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Morphogenic responses of two potato cultivars explants to sucrose, photoperiods and growth regulators

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ABSTRACT

The effects of sucrose concentration, photoperiod and growth regulators on stem nodal segments of potato (*Solanum tuberosum*) cultivars (Lady Rosetta and Chara) were studied. Firstly, stem nodal segments were cultured on Murashige and skoog's culture media (MS) containing sucrose concentrations equal to 3, 6, 9 and 12%. The highest microtubers number (7.50 ±1.0 and 1.83±0.30 / single nodal segment for lady rosetta and Chara respectively was obtained using sucrose concentration (9%) and this is why MS culture medium augmented with 9% sucrose was used to detect the effect of light/darkness photoperiod regime (16/8, 8/16 and 0/24) on explants. The best photoperiod regime is probably 16/8 over the other regimes where the numbers of microtubers per single explant reached up 7.33±1.45 and 2.33±0.33 for Lady Rosetta and Chara, respectively. The importance of light can be understood from the result that complete and continuous darkness significantly inhibited microtuber production in both cultivars. For the third type of treatments in this study MS augmented with the best sucrose concentration (9%) and the best photoperiod regime 16/8 were fixed while the effects of thidiazuron (TDZ) /dichlorophenoxy acetic acid (2,4-D) at 1and 5 ppm (individually and/or combined) were studied. In most cases, TDZ or 2, 4-D and their combinations, induced callus formation rather than microtuberization. Calli were almost compact, nodular brownish, yellowish or greenish and the highest calli fresh weights were 2.22±0.85 and 1.25±0.64 gram for Lady rosetta and Chara respectively. However, both morphogenic responses have their own promising uses in modern plant biotechnology.

Key Words:

Callus, Growth regulators, Microtuber, Potato, Photoperiod, Sucrose

1. INTRODUCTION

Potato crop *(Solanum tuberosum* L.) represents the fourth global food following only rice, wheat and maize. It has many health and nutrition benefits because it is rich in carbohydrates (about 75% weight) and contains proteins, fiber, vitamin C, vitamin B6, vitamin B3 **(**Niacin**),** potassium, manganese, magnesium, phosphorus and antioxidants like flavonoids, carotenoids and phenolic compounds [1, 2].

Potato cultivars are commonly vegetatively propagated by seed tubers, however chronic viral and bacterial diseases may occur which causes deterioration of tuber quality over few clonal generation and great yield loss [3]. This problem could be solved by *in vitro* production of potato microtubers.

Microtubers have the advantages of being easier for handling, transport and storage, and less costly than conventional tubers. Potato microtuberization appeared to be controlled by a range of exogenous and endogenous factors including sucrose concentration, temperature, photoperiod, light intensity, hormones and cultivar type [4]. Sucrose is critical factor as an energy source, osmotic adjustment and conservation of cellular water, and as signaling molecule [5]. In this respect, Asmono *et al*. [6] observed that the addition of sucrose at 150 g l^{-1} to culture medium gave higher microtubers number in red potato (*Solanum tuberosum*, L. var Desiree) than the other used concentrations, whereas 90 g l^{1} was the best for microtubers fresh mass.

Microtubers initiation and early growth is sensitive to photoperiod length with some contradictory appeared in the literature about the optimum light duration. Optimum day length for microtuberization may be 16, 8 h or 0.0 light which corresponds to 8, 16 or 24h night, respectively [7]. This may be related to variation in genotype of explant, light intensity, spectral wave length and hormonal balance.

Growth bioregulators play a principal role in explants microtuberization, where cytokinins appeared to be the potent one by regulating the partition of assimilates towards the sink. Consequently, thidiazuron (TDZ) and kinetin as cytokinins were widely applied for micropropagation [8]. Additionally, auxins such as 2,4-dichlorophenoxyacetic acid which is known as 2,4-D was found to induce Microtuberization [9], however the dual impact of this growth regulator with TDZ on potato microtuberization was rarely studied.

Callus induction is a central process in plant tissue culture and biotechnology. Potato callus could be utilized for microtubers production, secondary metabolites biosynthesis and developing new potato lines [10,11]. Auxins and cytokinins, at an intermediate ratio, induce callus formation [12], however optimization of combined TDZ and 2,4-D levels for potato callus formation did not appear in the literature and is needed to be explored.

Egypt is ranked the fifteenth on the world in potato production, however tubers yield is low compared with Northern European countries due to environmental impact, spread of microbial diseases and seed cost [13]. Therefore, this study was undertaken to detect the effect of sucrose, photoperiod, TDZ and 2, 4-D bioregulators levels for microtuberization organogenesis in Lady Rosetta and Chara potato cultivars. Additionally, the effect of these plant growth regulators on callus formation and characteristics was explored.

