Available online at sjuoz.uoz.edu.krd

journals.uoz.edu.krd

Science Journal of University of Zakho

Vol. 5, No. 1, pp. 53 –56, March-2017

p-ISSN: 2410-7549
e-ISSN: 2414-6943

THE EFFECTS OF VITAMINS ON MICROPROPAGATION OF DESIREE AND MOZART POTATOES (*SOLANUM TUBEROSUM* L.)

Fareed K. Yaseen [a,*], Rafail S. Toma [b], Daniela Carbonera [c]

[a] Dept. of Biology, Faculty of Science, University of Zakho, Kurdistan Region – Iraq.
[b] Dept of Horticulture, College of Agriculture, University of Duhok, Kurdistan Region – Iraq.
[c] Dipartimento di Biologia e Biotecnologie 'L. Spallanzani', via Ferrata 1, 27100 Pavia-Italy.

Received: Nov. 2016 / Accepted: Mar. 2017 / Published: Mar. 2017 ***https://doi.org/10.25271/2017.5.1.301***

ABSTRACT:
An attempt was done to achieve a micropropagation protocol by determining the most optimal types and concentrations of vitamins including thiamine (B1), nicotinic acid (B3), pyridoxine (B6) and folic acid to induce shoot formation and rooting *in vitro*, as replacement vitamins in the culture medium. The four vitamins were included in the medium at two concentrations and the control treatment without vitamins. The highest number of shoots per explant (2.50 shoots/ explant) was achieved from the addition of 0.3 mgl^{-1}thiamin to Desiree cultivar grown on MS medium. Whereas, the highest mean length of shoots was recorded in the case of adding nicotinic acid at 3.0 mgl^{-1} to Mozart cultivar grown on MS medium. The highest number of leaves 10.50 leaves/ explant) was obtained from Mozart cultivar grown on a medium enriched with 0.6 mgl^{-1} pyridoxine. The highest number of roots (7.00 roots/ explant) was achieved from the control treatment from Desiree potato cultivar. The longest roots (12.50 cm) were produced by Desiree potato cultivar grown on MS medium supplemented with 0.5 mgl-1 thiamin. Mozart potato cultivar performed better than Desiree cultivar in concern to mean length of shoots, number of leaves, number of roots and mean length of roots. No significant differences were found between the both cultivars in concern to the number of shoots and roots.

KEYWORDS: Potato, in Vitro, Vitamins, Thiamine, Nicotinic Acid, Pyridoxyne, Folic Acid.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered as one of the important food crops in the world (Lemaga *et al*,. 2009). It comes first in the world from non-grain crops to ensure food security (Ethiopian variety registers, 2006). In plant tissue culture media, vitamins including thiamine, nicotinic acid, pyridoxine and myo-inositol are usually found in Murashige and Skoog (MS) medium at 0.1 mg·l^{-1}, 0.5 mg·l^{-1}, 0.5 mg·l^{-1}, and 100 mg·l^{-1} respectively are the most commonly used (Murashige and Skoog, 1962). Potato micropropagation requires various additives in the culture medium including inorganic salts and organic compounds like growth regulators, amino acids, carbohydrates and vitamins. Vitamins are compounds required by living organisms in very small amounts as necessary ancillary food factors. Their absence from the diet leads to abnormal growth, development and an unhealthy condition. Many of the same substances are also needed by plant cells as essential intermediates or metabolic catalysis but intact plants, unlike animals, are able to produce their own requirements (Abrahamian and Kantharajah, 2011).

Cultured plant cells and tissues can however become deficient in some factors; growth and survival is then improved by their addition to the culture medium. Plants synthesize vitamins endogenously and these are used as catalysts in various metabolic processes. When plant cells and tissues are grown *in vitro*, some essential vitamins are synthesized but only in suboptimal quantities. Hence, it is necessary to supplement the medium with required vitamins and amino acids to achieve the best growth of the tissue. Thiamine (B1), nicotinic acid (B3), pyridoxine (B6), calcium pentothenate (B5), and myo-inositol are used more often of these (Abrahamian and Kantharajah, 2011). Vitamins are divided into two main groups, the water-soluble (Ascorbic acid (C); thiamine (B1); riboflavin (B2); pyridoxine (B6); nicotinic acid; cobalamin (B12); folic acid; pan- tothenic acid (B5); biotin) and fat-soluble (A, D, E and K) vitamins (Horton, 2006). Thiamine is the basic vitamin required by all cells and tissues, and necessarily required by all cells for growth (Ohira, *etal*, .1976).

