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In vitro propagation and shootlets assessment for drought and salinity tolerance of traditional accessions of potato

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Abstract: Abiotic stresses, including heat, drought, and salinity, pose severe threats to agricultural yields, globally affecting essential crops like potatoes. The aim of this study is to establish an *in vitro* culture system for three potato accessions: Tal Amara 1 (TA1), Tal Amara 2 (TA2), and Tal Amara 3 (TA3) and to quantify their tolerance to temperature, drought, salinity, and combined stresses. The results demonstrated that MS0 (devoid of growth regulators) medium was the best for culture initiation, with a percentage of reactive meristems of 82.22%, whereas MS1 (0.35 mg L^{-1} Kin + 0.2 mg L^{-1} IAA + 0.1 mg L^{-1} GA_3) medium resulted in the highest multiplication rate of 5.5. The most heat tolerant accession was TA1, with shootlets lengths ranging from 2 cm to 4.4 cm at temperatures of 4°C and 38°C respectively. Concerning the effect of combined drought and temperature stresses, TA1 and TA3 showed tolerance to the different mannitol concentrations. Likewise, the most prominent accession in terms of combined salinity and temperature tolerance was TA2, with shootlets lengths of 3.2 cm (60 Mm NaCl, 22°C), 2.03 cm (60 Mm NaCl, 4°C) and 1.6 cm (60 Mm NaCl, 38°C).

1. Introduction

Belonging to the family of *Solanaceae*, potato (*Solanum tuberosum* L.) is considered as the most commonly cultivated tuber crop and is ranked the fourth most important food crop in the world, after wheat, rice and maize (Hussen, 2021). Potato is recognized as a crop of future and possesses a strong link in sustaining the global food security (Bakhsh *et al.*, 2023). It is cultivated in more than 158 countries worldwide (Muthoni and Shimelis, 2020). Considering its global importance, it is essential to

maintain its yield and sustain its productivity (Bakhsh *et al.*, 2023).

Potatoes grow perfectly in excellent climatic conditions with an optimal growth and yield at a temperature range of 15-20°C and ideally at a minimum rainfall 750-1000 mm. However, potato tuber growth is strongly affected by temperature fluctuations <5°C and >30°C. Temperatures above 30°C can negatively impact the potato production, especially by reducing, the tuber growth starch partitioning and dormancy and increasing disease incidences. Such increase in temperature can adversely reduce the growth performance, the yield of crops and thus the weight of tubers. Moreover, at shallow temperatures, potatoes become susceptible to frost damage and this in turn, causes reduced growth and damage to tubers (Mwakidoshi *et al.*, 2021). Besides, drought stress delays the emergence, slows the plant development, and reduces the plant mass weight as well as the tuber number, size and yield (Zaki and Radwan, 2022). Alternatively, salt stress negatively impacts crop yield by changing the plant metabolism and inducing substantial alterations in both biochemical and molecular processes (Abdelsalam *et al.*, 2021).

Seed production of potato is normally vegetatively propagated through the use of potatoes that have been previously propagated by harvesting and replanting the tubers in the field (Singh *et al.,* 2012; Shiwani *et al*., 2021). However, this conventional seed plant potato production has proved to be prone to pests and disease infestations where fungal, bacterial and viral disease agents can be transmitted easily through the tubers (Morais *et al.,* 2018; Shiwani *et al.*, 2021). In this event, plant tissue culture techniques and more specifically micropropagation offers a great potential to complement conventional breeding methodology for potato improvement and production (Singh *et al.*, 2012). Micropropagation is generally referred to the production of a large number of *in vitro* plants on a defined nutrient media under aseptic conditions within a limited space and time. This term includes the use of different techniques in potato production such as, shoot-tip culture, meristem culture, single-node culture and micro-tuberization (Shiwani *et al.*, 2021).

Potato production in Lebanon is very important for sustaining the food security from one side, and as a source of revenue in rural areas (Dalleh *et al.,* 2023). This strategic crop, covers around 19,000 ha in the Bekaa plain, with a production reaching 300,000 tonnes per year, and is considered as the greatest field crop tonnage in Lebanon (Choueiri *et al.,* 2017; Dalleh *et al.,* 2023). Seventy percentage of the Lebanese total potato cultivated area is concentrated mainly in the Bekaa valley at 900-1000 m above sea level (Dalleh *et al.*, 2023). Its cultivated for direct consumption and processing product with a part being exported. Despite its importance, a number of production constraints are hindering the full export potential of potato production in Lebanon such as, climate change, the use of low yielding varieties and the occurrence of bacterial diseases and viral infections (Choueiri *et al.*, 2017). Furthermore, potatoes during the summer are vulnerable to drought and salinity stresses due to insufficient irrigation water and temperature extremes (Verner *et al.*, 2018). Therefore, the need for identification of heat, drought and salinity tolerant potato genotypes for breeding by early selection is immense.

The aim of this present investigation is to establish a micropropagation system and screen *in vitro* initial explants of three potato accessions for temperature, drought and salinity tolerance: Tal Amara 1 (TA1), Tal Amara 2 (TA2) and Tal Amara 3 (TA3).

2. Materials and Methods

Plant material

This study for *in vitro* micropropagation was conducted at the Lebanese Agricultural Research Institute (LARI, Tal Amara Station). Clean tubers of three high yielding potato accessions namely Tal Amara 1 (TA1), Tal Amara 2 (TA2) and Tal Amara 3 (TA3) were kept under heating room conditions at 38°C for 30 days and used as a source for explants throughout the experiment. Four-weeks old healthy sprouts extracted from the three accessions, were surface sterilized using 70% ethanol for only 1 minute then dipped in 20% (v/v) sodium hypochlorite for 10 min. Following that, explants were rinsed four times with sterile distilled water for 20 min.

