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The Role of Plant Growth Regulators in Micropropagation of Potato (*Solanum tuberosum* L.) for Achieving Sustainable Food Security

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ABSTRACT

Background: This study refers to selecting the influence of various EGRs (explant growth regulators) and their specific concentrations at *in vitro* micropropagation efficiency (including shoot propagation and rooting) of potato and subsequently, to evaluate the resulting micro propagated plantlets capacity to tolerate drought stress under controlled conditions.

Methods: The study was directed to determine the optimal concentrations of BA (benzyl adenine) with (0, 0.5, 1 and 1.5 mg/L), GA3 (gibberellic acid) with (0, 0.1, 0.2 and 0.3 mg/L) and Kin (kinetin) with BA (0, 0.5, 1 and 1.5 mg/L) to stimulate micro tuber formation in the potato varieties Borin and Naima and induction drought was PEG with (0, 0.5, 1 and 1.5 g/L) used. The work was carried out by complete randomized design (CRD) where the number of samples by which the analysis was performed was 112 unit experimental that resulted from the use of two varieties of potato and three replications.

Result: The results revealed that BA at 0.5 mg/L exhibited best shoot growth number reached 10.8 shoot/explant in Naima. BA in 0.5 mg/L and Kin of 1 mg/L resulted in the best number and dimeter of micro tuber reached to 18.5 tuber/explant in Naima.

Key words: Acclimatization, BA, Borin, GA3, Kin, Micro tubers, Naima, Tissue culture.

INTRODUCTION

The potato variety (*Solanum tuberosum* L.) has immense global growing importance and has been reported as the fourth most essential food crop all over the world (Khierallah and Jawad, 2017). Due to its high nutritional value, energy density and fundamental yield (*Solanum tuberosum*) is considered as a very important crop product of global food security, especially in the developing nations (Toma, 2022; Sahay *et al.,* 2026). Abiotic stresses, drought and water lack confuse a remarkable threat to the stability of the yield (Hassan *et al*., 2018) this appeared especially in the agricultural systems that obeyed dry and semi-dry methods (Bahar *et al*., 2025). Drought tolerance decreases the physiological key processes that include photosynthesis, stomatal behavior and nutrient up taking leads to yield loss normally exceeding 50% (Qiao *et al*., 2024). This susceptibility assures the urgent need for the development of drought-tolerant potato varieties. The solution to overcome these problems is by micropropagation the technique of *in vitro* plant tissue culture which present an efficient and Necessary planning (Abdelhafez *et al.,* 2024; Patel *et al.,* 2026). This method enables us to work for rapid and high levels production of genetically regular and disease-free plants. This can serve in the spreading of the better and improved potato varieties (Altindal and Karaca, 2019; Waheeb *et al.,* 2025). The efficacy of micropropagation conditions is precisely regulated by the chemical composition and percentage presented in the culture media specifically amount and type of (EGRs) (Nasrallah *et al.,* 2015; Kadhim *et al.,* 2023). Phytohormones like auxins, cytokinin's and gibberellins have crucial role in the adjustment of the division of cells, difference and morphogenesis (Hamza and Ali, 2017), so by controlling *in vitro* stages of shoot propagation, elongation and root formation (Asakaviciute, 2023; Sufiyan, 2025).

The exact balanced ratio between cytokinin's and auxins orders the success of both multiplication and rooting stages (Shoyeb *et al.,* 2017). Understanding how specific\tuberosum varieties respond to different EGR systems is essential for inquiry of maximum *in vitro* efficacy (Zaki and Khlode, 2022; Al-Jubori and Mohammed, 2024). This method can serve for the evaluation of a lot of genotypes in a controlled environment, reducing up the

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time and resources related to the field cases. Polyethylene glycol (PEG) is commonly good osmotic agent used to simulate drought conditions *in vitro* (Hadi *et al.,* 2019). PEG is non-toxic and cannot be used by the explant and only works to reduce the water potential of the medium. Studies have shown that exposing potato micro plants to different PEG levels can effectively show many differences in features related to osmotic adjustment, water-use efficiency and tolerance to stress of oxidation (Sahat *et al.,* 2022; Lama and Neamah, 2023; Akhtar *et al.,* 2024). Fusing EGR optimization with PEG-induced stress is considered a novel approach to selecting high propagation and drought resisted potato lines, thus directly addressing the challenge of achieving sustainable food security.

