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The Effect of Salicylic Acid on the Growth and Microtuberization of Potato (Solanum tuberosum L.) cv. Arizona Propagated in Vitro

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Abstract
Salicylic acid was employed in this study at levels (0, 50, 100, 150, 200 mg/L) in culture media for shoot development from the culture of single nodal segments, the results showed that the level (100 mg/L) was the best significantly in shoot number, leaf number, leaf area, root length and number, the significant effect of (100 mg/L) was also in total soluble protein (16.78 mg/gm) in shoots, whereas the levels (100,150, 200 mg/l) were the best significantly in chlorophyll content of the shoot leaves, the level (100 mg/L) was also affected significantly in the means of microtuber weight and number(1.094), (7.125) and total soluble protein (5.146 µg /gm) whereas the levels (100 and 150 mg/L) of salicylic acid in starch percentage (12.97, 12.99 %) were better significantly than control treatment (2.55 %).
Keywords: Salicylic acid, Potato. Microtuberization, *InVitro*

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most cultivated food crop after wheat, rice and maize (Moeinil *et al*, 2011).

Potato as a crop of high biological value for its protein and substantial amount of vitamin, minerals and trace elements, it is undoubtedly a very important crop in many countries (Gebre and Sathyanarayana, 2001). Some studies pointed out to the impotence of the presence of BA in the medium for the production large number of shoots and cause vegetative proliferation for a wide range of plants. (Hussey and Stace, 1981; Fandino *et al.*, 1990; Rosell *et al.*, 1987).

Al- Rubayi (2011) found the significant effect of kinetin, BA and 2,ip, each alone or in a combination with IAA (I mg/L) on the initiation of potato plantlets I*n Vitro*. Khadiga *et al* (2009) employed BA alone or in combination with NAA and using nadal explant of four cultivars Diamant, Agria, Alpha, almera cultured on MS medium. Salicylic acid which is a type of phenolic acid and a beta functions as plant hormone applied to induce flowering and tuberization (Jahanbazi *et al.*, 2014). Salicylic acid induces microtuberizationin potato (Koda, 1992)

Materials &Methods

1. Plant materials

Potato tubers of certified cv. Arizona were bringing from Basra directorate culture, the tubers were washed thoroughly, incubated in 20 –22 c° at 16 hours' photoperiod.

2. Preparation of media

A) Shoot initiation:
Liter of medium is prepared from MS basal salts Murashige & Skoog (1962) and following constituents were added in Meso- inositol (100 mg/L) and thiamin- Hcl (0.5). Sucrose (30000), glycine (2), Nicotinic acid (2), adenine sulfate (40), and agar (8000). The same media were used for shoot proliferation.

B) Media for salicylic acid treatments:
Liter of MS medium was prepared by the addition of MS basal salts supplemented with BA (0.5 mg/L) and IAA (0.5 mg/L), and then the solution is dispensed in to five parts for the different treatments of salicylic acid which was added in the following concentration: 0, 50, 100, 150 and 200 mg/L. The medium was dispensed in to tubes (12 replicates) for each treatment closed and incubated, cooled and stored for culture.

C) Media for microtubrization:
Liter of MS basal salts in addition to BA (3mg/L) sucrose (60 gm/L) was prepared and dispensed in 5 parts followed by the addition of salicylic acid (0.50,100,150, 200 mg/L) as five treatments.
Media were dispensed in to 100 ml-flasks, closed and autoclaved, cooled for culture.

3. Explant culture

A) The sprouts (5 mm) were sterilized with sodium hypochlorite 10% for 15 min, and washed three times and cultured on MS medium (A) in tubes (one sprout /tube) after incubation under 16 hrs. photoperiod at 24°C when shoots became 7–9 cm length, they were cut into single nodal segments (1cm) and cultured on the same medium for increasing the number of shoots.
B) Single nodal segments (1cm) were cultured on media (B), after two months, the shoots were 7 – 9 cm length,

plant height, shoot number, leaf number, leaf area (according Lutfi, 1986) length and number of roots were measured.

C) Single nodal segments (1cm) were cultured on media(c), microtubers were integrated after 75 days of culture period and the number, weight and diameter of microtuber were measured.

