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In VITRO Microtuberization of Two Potato Cultivars Under Sea Water Stress

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***Abstract* – Plant nodal cuttings as single node explants from previously selected plantlets on innovative exchangeable cycles of selection *in vitro* of two potato verities named Lady Rosetta and Cara were used for induction of microtubers *in vitro*. The nodal cuttings were cultured on solidified MS medium free of plant growth regulators and supplemented with 8% sucrose and two doses of sea waters (8000 and 9000 ppm for Lady Rosetta and 7000 and 8000 ppm for Cara). The results cleared that the tested plantlets under higher saline concentration (9000 ppm for potato lady Rosetta and 8000 ppm for potato Cara) gave higher numbers of microtubers (6.7 and 6 respectively) as compared to the control plantlets which gave (5.7 and 5.3 respectively) and grown on medium free of sea water. However, data cleared that the fresh matter and microtuber diameter of both tested cultivar showed a slight increase over the control. The presented work brings an efficient easy protocol for production of potato microtubers *in vitro* under saline conditions.**

***Keywords* – Microtuber Production, Nodal Cuttings, Salinity, Solanum Tuberosum.**

I. Introduction

Plant cells and tissue culture is one of the most vital techniques to improve desired plant quality. The use of plant nodal cuttings as single node explants with innovative exchangeable cycles of *in vitro* selection [1] is one of the most important ways that leads to selection of plantlets with genetic stability under stress conditions, such as one of them the high salinity, especially sea water.

Solanum tuberosum L. (potato) is one of the most important vegetable feeding crops in Egypt and all over the world. Potato plant is an annual herbaceous plant and mostly propagates *via* complete or segment tubers and rarely by seeds. Potato Microtubers are very small tubers developed "*in vitro*" and represent a mediator phase between "*in vitro*" plantlets and minitubers production *in vivo*. Hoque [2] said that the process of the tuber creation in potato is a significantly complicated, which may be modified in various ways. Tuberization can be created under *in vitro* circumstance. *In vitro* propagation of potatoes is the best module for advanced rapid propagation, and production of free-pathogens microtubers which are needed for the production of minitubers, that required for the main cultivation yield [3]-[5]. Extensive physiological research has brought out that tuberization is judged by several factors, such as growth regulators combination, ratio of photoperiodism, nourishing constitutions, salinity, etc.[6]-[14]. However, microtuber production was affected by different levels of sucrose, 6-benzyl aminopurine (BAP) and genotypic variation of potato genotypes [15], [16]. On the other hand, under stress such as salinity, the endogenous hormones strongly affect and led to accumulate the gibberellic acids in the plant cells that strongly connected with plant growth development and that will help the plant to stand front the stresses and put the plant under healthy physiological processes [17]-[19]. Microtuber production represents in the same time an efficient method for obtaining a healthy material, by which the process of potato production is lessened by 3-4 years [20].

The objective of this study was to produce potato microtubers *in vitro* under salinity stress of different sea water levels from single node cuttings of selected tolerant *in vitro* plantlets on various sea water concentrations.

II. Materials And Methods

The experiment was conducted in Egypt at the Plant Biotechnology Department, National Research Centre and incorporation with Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University.

Micropropagated plantlets of two potato cultivars named Lady Rosetta and Cara, which were brought from Egyptian Ministry of Agriculture, were used [21]. The plantlets were previously exposed to exchangeable cycles of selections under various sea water concentrations [22]. This experiment occurred with *in vitro* control plantlets and with selected tolerant plantlets with two saline levels of sea water.

Single nodes were taken from 21 days old plantlets from the selected tolerant micropropagated ones of both cultivars under saline solutions of seawater (8000 and 9000 ppm for Lady Rosetta and 7000 and 8000 ppm for Cara). Each jar was contained 10 nods; then the plantlets that reached about 8-10 cm (as seen in Fig 1) were transferred and cultured in glass jars containing MS medium [23] supplemented with 8 g/l agar and 80 g/l sucrose and the same saline concentrations previously mentioned. Each treatment was replicated 3 times. The nodal cultures were incubated in a growth chamber supplemented with controlled air conditioner at 20 - 22°C for 60 days at a 16 hours photoperiod of 70 µmol $m^{-2}s^{-1}$ initiated from illuminated fluorescent lamps.

Fig. 1. Potato plantlets reached about 8-10 cm in length.

Analyses averages and standard errors of the recorded data were performed using the online Statistics Calculator (http://www.numberempire.com/statistics_calculator.php). Completely randomized experimental design was performed.

III. Results And Discussion

In general, microtuber numbers, their fresh matter and diameters increased by increasing the levels of sea water as compared to control, as shown in Table (1) and Fig. (2). Differences among potato cultivars tested in the presented work were detected in their microtuber characteristics under different salinity levels. Data in (Table I) clear that the tested plantlets under higher saline concentration of sea water (9000 ppm for potato Lady Rosetta and 8000 ppm for potato Cara) gave higher numbers of microtubers (6.7 and 6 microtubers respectively) as compared to the control plantlets which gave 5.7 and 5.3, microtubers respectively and grown on medium free of sea water. However, data cleared that the fresh matter and microtubers diameter of both tested cultivar showed a slight increase over the control.