MATERIALS AND METHODS

Explant material (explant parts)

The study was conducted in the Department of Desertification Control Laboratory and the Crop Physiology Laboratory of the College of Agricultural Engineering Sciences, University of Baghdad at period 2025/2026, potato tubers of the Borin and Naima varieties were obtained from the Horticulture Department of the Ministry of Agriculture. The tubers were screened for disease before being explanted in trays containing potting soil (as shown in (Fig 1) (Al-Jubori and Al-Shamari, 2025). The growing tips of the shoots growing from these tubers were used as the source of explant parts. Explant parts were sterilized by immersing them in 10% sodium hypochlorite solution for 10 minutes (Al-Jubori and Al-Amery, 2022), then washed by sterilized distilled water three times to remove remains of the sterile solution (Al-Jubori *et al.,* 2023).

Tissue culture establishment

Bud formation was induced from the shoot tips using modified (MS) media (Murashige and Skoog, 1962) containing 3% sucrose. Plant growth regulators added to the media, including (NAA) at a fixed 0.2 mg/L and (BA) at several amounts (0, 0.25, 0.5 and 1.0 mg/L). Plants cultured are incubated in a chamber used for growth study at 25±2°C, using light cycle about 16 hrs and 8 hrs in dark for 4 weeks.

Micro tuber induction experiments

The first experiment

Media from Murashige and Skoog (MS) with a low nitrogen concentration was used, modified by halving the concentration of macronutrients and complemented with (GA3) using 0.0, 0.1, 0.2 and 0.3 mg/L and mannitol at a concentration of 8%.

The second experiment

MS media with a low nitrogen concentration was used and complemented with 50 g/L sucrose and (Kin) amounts of 0, 0.5 and 1 mg/L. The micro-tuber cultured plants are incubated using low light intensity and at a temperature of 18±2°C for 4 weeks.

PEG treatments

Which was 0.1 mg/L of GA3 and 1 of Kin with 0.5 of BA mg/L for the nutrient media containing PEG at 0, 0.5, 1 and 1.5 g/L and the cultured plant incubated at of 25±2°C and using light of 1000 lux to16 hrs of lighting and 8 hrs of dark (Al-Rubaie and Al-Jubouri, 2023). Observations were taken after four weeks of cultivation and then the stages of rooting and acclimatization shown in (Fig 2).

Acclimatization of tissue cultures

The tissue cultures were transferred to pots that contain a sterile culturing mixture, peat moss and perlite at 1:1:2 ratio of (Alogaidi *et al.,* 2019).

Measurement of plant relative growth rate

Physiological properties were measured as the average as described by Farshadfar *et al.* (2014):

$$\frac{W2-W1}{W1}$$



Fig 1: Potato tubers grow in trays as a source of explant parts.

Statistical design and analysis

The analysis of data was carried out by a factorial design with a complete randomized design (CRD) and 5 replicants of each other's treatments, mass and diversity the total experiments. The growth averages are examined using LSD test at 0.05 odds level applied in Genestat 2007 (Lamidi *et al.,* 2024).

RESULTS AND DISCUSSION

Effect by using BA on the growth of vegetative shoots number in potato

The results in (Table 1) indicate that the higher number of vegetative shoots was recorded in the Naima 10.8 shoot/explant compared to the Borin, which recorded a lower rate 5.1 shoot/explant. The higher rate of shoots recorded at 0.5 of BA which recorded 12.9 shoot/explant compared to the control treatment, which recorded several vegetative shoots 3.3 shoot/explant. Regarding the interaction between varieties and BA concentrations, Naima recorded a higher rate at a concentration of 0.5 which recorded 17.7 shoot/explant than the Borin in the control treatment which recorded 2.3 shoot/explant, as in (Fig 3).

