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Potato (*Solanum tuberosum* L.) Nodal Regeneration as Affected by Different Concentrations and Immersion Time of Commercial Clorox, Sugar Concentration and Murashige and Skoog (MS) Salt Strength

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Abstract

A study was conducted to investigate the effects of different concentrations and immersion time of commercial Clorox, sugar concentration and Murashige and Skoog (MS) salt strength on nodal regeneration of potato (*Solanum tuberosum* L.). Results indicated that commercial Clorox containing sodium hypochlorite at a concentration of 10% for a period of 10-20 minutes immersion, despite its preference over higher concentrations, was not very effective in sterilizing plant parts, as the percentage of survived and free of contaminants cultures did not exceed 50% at the end of the experiment. Sugar concentration of 3% gave the highest number of leaves and roots compared with the lowest concentrations. Three quarters concentration of MS salts gave the highest number of leaves and the highest number of roots in in vitropotato explants.

Keywords: Potato, Disinfection, Nodal regeneration, Sugar , Salt Strength

التجدد العقدي للبطاطس وتأثره بالتركيز وفترة الغمر للكلوروكس التجاري وتركيز السكر وقوة املاح مورشيجي واسكوج

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المستخلص

أجريت دراسة لمعرفة تأثير التركيزات المختلفة وفترة الغمر لمحلول الكلوروكس التجاري وتركيز السكر و املاح موراشيجي وسكووج (MS) على التجدد العقدي للبطاطس (*Solanum tuberosum* L). أشارت النتائج إلى ان الكلوروكس التجاري المحتوي على هيبوكلوريت الصوديوم بتركيز 10٪ لمدة 10-20 دقيقة من الغمر ، بالرغم من افضيلته على التركيزات الأعلى ، لم يكن فعالاً جداً في تعقيم أجزاء النبات ، حيث أن النسبة المئوية للمزارع الباقية علي قيد الحياة والخالية من الملوثات لم تتجاوز 50٪ في نهاية التجربة. أعطى تركيز السكر 3 % أعلى عدد من الأوراق والجذور مقارنة بالتراكيز الاقل. أعطت ثلاثة أرباع تركيز أملاح موراشيجي وسكووج أعلى عدد من الأوراق وأعلى عدد من الجذور في نبيتات البطاطس داخل المختبر.

كلمات مفتاحية: البطاطس، تعقيم ، التجدد العقدي، السكر، قوة الاملاح

Introduction

Microbial contamination is one of the most injurious problems affecting tissue culture.The sources and causes of microbial contamination in the laboratory vary, but one of the most possibilities of its source is plant to be cultured, especially if it comes from field conditions. The surface sterilization of the cultured plant is one of the key steps of tissue culture (Onwubiko, *et. al*, 2013). Surface sterilization is a vital step in preparing healthy and viable *in vitro* plants because plants in the field are highly susceptible to microbial contamination. Most surface contaminants as bacteria and fungi can be eliminated by plant surface sterilization using an appropriate disinfecting agent (Mahmoud and Al-Ani, 2016). Surface sterilization of plant materials is complex but is a very important step in the formation of healthy plant tissues within the protocol of cultures before being placed on sterile media (Bello Oluwakemi *et al*., 2018.). Therefore, a specific and successful standard sterilization protocol must be developed for sterilizing the cultures to prevent microbial contamination bearing in mind that laboratory environment and presence of nutrients can encourage the growth of microbes. Determining the time of sterilization without causing negative effects on the culture with the chemicals used in the sterilization processes also is an important factor to produce healthy plants.

Clorox (commercial bleach) often contains 5% of sodium hypochlorite. In this case, the use of double the dose (2 ml of bleach per 100 ml of water) gives a concentration of 10% sodium hypochlorite and dipping the plants in it for a period ranging 20- 30 minutes is sufficient to disinfect Plant parts (Ali and Abdalla, 2010).

