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Regeneration of selected callus of three potato cultivars (*Solanum tuberosum*.L) and studying their tolerance to drought stress

Lama Abdullah Laila[1], Salim Hussein Zaid[1], Fahed Al-Biski[2] and Abdulkarim Dakah[3*]

Abstract

Drought stress poses a significant threat to potato production in Syria, necessitating the development of resilient cultivars. This study, conducted at the Plant Biotechnology Laboratory of the National Commission for Biotechnology in Damascus, investigated the impact of drought stress on key growth parameters of regenerated somaclones derived from selected callus of three potato cultivars (*Solanum tuberosum* L.): Salvador, Yalas, and Arizona. The research revealed striking differences in regeneration efficiency among cultivars. Yalas exhibited the highest somaclone regeneration rate at 32.1% when cultured on medium supplemented with 25 mg·L^{-1} adenine sulfate and 1.5 mg·L^{-1} BAP. Salvador and Arizona followed with regeneration rates of 21.4% and 14.5%, respectively, using 15 mg·L^{-1} adenine sulfate plus 1.5 mg·L^{-1} BAP. Comprehensive analysis of growth indicators (including plant height, leaf number and area, and root number and length) under drought stress conditions identified superior drought-tolerant somaclones. Notably, Y3 and Y2 (Yalas), S3 and S6 (Salvador), and A1 (Arizona) demonstrated exceptional drought resilience across multiple growth parameters. These findings provide valuable insights for potato breeding programs aimed at enhancing drought tolerance, potentially mitigating the impact of water scarcity on potato production in Syria and similar arid regions.

Keywords Potato, Drought stress, Sorbitol, Regeneration, Callus, Adenine sulfate

Introduction

Potato (*Solanum tuberosum*. L) is one of the most important food crops in the world [1], the fourth most cultivated crop after wheat, rice and maize, and the most important dicotyledonous crop [2]. It is grown in about 150 countries [3] and is the most important non-cereal food crop in the world [4, 5]. Their global significance is underscored by widespread cultivation, demonstrating their adaptability to diverse climates and agricultural systems, and in 2016, the potato industry generated a remarkable gross production value of 63.6 billion US dollars, highlighting its substantial contribution to global agriculture and food economies, also the year 2018 saw an impressive annual potato production of 368 million tons, illustrating the crop's critical role in global food security and agricultural output [4]. Biotic (insects, viral and fungal diseases) and abiotic (drought, salinity, frost, temperature, etc.) stresses affect the growth and production rate of many crops, including potatoes, causing huge losses estimated at billions of dollars as a result of plants being exposed to stresses [6, 7]. Drought is one of the most important abiotic stresses that limit plant production in most parts of the world, as it affects all plant

*Correspondence:
Abdulkarim Dakah
abdu83alkarim@yahoo.com
[1] Department of Plant Biology, Faculty of Sciences, Damascus University, Damascus, Syria
[2] Researcher at the National Commission for Biotechnology, Damascus, Syria
[3] Department of Biotechnology, Faculty of Sciences, University of Kalamoon, Deir attyah, Syria

functions [8] and thus negatively affects the productivity of many crop species, as a result of the annual exposure of large areas of the Earth to short or long waves of drought coinciding with high temperatures [9]. Potatoes that are plants that sensitive to drought stress [10]. Many studies have shown that potato species have genetic variations in their tolerance to stress, and thus these genetic variations can be exploited by creating them through laboratory tissue culture technology, which can lead to increased production, improved quality, and disease resistance [11]. Callus induction is an important step in bringing about genetic changes that are necessary in breeding programs, and it also plays an important role in producing a large number of laboratory plants easily [12, 13]. The importance of somatic genetic variation lies in enhancing genetic improvement programs for potato crops, and introducing mutations that ultimately give plants that differ from the mothers plants [14]. Somatic phenotypic variations vary according to the plant material (varieties), the cultivation period, the basic chromosome group and its multiplications, or according to the stress-causing factors, or the composition of the medium from plant growth regulators [15]. Somaclones with new genetic compositions are called Calli Clones because they result from callus differentiation under laboratory culture conditions. These somaclones are known as calli somaclones [16]. The somatic genetic variations can vary depending on the genotype or the basic chromosome set and its multiplications, or on the plant growth regulators included in the nutrient medium, or the cultivation period, or the chemical compounds that cause salt stress, such as sodium chloride (NaCl), or drought stress, such as polyethylene glycol (PEG) or sorbitol [15, 17]. The medium having growth regulators plays an important role in shaping somatic variations [18] because callus production is primarily linked to the type of growth regulators, which varies according to the type of explant taken and the type studied, as genetic programs differ according to the auxin/cytokinin ratio [12]. Hussain et al. [19] studied the possibility of regeneration of three potato varieties from apical cuttings, leaf parts and internodes, and it was found that the best medium was the MS medium supplemented with (2 $mg.l^{-1}$)BAP +(0.5 $mg.l^{-1}$) IAA Indole acetic acid, and therefore the idea of the research was to regenerate the somaclones of the selected callus of three potato varieties, and to study their tolerance to drought stress and to renew the superior somaclones of them.

