

**EFFECTS OF LEMON JUICE ON PLANT GROWTH FOR DIFFERENT
VARIETIES OF POTATO (SOLANUM TUBEROSUM L.) IN VITRO**

AL-ZUBAIDI ALI HASSEN ALI^{1,2}
LIPING JIN¹
JIANFEI XU¹

^{1,2}Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China, Ministry of Higher Education and Scientific Research, Foundation of Technical Education, Iraq

¹Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China

¹Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China

ABSTRACT

Lemon juice concentration (0, 1, 2 and 3 ml/L) are used in this study with three varieties of potatoes (*Solanum tuberosum* L.) to determine the optimal environmental conditions and their effect on the growth of vegetative for potatoes in vitro. The results showed that the variety (Zhongshu20) surpassed on the other varieties and the highest rate for variety in qualities (plant length, root's length, number of leaves, node number, wet weight) amounted (10.06cm, 11.36cm, 12.62, 10.68 and 125.02mg) respectively, also surpassed superiority in 2ml/L concentration on a number of roots and leaf size as shown in the results. The main aim for using lemon juice in our study; is to use a suitable alternative regulator for industrial growth regulators with low cost also the difficulty to obtain industrial growth regulators in some of countries.

KEYWORDS: Lemon juice, potato's varieties, Growth regulators, *Solanum tuberosum* L., Tissue culture.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the solanaceae family which grown worldwide, most important food for the largest number of peoples in the world. Potato is usually propagated asexually by tubers. However, regeneration of potato in vitro is easily done from different explants on Murashige and Skoog MS media (Murashige and Skoog 1962) supplemented with different auxin and cytokine in for diseases free good-quality seeds and pathogen Virus-free planting materials (Hossain. 1994; Parmar et al 2012, Devi, M., Dhaliwal et al. 2008). Both callus induction and plant regeneration from explants need acceptable mixtures and concentrations of plant growth regulators within the culture MS media (Ehsanpour and Jones 2000). The growth of plants tissue and organ culture systems used by forest species make use of natural or artificial plant growth regulators (A. Ghaffoor, G.B. Shah and K. Waseem 2003).. Without added hormones, most tissues do not remain viable; and much less grow after the manner. Unfortunately, few studies have dealt with the

mechanism of hormone action and effective hormones and their concentration have been derived empirically. A wide range of hormones has been applied to a number of species with sometimes conflicting results (Rabbani, Askari et al. 2001). The tissues and organs are in sterile conditions supported by an appropriate culture media which normally contain a mixture of major and minor salts, vitamins, sugars as a carbon source and plant growth regulators used formulations to enhance maintenance of plant growth quality of in vitro. While, the propagation process in vitro is facing a lot of difficulties including high prices of growth regulators, low efficiency and changes in high temperatures sterilization process, and the valuable materials from natural plant are not discovered. A method for culturing plant cells and tissues should provide new means for the commercial processing of even rare plants and the chemicals they produce (Murashige and Skoog 1962). These technologies are extended and enhance the usefulness of plants as renewable resources of valuable chemicals. Biologically active plant-derived chemicals can be expected to play an increasingly significant role in commercial development on new products for regulating plant growth. Fruit juice, as one of plant-derived chemicals, is studied on its effects on plant growth in vitro in containing salt's MS media. Plant variation of cultivars studied before was showed in their response to the growth regulators. The highest number of roots per shoot was obtained on MS media supplemented with 1.0 mg/L indole 3-butyric acid (IBA). Regenerated plants were successfully acclimatized and eventually transferred to the green house with higher survival rate. All the plants appeared morphologically uniform with normal leaf form, shape and growth pattern. The described protocol may be useful to establish micro propagation industry of potato that to help in overcoming difficulties of conventional vegetative propagation for the crop species (Khadiga, Elaleem et al. 2009). Multiplication velocity of plants differed in the amount of growth regulators. The lemon juice and condensed milk are used in the vegetative production for potato plants in vitro (Hassen 2011). Top sprouts of desert variety tubers was cut and cultured in M.S media that contains different lemon juice and coconut milk concentration (1,2 ml/L), with 2mg/L BA as control (Hassen 2011). It is also indicated that the use of pollen extracts, licorice and lemon juice in media of had effects on plant growth (Al-Kaaby and Hussein 2000). The addition of 6 cm³/L lemon juice had an encouraging effect on callus induction and growth. PGRs compounds and cultivars may produce the better results in comparison with others. There are also different reports that interaction of growth regulator and cultivar are significant. The main aim of this study is to get optimal level lemon juice. For reduce a cost without impact on the efficiency of the