Biochemical studies

A) Shoots

1. Total dissolved protein (TSP) was estimated according to (Cresser and Parsons 1979).
2. Chlorophyll was estimated according (Zaehringer *et al*. (1974)

B) Microtubers

1. Total dissolved protein (TSP) was estimated according (Cresser and parsons 1979).
2. Starch percentage % was estimated according the following formula:

Percentage % = 17.55 + 0.891(% dry material – 24.18) (Al–Khazaly, 2000)

Statistical analysis

Significance was determined with one-way ANOVA (a=0.05) using the GenStat Statistical Packages.

Results & Discussion

Green thrive shoots were appeared after (30 days) (Fig1). These shoots were cut into single nodal segments for the following experiment treatments, however Hussey and Stacey (1981) obtained rooted plantlets by the culture meristem tip on MS medium supplemented with Meso – Inositol (1mg/L), thiamine – Hcl (0.5mg/L), pyridoxine – Hcl (1mg/L). Nicotinic acid (0.5 mg/L) pantothenic acid (2.5 mg/L)

Fig (1): shoot development after the culture of sprout buds .

1. The effect of salicylic acid on shoot development

The results in table (1) there is no significant differences in shoot lengths among all treatments, however (100 mg /L) salicylic acid is the best significant level among all treatments in shoot number, leaf number, leaf area, root number, root length with exception of (150 mg/L) in leaf number, leaf area, root length and also (150 mg/L) and (200 mg/L) in root number.

The significance of salicylic acid (100 mg/L) level in the presence of BA (1 mg/L) and IAA (1mg/L) for shoot development were in agreement with Galal (2012) who found that lower concentration of salicylic acid in culture media improved the proliferation efficiency of *Ziziphus spina* Christi tissue culture, however Sakhanokho and Kelly (2009) revealed that the positive effect of salicylic acid on the proliferation of plant tissue cultures could be are flection of an increase in the number of meristematic cells; BA was useful as used in lower concentrations in this study for shoot proliferation and development, some authors agreed with this result; Roca *et al*. (1978) obtained large number of plants for 38 cultivars of potato by tissue culture by separation of shoot

tips and cultured them on MS containing BA(0.8 – 2.2µ).

AL–Salihi (1994) found that the addition of BA to multiplication medium at level (1.5 – 2mg/L) cause the increase the number of shoots for several cultivars of potato (Famosa, Marfona, Mirka, Desiree). Liljana *et al*. (2012) used MS medium containing (2 mg/L) BA for rooted shoots production; They used also MS medium containing (4 mg/L) kinetin and (1mg/L) IAA and MS medium supplemented with 2 mg/L BA and (1mg/L) (NAA) for nodal explants to affect the formation of plantlets and microtubers of potato, however, lower concentrations of cytokinins were better for lateral bud proliferation than higher ones. (Al–Sulaiman and Barakat, 2010); Al–Rubaiee (2011) found the significant effect of kinetin BA and 2ip each alone and with combination of IAA (1mg/L) on the initiation of potato plantlets In Vitro.

Hussain *et al.* (2005) stated that the most suitable medium was MS with (2 mg/L) BA and IAA (0.5mg/L) giving maximum regeneration.

Table (1) effect of SA on shoot development of potato

Growth parameter / SA. conc.	Plant Length Cm	Shoot number	Leaf number	Leafarea mm2	Roor Length cm	Root number
Control	a 11.512	b 1.6	c 7.25	b 10	b 6.72	b 7.125
50	a 11.37	b 1.73	c 7.75	b 11	b 8	b 9.125
100	a 12.51	a 4.00	a 13.33	a 14	a 10.43	a 18.12
150	a 11.188	b 1.62	b 11.87	a 14	a 9.033	a 16.45
200	a 10.43	b 2.00	c 6.25	b 10	b 7.125	a 16.250

Means with similar letters indicate no significant difference at P<0.05.

SA conc.: Salicylic acid concentration

(100 mg/L) salicylic acid treatment was significant in TSP estimation of shoots

2.The effect of salicylic acid on biochemical components in shoots:

A) Total soluble protein (TSP) (µg/gm) dry weight.

The results showed that (16.78 µg/gm) whereas the control treatment is the least (10.51µg/gm) (Fig 2).