Nasr El-Din *et al*., (2014) mentioned that a dose of 10-20% of commercial bleach contains often 5-10% sodium hypochlorite, in which the explant is dipped with shaking for a period of 5-30 minutes for disinfection. Mahmoud, (2007) also mentioned that 2-2.5% of sodium hypochlorite,

which is often equivalent to 5—20% of the commercial bleaching solution known as Clorox, is sufficient for sterilization, taking into account the type of plant tissue and the time of immersion.
Plants benefit from light to manufacture carbohydrates in the presence of chlorophyll pigment. Since the plants in tissue culture are weak in the vegetative system and grow under weak lighting, they will not have a full ability to manufacture energy materials. Therefore, plants are provided with sugar in tissue culture media, which is known as non-autotrophic (heterotrophic), and many scientists have suggested that raising the proportion of carbon dioxide in the atmosphere surrounding the plant will enforce it to become autotrophic instead of adding sugar to the environment. However, experiments proved a low feasibility for this.
Medium for plant tissue culture is commonly supplemented with MS salts and other organic and inorganic components, but optimum salts strength (concentration) is a subject of species and specific perpous. Ibrahim *et al*., (2016) found no significant differences in plant height observed in two potato cultivars as affected by MS salt strength, while there was a significant increase in the number of leaves when using the full MS, While, the lowest concentration (1/4 MS) resulted in a significant increase in root growth compared with higher MS salt strength. Abd- Elaleem *et al*., (2009) found that shoots regenerated from callus were rooted most effectively on half-strength MS medium containing 0.5 mg/l IBA.
The objective of this research was to investigate the effects of different concentrations and immersion time of commercial Clorox, sugar Concentration and Murashige and Skoog (MS) salt strength on nodal regeneration of potato (*Solanum tuberosum* L.).

Materials and method

This study was conducted at the tissue culture laboratory of Al-Rajhi Company in Berber city in order to identify the effect of Clorox as a disinfectant for potato cultures and the effect of each of the concentration of sugar and Murashige and Skoog salts on *in vitro*potato growth .

Planting material selection and surface sterilization

Bukhari cultivar was selected from the greenhouse of Al-Rajhi Agricultural Company as starting experimental materials. The growing top of the plant was taken after removing the surrounding leaves and this process was done outside the laboratory. The plant was washed well with soap and water, to get rid of the soap effect and for further sterilization, drops of Clorox and running water were used.

Media preparation and sterilization techniques

Murashige and Skoog (1962) medium (MS) was used by preparing working solution from stock solutions as described by Nasr El-din *et al*. (2014). Addition of growth regulators were made according to their heat stability and according to their need for each experiment, before autoclaving. Sucrose added, as specified in each experiment. Gelrite was added at rate of two grams per liter as media gelling agent. Medium (pH) was adjusted to 5.8 using both potassium hydroxide and hydrochloric acid followed by heating on hot plate till full blend. Finally, 30 ml of nutrient medium was poured into 250 ml containers and covered with a polypropylene cap. The containers were placed in the autoclave at a temperature of 121°C and a pressure of 15 psi for 20-30 minutes to sterilize the nutrient medium, then the containers were incubated at a temperature of 20-24 overnight before experimentation.

Experimentation

In the first experiment, Clorox was used as a sterilizing agent with different concentrations and exposure time (dipping time) to study its effect as a sterilization substance on potato cultures. Sterilization by Clorox was done at three concentrations (10, 20, 30%) for (10, 20, 30 minutes). To test for decontamination. A medium containing 3/4 strength of Murashige and Skoog salt (MS) supplemented with appropriate plant growth regulators (kinetin at a rate of 2 mg / liter) was used in the laboratory and placed in containers and monitored for four weeks. The pH of the medium was adjusted to 5.8 and the containers were then sterilized using an autoclave at 121 °C for 20 min. Ten explants were used for each sterilization treatment.One explant was cultured in each container. The cultures were placed in a growth incubator at 24 ± 1 °C for four weeks, with a 16/8-hour light/dark period under 1000 lux illumination provided by cool white fluorescent light. Aeration and humidity were also properly maintained in the growth incubator throughout the assay period. After four weeks, the percentages of contaminated, surviving and dead nodes were recorded.