process of micro propagation. Was lemon juice (Table 2), is one of the solutions to this problem as an alternative to commercial industrial growth regulators who increased their use of private studies in tissue culture, the remnant of this paper is organized as follows; Materials and method are explained in next section then the results and discussion are presented, finally the conclusions are given.

2. MATERIALS AND METHOD

This study was conducted in Institute and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS). Three potato varieties, Zhongshu 3 (early maturity), Zhongshu 20 (late maturity) and Atlantic (mid-maturity) are used in this study. The virus-free seedling was obtained from potato Research Centre. The nutrient media, was consisted of MS media (Murashige and Skoog 1962) for following ingredients: 4.43 g /L of MS powder, the medium contained 30 g /L sucrose and 5.89g /L, agar the pH of the media was adjusted to 5.8 ± 0.01 with 0.1 N either NaOH or 0.1 N HCl before adding plant agar prior to autoclaving (Bhatia. 2005).

2.1. Culture Media and Conditions

Required doses of growth regulators were added alternative to commercial industrial growth regulators lemon juice to the normal MS media (Table 2), (0, 1, 2 and 3 ml/L) respectively as included in below. Containing according to a potato nodal segment culture protocol. In addition, cut seedling a length of 1-2 cm (Fig 1). And Planting culture explants and prepared and sterilized under a laminar air flower with filtration method. Using a tongs length media sterilized vertical 3 pots/each variety, 15 explants /each pot, planted in pots containing control (standard media without the growth regulator) and of concentrations from lemon juice for each three varieties and closed the pots by cover. And they were incubated under controlled environmental conduit of $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$. 16/8 light /dark cycles and the illumination intensity at 1500 lx (Table 1), (Fig 1). The data are registered for the experiment after the 30 days from the date of planting and take the averages of the studied characteristics: (length of plant, length of root, branch number, leaf number, leaf size, number of nodes and wet weight).

Table 1. Preparation four treatments used are in this experiment

Treatment	Media	Quantity
0	Standard MS media without the growth regulators.	3 pots/each variety, 15 plantlets/each pot.
1	Standard MS media with 1ml/L lemon juice.	3 pots/each variety, 15 plantlets/each pot.
2	Standard MS media with 2ml/L lemon juice.	3 pots/each variety, 15 plantlets/each pot.
3	Standard MS media with 3ml/ L lemon juice.	3 pots/each variety, 15 plantlets/each pot.



Figure 1: Stages multiplication and plant growth in the laboratory under normal MS media and concentrations from lemon juice.

Table 2: The nutritional value per 100g in raw lemon without peel.(U.S. Department of Agriculture 2013).

Nutritional value per 100 g	
Protein	1.1 g
Thiamine (vit. B1)	0.04 mg(3%)
Riboflavin (vit. B2)	0.02 mg (2%)
Niacin (vit. B3)	0.1 mg (1%)
Pantothenic acid (B5)	0.19 mg (4%)
Vitamin B6	0.08 mg (3%)
Folate (vit Vitamin C. B9)	11 mg (3%)
Choline	5.1 mg (1%)
Vitamin C	53 mg (64%)
Calcium	26 mg(3%)
Iron	0.6 mg (5%)
Magnesium	8 mg (2%)
Manganese	0.03 mg (1%)
Phosphorus	16 mg (2%)
Potassium	138 mg (3%)
Zinc	0.06 g (1%)

2.2. Statistical analysis

Data are analysed using the Statistical Analysis System (SAS. 2010.) program. Plantlets samples tested are repeated three times under each treatment three samples are duplicated in each tested.