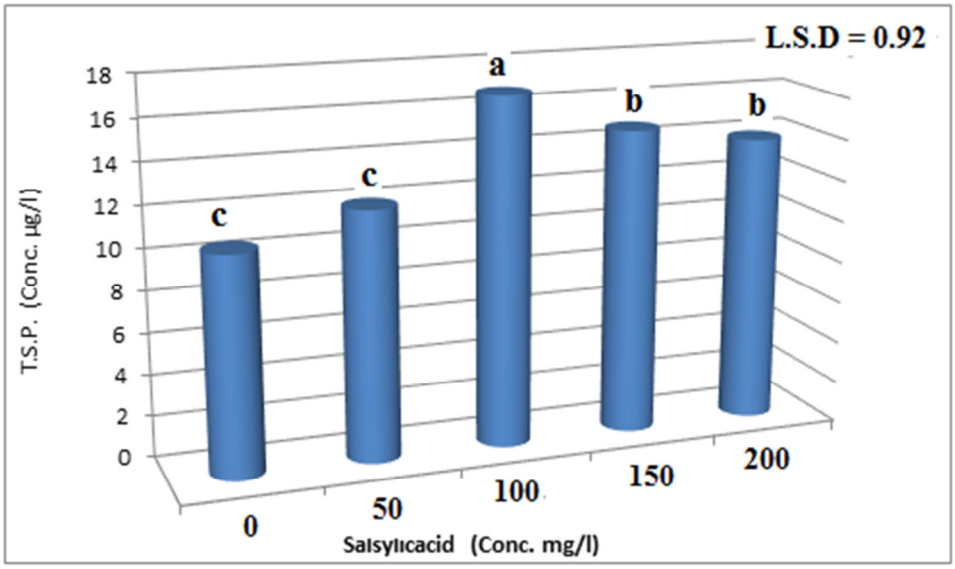

Fig (2): effect of salicylic acid on protein concentration in the plantlets leaves of potato cv. Arizona propagated *in vitro*.

B) Total chlorophyll content (mg/gm fresh weight) (100, 150, 200 mg/L) salicylic acid were the best significantly in total chlorophyll content in the leaves of shoots (1.58, 1.92, and 1.84 mg/gm) respectively, Fig 3.

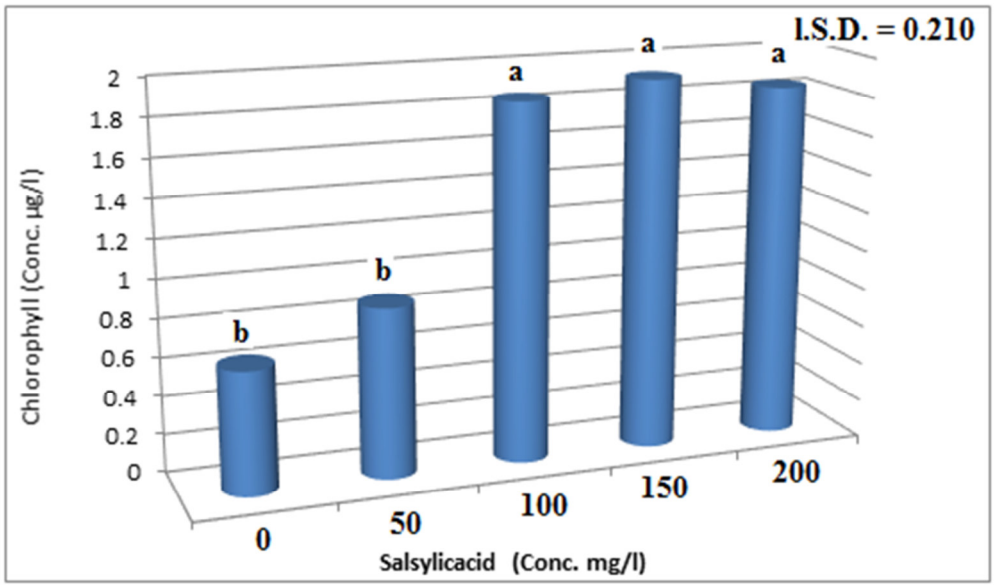

Fig (3): effect of salicylic acid on chlorophyll consent in the plantlets leaves of potato cv. Arizona propagated *in vitro*.

Generally, the significant effect of salicylic acid in agreement with Popova *et al*. (2008) who referred that the enhancement of the level of chlorophyll and carotenoid pigments, photo synthetic rate and modifying the activity of some of the important enzymes are the other roles assigned to salicylic acid which induces specific changes in leaf anatomy and chloroplast structure.

