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Effect of growth regulators and colchicine on regeneration of two potato cultivars *in vitro*

HASSAN ABU-QAOUD[1,*], BAKER BSHARAT[2] AND MUNQEZ SHTAYA[1]

[1]*Department of Plant Production and Protection*
Faculty of Agriculture and Veterinary Medicine
An-Najah National University, Nablus P.O. Box 707, Palestine
[*]*(e-mail: hassan@najah.edu)*

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ABSTRACT

The effect of different levels of Benzyl adenine (BA) and Naphthalene acetic acid (NAA) on *in vitro* regeneration of Spunta and Mondial potato cultivars were studied, in addition, the effect of different levels of colchicine administered either as part of MS media (0.0, 10 ì M, 100 ì M and 1 mM) or by soaking potato buds (0.0, 1, 2 and 3 mM) on *in vitro* regeneration of two potato cultivars was studied in the Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Tulkarm, Palestine. Higher shoot number was obtained when BA at 1 and 2 ppm were combined with 0.5 ppm NAA or without NAA, with variation between the two cultivars. Root number was significantly affected with the hormone levels, higher root number per bud in Spunta cultivar was obtained when NAA was utilized exclusively at 1 and 0.5 ppm respectively, however with Mondial cultivar, the higher number was observed at 1 ppm NAA. Significant effect of colchicine on shoot multiplication in both cultivars was observed, higher average shoot number for both cultivars (3.25 and 3.07) was recorded when higher level of colchicine (1mM and 100 ì M) was added to the media for "Spunta' and "Mondial" respectively. When potato buds were soaked in colchicine, no significant effect on regeneration ability of the two cultivars was observed. Our results indicated a positive effect of colchicine on *in vitro* regeneration of two potato cultivars, therefore, protocols of polypolidization of potato through *in vitro* culture could be implemented.

Key words: Colchicine, growth regulators, potato, regeneration

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's third most-produced food crop. It is an essential component of the human diet. It is considered one of the top promising crops to reduce human hunger and poverty in the world (Maiti and Singh, 2022; Naeem *et al*., 2023). Potato production has reached over 300 million tons annually (It is estimated that over 1 billion people use potato as a staple food (Iqbal *et al*., 2016). The annual cultivated area in Palestine was estimated to be around 1579 ha, with about 60,000 tons as annual production (FAOSTAT Agriculture, 2021), Potato is originated as a hybrid between an unknown wild plant genus *Ipomoea* (O'Brien, 1972). Potato is also an auto-tetraploid (2n = 4x =48), which makes breeding difficult (Mahbube *et al*., 2011). Biotechnology has long been used to accelerate potato plant breeding including tissue culture processes or polyploidy induction through mutagenic agents (Siregar *et al*., 2022). Polyploidization is an important approach in crop breeding for agronomic trait improvement, especially for biomass production (Naeem *et al*., 2023). Polyploidization enhances genotype diversity and increases ploidy level, as a result of the doubling of chromosome the size of the cells increases. An increase in cell size may result in the development of larger plants (Tiwari and Mishra, 2012).

Several methods are used to induce polyploidization, Colchicine is the most widely used agent for chromosome multiplication. Colchicine ($C_{22}H_{25}O_6N$) is a chemical mutagen used for the induction of polyploidy in many plants (Mncwango *et al*., 2019; Rodiansah and Puspita, 2020). To-date, there are at least 3281 registered mutant varieties

[2]Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

of major crop commodities. Among them, 46 are colchicine-induced polyploids (Eng and Ho, 2019). Immersion of plants in colchicine can increase ploidy, which affects the morphological characteristics of plants, causing the plants to grow larger, which is expected to increase productivity (Siregar *et al*., 2022). Significant differences in plant height and leaf size in Stevia mutants treated with cholchocine were observed (Azizan *et al*., 2020). Colchicine treatment of diploid potato (*Solanum chacoense*) plantlets resulted in tetraploid (Tet) and cytochimera (Cyt) lines with significantly increased in plant growth and biomass yields (Zhao *et al*., 2022). In addition, polyploidy would not only improve the quality and quantity of crop yields but also produce a unique plant which has a resistance to pests and others abiotic stress (Rodiansah and Puspita, 2020). Concentration and induction time are important parameters with clear interactions between the two factors (Sjahril *et al*., 2020). According to Syaifudin *et al*. (2013) colchicine will work effectively at concentrations of 0.01-1.00 % or 0.001- 1.00 % with the duration of soaking colchicine ranging from 3-24 hours.

