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Production of Potato Tubers and Seedlings using Plant Tissue Culture and Aeroponic for Potato Cultivation

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Abstract

Micro-propagation is a promising and effective technique for propagation of healthy virus-free potato tubers. This study aimed to produce potato mini- and micro-tubers using micro-propagation and in vitro tuberization for two potato varieties, Lady Rosetta and Spunta. Six Combinations and concentrations of benzyl aminopurine (BAP), kinetin, and indole acetic acid (IAA) growth regulators were tested with in vitro tuberization, while BAP and kinetin levels of 0, 0.5, 1, 1.5, and 2 mg/L were tested for in vitro micro-propagation. Results showed that attributes of explants' micro-propagation transformation into plantlets varied with variable combinations and concentrations of BAP, kinetin, and IAA. Impact of kinetin on number of shoot-less explants, root lengths and cell division were lower than BAP, regardless of concentration. Treatment with BAP 1.5 mg/L + IAA 1 mg/L resulted in a higher proportion (60%) of tuberization than other treatments. Tubers' size increased from 0.7 cm to 1.2 cm, which is the proper size for planting using aeroponic technology. The study showed the applicability of the applied technique for in vitro potato micro-tuberization and multiplication, as well as potential for production of virus-free potato tubers.

Keywords: Potato, in-vitro propagation, Tuberization, Microtubers, Minitubers, Aeroponic, Growth regulators.

1. Introduction

Potato (Solanum tuberosum L.) is one of the most important and economical non-cereal crops and a primary source of nutrition for humankind worldwide, and is the second economical crop, after tomato, in Egypt [2].Propagation of potato is traditionally conducted asexually, using tubers, which is a risky procedure since it facilitates spreading pathogens and diseases to new cultivation areas. This compromises genotype preservation for use in breeding or commerce in addition to reducing the solanaceous plant's productivity [1]. Egypt is the top producer of potatoes in Africa, but by controlling diseases that lower yield, the production may increase by more than a 25% [2]. Due to the challenges of using botanical seeds, traditional methods of conserving and exchanging t-his species' genetic material are not feasible. To ensure crop output, high-quality tubers have been essential in every region that grows potatoes. Biotechnological methods based on tissue culture utilize in vitro techniques to synthesize and multiply high-quality materials, offering an alternative method of propagation [3-4]. This biotechnological approach, in addition to clonal multiplication, makes initial indexed material accessible to breeding initiatives and certified seed potatoes, as well as aiding the exchange and conservation of germplasm. Incorporating these processes into the potato production chain will benefit farmers by producing high-quality propagules right away for all the reasons [5].Effects of cytokinin on the induction and multiplication of shoots nodal explants of Ceropegia maculata Bedd. plant, grown on MS basal media supplemented with a variety of cytokinins, showed to be dependant on the type and dosage of the chemical used for shoot bud induction. Results of this study showed that the maximum of 2.43 shoots per nodal explant were produced after 45 days of culture in MS medium supplemented with BA 1.5 mg L1 [6]. Photoperiod showed to have significant influence on creating microtubers. 8-hour dark, 16-hour light, were tested in in vitro culture of potato "Floresta" cultivar., with full-strength Murashige and Skoog medium used as a control. Results of this study showed that treatment with 9.3 mg/L boric acid was the best to increase the quantity and biomass of the produced microtubers and biomass was higher in the dark than in light conditions. Nevertheless, it is advised to use illumination settings to encourage larger and greener microtubers [7], results showed that best shoot initiation was obtained with MS medium supplemented with 1.5 mg/L BAP + 3.0 mg/L NAA for gudiene variety, and 1.0 mg/L BAP and 2.0 mg/L NAA for Belete variety. Initiated shoots of the two potato varieties increased two- to three-fold upon sub-culturing on the MS medium fortified with varying concentrations of BAP and Kinetin and the highest numbers of multiple shoots were obtained in the MS medium containing 2.5 mg/L Kinetin. This study also suggested that successful in vitro potato micropropagation can be achieved using lateral buds as explants in plant tissue culture [4], different potato varieties of sprouting