3. The effect of salicylic acid on the microtuberization

A) Parameters of the growth:

Results showed there is no significant differences among the treatments of salicylic acid (0, 50, 100, 150, 200 mg/L) in tuber diameter where as there is significant difference of (100 mg/L) level in microtuber weight and number (1.094), (7.125) respectively in table (2).

Table (2) the effect of salicylic acid on the growth parameters of potato cv. Arizona microtubers *In Vitro*

Growth parameters / **Salicylic acid conc.**	**Tuber weight gm**	**Tuber diameter mm**	**Tuber number Means**
0	b 0.556	a 9.78	c 3.50
50	b 0.620	a 10.2	c 4.250
100	a 1.094	a 10	a 7.125
150	a 1.025	a 9.85	a 6.37
200	b 0.865	a 9.9	b 3.625

Means with similar letters indicate no significant different at P< 0.05.

This result was in agreement with the finding of Al- Himaree (2005) who was found that BA (1.5 mg/L) and sucrose 10% were necessary for the microtuberization by giving the highest means of fresh weight, size and number of microtubers.

Promotion of microtuberization on cultured shoots by cytokinins (Benzyl adenine) has been demonstrated by (Hussey and stacy, 1984) IAA supported indirectly microtuber formation (Vreugdenhil *et al*., 1994) by decreasing the stolon elongation a result of ethylene production.

The treatment of potato cultures with IAA (0.1 mg/L) in addition to some cytokinin and high levels of sucrose increased the production of microtubers (Suttle, 1998).

Nistor *et al*. (2013) found that the highest value for average weight of microtubers of 0.61gm was obtained in medium containing salicylic acid in medium of microtuberization and the lowest average weight for microtuberization medium containing acetylsalicylic acid (0.24 gm) both values was for Christian variety of potato.

B) The effect of salicylic acid on the biochemical components in microtubers:

1. Total soluble protein (TSP) µg/gm dry weight:

There is a significant difference in TSP of microtuber, that (100 mg/L) salicylic acid was the best significantly (5.146 µg/gm) among other concentrations Fig. 4.

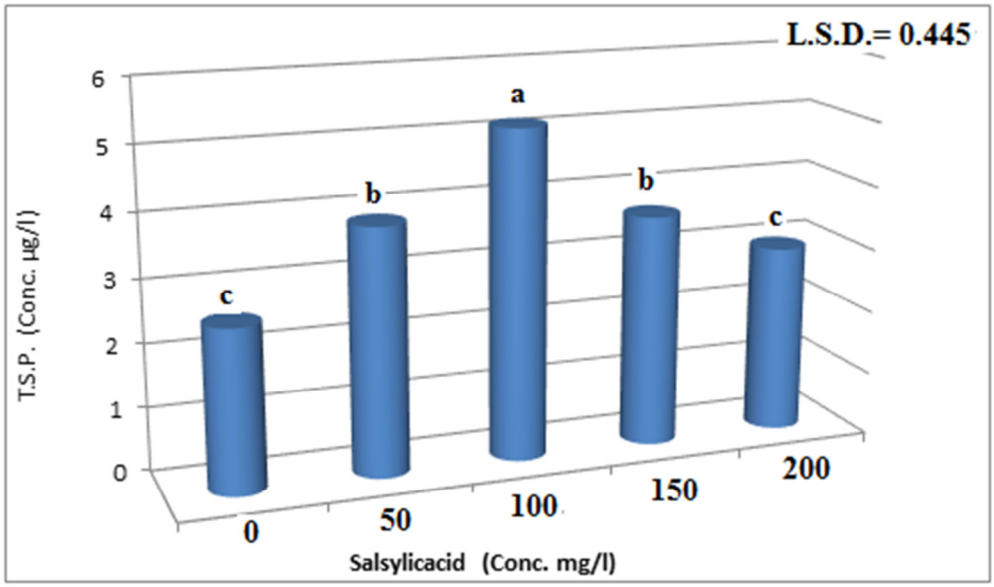

Fig (4): Effect of salicylic acid on protein concentration in microtuber of potato cv. Arizona *in vitro*

2. Starch percentage estimation of potato microtubers:

The results showed that the salicylic acid at levels (100,150 mg/L) and better significantly (12.97, 12.99% starch %) respectively than the control treatment (2.55% starch percentage %) Fig.(5).

