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Interaction between sowing date and mulching is important for better growth and productivity of carrot in a weather-vulnerable area of Ethiopia

OPEN ACCESS

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Abstract: Inadequate agronomic practices and unfavorable weather conditions often hinder carrot cultivation. Therefore, this experiment evaluated the effects of sowing date and mulching on the growth and yield of carrots during the 2023/2024 main cropping season at Kersole, Legambo District. The experiment involved three sowing dates (early, mid, and late in July) and four mulching materials (no mulch, sawdust, straw, and dried grass), utilizing a Randomized Completely Block Design (RCBD) with three replications. Except main effects of sowing date, and interaction effects on days to 50% emergence and root diameter, all other parameters were significantly ($P \leq 0.05$) affected. Early sowing combined with either sawdust or dried grass mulch resulted in the highest marketable root yields respectively. Late sowing without mulch and with dried grass mulch showed the lowest marketable root yield and minimum weed density respectively. Early sowing with sawdust mulch also provided the highest net benefit, while early sowing with dried grass mulch exhibited the highest marginal rate of return. Therefore, early sowing with dried grass mulch can be recommended for carrot cultivation in study areas and similar agroecologies. However, for optimal results, it is necessary to carry out the experiment using several mulching materials and various sowing dates across seasons and locations.

1. Introduction

Carrot (*Daucus carota* L.) is a commonly important root crop of the *Apiaceae* family, widely distributed worldwide. Carrots are herbaceous dicotyledonous plants that grow upright, reaching a height of 20-50 cm when mature. The fleshy taproot is its primary edible part, which typically

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exhibits a straight, conical, and cylindrical shape. It was originally wild in different parts of Asia and Europe. It was primarily domesticated in rich Afghanistan, considered the first center of origin, and Turkey is believed to be the second center of origin. From these centers of diversity, carrots gradually spread across Europe, the Mediterranean, and numerous countries in Asia. Over time, it was cultivated and introduced to local wild varieties across the globe (Stolarczyk and Janick, 2018). Carrot roots are highly valued for their abundance of carotenoids, which serve as precursors to vitamin A (Tabor and Yesuf, 2012). Additionally, carrots contain flavonoids, vitamins, and minerals, making them a nutritious crop that contributes to overall health and well-being (Zeleke and Derso, 2015). China is the leading global producer, with Europe emerging as the rapidly advancing carrot market. Notably, North America, particularly the USA and Canada, boasts the most significant shares in the carrot markets. As of 2020, the world consumed 46.3 million metric tonnes of turnips and carrots globally, according to FAOSTAT 2021 data.

Ethiopia has a relatively low production compared to the global average. Its production has not been adequately exploited as it faces several constraints such as limited research activities on the topic, unfavorable weather conditions, and poor agronomic practices such as unplanned sowing dates, lack of mulching practices, and improper weeding. In the study area, the quality of carrot roots is also compromised due to suboptimal agronomic practices, thus discouraging farmers from engaging in carrot production. Typically, farmers in the study area sow carrots on bare beds without applying mulches. Similarly, they often sow carrots in late July, thus leading to poor seed germination, inadequate growth, and development as well as exposure to severe winter conditions (soil moisture deficit and lack of rainfall) towards the end of summer. The research by Mengistu (2009) reveals that the pre-termination of rainfall, occurring during critical developmental stages of the crop, especially the root initiation stage, results in both quantitative and qualitative reductions in yield. Farmers relying on subsistence agriculture within Legambo District have faced recurrent droughts and famines resulting from severe weather occurrences associated with climate change, such as erratic rainfall, frost threats, and hailstorm flooding (Cafer and Rikoon, 2017).

Within subsistence agriculture, the occurrences and regularities of climate extremes and variabilities pose significant challenges comparable to average annual shifts. The magnitude and implications of climate variability are not adequately examined in Ethiopia. For subsistence agriculture, occurrences and frequencies of climate extremes and variabilities are equally affecting as of mean annual changes. However, despite its climatic constraints, the region still holds promise as a viable location for cultivating carrots, especially through strategic adjustments in sowing dates and the application of mulching techniques. The importance of adjusting the cropping schedule is appropriate to adapt a weather and climate variations in the particular region (Desta *et al*., 2020). The timing of sowing is essential as it aligns with favorable climatic conditions and has been demonstrated to impact the growth and yield of carrots (Gagopale, 2019). Moreover, the use of mulch helps to regulate temperature extremes (Rajasekar *et al*., 2020). Furthermore, cultivators must consider the appropriate sowing date and mulching as one of the most significant factors to maximize productivity and quality, minimize weed occurrence, and maintain soil health. Consequently, this study was conducted to evaluate the effect of sowing date and mulching on the growth and yield of carrots in Kersole, Legambo district.

2. Materials and Methods

Description of the study area

The experiment was conducted at Kersole Kebele, Legambo district of agriculture and temperate fruit nursery site, South Wollo Zone, Ethiopia in the 2023/2024 main cropping season (from July to November). The site is located at 10°51’ N and 39°11’ E (Fig. 1) with an altitude of 2800 meters above sea level (m a.s.l.). The area is situated 501 kilometers north of Addis Ababa and 372 kilometers east of

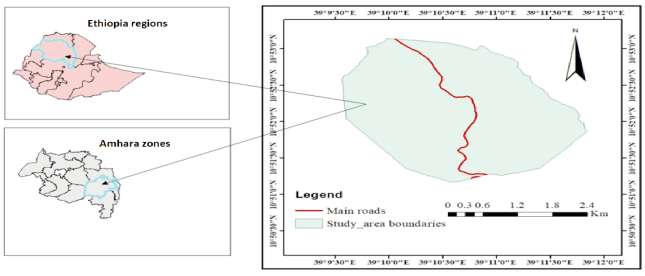

Fig. 1 - Map of the study area.

Bahir Dar. The area experiences an annual rainfall ranging from 700 to 1300 millimeters. The site has an average minimum temperature of 3°C and an average maximum temperature of 18°C. The predominant soil type in the area is clay soil, characterized by a pH of 5.57 (Regassa *et al.*, 2023) (Fig. 2). The region is predominantly characterized by its rolling terrain, (Regassa *et al.*, 2023). Approximately 68.54% of the district features a temperate (Dega) agroecology, with the next largest portion comprising alpine (Wurch) landscapes at 29.53%. A small percentage of 1.93% and 0.0016% of the district area is covered by subtropical (Woinadega) and tropical (Qolla) agroecology, respectively.

Treatments and experimental design

The experiment contained two factors (sowing date x mulching material). Sowing dates comprised three levels (Early - July = 10th July (S1); Mid - July = 20th July (S2) and Late - July = 30th July (S3)). Four levels of mulching materials were: no mulch (M0), sawdust (M1), straw (M2) (Fig. 3), and dried grass (M3). The experiment arrangement used a Randomized Completely Block Design (RCBD) in three replicates. The whole experimental area was 218.3 m^2 (29.5 m x 7.4 m) in length and width respectively. This area was divided into three blocks, and each block was further subdivided into 12 plots. As a result, there were a total of 36 unit plots. The treatments were assigned randomly within each plot of the block. Each unit plot had a net size of 3.6 m^2 (2 m x 1.8 m) which consisted of 9 rows and 40 plants per row. The distance between adjacent blocks was 1.0 meters, while the distance between plots within a block was 0.5 meters.

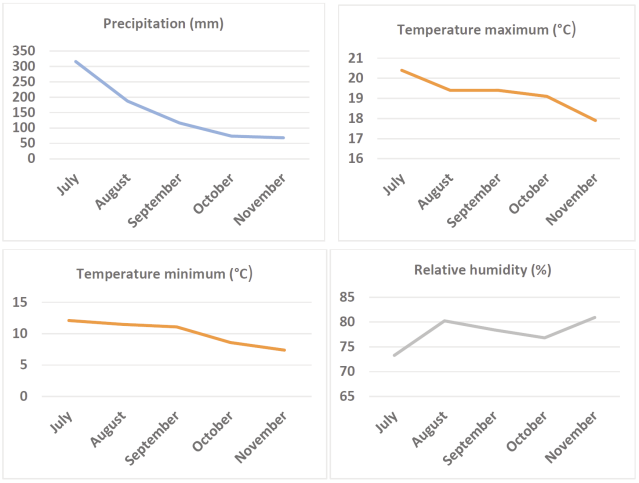

Fig. 2 - Monthly average weather data of the study area during the experimentation period (2023/2024). Source: https://power. larc.nasa.gov/data-access-viewer/

Experimental materials and procedures

'Nantes' variety of carrots was used for the experiment. The seeds of this particular variety were sourced and acquired from the Debrezeit Agricultural Research Center. Farmers are highly interested in this variety of carrots for their good adaptation, high marketing demand, and better root quality in the study area. The experimental field was readied using traditional tillage methods and cultivated using oxen to loosen the soil, catering to the deep and well-drained soil requirements favored by carrots. The soil was molded into raised beds to enhance drainage, promote extensive root growth, ensure uniformity, and minimize soil compaction. After this, a field layout was implemented, and each treatment was assigned randomly to the experimental plots. Seeds were sown as per each sowing date time on a 20 cm height raised bed with 20 cm x 5 cm spacing between rows

Fig. 3 - Pictures of mulch during a field trial.

and plants respectively. The straw and dried grass mulch were cut into small pieces approximately 5-10 cm by machete, following the method outlined by Olfati *et al*. (2008). After sowing the seeds on each particular sowing date, mulching materials were applied at about a rate of 4 t ha^{-1} sawdust and 6 t ha^{-1} straw and dried grass. Fully rate of phosphorus (175 kg P_2O_5 ha^{1}) was applied at the time of each sowing date, while the nitrogen in the form of urea (150 kg ha^{1}) was applied in a split way: half of the rate was applied at the time of each sowing date, and the remaining half was topdressed in the spaces during the active vegetative crop stage, which occurred 5 weeks after emergence (WAE). Weeds were lifted manually and removed from the crop fields, while harvesting was done at maturity by using a hand cultivator.

Data collection

Days to 50% emergence and 90% physiological maturity were obtained by counting the number of days to days when 50% of the seeds emerged and 90% of the plants attained physiologically matured, respectively. Carrot plants were physiologically matured when their lower leaves turned yellow and roots were at the harvestable size with their crown attaining a diameter ranging from 2-3.8 cm as described by UNECE (2018).

At physiological maturity stage, the plant height and number of leaves per plant were obtained from ten randomly selected plants from each unit experiment. The height of ten randomly selected plants from the ground level to the end of the uppermost parts was measured using a ruler.

After harvesting, the root length and diameter of ten randomly selected marketable carrot roots from each unit experiment was recorded using a digital caliper. The root diameter was obtained approximately two centimeters below the root collar (at middle) according to Zelalem (2019). Then the root fresh weight was recorded by weighing ten randomly selected marketable carrot roots from each net plot area and each replication after harvesting using a sensitive balance and the mean values were computed for further analysis. However, marketable carrot roots were those that were free from mechanical damage, disease, insect pest attack, undersized (<50 g), and cracks. The weight of those roots obtained from the designated plot was measured in kilograms using a scale balance and the yield was expressed as a ton per hectare. Carrot roots that were diseased, insect pest-damaged, cracked, branched, and undersized (<50 g) were considered unmarketable roots and their weight was measured in kilograms using a scale balance and their yield was also expressed as a ton per hectare.

Weed density was assessed determining the number of weeds in each plot during the second and fourth weeks after sowing. Samples were collected from a designated area measuring 0.25 square meters (0.5 m x 0.5 m) at two randomly selected points within each plot. The mean values were computed for further analysis and expressed as a quantity per square meter.

Data analysis

All the collected data were subjected to analysis of variance (ANOVA) using the statistical procedures described by Gomez and Gomez (1984) with the help of R-software version 4.2 and package Agricolae (R institute, 2022). The mean separation was conducted using the Least Significant Difference (LSD) at 5% of the required probability level. A simple correlation analysis was conducted to determine the relationship between growth and yield parameters of carrots as influenced by sowing date and mulching. Moreover, Partial budget analysis was conducted to evaluate the economic feasibility of sowing date and mulching, following the procedures below described by CIMMYT (1988). In brief:

Total root yield (TRY): The average yield of each treatment, measured in tons per hectare.

Adjusted total root yield (AjTRY): The average yield was decreased by 10% to accommodate the tendency for experimental yields to exceed what farmers could attain using identical treatments. In economic assessments, farmer yields are typically adjusted to be 10% lower than research findings to align with practical expectations.

Adjusted total root yield (AjTRY) = TRY x (1 - 0.1)

Gross field benefit (GFB): Determined by multiplying the farm/ field gate price received by farmers for the carrots when sold by the adjusted marketable yield.

Gross field benefit (GFB) = AjTRY x price of carrot at farm gate

Total variable costs (TVC): The expenses for mulch, the application cost of mulch, and labor costs for weeding varied across the treatments. The costs of additional inputs and production practices like land

preparation, sowing, fertilizing, and harvesting were either consistent or negligible across the treatments.

Net benefit (NB): Obtained by deducting the total variable costs for inputs (TVC) from the gross field benefit (GFB).

Net benefit (NB) = Gross field benefit (GFB) - Total variable costs (TVC)

Marginal rate of return (MRR%): Calculated as the division of the change in net benefit by the change in total variable cost.

Marginal rate of return (MRR%) = [Change in net benefit (ΔNB)]/[Change in total variable cost(ΔTVC)] x 100

3. Results

Phenological parameters

The analysis of variance (ANOVA) showed that except for the effects of sowing date on days to 50% emergence, days to 90% maturity were significantly affected ($P \leq 0.05$) by sowing date, mulching, and their interactions (sowing date with mulching materials). In the case of mulching materials, sawdust mulch (M1) resulted in a minimum of 13.00 days, while bare soil (M0) led to a maximum of 15.00 days for 50% emergence. A maximum (101.66 days) number of days to achieve 90% maturity was observed from late sowing in July with growing carrots on bare soil (S3M0). Whereas, the minimum (97.00 days) number of days to 90% maturity was recorded from early sowing in July with sawdust mulch (S1M1) (Table 1).

