American-Eurasian J. Agric. & Environ. Sci., 13 (8): 1129-1134, 2013
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2013.13.08.2614

An Evaluation of Effects of Plant Growth Regulators and Light on Callus Induction for Varieties of Potatoes

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Abstract: In this research, three types of potatoes (*Solanum tuberosum* L.) including Sante, Agria and Savalan are studied in order to review the effect of plant growth regulators and light on callus induction. In this study, factorial test is used in terms of absolutely random design with 18 different treatments and three repeat in any treatment. Plantlets of these figures after reproducing by *in vitro* method, were placed at MS culture environment under different hormonal treatments include 2,4-D at three levels (0, 2 and 5 mg per liter) and Kinetin in three levels (0, 5/0 and 2 mg/L) and different photoperiods (light and dark) to produce callus. Under study traits include fresh callus weight, callus diameter, seedling length and percentage of callus induction. Results showed that there is a very significant different between numbers and different hormonal treatments in terms of all traits studied. Also there is a very significant difference between different photoperiods in terms of all traits, except for callus fresh weight. Calluses produced under dark conditions and hormonal treatments of 5 mg/L 2,4-D and 2 mg/L Kinetin showed superiority among under study cultivars over other treatments. In under study cultivars, more 2,4-D Hormone concentration in cultivate environment cause to more cell divisions and producing larger calluses. Generally, results showed that the highest and lowest callus induction percent was seen in Santer and Agria cultivars, respectively.

Key words: Potato · Tissue culture · Callus · Hormone · Light

INTRODUCTION

Potato (*Solanum tuberosum* L) is the most important nutrition supply for human being and it is the fourth food from nutritional value viewpoint in the world. Also in terms of spread, after maize, it has the widest distribution in the world. This plant has various applications, such as supply and energy production and as a food source it has the best balance of essential amino acids, [1]. As a first step in many experiments in tissue culture, callus induction of prototype is necessary. This fine plantlet may be a new seedling, root, stem, leaf or sterilized reproductive organs. Callus tissue is a result of wounding in plant. All cells of micro plantlet are not involved in callus formation and more importantly, some of callus cells are competing regenerated. Certain other cell types of callus do not compete for expressing Totipotency. It is usually necessary to select first observation for selecting regeneration cells. Levels of plant growth regulators (Auxine, Cytokine, Gibberellin, ethylene, etc) are important factors that control callus formation in culture environment. Concentration of plant growth regulators is different for any plant and it depends on source of micro plantlet or determined plant. Culture conditions (temperature, solid culture condition versus semi-solid, light, etc.) are also important in formation and development of callus. When Callus is produced, it can be used for a range of different tests. Callus cultures are used for isolation of protoplast, cell type; cell selection, somatic embryogenesis, organogenesis and production of secondary products [2]. Potato is from plants that most culture techniques are done *in vitro* about it. For *in vitro* cultivation of this plant, micro plantlet gland, branch, meristem, shoot and pollen are used and in some cases, protoplasts are used for plant regeneration [2, 3], regulating hormone concentrations, report 50-fold increase in fresh weight of cultured tissue over 5 weeks and this was the first report on potato tissue culture [4]. Studied the effect of growth regulators concentrations (2, 4-D and Kinetin) and light on callus induction of under

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study cultivars. In this experiment, there was a significant different in terms of callus induction percentage and number of roots on callus, so that the highest percentage of callus induction and roots on callus obtained at 1 mg in 2,4-D liter. [5] also reviewed effect of micro plantlet and genotype on callus induction and regeneration. Plants in terms of physical conditions such as light and temperature requirements for callus are different. Some plants in light and some plants in the dark can produce more calluses. Using stem small components micro plantlet small pieces with terminal or side shoots of potato varieties in liquid or semi-solid culture medium provides a good growth response. MS base culture medium induces the most stem growth [5].