2. METHODS AND MATERIALS

2.1 Plant Materials and source of explants

All experiments were undertaken at the Plant Biotechnology Lab., Faculty of Science, Port-Said, Egypt. Two potato (*Solanum tuberosum* L.) cultivars (Lady Rosetta and Chara) were used in this study, where their diseases-free *in vitro* plantlets were obtained from the Tissue Culture Laboratory, Faculty of Science, Al-Azhar University. These plantlets were used to provide explant materials along the study period.

2.2 *In vitro* multiplication of plantlets

For micropropagation, Murashige and Skoog (MS) medium was used and supplied with sucrose concentration 3%, myo-instol 0.01% and solidified with agar 0.6% [14]. The medium pH was adjusted to be between 5.6-5.8, using 1.0 M HCl or 1.0 M KOH. The plantlets were carefully cut under aseptic conditions in a Laminar flow Cabinet to get explants with two-stem nodes. After that, these explants were cultured aseptically in standard transparent sterilized jars containing 40 cm^3 of sterilized culture medium. These jars were transferred to the culture room at 20±2 ºC with 16 hours illumination and 8 hours dark,

where the light was provided by florescent tube lamps at 2,000 lux intensity [15]. Subculturing was carried out every six weeks interval to obtain explants for experimentation.

2.3 *In vitro* production of microtubers and callus

The above basal culture medium and conditions were used in three sequential experiments about potato microtuberization and callus formation. In first experiment, four sucrose levels 3%, 6%, 9% and 12% were supplemented to the medium. In second experiment, the effect of photoperiod duration was investigated, where three light/dark regimes were used : 16/8 and 8/16 day/dark, and continuous dark periods at optimum sucrose level as recorded from the 1st experiment. In third experiment, the influence of 2,4-D and TDZ bioregulators on microtubers and callus formation was observed. In this case, the culture medium was supplied with 0, 1 or 5 ppm 2,4-D and TDZ either alone or in combination at the best sucrose concentration and photoperiod length as appeared from 1st and 2nd experiments. In all experiments, microtubers and callus were harvested for analysis and characterization after 90 days incubation period.

2.4 Evaluated characteristics

The harvested microtubers and callus were kept in clean and dry Petri dishes. Then Growth aspects such as number of microtubers / explant, dimensions, fresh and dry biomass and moisture content were recorded. Dry biomass was obtained after oven-drying for 4 days at 65° C [16].

2.5 Data analyses

Results are presented by their mean ± SE (3 replicates or more). The General Linear Model (GLM mode) was used for analysis of variance with two factors during each test (Cultivar, photoperiod, sucrose and growth regulator). The significant difference between means at $p< 0.05$ was evaluated by LSD test. All statistical analyses were achieved by SPSS software version 19.

3. RESULTS AND DISCUSSION

Effect of sucrose concentrations on potato microtuberization

Influence of different sucrose levels on microtuberization (MT) induction of the two potato cultivars (Lady Rosetta and Chara) is shown in table 1 and fig.1. The effect of both factors, cultivar and sucrose, was significant on microtuber number. Lady Rosetta appeared to produce more microtubers than Chara was about 2-flod increase. Usage of sucrose at 90 g/l enormously increased microtuber numbers in relation with the other sucrose concentration (30, 60 and 120 g/l). It appeared that nodal segment that was cultured on MS medium and–provided with 90 g/l sucrose produced about 8 and 2 microtuber /explant in Lady Rosetta and Chara, respectively .

The main effect of cultivar was not significant on microtuber fresh and dry masses, length, and width, whereas sucrose effect was significant for theses parameters. However, at sucrose 90 g/l, Chara appeared to have higher microtuber fresh and dry masses, and dimensions than Lady Rosetta by about 100 - 200% increase. The length and width of Chara *in vitro* tubers at 90 g/l were about 12 and 8 mm, respectively, so they can be considered minitubers .Usage of 90 g/l sucrose concentration induced more increase in microtuber fresh and dry mass, length, and width than the other sucrose concentrations (30, 60 and120 g/l).

These results were compatible with others who found that the best sucrose concentration for potato microtuberization was 90 g/l [17, 18]. This is mainly due to sucrose acts not only as a carbon source but also as osmotic regulators and its ability to generate signals for formation of microtuber [5, 19]. In this respect, Islam *et al* [20]reported a strong correlation between sucrose concentration and microtuberization as the concentration increases the number of microtuber increases until certain level then start to decrease. The reduction of microtuber formation at high sucrose concentration might be due to the effect of super

optimal level of sucrose that can result in an unfavorable osmotic condition for water uptake, and thus affecting microtuber formation [21].

