارب البحث في مختبرات الزراعة النسيجية لوحدة ابحاث النخيل في كلية الزراعة / جامعة بغداد للفترة من كانون الثاني 2012 ولغاية تموز 2014 . تضمنت التجارب اضافة الكاينتين بالتراكيز (0 و3 و5 و7 ملغم/لتر) الى وسط MS الحاوي على 80 غرام /لتر سكروز بالنسبة لانتاج الدرنات الدقيقة واما بالنسبة للنمو البطئ فقد استعمل المانتول (40 و20 و10 و0) غرام /لتر) و ABA (2 و0 و4 ملغم/لتر) طبقت التجربة على ثلاثة اصناف من البطاطا هي ايما وسانتي وارنوفا. ان افضل المعاملات كانت باستعمال الكاينتين بتركيز 7 ملغم/لتر لجميع الصفات قيد الدراسة (عدد الدرنات ووزن الدرنات وقطر الدرنات والنسبة المئوية للوزن الجاف للدرنات والنسبة المئوية للنشا والنسبة المئوية للبروتين). اما الاصناف فقد اختلفت في الصفات قيد الدراسة. حصدت الدرينات الدقيقة وحفظت بدرجة 4 °م لثلاثة مدد 2 و4 و6 اشهر (بعد ان وضعت الدرينات في اوعية زجاجية وغطيت بالقطن الطبي) بعد انتهاء فترة الحفظ زرعت الدرينات في اصص بلاستيكية لاجل النمو بالنسبة لمعاملات النمو البطئو حظنت الاجزاء النباتية لمدة 6 و4 و2 شهر بعدها نقلت الى وسط اعادة نمو (MS بدون هرمونات) لمدة اربعة اسابيع ايضا (النتائج غير معروضة) بعدها تم زراعتها في البيت الزجاجي المكيف اخذت القياسات للمجموع الخضري قبل جفافه واصفراره. حصدت الدرنات بتاريخ 2013/6/1 وحسبت قياسات الحاصل افضل طريقة لانتاج التقاوي كانت باستخدام طريقة الحفظ بالنمو البطئ مقارنة باستخدام الدرينات الدقيقة ولجميع الصفات قيد الدراسة.

الكلمات المفتاحية: حفظ المصادر الوراثية، الزراعة النسيجية.
البحث مستل من اطروحة دكتوراه للباحث الاول.

INTRODUCTION

Some of in vitro techniques have been sophisticated in the last few 10 years. The systematic cultures have a high degree of genetic stability and are more likely to be of importance for germplasm conservation, especially the shoot tips or meristem cultures. Potato plant was distributed and utilized all over the world in in vitro and in vivo (1) and (37). In vitro techniques are applied to eliminate diseases and pests. However, some viruses particularly are not necessarily eliminated or even detected and can readily multiply in tissue culture (32). These can be discarded by meristem or shoot tip cultures possibly in combination with both heat and cold treatment. CIP maintains the potato germplasm as an in vitro plantlets or microtubers. Potato germplasm is being conserved at CIP through in vitro techniques using meristem and shoot tip culture for international exchange of. Now, with in vitro techniques, it is possible to provide a germplasm preservation protocols which uniquely combines the possibilities of disease disposal and clonal propagation (16). Further, the virus-tested cultures could provide ideal material for international exchange and distribution of germplasm as they will be acceptable to plant quarantine power (26) and comply with international quarantine regulations. Minimal growth conditions for short to medium term preservation can be followed in many ways such as reduced temperature and/or light, induction of osmotic stress with sucrose or mannitol, incorporation of sub lethal levels of growth retardants ((11); (12); (31)), and maintenance of cultures at a reduced nutritional status particularly reduction of gas pressure over the cultures, reduced carbon, dehydration and mineral oil overlay. The advantage of this process is that cultures can be readily brought back to normal culture conditions to produce plants on request. (25). In vitro preservation based on slow growth techniques has been indicate as alternative strategies for preservation of plants genetic resources. In particular, it is useful where the seed banking is not possible, such as vegetatively propagated plants, recalcitrant seed species, and plants with unavailable or non-viable seeds due to damage of gazing or diseases, and large and fleshy seeds (18). Some species preserved at in vitro conditions are *Allium spp*., *Cocos nucifera*, *Theobroma cocoa*, *Vitis spp*, *Primus spp*, *Citrus spp*., *Saccharum*, *Solanum spp*., *Musa spp*., *Colocasia esculentum*, *Manihot spp*., and *Ipomaea batatas*, *E. chlorocoiymbos Schltr* ((38): (34); (4); (35); (7): (10); (27); (30); (2); (14): (20)). In vitro storage techniques include the medium-term conservation using slow growth protocol (5) or synthetic seed production ((29); (15); (13)), and long-term conservation using cryopreservation. Generally, there are three recognized methods for reducing in vitro growth rates, including physical, chemical, and a combination of the two (8). The endangered species preserved by medium-term tissue culture are *Artemisia tschernieviana*, *Astragalus pseudopurpureus* ,*Cerastium transsilvanicum*, *Di an thus callizonus*, *D. spiculifolius*, *D.tenuifolius*, *Erigeron nanus*, *Hieracium pojoritense*, and *Marsilea quadrifolia* (28). Ornamental plants are preserved just a few degees above the freezing (mainly, at 4-5°C) (24). Loureiro da Silva and Scherwinski-Pereira Explained the objective of their study was to evaluate in vitro preserve of Piper achmcitm and P. hispiclinenntm under slow-growth conditions (21). Shoots were preserved at low temperatures (10, 20 and 25°C), and the culture medium was supplemented with osmotic agents (sucrose and mannitol - at 1, 2 and 3%). After six-months of storage, shoots were evaluated for survival and regrowth. Low temperature at 20°C was effective for the in vitro preservation of *P. cichincum* and *P. hispidinervum* shoots. In vitro cultures maintained at 20°C on MS medium showed 100% survival with slow- growth shoots. The presence of mannitol, in the culture medium, negatively affected shoot growth, which is evidenced by the low rate of recovered shoots. In potato, The production of microtubers in in vitro culture as an alternative methods for mid-term preservation of potato cultivar has also been evaluated at (NCGRP) (17) and (CIP) (9). microtubers can be preserved at 4-10°C up to 10 month.