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plants produced tubers of the same size and quantity, but with variations in fresh weight, which led to variations in pot production. The average diameter of the tubers was 3.7 cm [8]. Micro-propagation showed to be an effective method for direct shoot induction from leaf explants, where shoot initials were produced on 90% of nodal explants within 7 days of culture on MS; medium supplemented with 2.22 M BAP. The produced shoots bear satisfied leaf explants after 45 days of development [9], mini-tuber production by aeroponic is one of the fastest ways to propagate potato seeds, which is conducted without soil or other agglomerated media. Aeroponic is the practice of growing plants in an atmosphere of air with mist, where crops are grown by suspending their roots in a misted nutrient mixture. Minitubers are tubers offspring developed on plantlets. Minitubers can range in size from 5 to 25 mm [10], [11], and requires monitoring nutrients and pH, by precisely calculating the crop's plant nutrient needs, reducing the need for fertilizer and lowering the likelihood that excessive fertilizer residues would enter the subterranean water table [12]. Therefore, the current study was proposed to produce potato mini- and micro-tubers, employing micro-propagation and in vitro tuberization techniques on two potato varieties, Lady Rosetta and Spunta, with different combinations and concentrations of the growth regulators BAP, kinetin, and IAA.

2. Materials and Methods

2.1 Plant materials

The experiment was carried out in the Plant Tissue Culture Lab at the Faculty of Organic Agriculture, Heliopolis University for Sustainable Development, and in the aquaponics greenhouse.

Apical/axillary growth tips (0.1 to 0.3 mm) are dissected and allowed to develop into plantlets on artificial nutritional media under controlled conditions (22 to 25°C temperature and 50 to 60 E/m^2/s light intensity for 16 hours/day).

2.2 Media

Ms medium (Murashige & Skoog 1962) [13] was used for micro-propagation of potato explants (Table 1).

Table 1: MS medium (Murashige & Skoog 1962) [13]

ingredient	conc. (mg/L)	ingredient	conc. (mg/L)
Macronutrients		**Micronutrients**	
NH_4NO_3	1650	KI	0.83
KNO_3	1900	H_3BO_3	6.20
$CaCl_2.2H_2O$	440	$MnSO_4.4H_2O$	22.30
$MgSO_4.7H_2O$	370	$ZnSO_4.7H_2O$	8.6
KH_2PO_4	170	$Na_2MoO_4.2H_2O$	0.25
		$CuSO_4.5H_2O$	0.025
		$CoCl_2.6H_2O$	0.025
		Na_2EDTA	37.3
		$FeSO_4.7H_2O$	27.8

2.3 Tuber and explant sterilization.

Potato tubers (12 day-old buds), used as initial explants, were thoroughly cleaned with tap water then immersed in soap solutions (NaOH 13%) for 20 minutes.

The isolated apical buds were sterilized with 0.01% $HgCl_2$ for five minutes, cleaned with 70% ethanol for 4 minutes, then rinsed five times with sterile distilled water [13,14].

2.4 Proliferation and tuberization

To evaluate the effect of benzyl aminopurine (BAP), kinetin and indole acetic acid (IAA) on the proliferation of potato explants, initial explants were grown on MS medium with 6 % sucrose and 0.7 % agar for 25 days, and combinations of three concentrations of each hormone were added to the growth medium (Table 2, Fig 1), where each treatment had three replicates. The pH was stabilized at 5.7 using KOH and HCl before sterilization for 20 minutes at 121°C.

Fig 1: Stages of *in vitro* potato tubers sprouting

Table 2. Treatments for *in vitro* proliferation and tuberization stages.

Treatment	Growth stage	BAP (mg/L)	Kinetin (mg/L)	IAA (mg/L)
T1	Inoculation and proliferation	0.5	0	0
T2		1	0.5	0
T3		1.5	1	0
T1-1	Tuberization	1.5	0	0.5
T1-2		2	0	1
T1-3		1.5	0	0.5
T1-4		1.5	0.5	1
T1-5		0	0.5	0.5
T1-6		0	1	1
BAP: benzyl aminopurine, IAA: indole acetic acid				

2.5 Micro tuberization experiment

The proliferating explants were placed into in vitro sterile MS medium 28 days after the start of the in vitro culture. Six hormonal mixture combinations of the kinetin/IAA and BAP/IAA ratios were tested for their effect on proliferation and tuberization, T1-1 to T1-6, shown in (Table 2 and Fig 1). All cultures were kept in the growth room with the controlled physical conditions (25°C and 16h/8h light/dark routine with white, fluorescent light of 43.4 molm-2/s intensity).

2.6. Acclimatization Stage

The hardened in vitro plants were removed from culture vessels by sterilized forceps, cleaned to remove clinging material and plants were weaned. Plants were hardened in a greenhouse for 28 days on a substrate composed of Peat Moss and Sand 1:1.

2.7 Aeroponic technique and system design

Regularly, plants' hanging roots were misted with water containing organic fertilizers every 3 minutes for 30 seconds, at 30°C, using 0.5 hp pump.