Effect of photoperiod length on potato microtuberization

The best sucrose level, 90 g/l, was supplemented to the culture medium of this experiment. Statistical analyses of obtained results displayed that the effect of photoperiod was significant on microtubers number, fresh and dry masses, and dimensions. Usage of photoperiod 16h light/8h dark was more promotive to microtubers number and growth than the other photoperiods (8/16h and 0/24h day/dark period length). Additionally, there was also a significant cultivar diverse, where Lady Rosetta produced higher microtubers/ explant than Chara and this was consistent with the results of first experiment. Lady Rosetta produced about 7 microtubers (MT)/ explant, whereas Chara manifested 2 MT only in response to this photoperiod (Table 2). Conversely, Chara MT fresh and dry masses, length and width was 2- 3-fold higher than those of Lady Rosetta and this was also compatible with the findings of first experiment.

Noteworthy, continuous darkness induced the lowest MT number in Chara and inhibited MT production in Lady Rosetta (0 MT/explant). These results were not far from those of AL-Hussaini *et al* [7]. Conversely, others found that 0/24h day/ dark period was the best for microtubers production [22, 23]. This variation in response of potato microtuberization to light duration could be attributed to the cultivar type, light intensity, and endogenous hormonal balance.

Effect of plant growth regulators on potato microtuberization

The effects of TDZ and 2,4-D plant growth regulators on potato microtuberization were investigated with 90 g/l sucrose level and 16/8 h day/dark photoperiod, and the results are shown in table 3 and fig. 2. Cultivar effects, in general, did not differ from those of the first and second experiments. Growth regulator had a significant impact on potato microtuberization, whereas all concentrations (1 & 5 ppm) of TDZ and 2,4-D and their combinations significantly reduced MT numbers and associated growth parameters as compared with control. The best treatment in this experiment was control (0.0 of TDZ and 0 2, 4-D) which gave 1-3 microtubers/ explant.

Although the addition of 1 TDZ + 0 2, 4- D to the culture medium appeared to improve to some extent MT fresh and dry masses, and dimensions in both cultivars, this effect was not significant in relation with control. These results clarified that the used TDZ and 2,4-D concentrations were not optimum for microtuberization in Lady Rosetta and Chara potato cultivars at 90 g/l sucrose. Conversely, others reported the beneficial effect of either TDZ or 2,4-D on in vitro potato microtuberization [24, 25], but ours may be the first report about the combined effect of TDZ and 2,4-D. .

Effect of plant growth regulators on potato callus formation and characteristics

Statistical analyses manifested that the main effect of both factors, cultivar and growth regulators, on callus number was significant. Lady Rosetta produced more callus by about 66% than chara (Table 4 and Fig.3). On many occasions, supplemented plant growth regulators to culture medium enhanced callus production as compared with control (0 callus/ explant). The best treatment for obtaining callus number was 1 ppm TDZ + 1 ppm 2, 4-D which displayed 1 callus/explant and this mean that all explants formed callus (100% callus). No significant cultivar diverse was observed regarding callus fresh and dry masses. However, the effect of plant growth regulator on these parameters was more comparable with that of callus number/ explant. These results were in accord with those of Chung and Ouyang [26] who observed that the combination of TDZ and 2,4-D at low concentrations induced 100% callus formation in leaf explants of wild Strawberry (*Fragaria vesca*) and this was better than each one alone. This could confirm that TDZ as a cytokinin and 2,4-D as an auxin, at appropriate levels, regulates callus formation probably by acting as a signals which promotes the expression of specific genes leads to cell proliferation [12].

Callus visual characteristics are shown in table 5 and fig.3. It can be noted that not all treatments were able to produce callus, and TDZ and 2,4-D appeared as the potent treatments for callus initiation. Callus color was mainly yellowish, brown and brownish. These colours could be attributed to the biosynthesis of secondary metabolites such as phenolic compounds which was induced by supplementation of TDZ

and/or 2,4-D to the culture medium [27]. Additionally, callus surface was nodular, with a hard (compact). This compact texture of callus could display the high dense cells and rapid proliferation due to application of TDZ and 2,4-D growth regulators in the culture medium.

4. CONCLUSION

The optimum sucrose and photoperiod duration for *in vitro* microtuberization of explants from two potato cultivar, Chara and Lady Rosetta, were 90 g/l sucrose and 16/8 h day/dark, respectively. Supplementation of the culture media with TDZ and 2, 4-D unfortunately did not increase the microtuber number than control, however they induced callus formation mainly with the dual treatment 1 ppm TDZ + 1 ppm 2, 4-D. This may be the first study which disclosed the impact of these two growth regulators, in combination, on potato microtuberization and callus formation and consequently potato morphogenesis.