Effect of GA3 on the number of micro tubers in potato

The results from (Table 2) indicate that the higher number of micro tubers recorded in the Naima 3.4 tuber/explant compared to the Borin, which recorded a lower rate of 2.3 tuber/explant. The highest rate of number of micro tubers was recorded at 0.1 of GA3 which recorded 4.0 tuber/explant



Fig 2: Stages tissue culture.



Fig 3: BA on the growth of vegetative shoots number in Naima.

Table 1: Effect of BA on the number of growing vegetative shoots in potato (shoot/explant) varieties Borin and Naima.

Variety	Conc. of BA mg/L				Average
	0.0	0.25	0.5	1.0	
Borin	2.3	3.7	8.0	6.7	5.1
Naima	4.3	7.3	17.7	14.0	10.8
LSD 0.05		1.9			0.9
Average	3.3	5.5	12.9	10.4	
LSD 0.05		1.3			
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Var.	1	216.0	216.0	185.14	<.001
BA	3	304.33	101.44	86.95	<.001
Var. BA	3	72.333	24.111	20.67	<.001
Residual	16	18.667	1.167		
Total	23	611.33			
S.e.	1.08				
Cv%	13.8				

by comparison to the control treating recorded several numbers of micro tubers 1.7 tuber/explant. Regarding the interaction between varieties and GA3 concentrations, Naima recorded a higher rate at a concentration of 0.1 which recorded 5.0 tuber/explant than the Borin in the control treatment which recorded 1.3 tuber/explant.

Effect of kin and BA on the number of micro tubers in potato

Table 3 showed a high difference in the two potato varieties. The Naima showed a higher average number of micro tubers to 12.9 tuber/explant. The Borin reached a lower average number of micro tubers at 8.6 tuber/explant. The BA afforded high progress of the 0.5 mg/L, with the higher average number of micro tubers that reaching 12.1 tuber/explant, however the control treating indicated lower shoots average number reached to 8.4 tuber/explant. The 1 mg/L of Kin significantly appeared higher average number of growing shoots, 13.4 tuber/explant, however the control treating afforded lower shoots average number of 8.7 tuber/explant. The relationship between varieties and BA mass was significant. The Naima at 0.5 mg/L gave higher average number of shoots to 14.5 tuber/explant, while lower average number of tubers reaching 6.4 tuber/explant was recorded when using Borin at 0 mg/L. The interaction between varieties and Kin concentrations Naima at 1 mg/L exhibited up a reliable average number of tubers 17.1 tuber/explant, the Borin in 0 mg/L of Kin gave a least average number of tubers 6.5 tuber/explant. Concerning interaction between the BA and Kin amounts, the higher average number of tubers was gained in 0.5 mg/L of BA and 1 mg/L of Kin, amounting to 16.0 tuber/explant and at 0 BA and Kin gave up a lower average number of tubers in 6.3 tuber/explant. Table 3 shown a considerable difference between the varieties and amounts of BA and Kin. The Naima was high in 0.5 mg/L of BA and at 1 mg/L of Kin and higher average number of tubers up to 18.5 tuber/explant however the Borin appeared with the low average number of tubers in 5.5 tuber/explant and in 0 mg/L of BA and Kin.

Effect of PEG on plant relative growth rate in potato

Table 4 showed results that indicate the higher plant relative growth rate was recorded in (4) 2.6 mg/day compared to the 1, which recorded a lower rate 1.6 mg/day. The highest plant relative growth rate was recorded at 0.5 g/L of PEG which recorded 3.7 mg/day compared to 1.5 g/L of PEG treatment, which recorded 1.0 mg/day. Regarding the interaction between varieties and PEG concentrations, 4 recorded a higher rate at a concentration of 0.5 which recorded 4.2 mg/day than the 1 in the 1.5 g/L of PEG treatment which recorded 0.7 mg/day.

