EFFECT OF CALCIUM PANTOTHENATE ON POTATO PLANTS GROWTH AND SHOOT TIP NECROSIS, CV.SPUNTA

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ABSTRACT

The aim of study is to detect the effect of Calcium pantothenate on potatoplants growth and Shoot tip necrosis, cv. Spunta. Calcium pantothenate deficiency had no effect on potato plantlets growth rate, but it increased the percentage of shoot tip necrosis which is eliminated absolutely at calcium pantothenate concentration 3mlg/l, while there is no effect of increasing calcium pantothenate concentration more than 3mlg/l. Shoot tip necrosis scale was 2.6 in case of the absence of Calcium pantothenate, while it was (1.4, 0.6, 0 and 0 in case of Calcium pantothenate concentrations (1,2,3 and 4mg/l respectively). Calcium deficiency leads to an increase in shoot tip necrosis. So, the best concentration of Calcium pantothenate to prevent shoot tip necrosis is 3mg/l, while if its concentration becomes 4mg/l, there is no any toxic effect of Calcium pantothenate. There is no any significant difference among all treatments concerning all growth rate parameters, but, there is an evident significant difference between treatment 4(3 mg/l Calcium pantothenate) and all treatments of Calcium pantothenate except treatment 5(4mg/l Calcium pantothenate) concerning shoot tip necrosis. So, the concentration of (3mg/l Calcium pantothenate) is the best one because it is economically better.

Keywords: *Calcium pantothenate - Potato - Shoot tip necrosis – Micro propagation – In vitro.*

1. INTRODUCTION

Potato (*Solanum tubersoum L.*) is the most important non-cereal world food plant, and considered to be the fourth important crop in the world after

wheat, maize, and rice, **(Anon., 1996),** it is grown in 180 countries worldwide, (**Liljana *et al*., 2012**).

Potato tuber is a source of food in almost every nation in the world. The principal cultivated potato is a tetraploid species (2n = 4x = 48). Potato is one of the most important vegetable crops grown in Libya. The total cultivated area with potato was 15273 hectares in 2010 with a total production of 296068 tonnes, whilethe harvested area in 2016 was 17235 hectares with a total production of a total production of 337465 tonnes **(FAO Organization, 2018).**

Tubers contain around 75-80% water, 16-20% carbohydrates, 2.5-3.2 % crude protein, 0.8-1.2 % mineral nutrients, 0.1-0.2 % crude fats 0.6 % crude fiber and some vitamins **(Hooker, 1981).**

Potato is grown in a wide variety of climates and humidity ranges. Potato is from the economic point of view clearly an important food crop not only in terms of production but also in terms of nutrition and rural income. Also, it is considered as a good source of carbohydrates than any other food crop. However, its vulnerability to widely distributed diseases makes potato the heaviest user of chemical pesticides among the major crops **(Raman, 1994).**

The center of origin of potato in the high region of Anden in Lima, Peru, where the earliest attempts at cultivation were probably made 18 centuries B. C. **(Burton 1989).**

As for diseases, virus diseases, particularly leaf roll (PLRV) and PVY, are severe problems in Egypt and their aphid vectors is a primary goal of the seed import and production practices.

Salazar, (1977)reported that viruses and related agents seem to fall into two well-defined groups:

A- Viruses that depend on potato for their survival and spread generally have a restricted or moderate host range. Most of these viruses, however, appear to occur as many distinct strains in potato or host in the Solanaceae family, so there is probably a close association between them. This variability is commonly found in the Andean region, the main center of origin of potato.

B- Viruses that don't depend on potato for survival and spread and display a wide host range, only a few of their strains have been found in potato, these usually cause economically important diseases in other crop.

Tissue culture technique was accepted as successful mean of developing virus-free plants from stock of infected plant materials. This technique based on excising or isolating a virus free tissue that will be regenerate into a rooted plant.