Table 1 - Effects of sowing date and mulching on days to 50% emergency (DE) and 90% maturity (DM) Asian economic status

Factors	DE (no.)	DM (no.)
Sowing dates (S)		
S1	13.83 a	99.50 c
S2	13.91 a	100.25 b
S3	14.00 a	101.00 a
Mulching (M)		
M0	15.00 a	101.55 a
M1	13.00 c	98.44 d
M2	13.88 b	100.77 b
M3	13.77 b	100.22 c
Interaction (S X M)		
S1M0	15.00 a	101.33 a
S1M1	13.00 c	97.00 d
S1M2	13.66 b	100.00 b
S1M3	13.66 b	99.66 b
S2M0	15.00 a	101.66 a
S2M1	13.00 c	98.33 c
S2M2	14.00 b	101.00 a
S2M3	13.66 b	100.00 b
S3M0	15.00 a	101.66 a
S3M1	13.00 c	100.00 b
S3M2	14.00 b	101.33 a
S3M3	14.00 b	101.00 a
LSD (0.05)	0.25	0.84
CV (%)	1.87%	0.49%

Mean values within rows and columns followed by a different letter(s) are significantly different at a 5% probability level. CV = coefficient of variation. LSD = least significant difference.

Growth and yield parameters

ANOVA revealed that all the growth and yield parameters were significantly ($P \leq 0.05$) affected by sowing date, mulching, and their interactions. However, root diameter was not significantly influenced by the interaction effects of sowing date and mulching. The maximum plant height (23.94 cm), and number of leaves (9.80) were observed from early sowing with sawdust mulch (S1M1). Conversely, minimum plant height (18.65 cm) and leaf numbers (6.80) were obtained from late sowing without mulch (S3M0) (Table 2). The maximum root length (17.43 cm), and root fresh weight (106.78 g) were observed from early sowing with sawdust mulch (S1M1). While, minimum root length (14.00 cm) and root fresh weight (54.87 g) were obtained from late sowing without mulch (S3M0). Moreover, root diameter showed a linear decrease as the sowing date was delayed in which the maximum (2.67 cm) and minimum root diameter (2.39 cm) were recorded from growing carrots on sawdust mulch (M1) and without mulch (M0) respectively (Table 2).

On the other hand, early sowing with sawdust mulch (S1M1) resulted in the highest marketable root yield (26.19 t ha^{-1}) and the minimum (0.71 t ha^{-1}) unmarketable root yield. Following closely, the early sowing with dried grass mulch (S1M3) recorded a marketable root yield of 23.57 t ha^{-1}. In contrast, late sowing without mulch (S3M0) resulted in the lowest marketable root yield of 13.19 t ha^{-1} and the maximum (2.12 t ha^{-1}) unmarketable root yield. Furthermore, marketable root yield showed a linear decrease as the sowing date was delayed. Conversely,

Table 2 - Effects of sowing date and mulching on plant height, number of leaves, root length, root diameter, and root fresh weight

Factors	Plant height (cm)	Leaves (No.)	Root lenghts (cm)	Roort diameter (cm)	Root fresh weight
Sowing dates (S)					
S1	21.94 a	8.20 a	16.14 a	2.68 a	80.65 a
S2	21.06 b	7.82 b	15.41 b	2.51 b	65.82 b
S3	20.30 c	7.49 c	14.86 c	2.45 b	60.40 c
Mulching (M)					
M0	19.63 d	7.23 c	14.75 c	2.39 c	60.87 c
M1	22.49 a	8.76 a	16.32 a	2.67 a	80.55 a
M2	20.89 c	7.50 b	15.35 b	2.53 b	66.13 b
M3	21.38 b	7.79 b	15.46 b	2.61 ab	68.28 b
Interaction (S X M)					
S1M0	20.25 ef	7.45 c	15.47 c	2.50 cde	63.98 cd
S1M1	23.94 a	9.80 a	17.43 a	2.86 a	106.78 a
S1M2	21.68 bc	7.65 c	15.76 bc	2.64 bc	73.45 b
S1M3	21.88 bc	7.90 bc	15.90 b	2.74 ab	78.37 b
S2M0	19.99 f	7.45 c	14.78 e	2.35 ef	63.77 cd
S2M1	22.39 b	8.35 b	16.02 b	2.62 bcd	67.62 c
S2M2	20.34 ef	7.60 c	15.39 cd	2.48 def	65.29 cd
S2M3	21.53 c	7.90 bc	15.44 c	2.60 bcd	66.61 c
S3M0	18.65 g	6.80 d	14.00 f	2.32 f	54.87 e
S3M1	21.15 cd	8.15 b	15.51 c	2.54 cd	67.24 c
S3M2	20.66 def	7.45 c	14.92 e	2.47 def	59.66 de
S3M3	20.74 de	7.58 c	15.04 de	2.49 cde	59.84 de
LSD (0.05)	0.74	0.49	0.38	0.09	5.57
CV (%)	2.09%	3.75%	1.46%	3.72%	4.93%

Mean values within rows and columns followed by a different letter(s) are significantly different at a 5% probability level.
CV = coefficient of variation. LSD = least significant difference.

unmarketable root yield showed a gradual increase as the date of sowing was delayed (Table 3).

Weed density

ANOVA revealed that sowing date, mulching, and their interactions significantly affected weed density ($P \leq 0.01$). The density of weeds showed a progressive decrease as the date of sowing was delayed in which the maximum (109.36 n m^{-2}) and minimum (68.00 n m^{-2}) densities of weeds were recorded from early (S1) and late (S3) sowing in July respectively. Whereas, in the cases of mulching maximum (179.87 n m^{-2}) and minimum (35.62 n m^{-2}) densities of weeds were recorded from growing carrots on bare soil and dried grass mulch (M3) applications respectively (Table 3).

Correlation

The correlation analysis using Pearson correlation coefficients (r) was performed to assess the relationship between the growth and yield parameters of carrots, considering the effects of sowing date and mulching. The results showed a significant positive correlation among all the growth parameters of the carrots, indicating a direct relationship where the effect of one parameter depends on another. However, the growth parameters, namely plant height and leaf number, exhibited a negative correlation with weed densities (WD). Accordingly, the total yield of carrots positively correlated with plant height (r = 0.77**), number of leaves per plant (r = 0.61**), root length (r = 0.75**), root diameter (r = 0.69**), root fresh weight (r = 0.64**), and marketable root yield (r = 0.6**). Furthermore, except for the unmarketable root yield (URY), all the yield parameters were negatively correlated with weed densities (Table 4).

Partial budget analysis

The minimum (375 USD ha^{-1}) and maximum

Table 3 - Effects of sowing date and mulching on marketable root yield (MRY), unmarketable root yield (URY), and weed density (WD)

Factors	MRY (t ha^{-1})	URY (t ha^{-1})	WD (n m^{-2})
Sowing dates (S)			
S1	23.40 a	0.96 b	109.36 a
S2	21.65 b	1.48 a	86.01 b
S3	19.16 c	1.56 a	68.00 c
Mulching (M)			
M0	17.60 c	1.75 a	179.87 a
M1	23.74 a	0.99 d	46.42 c
M2	21.79 b	1.36 b	89.25 b
M3	22.49 ab	1.23 c	35.62 d
Interaction (S X M)			
S1M0	20.57 cd	1.31 de	231.03 a
S1M1	26.19 a	0.71 i	61.35 f
S1M2	23.31 b	0.97 gh	93.03 d
S1M3	23.57 b	0.85 hi	52.02 g
S2M0	19.04 d	1.82 b	172.50 b
S2M1	23.25 b	1.07 fg	45.63 g
S2M2	22.1 bc	1.62 c	90.40 de
S2M3	22.23 bc	1.44 cd	35.51 h
S3M0	13.19 e	2.12 a	136.10 c
S3M1	21.79 bc	1.20 ef	32.3 h
S3M2	19.97 cd	1.49 cd	84.3 e
S3M3	21.69 bc	1.42 d	19.33 i
LSD (0.05)	2.45	0.19	8.12
CV (%)	6.76%	8.76%	5.46%

Mean values within rows and columns followed by a different letter(s) are significantly different at a 5% probability level.
CV = coefficient of variation. LSD = least significant difference.

(863.33 USD ha^{-1}) total variable cost (TVC) was obtained from early sowing in July with no mulching (S1M0) and late sowing in July with sawdust mulching (S3M1) respectively and all the remaining treatments were confined between these two ranges (Table 5). According to the results of the partial budget analysis, early sowing with sawdust mulch (S1M1) yielded the highest net benefits of 11266.67 USD per hectare with a remarkable marginal rate of return (799.4%). Following closely was early sowing in July with dried grass mulch (S1M3), which had a net benefit of 10555.67 USD per hectare and the highest marginal rate of return (12,199.7%). On the other hand, late sowing in July with no mulching (S3M0) resulted in the lowest net benefit of 6489.5 USD per hectare and an unacceptable marginal rate of return (MRR%) (Table 5).

4. Discussion and Conclusions

Phenological parameters

Despite the sowing date, mulching regulates important seed emergence factors. In line with a study conducted by Mengistu and Yamoah (2010), most carrot varieties exhibited a typical emergence period ranging from 10 to 15 days. Mulching regulates essential factors for seed germination and emergence, including soil moisture, temperature, and air conditions. This optimized environment created by mulching can contribute to faster and

Table 4 - Correlation analysis of growth and yield parameters of carrot as influenced by sowing date and mulch

Par	DE	DM	PH	NL	RL	RD	RFW	MRY	URY	TRY	WD
DE	1	0.77 **	-0.76 **	-0.66 **	-0.67 **	-0.65 **	-0.54 **	-0.72 **	0.66 **	-0.70 **	0.77 **
DM		1	-0.85 **	-0.86 **	-0.84 **	-0.73 **	-0.79 **	-0.60 **	0.74 **	-0.56 **	0.49 **
PH			1	0.84 **	0.88 **	0.85 **	0.81 **	0.80 **	-0.85 **	0.77 **	-0.50 **
NL				1	0.86 **	0.70 **	0.83 **	0.64 **	-0.70 **	0.61 **	-0.40 *
RL					1	0.82 **	0.85 **	0.78 **	-0.86 **	0.75 **	-0.32 NS
RD						1	0.73 **	0.72 **	-0.79 **	0.69 **	-0.4 2*
RFW							1	0.67 **	-0.72 **	0.64 **	-0.23 NS
MRY								1	-0.77 **	0.60 **	-0.42 *
URY									1	-0.72 **	0.40 *
TRY										1	-0.41 *
WD											1

Par = parameters, DE = days to 50% emergence, DM = days to 90% maturity, PH = plant height, NL = number of leaves, RL = root length, RD = root diameter, RFW = root fresh weight, MRY = marketable root yield, URY = unmarketable root yield, TRY = total root yield, WD = weed densities, ** = highly significant ($p \leq 0.01$), * = significant ($p \leq 0.05$), NS = not significant.

Table 5 - Partial budget and marginal rate of return (MRR) analysis for a response of carrot to sowing date and mulching

Treatment combinations	Total root yields (Kg ha^{-1})	Adjustable total root yield (Kg ha^{-1})	Growth field benefit (USD)	Total variable cost (USD)	Net benefit (USD)	Marginal rate return (%)	Rank
S_1M_0	21880	19692	9846	375	9471	--	
S_2M_0	20860	18774	9387	387.5	8999.5	D	
S_3M_0	15310	13779	6889.5	400	6489.5	D	
S_1M_3	24420	21978	10989	433.33	10555.67	12199.7	1
S_2M_3	23670	21303	10651.5	445.83	10205.67	D	
S_3M_3	23110	20799	10399.5	458.33	9941.16	D	
S_1M_2	24280	21852	10926	541.66	10384.33	531.8	3
S_2M_2	23720	21348	10674	554.16	10119.83	D	
S_3M_2	21470	19323	9661.5	566.66	9094.83	D	
S_1M_1	26900	24210	12105	838.33	11266.67	799.4	2
S_2M_1	24320	21888	10944	850.83	10093.17	D	
S_3M_1	22990	20691	10345.5	863.33	9482.16	D	

D = dominated, selling price of carrot at farm gate = 0.5 USD kg^{-1}, labor cost = 2.5 USD Man per day.

more efficient seed emergence. The moisture content should be sufficient for the seeds to absorb water and germinate. Low soil moisture content delays or inhibits seed germination in the field, reduces uniformity of seedling performance, and total stand establishment, and ultimately reduces the yield of carrots (Muhie *et al*., 2024). The delayed maturity observed in late sowing (S3) could be attributed to unfavorable weather conditions typically experienced towards the end of summer (August). Insufficient rainfall during the critical growth stages such as root development may adversely affect plant phenology. Throughout the vegetative growth stages of the late sowing date, minimum temperatures were received. This might have requested a prolonged time to reach 90% maturity. In contrast, the vegetative growth periods (July and August) associated with early sowing in July benefited from sufficient rainfall and relatively favorable temperatures (Fig. 2). Indeed, this promoted better vegetative growth, which is vital for achieving early 90% maturity. Sandler *et al*. (2015), support the current finding, that a proper sowing date could help to minimize damage from cold, moisture deficit, weeds, pests, and diseases. Similarly, the accelerated maturity of carrot roots might be associated with the easy uptake of nutrients as the available water helps the plant to dissolve the nutrients and move through transpiration pull which in turn helps carrot roots to mature early (Muhie *et al*., 2024). In contrast to growing carrots without mulch, the practice of mulching generally enhanced the likelihood of achieving early maturity. Mulching achieved this by providing organic matter, effectively regulating soil temperature and moisture levels. In agreement with this experiment, Singh and Jaysawal (2021) observed that sawdust mulching has a significant influence on the harvesting period (maturity).

Growth and yield parameters

The higher plant height and leave numbers observed from the early sowing in July (S1) could be attributed to maximum rainfall and temperature in July and August (Fig. 2). Moreover, they contributed to improving various vegetative aspects, including plant height and leaf number. Additionally, carrots require a sufficient amount of water for proper growth and development consequently, these conditions play a crucial role in accelerating the crop's physiological processes. Considering the combined effect of precipitation and temperature, carrot farmers must plan their planting and cultivation schedules accordingly. Sowing during periods of adequate rainfall can improve the chances of successful growth. This result is consistent with Kabir *et al*. (2013), who observed that all environmental conditions, especially temperature, facilitated vegetative growth. Furthermore, researchers have observed that early sowing may result in maximal photosynthesis and a longer growth period than late sowing, which encountered

harsh winter months immediately following sowing and thus diminished growth (Lavanya *et al*., 2017). However, mulching offers vital soil microclimate elements such as moisture, temperature, nutrients, aeration, and weed control, which can enhance crop growth and development.

Acharyya *et al*. (2020) verified this result, sawdust mulch maintains ideal soil temperature, which promotes vegetative development and overall crop yields to a satisfactory level. Based on the trends shown in figure 2, the fluctuations in precipitation and temperature can impact the enhancement of root growth (root length, diameter, and weight) throughout the entire growing season. Crops that were sown early have a greater opportunity to experience relatively optimal temperatures and precipitation throughout their entire growing periods, consequently, this favorable condition of early sowing can result in increased leaf production and canopies, which have the potential to capture more sunlight. This can contribute to improved root growth and ultimately lead to higher crop yields. On the other hand, the root developmental periods of late sowing in July (September and October) were characterized by lower maximum (19.1°C) and minimum (8.6°C) temperatures (Fig. 2). These were below the physiological range of temperatures (15-20°C), and might potentially hinder root growth by disrupting the normal physiological processes of crops. This experimental result is supported by the findings of different researchers, who reported that carrots are a temperature-sensitive root crop and their root growth was developed under suitable environmental conditions (Kabir *et al*., 2013).