Effect of PEG on Tolerance index in potato

Fig 4 showed results that indicate higher tolerance index was recorded in (4) 32.1 compared to the 1, which recorded a lower rate of 22.9. The higher tolerance index recorded at 0.5 g/L of PEG which recorded 50.8 compared to 1.5 g/L of PEG treatment, which recorded 18.5. Regarding the interaction between varieties and PEG concentrations, 4 recorded a higher rate at a concentration of 0.5 which recorded 64.3 than the 1 in the 1.5 g/L of PEG treatment which recorded 13.7.

Cytokinin's are plant hormones primarily that are responsible for promoting the division of cells and difference in the shoots. They are mostly acted to break apical dominance, stimulating the growth and development of axillary buds to new shoots (Solano-Campos *et al.,* 2019). The mass of 0.5 mg/L is known as the optimal balance where the growth-promoting effects of the hormone are high without reaching optimum levels that could induct inhibitory effects or undesired physiological conditions (Asakaviciute *et al.,* 2023; Shoyeb *et al.,* 2017). This maximum outcome assures that the genetic potential of the Naima variety is fully expressed only when combined with the optimized signal of hormones. The high yield is

Table 2: Effect of GA3 on number of micro tubers (tuber/explant) in potato varieties Borin and Naima.

Variety	Conc. of GA3 mg/L				Average
	0.0	0.1	0.2	0.3	
Borin	1.3	3.0	2.7	2.0	2.3
Naima	2.0	5.0	4.0	2.7	3.4
LSD 0.05		1.5			0.7
Average	1.7	4.0	3.3	2.3	
LSD 0.05		1.1			
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Var.	1	8.1667	8.1667	10.89	0.005
GA3	3	19.3333	6.4444	8.59	0.001
Var. GA3	3	1.8333	0.6111	0.81	0.504
Residual	16	12.0	0.75		
Total	23	41.3333			
S.e.	0.38				
Cv%	11.8				

not just an additive effect but a cooperative one, demonstrates that an efficient micropropagation protocol must be variety specific. In contrast, the lowest rate obtained (Borin in control, 2.3 shoot/explant) sets the baseline for the non-responsive variety in the absence of the stimulatory hormone (Pasternak and Steinmache, 2024).

Table 3: Effect of BA and Kin on the number of micro tubers (tuber/explant) in potato varieties Borin and Naima.

Varieties	Conc. of BA mg/L	Conc. of Kin mg/L				Average of var. × BA
		0	0.5	1	1.5	
Borin	0	5.5	6.5	7.5	6.0	6.4
	0.5	6.5	8.0	13.5	10.0	9.5
	1	6.0	7.5	11.5	9.5	8.6
	1.5	5.0	6.5	10.0	8.0	7.4
Naima	0	7.0	8.5	14.5	11.0	10.3
	0.5	11.5	13.5	18.5	14.5	14.5
	1	10.0	11.5	17.5	13.5	13.1
	1.5	9.5	10.5	13.0	12.5	11.4
LSD 0.05			1.8			0.9
Varieties			**Kin × varieties**			**Varieties average**
Borin		6.5	7.9	11.1	9.0	8.6
Naima		9.8	11.3	17.1	13.4	12.9
LSD 0.05			0.9			0.5
BA conc.			**Kin × BA**			**BA average**
0		6.3	7.5	11.0	8.8	8.4
0.5		9.0	10.8	16.0	12.3	12.1
1		8.5	10.0	14.5	11.5	11.1
1.5		8.1	9.0	11.4	10.3	9.7
LSD 0.05			1.3			0.6
Kin average		7.8	9.2	13.4	10.7	
LSD 0.05			0.6			
Source of variation		**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Var.		1	289.0	289.0	369.92	<.001
BA		3	125.5	41.833	53.55	<.001
Kin		3	318.13	106.04	135.73	<.001
Var. BA		3	3.5	1.1667	1.49	0.235
Var. Kin		3	19.375	6.4583	8.27	<.001
BA. Kin		9	9.875	1.0972	1.4	0.227
Var. BA. Kin		9	11.625	1.2917	1.65	0.142
Residual		32	25.0	0.7812		
Total		63	802.0			
S.e.		0.884				
Cv%		8.2				