MATERIALS AND METHODS

All Research's experiments were carried out in plant tissue culture labs., Date Palm Research

Unit, College of Agiculture, University of Baghdad, Iraq.The experiments were implemented during the period from Jun 2012 to July 2013. Three commercial potato *(Solanum tuberosum*) cultivars were used in this investigation. Cultivars were obtained from the General Company for Horticulture and Forestry, Ministry of Agiculture,Iraq .These tuber seeds were imported at Elite stage from IPM company for Emma and Agico company for Sante and Arnova. Three selected cultivars (Emma, Santa and Arnova) were tested in the General Authority for Examination and Certification of Seeds, Ministry of Agiculture, Iraq, to make sure they are free of viral etiology and pathogenesis. Tubers Seeds washed with tab water to get rid of 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 (36). After the arrival of the buds grow to the length of 1.5-2.5cm they were isolated to conduct sterilization. Isolated grown sprouts were cut in nodal segments and endings 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 cultured medium All these steps were done inside the laminar air flow cabinet. Pre-mixed (23) (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 150 x 25mm test tubes at 15 ml /tube using media automated pump dispenser and capped with polypropylene caps before autoclaved at 121 °C, 1.04 kg/cm^2 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 for four weeks. Plantlets were then cut to hold onto single nods 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).

Preservation using slow growth culture preservation under 4°C. The explants (shoot tips and single nods) were cultured in test tubes contain hormone free MS media and incubated in full darkness at 4°C for 2, 4 and 6 months.

Preservation under low temperature and osmotic compound effect. The explants (shoot tips and single nods) were cultured in test tubes contain MS media supplemented with Mannitol at 0, 10, 20 and 40g/L). Cultures were incubated in 16h photo period and 1000 lux light intensity at 6-8°C for 2, 4 and 6 month.

Preservation under Growth retardant effect. The explants (shoot tips and nods) were cultured in test tubes contain MS media supplemented with ABA at 0, 2.0 and 4.0 mg/L. Cultures were incubated in 16h photo period and light intensity about 1000 lux at 6-8°C for 2, 4 and 6 month. After the above preservation methods, cultures were transferred to a new fresh hormones free MS media and then transferred to standard culture media and conditions.

Microtubers preservation. Shoots (for three cultivars) consisted of 3 nods were cultured onto MS media supplemented with 80g/L sucrose and kinetin at 7 mg/1. 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.

Hardening off, acclimatization and microtubers transplanting. Plantlets were hardening and acclimatization from each preservation methods and periods for all cultivars. Plantlets toke off from the test tubes, washed thoroughly with running tab water to remove remains stuck media and treated with fungicide Beltanol at ml/Lfor 10 mints. Then they gown in pots filled with 2 peatmoss:l perlit (v:v) and watered with 1/4 MS solution. Pots placed in the greenhouse and under plastic tunnel (2.5m lenght, 1.2m width and 0.6mhight). Pots watered with tap water as

needed for a month and plastic cover were removed gradually. The experiment were conducted with 10 replicates (plant/pot/treatment).Indicator that are being studied include the percentage for the survival planlet.