Table 1. Effect of different sucrose concentrations on microtuberization of two potato cultivars. Values are mean ±SE. Basal culture media used is MS. Means, for each parameter, with similar letter (s) do not display significant difference.

Variety	Sucrose concentration (g/liter)	Microtuber number/ Explant	Microtuber fresh mass (g)	Microtuber dry mass (g)	Microtuber length (mm)	Microtuber width (mm)
Lady Rosetta	3	Zero b	Zero b	Zero c	Zero c	Zero c
	6	Zero b	Zero b	Zero c	Zero c	Zero c
	9	7.50 ±1.02a	0.30±0.13b	0.07±0.03bc	7.00±1.19b	4.37 ±0.53b
	12	5.67±2.40a	0.11±0.02b	0.02±0.005c	5.52±0.70b	3.63 ±0.47b
Chara	3	Zero b	Zero b	Zero c	Zero c	Zero c
	6	1.0±0.0b	0.10±0.04b	0.01±0.005c	3.33±1.33bc	3.33±1.33b
	9	1.83±0.30 b	0.88±0.17 a	0.17±0.03a	12.06±1.83a	7.41±0.95a
	12	1.33± 0.33 b	0.39±0.15 b	0.14±0.05ab	5.0±2.0b	4.16 ± 1.6b
LSD at p≤ 0.05		3.01	0.46	0.11	4.86	2.9

Table 2. Effect of different photoperiods on microtuberization of two potato cultivars. Culture media used MS plus 9% sucrose.Values are mean ±SE. Means, for each parameter, with similar letter (s) do not display significant difference.

Variety	Photo period (Light/Dark)	Microtuber number/ explant	Microtuber fresh mass (g)	Microtuber dry mass (g)	Microtuber length (mm)	Microtuber width (mm)
Lady Rosetta	16h\8h	7.33±1.45 a	0.48±0.22ab	0.12±0.06 a	8.6±1.9 a	5.07±0.77 a
	8h\16h	3.33±2.33 bc	0.01±0.007b	0.003±0.001b	2±0.8 bc	1.52±0.6 b
	0h\24h	Zero c	Zero b	Zero b	Zero c	Zero b
Chara	16h\8h	2.33±0.33 bc	0.90±0.13 a	0.17±0.03 a	13.4±3.08 a	7.6±0.88 a
	8h\16h	2.00±1.00 ab	0.48±0.17 ab	0.08±0.02 ab	9.9±3.04 a	5.5±1.25 a
	0h\24h	1.00±0.00c	0.47±0.28 ab	0.07±0.04 ab	8±1.15 ab	6.2±1.9 a
LSD at p≤ 0.05		3.7	0.53	0.113	6.26	3.32

Table 3. Influence of 2,4-D and TDZ on callus formation from stem nodal segments of two potato cultivars. Culture media used is MS + 9% sucrose and 16/8 photoperiod. Values are mean±SE. Means, for each parameter, with similar letter (s) do not display significant difference.

Variety	Plant growth regulator			Microtuber number/ explant	Microtuber fresh mass (g)	Microtuber dry mass (g)	Microtuber length (mm)	Microtuber width (mm)
	Type	Concentration (ppm)						
		TDZ	2,4-D					
Lady Rosetta	Control	0	0	5.67±0.66a	0.47±0.24bc	0.11±0.07ab	7.97±2.39c	4.37±1.03bc
	2,4-D	0	1	Zero e	Zero d	Zero c	Zero d	Zero d
	2,4-D	0	5	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ	1	0	1.33±0.33cd	0.75±0.3ab	0.17±0.06a	8.16±3.16bc	5.3±2.33abc
	TDZ	5	0	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ+2,4-D	1	1	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ+2,4-D	1	5	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ+2,4-D	5	1	1.67±0.66cd	0.22±0.66cd	0.01±0.004c	3.66±1.66cd	2.55±0.55cd
	TDZ+2,4-D	5	5	Zero e	Zero d	Zero c	Zero d	Zero d
Chara	Control	0	0	3±0.577b	0.91±0.13ab	0.17±0.03a	13.4±3ab	8.33±0.33a
	2,4-D	0	1	1.33±0.33cd	0.27±0.1cd	0.04±0.01bc	6.6±2.66c	3.3±1.33cd
	2,4-D	0	5	1±0de	0.29±0.12cd	0.04±0.01bc	5.3±2.33cd	3.66±1.67c
	TDZ	1	0	2.33±0.88bc	1.01±0.26a	0.19±0.04a	15.1±2.31a	8.11±0.48a
	TDZ	5	0	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ+2,4-D	1	1	1.0±0.0de	0.08±0.03cd	0.13±0.005c	4.33±1.33cd	3.33±1.33cd
	TDZ+2,4-D	1	5	1.0±0.0de	1.02±0.4a	0.17±0.06a	9.0±4.0bc	7.3±3.33ab
	TDZ+2,4-D	5	1	Zero e	Zero d	Zero c	Zero d	Zero d
	TDZ+2,4-D	5	5	Zero e	Zero d	Zero c	Zero d	Zero d
	LSD at p≤ 0.05			1.005	0.44	0.09	5.4	3.35