In vitro propagation

Meristem tips of the three potato accessions were dissected from apical and lateral buds of the disinfected sprouts. The size of the meristem ranged from 0.5 to 1 mm. The dissected meristems were placed on petri dishes containing three MS basal

media (Murashige and Skoog, 1962); "MS0" without growth regulators, "MS1" containing Kinetin (Kin) 0.35 mg L^{-1} in combination with Indole-3-acetic acid (IAA) 0.2 mg L^{-1} and Gibberellin (GA3) 0.1 mg L^{-1} and "MS2" containing 6-Benzylaminopurine (BAP) 1 mg L^{-1} and Gibberellin (GA3) 0.5 mg L^{-1} (Salem and Hassanein, 2017). The three MS media contained MS macroelements, MS microelements, MS vitamins and MS Ferrous with 30 g L^{-1} of sucrose and 7.6 g L^{-1} agar. All the prepared media were adjusted to a pH 5.7-5.75, then were autoclaved at 121°C and 0.103 MPa pressure for 20 minutes and cooled to 60°C. Each treatment was performed in ten replicas with 15 meristems per replica for testing. The cultures were then placed in the culture growth room under the following conditions (Temperature 22°C, Photoperiod 16:8 h light:dark, Relative Humidity 50%, Illumination of 3000-4000 lux). Thirty days later, the number of reactive meristems was recorded. Shoots derived from meristems were further multiplied by nodal cuttings. Nodal segments were cultured on fresh media using the same three prepared MS media (MS0, MS1 and MS2). Eight shoots, derived from nodal segment, per jar were inoculated and five replications for each treatment were conducted. Every 30 days, shoots were aseptically taken out and inoculated on the multiplication medium marking a new subculture, three subcultures were examined. For every subculture the following parameters were registered: the multiplication rate (Number of new shootlets/Number of initial shootlets) and shootlets height. The heights of shootlets were measured from their base to the tip.

Sanitary control

For each accession, 30 samples of shootlets of the third subculture were tested for six potato viruses, PVS, PVM, PVX, PVY, PVA and PLRV at the Plant Protection Laboratory (LARI Tal Amara) by using BIOREBA kit of double Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (Ren *et al.,* 2022).

Screening for stress tolerance

Plants from four subcultures on MS0 medium were used in the following experiments: heat, drought, salinity, and combined drought-heat and salinity-heat tolerance of the tested accessions were screened.

Heat tolerance assay. Individual nodal segments were cultured in test tubes each containing MS0 medium with 30 g L^{-1} sucrose. This *in vitro* assay employed three temperature treatments T1 (22°C control, Tal Amara Culture Room, 16:8 h photoperiod and 3000-4000 lux), T2 (4°C, Tal Amara Fridge Room) and T3 (38°C, Tal Amara Heating Room).

Drought adaptive screening. In order to assess drought tolerance, nodal segments were cultured on MS0 medium supplemented with mannitol. Four replications were conducted for each treatment in test tubes, with three nodal segments per replicate. Mannitol concentrations (C1: 403, C2: 807, C3: 1210 mM) were added to the media to reduce the water potential of the media to -1, -2 and -3 MPa. The water potential was calculated according to van't Hoff equation: $\pi=i\times M\times R\times T$, where π is the osmotic potential of the media, i is the van't Hoff factor for solute (mannitol), M is the molarity of the solution, R is the gas constant (=0.0083 MPa g/L^{-1} K^{-1}), and T is the temperature in Kelvin (Pant *et al.*, 2014). All subcultures were maintained under 22°C with 16:8 h photoperiod and 3000-4000 lux, as a light intensity.

Salinity tolerance evaluation. To screen for salt tolerance, nodal segments were grown in test tubes on an MS0 medium supplemented with various concentrations of NaCl (C1: 40 mM and C2: 60 mM) at 22°C for 3 weeks (Garramone *et al.*, 2023) with four replications per treatment and three nodal segments per replicate.

Combined drought-temperature stress. In order to induce a combined drought and heat stress, nodal segments were placed in test tubes on an MS0 medium containing the three different concentrations of mannitol mentioned above (C1: 403, C2: 807, C3: 1210 mM) at temperatures of 4 and 38°C for 3 weeks (Handayani and Watanabe, 2021) with four replications per treatment and three nodal segments per replicate.

Combined salt-temperature stress. Coupled treatments of salt and heat stresses were induced and single nodes of each accession were placed in test tubes on an MS0 medium supplemented with the two concentrations of NaCl mentioned above (C1: 40, and C2: 60 mM) at temperatures of 4 and 38°C for 3 weeks (Nahar *et al.,* 2022). Similarly, four replications per treatment were conducted and three nodal segments per replicate were used.

Statistical analysis

Four replications per accession were studied using a completely randomized design (CRD) and evaluated under each treatment for stress tolerance. Growth and morphological changes as a result of the

different stresses in culture were observed and recorded after 30 days of *in vitro* propagation and 3 weeks of stress tolerance assays. Data for various shoot and root characteristics were recorded based on the study of Albiski *et al.* (2012): Shootlets height (cm), number of shootlets leaves, number of shootlets roots, shootlets fresh and dry weights (oven dried plants at 70°C for 48h) (g) and plant water content (PWC%) = [(fresh weight - dry weight)/fresh weight] x 100.

