



Production of Minitubers of Potato (*Solanum tuberosum* L.) Cv. Arizona Class a *in vitro*

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ABSTRACT

Background: The propagation of potato (*Solanum tuberosum* L.) through tissue culture provides a rapid and reliable means of producing disease-free plantlets for commercial cultivation. The efficiency of this process largely depends on the type and concentration of growth regulators used during different developmental stages. This study aimed to develop an optimized tissue culture protocol for potato cv. Arizona Class A by evaluating the effects of BA, Zeatin, and IBA across initiation, multiplication, rooting, and minitubers formation stages.

Methods: Murashige and Skoog (MS) medium was supplemented with different concentrations of BA, Zeatin and IBA to determine their effects on shoot initiation, multiplication, rooting, and minituberization. Shoot number, root formation, and minituber production were recorded at each stage to identify the most effective hormonal combinations for *in vitro* propagation and subsequent greenhouse growth.

Result: The addition of 1.0 mg L⁻¹ BA to the MS medium resulted in the highest mean number of shoots (4.5 shoots explant⁻¹) during initiation, while 2.0 mg L⁻¹ BA significantly enhanced multiplication (17.2 shoots explant⁻¹). Zeatin at 0.5 mg L⁻¹ produced the best response in shoot number during initiation (2.0 shoots explant⁻¹), whereas 0.75 mg L⁻¹ Zeatin yielded the highest multiplication response (5.3 shoots explant⁻¹). Rooting percentage reached its maximum (90%) with 0.5 mg L⁻¹ IBA and concentrations between 0.5 and 0.75 mg L⁻¹ significantly increased root number and length. During the minituberization stage under greenhouse conditions, plantlets derived from BA at 0.5 mg L⁻¹ produced the highest number of minitubers, while Zeatin at 0.75 mg L⁻¹ also promoted strong minitubers formation. These results highlight the crucial role of growth regulator selection at each developmental stage, with BA showing high efficiency during multiplication and minituberization, and Zeatin demonstrating moderate effects on multiplication but good potential in minitubers formation.

Key words: Arizona cultivar, BA, Minitubers, Potato *Solanum tuberosum* L., Shoot multiplication, Zeatin.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetatively propagated crops worldwide, ranking fourth after wheat, rice and maize in economic and nutritional importance and is classified as the most significant dicotyledonous tuber crop (World Book, Potato, 2000). It belongs to the family *Solanaceae* and originates from South America. Due to its wide economic and nutritional significance, potato has been a major focus of numerous studies in plant biotechnology. The success of these biotechnological applications largely depends on the development of efficient tissue culture systems that enable plant regeneration from cultured cells and tissues. Scientific studies have shown that the response of cultured plant explants is often genotype-specific, which necessitates the establishment of precise regeneration protocols tailored for each cultivar (Campos *et al.*, 2016; Tara, *et al.*, 2017; Yadav and Sticklen, 1995). Traditionally, potato is propagated by planting sprouts or "eyes" present on seed tubers. However, *in vitro* micropropagation provides an efficient means of producing large numbers of clonal, disease-free plantlets within a short period under controlled aseptic conditions (Copeland, 1982; Espinoza *et al.*, 1986). *In vitro* propagation using vegetative buds has proven to be more reliable for maintaining the genetic integrity of

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clonal germplasm (Wang and Hu, 1982). Sharma *et al.* (2023) reported that the highest shoot induction percentage (100%) and an average of 3.75 shoots per explant were achieved from culturing potato (*Solanum tuberosum* L.) cv. Kufri Sindhuri shoot buds on MS medium supplemented with 1.5 mg L⁻¹ BA and 0.1 mg L⁻¹ NAA. Chandana *et al.* (2024) successfully cultured shoots of potato (*Solanum tuberosum* L.) cv. Kufri Surya on MS medium provided with 3.0 mg L⁻¹ BAP and 5.0 mg L⁻¹ Kin, achieving the highest buds induction rate (93.0%), with a mean of 3.47 shoots explant⁻¹, maximum shoot length (7.17 cm) and the highest