Materials and methods

Plant material

This research was carried out in the laboratories of the National Commission for Biotechnology in Damascus during the period (2024). Three varieties of introduced potatoes were used in this research, which were obtained from the Seed Multiplication Foundation in Damascus, namely (Salvador, Yalas, Arizona). These varieties are distinguished by their quality and productivity.

Callus induction

They were introduced and multiplied in the laboratory. Callus was initiated from internodes and young leaves of the three potato varieties by culturing them on MS (Murashige and Skoog) nutrient medium supplemented with 3 $mg{\cdot}L^{-1}$ auxin 2,4-D (Dichlorophenoxyacetic acid) [20]. The callus was subcultured three consecutive times by dividing and transferring it to fresh medium with the same composition as the initiation medium.

Drought stress treatment and obtained selected callus

The callus was transferred after reaching a suitable size to the drought stress medium, which is a medium similar in composition to the initiation medium, but with the addition of the drought stress factor (sorbitol) at varying concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400 mM). Plates were incubated in the dark at 25 ± 1 °C. The semi-lethal concentration (causing a 50% reduction in cell viability and relative growth rate) was determined for each variety after 60 days of the three studied varieties [21].

Regeneration of callus

Selected callus from semi-lethal concentration media was transferred to regeneration medium (MS medium) supplemented with BAP (Benzylaminopurine) at different concentrations (0.5, 1, and 1.5 $mg{\cdot}L^{-1}$) and $AdSO_4$ (adenine sulfate) at two concentrations 15 and 25 $mg{\cdot}L^{-1}$ (Table 1).

Drought stress selection

Regenerated somaclones were subjected to sorbitol stress at semi-lethal concentrations (250–300 mM) for further selection. Growth indicators were measured after 45 days of cultivation alongside a control group free of stress factors.

Experimental design and statistical analysis

The experiment was designed according to a complete randomized block design, where measurements were taken after 45 days of applying the stress treatments and at a rate of three replicates of each stress treatment and the control. The results were analyzed using the statistical program (XLSTAT) to compare the averages and calculate the least significant difference (L.S.D) at a significance level of 1%.

Table 1 Nutrient media that used in the induction, propagation and regeneration of callus

Compound	Callus induction ($mg.l^{-1}$)	Callus propagation ($mg.l^{-1}$)	Regeneration ($mg.l^{-1}$)
Nutrition medium	MS	MS	MS
Thiamin	0.5	0.5	0.5
Pyridoxine	0.5	0.5	0.5
Nicotinic acid	0.5	0.5	0.5
Folic acid	0.5	0.5	0.5
Biotin	0.05	0.05	0.05
Glycine	2	2	2
Myoinositol	100	100	100
Glutamine	2	2	2
Dichlorophenoxy-acetic acid 2,4-D	5,3,1.5,1,0	0.5	-
$AdSO_4$ + BAP	-	-	(15, 25) + (0.5, 1, 1.5)
Sucrose	30,000	30,000	30,000
High purity agar	7000	7000	7000
pH	5.8	5.8	5.8

Results

Regeneration of selected callus by the effect of growth regulators BAP and $AdSO_4$

The results indicate that the relative growth rate of callus was good at the low level of sorbitol, and the increasing concentration of sorbitol in the medium led to a decrease in the relative rate of callus. Regeneration observations were recorded after a month and a half of culturing, and it was found that there was no regeneration of the callus of the Internodes, but rather the regeneration was from the callus of the leaves for the three studied varieties. The results of (Table 2) showed that the highest regeneration rate reached 32.1% in the Yalas variety when grown on the medium supplied with 25 $mg.l^{-1}$ adenine sulfate and 11.5 $mg.l^{-1}$ BAP, followed by the Salvador variety with a rate of 21.4%, then the Arizona variety with 14.5% using the treatment (15 $mg.l^{-1}$ adenine sulfate) + (11.5 $mg.l^{-1}$ BAP). The results showed that the importance of adenine sulfate and BAP in callus regeneration, as shown in Fig. 1.