Callus induction and organogenesis of that in many fine plantlet such as leaves, stems and flowers have been done [6, 7], to induce callus from Almera cultivar in potatoes, cultivate them in a MS cultivate medium contain 2,4-D concentration and they conclude that micro plantlet in a MS cultivate medium is able to produce callus with or without 2,4-D. [7] reported that high concentrations of 2,4-D (3 mg/lit) are the most effective concentration for callus induction in all cultivars. Light is an important factor *in vitro* cultivation. Time of light exposure, light intensity and its spectral composition is of particular importance. Response to light varies depending on the source of explants [8] Light existence is not necessary for callus growth and in most reports, callus induction are done in the dark [9-11] But there are some reports that they use light for callus proliferation and maintenance [12]. Unlike callus induction, light plays an important role for plant regeneration and for differentiation of organs and tissues, the presence of light is required.

For regeneration, generally, photoperiod of 16 hours of light and 8 h dark is used [13, 14]. Basically the best photoperiod for *in vitro* condition is light over a day that happen for a complete plant in a natural condition, but intense light is harmful to *in vitro* plants. Because intense light cause greenhouse effect in culture thus increasing the temperature of the medium that it is a deterrent factor to growth [15, 16] reported that in potato, between two micro sample of leaf and node, leaf micro sample has callus genesis greater speed and has fewer roots so it is more preferable. Any stages of callus genesis, reproducing, organogenesis and plantlet formation requires a specific combination of growth regulators [17]. So that in regeneration process, greater amount of Cytokinin and little amount of auxine is required in callus genesis and usually in regeneration medium, a weak Auxine like IAA or NAA instead of 2,4-D, (2,4-D) that is a strong Auxine is used [1, 18-21]. Also, different Cytokinin compounds are used for regeneration from which the most important are BAP and Kinetin [1]. Potato has many improvements in producing callus in recent years [22]. Callus tissues derived from plants micro plantlet may appear to be different from each other as well as reproducibility and regeneration. Thus callus are divided into two categories of embryonic and non-embryonic calluses. Groups of callus that have the capability of producing plant through the processes of organogenesis and embryogenesis are called embryonic callus. First visible indicator in development of embryonic callus is often grain, color and surface morphology. Embryonic callus are dense and with node and often brittle [23, 24]. General properties growth of a callus depends on complex relationship between cultured micro sample, culture content and culture conditions at the time of *in vitro* culture [25]. Tissue culture technology has a wide range of techniques that are potentially useful in all of which cells with plant tissues are cultured in an artificial food condition so through this, whole plants are produced from tissue or isolated cells. Different species, varieties within each species, or even different parts of a plant do not have the same reaction to *in vitro* culture. This progress and making homogeneity ways that are commonly used in breeding programs. Therefore, for using these methods in breeding program, the optimal synthetic medium combination should be identified for used genetic material. Callus production and somatic embryogenesis and thus plant regeneration from somatic embryos are considered the main problem in application of biotechnology techniques in breeding programs. Some researchers consider such differences in response to culture tissue for different combinations and concentrations of within hormones and different sensitivity to added synthetic hormones to culture medium. In addition to plant genotype and medium composition, the type of tissue that is used for production of callus has also contributed to the response for culture *in vitro*. Thus aim of this study was to evaluate the effects of plant growth regulators and light on callus production of potato varieties Sante, Agria and Savalan and determining the best conditions for callus genesis.

MATERIALS AND METHODS

In this study, potato varieties Agria, Sante and Savalan and MS medium were used. Concentration of sucrose and agar used in culture medium are 30 and 6.7 g/L, respectively. PH Solution is set in 5.7 and autoclave

is used for sterilizing culture medium at a temperature of 121°C and a pressure of 2.1 atmospheres for 15 min. In this experiment, the effect of hormonal treatments 2,4-D at three levels (0, 2 and 5 mg per liter) and Kinetin at three levels (0, 0.5 and 2 mg per liter) are reviewed in different photoperiod conditions (light and dark) on callus genesis of under study potato as factorial experiment in terms of a completely random design with 18 different treatments and three replicates of each treatment. It should be noted that each replicate consists of 5 test tubes which each tube has also a micro sample. At first, sterile seedlings should be divided into micro samples containing a bud with a length of about 5 mm in sterile conditions and then they should be placed in medium containing different hormonal concentration for production of callus. Micro samples for callus genesis after culture are transferred to growth room under two light photoperiod (16 h light and 8 h dark with light intensity of 5000 lux) and darkness and 25±2 C. Four weeks after culture of micro samples, traits such as callus fresh weight, callus diameter, percentage of callus genesis and regenerated seedling length was measured. Statistical calculations and graph drawing was done by using SAS and Excel software, respectively. Average comparing was also done with software MSTATC and using Duncan test at probability of level 5%.