In the second experiment, the effect of sucrose concentration on potato nodal explant growth was studied. The explants were cultured on a medium containing ¾ Murashige and Skoog salt strength.Growth regulator (kinetin at a concentration of 2 mg / liter) and gelrite as a gelling agent at rate of two grams per liter were added. Sucrose concentrations were 10, 20, 30 grams per liter. Ten culture samples were planted in four replicates, and the experiment was statistically analyzed according to a randomized complete block design (RCBD) with means separated by LSD using SAS statistical computer package. The readings namely, the appearance of leaves and roots were taken in different periods of the experiment as shown in the results tables, At the end of each 7days for four weeks the number of leaves and the number of roots in the culture medium were registered.

In the third experiment, the effect of Murashige and Skoog (MS) salt strength on the growth and development of potato cultures inside the tissue culture laboratory was studied. Sugar was used at a rate of 30 grams per liter. Growth regulator (kinetin at a concentration of 2 mg per liter) and gelrite as a gelling material at a rate of two grams per liter were added. Ten cultures in four replicates were studied, and the experiment was statistically analyzed according to a randomized complete block design (RCBD) with means separated by LSD using SAS statistical computer package. Parameters namely, the appearance of leaves and roots were investigated at different periods of the experiment as shown in the results tables. At the end of each 7days for four weeks, the number of leaves and the number of roots in the culture medium were registered.

Results and Discussion

The best results from sterilization with commercial Clorox were obtained when using it at a concentration of 10% with the survival of 80% or more of the plants at the end of the experiment time after four weeks of culture for the three immersion times. Also, immersion for 10 minutes had better results compared to the longer times. Fifty % of the plants are free of contamination four weeks after planting. Concentrations of 20 and 30% of the sterilization solution showed in terms of the percentage of contamination and the explant survival. Explant survival rate decreased with the increase in the duration of immersion (Table 1).

Results obtained seem to be in line with many researchers findings. From them Badoni and Chauhan, (2010) who for sterilization of the potato varieties Kufri and Himalinimadea compared between sodium hypochlorite and mercuric chloride for three immersion periods of 2, 5 and 8 minutes to detect the non-growing, infected and healthy cultures with no contamination. Results indicated that hypochlorite (NaOCl) was better in controlling the infection and it did not have any negative effect on the explants even in the long term. It was shown that sodium hypochlorite for 8 minutes was a suitable chemical for sterilization used after 5 minutes of washing with saflon, immersion for 30 seconds in ethanol and finally washing with double distilled water.
Amissah, *et al*., (2016) obtained best results from the use of 70% ethanol for 3 minutes, followed by 20% sodium hypochlorite for 10 minutes, where 90% of the cultures remained intact from contamination after four weeks of cultivation when three methods were tried for surface sterilization of sweet potato, including the addition of 70% ethanol for 1 minute, followed by 10% sodium chloride for 15 minutes and 70% ethanol for 3 minutes, followed by 20% sodium chloride for 10 min and 90% ethanol for 3 min, followed by 30% sodium chloride for 10 minplus control where only distilled water was used to rinse the explants.

Unlike others Onwubiko *et al*., (2013) studied the effect of different levels of sodium hypochlorite concentration and exposure time on sweet potatoes. His results showed that sodium hypochlorite was not a very good sterilizer as a very high percentage of the cultures were contaminated in all the experiments conducted. But a low percentage (40%) of the clean cultures was observed at 20% concentration, with a duration of 20 minutes as exposure time. It seems that decontamination effect of hypochlorite will be magnified when supported by use of other disinfectant, while too long immersion period may harm treated explants.

Table 1: Performance of potato explants(*in vitro*) as affected by Clorox concentration and immersion time

Hypochlorite conc.	Clorox conc. %	Time of immersion (minutes)	contaminated plants No.	Contaminated plants %	No of dead plants	Plants survival %	Response Rate%
0.5	10	10	5	%50	1	90 %	90%
		20	8	%80	1	90 %	90%
		30	8	%80	2	80 %	80%
1	20	10	7	%70	10	0%	0%
		20	10	%100	4	60%	60%
		30	10	%100	7	30%	30%
1.5	30	10	10	%100	7	30%	30%
		20	10	%100	9	10%	10%
		30	0	0	10	0%	0%

Response rate include survived plants even if with microbial contaminants by the end of four weeks

Significant differences in the number of leaves as affected by sucrose concentration were noticed(Table 2). The greatest number of leaves resulted from 30 g/l sucrose across the four weeks readings (22.20, 30.00, 38.60 and 59.20 leaves in the first, second, third and fourth week respectively). The least number of leaves resulted from 20 g/lsucrose (3.50) in the first reading at the end of the first week, from 20 and 10 g/l, respectively (7.40 and 7.10) by the end of the second

week, 10 g/l (9.60) in the third week. and from 10 and 20 g/l, respectively in the fourth week (41.40 and 42.00). Results indicated the positive effect of high sugar concentration in increasing leaves numbers. Ibrahim *et al*., (2016) obtained similar results.