The remaining regenerated somaclones of the studied varieties were propagated, as the resulting plants were divided into small cuttings of 1.5–1 cm in length containing a lateral bud with a leaf, and were planted in MS medium to obtain a sufficient amount of plants to continue the remaining experiments.

Effect of sorbitol concentration on growth indicators of callus-regenerated plants grown in the laboratory

The callus-regenerated somaclones of the three studied potato varieties were planted in (MS) medium to which the drought stress factor (sorbitol) was added at two concentrations (250 or 300 mM), and the readings were taken after 45 days of planting. Table 3 shows that all the 10 regenerated somaclones (4 salvador, 5 Yalas, 1 Arizona) and their controls outperformed the mothers of the three varieties in terms of the growth indicators

Table 2 Effect of growth regulator BAP and adenine sulfate $AdSO_4$ on callus regeneration from leaves of potato varieties

Explants	Leaves								
Media	Varieties								
BAP + adeninsulphat (mgl^{-1})	Salvador			Yalas			Arizona		
	Regeneration %	Number of days required for regeneration	Average number of growths formed/ callus	Regeneration %	Number of days required for regeneration	Average number of growths formed/ callus	Regeneration %	Number of days required for regeneration	Average number of growths formed/ callus
0	0e	-	-	0e	-	-	0e	-	-
0.5 + 15	0e	-	-	0e	-	-	0e	-	-
1 + 15	0e	-	-	0e	-	-	0e	-	-
1.5 + 15	21.4b	40	2	0e	-	-	14.5 d	45	1
0.5 + 25	0e	-	-	0e	-	-	0e	-	-
1 + 25	0e	-	-	18.5c	40	1	0e	-	-
1.5 + 25	0e	-	-	32.1a	45	2	0e	-	-
Average	3.06A			7.23 A			2.07A		
$LSD_{0.01}$ Treatment	0.62								
$LSD_{0.01}$ Variety	5.49								

The difference in capital letters within rows indicates significant differences between the varieties, while the difference in lowercase letters in the columns indicates significant differences between the means at the 99% confidence level

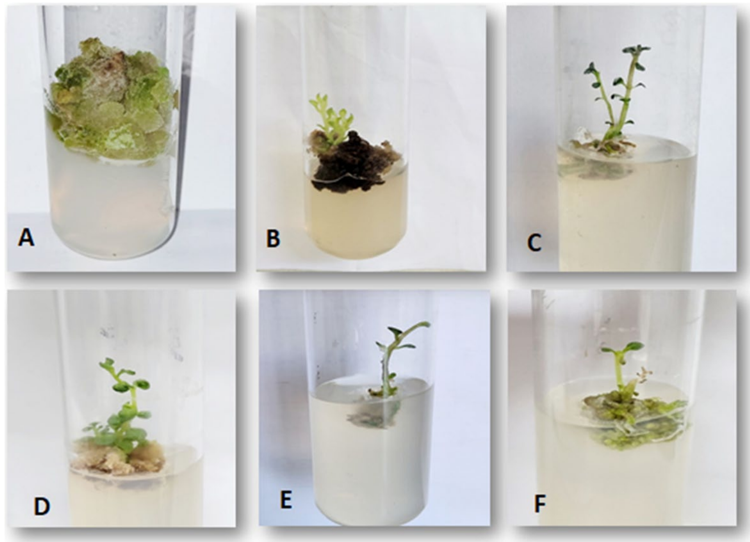

Fig. 1 Regeneration of growths from selected callus of the studied varieties, (**A**) Formation of vegetative buds on selected callus, (**B**-**C**-**D**-**E**–**F**) Regenerative somaclones

studied at all the studied concentrations (250 or 300 mM) of sorbitol.