RESULT AND DISCUSSION

Results of variance analysis showed that the effects of number and different hormonal combinations are significant on all under study traits in 1% level. Moreover photoperiod effect on all traits is significant at 1% level, except for callus fresh weight. Also the interaction of cultivar × hormone × photoperiod is insignificant on all traits except for percentage of callus genesis (Table 1).

Comparison of the effect of different hormone combinations on callus diameter indicates the maximum diameter of callus related to hormone combination of 5 mg/l and 2 mg/l of kinetin with mean of 1.198 cm and the minimum is related to control treatment (non-hormone) with a mean of 0.096 cm. The highest callus fresh weight was also associated with combined hormone of 5 mg per liter and .5 mg/l of Kinetin with an average of 0.426 mg and a minimum is related to control treatment (no hormone) with a mean of 0.013 g. In addition, the highest seedling length is related to combined hormonal of 0 mg/l 2,4-D and 0.5 mg/l in kinetin with a mean of 2.591 cm a minimum is related to 5 milligrams per liter 2,4-D and 2 mg/l of kinetin with an average of 0.52 cm. In this experiment, the highest percentage of callus genesis is related to hormone combination of 5 mg/l 2,4-D and 2 mg/l of kinetin with an average of 90% and the minimum is related to control treatment with a mean of 17.952 percent (Table 2).

Comparison of averages effects of potato varieties on callus diameter indicates that the maximum diameter of callus is related to Savalan with average of 1.121 and the minimum is related to Agria with an average of 0.41 cm. the highest callus fresh weight was also attributed to Savalan with an averave of 0.425 g and the lowest is related to Agria with an average of 0.043 g. In addition, maximum length of seedling is related to Savalan with an average of 2.310 cm and the minimum is related to Agria with an average of 0.797 cm. In this study, the highest percentage of callus genesis is related to Sante with an average of 68.413% and the lowest is related to Agria with an average of 50.592% (Table 3).

Comparison of averages effects of photoperiod on callus diameter indicates that the maximum diameter of callus is related to dark condition with average of 0.709 p and the minimum is related to light condition with an average of 0.637 cm. The highest length of plantlet was

Table 1: Results of variance analysis of effects of number, hormone and light on under study traits

Source of Variation (S.O.V)	Mean-square				
	Degrees of freedom	Callus diameter	Fresh weight of callus	Seedling length	Percentage of callus genesis
Cultivar		8.196 *	2.000 *	33.058 *	4392.777 *
Hormone	2	3.311 *	0.534 *	10.183 *	16316.374 *
Photoperiod	8	.214**	0.004ns	6.820**	1100.221**
Cultivar × Hormone	1	.849**	0.312**	1.687**	1337.184**
Cultivar ×Photoperiod	16	0.103*	0.024*	2.022**	397.431*
Hormone × Photoperiod	2	.034 ns	.008 ns	.159 ns	403.145**
Cultivar × Hormone× Photoperiod	8	.042 ns	.007 ns	.476 ns	517.515**
Test error	16	.031	.007	.439	111.940

** Significant at 1%; * Significant at 5% and ns is insignificant

Table 2: Comparison of effect average of different hormone combinations on traits