In vitro system depends on regeneration system, mutagenic agent have a direct effect on regeneration. Ahmad *et al*. (2010) reported that the regeneration ability of different explants of three potato cultivars was affected by colchicine treatment therefore, the effect of these chemicals on regeneration must be studied before proceeding with polyploidaization protocol. The objective of this study was to investigate the effect of different concentrations of colchicine, supplemented with two different levels of BA and NAA, using two application methods, on the *in vitro* regeneration of two potato cultivars.

MATERIALS AND METHODS

The study was carried out in the Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Tulkarm, Palestine during the 2020-2021 growing season. Tow potato cultivars (Spunta and Mondial) were used in the experiment.

In vitro Establishment

Fifty tubers of Spunta and Mondial potato cultivar were cleaned with running tap water, then dried and wrapped with 70% alcohol before being incubated in a growth chamber at 23°C and 16 h/8 h (light/dark) at an irradiance of 40 µmol/m^2/s. After 3 week, sprouted buds were excised and soaked in a 0.2% sodium hypochlorite (V/V) solution for 15 minutes, followed by 30-second washing with 70% alcohol, followed by three times washing (5 minutes/time) with sterile distilled water. The buds were then planted onto a solid MS (Murashige and Skoog, 1962) multiplication media. In this study, two separate experiments were conducted. Experiment 1 (multiplication experiment) aimed to investigate the effects of three factors, namely BA, NAA, and cultivar, on the *in vitro* regeneration of potato. While in experiment 2 (colchicine experiment), the effect of 2 two factors, cultivar and colchicine concentration on *in vitro* potato regeneration was investigated.

Multiplication Experiment

Disinfested buds of both cultivars were cut at the base and transferred to MS media supplied with different combination of NAA and BA, the cultures were kept in a growth chamber at 22 ± 1°C and 16 h/8 h (light/dark) at an irradiance of 40 µmol/m2/s). The experiment was arranged as a factorial treatment with two factors (BA and NAA) for each cultivar, treatments were the combination of both levels of BA (0.0, 1.0 and 2.0 ppm) and NAA (0.0, 0.5, and 1.0 ppm) were arranged in a completely randomized design (CRD) with 20 replicate per treatment. At the end of the experiment, number of multiplied shoots, leaf number per shoot and number of roots were recorded for each replicate (tube).

Colchicine Experiments

Fifty tubers of each cultivar were incubated as before, 200 sprouted buds were cut off, disinfested as in the multiplication experiment, one hundred were disinfested as before and transferred to MS basal media supplied with 1 ppm BA and 0.5 ppm NAA as a multiplication medium, based on the results of experiment 1, where different levels of BA and NAA were tested. The medium was divided into four different media supplied with 4 different levels of colchicine (0.0, 10 ì M, 100

ì M and 1 mM) with 20 buds per level. The remaining 100 buds were soaked with different levels of colchicine (0.0, 1, 2 and 3 mM) for 24 hours under dark aseptic condition, 25 buds per level, the buds were then transferred to the culture media. All tubes were incubated at 22 ± 1°C and 16 h/8 h (light/dark) at an irradiance of 40 µmol/m^2/s. After four weeks, the number of shoots, leaf and root per shoot were recorded for each tube, each experiment was arranged as a factorial treatment (cvs and colchicine levels) in a completely randomized design with 20 replicate (tube) per treatment.