3. RESULTS

3.1. Length of plants

Plant length was affected significantly by different lemon juice level on the whole (Table 3). Nevertheless, with the increase of lemon juice concentration, plant length of each variety showed not same reaction to lemon juice. Plant length of Atlantic was affected most significantly that it increased 2.18 cm in MS media with 3 ml/L lemon juice in comparison to normal media. While, lemon juice induced slight effects on plant length of Zhongshu 3 that just increased 0.67 cm in comparison to the normal media .The results indicated that effects of lemon juice on plant length differ from potato varieties. The plant morphology of each variety is shown as in (Fig 2, A, B and C).

Table 3. Effects of different lemon juice concentrations on plant length (cm).

Variety	Plant length (cm)				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	7.99	8.04	8.4	8.66	8.27	0.67
Zhongshu 20	8.22	8.05	9.65	10.06	8.99	1.84
Atlantic	6.13	6.87	7.56	8.31	7.22	2.18
Mean	7.45	7.65	8.54	9.01	---	1.56

Note: LSD, variety 0.359* , level 0.415* , variety× level 0.719* . *significant at P<0.05.

(A) Zhongshu 20 (B) Zhongshu 3 (C) Atlantic

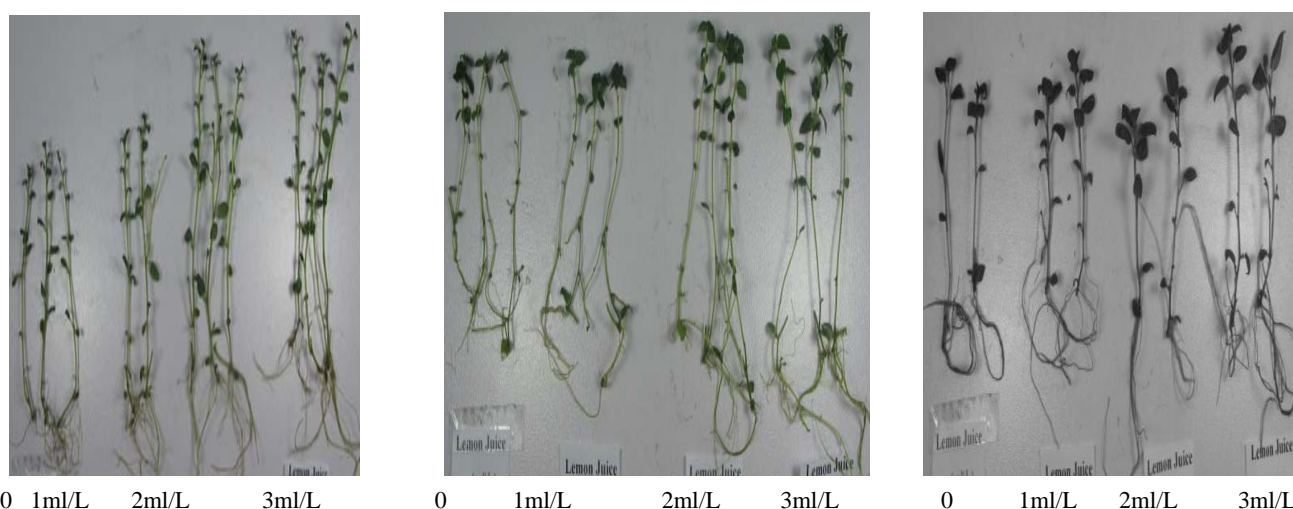


Fig 2. Effects of three concentration of lemon juice (0, 1, 2 and 3 ml/L), on potato varieties (A) Zhongshu 20 variety, (B) Zhongshu 3 variety and (C) Atlantic variety.