Evaluate the field performance of the *ex vitro* plants derived from each preservation method. This experiment was conducted in the date palm research unit greenhouse which supplemented with cooling (fan and pad) system at 1/3/2013. The planting bed was prepared by covering the old soil with black polyethylene adding new soil and peatmoss mixture, bed configuration, soften, settle and sterilization. The potted *ex vitro* plants derived from each preservation methods and periods for all cultivars were then planted on three panels (4.0m length and 2.0m width), at 20x20 cm planting dimensions. Drip irrigation system was extended, A preventive program to combat some fungal and insect infections was conducted asrecommended.Furthermore, all agricultural operations were done as recommended for potato crop (22).until it reaches the production stage. Before the shoots dry and yellowing, vegetative growth parameters were measured as : Average of Number of leaves/plant, Average of Number of stem/plant, Average of plant height (cm), Plant Leaf Area.(d. cm^2 / plant). It was calculated as (33), Dry weight of shoots (g) It was measured two weeks before tuber harvesting. Plants were cut from crown area and placed in a ventilated room for air drying, followed by heat drying in electric oven (60°C) until weight constancy. Then dry weight of plants was determined. Tuber harvesting has been done in 1/6/2013 and yield parameters were calculated: Number of tubers /plant extracted from dividing the number of tubers /tuber number and excluded infected and distorted tubers and small diameter tubers less than 2.5 cm, Average of tubers weight (g/tuber). It was calculated by dividing the weight of quotient by the number of tubers of plants, Yield of tubers weight (g/plant). It was calculated by dividing the weight of quotient by the number of plants. The experiments were designed as split plot with using Complete Randomize Block Design (RCBD). Results were analyzed using the statistical program Genstat Discovery 4th Edition, 2012 and comparing of means using Less Significant Difference (LSD) at the level of 5% probability (6).

RESULT AND DISCUSSION

Acclimation: Acclimatization results gave the highest rate of traits under study for seedlings resulting from cultured on MS media supplemented 40g/l mannitol (results not shown).

Evaluation of the field performance and productive of seedlings:

1. Number of leaves/plant

Results in table (1) show that there were no significant differences between the preservation periods under study in plant Number of leaves/plant, and there were no significant differences between the cultivars Sante and Emma that gave 22.51 and 22.57 leaves/plant respectively, we also noted that Arnova significant superiority in number of leaves and gave 24.50 leaf /plant. Also results show that there were significant different between the two preservation methods, Slow growth and Microtuber. The first one significant superiority in the plant Number of leaves/plant and gave 25.94 leaf/plant, while the second one gave the lowest value 19.74 leaf /plant. For the interaction between the cultivars and preservation periods, we noted that Arnova preserved for 2 months distinction and gave highest leaf number average 25.14 leaf/plant. As for the interaction between preservation periods and the preservation methods on Number of leaves/plant, the results suggest that plants result from slow growth preservation method for 6 months gave highest leaf number average 28.37 leaf/plant which wasn't significant differences with plants result from slow growth preservation method for 4 months 25.86 leaf /plant. While plants result from microtuber preservation method for 6 months lowest leaf number average 17.87 leaves /plant. For the interaction between the cultivars and preservation methods in the plant Number of leaves/plant, the results suggest the highest leaf number average for Arnova preserved by slow growth method (30.03 leaf /plant) which was significant differences with other interactions. While Emma preserved by microtuber lowest leaf number average 17.05

leaf/plant. According to triple interaction, the results in table (1) show that high leaf number average for Arnova plants preserved by slow growth method for 6 months 30.77 leaf /plant, which wasn't significant differences when preserved the same cultivar by the same method for 4 months 29.10 1eaf/plant, and for 2 months (30.22 leaf/plant) and Emma plants preserved by same method for 6 months (30.27 leaf/plant) but they were significant different with all other interactions. While Emma plants preserved by microtuber for 6 months lowest leaf number average (13.17 leaf/plant) which wasn't significant differences when preserved the same cultivar by the same method for 4 months (16.25 1eaf/plant).