Nicotinic acid and pyridoxine are usually added to culture media but may not be essential for cell growth in many species (White, 1943). Other vitamins such as biotin, folic acid, ascorbic acid, pantothenic acid, vitamin E (tocophenol), riboflavin and p-aminobenzoic acid are also invariably used, particularly when cells are grown at very low population densities (Schneider,2005).

Their requirement by plant cell or tissue cultures is apparently negligible. Generally, these vitamins are added in the range of 0.1 to 10.0 mg l^{-1}. Vitamins (thiamine, pyridoxine, nicotinic acid, folic acid, and biotin) from a Bourgin and Nitsch (Bourgin, and Nitsch, 1967) media *in vitro* did not affect 16 cultivars, except one, of *Begonia x hiemalis* shoot and root formation (Welander, 977). In tissue culture media, effect of vitamins on *in vitro* organogenesis of Plant, thiamine nicotinic acid, pyridoxine and myo-inositol found in Murashige and Skoog (Murashige, and Skoog, 1962) (MS) medium at 0.1 mg.l^{-1}, 0.5 mg.l^{-1} and 100 mg.l^{-1} respectively are the most commonly used, while the addition of other essential vitamins to media is uncertain. Myo-inositol remains a controversial compound being classified either as a water-soluble plant vitamin or as a sugar alcohol (George, *et al*., 2008).

* Corresponding author

Earlier studies in pea embryos done by Ray (Ray, 1934) have shown that it is possible to achieve good *in vitro* growth by increasing vitamin C content. This finding cannot be broadly applied as some plants are less receptive to increasing concentrations of vitamin C, indicating more autotrophism than other plants (tomato and oat) (Bonner, 1937). It has also been noticed that adding biotin increased the shoot dry weights of peas, similar to the response observed in Ricciocarpus plants treated with pantothenic acid. Unlike other vitamins, thiamine additions to pea embryos *in vitro* affect rooting and shoot growth simultaneously. *In vitro* studies have shown that tomato roots are capable of exhibiting prolonged thiamine dependency (George *et al*., 2008).

2. MATERIALS AND METHODS

The nodal explants were divided into 1 -2 cm long segments, each with one leaf and an axillary bud. The explants were thoroughly disinfested by immersing in sodium hypochlorite (2.5%) for 10 minutes. The segments were transferred to culture jars containing the MS nutrient medium. The vitamins thiamine (B1), nicotinic acid (B3), pyridoxine (B6) and folic acid were tested for their ability to induce shoot formation and rooting *in vitro*, as replacement vitamins in the culture medium. The four vitamins were included in the medium at two concentrations. The concentrations of thiamine (B1) were 0.3 and 0.5 mgl^{-1}, nicotinic acid at 1 and 3 mgl^{-1}, pyridoxine and folic acid at 0.4 and 0.6 mgl^{-1} in addition to control treatment free of vitamins (Figure 1).

Moreover, all nodal segments (explants) were rooted with all treatments exploited. *In vitro* derived plantlets were germinated in pots containing compost, perlite and vermiculate (1:1:1) under mist conditions during 4-5 days. Then, the hardened plantlets were transferred to the greenhouse. The experiment was arranged as factorial based on completely randomized design (CRD). After 6 weeks. Data were analyzed using SAS software Ver.9.1. The means of treatments were compared using Duncan's Multiple Range Tests at 5 % probability level using a computerized program of SAS (SAS, 2001).