Abbreviations: 2,4-D and TDZ are 2,4 dichlorpphenoxyacetic acid and thidiazuron, respectively.

Table 4. Influence of some growth regulators on callus formation from stem nodal segments of two potato cultivars. Culture media used MS plus 9% sucrose and 16/8 photoperiod. Values are mean ±SE. Means, for each parameter, with similar letter (s) do not display significant difference.

Variety	Growth regulators			Callus number/ explant	Callus fresh mass/ explant (g)	Callus dry mass/ explant (g)
	Type	Concentration (ppm)				
		TDZ	2,4-D			
Lady Rosetta	Control	0	0	Zero c	Zero d	Zero d
	2,4-D	0	1	Zero c	Zero d	Zero d
	2,4-D	0	5	1.33±0.33ab	0.24±0.03cd	0.03±0.005cd
	TDZ	1	0	Zero c	Zero d	Zero d
	TDZ	5	0	Zero c	Zero d	Zero d
	TDZ+2,4-D	1	1	1.33±0.33ab	0.81±0.12bc	0.09±0.01bc
	TDZ+2,4-D	1	5	1.00±0.0b	0.32±0.04cd	0.03±0.005cd
	TDZ+2,4-D	5	1	Zero c	Zero d	Zero d
	TDZ+2,4-D	5	5	1.0±0.0b	2.22±0.85a	0.30±0.12a
Chara	Control	0	0	Zero c	Zero d	Zero d
	2,4-D	0	1	Zero c	Zero d	Zero d
	2,4-D	0	5	Zero c	Zero d	Zero d
	TDZ	1	0	Zero c	Zero d	Zero d
	TDZ	5	0	Zero c	Zero d	Zero d
	TDZ+2,4-D	1	1	1.67±0.33a	1.25±0.64b	0.13±0.05b
	TDZ+2,4-D	1	5	Zero c	Zero d	Zero d
	TDZ+2,4-D	5	1	1.33±0.33ab	0.32±0.04cd	0.01±0.002cd
	TDZ+2,4-D	5	5	Zero c	Zero d	Zero d
LSD at p≤ 0.05				0.503	0.75	0.09

For abbreviations, see table 3.

Table 5. Influence of some growth regulators on morphological features of callus of explants from two potato cultivars.

Cultivar	Growth regulator			Color	Surface	Texture
	Type	Concentration				
		TDZ	2,4-D			
Lady Rosetta	Control	0.0	0.0	- ve	- ve	- ve
	2,4-D	0	1	- ve	- ve	- ve
	2,4-D	0	5	Brown	Nodular	compact
	TDZ	1	0	- ve	- ve	- ve
	TDZ	5	0	- ve	- ve	- ve
	TDZ+2,4-D	1	1	Brown	Nodular	compact
	TDZ+2,4-D	1	5	Brownish	Nodular	compact
	TDZ+2,4-D	5	1	- ve	- ve	- ve
	TDZ+2,4-D	5	5	Yellowish	Nodular	compact
Chara	Control	0.0	0.0	- ve	- ve	- ve
	2,4-D	0	1	- ve	- ve	- ve
	2,4-D	0	5	- ve	- ve	- ve
	TDZ	1	0	- ve	- ve	- ve
	TDZ	5	0	- ve	- ve	- ve
	TDZ+2,4-D	1	1	Greenish	Nodular	compact
	TDZ+2,4-D	1	5	- ve	- ve	- ve
	TDZ+2,4-D	5	1	Brownish	Nodular	compact
	TDZ+2,4-D	5	5	- ve	- ve	- ve

For abbreviations, see table 3.