Rooting and Acclimatization

Regenerated shoots of about 2-3 cm long were isolated and transferred to multiplication media for more proliferation, after 4 weeks developed shoots were cut (2-4 cm) length dipped in IBA rooting powder of 8000 ppm (T8) before being transplanted to 10 cm diameter pots filled with sterile vermiculite and peat moss mixture (1:2; v/v) (Abu-Qaoud *et al*., 2022). The pots were sealed with thin plastic sheet and irrigated with sterile water before being placed in the growing conditions described above. The sheets were gradually removed one week later. Shoots with well-formed roots were transplanted into 30 cm diameter pots filled with both vermiculite and peat moss mixture (1:2; v/v) and kept in a greenhouse with partial shade (30-40% of the time). Rooted shoots from different treatments of the two colchicine administration protocol (in media and soaking) were kept in the greenhouse for further growth.

Data collection and Statistical Analysis

In all experiments, the culture was evaluated after four weeks, shoot, root and leaf number per explant were recorded, data were analyzed using Minitab (18) software, for multiplication experiment the data for each cultivar were analyzed separately. ANOVA was conducted followed by mean separation using Fisher least significant test (LSD) at 0.05 probability level.

RESULTS AND DISCUSSION

Multiplication Experiment

The effect of different hormones on shoot multiplication of “Spunta” and “Modial” potato cultivars are presented in Table 1. Significant differences were observed in the average number of shoots multiplied per explants in response to the different hormone combinations and concentrations. Maximum shoot number (5.0) with Spunta cultivar was observed with medium supplied with 1 ppm BA and 0.5ppm NAA, however, without significant

Table 1. Effect of different levels of NAA and BA on regeneration of Spunta and Mondial potato cultivars *in vitro*

Growth regulator		Spunta			Mondial		
BA	NAA	No. of shoots	No. of leaves	No. of roots	No. of shoots	No. of leaves	No. of Roots
0	0.0	1.83^{e}	4.00^{cd}	6.50^{c}	1.50^{d}	5.33^{ab}	3.75^{bc}
	0.5	4.67^{ab}	5.72^{abc}	28.33^{ab}	4.00^{ab}	6.48^{ab}	5.70^{bc}
	1.0	4.00^{abc}	6.33^{ab}	31.67^{a}	3.50^{bc}	7.13^{a}	15.94^{a}
1	0.0	2.50^{cde}	5.74^{abc}	1.33^{c}	5.00^{a}	3.90^{b}	0.67^{c}
	0.5	5.00^{a}	3.39^{d}	26.00^{b}	4.00^{ab}	4.60^{b}	0.00^{c}
	1.0	2.00^{de}	4.50^{bcd}	0.00^{c}	4.00^{ab}	3.20^{b}	11.23^{ab}
2	0.0	2.67^{bcd}	4.00^{bcd}	0.00^{c}	1.50^{d}	5.34^{ab}	0.67^{c}
	0.5	4.17^{abc}	3.17^{d}	0.00^{c}	2.17^{cd}	4.10^{b}	0.00^{cd}
	1.0	2.17^{bcd}	6.67^{a}	0.00^{c}	3.50^{b}	4.42^{b}	2.67^{c}
P-Value							
NAA		<0.0001	0.012	0.0001	0.050	0.962	0.000
BA		0.1450	0.298	<0.0001	0.000	0.014	0.000
NAA*BA		0.0468	0.003	<0.0001	0.005	0.316	0.000

Means within each column that do not share a letter are significantly different at P=0.05 probability level according to LSD test.

difference from media supplied with 2 and 0.5ppm, of both BA and NAA. In Mondial cultivar, maximum shoot number per explant was observed with media supplied with 1 ppm BA, but without significant difference from media supplied with 0.5 or 1 ppm NAA, or medium with only 0.5 NAA, the least number of shoots (1.5 and 1.83) was observed in media without hormones for both Mondial and Spunta cultivar respectively. Regarding the average number of leaves per regenerated shoot, no clear trend was observed in both cultivars, however, in Spunta the higher number was observed in medium supplied with 2 and 1 ppm of BA and NAA (6.67), however this number was significantly similar to media supplied with only NAA at 0.5 or 1.0 ppm or that with media with 1 ppm BA only for Mondial cultivar, the highest average leaf number (7.13) was obtained in medium supplied with 1 ppm NAA but without significant difference from media without hormone or those supplied with either 2 ppm BA or 0.5 ppm NAA only. The number of roots was significantly influenced by the hormonal levels during the multiplication stage. In the Spunta cultivar, a higher number of roots per bud (31.67 and 28.33) was observed when NAA was used without BA at 1 and 0.5 ppm, respectively. However, in the Mondial cultivar, the highest number of roots (15.94) was observed at 1 ppm NAA, which did not significantly differ from the number obtained with media supplemented with 1 ppm of both NAA and BA. This indicates that hormonal supplementation of the shooting media in MS significantly affects rooting in media supplemented with IBA.