The length of the plant stem decreased with increasing the sorbitol concentration in the MS growth medium in all the studied somaclones. There were significant differences in stem length at the two concentrations (250 and 300) mM of sorbitol, compared to the control treatment. The plant length in the Y2 somaclone was (3.9 and 4.1 cm) respectively compared to the control (15.8 cm). In the S6 somaclone (5.8 and 6.2 cm) respectively compared to the control (16.4 cm), and in the A1 somaclone (2.9 and 3.2 cm) respectively compared to the control (13.0 cm). In terms of the effect of the variety, no significant differences were observed in the average stem length in all the studied somaclone. Figure 2. We also note from (Table 3) a decrease in the number of leaves and leaf area on the plant in the studied somaclones due to the effect of increasing the concentration of sorbitol in the medium, as the decrease from the control was significant at all sorbitol concentrations used in all the studied somaclones. It is noted that there were no significant differences between the somaclone in the number of leaves and leaf area, as the number of leaves in the Y2 somaclone was (6.9 and 8.0) leaves respectively compared to the control (15 leaves). In the S6 somaclone (7.5–9) leaves were found compared to the control (15.0 leaves), and in the A1 somaclone (3.9–3 leaves respectively compared to the control (13.7) leaves. Table 3 shows the difference in the root length of the studied somaclones in terms of their response to drought stress, as the length of the roots formed on the renewed somaclones decreased significantly with increasing severity of drought stress, where the highest value of root length was recorded in the Yalas somaclones at the Y2 somaclone (4.3–5.9 cm) respectively compared to the control (8.4 cm). In the Salvador somaclones at the S6 somaclone (4.2–5.7 cm) respectively compared to the control (9.8 cm). As for the Arizona variety lineage, at lineage A1 (1.3–2.5 cm) respectively compared to the control (5 cm). The results in Table 3 also showed a difference in the number of roots of the studied lines in terms of their response to drought stress, as the number of roots formed on the renewed lines decreased significantly with increasing severity of drought stress, where the lowest value for root length was recorded in the Yalas variety lines at lineage Y8 (2.9–3.5 roots/plant respectively compared to the control (10.1) roots/plant. And in the Salvador variety lines at lineage S4 (5.1–5.3) roots/plant respectively compared to the control (8.2) roots/plant. As for the Arizona variety lineage at lineage A1 (1.8–2.0) roots/plant respectively compared to the control (6.1) roots/plant. We find in comparison that all lines outperformed the plants of the parent varieties in the length and number of roots per plant. While the value of the root length and number in the controls outperformed its somaclones. It is noted from all the results that the somaclones Y3, Y2 of the Yalas variety, the somaclones S3, S6 of the Salvador variety, and the somaclone A1 of the Arizona variety are superior to the rest of the studied somaclones in terms of their high ability to withstand drought stress in various growth indicators, especially at a concentration of 300 mM of sorbitol, Fig. 2.

Table 3 Effect of drought stress treatments on growth indicators of regenerative potato somaclones