The observed maximum root length, root diameter, and root weight in the sawdust mulch could be attributed to several factors such as mulch contributes to the improvement of soil structure and aeration, this creates a favorable environment for root development, allowing roots to penetrate the soil more easily and access nutrients effectively. In addition, the mulch layer helps to maintain the temperature of the soil by providing insulation, which can mitigate extreme temperature fluctuations. This stable soil temperature promotes optimal root growth and function. Furthermore, mulch supports beneficial microbial flora in the soil. These contribute to nutrient cycling and availability, promoting nutrient uptake by roots and enhancing root growth. Whereas, the cultivation of carrots on bare soil encounters unfavorable soil conditions, leading to the production of carrots with poor root quality. Findings of this experiment align with, Shahadot (2021), indicating that the application of sawdust mulch is likely associated with the provision of consistent moisture and nutrients to the root zone. This favorable supply of moisture and nutrients promotes rapid cell division and cell elongation, ultimately leading to the production of long and thicker roots. In addition, consistent findings of the maximum fresh weight of roots were reported in multiple studies using sawdust mulch, including those conducted by Ladumor *et al*. (2020), Acharyya *et al*. (2020), and Paunović *et al*. (2020).

Figure 2 reveals a decrease in precipitation levels from July to November. Carrots require a sufficient amount of water for proper growth and development. Inadequate rainfall during critical stages, such as root development, can lead to stunted growth and reduced yields. The success of crop establishment, yield, and profitability could be attributed to the favorable precipitation and temperature observed during the vegetative growing periods (July and August) of early-sown crops. An optimal environment for the crops, promotes healthy growth and development, ultimately leading to higher yields and increased profitability. Furthermore, improved vegetative performance, characterized by increased net photosynthetic rate, stomatal conductance, and leaf chlorophyll content, plays a vital role in enhancing root quality. Conversely, late-sown crops faced challenges due to inadequate rainfall and lower temperatures experienced during the vegetative growing periods (August and September) (Fig. 2), thus unfavorable conditions during critical growth stages might have negatively impacted crop performance, leading to potential yield reductions.

Mulching offers a comprehensive supply of essential resources, thereby enhancing the quality of roots for the market. This study is in line with, Hasan *et al*. (2018), who reported that the use of mulch resulted in the highest marketable root yield. The maximum unmarketable root yield observed in bare soil could be attributed to fluctuating soil moisture, temperature, and inadequate soil aeration. These contribute to the development of poor-quality roots, characterized by branching, cracking, forking, under-sizing, underweight, and green shoulder roots. Whereas, minimum non-marketable root yield observed in carrot cultivation with sawdust mulch could be attrib-

uted to the regulating effect of mulching on fluctuating soil conditions, especially moisture and temperatures. By maintaining more stable soil conditions, mulching reduces the occurrence of root branching and cracking, resulting in roots that are highly desirable to both consumers and the market. Furthermore, mulching contributes to the production of fewer green shoulder roots by protecting the soil against cracking and direct exposure to light. Green shoulder roots, which have a bitter taste, negatively impact root appearance and quality, unsuitable for consumption and market. The present experimental result is consistent with Paunović *et al*. (2020) who revealed that various mulching materials such as sawdust affected the availability of nutrients to the plants. The application of sawdust mulch reduces the loss of phosphorous due to excessive precipitation, thus leading to an increase in the production of quality roots (Sarolia and Bhardwaj, 2012).

Weed density

The reason for the density of weeds showing a progressive decrease as the date of sowing was delayed could be, that at the onset of summer (July), there was a higher amount of rainfall and a faster rate of temperature rise compared to the end of summer (Fig. 2). Consequently, these conditions contribute to the proliferation of weeds, resulting in higher weed growth and densities. The availability of water resources encourages weed species to flourish and compete with desired plants for resources. The result is consistent with Singh *et al*. (2019), who found that weed emergence is comparatively weaker during the latter part of summer and early autumn compared to the early summer and spring periods. The reason for minimum weed density within dried grass mulch might be attributed to its slow decomposition rate, which has the potential to suppress weed growth and promote positive plant growth supported by Hayati *et al*. (2023). Among the different mulch materials studied, the straw mulch was the least effective in terms of weed suppression potential. This can be attributed to the loose nature of straw mulch, which does not provide tight coverage of the soil. As a result, straw mulch does not offer effective weed control efficacy when compared to dried grass and sawdust mulch. Furthermore, the current investigation aligns with Ossom *et al*. (2019), who suggested that mulches effectively inhibit weed growth by blocking the penetration of light or excluding specific wavelengths of light required for weed germination and growth. Additionally, Biswas and Das (2019) reported that straw mulch decomposes rapidly, leading to a short duration of weed control efficiency.

Correlation

This finding suggests that both the application of sowing date and mulching positively impacted the yield of carrots by influencing important yield components of the crop. As a result, the yield of carrots was increased. This could be attributed to the fact that increased weed presence leads to a reduction in crop growth and yield. Weeds compete with the crop for essential resources such as nutrients, water, space, and light, as supported by the findings of Manthy *et al*. (2020). In general, there was a positive correlation between the total yield of carrots and the growth parameters. Enhanced vegetative growth such as plant height and leaf performance, contributed to the production of higher quantities of photoassimilates, consequently leading to increased root yield. This concept is supported by the findings of Acharyya *et al.* (2020), who reported that the use of organic mulching promotes improved vegetative growth, ultimately resulting in increased root yield.

Partial budget analysis

From the economic point of view, all treatments with a marginal rate of return higher than the minimum rate of return are considered advantageous and economically viable. The results indicated that the most economically productive treatment combination, offering the highest marginal rate of return, was early sowing with dried grass mulch (S1M3), making it an ideal choice for small-scale farmers. Additionally, for resourceful cultivators or investors, the application of early sowing in July with sawdust mulch (S1M1) proved to be profitable despite its higher cost, resulting in the highest net benefit among all the treatments.

In the study area, characterized by a temperate climate, farmers typically cultivate carrots on bare beds and frequently sow carrots in late July, attributed to severe winter conditions, such as soil moisture deficit due to insufficient rainfall, at the end of summer. This leads to suboptimal yield and, as a result, farmers are discouraged from engaging in carrot cultivation. For this reason, this study focused on implementing management practices such as proper sowing dates and mulching to minimize adverse effects on root yield and suppress weed growth. The result showed that sowing date and mulching had a

significant influence on almost all parameters except for days to 50% emergence. Early sowing with sawdust mulch resulted in the maximum plant height, number of leaves, root length, root fresh weight, and marketable root yield. Additionally, early sowing with no mulch (S1M0) resulted in the maximum weed density. While late sowing with dried grass mulch (S3M3) had a minimum weed density. The correlation analysis showed that the growth parameters and the majority of the yield parameters of carrots exhibited positive correlations with both marketable and total root yields. Based on the partial budget analysis, early sowing in July with sawdust mulch (S1M1) resulted in the highest net benefit. However, the highest marginal rate of return was recorded from early sowing in July with dried grass mulch (S1M3). This research evidences that early sowing with sawdust mulch resulted in the highest marketable root yield and net benefit, despite the associated higher costs. For resource-full producers, it can be recommended as the second-best alternative. However, for the economical production of carrots, a temporary recommendation is to utilize an early sowing in July with dried grass mulch. This particular combination exhibited the highest marginal rate of return, making it the most desirable agronomic management practice for small-scale farmers in the study area. However, this investigation specifically emphasizes agronomic practices. In addition, it is a one-time experiment. Therefore, it is necessary to carry out the experiment using several mulching materials under various sowing dates and locations. This comprehensive approach will lead to efficient results and sound recommendations.

References

ACHARYYA P., BANERJEE A., MUKHERJEE D., MANDAL J., SAHOO B., 2020 - *Impact of different types of organic mulch on growth, yield, soil dynamics and weed infestation in beetroot (*Beta vulgaris *L. cv. Detroit Dark Red) Plots.* - Int. J. Curr. Microbiol. App. Sci., 9(7): 1419-1427.

BISWAS S., DAS R., 2019 - *Role of organic mulch in weed management*. - Sci. Agric. Allied Sector, 3(5): 8-12.

CAFER A., RIKOON S., 2017 - *Coerced agricultural modernization: a political ecology perspective of agricultural input packages in South Wollo, Ethiopia*. - J. Rural Social Sci., 32(1): 77-97.

CIMMY T., 1988 - *From agronomic data to farmer recommendations*: *An economics training manual*. - CIMMY, International Maize and Wheat Improvement Center, Mexico D.F., Mexico, pp. 84.

DESTA B.T., GEZAHEGN A.M., TESEMA S.E., 2020 - *Planting time effects on the productivity of Tef* [Eragrostis tef (Zucc.)] varieties in Ethiopia. - Amer. J. Life Sci., 8(3): 34-40.

FAOSTAT, 2021 - *Crops and livestock products*. - https://www.fao.org/faostat/en/#data/QCL.

GAGOPALE B., 2019 - *The critical period for thinning carrot* (Daucus carota *L.).* - World J. Agric. Res., 7(1): 8-13.

GOMEZ K.A., GOMEZ A.A., 1984 - *Statistical procedures for agricultural research. 2nd Edition*. - Wiley and Sons, NY, USA, pp. 690.

HASAN M.M., ALI M.A., RUBEL M.M.K., SHAH M., ALZAHRANI Y., HAKEEM K.R., 2018 - *Influences of vermicompost and organic mulching on growth, yield, and profitability of carrot* (Daucus carota *L.).* - J. Agric. Biotech., 3(1): 19-31.

HAYATI P.K.D., ABIDAH P.A., JUNIARTI, SYARIF A., 2023 - *Cogon grass* (Imperata cylindrica *L.*) *mulch effect on microclimate, weed management, and yield of upland rice*. - IOP Conference Series: Earth and Environmental Science, 1160(1): 1-7.

KABIR A., ARFAN A., WALIULLA M.H., MEN-UR RAHMAN M.M., RASHID A., 2013 - *Effect of spacing and sowing time on growth and yield of carrot (*Daucus carrota *L.).* - Inter. J. Sust. Agri., 5(1): 29-36.

LADUMOR R.G., NANDRE B.M., SHARMA M.K., WANKHADE V.R., JOSHI P.C., 2020 - *Effect of different dates of sowing on growth, yield, and quality of carrot* (Daucus carota *L.).* - Int. J. Chem. Studies, 8(1): 1517-1520.

LAVANYA A.V.N., VANI V.S., REDDY P.S.S., CHAITANYA K., 2017 - *Effect of sowing dates and spacing on growth and root yield of radish cv. Pusa Chetki*. - Int. J. Pure App. Biosci., 5 (4): 1774-1779.

MANTHY A.D., ABDULHAMID S., ADAMU I.K., 2020 - *Effectiveness of mulching and inter-row spacing on weed suppression and yield of upland rice*. - J. Sci. Multidisciplin. Res., 12(3): 74-87.

MENGISTU D.K., 2009 - *The influence of soil water deficit imposed during various developmental phases on physiological processes of tef* (Eragrostis tef). - Agric., Ecosystems and Environment, 132, 283-289.

MENGISTU T., YAMOAH C., 2010 - *Effect of sowing date and planting density on seed production of carrot (*Daucus carota *var.* sativa*) in Ethiopia*. - Afr. J. Plant Sci., 4: 270-279.

MOHAMMED W., BEZU T., DECHASSA N., WOLDETSADIK K., HAILU M., ABEBIE B., 2014 - *Registration of "Haramaya I" carrot* (Daucus carota *L.) variety*. - East Afr. J. Sci., 8(1): 65-70.

MUHIE S.H., AKELE F., YESHIWAS T., 2024 - *Phenological and yield response of primed carrot* (Daucus carota *L.) seeds under deficit irrigation*. - Adv. Hort. Sci., 38(2):

119-127.

OLFATI J.A., PEYVAST Gh., NOSRATI-RAD Z., 2008 - *Organic mulching on carrot yield and quality*. - Int. J. Veg. Sci., 14(4): 362-368.

OSSOM E.M., PACE P.F., RHYKERD R.L., RHYKERD R.C., 2019 - *Effect of mulch on weed infestation, soil temperature, nutrient concentration, and tuber yield in* (Ipomoea batatas *L.) Lam. in Papua New Guinea*. - Tropical Agric., 78(3): 144-151.

PAUNOVIĆ S.M., MILINKOVIĆ M., PEŠAKOVIĆ M., 2020 - *Effect of sawdust and foil mulches on soil properties, growth and yield of black currant.* - Erwerbs-Obstbau, 62: 429-435.

RAJASEKAR M., UDHAYA NANDHINI D., SUGANTHI S., 2020 - *Soil management with mulches for sustained crop production*. - Indian Farmer, 6(1): 28-34.

REGASSA A., ASSEN M., ALI A., GESSESSE B., 2023 - *Major soil types*, pp. 77-11. - In: BEYEN S., A. REGASSA, B.B. MISHRA, and M. HAILE (eds.) *The soils of Ethiopia*. Springer, Cham, Switzerlands, pp. 326.

SANDLER L., NELSON K.A., DUDENHOEFFER C., 2015 - *Radish planting date and nitrogen rate for cover crop production and the impact on corn yields in Upstate Missouri*. - J. Agri. Sci., 7(6): 1-13.

SHAHADOT M.H., 2021 - *Effect of mulching and potassium on growth and yield of carrot* (Daucus carota *L.).* - Thesis, Faculty of Agriculture, Sher-e-Bangla Agricultural University Dhaka, pp. 78.

SINGH G., JAYSAWAL N., 2021 - *Influence of different mulches on growth, yield, and quality of carrot* (Daucus carota *L.).* - Annals Biol., 34(2): 181-186.

SINGH M.K., MISHRA A., KHANAL N., PRASAD S.K., 2019 - *Effects of sowing dates and mulching on growth and yield of wheat and weeds* (Phalaris minor *Retz).* - Bangladesh J. Bot., 48(1): 75-84.

SAROLIA D.K., BHARDWAJ R.L., 2012 - *Effect of mulching on crop production under rainfed condition - A Review.* - Int. J Res. Chem. Environ., 2: 8-20.

STOLARCZYK J., JANICK J., 2018 - *Carrot: history and iconography*. - Chronica Horticulturae, 51(2): 1-7.