3.2. Length of Root

Results showed in (Table 4) in the Zhongshu 20 surpassed significantly on Atlantic and didn't surpass on variety Zhongshu 3, where it didn't record any significant differences, where it recorded the highest rate for root's length 10.02cm, and the lowest rate of described 9.08 cm. As best responses to regeneration were at 3ml/L concentration recorded a significant difference on the rest of the concentration recording 10.40 cm, and lowest length for roots was at (0) concentration, has recorded less value 8.70 cm, and as is the case in plant height, for lemon juice slight effects on root's length of Zhongshu 3 that just increased 0.69cm in comparison to the normal MS media. Maximum percentage of difference between 0 and 3 more length of root formations was recorded of Zhongshu 20 and Atlantic from lemon juice, (Fig2, A,B and C).

Table 4. Effects of different concentrations of lemon juice on length of root (cm).

Variety	length of root (cm)				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	9.33	9.99	10.37	10.02	9.93	0.69
Zhongshu 20	9.25	9.75	9.68	11.36	10.01	2.11
Atlantic	7.51	9.22	9.77	9.81	9.08	2.30
Mean	8.70	9.65	9.94	10.40	---	1.70

Note: LSD: Variety 0.410* , Level 0.473* , Variety x Level 0.820* * (P<0.05)..

3.3. Number of root

(Table 5), showed that the Zhongshu 20 surpassed on the other significantly superiority, where it recorded highest rate for the root's number, which has reached to 5.68 in the control, while the lowest rate has recorded by Atlantic, which is 4.98. For transaction showed that the level 2ml/L of Zhongshu 20 surpassed significant superiority on the rest of the transactions, which has recorded the highest number 6.13, also level 1ml/L Zhongshu 3 have recorded the lowest number of roots. Whereas it was, application of lemon juice was not effective for increasing the number of roots for Zhongshu 20 and improving the quality as compared to other the concentrations that recorded -0.56.

Table 5. Effects of different concentrations of lemon juice on the number of roots

Variety	number of roots				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	5.13	4.40	5.57	5.35	5.11	0.22
Zhongshu 20	5.84	5.46	6.13	5.28	5.68	-0.56
Atlantic	5.02	4.66	5.20	5.04	4.98	0.02
Mean	5.33	4.84	5.63	5.22	---	-0.11

Note: LSD: Variety 0.270* , Level 0.312* , Variety x Level 0.541* * (P<0.05).

3.4. Number of leafs

(Table 6), we saw the significant increase in the number of leafs compared to the control. On the other hand, at the level of 3ml/L for the three varieties, Zhongshu 20 surpassed on the rest of the varieties and recorded the highest rate for a leaf's number, which recorded to 11.66, While, Atlantic variety has recorded the lowest rate for a leaf's number, which has reached to 9.56 (Fig 2, A and C). Zhongshu 20 have recorded at level 3 ml/L and significant difference on the rest levels which have reached to 12.62, didn't show the difference for

lemon juice concentration, and in comparison to the normal media induced slight effects on the number of leaflets of Zhongshu 20 that just increased 0.62 cm .

Table 6.Effect of different concentrations of lemon juice on a number of leaflets.

Variety	number of leaflets				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	9.60	10.75	10.95	11.13	10.61	1.53
Zhongshu 20	12.00	9.68	12.33	12.62	11.66	0.62
Atlantic	8.44	10.11	9.53	10.17	9.56	1.73
Mean	10.01	10.18	10.94	11.31	---	1.30

Note: LSD: Variety 0.432*, Level 0.499*, Variety x Level 0.865* * (P<0.05).

3.5. Number of node

Zhongshu 20 surpassed significantly on the rest of transaction as shown in (Table 7), whereas the highest rate recorded 8.52 nodes. There is no significant difference between Zhongshu20, Zhongshu3. The Atlantic recorded the lowest rate 7.27. The results didn't indicate that there are presents a difference between the third level and comparative transaction, whereas level 2ml/L recorded lowest rate for the number, which is 7.08 node(Fig 2, A, B and C). As the results showed that there was no effect for lemon juice concentration of nodes the Atlantic that recorded -2.27at difference between control and lemonjuice concentration.

Table 7.Effects of different concentrations of lemon juice on the number of nodes.

Variety	number of nodes				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	7.40	7.46	7.35	7.55	7.44	0.15
Zhongshu 20	7.64	7.51	8.26	10.68	8.52	3.04
Atlantic	8.91	7.93	5.62	6.64	7.27	-2.27
Mean	7.98	7.63	7.08	8.29	---	0.31

Note: LSD: Variety 0.354*, Level 0.409*, Variety x Level 0.708* * (P<0.05).