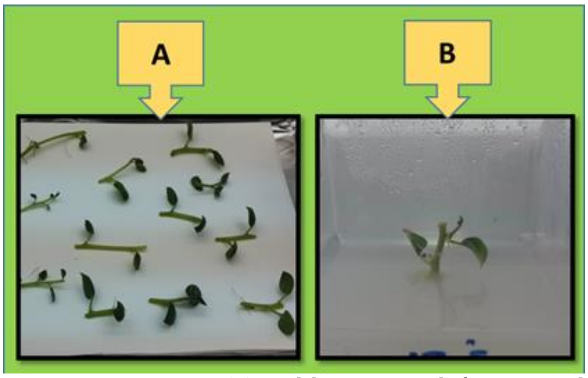

Figure 1. Initiation stage: (A); Nodal segments ready for *in vitro* culture. (B); Successfully established explants.

3. RESULTS AND DISCUSSION

Table (1) shows that the highest number of shoots per explant (2.50 shoots/ explant) was achieved from the addition of 0.3 mgl^{-1} thiamin to Desiree cultivar grown on MS medium. Whereas, the highest mean length of shoots was recorded in the case of adding nicotinic acid at 3.0 mgl^{-1} to Mozart cultivar grown on MS medium. The highest number of leaves 10.50 leaves/ explant) was obtained from Mozart cultivar grown on a medium enriched with 0.6 mgl^{-1} pyridoxine. The highest number of roots (7.00 roots/ explant) was achieved from the control treatment from Desiree potato cultivar. The longest roots (12.50 cm) were produced by Desiree potato cultivar grown on MS medium supplemented with 0.5 mgl^{-1} thiamin (Figures 2 and 3).

Table 1. Effect of various vitamins at different concentrations on Desiree and Mozart potatoes growth under *in vitro* conditions after 6 weeks.

Cultivars	Vitamins	Con. (mgl-1)	Number of Shoots/ Explant	Mean Length of Shoots	Number of Leaves/ Explants	Number of Roots/ explant	Mean Length of Roots (cm)
Mozart	Thiamin	0.3	1.75 ab	9.00 a-d	8.00 b-d	3.75 c-e	9.00 c-e
		0.5	1.75 ab	7.00 e-g	7.75 cd	2.75 e-g	9.50 b-e
	Nicotinic acid	1	1.75 ab	7.00 e-g	7.75 cd	3.75 c-e	11.50 ab
		3	1.50 ab	10.50 a	9.00 a-c	3.00 d-g	10.00 b-d
	pyridoxine	0.4	1.75 ab	6.75 fg	8.00 b-d	3.50 c-f	10.00 b-d
		0.6	1.75 ab	8.50 b-e	10.50 a	4.50 b-d	11.75 ab
	Folic acid	0.4	1.50 ab	9.50 a-c	10.25 ab	3.75 c-e	9.50 b-e
		0.6	1.50 ab	9.00 a-d	6.50 de	3.00 d-g	9.75 b-d
	Control	0	1.50 ab	8.50 b-e	6.25 de	5.50 ab	10.75 a-c
Desiree	Thiamin	0.3	2.50 a	8.00 c-f	8.50 a-d	3.25 d-g	6.25 fg
		0.5	1.25 b	9.75 ab	8.25 a-d	5.00 bc	12.50 a
	Nicotinic acid	1	1.50 ab	5.50 gh	3.75 fg	1.75 g	5.50 g
		3	1.25 b	5.25 h	3.00 g	1.75 g	6.50 fg
	pyridoxine	0.4	1.00 b	7.00 e-g	6.25 de	3.50 c-f	7.25 e-g
		0.6	1.50 ab	8.75 b-d	4.25 e-g	2.00 fg	9.75 b-d
	Folic acid	0.4	2.00 ab	7.75 d-f	6.00 d-f	3.75 c-e	7.75 d-g
		0.6	1.75 ab	7.00 e-g	3.75 fg	2.00 fg	8.25 d-f
	Control	0	1.50 ab	7.75 d-f	6.00 d-f	7.00 a	11.00 a-c

Mean within a column, row and their interaction following with the same latter are not significantly different according to Duncan multiple range test at the probability of 0.05 levels.