2. Leaf Area (d.cm^2/ plant)

Results in table (2) show that there were no significant differences between the preservation periods under study in plant leaf area, and Arnova significant superiorited in leaf area and gave 29.30 d.cm^2/plant there were no significant differences between the cultivars Sante and Emma that gave leaf area 28.44 and 28.10 d.cm^2 /plant respectively. Also results show that there were significant difference between preservation methods, slow growth, microtuber and control. Slow growth method significant superiority in the plant leaf area (33.41 d.cm^2/plant) and microtuber method gave the lowest leaf area (24.86 d.cm^2/plant). For the interaction between the cultivars and preservation, we noted that there were no significant different between all interactions. As for the interaction between preservation periods and the preservation methods, the results suggest that plants result from slow growth preservation method for 6 months gave highest leaf area 33.63 d.cm^2/plant which wasn't significant differences with plants result from slow growth preservation method for 4 months and 2 months 33.34, 33.26 d.cm^2/plant respectively. While plants result from microtuber preservation method for all preservation periods under study significant different with control and slow growth preservation and plants preserved for 6 months gave lowest leaf area 24.61 d.cm^2/plant. For the interaction between the cultivars and preservation methods, the results suggest the highest leaf area for Arnova preserved by slow growth method 34.18 d.cm^2/plant which was significant differences with other interactions. While sante cultivar preserved by microtuber lowest leaf area 24.72 d.cm^2/plant which wasn't significant differences with Emma and Arnova preserved by the same method. According to triple interaction, the results in table (2) show that high leaf area for Arnova plants preserved by slow growth method for 2 and 4 months 34.34 d.cm^2 /plant for both, which weren't significant differences than preserved the same cultivar by the same method for 6 months 33.87 d.cm^2/plant, and sante preserved by slow growth for 4 months 33.71 d.cm^2/p!ant and Emma preserved by same method for 6 months 33.38 d.cm^2/plant While Emma plants preserved by microtuber for 6 months had lowest leaf area 24.04 d.cm^2/plant.

3. Plant height (cm):

Results in Table (3) show that plants preserved for 2 months significant superiorited in plant height it average 83.48 cm which wasn't significant different with plants preserved for 4 months (82.20cm) but they show significant different with plants preserved for 6 months (77.73 cm) , We also note that there were no significant differences between Arnova and sante they were significant superiorited in plant flight (87.45 cm and 85.71 cm respectively), Emma gave lowest significant value average 70.24 cm in plant height. Also results shown that there were significant difference between preservation methods. Slow growth method significant superiorited in the plant height (88.25cm) with control (79.32 cm) and microtuber method (75.33 cm) which gave the lowest average. For the interaction between the cultivars and preservation periods in the plant height, we noted that Arnova preserved for 2 months superiorited in plant height and gave 92.29 cm which wasn't significant different with plants of same cultivar preserved for 4 months (87.80cm) and 6 months (82.27cm) ,and sante cultivar preserved for 2 months (88.85 cm) and 4 months (87.96 cm), while Emma preserved for 2 months gave lowest average (69.30 cm) which wasn't significant different with plants of same cultivar preserved for 4 months (70.83cm) and 6 months (70.60cm). As for the interaction between preservation periods and

the preservation methods on the plant height, the results shown that the highest value of plant height was in slow growth preservation method for 4 months it average 90.36 cm. While the lowest value of plant height was in microtuber preservation method for 4 months and 6 months (76.90, 66.10cm respectively). For the interaction between the cultivars and preservation methods, the results suggest the highest average for Arnova preserved by slow growth method (101.37 cm) which was significant differences with other interactions. While Emma preserved by microtuber lowest average (48.11 cm). According to triple interaction, the results in table (3) shown that high average of plant height for Arnova plants preserved by slow growth method for 2 months (103.50 cm), which weren't significant different when preserved the same cultivar by the same method for 6 months (101.35cm) and 4 months (99.27cm), and same cultivar plants preserved by microtuber for 2 months (97.75 cm).While Emma plants preserved by microtuber for 6 months geve lowest average of plant height (43.72cm).

Table1. Effect of preservation methods and preservation periods for three potato cultivar on the Number of Leaves/plant

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	23.77	30.27	13 17	22.40	23.11
	Sante	24.07	24.07	19.90	22.68	
	Arnova	21.40	30 77	20.55	24.24	
4 months	Emma	23.77	23.02	16 25	21.01	22.70
	Sante	24.07	24.92	19.92	22.97	
	Arnova	21.40	29.10	21.85	24.11	
2 months	Emma	23.77	20.02	21.72	21.84	22.95
	Sante	24.07	21.10	20.50	21.89	
	Arnova	21.40	30.22	23.80	25.14	
LSD 5%		3.34			3.61	1.11
Preservation methods average		23.08	25.94	19 74	cultivars average	
LSD.5%		1.11				
Cultivars x Preservation methods	Emma	23.77	24.44	17.05	21.75	
	Sante	24.07	23.36	20.10	22.51	
	Arnova	21.40	30.03	22.06	24.50	
LSD.5%		2.44			NS	
Preservation methods x Preservation Periods	6 months	23.08	28.37	17 87		
	4 months	23.08	25.68	19.34		
	2 months	23.08	23.78	22.00		
LSD 5%		2.80				

Table2. Effect of preservation methods and preservation periods for three potato cultivar on Leaf area (d.cm^2/plant)