3.6. Number of branch

The results in (Table 8), observed that there are no differences between Zhongshu 20 and Zhongshu 3. While the Atlantic is recorded the lowest rate for the branch's number, which has reached to 1.18. For level's lemon juice, control (0) recorded significant difference on the level 2ml/L and 3ml/L, while there is no significant difference between control treatments (0) and where this transaction recorded highest rate for the branch's number,

which has reached to 1.21 branch. Transaction results indicated that the Zhongshu 20 significantly on the rest of control treatments at the level (0), which recorded the highest rate of the branch's number, which has reached to 1.93 also Atlantic recorded lowest rate at the level (0) which is reached to 1.11. There was no effect for lemon juice concentration on Zhongshu 20 and Zhongshu 3 in a number of branches, however has shown that slight effects on Atlantic that recorded just increased 0.17 lemon juice concentration.

Table 8. Effects of different concentrations of lemon juice on the number of branches

Variety	number of branches				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	1.26	1.17	1.24	1.11	1.20	-0.15
Zhongshu 20	1.93	1.48	1.24	1.26	1.48	-0.67
Atlantic	1.11	1.20	1.15	1.28	1.18	0.17
Mean	1.43	1.29	1.21	1.22	---	-0.21

Note: LSD: Variety 0.133*, Level 0.153*, Variety x Level 0.266* * (P<0.05).

3.7. Weight of plant

The results indicated in (Table 9), for wet weight, Atlantic variety surpassed on the Zhongshu 3, which recorded the highest rate recorded 110.10 mg, while there is no revealed any difference between Zhongshu 20 and Atlantic. Also Zhongshu 3 recorded the lowest rate for wet weight 98.03 mg. For concentration, Zhongshu 3 is recorded the lowest rate of lemon juice, and has reached significant difference on control treatment (0) reached to 101.14 mg. For treatments, Zhongshu 20 at third-level significant difference which has reached 125.02 mg on Zhongshu 3 varieties, while there is a significant difference with Atlantic and concentration 3ml/L. The results of comparing a difference between 0 and 3 level, 3 ml/L between concentration on wet weight of plant of Zhongshu 3 showed that there is not a significant effect were reached -2.42.

Table 9. Effects of different concentrations of lemon juice on wet weight of plant (mg).

Variety	weight of plant (mg)				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	101.06	95.06	97.35	98.64	98.03	-2.42
Zhongshu 20	95.60	100.53	116.93	125.02	109.52	29.42
Atlantic	106.77	108.04	107.73	117.84	110.10	11.07
Mean	101.14	101.21	107.34	113.84	---	12.70

Note: LSD: Variety 7.744*, Level 8.943*, Variety x Level 15.49* * (P<0.05).

3.8. Size of leaf

The Atlantic is surpassed significantly on the rest of varieties, which has recorded the highest rate 3.17, whereas no significant differences between Zhongshu 3 and Zhongshu 20 as shown in (Fig 2, A, B and C) and (Table 10), For lemon juice, there was no longer any significant difference inter MS of transaction, level 2ml/L recorded the highest rate for Zhongshu 20, which have reached 4.33, also Zhongshu 3 recorded control at (0) level lowest rate has reached to 2.00. However, the percentage response was found varying, depending on the concentration of lemon juice concentration in the MS media. From the difference between 0 and 3, it is clear that with an increase in the concentration of lemon juice, there was a gradual increase the response simple for size of leaf.

Table 10. Effects of different concentrations of lemon juice on size of leaf.

Variety	size of leafs				Mean	Difference between 0 and 3
	Lemon juice concentration(ml/L)					
	0	1	2	3		
Zhongshu 3	2.00	2.33	4.00	3.66	3.00	1.66
Zhongshu 20	2.06	2.33	4.33	3.33	3.01	1.27
Atlantic	2.66	2.66	3.66	3.66	3.17	1.00
Mean	2.24	2.44	4.00	3.55	---	1.31

Note: LSD: Variety 0.120*, Level 09.139*, Variety x Level 0.241* * (P<0.05).