In this study higher shoot number was obtained when BA at 1 and 2 ppm were combined with 0.5 ppm NAA or without NAA, with variation between the two cultivars, this was in agreement with other researchers who reported good shoot regeneration of potato plant from callus and meristem by using the, BAP and NAA at the rate of 3.0 mg/L and 2.0 mg/L, respectively (Pawar *et al*., 2019), others found higher regeneration with opposite hormone combination (high auxin to low cytokinin, with variation between cultivar). The outcome of present study with respect to variation between the two cultivars supports the previous study reported by Thornton *et al*. (2013) that genotype played a very significant role in potato micropropagation. Hajare *et al*. (2021) also reported that culture initiation was obtained on MS medium supplemented with 1.5 mg/l BAP + 3.0 mg/l NAA for Gudiene variety, whereas 1.0 mg/l BAP and 2.0 mg/l NAA produced more shoots in Belete potato variety. *In vitro* regeneration and multiplication potential was different among three potato cultivars (Iqbal *et al*., 2016). With Mondial cultivar, in this study, no significant difference in shoot number between media with 0.5 ppm NAA only and that containing 0.0, 0.5 and 1 ppm NAA combined with 1 ppm BA. Both auxins and cytokinins are important for shoot regeneration, it has been reported that exogenous application of different cytokinins is an obligation for the induction of multiple shoots in many plants (Kishor and Devi, 2009), this does not ignore the endogenous auxin level. No clear trend was observed regarding the leaf number, but it was higher with the higher BA levels. Leaf number is mainly correlated to shoot length which was not measured in the study, however, Badoni and Chauhan, (2009) reported higher potato shoot height when Kinetin and NAA were used at 1 mg/L Kinetin and 0.1 mg/L NAA combination.

Colchicine Experiments

The effect of colchicine on *in vitro* regeneration of both potato cultivars is presented in Tables 2 and 3.

Colchicine in the Media

The effect of supplementing different levels of colchicine in the media is shown in Table 2. Significant effect of colchicine on shoot multiplication in both cultivars was observed. In Spunta cultivar, the higher significant shoot number (3.25) was obtained with colchicine at 1 mM, however, with Mondial cultivar, the higher shoot number was observed with both 100 µM and 1mM levels (3.07 and 2.93 shoots) respectively, this was not differ from the control, there was no significant interaction between colchicine and cultivar (P = 0.234). Regarding the average leaf number per regenerated shoots, significant interaction was observed between the two factors with the higher leaf number (9.71) obtained in medium supplied with 100 µM colchicine without significant difference from media containing 10 µM and 1 mM in both cultivars. In case of rooting, the higher root

Table 2. Effect of different levels of colchicine in the media on regeneration of Spunta and Mondial potato cultivars *in vitro*

Cultivar	Colchicine	No. of shoots	No. of leaves	No. of roots
Spunta	0.0	2.00^{c}	6.38^{bcd}	4.00^{c}
	10 µM	1.94^{c}	4.94^{de}	2.10^{d}
	100 µM	2.00^{c}	2.25^{e}	6.61^{a}
	1 mM	3.25^{a}	7.05^{cd}	3.833^{c}
Mondial	0.0	2.50^{abc}	6.00^{cd}	5.95^{ab}
	10 µM	2.18^{c}	8.59^{ab}	5.22^{abc}
	100 µM	3.07^{ab}	9.71^{a}	4.90^{bc}
	1 mM	2.93^{ab}	8.07^{abc}	6.43^{a}
P-Value				
Cv		0.1170	0.1560	0.000
Colchicine		0.0010	0.0000	0.004
Cv*Colcicine		0..234	0.0010	0.000

Means within each column that do not share a letter are significantly different at P=0.05 probability level according to LSD test.

number (6.61) was recorded in media with 100 µM with Spunta cultivar, this number was not statistically different from treatment received 10 µM, 1 mM or the control in Mondial cultivar.