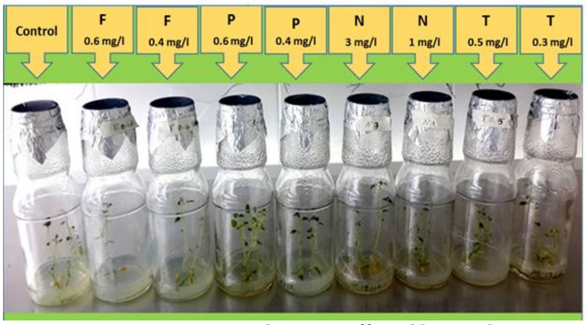

Figure 2. Desire potato cultivars as affected by pyridoxine, nicotinic acid, folic acid, thiamine (B1) vitamins grown on MS medium.

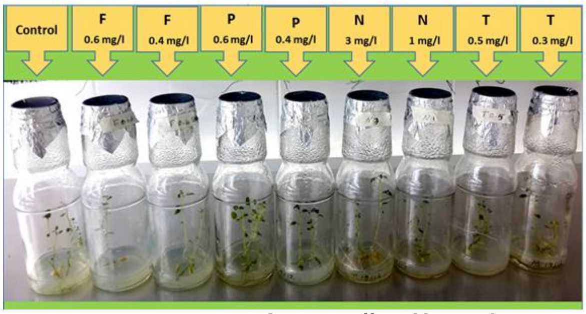

Figure 3. Mozart potato cultivars as affected by pyridoxine, nicotinic acid, folic acid, thiamine (B1) vitamins grown on MS medium.

Data in Table 2 declare that Mozart potato cultivar performed better than Desiree cultivar in concern to mean length of shoots, number of leaves, and number of roots and mean length of roots by recording 8.44 cm shoots, 7.33 leaves/ explant, 4.08 cm roots and10.33 roots/ explant respectively. Whereas, no significant differences were found between the both cultivars in concern to the number of shoots and roots.

Table 2. The response of Desiree and Mozart Potato Cultivars to in vitro Propagation

Cultivars	Number of Shoots/ Explant	Mean Length of Shoots (cm)	Number of Leaves/ Explants	Number of Roots/ explant	Mean Length of Roots (cm)
Mozart	1.60 a	8.44 a	7.73 a	4.08 a	10.33 a
Desiree	1.56 a	7.50 b	5.65 b	4.25 a	8.98 b

At acclimatization stage, the produced plantlets of both cultivars were successfully shifted from sugar-depend stage to autotrophic stage by following an accurate procedure for gradually moving the plantlets from the lab condition into out-air conditions. A survival rate of 100% was recorded from the successful acclimatized plants (Figure 4).

These results are in agreement with those published by Abrahamian and Kantharajah (2011) on the effects of vitamins on different plant species organogenesis *in vitro*.

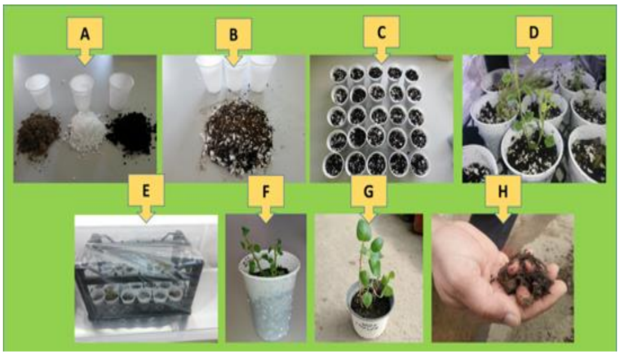

Figure 4. Potato acclimatization stage: (A); premixed culture medium (silt, styrophoam and peatmoss). (B); mixed culture medium. (C); pots filled with mixed culture medium. (D); initially acclimatized plantlets shifted into pots. (F); and (G); normally grown acclimatized plantlets. (H); microtubers produced by the plantlets.