4. DISCUSSION

The present investigation was conducted to find out the effects of alternative growth regulators on direct regeneration of potato. Data on seedling visible :(length of plant, length of root, branch number, leaf number, leaf size, number of nodes and wet weight). After 30 days, the discussions of the study have been presented below: The showed wide variation in their response to the lemon juice concentration. The concentration 3 ml/L, recording the highest rate for variety in qualities (plant length, root's length, number of leafs, node number and wet weight) amounted respectively (10.06 cm, 11.36 cm, 12.62, 10.68 and 125.02 mg), The results of the study reflected interaction effects between lemon juice concentration and potato cultivars , this can be justified by that the different concentrations of lemon juice plays a positive role in to promote the cell division growth, and stimulate the activity of some enzymes, the nutrients be transported to the parts of plant more easily. And on the other hand increase the growth of vegetative and weight at the same time. Lemon is the major component than bioactive compounds and presence of flavonoids and vitamin C polysaccharides and acids (Table 2). As in The results from previous research on this subject

have that the effect of lemon juice on in vitro propagation of potato seedlings and also which corroborates findings for this study. (Asmaiel, K. A.2002, Faridud in et al 2004, hassen 2011).And the surpassed significant superiority at 2 ml/l concentration number of roots and leaf size, which has recorded the highest rate (6.13 and 4.33) growth regulators would effect on growth plant in addition because of genotype effect and are widely used in vitro multiplication through to change the behaviour the plants, thus optimizing the vegetative growth reported by many researchers.(Prat 2004; Zhang et al., 2005; Chaudhary, Z., Afroz, A., and Rashid, H 2007, Ghavidel et al., 2012) . It may be mentioned here that among the three potato varieties used in the present investigation showed best zhongshu 20 variety surpassed increment of most qualities significant superiority attributed difference genotype the and also variable responses of different potato varieties due to genetic makeup and physiological effects on plant cells and this result possibly is based on the hypothesis that plant hormones responsible for cell growth the greatest cellular growth when had the concentrations of plant growth regulators used .Where this result is in line with(A. Rabbani, B. Askari 2001, Ehsanpour, A. A. and M. G. K. Jones 2000).

5. CONCLUSION AND RECOMMENDATION

From the above study, lemon juice growth regulators had significant influence on three varieties from the potatoes in vitro. It gave the best response the Zhongshu 20 variety significant surpassed from Zhongshu 3 and Atlantic varieties. As 3 ml/Llemon juice concentration gave the best response for of the studied characteristics. Therefore, recommend the addition of lemon juice for MS media to an increase in growth, and cost less productivity for MS media.

ACKNOWLEDGMENT

This project is supported by the Key Laboratory of Biology and Genetic Improvement of Tuber and Root Crop, Ministry of Agriculture, P.R.China and funded by the earmarked fund for Modern Agro-industry Technology Research System (CARS-10).

REFERENCES

1. Ghaffoor, G.B. Shah and K. Waseem. In vitro response of potato (*Solanum tuberosum* L.) to various growth regulators. *Biotechnology*.2003, 2: 191-197.
2. Rabbani, B. Askari, N.A. Abbasi, M.Bhatti, and A. Quraishi. Effect of growth regulators on in vitromultiplication of potato. *International J. Agric. and Biol.* 2001, 3(2):181-182.
3. Al-Kaaby, H. K. and W. M. Hussein "Effect of lemon juice Citrus sinensis extracts on callus induction and development of two local potato (*Solanum tuberosum* L.) " varieties College of Education, University of Basrah ISSN. 2001, 1817-2695.