The system was composed of a nutritional solution tank, a culture channel, a pump, a spraying tube, and a timer. Through a culture tunnel, tubes with numerous nozzles mist nutrient solution to the root zone. The top of the culture channel was detachable with openings for potato plants, in which plantlets were placed. As plants' growth progresses, spraying interval was extended to once every 15 minutes. The minitubers were kept in the refrigerator at 4°C and used as seedlings for the next growing season.

3. Results and Discussion

3.1 Proliferation during first and second stages

Micro-propagation of potato explants were carried out in MS medium supplemented with three combinations of BAP, Kinetin and IAA. Results showed that 15 explants grown in MS medium revealed growth of up to 2 branches/plant after 10 days and 3 branches after 25 days **(Figure 2 and 3**). A maximum of 2, 6, 11 and 16 nodes after 1, 2, 3 and 4 weeks, respectively, and shoot growth to 12 cm was recorded.

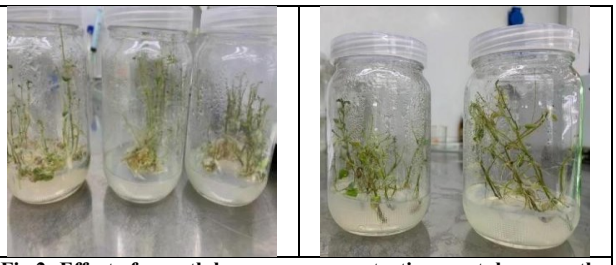

Fig 2: Effect of growth hormones concentrations on tuber growth after 3 weeks.

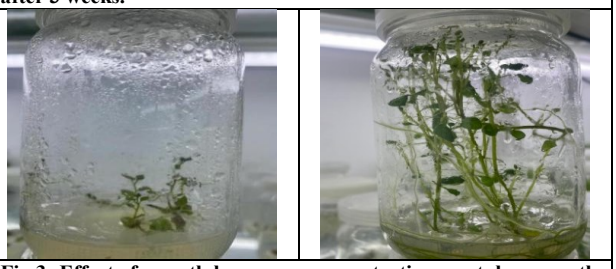

Fig 3: Effect of growth hormones concentrations on tuber growth after 4 weeks.

MS media containing hormones was utilized to proliferate the plantlets, and after two weeks, shoot growth reached 6-8 cm. For the initial subculturing, 30 explants were selected from the existing mother plant cultures based on their highest growth. 52% of the cultures in the first subculture had three branches, whereas 37% had up to 18 nodes.

Shoots length reached 4-6 cm after three weeks of subculturing. In MS medium supplemented with BAP for shoot induction and multiplication, tissues were able to regenerate following T2 treatment, while rooting was accomplished using IAA and BA which were added in various concentrations and combinations to the modified MS medium in which the potato tuber explants were cultivated **(Table 2).**

In similar study, aimed to develop an efficient *in vitro* propagation protocol for Belete and Gudiene potato varieties, using lateral bud as explants, buds were inoculated on full-strength MS medium fortified with variable concentrations of BAP and NAA. Results showed that best shoot 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 variety, and the highest numbers of multiple shoots were obtained in the MS medium containing 2.5 mg/L Kinetin [16].

3.2 Proliferation during subculturing

Before transferring the plantlets to the pre-tuberization medium, observations were made and recorded. As illustrated in **Table 3**, the highest number of branches was observed when using a growing medium composed of MS medium, a blend of vitamins and minerals.

Table 3 Effect of growth hormones on no. of branches, no. of nodes and shoot length during the production and in sub-culturing stages.

Treatment	No. of Branches/ plantlet	No. of nodes/ plantlet	Shoot length (cm)
T1	4	3	3
T2	8	12	6
T3	6	6	4

This medium also included a carbon source, such as sucrose, at a concentration of 6% as well as a coagulant like agar-agar at 0.7% (w/v) to create a semisolid medium suitable for potato micro-propagation.

The combination of BAP (0.5-2 mg/L) and kinetin (0.5-1 mg/L) showed to enhance growth in explants due to their slower growth rate. This combination is effective when used under sterile conditions, where the meristematic dome is carefully excised from the mother plant using hypodermic needles.

The most used method for producing explants *in vitro* is by harvesting explants from shoot meristem tips. This technique allows for the cultivation of explants in a controlled environment.