Table I. Effect of salinity levels on the *in vitro* microtuber characteristics of Lady Rosetta and Cara potato cultivars.

Salinity levels (ppm)	Numbers of microtubers	Average of fresh matter (g)	Diameter average (cm)
		Lady Rosetta	
control	5.7 ± 0.333	1.23 ± 0.039445	1.2 ± 0.037048
8000	6.3 ± 0.88	1.3 ± 0.031311	1.26 ± 0.032562
9000	6.7 ± 0.333	1.4 ± 0.019803	1.3 ± 0.019803
		Cara	
control	5.3 ± 0.333	1.1 ± 0.048507	0.9 ± 0.049836
7000	5.7 ± 0.333	1.188 ± 0.045653	1 ± 0.056011
8000	6 ± 0.58	1.194 ± 0.043890	1.03 ± 0.057055

(a)

(b)

Fig. 2. *In vitro* microtubers from Cara (a) and Lady Rosetta (b) potato cultivars produced on 8000 ppm and 9000 ppm of seawater salinity respectively.

Potato Microtubers are healthy petite tubers produced "*in vitro*" and they intercessor between stage of "*in vitro*"plantlets and minitubers that created *in vivo*.

According to the fact that most potato species are polyploidy, therefore, tissue culture of potatoes is the best module for rapidly propagation, genetic manipulation with advanced techniques and production of free-pathogens microtubers, which are needed for the production of minitubers that required for the main cultivation yield [2]-[5].

Microtubers of potato consider as the first regenerative seeds during the tissue culture processing. Potato microtubers are the best solution to solve the problems initiated through the transplanting of the plantlets from *in vitro* condition to *in vivo* circumstances. The miniature size and lessened weight of the microtubers consider an advantage offer to microtubers storage, transport and mechanization. Microtubers can be found all year time. However, they can be seeded directly in the cultivated land. Physically, they have the same characteristics with traditionally produced tubers. *In vitro* microtubers are used for conserving valuably important potato stocks. Such potato microtubers along with plantlets and minitubers which created through nodal cuttings *in vitro*, consider important creatures for handling, storage and exchange of a healthy germplasm that will be used in potato production elsewhere with lessened cultivation time about 3-4 years [20]. In this respect, Kanwal *et al.*, [15] reported that *in vitro* microtuberization occurred on MS medium supplemented with 8% sucrose without growth regulators and produced microtubers in a minimum period (29 - 30 days), and 96.6% of cultures showed microtuberization with 5 - 6 microtubers per culture with average fresh matter of microtuber of about 0.115 g, which generally agreed with the presented work. Moreover, the production of microtubers physiologically affected by many factors especially the endogenous hormones such as GA_3, other plant growth regulators, nutrition and photoperiod [6],[9],[11],[13],[14].

However, Zhang and Donnelly [7] reported that total microtuber numbers were decreased in some potato cultivars, but increased in others and that agreed with the presented data. On the other hand, Zhang *et al.* [10] found that the growth of *in vitro* plantlets and microtuberization process severely inhibited by increasing salinity level. Also, Pour *et al.* [12] said that microtuberization showed higher increase in the absence of salinity, Ghosh *et al.*[8] detected that total and marketable tuber yield decreased with increasing salt level. According to the presented work based on the exchangeable cycles of selection in which the tested plantlets were selected bring valuable system for *in vitro* production of healthy microtubers under stress of different concentrations of sea water with equal or higher numbers of microtubers than on stressless medium. In this respect, gibberellic acids (also called Gibberellin A_3, GA, and GA_3) are generally involved in growth and development of plants. Additionally, gibberellins interact with other hormones to regulate various metabolic processes in the plants [11]. The biosynthesis of GAs is regulated by both endogenous and exogenous stimuli [19]. However, exposition to biotic [17] and abiotic stresses [18] strongly affect the accumulation of Gibberellic acid (GA). Therefore, the tested plantlets under sea water stress showed equal or better growth than in the control treatment rather in numbers of produced microtubers and their characteristics.

IV. Conclusion

In conclusion, sea water stress tolerance of potato genotypes can be evaluated *in vitro*, and screening of growth parameters can be done at various salinity levels for *in vitro* and *ex vitro* potato genotypes. Many potato genotypes could be easily evaluated by exchangeable cycles of selection method [1], [22] for the identification of suitable potato lines with improved stress tolerance. The results of the presented study cleared that screening of potato genotypes under sea water circumstances provide a high efficacy of an *in vitro* screening method for salinity tolerance. The effectiveness of this approach was tested on these potato genotypes through the production of microtubers and measuring the performance of their characteristics in relation to salinity-tolerance under *in vitro* circumstances.

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