All experimental results were expressed as mean values ± Standard Deviation. The data were tested for normal distribution using Shapiro-Wilk test, an analysis of variance (ANOVA) was calculated to assess accessions and treatments effects and interactions, and means were compared pairwise by Tukey tests at $p<0.05$ using the extension XLSTAT 2016 from Microsoft Excel (Addinsoft, 2016).

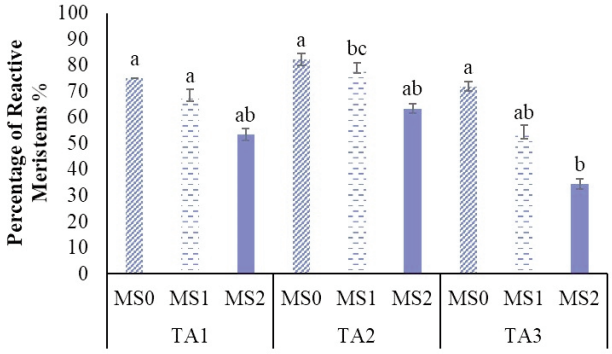

Fig. 1 - Percentage of reactive meristems of the three potato accessions TA1 (Tal Amara1), TA2 (Tal Amara2) and TA3 (Tal Amara3) during culture establishment after 30 days on the different MS media (MS0: 0 hormones, MS1: Kinetin 0.35 mg L^{-1}+ IAA 0.2 mg L^{-1} + GA3 0.1 mg L^{-1} and MS2: BAP: 1 mg L^{-1} + GA3 0.5 mg L^{-1}). Histograms surmounted by same letters are not significantly different ($p<0.05$) according to Tukeys' test.

3. Results and Discussion

In vitro propagation

Isolated meristems from the three potato accessions, TA1, TA2 and TA3, were subjected to three different media, MS0, MS1 and MS2. Among the three media, MS0 demonstrated a superior effect on meristem culture with a percentage of reactive meristems ranging between 71.83% to 82.22%, while on MS1 the percentage of reactive meristems ranged between 54.44% and 78%, and on MS2 between 34.44% and 63.33% (Fig. 1).

Among the three accessions, TA2 (82.22%) exhibited the best response when established in the control media. There were no significant differences observed in the number of reactive meristems on MS0 and MS2 among the accessions (except for TA3). These findings are similar to those reported by Dalleh *et al.* (2023) where the highest percentage of reactive meristems in Spunta variety occurred on an MS hormone-free media. Xhulaj and Gixhari (2018) also reported that potato explants established on MS media supplemented with phytohormones resulted in high proliferation rates which is consistent to our results, where MS1 and MS2 supplemented with hormones also yielded high rates of reactive meristems, reflecting the importance of using Murashige and Skoog medium during the establishment phase of potato.

Established shootlets of TA1, TA2 and TA3 were transferred onto the three fresh MS media (MS0, MS1 and MS2) previously utilized for the cultures' establishment. Detailed data on the multiplication rate and shootlets' height was recorded over three subcultures as presented in Table 1. The highest multiplication rate (8 shootlets) was observed with TA1 using MS1 medium while the lowest (2.27 shootlets) was observed with TA3 using MS2 medium along the 1st subculture. However, regarding the shootlets height, TA2 demonstrated the highest shootlets height on MS1 (8.85 cm) at the 3rd subculture, while the lowest height (1.94 cm) was also observed by TA2 at the 1st subculture on MS2 (Fig. 2). Besides, MS1 resulted in the highest multiplication rates among the three accessions during the three subcultures, and achieved the highest shootlet height during the first and third subcultures. These results are in accordance with Dalleh *et al.* (2023) where the highest number of shootlets per plant was obtained on a medium containing 0.4 mg L^{-1} Kin, 0.5 mg L^{-1} GA3 and 0.5 mg L^{-1} IBA for the Spunta potato variety. Emaraa *et al.* (2017) also reported that the highest multiplication rate of the Lady-Rosetta potato variety was obtained on an MS media supplemented with Kin 0.2 mg L^{-1} in combination with NAA 0.2 mg L^{-1}. On the other hand, Xhulaj and Gixhari (2018) demonstrated the importance of combining GA3 and BAP to improve the number of shoots of Bergerac potato cultivar. Similarly, Dessoky *et al.* (2016) revealed that MS medium containing 3 mg L^{-1} GA3 and 0.1 mg L^{-1} Kin resulted in the highest multiplication rate in Diamant potato cultivar.

Table 1 - Effect of the three media (MS0: 0 hormones, MS1: Kinetin 0.35 mg L^{-1} + IAA 0.2 mg L^{-1} + GA3 0.1 mg L^{-1} and MS2:BAP:1 mg L^{-1}+ GA3 0.5 mg L^{-1}) on the multiplication rate and shootlets height measured during *in vitro* propagation of the three potato accessions; Tal Amara 1 (TA1), Tal Amara 2 (TA2) and Tal Amara 3 (TA3) along 3 subcultures