The different response of potato cultivars to the different kinds and concentrations of vitamins might be due to the genotypes tested that usually responses variously to the endogenous additives in their growth and development. Being essential intermediates in biochemical reactions and as catalysts in various metabolic pathways in plant growth and development, vitamins usually differ in their effect on plant tissue culture performance according to type and concentration. Vitamins in tissue culture media must be further investigated in order to justify their essential addition. For example, a little bit is known about vitamin E (α-tocopherol), a phenol anti-oxidant, presence in tissue culture media. Recently, few research workers have been worked on certain vitamins, such as biotin and pantothenic acid. Potato cultivars require different amounts of vitamins, while others do not need any at all (Ohira *et al*. 1976).

REFERENCES

Abrahamian, P and A. Kantharajah (2011). Effect of vitamins on *in vitro* organogenesis of plants. *American Journal of Plant Sciences*, 2, 669-674

Bonner, J. (1937). “The Role of Vitamins in Plant Development,” Botanical Review, Vol. 3, No. 12, pp. 616-640.

Ethiopian variety registers (2006) Workshop on opportunities and challenges for promotion and sustainable production and protection of the potato crop in Vietnam and elsewhere in Asia. FAO, Addis Ababa, Ethopia 25-28.

Horton, R. (2006). “Coenzymes and Vitamins,” *Principles of Biochemistry*, Pearson Education International, Upper Saddle River,.

George, E.; Hall, M. and Klerk, G. (2008). “The Components of Plant Tissue Culture Media II,” Plant Propagation by Tissue Culture, Springer, Dordrecht, pp. 115-119.

Bourgin, J. and Nitsch,J. (1967). “Obtention de Nicotiana Haploïdes à Partir d’Étamines Cultivées *In Vitro*. (Produc- tion of Haploid *Nicotiana* from Excised Stamen),” *Annales de Physiologie Végétale*, Vol. 9, pp. 377-382.

Lemaga, B. Kakuhenzire, R. Gildemacher, P. Borus, D. Gebremedhin, WG. (2009) Current status and opportunities for improving the access to quality potato seed by small farmers in Eastern Africa. Symposium 15th triennial of the symposium of the International Society for Tropical Root Crops, Africa.

Murashige, T. and Skoog, F. (1962). “A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures” Physiologia Plantarum, Vol. 15, No. 3, pp.473-497.

Ohira, K.; Makoto, I. and Ojima, K. (1976).Thiamine requirements of various plant cells in suspension culture. Plant Cell Physiol.; 17(3): 583-590.

Ray, S. (1934). “On the Nature of the Precursor of the Vitamin C in the Vegetable Kingdom. I. Vitamin C in the Growing Pea Seedling,” Biochemical Journal, Vol. 28, No. 3, pp. 996-1003.

SAS. (2001). SAS/ STAT User’s Guide for Personal Computers. Release 6.12. SAS Institute Inc. Cary, NC, USA.

Schneider, C. (2005). Chemistry and biology of vitamin E. Mol Nutr Food Res 49:7–30

Welander, T. (1977). "*In Vitro* Organogenesis in Explants from Different Cultivars of Begonia x Hiemalis," Physiologia Plantarum, Vol. 41, No. 2, pp. 142-145.

White, P. (1943).Nutrient deficiency studies and improved inorganic nutrients for cultivation of excised tomato roots. Growth; 7: 53- 65.

کارتێکرنا ڤیتامینا ل سەر زێدەکرنا هویر یا بتاتێن (Solanum tuberosum L.) دیزیریە وموزارت

کورتیا لێکولینێ:

دئەڤێ ڤەکولینێ دا، هەولەك هاتە کرن ژبو بدەستڤەئینانا پروگرامەکێ زێدەکرنا هویر بو رووەکێ پتاتێ درێکا دیارکرنا باشترین جۆر وتیراتییێن ڤیتامینێن دهێنە زێدەکرن دناڤ بیاڤێ چاندنێ دا ئەوژی هەر ئێك ژ سیامین (B1) وترشێ نیکوتنیك (B3) وپیرودوکسین (B6) وترشێ فولیك ژبو پالدانا چێکرنا چقێن کەسك وریهدانێ دەرڤەی لەشێ زیندی. هەر چوار ڤیتامین هاتنە زێدەکرن دناڤ بیاڤێ چاندنێ دا ب دوو تیراتیا دگەل سەرەدەریا کونترولی. مەزنترین ژمار چقا (2.5 چق/ پارچا رووەکی) هاتنە بدەستڤەئینان ددەمێ بکارئینانا 0.3 ملگم/ لتر ژسایمینی بو پتاتێن دیزیریە ئەوێ هاتیە چاندن دبیاڤێ MS دا. درێژترین چق هاتنە تومارکرن ددەمێ بکارئینانا ترشێ نیکوتینیك (3.0 ملگم/ لتر) بو پتاتێن موزارت ئەوێ هاتیە چاندن دبیاڤێ MS دا. مەزنترین ژمارا بەلگان (10.5 بەلگ/ پارچا رووەکی) هاتنە بدەستڤەئینان ددەمێ چاندنا پتاتێن موزارت دبیاڤێ هاتیە زێدەکرن بـ 0.6 ملگم/ لتر ژپیرودوکسینی. مەزنترین ژمارا رویها (7.00 رویە/ پارچا رووەکی) هاتنە تومارکرن ژلایێ پتاتێن دیزیریە دگەل سەرەدەریا کونترولی. ودرێژترین رویە (12.5 سم) هاتنە تومارکرن ژلایێ پتاتێن دیزیرە هاتیە چاندن دناڤ بیاڤێ هاتیە بهێزکرن بـ 0.5 ملگم/ لتر ژسیامین. ب گشتی، پتاتێن موزارت دباشتربوون ژدیزیریە دسالوخەتێن رێژەیا درێژاهیا چقا وژمارا بەلگان وژمارا رویها ورێژەیا درێژاهیاوان. چ جیاوازیێن بەرچاڤ نەهاتنە تومارکرن دناڤبەرا هەردوو جۆرێن پتاتا دژمارا چقا ودیسان دژمارا رویهان دا.

تأثير الفيتامينات في الاكثار الدقيق لصنفي البطاطا (*Solanum tuberosum* L.) ديزريه وموزارت

خلاصة البحث:

تم من خلال هذه الدراسة محاولة الحصول على برنامج اكثار دقيق متكامل للبطاطا من خلال اختبار التوصل على أنسب أنواع وتراكيز الفيتامينات المضافة الى أوساط الزراعة والتي تضمنت الثيامين (B1) وحامض النيكوتينيك (B3) والبيرودوكسين (B6) وحامض الفوليك لتحفيز تكوين الأفرع الخضرية والتجذير خارج الجسم الحي. الفيتامينات الأربعة تمت اضافتها الى وسط الزراعة بتركيزين فضلاً عن معاملة المقارنة بدون اضافتها. تم الحصول على اكبر عدد من الأفرع (2.5 فرع/ جزء نباتي) عند اضافة 0.3 ملغم/ لتر من الثايمين لصنف ديزريه النامي في وسط MS. بينما تم تسجيل أطول الفروع في حالة اضافة حامض النيكوتينيك بتركيز 3.0 ملغم/ لتر لصنف موزارت النامي في وسط MS. أما أكبر عدد من الأوراق (10.5 أوراق/ جزء نباتي) فتم الحصول عليها من صنف موزارت النامي في الوسط المقوى بـ0.6 ملغم/ لتر من البيردوكسين. أكبر عدد من الجذور (7.00 جذور/ جزء نباتي) تم الحصول عليها من صنف ديزيريه عند معاملة المقارنة. أما أطول الجذور (12.5 سم) فقد تم تسجيلها لصنف ديزيريه النامي في الوسط المقوى بـ0.5 ملغم/ لتر من الثيامين. عموماً، فإن صنف موزارت قد قَدَّمَ أداءً أفضل من الصنف ديزيريه للمقاييس معدل طول الفروع وعدد الأوراق وعدد الجذور ومعدل أطوالها. لم يتم تسجيل أية فروقات معنوية بين كلا الصنفين لصفتي عدد الفروع أو الجذور.