leaves number (10.72 leaves explant⁻¹) after 30 days of culture (initiation stage). The highest multiplication rate was obtained with a mean of 9.30 shoots explant⁻¹ and maximum shoot length (7.20 cm) after 21 days of culture (multiplication stage). Similarly, Silva *et al.* (2024) investigated the effect of different concentrations of BAP and Zeatin on the micropropagation of potato (*Solanum tuberosum* L.) cv. Cecilia. They reported that nodal explants cultured on MS medium complemented with 0.3 or 0.4 mg L⁻¹ BAP achieved the highest shoots number (2.5 shoots explant⁻¹) with shoot lengths of 3.2 and 3.1 cm and leaves numbers of 5.7 and 8.2 leaves explant⁻¹, respectively, after 21 days of culture. Meanwhile, culturing on MS medium with 0.05 or 0.1 mg L⁻¹ Zeatin resulted in 2.2 and 2.4 shoots explant⁻¹, with shoot lengths of 4.9 and 5.3 cm and leaves number of 7.9 and 7.3 leaves explant⁻¹, respectively, after 21 days. Eid *et al.* (2025) obtained the highest shoots number (8.0 shoots explant⁻¹) and shoot length (6 cm) by culturing potato shoots on MS medium supplemented with 1.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ Kin for 25 days. Bandyopadhyay *et al.* (2025) found that the highest rate of shoot formation was obtained when potato (*Solanum tuberosum* L.) cv. Pilak shoot buds were cultured on MS medium supplemented with 1.5 mg L⁻¹ zeatin. For rooting, Othman *et al.* (2016) reported that culturing potato shoots derived from tissue culture on MS medium enriched with 1.0 mg L⁻¹ IBA combined with 0.5 mg L⁻¹ NAA resulted in the highest mean roots number (16.80 roots explant⁻¹) and root length (9.46 cm) in cv. Lady Balfour, while cv. Bellini achieved the highest mean root number (18.20 roots explant⁻¹) and root length (9.64 cm) after four weeks of culture on the same medium. Hajare *et al.* (2021) recorded the highest average root count 21.12 roots per shoot and root length 7.87 cm for the *Solanum tuberosum* L. Gudienne

cultivar when explants were cultured on MS medium augmented with 1.0 mg L⁻¹ IBA and 0.5 mg L⁻¹ IAA. Molla *et al.* (2022) achieved 100% rooting by culturing potato buds on MS medium supplemented with 1.0 mg L⁻¹ IBA, recording the highest mean roots number (22.35 roots explant⁻¹) and root initiation within 5-6 days after 28 days of culture. Xhulaj and Gixhari (2018) demonstrated differential acclimatization responses in potato plantlets derived from cv. Excusita and Bergerac, where only the plantlets of cv. Excusita survived after acclimatization in a pre-prepared soil-sand mixture in the greenhouse and successfully produced minitubers with an average weight of 10-12 g.

The present study aims to produce minitubers of potato (*Solanum tuberosum* L.) cv. Arizona Class A from the multiplication of vegetative buds derived from dormancy-broken mother tubers.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Cells and Plant Tissue Culture at Department of Horticulture and Landscape Design, Collage of Agriculture and Forestry, Mosul University, during the period from February 2024 to January 2025. This experiment was conducted using vegetative buds excised from seed tubers of potato (*Solanum tuberosum* L.) cv. Arizona Class A. The tubers were stored in darkness at 23-24°C for two months to break dormancy and promote sprout initiation and elongation. Sprouts of 2-3 cm in length were carefully excised from the tubers using a sharp, sterile scalpel. The explants were first rinsed under running tap water for 10-15 minutes to remove soil particles and surface microbes. They were then washed in a commercial detergent solution ("Zahi," locally available) for 5 minutes with continuous agitation, followed by thorough rinsing under running tap water. For