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	26.76	37.38	24.04	28.06	28.60
	Sante	27.29	33.64	24.69	28.54	
	Arnova	28.65	33.87	25.10	29.21	
4 months	Emma	26.76	31.96	25.16	27.96	28.52
	Sante	27.29	33.71	24.51	28.50	
	Arnova	28.65	34.34	24.33	29.11	
2 months	Emma	26.76	32.86	25.19	28.27	28.71
	Sante	27.29	32.57	24.98	28.28	
	Arnova	28.65	34.34	25.75	29.58	
LSD 5%		1.03			NS	NS
Preservation methods average		27.57	33 41	24.86	cultivars average	
LSD.5%		0.34				
Cultivars x Preservation methods	Emma	26.76	32.73	24.80	28.10	
	Sante	27.29	33.31	24.72	28.44	
	Arnova	28.56	34.18	25.06	29.30	
LSD.5%		0.63			0.34	
Preservation methods x Preservation Periods	6 months	27.57	33.63	24.61		
	4 months	27.56	33.34	24.66		
	2 months	27.56	33.26	25.31		
LSD 5%		0.78				

Table 3. Effect of preservation methods and preservation periods for three potato cultivar on Plant height (cm)

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	86.25	81.82	43 72	70.60	77.73
	Sante	76.10	80.12	84.72	80.31	
	Arnova	75.62	101.35	69.85	82.27	
4 months	Emma	86.25	80.10	46.15	70.83	82.20
	Sante	76.10	91 72	96.07	87.96	
	Arnova	75.62	99.27	88.50	87.80	
2 months	Emma	86.25	67.20	54 47	69.30	83.38
	Sante	76.10	93 70	96.77	88.85	
	Arnova	75.62	103.50	97.75	92.29	
LSD 5%			6.69		11.41	2.23
Preservation methods average		79.32	88.75	75.33	cultivars average	
LSD.5%			2,23			
Cultivars * Preservation methods	Emma	86.25	76.37	48.11	70.24	
	Sante	76.10	88.51	92.52	85.71	
	Arnova	75.62	101.37	85.36	87.15	
LSD.5%			5.83		2.23	
Preservation methods x Preservation Periods	6 months	79.32	87.76	66.10		
	4 months	79.22	90.36	76.90		
	2 months	79.32	88.13	83.00		
LSD 5%			12.10			

4. Number of stem/plant

Results in Table (4) show that there were significant difference between all preservation periods under study, the plants that preserved for 4 months gave the highest Number of stem/plant (4.30) and plants preserved for 2 months lowest number of stem/plant (3.65), We also note that there were significant differences between all cultivars under study, sante superiorited in Number of stem/plant (4.53), while Arnova gave lowest average of stems number (3.49). Also results show that there were significant different between preservation methods, Control treatment significant superiorited in the Number of stem/plant (4.94) than slow growth method (4.31) and microtuber method (2.84) which gave the lowest average. For the interaction between the cultivars and preservation periods, we noted that sante preserved for 4 months superiorited in Number of stem/plant and gave 4.94 which wasn't significant different with plants of same cultivar preserved for 2 months (4.53) and 6 months (4.11),and with Emma preserved for 2 months (4.48stem) and 4 months (4.15stem). while Arnova preserved for 6 months gave lowest average (3.28 stem). As for the interaction between preservation periods and the preservation methods, the results shown that plants from slow growth preservation method for 4months gave highest average (4.95) which wasn't significant different with control plant. While plants from microtuber preservation method for 6 months gave lowest average 2.29 stem/plant. For the interaction between the cultivars and preservation methods, the results shown the highest average for control Emmas (5.62) which wasn't significant different with sante (5.50), While Arnova preserved by microtuber lowest average (2.46stem/plant). And all cultivars under study preserved by slow growth methods were significant different between themselves. According to triple interaction, the results in table (4) show that high average of plant height for sante plants preserved by slow growth method for 4 months gave 5.77 stem/plant, which weren't significant difference with control same cultivar (5.50)and Emma control (5.62 stem/plant). While Arnova plants preserved by microtuber for 6 months gave lowest average 1.97 stem/plant which wasn't significant different with sante preserved by same method for same period (2.35 stem/plant).