Treatments	Multiplication rate			Shootlets height (cm)		
	TA1	TA2	TA3	TA1	TA2	TA3
Subculture 1						
MS0	2.53±0.66 d	2.64±0.78 d	2.33±0.65 d	4.19±1.82 abc	2.62±1.61 d	2.87±1.21 cd
MS1	8.00±0.63 a	3.46±0.51 c	6.20±0.78 b	5.75±1.48 a	2.60±1.04 d	3.18±0.96 bcd
MS2	5.85±0.89 b	2.38±0.50 d	2.27±0.46 d	5.02±0.52 ab	1.94±0.97 d	2.50±0.77 d
Subculture 2						
MS0	3.97±0.75 de	3.37±0.49 f	3.43±0.50 ef	7.80±1.48 a	6.06±1.58 b	6.62±1.31 b
MS1	4.91±0.73 bc	5.37±0.50 ab	5.55±0.51 a	4.37±1.68 c	4.43±1.76 c	3.87±0.95 c
MS2	3.88±0.76 def	4.68±0.74 c	4.33±0.49 cd	2.55±0.75 d	2.31±1.09 d	2.20±0.74 d
Subculture 3						
MS0	3.60±0.50 d	3.66±0.66 d	4.26±0.45 c	6.87±1.20 b	4.67±1.25 cde	5.83±1.45 bcd
MS1	4.91±0.84 ab	5.40±0.50 a	4.83±0.78 abc	8.57±2.68 a	8.85±1.81 a	4.88±1.91 cde
MS2	4.33±0.48 bc	3.38±0.50 d	3.42±0.51 d	6.40±2.31 bc	4.32±1.56 de	3.78±1.03 e

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

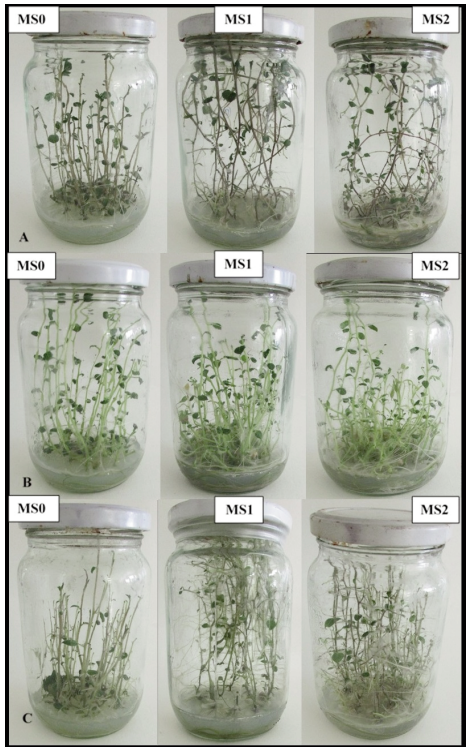

Fig. 2 - Shootlets proliferation in A: Tal Amara1, B: Tal Amara2 and C: Tal Amara3 accessions of potato cultured on different MS media (MS0: 0 hormones, MS1: Kinetin 0.35 mg L^{-1}+IAA 0.2 mg L^{-1}+ GA3 0.1mg L^{-1} and MS2: BAP: 1 mg L^{-1}+ GA3 0.5 mg L^{-1}).

Effect of accession

Evaluating the effect of potato accession on multiplication rate and shootlets' height, the results are depicted in figure 3. When combining subcultures and media together, no statistically significant distinctions were noted in multiplication rate and shootlets' height across the three tested potato accesssions (TA1, TA2 and TA3). Tal Amara 1 showed the highest multiplication rate (4.66) followed by Tal Amara 3 and Tal Amara 2. TA1 also exhibited the highest average shootlets' height at 4.93 cm, followed by TA2 at 3.64 cm and TA3 at 3.17 cm.

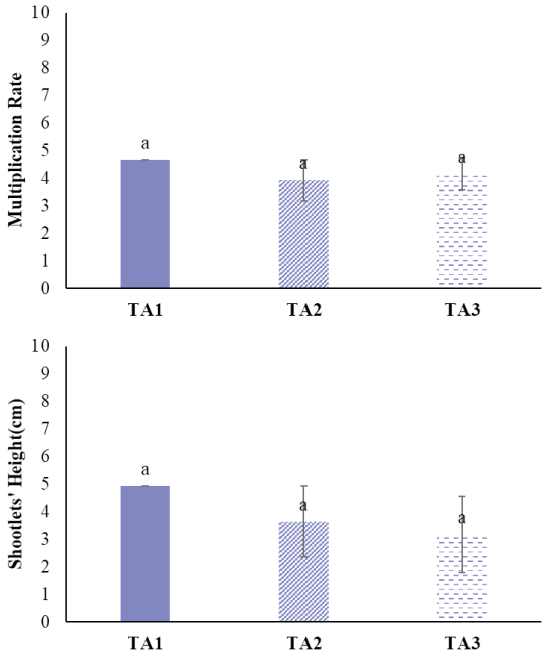

Fig. 3 - Effect of potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) on the multiplication rate and shootlets height of plants; media and subcultures are combined. same letters indicate not significantly different ($p<0.05$) according to Tukeys' test.