As for the roots, in the first week, the differences were not significant. The concentration of 20 g/l of sucrose gave the highest number of roots (2.20) and no roots appeared for the lower sugar concentration (10 g/l). In the second week, the high concentration of sugar (30 g/l) resulted in a significant increase in number of roots (8.40), and the lowest number resulted from lowest sugar concentration (1.70). In the third week, the two highest concentrations of sugar 30 g/L and 20 g/l gave a significant increase in the number of roots (23.00 and 9.60, respectively) and the lowest number of roots resulted from the lowest concentration (2.10). In the fourth week, the higher sugar concentrations (30 and 20 g/l) gave a significant increase in number of roots (60.90 and 28.80, respectively) and the lowest roots resulted from lowest concentration (4.90).

It seems that at the beginning, root growth did not follow the same trend of leaf growth, however, later higher sugar concentrations seem to increase root development.

Table 2: Potato *in vitro* nodal regeneration manifested in leaf and root number as affected by MS Sugar concentration

Weeks	Sugar conc. (g/l)	Root No.	Leaf No.
First	10	0.00	4.80
	20	2.20	3.50
	30	0.70	22.20
Second	10	1.70	7.10
	20	4.60	7.40
	30	8.40	30.30
Third	10	2.10	9.60
	20	9.60	36.60
	30	23.00	38.60
Forth	10	4.90	41.40
	20	28.80	42.00
	30	60.90	59.20
CV %		97.28	37.17
P sugar		***	***
P weeks		***	***
P interaction		***	***
LSD		5.28	6.09

Regarding salt strength effects, there were significant differences in the number of leaves between all concentrations used for salt strength through the four weeks period (Table 3). Salt strength of three quarters concentration gave the highest number of leaves (22.20, 30.70, 36.00 and 59.20in the first ,second, third and fourth week respectively), while the lowest number of leaves resulted from half salts strength (2.80, 4.50,6.50 and 9.10 in the first ,second, third and fourth week respectively). Results obtained resemble that obtained by Ibrahim *et al*., (2016). Root numbers followed the same trend as in leaf number as affected by salt strength except the first week,where

3/4 salt strength recorded the highest root number in the three later weeks (9.00, 23.00, 60.00 in the second, third and fourth week respectively). The result obtained resemble that obtained by Abd-Elaleem *et al*., (2009). Result obtained indicated that full MS salt did not record the highest root number. High osmotic potential resulted from full MS salts might reduce root number compared to 2/4 and ¾ salt strength.

Table 3: Potato *in vitro* nodal regeneration manifested in leaf and root number as affected by MS salt strength

Weeks	MS salt conc. (quarters)	Root No.	Leaf No.
First	4/4	2.40	9.90
	¾	1.40	22.20
	2/4	1.00	2.80
Second	4/4	6.70	16.50
	¾	9.00	30.00
	2/4	2.00	4.50
Third	4/4	10.00	22.30
	¾	23.00	36.90
	2/4	5.00	6.50
Forth	4/4	41.90	42.30
	¾	60.90	59.20
	2/4	12.00	9.10
CV %		90.33	51.18
P sugar		***	***
P weeks		***	***
P interaction		***	***
LSD		5.85	4.9

Conclusion

Commercial Clorox containing sodium hypochlorite at a concentration of 10% for a period of 10-20 minutes immersion, despite its preference over higher concentrations, was not very effective in sterilizing plant parts, as the percentage of survived and free of contaminants cultures did not exceed 50% at the end of the experiment. Therefore, it is preferable to be used with other sterilizers.

Sugar concentration of 3% gave the highest number of leaves and roots compared with the lowest concentrations.

Three quarters concentration of MS salts gave the highest number of leaves and the highest number of roots in *in vitro* potato explants.

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