5. Dry weight of shoots (g)

Results in table (5) show that there were no significant difference between all preservation periods under study in dry weight of shoots (g), We also note that Arnova significant superiority in dry weight of shoots (45.30 g), while there were no significant different between sante and Emma they gave 43.12 g and 43.07 g respectively. Also results shown that plants preserved by slow growth significant superiorited in dry weight of shoots (51.70 g) while there were no significant difference between control and microtuber

preservation they gave the lowest value of dry wieght of shoots. For the interaction between the cultivars and preservation periods in dry weight of shoots, we noted that there were no significant different between all interactions. As for the interaction between preservation periods and the preservation methods, the results shown that result plants from slow growth preservation method for 6 months gave highest average of dry weight of shoots (53.11 g) which wasn't significant different when preserved by same method for 4 months (52.00g). While result plants from microtuber preservation method for 6 months gave lowest average of dry weight of shoots (38.74 g). For the interaction between the cultivars and preservation methods, the results shown in Table (35) suggest the highest average of dry weight of shoots for Arnova preserved by slow growth (53.92g) which was significant different with all interactions. While control sante gave lowest average of dry weight of shoots (38.86g). According to triple interaction, the results in table (5) show that high average of dry weight of shoots for Arnova plants preserved by slow growth method for 6 months (55.23g), which was significant different with all interactions, While Emma plants preserved by microtuber for 6 months gave lowest average of dry weight of shoots (38.62 g). Carbohydrates works to increase leaf area through its positive role in the growth and development of vegetative and increase the number of branches, which consequently leads to increase in the leaves area and consumption the surplus of nutrients in the vegetative growth which involved in many biological processes and stimulate physiological or to do that which have a relationship within the plant manufactures food or stimulating cell division and elongation and installation of cellular membranes which lead to increased vegetative growth and leaf area and plant dry weight. The increase in dry weight of shoots may return to the role of sugars as it gives an opportunity to the accumulation of these elements and materials manufactured process of photosynthesis, such as carbohydrates and proteins in the tissues of the plant, which is one of the components of the dry matter in the plant, which, leading to increased dry weight of vegetative growth. The increase in the number and lengths of the branch of plants as well as increased vegetative growth and roots will increase the plant uptake of nutrients and thus increase the efficiency of the process of representation and increased carbon-manufactured materials accumulated in the plant like starch and sugars, thereby increasing the total dry weight of vegetative potato.

Table 4. Effect of preservation methods and preservation periods for three potato cultivar on number of stem/plant.

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	5.62	2.52	2.55	3.56	3.65
	Sante	5.50	4.50	2.35	4.11	
	Arnova	3.70	4.17	1.97	3.28	
4 months	Emma	5.62	4.05	2.80	4.15	4.30
	Sante	5.50	5.77	3.55	4.94	
	Arnova	3.70	5.05	2.65	3.80	
2 months	Emma	5 62	4.22	3.60	4.48	4.14
	Sante	5.50	4.77	3.32	4.53	
	Arnova	3.70	3.75	2.77	3.40	
LSD 5%			0.43		0.92	0.14
Preservation methods average		4.94	4.31	2.54	cultivars average	
LSD.5%			2.23			
Cultivars x Preservation methods	Emma	5.62	3.60	2.98	4.06	
	Sante	5.50	5.01	3.07	4.53	
	Arnova	3.70	4.32	2.46	3.49	
LSD.5%			0.44		0.14	
Preservation methods x Preservation Periods	6 months	4.94	3.73	2.29		
	4 months	4.04	4 95	3.00		
	2 months	4.94	4.25	3.23		
LSD 5%			0.62			

Table 5. Effect of preservation methods and preservation periods for three potato cultivar on Dry weight of shoots (g)

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	39.17	51.71	38.62	43.17	43.89
	Sante	38.86	52.40	38.70	43.32	
	Arnova	41.40	55.23	38.91	45.18	
4 months	Emma	39.17	50.97	39.14	43.09	43.81
	Sante	38.86	51.35	39.54	43.25	
	Arnova	41.40	53.68	40.13	45.07	
2 months	Emma	39.17	48.71	41.07	42.97	43.80
	Sante	38.86	48.41	41.08	42.78	
	Arnova	41.40	52.86	42.68	45.65	
LSD 5%			1.49		NS	NS
Preservation methods average		39.81	51.70	39.98	cultivars average	
LSD.5%			0.49			
Cultivars x Preservation methods	Emma	39.17	50.46	39.59	43.07	
	Sante	38.86	50.72	39.77	43.12	
	Arnova	41.40	53.92	40.57	45.30	
LSD.5%			1.19		0.49	
Preservation methods x Preservation Periods	6 months	39.81	53.11	38.74		
	4 months	39.81	52.00	39.60		
	2 months	39.81	50.00	41.59		
LSD 5%			1.27			