These results contradict with Asnake *et al.* (2023) and Tessema *et al.* (2021) who reported that variety significantly influence the growth parameters of potato, and this discrepancy could be correlated to the duration between subcultures and nutrient media used

Effect of culture media

The effect of culture medium on the multiplication rate and height of potato shootlets is illustrated in figure 4. When combining accessions and subcultures, noteworthy is the absence of any significant differences in shootlets' height among the three tested media. However, the control medium (MS0) yielded the greatest shootlets' height at 5.28 cm, succeeded by MS1 at 5.16 cm and MS2 at 3.44 cm. Ebad *et al.* (2015) reported that MS medium supplemented with vitamins without exogenous plant growth regulators can be used for mass propagation of potatoes. The highest multiplication rate was observed with MS1 medium (5.4) followed by MS2 and control (MS0). Hajare *et al.* (2021) also reported that the highest multiplication rate was obtained in MS medium containing Kinetin (2.5 mg L^{-1}). Moreover, Emaraa *et al.* (2017) revealed that the highest multiplication rate was noticed on MS media supplemented with 0.2 mg L^{-1} NAA together with 0.2 mg L^{-1} Kin while, Othman *et al.* (2016) reported that medium augmented with 2.0 mg L^{-1} BA and 0.250 mg L^{-1} NAA was the most favorable for the multiplication

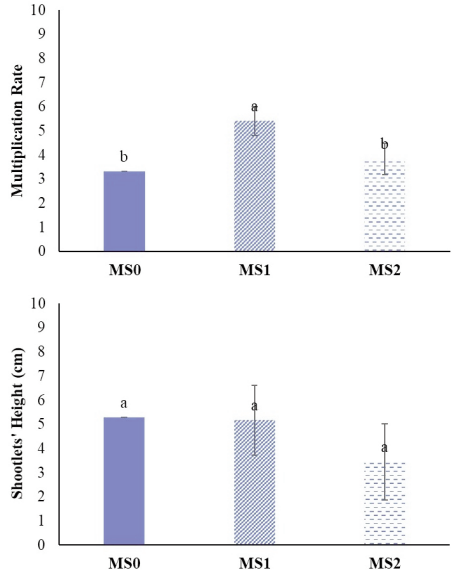

Fig. 4 - Effect of culture media (MS0: Control, MS1: Kinetin 0.35 mg L^{-1} +IAA 0.2 mg L^{-1}+ GA_3 0.1 mg L^{-1} and MS2: BAP: 1 mg L^{-1}+ GA3 0.5 mg L^{-1}) on multiplication rate and shootlets height of potato plants; accessions and subcultures are combined.

of Lady Balfour and Bellini cultivars. This thus elucidates the importance of both Kinetin BA and BAP in the multiplication of potatoes.

Effect of subculture

Sequential to the systematic exploration of factors influencing shootlets characteristics, this study transitions to investigate the effect of subculture on multiplication rate and shootlets' height. The experimental findings, regardless of accessions, are graphically represented in figure 5. By combining accessions and media, no statistically significant difference of multiplication rate was observed between subcultures. Subculture 3, however, showed the highest multiplication rate (5.19). A noticeable upward trend in shootlets' height was evident with successive subcultures, reaching a significant value of 6.01 cm at the 3rd subculture. These findings align with the results of Muthoni *et al.* (2014) where there was an increase in the multiplication rate of all potato cultivars with subcultures. He also noted that subculture 3, gave more cuttings than the first two.

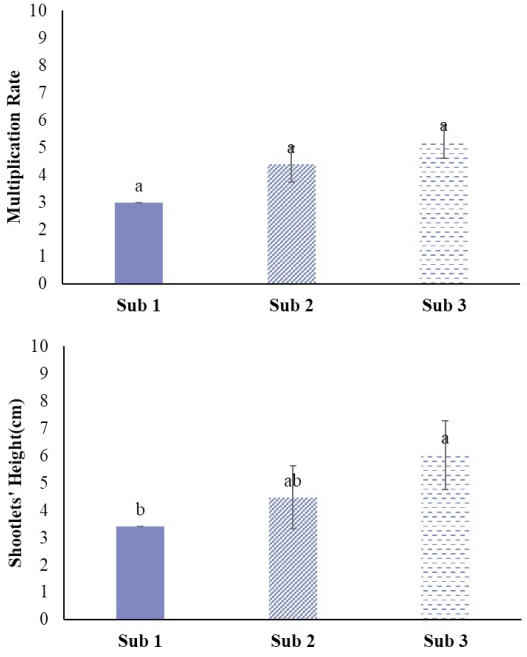

Fig. 5 - Effect of subcultures (sub1, sub2 and sub3) on multiplication rate and shootlets height of potato plants; media and accessions are combined. same letters indicate not significantly different ($p<0.05$) according to Tukeys' test.

Sanitary control

A total of ninety plant samples belonging to the three potato accessions (TA1, TA2 and TA3) were

tested for potato viruses; PVS, PVM, PVX, PVY, PVA and PLRV; using DAS-ELISA. It was shown that all the *in vitro* shootlets were 100% free from the 6 tested viruses, and no significant differences were observed between the three accessions. These findings are consistent with several studies showing that the meristem culture method is effective in producing disease-free plants in potato (Spunta) and other crops (Pradhan *et al.*, 2016; Dalleh *et al.*, 2023). Additionally, the size of the meristem explant is important for the efficient elimination of viruses (Azad *et al.,* 2020).

Temperature treatments

The *in vitro* effect of temperature stress on the growth and development of three potato accessions revealed that high and low temperatures had a significant impact on the evaluated growth parameters, such as shootlets height, the leaf and root number, and the plant water content percentage (Table 2). Low temperature (4°C) and high temperature (38°C) treatments indicated significant low values of shootlets height ranging from 1.6 (TA3) to 2 cm (TA1) at 4°C and 2.50 (TA2) to 4.40 cm (TA1) at 38°C when compared to the control treatment (22°C, ranging from 4.9 for TA2 to 10.9 cm for TA1).

Shootlets at the control treatment (22°C), presented a greater number of leaves (ranging between 6 leaves and 11.5) compared to those exposed at high temperature (from 4 to 7.5 leaves) and low temperature (ranging 2 and 4.5 leaves), with the exception for the accession TA3, where the high temperature showed more leaves (7.5) than the control treatment (6.0).