Hormon treatments (Mg/ lit)	Callus diameter (Cm)	Fresh Weight callus (G)	Seedling length (Cm)	Percentage callus
Control (no hormone)	0.096 d	0.013 c	2.427 ab	17.952 e
2 2,4-D + 0 Kinetin	0.832 c	0.204 b	1.486 c	72.751 c
5 2,4-D + 0 Kinetin	0.97 b	0.418 a	1.096 cd	77.751 bc
0 2,4-D + .5 Kinetin	0.086 d	0.16 c	2.519 a	17.443 e
2 2,4-D + .5 Kinetin	0.878 bc	0.220 b	1.081 de	79.096 bc
5 2,4-D + .5 Kinetin	0.936 bc	0.426 a	0.618 ef	79.737 bc
0 2,4-D + 2 Kinetin	0.174 d	0.043 c	2.017 b	26.916 d
2 2,4-D + 2 Kinetin	0.886 bc	0.238 b	1.168 cd	81.143 b
5 2,4-D + 2 Kinetin	1.198 a	0.419 a	0.52 f	90 a

Numbers in each column with common letters are not significantly different (Duncan and $\alpha = 5\%$).

Table 3: Comparison of effects average of Cultivar on traits

Cultivar	Callus diameter (Cm)	Callus fresh weight (g)	Seedling length	Callus genesis percent
Sante	.482b	.197b	1.207b	68.413a
Savalan	1.121a	.425a	2.310a	31.924b
Agria	.410b	.043c	.797c	50.592c

Numbers in each column with common letters are not significantly different (Duncan and $\alpha = 5\%$).

Table 4: Comparison of effects average of photoperiod on traits

Photoperiod	Callus diameter (Cm)	Callus fresh weight (g)	Seedling length	Callus genesis percent
Light	.637b	.217b	1.643b	57.704a
Dark	.709a	.227a	1.233a	62.916a

Numbers in each column with common letters are not significantly different (Duncan and $\alpha = 5\%$).

also attributed to light condition with an average of 2.310 cm and the lowest length of plantlet is related to dark condition with an average of 0.797 g. In addition, maximum callus genesis percent is related to dark condition with an average of 62.92 percent and the minimum is related to light condition with an average of 57.7 percent. It should be mentioned that the highest callus fresh weight is related to dark condition with an average of 0.227 g and the lowest is related to light condition with an average of .217 g (Table 4).

Comparison of effects average of interactions cultivar × hormone × photoperiod shows that Sante cultivars in a combined hormone 2 mg per liter 2,4-D and 0.5 mg/l of Kinetin and in lighting situations have more callus genesis percentage (90%) compared with other hormone combinations. While in dark conditions, mentioned figure in all hormone treatments have high callus genesis percent except 0.5 mg/l of Kinetin without 2,4-D and Treatment of 0.5 mg/l of Kinetin and 2 mg per liter 2,4-D. Savalan and Agria cultivars in lighting conditions compared with dark in different hormonal treatments may have a greater percentage of callus geneses. While Sante in darkness condition compared with lightening in different hormonal treatments have a higher percentage of callus genesis (Table 5 and 6).

Table 5: Comparison of effects average of interaction of photoperiod × figure × hormone on callus genesis percent on lightening photoperiod

Cultivar	Hormone (mg/l)	Callus genesis percent
Sante	Control (no hormone)	17.71 jklm
	2 2,4-D + 0 Kinetin	54.99 cdef
	5 2,4-D + 0 Kinetin	51.14 defg
	0 2,4-D + .5 Kinetin	72.29 abc
	2 2,4-D + .5 Kinetin	90a
	5 2,4-D+ .5 Kinetin	81.14ab
	0 2,4-D + 2 Kinetin	30.79 higk
	2 2,4-D + 2 Kinetin	47.30 efgh
	52,4-D + 2 Kinetin	51.14 defg
Savalan	Control (no hormone)	90a
	2 2,4-D + 0 Kinetin	90a
	5 2,4-D + 0 Kinetin	81.14ab
	0 2,4-D + .5 Kinetin	63.34 bcde
	2 2,4-D + .5 Kinetin	59.21 cdef
	5 2,4-D + .5 Kinetin	81.14ab
	0 2,4-D + 2 Kinetin	90a
	2 2,4-D + 2 Kinetin	90a
	5 2,4-D + 2 Kinetin	90a
Agria	Control (no hormone)	0m
	2 2,4-D + 0 Kinetin	0m
	5 2,4-D + 0 Kinetin	90a
	0 2,4-D + .5 Kinetin	90a
	2 2,4-D + .5 Kinetin	90a
	5 2,4-D + .5 Kinetin	90a
	0 2,4-D + 2 Kinetin	8.857lm
	2 2,4-D + 2 Kinetin	8.857lm
	5 2,4-D + 2 Kinetin	81.14ab