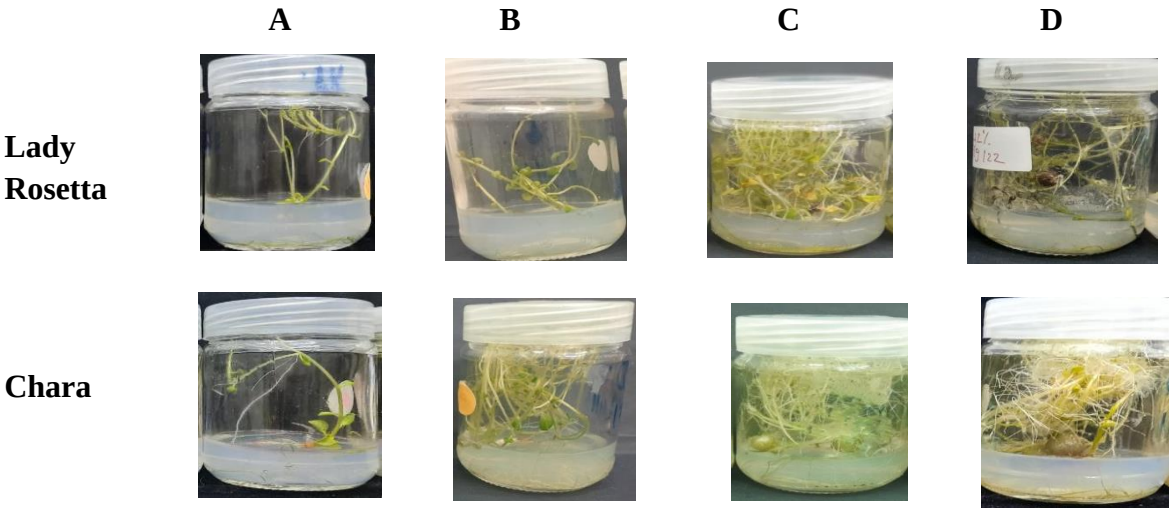

Fig1. Effect of sucrose concentration on microtuberization of two potato cultivars (Lady Rosetta and Chara). A, B,C and D are with sucrose 30 g/l, 60 g/l, 90 g/l and 120 g/l, respectively.

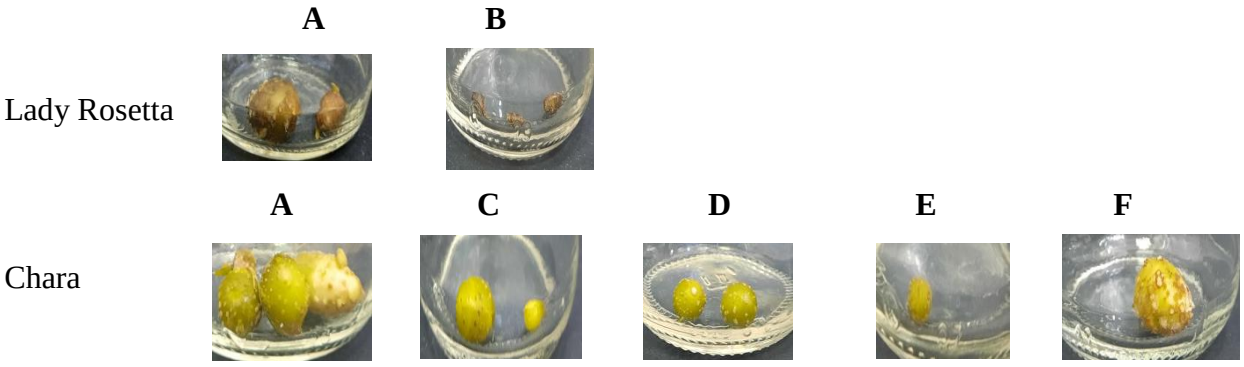

Fig.2. Effect of growth regulators on microtuberization of two potato cultivars (Lady Rosetta and Chara).

Abbreviations:

A, 1ppm TDZ + 0ppm 2, 4-D; B, 5ppm TDZ +1ppm 2 , 4-D; C, 0ppm TDZ +1ppm 2, 4-D; D, 0 ppm TDZ +5ppm 2, 4-D; E, 1ppm TDZ +1ppm 2, 4-D; F, 1ppm TDZ +5ppm 2, 4-D.