Similarly, plants subjected to the control treatment (22°C) and high temperature treatment developed more roots per shootlet ranging from 6 roots (TA3) to 13.5 roots (TA2) and 5.5 roots (TA2) to 8.5 roots (TA1) respectively as compared to the low temperature which indicated a number of roots ranging from 2.5 roots (TA3) to 4.5 roots (TA1). On the other hand, no significant percentage of the plant water content was recorded. It is fluctuated between 91.07% and 94.17% at high temperature, between 91.67% and 92.16% at low temperature, compared to the control (93.41% to 94.09%).

These observations demonstrate that the greatest tolerance under both high and low temperature conditions was exhibited by TA1 potato accession, with TA3 showing the next highest tolerance at high temperature. The distinct responses of potato accessions to varying temperatures indicate genotype-specific differences in growth parameters, consistent with studies reporting enhanced growth at higher temperatures (Mohamed *et al*., 2016). The reduction in nutrient absorption through the roots influence the shoot development under suboptimal temperature conditions, resulting in an immediate impact of temperature on shoot growth. Furthermore, leaf growth reacts promptly with various environmental stresses; including low and high temperatures; which could generally explain the decline in leaf number at low temperature associated with the hindrance of leaf initiation rates, leading to a direct reduction in both leaf cell division and elongation. The overall root system encountered likewise a significant decrease under low temperatures compared to high temperatures

Table 2 - Effect of *in vitro* heat stress on shootlets height, number of leaves, number of roots and plant water content percentage of 3 potato accession (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3)

Accession	Temperature (°C)		Shootlets height (cm)	No of leaves	No of roots	Plant water content PWC (%)
TA1	T0	22 (Control)	10.90±0.28 a	11.00±0.00 a	9.50±0.70 ab	93.41±0.08 a
	T1	4	2.00±0.70 c	4.50±0.70 b	4.50±2.12 cd	92.16±1.93 a
	T2	38	4.40±0.14 b	7.00±2.82 ab	8.50±0.70 bc	94.17±1.79 a
TA2	T0	22 (Control)	4.90±0.14 b	11.50±0.70 a	13.50±0.70 a	94.09±1.48 a
	T1	4	1.75±0.07 c	3.50±0.70 b	3.50±0.70 d	91.67±0.82 a
	T2	38	2.50±0.70 c	4.00±0.00 b	5.50±0.70 bcd	91.07±1.56 b
TA3	T0	22 (Control)	5.20±0.35 b	6.00±1.41 ab	6.00±0.00 bcd	94.09±0.59 a
	T1	4	1.60±0.14 c	2.00±1.41 b	2.50±0.70 d	92.02±0.96 a
	T2	38	4.25±0.35 b	7.50±2.12 ab	6.50±2.12 bcd	93.08±0.28 a

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

attributed to the cessation of all root growth and developmental parameters. Similarly, Chen *et al.* (2024) described the effect of low temperature on hindering the growth of potatoes, where a temperature below 7°C can cease the seedlings growth, which illustrates the low shootlets height, low number of leaves and roots observed at 4°C. Besides, at elevated temperatures, plants tend to close their stomata to minimize the water loss (Marchin *et al.*, 2021; Reddy *et al.*, 2021). This phenomenon elucidates the relatively high percentage of plant water content observed in TA1 and TA3 at high temperatures.

Drought treatments

The response of potato accessions to drought stress, indicated the absence of significant difference in shootlets' height, leaf number, and root development as mannitol concentrations increased across the three tested temperatures (22, 4, and 38°C) (Table 3, Table 4 and Table 5). However, a significant reduction in plant water content percentage was observed with escalating mannitol concentrations at the tested temperatures.

At 22°C the highest shootlets' height (1.95 cm), leaf (4) and root number (3), and plant water content percentage (81.06%) were observed by TA2 at the low mannitol concentration (C1: 403 mM), followed by TA1 and then TA3 (Table 3). However, with the increase in mannitol concentrations reaching C3 of 1210 mM, TA3 presented a stabilized response of 0.90 cm height, average number of leaves and roots of 1 and 59.55% plant water content. TA1 and TA2 on other hand didn't develop any roots at C3, due to

Table 3 - Effect of *in vitro* drought stress on shootlets height, number of leaves, number of roots and plant water content of 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at normal laboratory temperature (22±2°C)

Accession	22°C					
	Mannitol concentration (mM)		Shootlets height (cm)	Number of leaves	Number of roots	Plant water content PWC%
TA1	C1	403	1.53±0.11 abcd	1.66±0.57 a	2.00±0.00 ab	76.14±1.69 abcde
	C2	807	1.10±0.00 bcd	1.00±0.00 a	1.66±1.52 ab	70.87±2.67 defgh
	C3	1210	1.00±0.42 ab	1.00±0.70 a	0.00±0.00 b	59.50±2.36 j
TA2	C1	403	1.95±0.07 cd	4.00±1.41 a	3.00±1.41 ab	81.06±1.77 ab
	C2	807	1.60±0.07 abcd	1.50±0.00 a	0.00±0.00 b	69.75±0.17 efgh
	C3	1210	1.20±0.14 bcd	1.00±0.00 a	0.00±0.00 b	60.04±2.12 j
TA3	C1	403	1.20±0.14 bcd	1.00±0.00 a	2.00±1.41 ab	75.92±0.49 abcde
	C2	807	1.10±0.21 abc	1.00±0.00 a	1.00±1.41 ab	67.14±0.05 ghi
	C3	1210	0.90±0.26 abcd	1.00±0.00 a	1.00±0.57 ab	59.55±1.24 j