In each column, numbers with common letters have no significant difference (Duncan and $\alpha = 5\%$)

Table 6: Comparison of effects average of interaction of photoperiod×cultivar× hormone on callus genesis percent in dark photoperiod

Cultivar	Hormone (mg/l)	Callus genesis percent
Sante	Control (no hormone)	90a
	2 2,4-D + 0 Kinetin	90a
	5 2,4-D + 0 Kinetin	90a
	0 2,4-D + .5 Kinetin	0m
	2 2,4-D + .5 Kinetin	25.78 ijkl
	5 2,4-D + .5 Kinetin	90a
	0 2,4-D + 2 Kinetin	90a
	2 2,4-D + 2 Kinetin	90a
	52,4-D + 2 Kinetin	90a
Savalan	Control (no hormone)	0m
	2 2,4-D + 0 Kinetin	35.01 ghij
	5 2,4-D + 0 Kinetin	90a
	0 2,4-D + .5 Kinetin	43.08 fghi
	2 2,4-D + .5 Kinetin	51.14 kefg
	5 2,4-D + .5 Kinetin	64.22 bcde
	0 2,4-D + 2 Kinetin	0m
	2 2,4-D + 2 Kinetin	8.857 lm
	5 2,4-D + 2 Kinetin	90a
Agria	Control (no hormone)	72.29 abc
	2 2,4-D + 0 Kinetin	59.21 cdef
	5 2,4-D + 0 Kinetin	68.07 bcd
	0 2,4-D + .5 Kinetin	0m
	2 2,4-D + .5 Kinetin	31.08 klm
	5 2,4-D + .5 Kinetin	72.29 abc
	0 2,4-D + 2 Kinetin	63.43 bcde
	2 2,4-D + 2 Kinetin	90a
	5 2,4-D + 2 Kinetin	90a

In each column, numbers with common letters have no significant difference (Duncan and $\alpha = 5\%$)

DISCUSSION

The results of this study showed that there is a very significant difference ($p \leq 0.01$) among the studied cultivars, different hormonal treatments and photoperiod for all traits. Besides hormonal treatments 5 mg/l 2,4-D and 2 mg/l Kinetin was superior in terms of callus genesis percentage and callus diameter compared with other hormonal treatments. Savalan cultivar also among the under study cultivars had superiority in terms of all traits except for callus genesis percent. Dark photoperiod also showed significant superiority compared with lightening in terms of callus genesis percentage and callus diameter. These results correspond with results obtained by [26].

[4] showed with reviewing the effect of concentration of growth regulators (2,4-D and Kinetin) and light on varieties of potato callus genesis that a significant difference was observed in terms of percentage of callus genesis and roots on callus in 1 ml/l 2,4-D. They also reported that potato produce callus both in the light and in the dark condition and produced callus which are exposed to light are all yellow and firm. While callus produced in the dark are brittle, some of them are yellow and some are yellow to white. MS based culture media induce the greatest growth of stem. Some researchers have reported that light is not necessary for callus growth and it also takes place in the dark [27, 21].

[16] reported that in leaf micro sample potato in terms of callus genesis speed are more and they have little root on middle node micro sample. [26] showed with reviewing the effect of different concentration of 2,4-D and Kinetin on callus genesis of potato that in high levels of 2,4-D, callus genesis percentage and root numbers on callus are reduced in potato [17] reviewed induction of callus from Almera cultivar of potato on MS medium containing various concentrations of different 2,4-D and they showed that micro sample in MS medium with and without 2,4-D are able to produce callus. Also high concentration of 2,4-D (3 mg/l) is the most effective concentration for callus induction. In general, the results showed that with increasing 2,4-D concentration in medium, more cell division and larger callus are producing. In addition, the highest and the lowest percentage of callus genesis were observed in Agria and Sante cultivars.

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