Virus-free plants may be produced by tissue culture technique alone or in combination with treatments of chemotherapy or thermotherapy**(Mellor and Stace- Smith 1977).** Shoot tip necrosis (STN) disorder occurs in the *in vitro* multiplication **(Nezami *et al*., 2015).**

2. MATERIALS AND METHODS

Meristem culture and production of virus-free plantlets

First of all, potato tubers (cv. Spunta) were brought from the General Potato Growers Co-Operative (G.P.G.C.O) in Cairo, Egypt. Potato tubers were immersed in a solution of 20 ppm gibberellic acid (GA3) to break the dormancy phase of tubers for 10 minutes, then, they were stored in a growth room at 5°C- 8°C and 60- 70% relative humidity in dark. After about 2 months – sprouts, were grown (photo1). Potato tubers then were tested serologically to detect PVX, PVY and PLRV using [Enzyme-Linked Immuno Sorbent Assay (ELISA test)], according to the method described by Clark and Adams (1977),in the unit of plant virus detection (Toriel lab.) in the ministry of Agriculture in Giza, where, they were infected with PVX, PVY and PLRV. After ELISA test, sprouts were cut carefully from the same tested marked area on the tuber. These sprouts were washed using sterile distilled water, then sterilized under the aseptic conditions inside the culturecabinet (Laminar Air flow). Then, the sprouts were rinsed in 1% sodium hypo chloride for 20 minutes and washed about 5 times using sterile distilled water.

Then the sprouts were put under the binuclear microscope to separate themeristems (300-500um) with 2 leaf primordia

Figure 1. Sprouted tuber of potato (*Solanum tuberosum L.* cv. Spunta).

Preparation of culture room

To reduce contamination during culturing the following precautions were carried out: -

(1) U.V. light of the hood was lighted for about ½ hour before working.

(2) The inner surface of the hood was sterilized by ethyl alcohol.

(3) Scalpels and forceps were sterilized in the autoclave and then by ethyl alcohol and were flamed before and during culturing.

(4) Papers on which plants were cut must be sterilized in the autoclave before culturing.

Preparation of media on which meristems will grow

The basal nutrient medium which contains macro and microelements, vitamins and amino acids was prepared according to Murashige and Skoog (1962) as shown in Table (1).

The medium which is known as (MS medium) must be supplemented with 30 g/l Sucrose + 100 mg/l Myo-Inositol. The pH of the medium was adjusted at 5.7 $\pm$0.1 prior addition of Gelrite (1.75g/l), after putting media in tubes (25x150 mm), they must be autoclaved at 121°C at 15lb/I n^2 for 20 minutes.

TABLE (1) CHEMICAL INGREDIENT OF MURASHIGE AND SKOOG'S MEDIUM (*)

Solution	Constituents	Conc. In stock Solution g/l	Vol. Of stock solution in final medium ml/L.	Final conc. in medium mg/l.
A	NH4NO3	82.5	20	1650.0
B	KN O3	95.0	20	1900.0
C	H3B O3	1.24	5	6.0
	KI	34.0	-	0.83
	KH2PO4	0.166	-	170.0
	Na2MoO4.2H2O	0.050	-	0.25
	CoCl2.6H2o	0.005	-	0.025
D	CaCl2.2H2O	88.00	5	440.0
E	MgSO4.7H2O	74.0	5	370.0
	MnSO4.4H2O	4.46	-	22.3
	ZnSO4.7H2O	1.72	-	8.6
	CuSO4.5H2O	0.005	-	0.025

F	Na2.EDTA	7.45	5	37.35
	FeSO4.7H2O	5.57	-	27.85
G	Thiamin- HCl	0.02	5	0.10
	Nicotinic acid	0.10	-	0.50
	Pyridoxine-HCl	0.10	-	0.50
	Glycine	0.40	-	2.00

(*) According to Murashige and Skoog, (1962).

Preparation of media containing Virazole

To eradicate viruses from meristems, Virazole (1-ß-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) was added to MS medium in different concentrations (80, 100,120,150mg/l) at pH5.7$\pm$ 0.1 in a solid medium which is put in tubes, then meristems were placed on this medium under the hood. After 21 days, plantlets must be tested to detect viruses X, Y and PLRV, using the previous (ELISA) test. Finally, data were recorded to detect the effect of virazole on potato plants growth rate *in vitro.* The required growth rate parameters are: plant length, number of leaves, plant strength, number of roots, root length and shoot tip necrosis. For both plant strength and shoot tip necrosis, degrees were given to the plants using the method described by Ewase *et al.,* (2013). In this method, the plants were rated from culture on 0 to 5 relative growth scale as shown in Table (2).

TABLE (2). DESCRIPTIVE GRADES USED FOR DETERMINATION OF THE *IN VITRO* RELATIVE GROWTH OF POTATO EXPLANTS.