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

Table 4 - Effect of in *vitro* drought stress on shootlets height, number of leaves, number of roots and plant water content of 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at low temperature (4±2°C)

Accession	4 °C					
	Mannitol concentration (mM)		Shootlets height (cm)	Number of leaves	Number of roots	Plant water content PWC %
TA1	C1	403	1.60±0.14 abcd3	2.00±0.00 a	2.00±0.00 ab	81.56±1.35 ab
	C2	807	1.60±0.07 abcd	1.50±0.70 a	2.00±0.00 ab	74.10±0.50 cdef
	C3	1210	1.20±0.07 bcd	1.00±0.00 a	1.00±1.41 ab	64.75±0.88 hij
TA2	C1	403	1.75±0.21 ab	3.00±1.41 a	0.00±0.00 b	82.35±0.57 a
	C2	807	1.50±0.14 abcd	3.50±0.70 a	0.00±0.00 b	68.81±0.39 efgh
	C3	1210	1.35±0.21 abcd	2.50±0.70 a	0.00±0.00 b	67.83±4.24 fgh
TA3	C1	403	1.10±0.05 bcd	1.00±0.00 a	1.66±0.57 ab	81.41±0.66 ab
	C2	807	1.10±0.63 bcd	1.50±0.70 a	1.50±0.70 ab	72.89±0.61 defg
	C3	1210	0.90±0.28 d	1.50±0.70 a	1.50±0.70 ab	59.74±0.03 j

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

impact of elevated drought on the inhibition of the key physiological and biochemical processes (Gervais *et al.,* 2021).

Transitioning to low and high temperature conditions at 4°C and 38°C, the trend observed at 22°C persisted (Table 4, 5). TA2 continued to exhibit the highest tested parameters, except for the number of roots at low temperature and high concentration of mannitol. Concurrently, TA1 and TA3 exhibited a constant response in the measured parameters with the increase in concentration of mannitol. This indicates that the shootlets height, number of leaves, root development, and plant water content percentage were significantly affected by drought stress for all three potato accessions. The results are consistent with other studies that show a decrease in Spunta shoot length and roots at mannitol concentrations of 200 mM and above (Sattar *et al.*, 2021). The concurrent interplay between mannitol and temperature stresses, contributed to the enhancement of the tolerance mechanisms in potato plants, which is in accordance with other findings that demonstrated the positive impact of combined stresses on plants' tolerance (Rafique *et al.,* 2019).

Notably, the drop in the plant water content, that appeared in response to increasing mannitol concentrations, highlights the water stress that is simulated when an osmotic agent is introduced to the growth medium. This acts at reducing the availability of nutrients that are crucial to plant growth and hinders the absorption of water through the roots (Tican *et al.*, 2021). Drought stress additionally reduce the number, mass, and growth of roots, which in turn limits the availability of nutrients and water for the plant shoots (Jafari *et al.*, 2019).

Salinity treatments

At 22°C, TA2 exhibited superior performance in terms of plant water content (%PWC), shootlets'

Table 5 - Effect of *in vitro* drought stress on shootlets height, number of leaves, number of roots and plant water content of 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at high temperature (38±2°C)

Accession	38 °C					
	Mannitol concentration (mM)		Shootlets height (cm)	Number of leaves	Number of roots	Plant water content PWC%
TA1	C1	403	1.65±0.07 abc	2.50±0.70 a	2.00±0.00 ab	76.80±2.00 abcd
	C2	807	1.33±0.07 abcd	1.50±0.70 a	2.00±0.00 ab	70.18±1.61 defgh
	C3	1210	1.20±0.00 bcd	1.50±0.70 a	2.50±0.70 ab	64.95±2.42 hij
TA2	C1	403	1.96±0.25 a	4.00±1.00 a	3.66±1.15 a	79.30±0.81 abc
	C2	807	1.95±0.14 a	3.50±2.12 a	0.00±0.00 b	69.46±1.19 efgh
	C3	1210	1.65±0.07 abc	2.50±0.70 a	0.00±0.00 b	65.33±0.47 hij
TA3	C1	403	1.75±0.07 ab	1.00±0.00 a	2.00±0.00 ab	75.26±0.96 bcde
	C2	807	1.20±0.00 bcd	1.50±0.70 a	2.00±0.00 ab	72.53±0.80 cdefg
	C3	1210	1.10±0.28 bcd	2.00±0.00 a	2.00±0.00 ab	60.75±0.12 ij

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

Table 6 - Effect of *in vitro* salinity stress on shootlets' height, number of leaves, number of roots and plant water content of 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at normal laboratory temperature (22±2°C)

Accession	22°C					
	Concentration of NaCl (mM)		Shootlets height (cm)	Number of leaves	Number of roots	PWC%
TA1	C1	40	2.50±0.14 abcd	9.50±0.70 abc	6.00±0.00 abc	88.67±0.30 ab
	C2	60	2.00±0.14 cde	4.50±2.12 abcde	2.50±2.12 cde	87.06±1.50 ab
TA2	C1	40	3.45±0.35 a	11.50±2.12 a	7.00±1.41 ab	89.85±0.20 a
	C2	60	3.2±0.07 cde	8.50±3.53 abcd	4.50±0.70 abcd	88.53±0.18 ab
TA3	C1	40	3.40±0.28 ab	9.00±0.00 abc	6.00±1.41 abc	88.82±2.26 ab
	C2	60	2.70±0.00 abc	8.50±3.53 abcd	3.50±0.00 bcde	88.28±0.73 ab

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

height, and the number of leaves and roots at both NaCl concentrations (C1 and C2)(Table 6).