4. Asmaiel, K. A.. "Use of some alternatives for plant growth regulators in the propagation of date palm in vitro " Master Thesis submitted to the Department of Horticulture and palm - College Agriculture - University of Basra. 2002, 24(1), pp.11-28.
5. Bhatia, P., Ashwath, N., and David, M., Effects of genotype, explant orientation, and wounding on shoot regeneration in tomato, *In Vitro Cell. Dev. Biol.: Plant.*, 2005, vol. 41, pp. 457–464.
6. Chaudhary, Z., Afroz, A., and Rashid, H. Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars (*Lycopersicon esculentum*), *Pak. J. Bot.* 2007 , vol. 39(3), pp. 857–869.
7. Devi, M., Dhaliwal, M.S., Kaur, A., and Gosal, S.S. . Effect of growth regulators on in vitro morphogenetic response of tomato, *Indian J. Biotechnol.*. 2008 , vol. 7, pp. 526–530.
8. Ehsanpour, A. A. and M. G. K. Jones. "Evaluation of direct shoot regeneration from stem explants of potato (*Solanum tuberosum* L.)" cv. Delaware by Thidiazuron (TDZ). *J. Sci. & Tech. Agric. & Nat. Resour., Isf. Univ. Tech., Isf., Iran* . 2000, 4(3): 47-54.
9. Faridudin.M/, Taher. A., Islam, S.M.A and Hossain, M.Z. Effect of variety and plant growth regulators in MS medium on shoot induction from virus infected calli of tomato. *Journal of Biological Sciences* . 2004, 4(4):52-526.
10. Ghavidel, R.A., A.R. Bolandi, H. Hamidi and S. Foroghian, Effects of plant growth regulators and photoperiod on in vitro microtuberization of potato (*Solanum tuberosum* L.) *Afric.J.I Biotech.* 2012, 11(53): 11585-11590.
11. Gubis, J., Lajchova, Z., Farago, J., and Jurekova, Z.. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.), *Biologia (Bratislava)* . 2004 vol. 59 (3), pp. 405–408.
13. Hassen, A.-z. a. "The effect of lemon juice and coconut milk on in vitro propagation of potato (*Solanum tuberosum* L.) " scientific journal published by: foundation of technical education. 2011, 24(1), 25-38.
14. Hossain., M. J. "In vitro propagation of potato (*Solanum tuberosum* L.)" *Plant Tissue Culture*. 1994, 4(1): 33-37.
15. Khadiga, G. A., R. Elaleem, et al. "Effect of Cultivar and Growth Regulator on In vitro Micropropagation of Potato (*Solanum tuberosum* L.)" *Journal of Sustainable Agriculture Am.-Eurasian J. Sustain.* 2009, 3(1): 487-492.
16. Murashige, T. and F. Skoog , "Arevised medium for rapid growth and bioassays with tobacco tissuesultarum " *physiologia plantarum* . 1962, 15: 473-497.
17. Osman, H. A. R. "Effect of organic salts and vitamins on emergence and growth of callus date palm off vivo message Master section gardening " *Agricultural Faculty of the University of Basra*. 1987, 2(1): 12- 21.
18. Parmar, P., Subramanian, R.B and Achakzai, A.K.K..Optimization of Growth Regulators for Shoot Induction and Regeneration of Tomato (*Lycopersicum esculentum* Mill) *Academic Journal of Plant Sciences*5. 2012, (4): 114-118.
19. Prat, S. Hormonal and day length control of potato tuberization. In PJ Davis (ed). *Plant Hormones: Biosynthesis, Signal Transduction, Action*. Kluwer Acad. Publ. Dordrecht, Netherlands. 2004 , pp: 538-560.
20. Rabbani, A., B. Askari, et al. "Effect of growth regulators on in vitro multiplication of potato." *International J. Agric. and Biol.* 2001, 3(2): 181-182.
21. SAS. *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA. . 2010 .
22. U.S. Department of Agriculture, Agricultural Research Service. *USDA National Nutrient Database for Standard Reference, Release 26*. Nutrient Data Laboratory, <http://www.ars.usda.gov/ba/bhnrc/ndl> . 2013.vol. 2004,59 (3), pp. 405–408.
23. Zhang, Z., W. Zhou, and H. Li, The role of GA, IAA and BAP in the regulation of In vitro shoot growth and micro tuberization in potato. *Acta Pysiol. Planta.*, 2005, 27(3): 363-369.