Varieties		Treatment Sorbitol mM	Mean of steam length (cm)	Mean of leaves number	Mean of leaf area (cm^2)	Mean of root length (cm)	Mean of root number
Salvador	S1	Control	16.4^{ab}	14.4^{ab}	195^{h}	9.2^{abc}	8.5^{cd}
		250	5.9^{fg}	8.5^{cde}	66.1^{n}	5 g^{hijk}	5.5^{efg}
		300	5.3^{fghi}	7^{defghi}	58.9^{qr}	3.6^{klmn}	5.3^{efgh}
		Mean	9.53^{A}	9.97^{AB}	106.67^{AB}	5.93^{A}	6.43^{A}
	S6	Control	17.3^{a}	15^{a}	225^{f}	9.8^{ab}	9^{bcd}
		250	6.2^{fg}	9^{c}	73.7^{k}	5.7^{efgh}	6^{e}
		300	5.8^{fg}	$7.5^{cdefghi}$	65.9^{n}	4.2^{hijklm}	5.8^{ef}
		Mean	9.77^{A}	10.5^{A}	121.8^{AB}	6.57^{A}	6.93^{A}
	S3	control	17.13^{a}	14.7^{ab}	210^{g}	10.17^{a}	8.7^{cd}
		250	6^{fg}	8.7^{cd}	68.3^{m}	5.3^{fghij}	5.8^{ef}
		300	5.6^{fgh}	$7.3^{cdefghi}$	61.2^{p}	3.9^{ijklmn}	5.5^{efg}
		Mean	9.58^{A}	10.23^{AB}	113.1^{AB}	6.46^{A}	6.67^{A}
	S4	control	16^{abc}	13.2^{b}	180^{i}	9^{abc}	8.2^{d}
		250	5.5^{fghi}	8.3^{cdef}	60.5^{pq}	4.9^{ghijkl}	5.3^{efgh}
		300	5^{fghij}	6.8^{efghij}	55.3^{s}	3.3^{lmn}	5.1^{efghi}
		Mean	8.5^{A}	9.43^{AB}	98.6^{AB}	5.73^{A}	5.87^{AB}
Yalas	Y3	control	15.4^{bcd}	14.4^{ab}	350^{b}	8^{cd}	11^{a}
		250	3.9^{jkl}	7.6^{cdefgh}	68.2^{m}	5.4^{fghi}	$4.3f^{ghij}$
		300	3.7^{jkl}	6.5^{fghij}	63.5^{o}	4^{ijklmn}	4^{ghij}
		Mean	7.66^{A}	9.5^{AB}	160.5^{A}	5.8^{A}	6.77^{A}
	Y2	control	15.8^{abc}	15^{a}	410a	8.4^{bcd}	11.3^{a}
		250	4.1^{hijkl}	8^{cdefg}	70.3^{l}	6.21^{a}	4.7^{efghi}
		300	3.9^{ijkl}	6.9^{efghij}	67.9^{m}	$4.3^{ghijklm}$	4.2^{fghij}
		Mean	7.93^{A}	9.97^{AB}	182.7^{A}	6.21^{A}	6.73^{A}
	Y7	control	14.6^{cde}	13.6^{ab}	300^{d}	7.2^{de}	10.4^{ab}
		250	3.4^{jkl}	6.9^{efjhij}	60.4^{pq}	$4.4^{ghijklm}$	3.8^{hij}
		300	3.2^{kl}	5.9^{ij}	57.4^{r}	3.4^{klmn}	3^{jkl}
		Mean	7.07^{A}	8.8^{AB}	139.2^{AB}	5^{AB}	5.73^{AB}
	Y8	control	14^{de}	13.1^{b}	280^{e}	6.9^{def}	10.1^{abc}
		250	3.1^{kl}	6.4^{ghij}	58.2^{r}	4^{ijklmn}	3.5^{ijk}
		300	2.8^{l}	5.5^{jk}	54.2^{s}	3^{mn}	2.9^{jkl}
		Mean	6.63^{A}	8.33^{AB}	130.8^{AB}	4.63^{AB}	5.5 AB
	Y10	control	14.9^{bcd}	13.9^{ab}	315^{c}	7.7^{cd}	10.8^{a}
		250	3.7^{ljk}	7.2^{defghi}	65.3^{n}	4.9^{ghijkl}	4 ghij
		300	3.5^{jkl}	6.1^{hij}	60^{pq}	3.7^{jklmn}	3.5^{ijk}
		Mean	7.37^{A}	9.07^{AB}	146.7^{AB}	5.43^{A}	6.1^{A}
Arizona	A1	control	13^{e}	13.7^{ab}	108.4^{j}	5^{ghijk}	$6.^{e}$
		250	3.2^{kl}	3.9^{kl}	37.3^{t}	2.5^{no}	2^{kl}
		300	2.9^{l}	3^{kl}	35.7^{t}	1.3^{o}	1.8^{l}
		Mean	6.37^{A}	6.87^{B}	60.5^{B}	2.93^{B}	3.3^{B}
$LSD_{0.01}$		Treatment	1.61	1.63	1.63	1.62	1.61
		Varity	5.19	3.57	99.74	2.15	2.67

Different letters within a column indicate significant differences at the confidence level 99%

Discussion

All results are consistent with some studies that confirmed that it is necessary to achieve a hormonal balance between auxin and cytokinin to activate the synthesis of cell division proteins and to control plant regenerated from callus, the cytokinin/auxin balance ratio

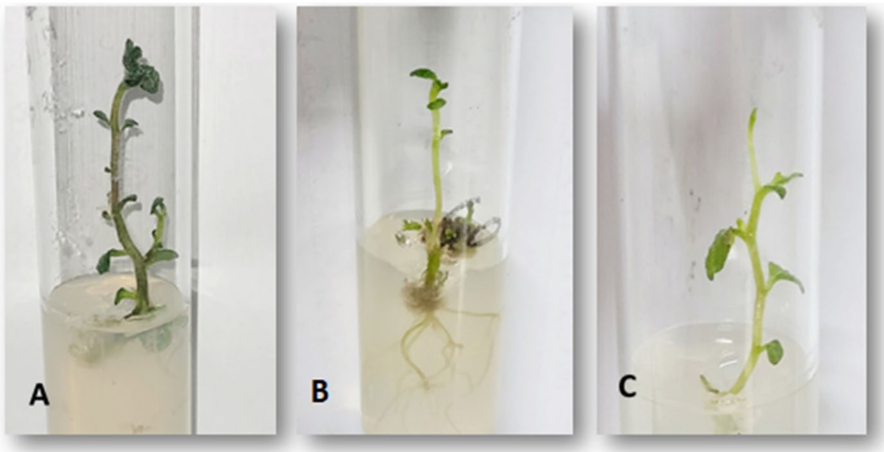

Fig. 2 Regenerated somaclones of Callus the three studied varieties are the most tolerant to drought stress (300 mM of sorbitol). **A** somaclone S6 of the Salvador variety (**B**) somaclone Y2 of the Yalas variety (**C**) somaclone A1 of the Arizona variety