6. Number of tubers (tuber/plant)

Results in table (6) show that preserved plants for 6 months significant superiorited in number of tubers and gave 5.49 tuber , while there were no significant different between the plants that preserved for 2 months (4.99 tuber) and for 4 months (4.89 tuber) , We also note that Emma significant superiorited in number of tubers and gave 5.28 tuber which wasn't significant different with Arnova (5.20 tuber), but it was significant different with sante that gave lowest average of number of tubers (4.89 tuber). Also results show that there were significant different between preservation methods. Slow growth method significant superiorited in the number of tubers (6.64 tuber) with control treatment (6.24 tuber) and microtuber method (2.49 tuber) which gave the lowest average. For the interaction between the cultivars and preservation periods in the number of tubers, we noted that there were no significant different between all interactions. As for the interaction between preservation periods and the preservation methods, the results shown that result plants from slow growth preservation method for 6 months gave highest average of number of tubers 7.56 tuber which was significant different with all interactions. While result plants from microtuber preservation method for 4 months gave lowest number of tubers 2.36 tuber. For the interaction between the cultivars and preservation methods in the number of tubers, the results shown the highest number of tubers for Emma preserved by slow growth (7.10 tuber) which wasn't significant different with sante and Arnova preserved by same method 6.42 tuber and 6.40 tuber respectively and control Arnova 6.90 tuber. While Arnova preserved by microtuber gave lowest number of tubers 2.31 tuber. All cultivars under study preserved by microtuber methods weren't significant different between themselves in number of tubers. According to triple interaction, the results in table (6) show that high respectively for sante plants preserved by slow growth method for 6 months (7.72 tuber). While sante plants preserved by microtuber for 2 months lowest number of tubers 2.05 tuber which wasn't significant different with Arnova and Emmas preserved by same method for same period (2.22 tuber and 3.05 tuber respectively) and all three cultivars under study preserved by same methods for 6 and 4 months.

7. Average of tubers weight (g/tuber)

Results in table (7) show that plants preserved for 6 month significant superiorited in average of tubers weight and gave 79.36 g which wasn't significant different with plant preserved for 4 months 78.48 g, while plants that preserved for 2 months gave lowest average 75.95 g, We also note that Emma significant superiorited in average of tubers weight (79.50 g), while there were no significant difference between sante and Arnova that gave 77.49 g and 78.81 g respectively. Also results shown that there

were significant different between preservation methods. Slow growth method significant superiorited in average of tubers weight (86.61 g) with control treatment (81.17 g) and microtuber method (66.02 g) that gave the lowest average. For the interaction between the cultivars and preservation periods, we noted that there were no significant different between all interactions. As for the interaction between preservation periods and the preservation methods, the results suggest that plants from slow growth preservation method for 6 months gave highest of tubers weight (88.99g) which wasn't significant different when preserved by same method for 4 months (88.64g). While result plants from microtuber preservation method for 2 months gave lowest of tubers weight (64.52 g). For the interaction between the cultivars and preservation, the results suggest the highest tubers weight for Emma preserved by slow growth (90.34g) which was significant different with all interactions. While sante preserved by microtuber method gave lowest average of tubers weight (64.99 g) which wasn't significant difference with Emma and Arnova preserved by same method (67.88g and 65.20 g respectively). According to triple interaction, the results in table (7) show that high average of tubers weight for sante plant preserved by slow growth method for 6 months (92.67 g) which wasn't significant different with Emma preserved by same method and same period (90.97 g) and with 4 months (91.80 g), 2 months (88.25 g) and arnova preserved by same method for 4 months (90.65 g) while sante plant preserved by microtuber for 2 months which gave lowest average of tubers weight (61.62 g).

8. Yield per plant (g/plant)

Results in table (8) show that plants preserved for 6 month significant superiorited in average yield per plant and gave 454.77 g, while there were no significant different between plants preserved for 4 months (400.02 g) and 2 months (392.36 g), We also note that Emma significant superiorited in average yield per plant (436.02 g) which was significant difference with sante that gave lowest average (393.68g) but it wasn't significant different with Arnova that gave 417.44 g. Also results shown that there were significant different between preservation methods. Slow growth method significant superiorited in average yield per plant (574.34 g) than control treatment and microtuber which gave 506.72 and 166.09 g respectively. For interaction between cultivars and preservation, we noted that there were no significant different between all interactions. As for the interaction between preservation periods and preservation methods, the results shown that plants from slow growth preservation method for 6 months gave highest yield per plant (673.38g) which was significant different with all interaction. While plants from microtuber preservation method for 4 months gave lowest average yield per plant (155.38g). For the interaction between cultivars and preservation methods in the yield per plant, the results shown the highest value yield per plant for Emma preserved by slow growth (640.43g) which was significant different with all interactions. While Arnova preserved by microtuber method gave lowest average yield per plant (155.31 g) which wasn't significant different with Emma and sante cultivars preserved by same method (186.57 and 156.40 g respectively). According to triple interaction, the results in table (8) show that high value of yield per plant for sante plants preserved by slow growth method for 6 months (717.36g), which wasn't significant different with Emma and Arnova preserved by same method and same period (681.63 and 677.64 g respectively). While sante plants preserved by microtuber for 2 months gave lowest value of yield per plant (125.96 g) which wasn't significant different with all cultivars for preservation periods under study by same methods. The increase in the average weight of tuber per increases with increasing mannitol concentration accompanies this increase is to increase the number of tubers formed on the plant thus reason to believe that an increase in nutrients as a result of increasing the amount of mannitol and provide a great surplus of the processed food that move around to the tubers, a storage places for materials like carbohydrates and starch. In addition to the increase leaf area and therefore action on the composition of vegetative group is able to carry out its functions, which works to increase the surplus to configure the number of