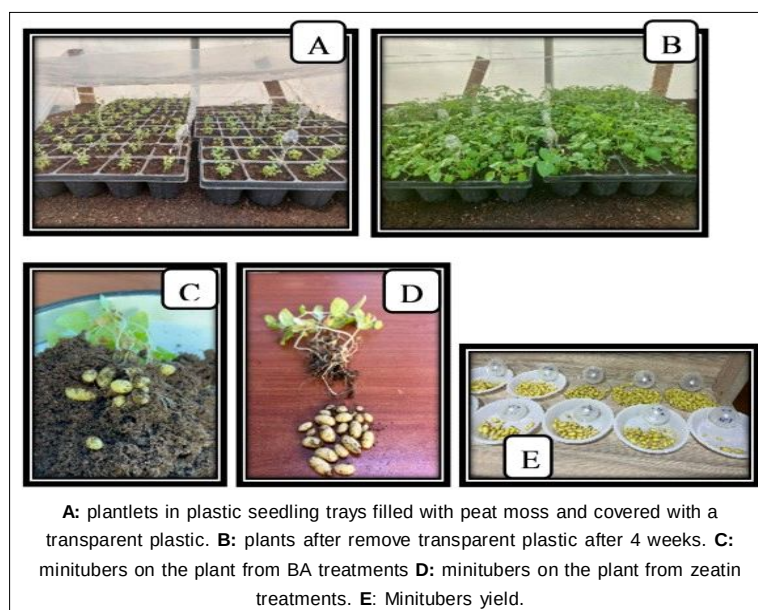


Fig 1: Stages of acclimatization plantlets of Arizona Class A potato plant produce *In vitro* and tuber formation.

surface sterilization, the explants were transferred to a laminar airflow cabinet and treated with 7% (v/v) commercial sodium hypochlorite (NaOCl) solution for 20 minutes with constant agitation. The sterilization solution was prepared from a household bleach containing 6% NaOCl and the sterilization step was enhanced using a vacuum pump to improve penetration. After sterilization, the explants were rinsed three times with sterile distilled water, each rinse lasting 5 minutes, as described by Kassab Bashi *et al.* (2023). The sterilized sprouts were trimmed to 2 cm in length and cultured on Murashige and Skoog (MS) basal medium with full salt strength, 3% sucrose and 6 g L⁻¹ agar, fortified with either BA at concentrations of 0.0, 0.25, 0.5, 1.0 and 2.0 mg L⁻¹ or Zeatin at concentrations of 0.0, 0.25, 0.5, 0.75 and 1.0 mg L⁻¹ for shoot initiation and multiplication. For rooting, buds were cultured on MS medium augmented with IBA at concentrations of 0.0, 0.25, 0.5, 0.75 and 1.0 mg L⁻¹. The pH was adjusted to 5.7±1. Then, they were distributed into bottles, with each bottle containing 40 ml and having a capacity of 210 ml. The bottle openings were covered with aluminum foil. Following a daily photoperiod of 16 hours of light followed by 8 hours of darkness, the cultures were

transferred to the incubation chamber, which was maintained at a temperature of 24±2°C and a light intensity of 3000 lux. The plantlets produced from the multiplication experiments were acclimatized under greenhouse conditions at the College of Agricultural Engineering Sciences, Duhok. The plantlets were transplanted into plastic seedling trays (55 × 30 cm) with 18 cells, each cell 9 cm deep, filled with peat moss as the growth substrate. The trays were initially covered with a transparent plastic lid to maintain high relative humidity during the early stages of acclimatization (Fig 1A). On the day following transplantation, the plantlets were irrigated by foliar spraying with quarter-strength MS nutrient solution, applied every two days for a total of five sprays. Approximately one week after transplanting, the plastic covers were removed and the plants were subsequently treated with foliar sprays of the commercial nutrient solution Trazex at 1 g L⁻¹, applied twice weekly (Fig 1B). Minutubers were harvested after the foliage had senesced and the tubers were collected from the plants (Fig 1C-E). The experiment was laid out in a completely randomized design (CRD) and treatment means were compared using Duncan's multiple range test at a 0.05 probability level. Each treatment was