must be greater than one [22]. The results showed that the most effective hormone to encourage the formation of shoots is BAP, and the reason is that cytokinins stop apical dominance and encourage the growth of lateral buds, stimulate division and lateral growth of them, and the formation and growth of shoots is enhanced by the presence of a combination of cytokinins and auxins in many species [23]. The results of this study are consistent with the results of [24] who showed the importance of adenine sulfate in callus regeneration, and this is because it stimulates cell division and participates in activating lateral buds, and stimulates the formation of lateral buds and encourages the formation of shoots from them. Its effectiveness also increases with the presence of cytokinins [25]. Many somaclones of selected callus of the three potato varieties were obtained, but they died due to their weakness, and many of them usually die in the early stages [26].

Effect of sorbitol concentration on growth indicators of callus-regenerated plants grown in the laboratory

Our results are consistent with those of Maryam and Kunwar [27, 28], as adding sorbitol to the growth medium led to a decrease in all studied growth indicators for five laboratory-grown potato varieties. The low concentration of sorbitol allows for an increase in the osmotic potential inside the plant cells; That is because the water potential becomes more negative compared to the surrounding medium, which leads to an increase in the rate of absorption of water and the mineral nutrients entering with the water current, which allows the formation of new plant cells, and the continued division and elongation of plant cells, as a result of maintaining the filling potential necessary to push the walls of the formed plant cells to elongate, so growth occurs, because growth is the result of irreversible division and elongation of plant cells, which leads to an increase in the wet weight of the callus and then an increase in its relative growth rate [29], and it is consistent with the results of [30], as the callus of the leaves was obtained on the highest renewal rate.

The decrease in the average root length and number, plant height, number of leaves and leaf area is attributed to the increase in the concentration of sorbitol in the growth medium, which leads to an increase in the turgor potential value in the growth medium, and then a decrease in the filling pressure inside the leaf cells, that causes a decrease in their elongation rate. The complete or total closure of the stomatal pores at higher turgor levels also disrupts the cooling effect of the transpiration water loss process, which leads to an increase in the temperature of the leaves, their burning and premature aging, which negatively affects the size of the green leaf surface effective in the photosynthesis process [31], which negatively affects the plant's metabolic efficiency and the amount of dry matter manufactured, so the growth rate of the plants and their development decreases [32]. As a response to drought, the plant tries to shorten its length and reduce the leaf area to reduce the rate of water loss by transpiration by reducing the leaf surface area, with the aim of maintaining the water content of the plant cells and the amount of water available in the soil for a period of time. Longer until plants can complete their life cycle, this mechanism helps avoid the harmful effects of water stress [33]. The researcher [34] also showed that potato varieties with deep and large roots were more tolerant to drought, as she found a positive and significant relationship between root length, leaf area of the plant, and tuber yield under drought conditions.

Conclusion

This study demonstrates that combining adenine sulfate with BAP significantly enhances callus regeneration efficiency under sorbitol-induced drought stress. The Yalas (Y3, Y2), Salvador (S3, S6), and Arizona (A1) strains emerged as superior performers, exhibiting robust drought tolerance across critical growth indices, a finding with direct implications for developing climate-resilient crops. These results establish callus culture technologies and in vitro cell selection as powerful tools for generating morphologically diverse, genetically enriched plant lines. The approach not only accelerates the breeding of drought-adapted varieties but also provides a scalable framework to address agricultural challenges posed by water scarcity.

Abbreviations

MS	Murashige and Skoog
IAA	Indole acetic acid
BAP	Benzylaminopurine
PEG	Polyethylene glycol
2,4-D	Dichlorophenoxyacetic acid
LSD	Least significant difference
AdSO4	Adenine sulfate

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Authors' contributions

L.L: Methodology, Software, Formal analysis, Writing original draft; S.Z: Data curation, Investigation, review; F.A: Data curation, Investigation, review; A.D: Writing, Data curation, review and editing manuscript.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author Abdulkarim Dakah on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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