TABOR G., YESUF M., 2012 - *Mapping the current Knowledge of carrot cultivation in Ethiopia*. - Carrot's Aid, Denmark, pp. 20.

UNECE, 2018 - *The UNECE standard FFV-10 concerns the marketing and commercial quality control of carrots*. - United Nations Economic Commission for Europe, Genève, Switzerland, pp. 8.

ZELALEM A., 2019 - *Effect of irrigation interval and number of rows per bed on gowth, root yield, and yield components of Carrot* (Daucus carrot *L.) at Koga irrigation scheme in Amhara region, Ethiopia*. - Dspace institution's institutional repository.

ZELEKE A., DERSO E., 2015 - *Production and management of major vegetable crops in Ethiopia*. - EIAR, Addis Ababa; KOPIA Ethiopian Center, Addis Ababa, Ethiopia, pp. 149.

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Butterhead lettuce growth under shallow water tables and its recovery on tropical urban ecosystem

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Key words: Excess water stress, groundwater level, hypoxia stress, post-water stress, tropical green vegie.

Abstract: Butterhead lettuce (*Lactuca sativa* var. capitata) is a nutrient-rich leafy vegetable beneficial for human health. Lettuce growth and yield performance hampered under water stress conditions. This study aimed to assess its growth and recovery under short-term shallow water conditions in the tropical urban ecosystem. A randomized block design was used with three water table treatments: 16.7 cm, 12.7 cm, and 9.7 cm from the substrate surface. The Results showed that butterhead lettuce is intolerant of excess water, with stunted growth at the 9.7 cm water level, by affecting leaf length, leaf width, leaf initiation, and canopy area. Substrate moisture also indicated excess water at this level. Optimal recovery was observed two weeks after water stress. Leaf length and leaf width were analyzed using zero-intercept linear regression and the results were reliable predictors of leaf area ($y = 0.6076LLxLW$; $R^2 = 0.9694$). In conclusion, butterhead lettuce is sensitive to excess water, as shown by morphological changes, and requires two weeks to recover after water stress.

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1. Introduction

The vegetables need of urban communities can be met through the optimization of cultivation in urban areas. Agriculture in urban areas is one of the efforts to support food sustainability (Abdoellah *et al.*, 2023). The benefits of urban farming to the food resilience of urban populations today are beginning to be recognized, especially after the COVID-19 pandemic (O'Hara and Toussaint, 2021; Murdad *et al.*, 2022). In addition, urban farming is an effort to preserve and enhance social space, green infrastructure, and biodiversity (Pradhan *et al.*, 2023). The optimization of urban farming is also essential, especially when reviewed from an aesthetic point of view. More thoroughly, Nicholas *et al.* (2023) stated that urban farming was very beneficial, especially in the environmental, social, and psychological contexts. The benefits of urban farming can also be seen from

the nutritional security and economic perspectives. Lal (2020) reported that beside being beneficial for improving environmental ecosystems, urban farming plays an important role in contributing to food and nutrition security as well as being economically beneficial. In line Ebenso *et al.* (2022) who emphasized that developing urban farming is important in supporting nutritional security. Furthermore, Yuan *et al.* (2022) mentioned that urban farming will increase community income thereby bringing economic benefits on both micro and macro scales.

Climate uncertainty is an issue that must be addressed, especially in tropical ecosystems. According to Sheldon (2019), climate change causes climate uncertainty that impacts ecology and evolution. As a result, this condition requires adaptation for several types of activities, one of which is activities related to agriculture. Climate change has a significant impact on the availability of water on agricultural land. Rainfall with high intensity is an impact of climate change. According to Eccles *et al.* (2019), excess water is one of the impacts of climate change that can occur in the tropics. This condition causes excess water availability, so plants experience excess water stress. In a riparian wet land, similar to this study site, excess water stress can occur through flooding. Several cases of excess water that negatively affect plant growth have been reported, such as tomatoes (Yin *et al.*, 2023) and *Brassica napus* (Guo *et al.*, 2020).

The efforts to find vegetable crops and their cultivation techniques under conditions of excess water stress continue to be developed. Susilawati and Lakitan (2019) reported that chickpea (*Phaseolus vulgaris* L.) plants were able to grow at a water table of 20 cm below the soil surface. Meanwhile, in other plants, such as tomato plants, 5 cm and 10 cm below media surface, did not reduce leaf growth rate, specific leaf weight, and leaf water content (Meihana *et al.*, 2017). Recovery is an effort to restore plant growth performance after experiencing excess water stress. Hud *et al.* (2023) stated that the recovery ability of white cabbage was considered satisfactory after experiencing excess water stress. However, each plant has its period to recover from excess water stress. Nazari *et al.* (2019) emphasized that the longer the recovery period, the better the changes after experiencing the effects of hypoxia, especially in the roots. Meanwhile, some plants show a better response after recovering from excess water stress, as has been reported in grass pea (Wiraguna *et al.*, 2021).

Experiments on the effect of a water table on vegetable growth, particularly on butterhead lettuce, have been few and far between. This validates the fact that vegetables are a kind of plant that is susceptible to stress. A shallow water table experiment on butterhead lettuce will provide an understanding, particularly of this lettuce's level of tolerance to climate uncertainty, particularly in excess water conditions. The study was aimed to evaluate the growth of butterhead lettuce on several shallow water tables as well as its ability to recover afterward.

2. Materials and Methods

Research site and agroclimatic conditions

The research was carried out at the Jakabaring Research Facility in Palembang, South Sumatra, Indonesia (104°46'44''E and 3°01'35'S). The study began on July 18, 2023, and ended on September 16, 2023. The study site is a lowland urban area with a tropical ecosystem. The study area has entered the dry season, which is characterized by low rainfall, but air humidity is high, often exceeding 70% (Fig. 1).

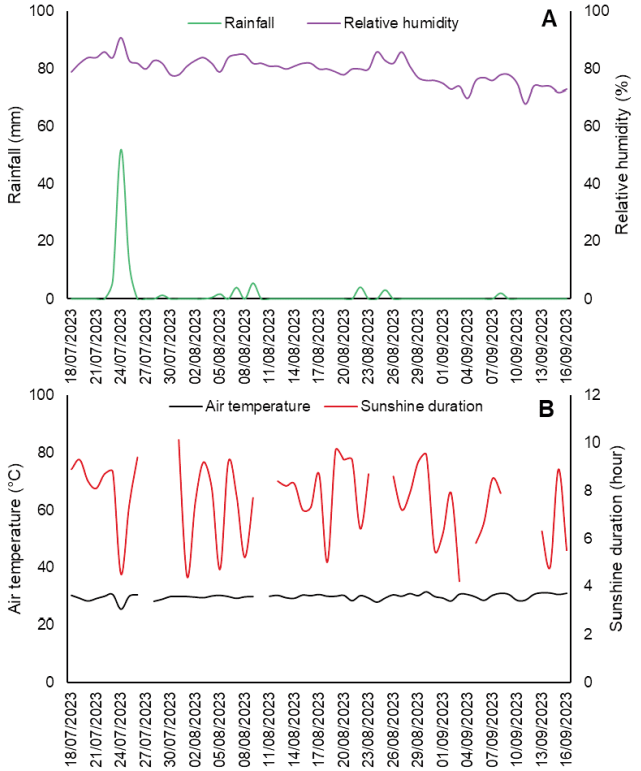


Fig. 1 - Daily rainfall-relative humidity (A) and air temperature-sunshine duration (B) at the research location during the research was carried out. Source: Meteorological, Climatological, and Geophysical Agency, 2023.

Research protocol

Twenty days old butterhead lettuce seedlings were used in the study. The seedlings were transplanted into pots (27.5 cm of height and diameter). As the growing substrate, the pots were filled with topsoil. The plants that had been transplanted to the growing substrate were placed in an open field until 4 weeks after transplanting. Fertilization was performed by NPK (16:16:16) fertilizer after 3 weeks, and watering was performed regularly in the afternoon when it was not raining.

In 4 weeks after transplanting, butterhead lettuce was treated with water maintaining 16.7 cm water tables (WT1), 12.7 cm (WT2), and 9.7 cm (WT3) from the substrate surface (Fig. 2). Each treatment was repeated 3 times. This stage was carried out in an experimental pond measuring 4 m (length) x 2 m (width) x 0.5 m (height). The pond was equipped with an outlet to allow water to flow out in the event of excessive rain. As a result, water level can be controlled based on the water table treatments. During this stage, the plants get their water from the bottom of the pot via capillary water movement, so no watering is required.

After 7 days of water treatments (WT1, WT2 and WT3) butterhead lettuce was return to the open area. As additional treatments, several recovery times were treated, including no recovery, one week of recovery, two weeks of recovery, and three weeks of recovery. During this phase, butterhead lettuce was watered minimally and only when there was no rain for three days in consecutive days.

Data collection

Butterhead lettuce growth data was collected consisting of individual leaf growth, canopy diameter, canopy area, fresh weight and dry weight organ. Individual leaf growth was monitored daily for length and width, starting when the leaf was fully unfolding. The canopy diameter was measured daily on the cross-sectional widest canopy to track canopy diameter growth. The butterhead lettuce canopy area and leaf number were measured weekly. The canopy area was measured using the image scanner Easy Leaf Area software for Android (Easlon and Bloom, 2014). Meanwhile, substrate moisture was measured using a moisture meter (Lutron Soil Moisture Meter PMS-714).

Destructive observation was conducted to collect fresh weight and dry weight data of plant organs. To obtain dry weight, each plant organ was thinned and then dried in an oven at 100°C for 24 hours.

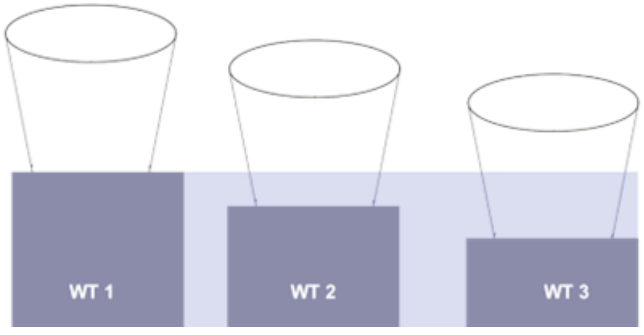


Fig. 2 - The illustration of the shallow water table treatments application. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

Experimental design and statistical analysis

The study used a randomized block design. The shallow water table treatments consisted of 16.7 cm (WT1), 12.7 cm (WT2), and 9.7 cm (WT3) from the substrate surface. All data collected were subjected to analysis of variance (ANOVA), then significance among treatments using the least significant difference (LSD) at $P<0.05$. The significance of differences among treatments was also tested using independent t-test at $P<0.05$. The analysis was performed using RStudio (v2023.06.0+421) for Windows 10 (Rstudio team, PBC, Boston, MA, USA). Meanwhile, data trend on the selected variables were analyzed using Microsoft Excel for Windows 10 (Microsoft Inc., Redmond, Washington, USA).

3. Results

Individual leaf growth

Butterhead lettuce leaf length increased up to 5 days after leaf unfolding (DAU). Furthermore, beginning at 8 DAU, leaf length gradually stagnated. The shallowest water table (WT3) affected the inhibited leaf length during the treatment (Fig. 3). Leaf widening was also observed with the shallow water table treatment. Butterhead lettuce grown in WT1 produced larger leaves. Meanwhile, butterheads planted at the shallowest water table (WT3) experienced inhibited leaf widening, resulting in narrower leaves. The butterhead lettuce leaves, on the other hand, continued to widen until 9 DAU. As a result, the width of the leaves stagnated and experienced senescence, which caused the tips of the

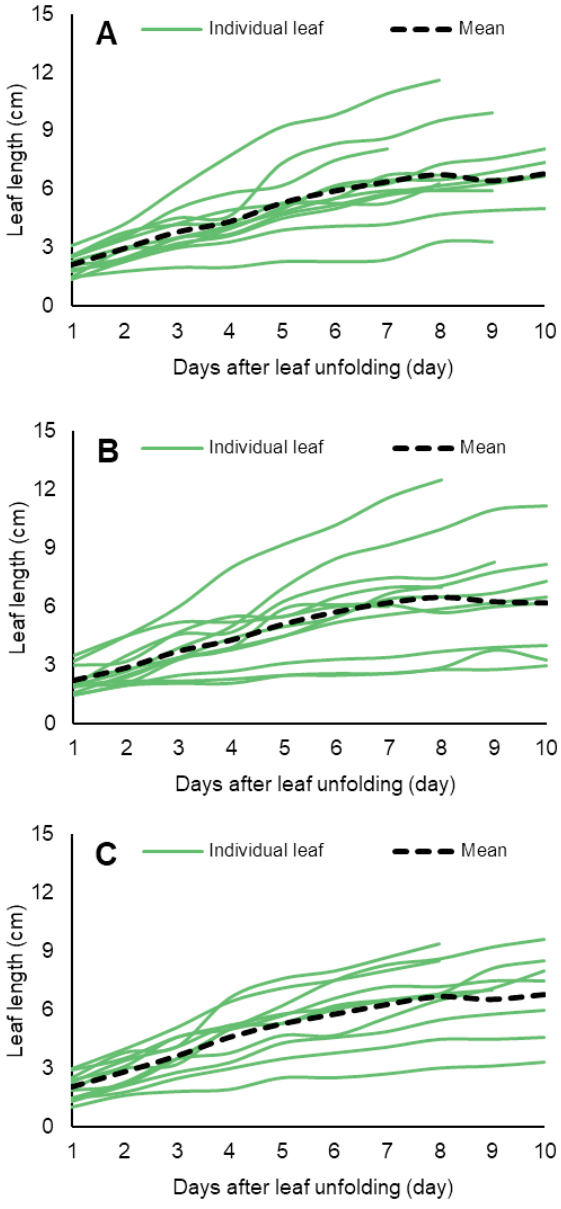


Fig. 3 - Daily leaf length of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

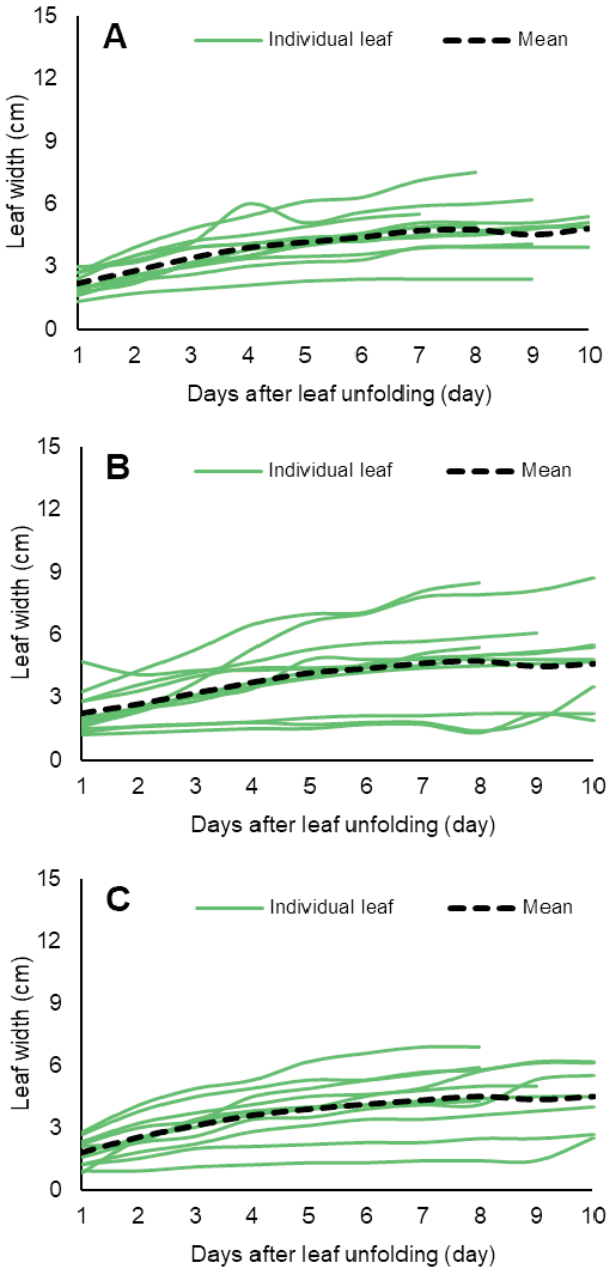


Fig. 4 - Daily leaf width of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

leaves to dry out, resulting in a decrease in leaf width (Fig. 4).