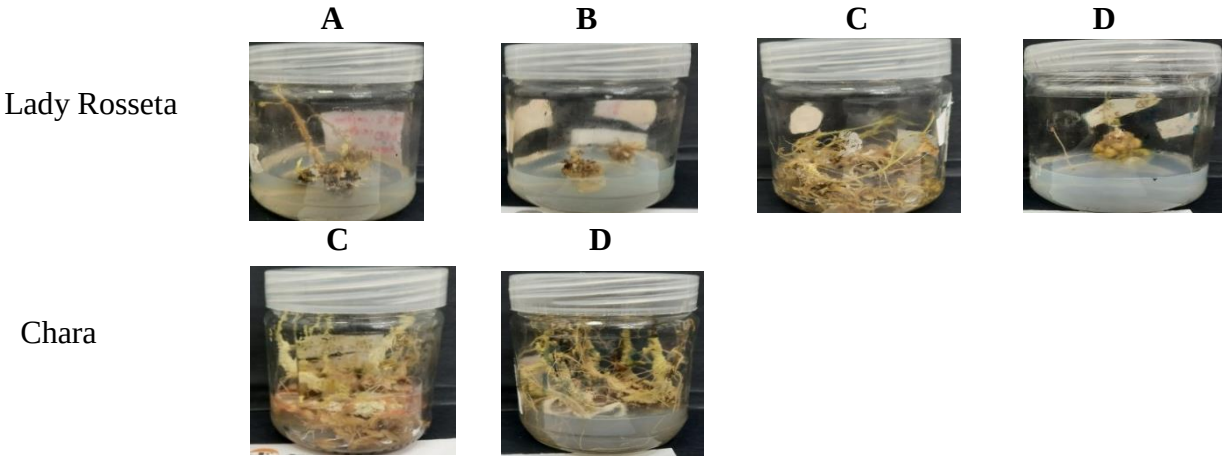

Fig3. Effect of growth regulators on callus formation in two potato cultivar (Lady Rosetta and Chara).

Abbreviations:

A, 0ppm TDZ +5ppm 2,4-D; B, 1ppm TDZ +5ppm 2,4-D; C, 1ppm TDZ +1ppm 2; D, 5ppm TDZ +5ppm 2,4-D; F, 5ppm TDZ +1ppm 2,4-D

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5. REFERENCES

[1] J. C. King and J. L. Slavin, "White potatoes, human health, and dietary guidance," Adv. Nutr., vol. 4, no. 3, 2013, doi: 10.3945/an.112.003525.

[2] E. R. Mwakidoshi, "Economic Importance, Ecological Requirements and Production Constraints of Potato (Solanum tuberosum L.) in Kenya," Int. J. Bioresour. Sci., vol. 8, no. 2, 2021, doi: 10.30954/2347-9655.02.2021.1.

[3] P. D. Shambhu and H. T. Lim, "Microtuberization of Potato (Solanum tuberosum L.) as Influenced by Supplementary Nutrients, Plant Growth Regulators, and In Vitro Culture Conditions, " Potato Research 55:97–108, 2012, DOI 10.1007/s11540-012-9212-y.

[4] H. A. Emaraa, E. M. Hamza, and W. A. Fekry, "In vitro propagation and microtuber formation of potato in relation to different concentrations of some growth regulators and sucrose," Middle East J. Agric. Res., vol. 6, no. 4, pp. 1029–1037, 2017.

[5] L. Herrera-Isidron, E. Valencia-Lozano, P.Y Rosiles-Loeza, M.G. Robles-Hernández, A. Napsuciale-Heredia and J.L.Cabrera-Ponce, Gene expression analysis of microtubers of potato Solanum tuberosum L. induced in cytokinin containing medium and osmotic stress. Plants 2021, 10, 876. https://doi.org/10.3390/ plants10050876.

[6] S L Asmono, V K Sari1 and Djenal, The effects of different concentration of sucrose and various auxin on in vitro shoot and microtuber formation of red potato (Solanum tuberosum, L. var Desiree). 1 st International Conference on Food and Agriculture 2018, IOP Conf. Series: Earth and Environmental Science 207. doi:10.1088/1755-1315/207/1/012002.

[7] Z.A. AL-Hussaini, SH. A. Yousif and , S.A AL-Ajeely, The role of sucrose and light duration on in vitro tuberization for two cultivars of potato Solanum tuberosum L. Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 277-283

[8] J.A. García-García, F. Solano-Campos , J.B Azofeifa-Bolaños and R. Orozco-Rodríguez, Effect of two cytokinins and a growth inhibitor on the in vitro tuberization of two genotypes of Solanum tuberosum L. cvs. Atlantic and Alpha, UNICIENCIA (2019) 33 (2), pp. 1-12.

[9] R. A. Ghavidel, A. R. Bolandi, H. Hamidi and S. Foroghian, Effects of plant growth regulators and photoperiod on in vitro microtuberization of potato (Solanum tuberosum L.), African Journal of Biotechnology (2012) 11(53), 11585-11590.

[10] A. M. Kumlay and S. Ercisli, "Callus induction, shoot proliferation and root regeneration of potato (Solanum tuberosum L.) stem node and leaf explants under long-day conditions," Biotechnol. Biotechnol. Equip., vol. 29, no. 6, pp. 1075–1084, 2015, doi: 10.1080/13102818.2015.1077685.