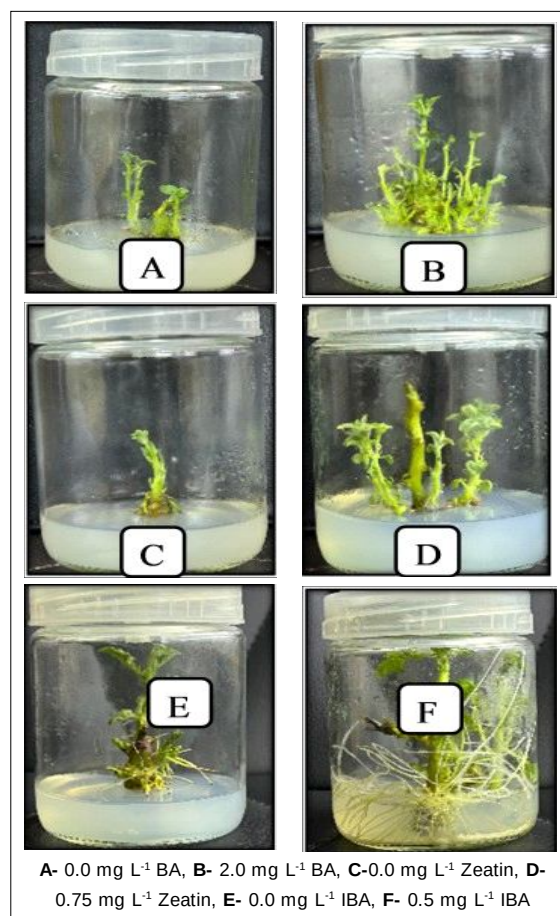


Fig 2: Effect of BA or Zeatin on shoot multiplication of Arizona Class A potato plants after 8 weeks of cultivation on MS medium or on IBA at rooting stage after 4 weeks.

Table 1: Effect of BA on shoots multiplication of (*Solanum tuberosum* L.) potato plant Arizona cv. grown on MS medium after 4 and 8 weeks planting.

BA mg.l ⁻¹	Establishment stage				Multiplication stage			
	Response %	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot	Response %	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot
0.0	80a	1.2b	1.18b	3.1c	100a	2.7c	2.3b	6.2c
0.25	100a	1.5b	2.4ab	7.0b	100a	4.8c	3.25b	8.9b
0.5	100a	1.6b	2.5ab	1.6a	100a	4.7c	3.2b	8.7b
1.0	100a	4.5a	3.9a	2.8a	100a	11.0b	3.55b	10.0ab
2.0	100a	3.7a	3.4a	1.7a	100a	17.2a	5.15a	12.3a
								2.1a

Table 2: Effect of zeatin on shoots multiplication of *Solanum tuberosum* L. potato plant Arizona cv. grown on MS medium after 4 and 8 weeks planting.

Ze mg.l ⁻¹	Establishment stage				Multiplication stage			
	Response %	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot	Response %	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot
0.0	80b	1.3a	0.92b	3.0b	100a	1.5b	1.28c	4.5a
0.25	100a	1.5a	1.96ab	8.9a	100a	3.6ab	2.35b	8.9a
0.5	100a	2.0a	1.76ab	9.2a	100a	4.5a	2.35b	9.4a
0.75	100a	1.5a	2.0b	8.6a	100a	5.3a	2.8ab	10.9a
1.0	100a	1.3a	2.6a	10.1a	100a	5.0a	3.45a	11.5a
								2.9ab
								5.8a

replicated ten times, with each replicate consisting of a single explant (Al-Rawi and Khalaf-Allah, 1980).