The leaf shape is represented by the leaf length-width ratio. Leaves with a length-width ratio over one indicate elongated leaf growth. If the leaf length-width ratio is less than one, it indicates the leaf growth has widened. There was no difference in the dynamics of changes in the shape of butterhead lettuce leaves in each water table treatment. Butterhead lettuce leaves widened as they aged as a whole (Fig. 5).

Different water table treatments influenced the growth canopy of butterhead lettuce. Butterhead lettuce with the shallowest water table (WT3)

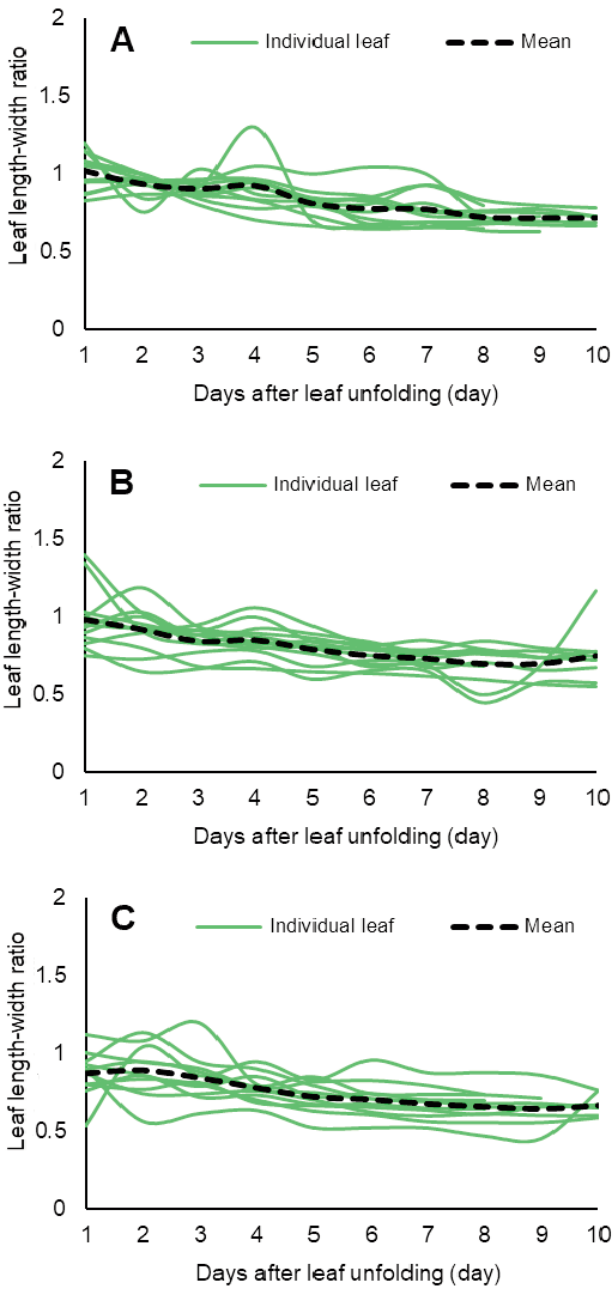


Fig. 5 - Daily leaf length-width ratio of butterhead lettuce on different shallow water tables. The shallow water tables (WT) consisted of WT1 (A), WT2 (B), and WT3 (C). The measurement was carried out when leaf was fully unfolded. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

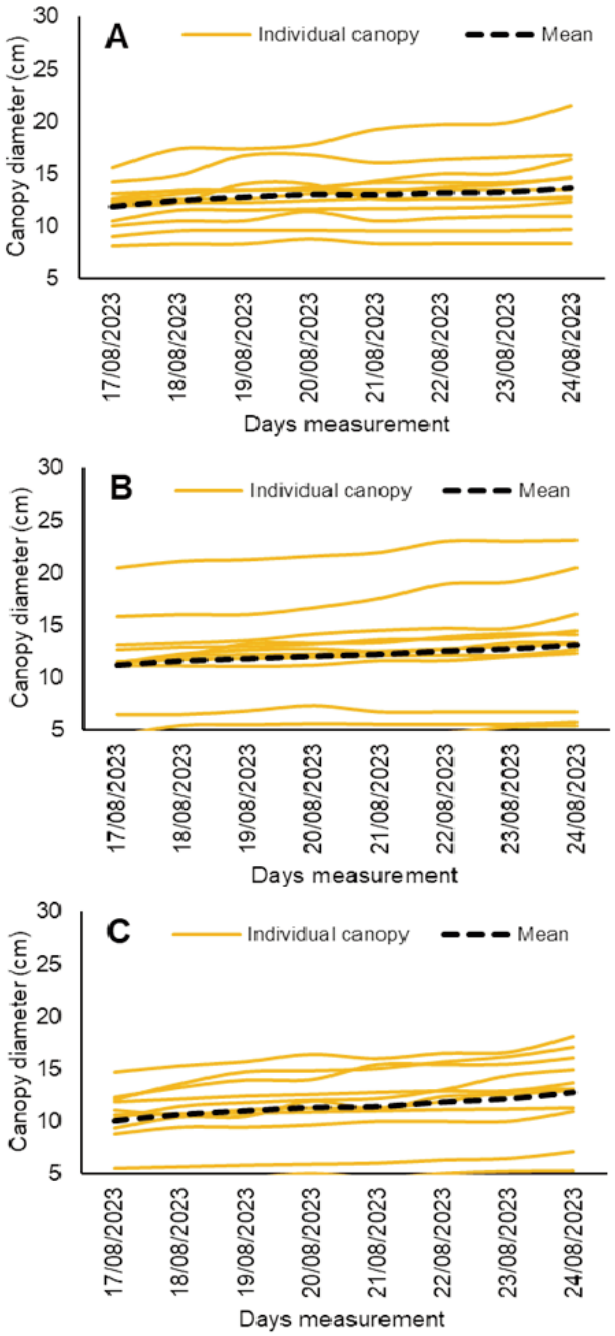


Fig. 6 - Daily canopy diameter of butterhead lettuce on different shallow water tables. The shallow water table consist of WT1 (A), WT2 (B), and WT3 (C). WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface.

exhibited lower canopy growth. The stunted growth of leaves in WT3 resulted in a low canopy width. In contrast, better leaf growth in WT1 and WT2, respectively, resulted in a wider canopy (Fig. 6).

Weekly growth of butterhead on different shallow water tables

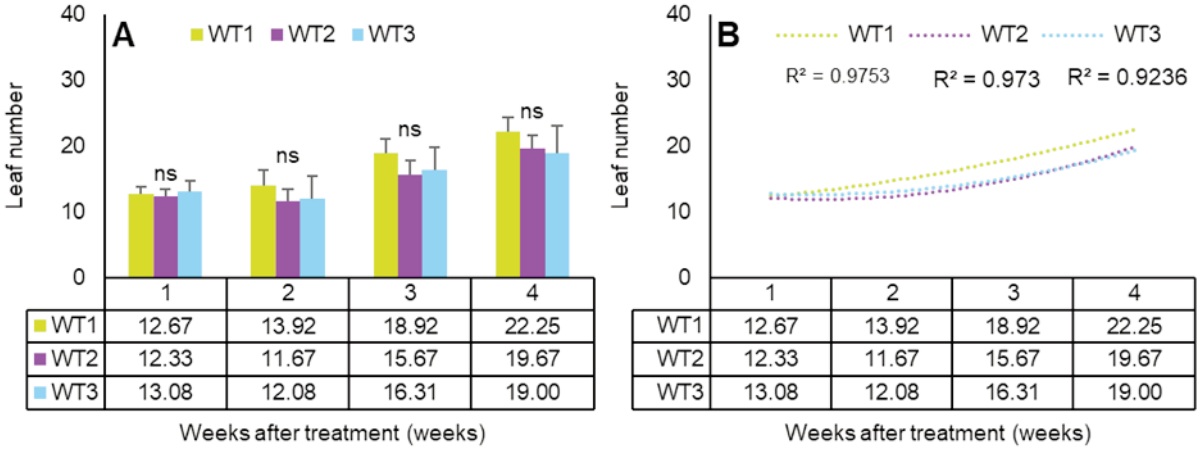
The WT1 exhibited a better trend for butterhead lettuce leaf initiation than the WT2 and WT3. However, statistically, no significant difference was found between the three treatments (WT1, WT2, and

WT3). The leaf number grows in line with the plant's age. The increase in leaf number follows a polynomial curve (Fig. 7).

The canopy area of butterhead lettuce differed among the treatments. During early vegetative growth (1 and 2 WAT), WT1 exhibited the most expansive canopy area, indicating significant differences in the canopy area. The canopy of butterhead lettuce showed no difference at later ages. However, when compared to the WT2 and WT3 treatments, the trend of canopy area growth of butterhead lettuce in WT1 remained higher, with canopy area growth following a polynomial curve. There are signs that WT1's canopy area growth has stagnated, especially after 4 WAT, when the WT1 butterhead lettuce canopy area is almost the same as the WT2 treatment (Fig. 8).

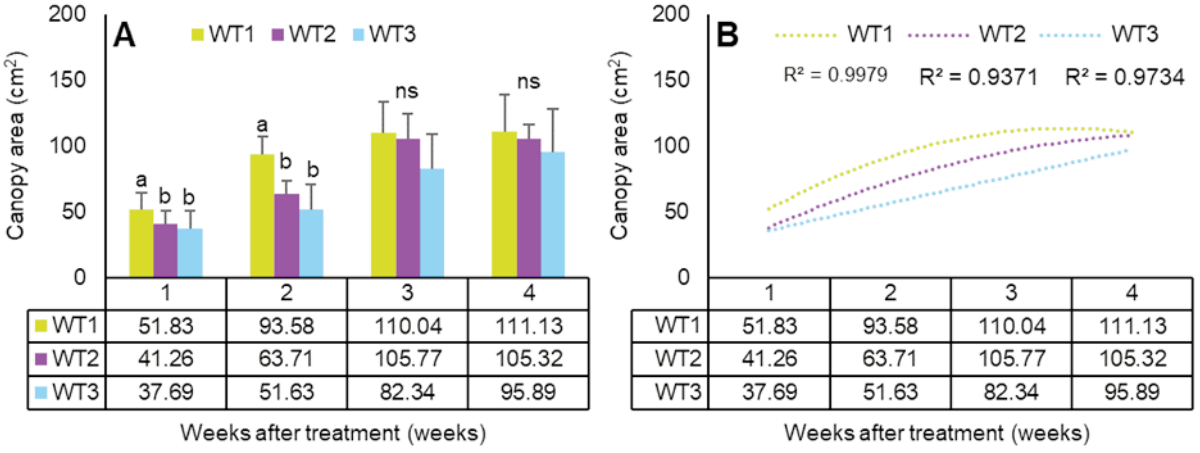
Butterhead growth performance during recovery time

After recovery from water stress, the production of edible and non-edible butterhead lettuce leaves fluctuated. All treatments showed peak edible leaf production at 2 weeks after recovery (WAR). At this time, butterhead lettuce in WT3 has the highest edible leaf production. Meanwhile, non-edible leaf production was highest in WT2 compared to the



	1	2	3	4
WT1	12.67	13.92	18.92	22.25
WT2	12.33	11.67	15.67	19.67
WT3	13.08	12.08	16.31	19.00

Fig. 7 - Leaf number of butterhead lettuce (A) and their trend (B) during on different shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The ns indicated each treatment non-significant at LSD0.05.



	1	2	3	4
WT1	51.83	93.58	110.04	111.13
WT2	41.26	63.71	105.77	105.32
WT3	37.69	51.63	82.34	95.89

Fig. 8 - Canopy area of butterhead lettuce (A) and their trends (B) on different shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The different letters on bar indicated each treatment significant different at LSD0.05. The ns indicated each treatment non-significant at LSD0.05.

other water table treatments (WT1 and WT3) (Fig. 9). Additionally, according to shoot fresh weight, butterhead lettuce shoots on all shallow water tables reached their peak growth at 2 weeks after recovery (WAR). Following the recovery time, the WT3 treatment showed improved shoot growth in comparison to WT 1 and WT 2 (Fig. 10).