[11] S. Ashrafzadeh and DW.M. Leung,_ Microtuber formation in potato callus,, ScienceAsia 41 (2015): 1–4, doi: 10.2306/scienceasia1513-1874.2015.41.001.

[12] M. Ikeuchi, K. Sugimoto, and A. Iwase, Plant Callus: Mechanisms of induction and repression, The Plant Cell, 25: 3159–3173.

[13] S. O. El-Abd, A. M. R. Abdel-Mawgoud, and S. D. Abou-Hussein, "Phenological and yield production comparative study of some new imported potato varieties under Egyptian conditions," Middle East J, vol. 2, no. 4, pp. 123–126, 2013.

[14] F. Murashige, T., Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," Physiol.Pplantarum, vol. 15, no. 3, pp. 473–497, 1962.

[15] A. K. Srivastava, L. C. Diengdoh, R. Rai, T. K. Bag, and B. P. Singh, "In vitro micropropagation and micro-tuberization potential of selected potato varieties," Indian J. Hill Farming, vol. 25, no. 2, pp. 14–17, 2012.

[16] I. M. Abu, H. I. A. Soliman, and E. M. R. Metwali, "Saudi Journal of Biological Sciences In vitro evaluation of some high yield potato (Solanum tuberosum L .) cultivars under imposition of salinity at the cellular and organ levels," Saudi J. Biol. Sci., vol. 29, no. 4, pp. 2541–2551, 2022, doi: 10.1016/j.sjbs.2021.12.040.

[17] S. Ali, N. Khan, F. Nouroz, S. Erum, and W. Nasim, "Effects of sucrose and growth regulators on the microtuberization ofcip potato (Solanum tuberosum) germplasm," Pakistan J. Bot., vol. 50, no. 2, pp. 763–768, 2018.

[18] A. El, S. Mohamed, and N. D. Girgis, "Factors affecting in vitro tuberization of potato," Bull. Natl. Res. Cent., 2023, doi: 10.1186/s42269-023-01056-3.

[19] A. M. Azar, S. Kazemiani, and F. Yarmohamadi, "Effect of Sugar / Osmotica Levels on in vitro Microtuberization of Potato (Solanum tuberosum L .) 1," vol. 39, no. 2, pp. 112–116, 2013, doi: 10.3103/S1068367413020146.

[20] M. S. Islam, M. Z. K. Roni, A. F. M. Jamal Uddin, and K. Shimasaki, "Tracing the role of sucrose in potato microtuber formation in vitro," Plant Omics, vol. 10, no. 1, pp. 15–19, 2017, doi: 10.21475/poj.10.01.17.282.

[21] M. M. Khalafalla, K. G. A. Elaleem, and R. S. Modawi, "Callus formation and organogenesis of potato (Solanum Tuberosum L.) cultivar Almera," In Vitro, vol. 2, no. 5, pp. 40–46, 2010.

[22] M. Fufa, M., & Diro, "The Effects of Sucrose on in vitro Tuberization of Potato Cultivars," Adv Crop Sci Tech, vol. 1, no. 4, p. 1:4, 2013.

[23] M. Dwiati and S. Anggorowati, "Induction of in vitro culture of potato microtuber by using alar and dark photoperiod application," Agrivita, vol. 33, no. 1, pp. 47–52, 2011.

[24] A. M. Soliman1, H. M. Abd El-Dayem, M. A. M. Mady, A. S. Ibrahim and R. S. M. Eid, "Effect of Thidiazuron and 6-Benzylaminopurine on in vitro microtuberization in potato cv. Spunta.," Ann. Agric. Sci. Moshtohor, vol. 60, no. 3, pp. 799–808, 2022, doi: 10.21608/assjm.2022.263821.

[25] H.M.S, Aboshama, S.T. El-Afifi, O.M. Abdel-Kafi and H.E. Abd Allah, "Microtuberization of potato (Solanum Tuberosum L.), 1-Protocol for production of microtuber in vitro" J. Agric. Sci. Mansoura Univ., 3 (9), 5909-5919 (2006).

[26] H.H Chung and H.Y.Ouyang, Use of Thidiazuron for High-Frequency Callus Induction and Organogenesis of Wild Strawberry (Fragaria vesca), Plants 2021, 10, 67. https://doi.org/10.3390/ plants10010067.

[27] H. Fitriani, P. D Aryaningrum and N. Sri Hartati, Proliferation of embryogenic callus of Satoimo taro (Colocasia esculenta var. antiquorum) in culture media with various levels of sucrose and gelling agent, Nusantara Bioscience, 8, (1), 316-320, 2016. DOI: 10.13057/nusbiosci/n080130.