RESULTS AND DISCUSSION

Table 1 shows that the buds cultured on MS medium supplemented with 1.0 mg L⁻¹ BA produced the highest mean number of shoots (4.5 shoots per explant) after four weeks of culture. In contrast, the treatment with 2.0 mg L⁻¹ BA showed a clear superiority during the multiplication stage after an additional four weeks, reaching the highest mean shoots number of 17.2 shoots per explant, surpassing all other concentrations used (Fig 2 A,B). This same treatment (2.0 mg L⁻¹ BA) also gave the greatest shoots length (5.15 cm per shoot) and the highest mean leaves number (12.3 leaves per shoot). The table also shows that there were no significant differences in the number of stolons among the treatments; however, the highest mean stolons number (9.7 stolons per explant) was recorded at 1.0 mg L⁻¹ BA.

From the data presented in (Table 2), it is observed that buds cultured on MS medium equipped with 0.5 mg L⁻¹ Zeatin produced the highest mean number of shoots (2.0 shoots per explant) after four weeks of culture. In contrast, the treatments with 0.5, 0.75 and 1.0 mg L⁻¹ Zeatin were significantly superior to the other treatments after an additional four weeks of subculture, recording mean shoots number of 4.5, 5.3 and 5.0 shoots per explant, respectively. The table also shows that the treatment with 1.0 mg L⁻¹ Zeatin produced the highest mean shoot length (3.45 cm) and the highest mean number of stolons (5.8 stolons per plant) as shown in (Fig 2C, D), whereas no significant differences were observed in the mean number of leaves among all treatments.

The results presented in (Table 1 and 2) indicate that the use of both BA and Zeatin, which are plant growth regulators belonging to the cytokinin family, is responsible for increasing the number of lateral shoots up to the optimal concentration, thereby suppressing apical dominance. The gradual increase in Zeatin concentration led to a reduction in apical dominance, resulting in an increase in shoot number and a significant superiority of certain treatments (Hopkins and Hüner, 2004; Wasfi, 1995). The physiological effect of cytokinins is explained by their role in promoting cell division and elongation, which in turn enhances growth characteristics, as well as their influence on protein and nucleic acid synthesis. This is reflected in the noticeable increases in shoot length, shoot number and leaf number achieved by the treatments (Hartmann *et al.*, 2002). The current study results exceeded those reported by Asma *et al.* (2001), with the highest number of shoots reaching 17.2 per plantlet at 2.0 mg/L BA, compared to 14 shoots per plantlet in the previous study.

Table 3 illustrates the effect of different concentrations of IBA on the rooting stage of potato (*Solanum tuberosum* L.) cv. Arizona Class A buds cultured on full-strength MS medium. The results showed that the treatment with 0.5 mg L⁻¹ IBA achieved the highest rooting percentage (90%), which was significantly superior to the other treatments except for the 0.75 mg L⁻¹ IBA treatment. Both 0.5 and 0.75 mg L⁻¹ IBA treatments were significantly superior in terms of root number and root length compared to the other treatments. Meanwhile, the treatment with 0.75 mg L⁻¹ IBA was significantly superior in terms of the mean shoots number (8.9 shoots per explant) this treatment in true produced the highest number of stolons reaching (30.7 stolons per explant) (Fig 2E, F). One possible explanation for these results is that IBA, as an auxin-type hormone,

Table 3: Effect of IBA on rooting of shoots of potato plant *Solanum tuberosum* L. cultivar Arizona Class A on MS medium full of salts concentration after 4 weeks of cultivation.

IBA mg.l ⁻¹	Rooting (%)	Root number	Length of the longest root (cm)	Number of stolons	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot
0.0	10d	0.2b	0.4b	21.1bc	3.1c	3.225bc	7.2bc
0.25	30cd	5.4b	1.05b	16.7bc	3.9bc	3.05bc	7.75b
0.5	90a	34.0a	506a	24.7ab	4.7bc	6.275a	10.9a
0.75	80ab	4.75a	4.30 b	3.7a	8.9a	4.30b	7.1bc
1.0	50bc	2.45b	2.725 c	15.1c	6.4ab	2.725c	5.3c

Table 4: Effect of BA on vegetative growth and minitubers of potato Arizona cv. Produced from multiplication and acclimatization stage after 60 days planting.