3.3 Tuberization

The standard method for producing microtuber involves mass-propagation of *in vitro* plants in flasks using liquid propagation material. In each container containing 20 ml of liquid propagation medium, stem segments (each with three to four nodes) are taken from five to six *in vitro* plants are cut and inoculated. The containers are then incubated stationary under typical potato propagation conditions (22 to 25 °C temperature and 50 to 60 $E/m^2/s$ light intensity for 16 hours). Several plantlets develop from axillary buds and fill the container in 15–20 days under these cultivation conditions. The liquid propagation media from the container was decanted under laminar flow conditions and swapped out for microtuber induction medium to produce microtubers.

Tuberization was achieved by multiplication of shoots in the pre-tuberization medium. Days to tuber

commencement, the number of microtubers, the average microtuber weight, yield, and the diameter of the largest microtuber were observed as function of BA treatments.

After 21 days of incubation, the liquid propagation medium was decanted from the Erlenmeyer flask in an aseptic conditions, then, 40 ml of microtuber induction medium were added. The microtuber induction medium consists of MS basal ingredients supplemented with 10 mg/L N6-benzyladenine (BA) and 8% sucrose as carbon source.

The induction cultures are incubated at a 20°C in total darkness. within 8-10 days, microtubers start to form epigeal shoots at the terminal or axillary ends of the shoots. Time required for microtubers to be ready for harvest varied from 60 to 90 days, depending on the genotype, and each jar typically produced 2 to 8 microtubers, with an average weight of 40 to 120 mg **(Table 4).**

Table 4 Observations of different parameters after tuberization

Treatment	No. of microtubers/ plant	average weight of microtuber (mg)	Diameter of largest microtuber(mm)
T1-1	2	40	25
T1-2	4	40	37
T1-3	5	40	30
T1-4	8	120	110.5
T1-5	2	95	65
T1-6	3	95	80

To prevent losses during storage, microtubers were kept to green for 10 to 15 days before harvest. This was done by exposing the microtubers to diffused or artificial light. Greening helps prevent shrinking, rotting, and excessive sprouting.

Depending on the genotype, microtubers can form epigeal shoots within one to two weeks, and typically harvested 60 to 75 days after incubation, and, additionally, produce tubers sized up to 1.2 cm **(Fig 4.).** However, before they can be utilized for planting, microtubers need to be stored for 3 to 4 months at a temperature of 5 to 6°C. This storage period is necessary to break their dormancy and ensure proper development, during storage [15].

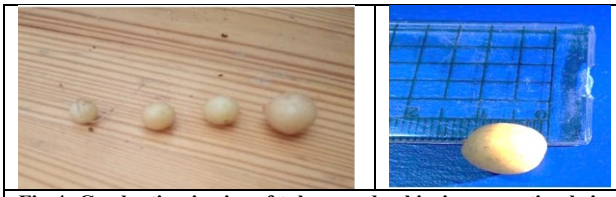

Fig 4: Graduating in size of tubers and achieving an optimal size suitable for planting in subsequent stage.

3.4 Minituber production from *in vitro* plants

This procedure involves leaving the culture tube plugs or the lids of the magenta boxes on while the cultures that are 15-20 days old are housed in glasshouses or polyhouses for 8–10 days to harden. To keep the soil moist and maintain humidity for the first week, three to four water sprays are applied daily with a sprayer.

Additional dirt substrate was put to the nursery beds to hide lower leaves as the plants grow more and more. This is crucial to maximizing the generation of minituber from buried axillary buds. On the nursery bed, the crop is allowed to mature, and minitubers are harvested.

Aeroponic systems have been designed to grow minitubers from *in vitro* plants, and in general, 80–90 % of cuttings establish and yield 8–12 minitubers per plant. These technologies enable year-round production and the implementation of phytosanitary requirements in addition to lowering the cost of manufacturing. High density plantings of aeroponic designed minitubers are created under strict hygienic guidelines. Plants growing in nutrient film are periodically harvested.

4. Conclusion

To produce high yield of potato with good quality, high-quality seeds must be used. The use of tissue culture or *in vitro* propagation is a well-established technology that is frequently utilized in the potato crop and has various advantages over traditional propagation techniques when considering the asexual propagation of the species. To produce healthy explants in large quantities, tissue culture is used in breeding programs, commercial farming, and fundamental research aimed at understanding tuber physiology. when using BA at 1.5 mg/L, Kin 0.5 mg/L and IAA 1 mg/L gained the best result to produce potato tubers, from where plant specifications, number of tubers, branches, side shoots, and weight. Finally, being aware of the many *in vitro* propagation techniques accessible enables one to choose the most appropriate and economical methods based on the needs of the propagating materials.

5. Acknowledgments

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6. Conflicts of interest

The author declares there is no conflict of interest.

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