Leaf estimation

The butterhead lettuce leaf has a morphology with pinnate veins. The pinnate leaf blade makes it possible to assign leaf length (LL) and leaf width (LW)

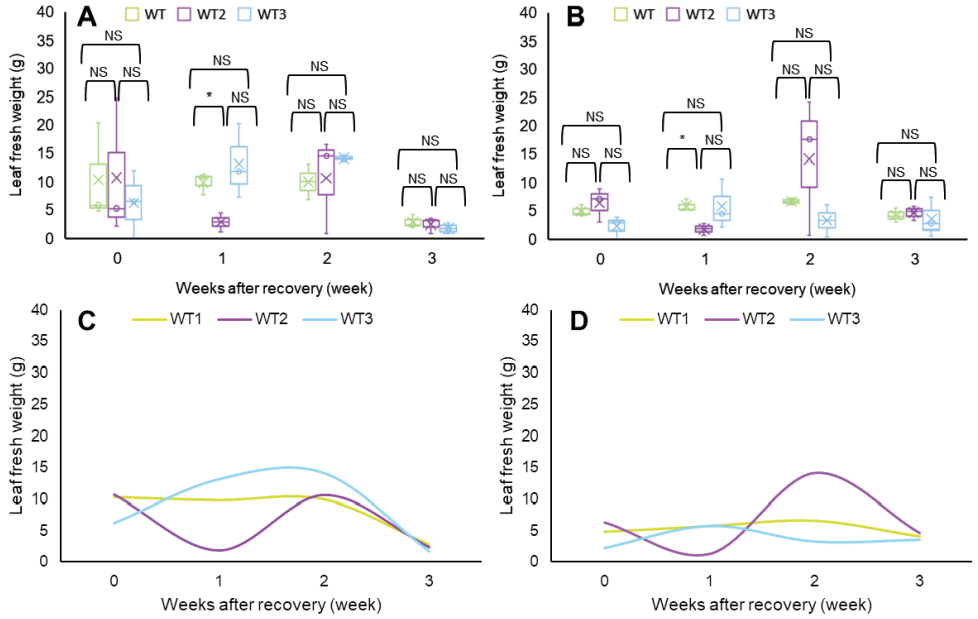


Fig. 9 - Edible leaf and non-edible leaf of butterhead lettuce (A-B) and their trends (C-D) on recovery from different water tables. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. NS: non-significance different based on independent t-test at P< 0.05; *: significance different based on independent t-test at P<0.05.

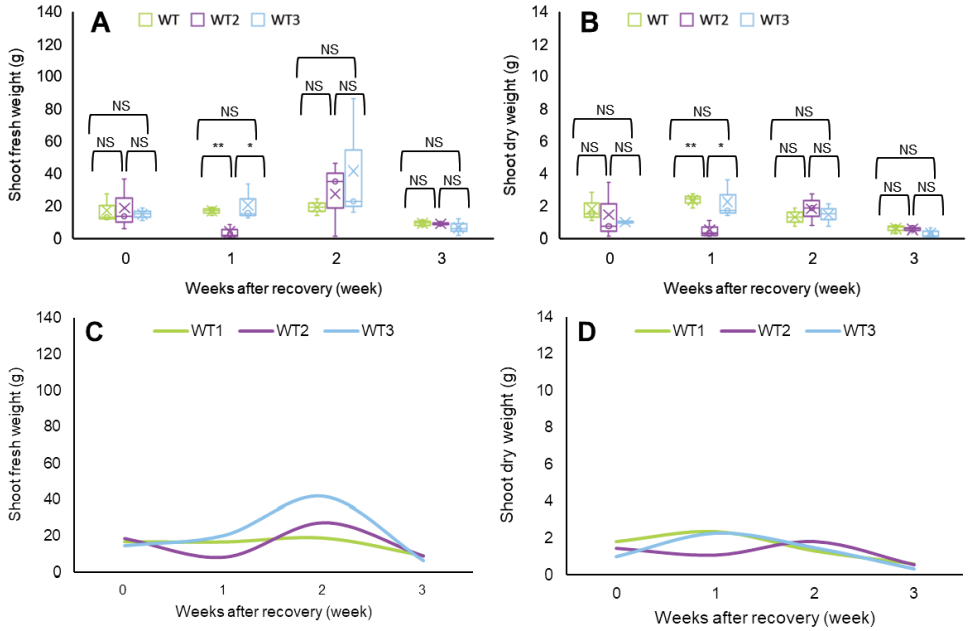


Fig. 10 - Fresh weight and dry weight of butterhead lettuce shoot (A-B) and their trends (C-D) on recovery from shallow water table. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. NS: non-significance different based on independent t-test at P< 0.05; *: significance different based on independent t-test at P<0.05; **: significance different based on independent t-test at P<0.01.

as primary predictors. The results showed that the combination of LL x LW using the zero-intercept linear regression type most reliably represented leaf area (R^2 = 0.9694) (Table 1).

The physiological capacity of a leaf is determined by its leaf area. The findings revealed that increased leaf fresh weight was linearly related to increased leaf area (R2=0.8744). This suggests that larger leaves have more biomass and water. The opposite condition occurred in narrow leaves (Fig. 11).

Water status on different water table treatment

Butterhead lettuce grown in the WT3 treatment receives more water than those grown in WT1 and WT2. This is an indication of excess water, as indicated by the moisture level of the substrate in each treatment. The shallow water table in WT3 causes water to fill the substrate pores faster, resulting in higher substrate moisture than in the other treatments. As a result of this condition, the aerobic space in the WT3 substrate is lower than in the WT1 and WT2 substrates (Fig. 12).

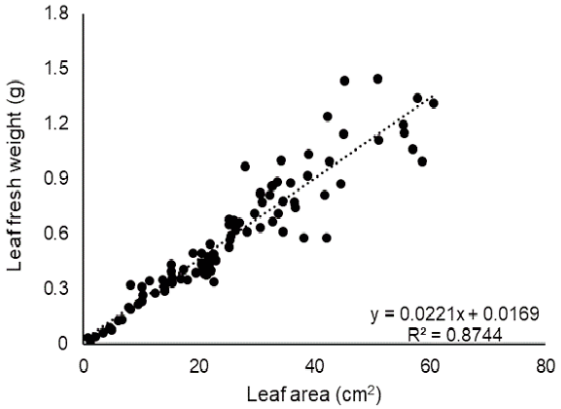


Fig. 11 - Relation between leaf area and leaf fresh weight of butterhead lettuce.

4. Discussion and Conclusions

Plant growth response under excess water conditions

The shallower the water table, the deeper the pot is submerged, hence the less aerobic space available to the plant. Aerobic space is incredibly beneficial to

Table 1 - Butterhead leaf estimation involve leaf length (LL), leaf width (LW), and LL x LW as predictors

Predictors	Regression type	Equation	R^2
Leaf length (LL)	Linear	Y= 5.0467(LL)-12.66	0.8674
	Exponential	Y= $2.6911e^{0.2656(LL)}$	0.7598
	Logarithmic	Y= 29.963ln(LL)-32.71	0.7534
	Quadratic	Y= $0.1327(LL)^2$+3.0079(LL)-5.7844	0.8752
	Power	Y= $0.6121(LL)^{1.7939}$	0.8681
	Zero intercept linear	Y= 3.5771(LL)	0.9491
	Zero intercept quadratic	Y= $0.2172(LL)^2$+1.5467(LL)	0.8715
Leaf width (LW)	Linear	Y= 9.0909(LW)-18.948	0.8464
	Exponential	Y= $1.8915e^{0.4829(LW)}$	0.6645
	Logarithmic	Y= 38.424ln(LW)-33.461	0.7777
	Quadratic	Y= $0.118(LW)^2$+7.908(LW)-16.239	0.8469
	Power	Y= $0.6148(LW)^{2.2685}$	0.8055
	Zero intercept linear	Y= 5.5761(LW)	0.9312
	Zero intercept quadratic	Y= $0.7056(LW)^2$+1.508(LW)	0.8310
LL×LW	Linear	Y= 0.5548(LL×LW)+2.9686	0.8813
	Exponential	Y= $6.8069e^{0.0267(LL×LW)}$	0.6010
	Logarithmic	Y= 17.35ln((LL×LW)-34.847	0.7874
	Quadratic	Y= $-0.0027(LL×LW)^2$+0.8335(LL×LW)-2.3609	0.9039
	Power	Y= $0.5508(LL×LW)^{1.0323}$	0.8780
	Zero intercept linear	Y= 0.6076(LL×LW)	0.9694
	Zero intercept quadratic	Y= $-0.002(LL×LW)^2$+0.7422(LL×LW)	0.9026

Coefficient of determination (R2) indicated strength level of each predictor and regression

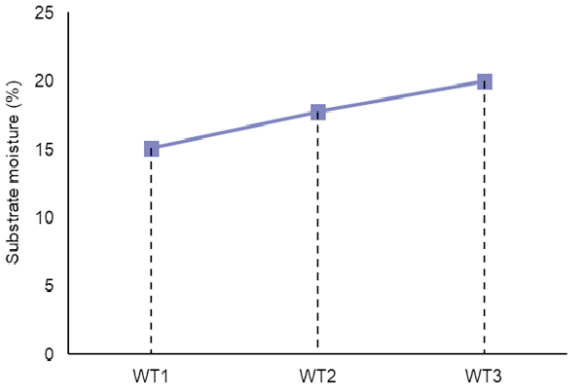


Fig. 12 - Substrate's water status on different shallow water table as indicated by substrate. moisture. The shallow water table consist of WT1, WT2, and WT3. WT1: 16.7 cm below the substrate surface; WT2: 12.7 cm below the substrate surface; WT3: 9.7 cm below the substrate surface. The measurement was conduct at 4 weeks after treatment.

plants as a source of oxygen for many kinds of crucial metabolic activities. Oxygen plays an essential role in several metabolisms in plants, including respiration, carbohydrate formation, protein synthesis, and nutrient solubility (Moreno Roblero *et al.*, 2020; Xu *et al.*, 2020). Oxygen plays an important role in the growth of some soil microorganisms (Wichern *et al.*, 2020). As a result, if the amount of oxygen in the pot is insufficient, plant growth will decrease.

Based on the results, butterhead lettuce grown at the shallowest water table (WT3) showed a stunted growth response. Actually, each plant has different tolerance abilities in excess water conditions. As a consequence of excessive water stress, each plant exhibits specific symptoms. In the case of butterhead lettuce, the plants exhibited stunted leaf and canopy growth. On other hand, plant growth performance was inhibited, as evidenced by data trend of fresh weight and dry weight of edible leaf and shoot on 0 week after recovery before recovery (WAR) (Fig. 9 and 10). Plants respond to excessive water stress by changing their physiology, anatomy, and morphology (Jia *et al.*, 2021; Kumar *et al.*, 2022). According to Zhou *et al.* (2020), plants grown under excess water, change metabolic energy, respiration, photosynthesis, and endogenous hormone regulation.

Hypoxia conditions further hinder plant growth. Some leafy vegetables, such as tomatoes (Tareq *et al.*, 2020) and broccoli (Casierra-Posada and Peña-Olmos, 2022), have been shown to have stunted growth. However, some plants, such as white cabbage, are potentially water-tolerant (Hud *et al.*, 2023). Thus, excess water is a problem for some crops, including butterhead lettuce. In response to excess water, several approaches have been tried, including enriching CO_2 in the substrate (Pérez-Romero *et al.*, 2019) and utilizing the role of ethylene (Khan *et al.*, 2020).

Recovery as an effort to restore plant growth performance

Recovery by returning to open areas was aimed to restore the butterhead lettuce growth after experiencing excess stress. Plant organ architecture and physiological regulation will improve as a result of recovery (Yin and Bauerle, 2017). Depending on the level of stress, each plant requires a different amount of time to recover and return to average or near-normal growth. Our observations indicate that butterhead lettuce takes 2 WAR to restore its growth performance after being grown in shallow water table treatments, was shown clearly in the WT3. The fresh weight of edible leaf and plant organs indicates this. In another case, Nazari *et al.* (2019) found that even 4 days after recovery, hypoxia did not affect *Cicer arietinum*.

After recovery, each plant treated with a different shallow water tables demonstrated a different level of endurance. Interestingly, the shallowest water table (WT3), which had stunted growth when treated with shallow water, had the best recovery growth. Because of the residual pretreatment, the water availability in WT3 was adequate, resulting in better growth. WT3, the shallowest water table, causes the most water retention in the substrate when compared to WT1 and WT2. Plants use excess water during the recovery process since they are rarely watered during this period. According to Bateman *et al.* (2019), substrates with adequate water storage will promote plant growth.

Leaf area estimation and leaf morphological characterization

The role of the length and width of butterhead lettuce leaf as a predictor is essential for a plant with a pinnate leaf shape. These predictors were also tested on leaves with similar leaf shapes, such as citrus (Muda *et al.*, 2023) and Swiss chard (Ria *et al.*, 2023). Furthermore, complex leaf shapes, such as *Amorphaphalus mullieri*, can be estimated by considering leaf morphology (Nurshanti *et al.*, 2022).

Furthermore, the choice of regression type influences predictor reliability in predicting leaf area. According to the findings, the zero-intercept linear regression with the LL x LW predictor was the most dependable. The logic behind zero intercept regression is that if the predictor is 0, the leaf area will also be 0 (Lakitan *et al.*, 2022). The use of zero intercept regression in estimating leaf area has been confirmed in cassava (Lakitan *et al.*, 2023) and chaya (Gustiar *et al.*, 2023).

Butterhead lettuce has been proven to be intolerant of excess water in growing environments, such as at a water table of 9.7 cm from the substrate surface. Butterhead lettuce that has experienced excess water stress needs recovery with the most optimal recovery time within 2 weeks. Butterhead lettuce has a pinnate leaf morphology, and leaf area can be estimated using the formula y = 0.6076 leaf length x leaf width.

References

ABDOELLAH O.S., SUPARMAN Y., SAFITRI K.I., MUBARAK A.Z., MILANI M., SURYA L., 2023 - *Between food fulfillment and income: Can urban agriculture contribute to both?* - Geography Sust., 4(2): 127-137.

BATEMAN A.M., ERICKSON T.E., MERRITT D.J., VENEKLAAS E.J., MUÑOZ-ROJAS M., 2019 - *Water availability drives the effectiveness of inorganic amendments to increase plant growth and substrate quality.* - Catena, 182: e104116.

CASIERRA-POSADA F., PEÑA-OLMOS J.E., 2022 - *Prolonged waterlogging reduces growth and yield in broccoli plants* (Brassica oleracea var. italica). - Gesunde Pflanzen., 74(2): 249-257.

EASLON H.M., BLOOM A.J., 2014 - *Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area* - Appl. Plant Sci., 2(7): e1400033.

EBENSO B., OTU A., GIUSTI A., COUSIN P., ADETIMIRIN V., RAZAFINDRALAMBO H., EFFA E., GKISAKIS V., THIARE O., LEVAVASSEUR V., KOUHOUNDE S., ADEOTI K., RAHIM A., MOUNIR, M., 2022 - *Nature-based one health approaches to urban agriculture can deliver food and nutrition security.* - Frontiers Nutr., 9: e773746.

ECCLES R., ZHANG H., HAMILTON D., 2019 - *A review of the effects of climate change on riverine flooding in subtropical and tropical regions* - J. Water Climate Change, 10(4): 687-707.

GUO Y., CHEN J., KUANG L., WANG N., ZHANG G., JIANG L., WU D., 2020 - *Effects of waterlogging stress on early seedling development and transcriptomic responses in* Brassica napus. - Mol. Breeding., 40: 1-14.