Colchicine (Soaking)

The results of soaking potato buds into different levels of colchicine are shown in Table 3. Higher levels of colchicine were used in soaking experiments with less exposure time. Shoot number was not affected significantly with the colchicine treatment with lower shoot number of all colchicine treatment than the control. The average leaf number per shoot did not also influenced by colchicine treatment, the higher significant leaf number (9.0) was obtained with the control treatment of Mondial cultivar, however, in Spunta, explants soaked with 1 or 2 mM colchicine exhibited significantly similar leaf number per shoot, higher level of colchicine (3 mM) significantly reduced the average leaf number (3.1) specially in Spunta cultivar. There was a clear interaction between the two factors in respect to leaf number. For root number, a significant interaction (P = 007) between cultivars and colchicine was shown, the higher average root number was observed with both cultivars in the control media (7.4 and 5.33) for Mondial and Spunta, respectively, but was not differ significantly from media with 3 mM in Modial and 2 mM in Spunta.

Table 3. Effects of soaking potato buds in different levels of colchicine on their *in vitro* regeneration of two potato cultivars

Cultivar	Colchicine	No. of shoots	No. of leaves	No. of roots
Spunta	0.0	2.50	5.71^{cd}	5.28^{ab}
	1 mM	2.00	4.50^{de}	1.80^{c}
	2 mM	2.25	6.00^{cd}	5.44^{ab}
	3 mM	2.00	3.10^{e}	4.10^{b}
Mondial	0.0	3.00	9.00^{a}	7.40^{a}
	1 mM	2.00	7.10^{bc}	5.00^{b}
	2 mM	2.00	5.00^{d}	3.90^{bc}
	3 mM	1.70	7.20^{bc}	5.33^{ab}
P-Value				
Cv		0.594	0.0001	0.018
Colchicine		0.622	0.0536	0.004
Cv*Colcicine		0.522	0.0002	0.007

Means within each column that do not share a letter are significantly different at P=0.05 probability level according to LSD test.

The result of this study indicated a positive effect of colchicine on shoot regeneration when colchicine was added to the media, however, when explants were soaked in colchicine solution a negative effect was observed, the high concentration used in the soaking experiment could be the reason of the outcome results, this hypothesis was supported by Chakraborti *et al*. (1998) who found that a negative effect of high colchicine concentration on shoot regeneration of African marigold explants. In addition, Ganga and Chezhiyan (2002) reported a negative effect of higher levels of colchicine (2.5, 5.0, 7.5 and 10.0 mM) on the number of multiple shoots regenerated in four banana cultivars, they also reported that the cultivars exhibited a genome-related response. The retarded growth could be due to reduced rate of cell division by colchicine. Similar observations in which shoot length decreased due to initial retardation of growth have also been reported (Abu-Qaoud and Shtaya, 2014). On the other hand, using colchicine at low concentration could have a positive effect on regeneration, this was supported by Siregar *et al*. (2023) who found that colchicine treatment resulted on variations of Olimpus potato shoot differently from control, colchicine with 0.04% for 24 hours immersion showed better growth of shoot and leaf number than the control, colchicine at 0.02% gave highest number of root. The higher concentration of colchicine

may have resulted in higher colchicine absorbed by cells so that root growth was inhibited.

In conclusion, the findings of this study demonstrate a positive effect of colchicine on shoot regeneration when added to the media. However, a negative effect was observed when explants were soaked in a high concentration of colchicine solution. The negative impact on growth and cell division is likely the result of colchicine-induced inhibition. On the other hand, the use of low concentrations of colchicine showed a positive effect on shoot, leaf, and root regeneration. Our results indicate successful acclimatization and growth of the rooted shoots. Further investigation is warranted to explore the effects of different colchicine levels on the regeneration process, aiming to optimize the efficiency of shoot regeneration in potato cultivars.

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