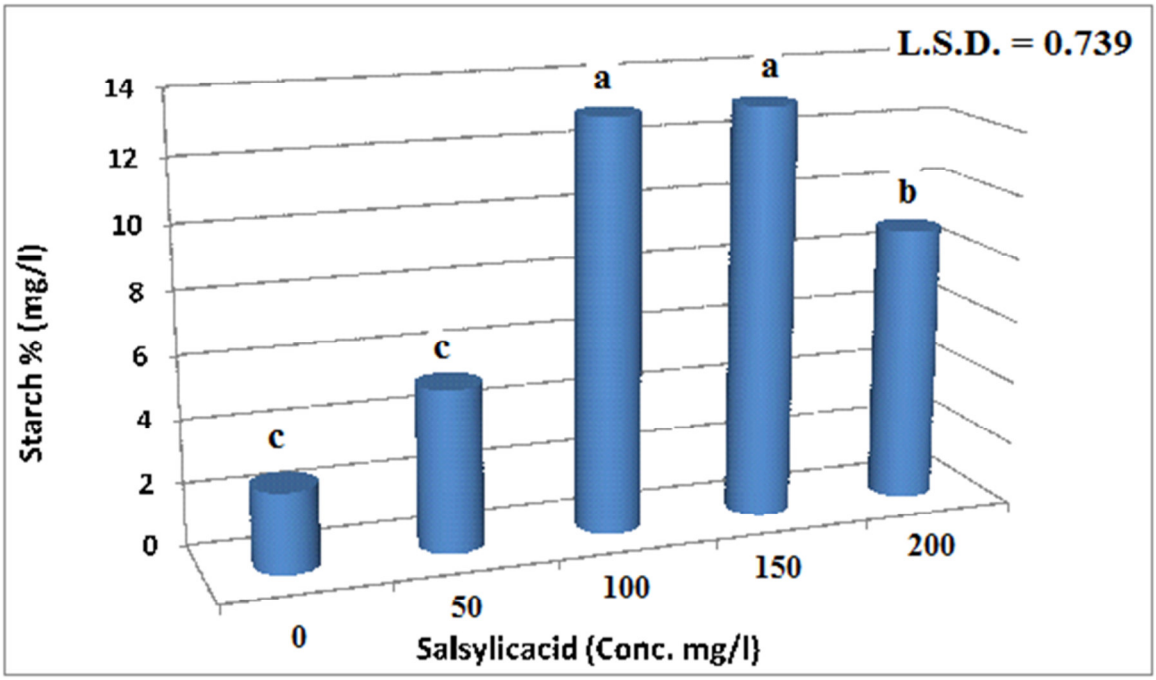

Fig (5): effect of salicylic acid on starch percentage in microtuber of potato cv. Arizona *in vitro*.

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تأثير حامض الساليسليك على نمو وتكوين الدرنات الدقيقة للبطاطا *Solanum tuberosum* L. المكثرخارج الجسم الحيصنفArizona

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الخلاصــة

في الوسط الزرعي لتطور الفروع من زراعة العقل ذات (0, 50, 100, 150, 200 mg L)استخدم حامض الساليسليك في هذه الدراسة بالتراكيز (افضلها معنوياً في عدد الفروع , عدد الأوراق ومساحة الورقة وعدد وطول الجذور باستثناء التركيز 100 mg)/ (L العقدة المنفردة فكان التركيز) لحامض الساليسليك في البروتين الذائب 100 mg/(L) في عدد ومساحة الأوراق وطول الجذر وكذلك التأثير المعنوي للتركيز 150 mg/(L) هي الأفضل معنوياً في محتوى الكلوروفيل لأوراق الفروع . كما 100, 150, 200 mg/L في الفروع وكانت التراكيز ((16.78 µg/gm)الكلي على التوالي. وفي البروتين الذائب الكلي 7.125))، (1.094) لحامض الساليسليك في معدل وزن وعدد الدرنات الدقيقة (100 mg/(Lاثر التركيز) افضل 12.97,12.99% لحامض الساليسليك في النسبة المئوية للنشأ (100,150 mg/(L). وكان التركيزان (gm/5.146 µgللدرنات الدقيقة ( ).2.55%معنوياً عن المعاملة المحايدة (