tubers with great weight in addition to large numbers of tubers formed. This result is consistent with what was said (19), which indicated that increasing the number of branch lead to increase the number of tubers formed on the plant and thus increase the amount of production per plant (3).

Table 6. Effect of preservation methods and preservation periods for three potato cultivar on Number of tubers (tuber/plant)

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	5.97	7.50	2.87	5.45	5.49
	Sante	5.85	7.72	2.57	5.38	
	Arnova	6.90	7.47	2.55	5.64	
4 months	Emma	5.97	6.12	2.35	4.81	4.99
	Sante	5.85	6.10	2.57	4.84	
	Arnova	6.90	5.97	2.17	5.01	
2 months	Emma	5.97	7.70	3.05	5.57	4.89
	Sante	5.85	5.45	2 05	4.45	
	Arnova	6.90	5.77	2.22	4.96	
LSD 5%			1.09		NS	0.36
Preservation methods average		6.24	6 64	2.49	cultivars average	
LSD.5%			0.36			
Cultivars x Preservation methods	Emma	5.97	7.10	2.75	5.28	
	Sante	5.85	6.42	2.40	4.89	
	Arnova	6.90	6.40	2.31	5.20	
LSD.5%			0.70		0.36	
Preservation methods x Preservation Periods	6 months	6.24	7.56	2.66		
	4 months	6.24	6.06	2.36		
	2 months	6.24	6.30	2.44		
LSD 5%			0.69			

Table 7. Effect of preservation methods and preservation periods for three potato cultivar on average of tubers weight (g / tuber).

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	80.30	90.97	66.92	79.40	79.36
	Sante	81.17	92.67	67.90	80.58	
	Arnova	82.05	83.32	68.97	78.11	
4 months	Emma	80.30	91.80	68.25	80.11	78.48
	Sante	81.17	83.47	65.45	76.70	
	Arnova	82.05	90.56	63.17	78.62	
2 months	Emma	80.30	88.25	68.47	79.00	75.97
	Sante	81.17	82.77	61.62	75.19	
	Arnova	82.05	75.62	63.47	73.71	
LSD 5%			4,56		NS	1,52
Preservation methods average		81.17	86.61	66.02	cultivars average	
LSD.5%			1.52			
Cultivars x Preservation methods	Emma	80.30	90.34	67.88	79.50	
	Sante	81.17	86.30	64.99	77.49	
	Arnova	82.05	83.20	65.20	76.81	
LSD.5%			3.42		1.52	
Preservation methods x Preservation Periods	6 months	81.17	88.99	67.93		
	4 months	81.17	88.64	65.62		
	2 months	81.17	82.21	64.52		
LSD 5%			3.38			

Table 8. Effect of preservation methods and preservation periods for three potato cultivar on Average yield per plant (g/plant)

Preservation periods	cultivars	Preservation methods			Preservation periods x cultivars	Preservation periods average
		control	Slow growth	micro tubers		
6 months	Emma	381.07	681.63	190.68	451.13	454 77
	Sante	475.48	717.30	174.28	455.71	
	Arnova	563.60	621.15	187.68	457.4S	
4 months	Emma	481.07	562.02	160.20	401.10	400.02
	Sante	475.48	510.61	168.96	385.02	
	Arnova	563.60	541.23	136.99	413.94	
2 months	Emma	481.07	677.64	208.83	455.85	392 36
	Sante	475.48	419.54	125.95	340.33	
	Arnova	563.60	437.90	141.25	380.92	
LSD 5%		90.10			NS	30.03
Preservation methods average		506.72	574.34	166.09	cultivars average	
LSD.5%		30.03				
Cultivars x Preservation methods	Emma	481.07	640.43	186.57	456.02	
	Sante	475.48	549.17	156.40	393.68	
	Arnova	563.60	533.43	155.31	417.44	
LSD.5%		65.49			30.03	
Preservation methods x Preservation Periods	6 months	506.72	673.38	184.21		
	4 months	506.72	537.95	155.38		
	2 months	506.72	511.69	158.68		
LSD 5%		63.34				

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