Rating	Description
0	The cultures turned brown and appeared to be invisible.
1	The culture stills green, showed no readily observable growth and development differentiation from the initially excised tissue.
2	One or two leaves were evident.
3	More than two leaves were evident.
4	The culture showed evidence of shoot formation and development.
5	Both shoots and roots have been developed.

Percentage of relative growth were calculated using the following formula:

$$\text{Relative growth \%} = \frac{\sum \text{ of individual ratings}}{\text{No. of explants assessed}} \times \frac{100}{5}$$

Nodal Cutting

After obtaining virus-free plants, these plants must be micro propagated to obtain a sufficient amount for carrying out all the required experiments. Excising nodal cuttings were about 2-3 cm in length with one or two axillary buds, these plantlets were obtained from the previous meristem culture. The plants were put in jars (350 ml) which contain MS medium (15

ml). Every 21 days it is preferable to carry out more nodal cuttings in jars or tubes (photo 2) because the plantlets will reach a high range of growth especially in lengths they may be subjected to death inside the jars.

Statistical analysis

Data of growth characters and micro tubers production were statistically analyzed by factor randomize. Complete designs and the means were compared using L.S.D. according to the method described by SAS (1990).

3. RESULTS AND DISCUSSION

Data in Table (3) indicated that Calcium pantothenate didn't have any effect on potato plant growth rate. This may be due to the fact that the Calcium amount in Calcium pantothenate is very small. The only effect of Calcium pantothenate deficiency in the media is an increase in shoot tip necrosis but in a percentage less than that in the case of Calcium chloride because the Calcium amount in Calcium chloride is much higher than that in Calcium pantothenate. Shoot tip necrosis was 2.6 in case of the absence of Calcium pantothenate, while it was (1.4, 0.6, 0 and 0.08 in case of Calcium pantothenate concentrations (1, 2, 3 and 4 mg/l respectively). Calcium deficiency leads to an increase in shoot tip necrosis as we discussed before in table (2) in the case of ($CaCl_2.2H_2O$). Data also showed that the best concentration of Calcium pantothenate to prevent shoot tip necrosis is 3mg/l, while if its concentration becomes 4mg/l, there is no toxic effect of Calcium pantothenate because the amount of Calcium in Calcium pantothenate is very little as we mentioned previously.

These results are similar to those of El-shobaky and Ibrahim (1999), who stated that Ca-Pantothenate had significant effect on shoot tip necrosis, 3mg/l produced the lower percentage of shoot tip necrosis, so, increasing Ca-Pantothenate concentration reduced shoot tip necrosis. Quite similar results were reported by (Sha*et al.*) 1985, Obordo and Zamora (1988) and Ammo *et al.* (1998).

There is no significant difference among all treatments concerning all growth parameters, but, there is an obvious significant difference between treatment concentration (3mg/l Calcium pantothenate) and all treatments of Calcium pantothenate except concentration 5 (4mg/l Calcium pantothenate) concerning shoot tip necrosis.

Also these results come in harmony with Ibrahim A. Ibrahim*et al*. (2016), who mentioned that the addition of 2 mg/l calcium pantothenate to MS medium promote potato plants growth.

TABLE (3). EFFECT OF CALCIUM PANTOTHENATE ON POTATO (*SOLANUM TUBEROSUM L.* CV. SPUNTA) PLANTS GROWTH *IN VITRO.*

Treatment concentration	Plant length (cm)	Leaves no.	Plant strength	Roots no.	Roots length (cm)	Shoot tip necrosis
Control (1)	9.4^{a}	9.8^{a}	4.0^{a}	7.1^{a}	8.5^{a}	2.6^{d}
(2) lmg/l Calcium	9.3^{a}	9.1^{a}	4.2^{a}	6.9^{a}	8.8^{a}	1.4^{c}

pantothenate						
(3) 2mg/l Calcium pantothenate	9.4[a]	9.2[a]	4.8[a]	7.2[a]	8.5[a]	0.6[b]
(4) 3mg/l Calcium pantothenate	9.2[a]	9.2[a]	5.0[a]	6.9[a]	8.2[a]	0.0[a]
(5) 4mg/l Calcium pantothenate	9.4[a]	9.6[a]	5.0[a]	7.4[a]	8.3[a]	0.0[a]
SE	0.31	0.33	0.03	0.32	0.24	0.08

Figure 2. Different potato plants growing *in vitro.*

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