GUSTIAR F., LAKITAN B., BUDIANTA D., NEGARA Z.P., 2023 - *Non-destructive model for estimating leaf area and growth of* Cnidoscolus aconitifolius *cultivated using different stem diameter of the semi hardwood cuttings.* - AGRIVITA, J. Agric. Sci., 45(2): 188-198.

HUD A., ŠAMEC D., SENKO H., PETEK M., BRKLJAČIĆ L., POLE L., LAZAREVIC B., RAJNOVIC I., UDIKOVIC-KOLIC N., MESIC A., PALIJAN G., SALOPEK-SONDI B., PETRIĆ I., 2023 - *Response of white cabbage (*Brassica oleracea *var.* capitata*) to single and repeated short-term waterlogging.* - Agronomy., 13(1): e200.

JIA W., MA M., CHEN J., WU S., 2021 - *Plant morphological, physiological and anatomical adaption to flooding stress and the underlying molecular mechanisms.* - Inter. J. Mol. Sci., 22(3): e1088.

KHAN M.I.R., TRIVELLINI A., CHHILLAR H., CHOPRA P., FERRANTE A., KHAN N.A., ISMAIL A.M., 2020 - *The significance and functions of ethylene in flooding stress tolerance in plants.* - Envir. Exp. Bot., 179: e104188.

KUMAR M.R., BAHADUR V., EKKA S.K., KUJUR R., 2022 - *Growing of vegetables under water deficiency and water logging*. - Pharma Innovation. J., 8: 1612-1615.

LAKITAN B., SIAGA E., FADILAH L.N., NURSHANTI D.F., WIDURI L.I., GUSTIAR F., PUTRI H.H., 2023 - *Accurate and non-destructive estimation of palmate compound leaf area in cassava (*Manihot esculenta *Crantz) based on morphological traits of its selected lobes.* - J. Agric. Techn., 19(1): 129-144.

LAKITAN B., SUSILAWATI S., WIJAYA A., RIA R.P., PUTRI H.H., 2022 - *Non-destructive leaf area estimation in habanero chili (*Capsicum chinense *Jacq.)*. - Inter. J. Agric. Techn., 18(2): 633-650.

LAL R., 2020 - *Home gardening and urban agriculture for advancing food and nutritional security in response to the COVID-19 pandemic.* - Food Security, 12(4): 871-876.

MEIHANA M., LAKITAN B., HARUN M.U., WIDURI L.I., KARTIKA K., SIAGA E., KRISWANTORO H., 2017 - *Steady shallow water table did not decrease leaf expansion rate, specific leaf weight, and specific leaf water content in tomato plants.* - Australian J. Crop Sci., 11(12): 1635-1641.

MORENO ROBLERO M.D.J., PINEDA PINEDA J., COLINAS LEÓN M.T., SAHAGÚN CASTELLANOS J., 2020 - *Oxygen in the root zone and its effect on plants.* - Rev. Mexicana Ciencias Agríc., 11(4): 931-943.

MUDA S.A., LAKITAN B., NURSHANTI D.F., GUSTIAR F., RIA R.P., RIZAR F.F., FADHILAH L.N., 2023 - *Morphological model and visual characteristic of leaf, and fruit of citrus (*Citrus sinensis*).* - AGRIUM: Jurnal Ilmu Pertanian, 26(2): 92-102.

MURDAD R., MUHIDDIN M., OSMAN W. H., TAJIDIN N. E., HAIDA Z., AWANG A., JALLOH M.B., 2022 - *Ensuring urban food security in Malaysia during the COVID-19 pandemic - Is urban farming the answer? A review*. - Sustainability, 14(7): e4155.

NAZARI M., MOSTAJERAN A., ZARINKAMAR F., 2019 - *Strong effect of recovery period between hypoxia events on roots of chickpea (*Cicer arietinum *L.).* - Rhizosphere, 11: e100163.

NICHOLAS S.O., GROOT S., HARRÉ N., 2023 - *Understanding urban agriculture in context: Environmental, social, and psychological benefits of agriculture in Singapore.* - Local Environment, 28(11): 1446-1462.

NURSHANTI D.F., LAKITAN B., HASMEDA M., FERLINAHAYATI F., NEGARA Z.P., SUSILAWATI S., BUDIANTA D., 2022 - *Planting materials, shading effects, and non-destructive estimation of compound leaf area in konjac (*Amorphophallus Muelleri*).* - Trends Sci., 19(9): 3973-3973.

O'HARA S., TOUSSAINT E.C., 2021 - *Food access in crisis: Food security and COVID-19.* - Ecological Economics, 180: e106859.

PÉREZ-ROMERO J.A., DUARTE B., BARCIA-PIEDRAS J.M., MATOS A.R., REDONDO-GÓMEZ S., CAÇADOR I., MATEOS-NARANJO E., 2019 - *Investigating the physiological mechanisms underlying* Salicornia ramosissima *response to atmospheric CO_2 enrichment under coexistence of prolonged soil flooding and saline excess.* - Plant Physiol. Biochem., 135: 149-159.

PRADHAN P., CALLAGHAN M., HU Y., DAHAL K., HUNECKE C., REUßWIG F., CAMPEN H.L., KROPP J.P., 2023 - *A systematic review highlights that there are multiple benefits of urban agriculture besides food*. - Global Food Security, 38: e100700.

RIA R.P., LAKITAN B., SULAIMAN F., YAKUP Y., 2023 - *Searching for suitable cultivation system of Swiss chard* (subsp.(L.) WDJ Koch) *in the tropical lowland.* - J. Hort. Res., 31(1): 81-90.

SHELDON K.S., 2019 - *Climate change in the tropics: Ecological and evolutionary responses at low latitudes.* - Annual Rev. Ecol, Evol., Systematics, 50: 303-333.

SUSILAWATI., LAKITAN B., 2019 - *Cultivation of common bean (*'Phaseolus vulgaris' *L.) subjected to shallow water table at riparian wetland in South Sumatra, Indonesia.* - Australian J. Crop Sci., 13(1): 98-104.

TAREQ M.Z., SARKER M.S.A., SARKER M.D.H., MONIRUZZAMAN M., HASIBUZZAMAN A.S.M., ISLAM S.N., 2020 - *Waterlogging stress adversely affects growth and development of Tomato.* - Asian J. Crop Sci., 2(1): 44-50.

WICHERN F., ISLAM M.R., HEMKEMEYER M., WATSON C., JOERGENSEN R.G., 2020 - *Organic amendments alleviate salinity effects on soil microorganisms and mineralisation processes in aerobic and anaerobic paddy rice soils.* - Frontiers Sustain. Food Syst., 4: e30.

WIRAGUNA E., MALIK A.I., COLMER T.D., ERSKINE W., 2021 - *Tolerance of four grain legume species to waterlogging, hypoxia and anoxia at germination and recovery.* - AoB Plants, 13(4): plab052.

XU C., CHEN L., CHEN S., CHU G., WANG D., ZHANG X., 2020 - *Effects of rhizosphere oxygen concentration on root physiological characteristics and anatomical structure at the tillering stage of rice.* - Ann. Appl. Biol., 177(1): 61-73.

YIN J., BAUERLE T.L., 2017 - *A global analysis of plant recovery performance from water stress.* - Oikos, 126(10): 1377-1388.

YIN J., NIU L., LI Y., SONG X., OTTOSEN C.O., WU Z., JIANG F., ZHOU R., 2023 - *The effects of waterlogging stress on plant morphology, leaf physiology and fruit yield in six tomato genotypes at anthesis stage.* - Veg. Res., 3(1): 1-9.

YUAN G.N., MARQUEZ G.P.B., DENG H., IU A., FABELLA M., SALONGA R.B., ASHARDIONO F., CARTAGENA J.A., 2022 - *A review on urban agriculture: technology, socio-economy, and policy.* - Heliyon, 8(11): e1183.

ZHOU W., CHEN F., MENG Y., CHANDRASEKARAN U., LUO X., YANG W., SHU K., 2020 - *Plant waterlogging/flooding stress responses: From seed germination to maturation.* - Plant Physiol. Biochem., 148: 228-236.

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Cocopeat-amended leaf mould compost yields quality potted dahlia specimens under shade net intercepting one-third sunlight

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

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Abstract: Growers in tropical and subtropical climates with higher afternoon solar intensities face challenges while cultivating potted dahlias. Dahlias that are cultivated in pots with limited capacity lose a great deal of their quality when it gets hot outside. Heat waves that occur suddenly damage dahlia plants' appearance by exposing dry petal margins and marginal leaf blistering. Despite these difficulties, no research has been done to yet to advise growers on the best organic growth medium for dahlias that can maintain high-quality flower production in an appropriate shadow regime. The dahlia cv. Babylon lila rooted cuttings were transplanted into five-gallon (18.92-liter) earthen pots that were filled with six distinct organic growing media formulations. According to our research, dahlia plants grown in a medium containing soil, leaf mould, and cocopeat (50:25:25 v/v) not only produced healthy tubers but also showed better vegetative growth and flowering characteristics. In order to produce higher-quality potted dahlia specimens in hot weather, the study especially advises growing dahlia under shade nets that intercept at least one-third of the incoming solar radiation in areas receiving scorching afternoon sun rays.

1. Introduction

Dahlia variabilis (Desf.), a member of the 'Asteraceae family, is a popular tuberous-rooted flower native to Mexico and parts of Central and South America (Dalda Sekere and Gülşen, 2016). People prize Dahlia flowers for their exquisite blooms, which display a variety of inflorescence styles and blossom colours (Evans, 1998; McClaren, 2004; Romer, 2008). Dahlia is commercially grown in Australia, France, Germany, Italy, Japan, Mexico, New Zealand, South Africa, the United States, and The Netherlands (Marina, 2015). Every year, the Dutch dahlia producers host the "Holland Dahlia Event" throughout the country to showcase the wide variety of flowers and export about 50 million dahlia tubers annually. Dahlia cultivation for the domestic flower market in India is restricted to

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the plains of the northwestern and central areas (De and Bhattacharjee, 2011; Bhattacharjee *et al*., 2019). The dahlia prefers temperatures between 18 to 23°C, relative humidity between 75% to 78% and well-drained, medium fertility sandy loam soils rich in organic matter with a pH range between 6.0 to 8.0 for their successful growth and reproductive development (De Hertogh and Le Nard, 1992; Marina, 2015). Dahlia cultivation in pots and as bedding has grown in popularity recently to beautify community parks and residential gardens (Yazici and Gunes, 2018). In addition to conventional field cultural practices, the capacity of potted dahlia to sustain growth and flower production is largely dependent on the growing media (GM) composition and the amount of sunlight received. The physical and chemical characteristics of the GM ingredients are essential for creating a favorable root zone environment that supports plant growth in a finite volume of pot.

The physical and chemical characteristics of GM have a substantial impact on the ability to retain water, provide aeration, and maintain nutritional status in a finite pot volume (Younis *et al*., 2014). Furthermore, throughout a plant's life cycle, the amount of light it receives influences its growth, development, and other physiological processes. The quality and intensity of light influence leaf expansion, stem length, branching patterns, flowering, and tuberization in dahlia, besides shaping the overall architecture of the plant (Hamrick, 2003). An adequate light intensity induces transition from vegetative to flowering phase and thereafter influences the presentable life of flowers with varying hues of colour (Paik and Huq, 2019).

Growers in tropical and subtropical regions face certain challenges in cultivating potted dahlias because of the higher light intensities (above 70,000 Lux) under open field conditions. Particularly during the afternoon, dahlia leaves exhibit scorching, fading of flower and leaf colour, and may often reveal temporary wilting of plants when exposed to sun for an extended time period. In subtropical locales, high air temperatures (exceeding 40^{o}C) coinciding with heat wave events throughout summers are prevalent. Because of the much lower humidity levels (less than 40%), these hot and dry weather conditions scorch dahlia leaf margins and attract thrips and mite attack. Although dahlias require full sunlight, they also benefit from moderate shade, provided by shade nets that block heat waves, particularly during the afternoon (Menzel, 2016).

The vegetative growth, reproductive development, and tuber production of dahlia are significantly influenced by edaphic factors, including the texture and structure of GM, nutrient composition, water retention capacity, total porosity, and air-filled porosity of medium in a finite pot volume (Reddy *et al.*, 2023). The purpose of this study is to examine how different levels of shade affect *Dahlia x hybrida* growth, flowering, and tuber formation in relation to different GM formulations. To date, there is no scientific research that supports the aforementioned claim.

Therefore, our study hypothesis suggests that growing potted dahlia in GM amended with organic manures will impact the plant's development, flowering, and ability to produce tubers at different shade levels. With this research, we hope to provide valuable insights and practical recommendations for improving potted dahlia cultivation in regions receiving higher sunlight intensity, inducing leaf scorching, especially during afternoon hours.

2. Materials and Methods

Experimental site

The experiment was carried out in the Punjab State in northwest India in the city of Ludhiana (30°45' N, 75°40' E). The city has a uniform topography, is 247 meters above sea level, and has sandy loam soil with a pH of 7.80. The city receives a mean annual rainfall of 640 mm, with sub-tropical exhibiting distinct seasonal variations throughout the year. It is divided into five distinct seasons: hot and dry summer (April to June), hot and humid monsoon (July to September), autumn (October), and winter (November to January). The distribution of the precipitation is irregular, with July through September accounting for around 70-80% of the total. The meteorological information, including air temperature, relative humidity, and light intensity during the period of study, has been provided in figure 1.

Treatment details

The terminal softwood cuttings of the *Dahlia x hybrida* 'Babylon lila' were derived from mother stock plants. The tubers that were planted on nursery beds on October 1, 2022, were used to raise the stock plants at the research farm of the department of Floriculture and Landscaping at Punjab Agricultural University, Ludhiana, Punjab, (India). The variety is

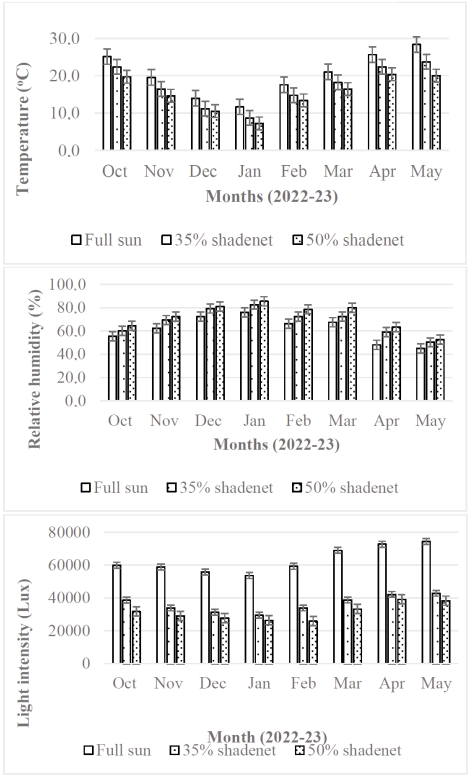

Fig. 1 - Meteorological data of air temperature (degree Celsius, °C), relative humidity (%) and light intensity, Lux) during the period of study (October 2022 to May 2023).

characterized by informal decorative inflorescence of purple colored ray florets. The cuttings were planted on raised nursery beds comprising sandy loam soil on October 22, 2022. The basal portion of cuttings was given a quick dip (5 sec) in 1500 mg L^{-1} IBA (powder initially dissolved in 20 ml of 70% ethanol). Thereafter, the cuttings were gently inserted for rooting on raised nursery beds composed of sandy loam soil. The cuttings were placed in sand beds for 35 days, during which time they grew roots and were subsequently transferred as a single rooted plant.