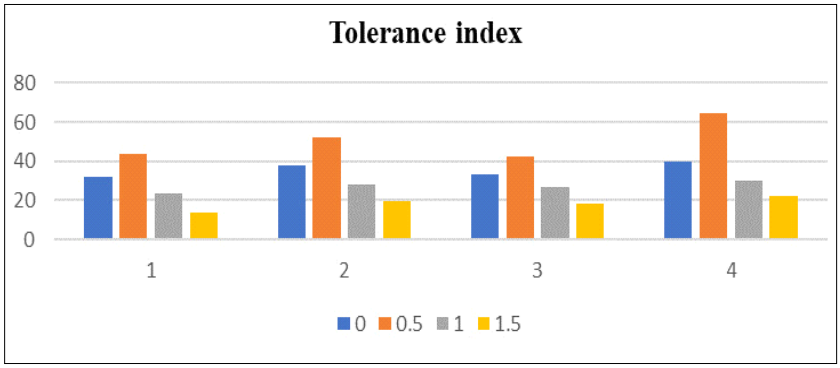


Fig 4: Effect of PEG on tolerance index in potato varieties Borin and Naima.

Table 4: Effect of PEG on plant relative growth rate (mg/day) in potato varieties Borin and Naima.

Variety	Con. PEG g/L				Average
	0	0.5	1	1.5	
1	2.0	3.2	0.9	0.7	1.6
2	2.3	3.8	1.3	1.0	2.1
3	2.2	3.6	1.1	0.9	2.2
4	2.4	4.2	1.4	1.2	2.6
LSD 0.05			0.13		0.06
Average	2.2	3.7	1.2	1.0	
LSD 0.05			0.06		
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Var.	3	4.5453	1.5151	63.95	<.001
PEG	3	61.589	20.53	866.46	<.001
Var. PEG	9	2.3556	0.2617	11.05	<.001
Residual	32	0.7582	0.0237		
Total	47	69.248			
S.e.	0.1539				
Cv%	6.3				

1. Borin treated with 0.1 mg/L of GA3.
2. Naima treated with 0.1 mg/L of GA3.
3. Borin treated with 1 of Kin and 0.5 of BA mg/L.
4. Naima treated with 1 of Kin and 0.5 of BA mg/L.

The tuber-inducing factors (like high sucrose levels in medium, short photoperiod and the hormone-like signaling protein StSP6A) are the dominant promoters and the low GA3 level successfully prevents the strong inhibitory effects that higher GA3 concentrations would exert. The GA3 concentration used here permits the overall tubergen signal to take effect (Rajeswar and Narasimhan, 2021; Bharath and Raju, 2023).

PEG is unable to penetrate by osmosis property used in tissue culture to simulate stress of water by lowering the water medium potential (Jan *et al.,* 2019; Kylyshbayeva *et al.,* 2024). When cells test low water potential, they initiate a protection mechanism known as Osmotic Adjustment. Proline is known as a key component of this adjustment. It is an amino acid that rapidly accumulates at stress conditions (Meena *et al.,* 2019; Kylyshbayeva *et al.,* 2025).

CONCLUSION

This study confirms the critical role of the explant growth regulators BA, Kin and GA3 in potato micropropagation. The optimum concentrations characterized in the work can be used to develop the production efficiency of high-quality, disease-free potato seedlings. These findings contribute to the development of sustainable agricultural technologies and enhance food security by providing improved explanting material.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect loss resulting from the use of this content.

Conflict of interest

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