BA mg.l ⁻¹	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot	Parts that formed tubers %	Number of minitubers	Wight minitubers (mg)	Size of minitubers (cm ³)
0.0	7.2a	17.25a	52.2bc	100a	6.0c	1.375a	1.945a
0.25	9.6b	11.55b	60.2b	100a	10.4ab	0.944ab	1.054b
0.5	13.0a	12.7ab	0.795b	100a	12.2a	0.705b	0.795b
1.0	6.0c	17.675a	46.0c	100a	7.2bc	1.088ab	1.245ab

Table 5: Effect of Zeatin on vegetative growth and minutubers of potato Arizona cv. Produced from multiplication and acclimatization stage after 60 days planting.

Zemg.l ⁻¹	Shoots number	Length of the longest shoot (cm)	Number of leaves for the longest shoot	Parts that formed tubers%	Number of minutubers	Wight minutubers (mg)	Size of minutubers (cm ³)
0.0	5.20a	15.250a	37.90a	100a	8.5ab	0.812a	1.795a
0.25	7.50a	17.950a	44.30a	100a	9.5ab	0.579ab	0.684b
0.5	6.50a	16.10a	46.45a	100a	9.2ab	0.734a	0.841b
0.75	7.50a	16.450a	49.70a	100a	12.1a	0.454b	0.565b
1.0	6.333a	13.611a	43.889a	100a	6.667b	0.780a	0.923b

plays a crucial role in stimulating roots growth by enhancing cell division and elongation in root differentiation zones, thus promoting the formation of adventitious roots. The addition of growth regulators enhances overall root biomass and mean root length up to the optimal concentration; however, further increases in growth regulator concentrations have an inhibitory effect (Muhammad and Al-Rayes, 1982).

The acclimatization and minutuber production stages

It is observed from (Table 4 and 5) that all BA and Zeatin treatments resulted in 100% minutubers formation, indicating the success of the acclimatization process and the induction of minutubers. The treatment with 0.5 mg L⁻¹ BA recorded the highest mean shoot number (13.0 shoots per plant), the longest mean shoot length (12.7 cm) and the highest mean leaf number (74.1 leaves per shoot). This treatment also produced the highest mean number of minutubers (12.2 minutubers per plant), which was significantly superior to the other treatments. This superiority is attributed to the effective role of the growth regulator BA in stimulating cell division and enhancing the biosynthesis of tubers (Aksenova *et al.*, 2012). The highest mean tuber weight and volume were recorded in the control treatment, reaching 1.375 g and 1.945 mL, respectively however, it did not differ significantly from most of the other treatments. In contrast, the different concentrations of Zeatin showed no significant differences in mean shoot number, shoot length, or leaf number of the longest shoot. However, the treatment with 0.75 mg L⁻¹ Zeatin produced the highest mean number of minutubers (12.1 minutubers per plant), while the highest mean tuber weight was recorded in the control (0.812 g) and the 1.0 mg L⁻¹ Zeatin treatment (0.780 g). The highest mean tuber volume (1.795 mL) was observed in the control treatment. This response may be attributed to the fact that Zeatin, as a naturally occurring cytokinin, plays an important role in promoting cell division, thereby contributing to tuber formation.

CONCLUSION

Tissue culture technology demonstrated high efficiency in the propagation of the potato cultivar Arizona Class A. The use of 2.0 mg L⁻¹ BA was superior in increasing the number of shoots, while 0.5 mg L⁻¹ IBA achieved a rooting percentage of 90%. Moreover, 0.5 mg L⁻¹ BA and 0.75 mg L⁻¹ Zeatin resulted in the highest mean number of minutubers, 12.2

and 12.1 minutubers per plant, respectively. This approach represents a reliable method for producing pure, high-quality potato seedlings.

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Conflict of interest

The writers claim that absolutely no disputes exist of interest concerning the publication of this work. No cooperation or financial support effect the research's arrangement, gathering of information, review, decide to publish, or Making the written work prepared.

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