Specifically, with the increase in NaCl concentration a reduction was experienced in growth, where at C2, TA2 achieved the highest %PWC at approximately 88.53%, surpassing TA3 (88.28%) and TA1 (87.06%), shootlets height of 3.2 cm with an average number of 8.5 leaves and 4.5 roots.

As the temperature dropped to 4°C , TA2 and TA3 showed a stabilized effect to the increase in NaCl concentrations (Table 7). Notably, TA2 continued to have the maximum numbers of leaves and roots as well as the highest shootlets height of 2.03 cm at C2.

On the other hand, TA1 reported the greatest PWC% at C2 (87.80%), followed by TA2 (87.05%) and TA3 (85.07%), the highest shootlets height (1.6 cm), number of leaves (5) and roots (3), when subjected to a temperature of 38°C (Table 8).

The exposure to salinity stress resulted in reductions in shootlets height, the number of leaves and roots, and the percentage of plant water content (% PWC). These reductions could be correlated to the modifications induced in terms of balance, water status, mineral nutrition as well as efficiency of photosynthesis (Abdelsalam *et al.*, 2021). These findings also align with prior research, indicating that Spunta exhibited growth variations with the escalating NaCl concentrations (40 to 80 to 120 mM), and the growth of various potato cultivars was affected, showing a decrease in both shoot and root length (Khenifi *et al.*, 2011). Notably, TA2 demonstrated resilience to salinity stress at both NaCl concentrations at 22°C. Under low-temperature conditions, TA3 exhibited the highest water content preservation at the elevated salt concentration, while TA2 displayed notable tolerance in terms of shootlets' height and the number of leaves and roots compared to other accessions. Nevertheless, at higher temperature and salt concentrations, TA1 maintained the water content whereas TA2 displayed highest values in leaves, roots, and height. These results demonstrate the tolerance displayed by TA2

Table 7 - Effect of *in vitro* salinity stress on shootlets' height, number of leaves, number of roots and plant water content of 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at low temperature (4±2°C)

Accession	4°C					
	Concentration of NaCl (mM)		Shootlets height (cm)	Number of leaves	Number of roots	PWC%
TA1	C1	40	1.60±0.14 de	1.00±0.00 e	0.00±0.00 e	90.92±0.16 a
	C2	60	1.50±0.14 de	1.00±0.00 e	0.00±0.00 e	87.94±0.51 ab
TA2	C1	40	2.35±0.21 cd	2.50±0.70 cde	2.00±0.00 cde	89.30±0.85 ab
	C2	60	2.03±0.05 cde	2.00±0.00 cde	1.33±1.15 de	88.53±1.48 ab
TA3	C1	40	1.55±0.07 de	1.50±0.70 de	1.50±0.70 de	89.09±0.36 ab
	C2	60	1.50±0.49 de	1.33±0.70 de	1.00±0.70 e	88.59±0.24 ab

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

Table 8 - Effect of *in vitro* salinity stress on shootlets height, number of leaves, number of roots and plant water content 3 potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) at high temperature (38±2°C)

Accession	38°C					
	Concentration of NaCl mM		Shootlets height (cm)	Number of leaves	Number of roots	PWC%
TA1	C1	40	2.45±0.35 bcd	7.50±0.70 abcde	3.50±0.70 bcde	88.75±1.46 ab
	C2	60	1.30±0.07 e	3.00±0.70 cde	2.00±0.00 cde	87.80±0.35 ab
TA2	C1	40	2.20±0.00 cde	7.50±0.70 abcde	2.00±0.00 cde	89.01±0.51 ab
	C2	60	1.60±0.14 de	5.00±0.00 abcde	3.00±1.41 bcde	87.05±1.82 ab
TA3	C1	40	1.65±0.07 de	1.50±0.70 de	2.00±1.41 cde	86.48±2.03 ab
	C2	60	1.35±0.63 e	3.50±0.70 bcde	2.00±0.00 cde	85.07±1.80 b

Data are expressed as the mean of the determinations ± S.D. Means followed by the same letter within a column are not significantly different ($p<0.05$).

when subjected to single salinity stress or combination of salinity-heat stresses.

4. Conclusions

In conclusion, medium devoid of hormones (MS0) was an optimal medium for initiation of potato. MS1 demonstrated efficacy in achieving substantial multiplication rates. Importantly, TA1 showed the best tolerance to high and low temperature treatments. TA2 exhibited tolerance to low drought stress (low concentration of mannitol), while the results of TA1 and TA3 indicated more stability in their tolerance at different concentrations of mannitol. TA2 also showed remarkable resilience under salinity and combined salinity-temperature stresses followed by TA3. These results highlight that TA1 accession is more relevant during temperature stress with no humidity stress, while during low drought stress TA2 performs the best and TA3 and TA1 show constant response when subjected to increasing drought stress. Moreover, TA2 is well suited in conditions of salinity and temperature stresses. This study emphasizes the importance of selecting resilient potato accessions to govern sustainable seed production, focusing on the interrelations that exist between abiotic stresses and the growth factors of potato, and underscores the importance of ongoing research to integrate laboratory findings with practical field assessments.

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