The rooted cuttings were transplanted in 5 gallon (18.92 litres) earthen pots filled with six different GM formulations with ingredients mixed in volumetric proportions. Soil, well-rotted farmyard manure (FYM), leaf mold (LM), and cocopeat (CP) were the main components of GM. The different ingredients of these media mixtures consisted of GM1 - Soil: FYM: CP (75:25:0) control; GM 2 - Soil: FYM: CP (50:25:25); GM 3 - Soil: 103 FYM: CP (25:25:50); GM 4 - Soil: LM: CP (75:25:0); GM 5 - Soil: LM: CP (50:25:25); and GM 6 - 104 Soil: LM: CP (25:25:50). The GM were filled by carefully tapping the pots to ensure consistent compaction levels. To ensure the establishment of young, tender cuttings, the potted dahlia plants were first kept in partial shade for 20 days. After that, they were exposed to morning sunlight (until 12:00 pm) for an additional 20 days before being placed under two separate green shade nets of 35% (S2) and 50% (S3) light transmittance with varying densities of mesh size (9 pores per square inch and 60 pores per square inch, respectively). The pore size of 35% shade net measured 5 mm x 7 mm, and for 50% shade net it was 4 mm x 2 mm. Until the completion of the trial, the potted dahlia in the control treatment was kept in full sunshine (S1, 0% shade).

Experimental design and statistical analysis

Using a factorial completely randomized design, a total of three pots per three replications were positioned in each of the treatments, for a total of nine pots per treatment. Two fixed factors comprising factor A, shade levels (3 levels), and factor B, growing media (6 levels), were assessed for their effects on vegetative growth, reproductive development, and tuber production in potted dahlia. Observational data were subjected to a two-way analysis of variance (ANOVA) in SPSS IBM statistical software (Version 26) to examine treatment effects through the general linear model (GLM) approach. Tukey's confidence intervals were used to determine the significance of mean differences at the $p \leq 0.05$ level of significance (Table S1).

Cultural practices

Fermented mustard cake was used as fertilizer for the potted dahlia plants in each treatment. An organic fertilizer, mustard cake contains essential plant micronutrients (zinc, calcium, sulphur, magnesium, manganese, iron, and copper) and nitrogen, phosphorus, and potassium in a 4-1-1 ratio. In an earthen pitcher, one kilogram of mustard cake was diluted with 10 liters of water. Using a wooden stick, the mixture was stirred once a day for up to 20 days, making sure the cake was properly dissolved into the slurry until the characteristic odor emanated, which indicated completion of fermentation, which took 21 days. This fermented mustard cake slurry was given as a soil drench by diluting in water (1:10 v/v), every two weeks, beginning one month after planting and continuing until the emergence of flower buds. No additional supply of inorganic fertilizers was made during the plant growth. The potted dahlias were maintained following necessary cultural practices (staking, removal of dead or dried leaves, spent flowers, and disbudding).

Measurements

During the vegetative phase, 110 days after transplanting, before bud initiation, observations were made on vegetative characters such as height (cm), stem internodal length (INL, mm), stem girth (S_G, mm), and stem length (SL, cm); leaf characters, such as leaf length (L_L, cm), leaf width (L_W, cm), number of leaf pairs (NoLP), leaf area (LA, cm^2), leaf dry weight (L_{DW}), and specific leaf area (SLA, cm^{-2} g^{-1} DM), computed as the ratio of LA to L_{DW}. The following flowering traits were also noted during the harvesting process: flower diameter (FD, cm), flower fresh weight (FFW, g), days to full bloom (DtFB), duration of flowering (DoF), number of tubers per plant (NoT), and tuber weight (Tw, g).

Physical parameters such as bulk density (g cm^{-3}), water holding capacity (%), total porosity (%), and air-filled porosity (%) were also analyzed for various GM using standard laboratory protocols. Chemical properties such as pH, EC, per cent nitrogen (N), per cent phosphorus (P), per cent potasssium (K), and per cent organic carbon (OC) were also examined (Table 1). The soil core method (Blake and Hartge 1986) was used to calculate the bulk density (Db, Mg m^{-3}). The water holding capacity (WHC) of the air-dried media samples was specifically determined using Keen's box, which had a perforated bottom, a filter paper disc fixed with a steel ring at the bottom end. The following formula was used to calculate the overall porosity:

$$\% f = (1- Db/Dp) \times 100$$

Where 'f' is total porosity (%), Db and Dp are bulk density and particle density (Mg m^{-3}).

The air-filled porosity (fa) was determined at container capacity moisture content as per the following equation:

$$fa = (f-\theta)$$

where 'f' is the total porosity (%) and θ is the volumetric moisture content at field capacity (%). The pH was determined in suspension by mixing 10 g of GM with 50 ml of distilled water. The EC was measured in the same suspension after 24 h in the supernatant solution. The estimation of N was determined by the alkaline potassium permanganate ($KMNO_4$) method described by Subbiah and Asija (1956). The available P was determined on a spectrophotometer at 760 µm wavelength after shaking the media with extractant and filtering the suspension. The K in the media was determined after digestion of the media mixture and filtering the suspension for recording readings on a flame photometer at 420 µm. The determination of OC was made with the standard procedure (Nelson and Sommers, 1982).

3. Results

Vegetative characteristics

With the exception of the number of leaf pairs, different GM had a substantial impact on the mean values for vegetative and leaf attributes of dahlia. When compared to the control group (GM1), the mean height of plants grown in LM-based media containing 25% CP (GM5) was 17.2% higher and substantially different. But in FYM-based formulations (GM2 and GM3) amended with 25% and 50% CP, as well as in LM-based media with 50% CP (GM6), no dis-

Table 1 - Physico-chemical properties* of different growing media mixtures used for potted dahlia production

Growing media (GM)	Physical properties				Chemical properties					
	Bulk density (g cm^{-3})	Water holding capacity (%)	Total porosity (%)	Air filled porosity (%)	pH	EC (ds m^{-1})	N (%)	P (%)	K (%)	OC (%)
GM1	1.10	46.81	47.2	22.3	7.38	0.12	0.51	0.19	1.61	0.58
GM 2	0.96	77.27	52.5	24.5	7.04	0.25	0.47	0.24	3.02	0.69
GM 3	0.44	115.31	62.1	30.4	6.75	0.37	0.42	0.37	3.54	0.78
GM 4	1.23	43.00	51.3	24.6	7.93	0.11	0.71	0.27	1.70	0.44
GM 5	1.14	58.83	59.4	28.7	7.48	0.38	0.42	0.34	3.17	0.59
GM 6	0.63	104.40	64.2	38.5	7.20	0.58	0.35	0.45	3.75	0.81

* Representative GM samples were estimated prior to the start of the experiment. GM1 - Soil: FYM: CP (75:25:0) control; GM 2 - Soil: FYM: CP (50:25:25); GM 3 - Soil: FYM: CP (25:25:50); GM 4 - Soil: leaf mould: CP (75:25:0); GM 5 - Soil: leaf mould: CP (50:25:25); GM 6 - Soil: leaf mould: CP (25:25:50).

cernible variation in height was seen (Table 2). The mean height of dahlia plants grown under different shade levels showed a statistically significant difference (Fig. 2). Growing under 50% shade produced plants that were 1.6 times taller than those exposed to full sun (0% shade) and growing in 35% shade produced plants that were 28.1% taller. The maximum stem INL was recorded in plants grown in LM-based media (GM6), with a significantly higher (15.1%) mean INL compared to the control (GM1). These differences in stem INL were particularly pronounced in plants grown under shaded conditions.

When plants were cultivated under 50% shade, their maximum mean stem INL was observed to be 2.7 times longer than when plants were grown under controlled conditions (S1). On the other hand, compared to plants grown under control, plants exposed to 35% shade showed a smaller (1.71 times) increase in INL compared to the plants that were cultivated under 50% shade, revealing a higher (2.7 times) increment in stem INL relative to the plants under full sun (S1). The maximum S_G in LM-based GM amended with 50% CP was reported, and this was considerably different from the S_G (which was measured to be 27.5% wider) in plants grown in GM1 (control). There were no appreciable changes in the S_G measured in any FYM-based GM when compared to control. Significant variations in mean S_G were also observed in dahlia plants subjected to different levels of shade. The plants exposed to 50% and 35% shade showed a greater increase (39.1% and 21.5%, respectively) in S_G, in comparison to plants exposed to full sun (control). The variation in mean SL varied from 6.76-7.26, which was determined to be non-significant. Following increased plant exposure to shade, the SL rose linearly. In comparison, the percentage increase in S_L for plants grown in 50% and 35% shade was 28.2% and 33.8%, respectively, compared to those plants that were exposed to full sun.

Leaf characteristics

In comparison to the plants grown in FYM and LM-based media mixtures, the mean L_L in media devoid of CP was found to be lower (Table 2).

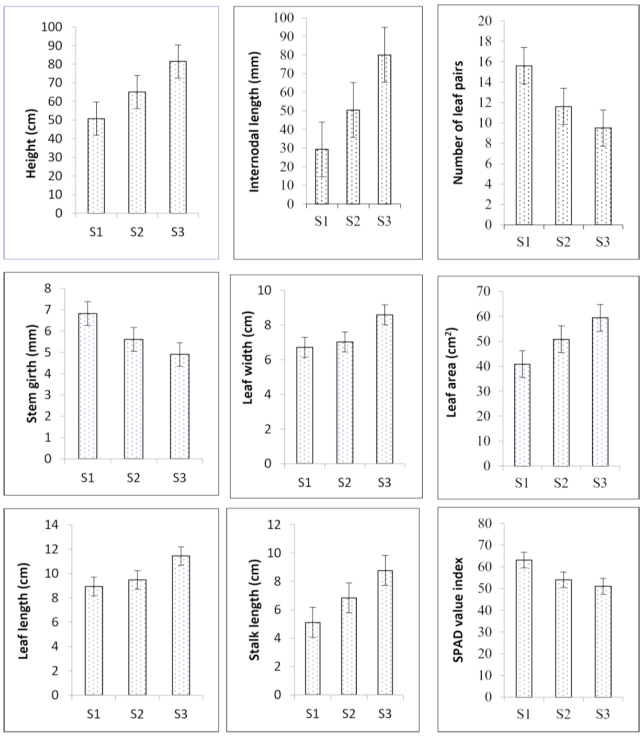

Fig. 2 - Effect of varying shade levels on vegetative growth parameters of potted dahlia. Values represent mean values (n=9) ± SEM for different observations. Letters above

Table 2 - Effect of growing media on vegetative and leaf characteristics of dahlia raised in earthen pots for the period 16 November 2022 to 10 May 2023

Growing media	Vegetative characteristics				Leaf characteristics					
	Height	INL (mm)	SG (mm)	SL (cm)	LL (cm)	LW (cm)	LP (No.)	LA (cm^2)	LDW (g)	SLA ($cm^{-1}\ g^{-1}$ DM)
GM1	59.78 a	48.78 a	5.16 a	6.44 c	9.61 a	7.1 a	10.74	47.4 a	0.080 a	584.32 ab
GM 2	65.70 ab	52.40 b	5.49 a	6.86 ab	10.31 b	7.6 ab	12.28	48.1 ab	0.084 ab	569.25 a
GM 3	70.09 b	55.24 c	5.73 ab	6.90 ab	10.10 ab	7.4 ab	12.52	51.6 bc	0.086 bc	596.29 ab
GM 4	61.47 a	52.34 b	5.43 a	6.76 ab	9.51 a	7.2 ab	12.89	46.3 a	0.082 ab	560.13 a
GM 5	69.12 b	54.82 bc	6.26 cd	7.26 a	10.10 ab	7.6 ab	12.59	54.5 c	0.081 ab	668.05 c
GM 6	68.62 b	56.18 c	6.58 d	7.23 a	10.03 ab	7.8 b	12.48	54.2 c	0.088 c	615.33 b

INL-stem internodal length; SG-stem girth; SL-stem length; LL- leaf length; LW- leaf width; NoLP-number of leaf pairs; LA-leaf area, LDW-leaf dry weight; SLA- specific leaf area; Mean values are representative of observations obtained from nine pots from each of the treatment. Values followed by different letters differ significantly within different growing media treatments, computed following Tukey's mean separation test at p = 0.05 level of significance.

Significant differences in L_L were seen between the various shade levels, and these differences grew as shade exposure increased while the percentage increment differed from the control. In comparison to plants grown in full sun, the mean L_L of plants exposed to 35% and 50% shade levels showed an increase of 6% and 28.1%, respectively, suggesting a higher degree of variability in L_L in plants exposed to higher shade levels. When comparing the maximum mean L_W of plants produced in LM-based media formulation modified with 50% CP to the mean L_W of plants raised in control, a statistically significant difference was observed, indicating a 9.8% increase in L_W. When compared to plants grown in full sun, the mean LW of plants exposed to 50% shade levels showed a 27.8% increase; however, the change was not determined to be significant for plants raised under 35% shade.

The research showed that dahlia exposed to 50% shade had a decreased NoLP count. There was a drop in the NoLP, with plants exposed to 50% shade showing a larger percentage decline (38.7%) than plants that were exposed to 35% shade (25.6%). Dahlia plants cultivated in various GM showed substantial variance in LA, with the highest value found in plants grown in LM-based media modified with 25% CP, which differed significantly from the control, which showed a drop (13.0%) in LA. Dahlia plants exposed to varying degrees of shade showed a substantial variance in LA, which rose linearly with higher exposure. In comparison to plants exposed to 35% shade, which reported an increase of 24.4%, plants exposed to 50% shade showed a higher percentage increment (45.5%) in LA, relative to those exposed to full light. Potted dahlia plants grown in GM amended with 50% CP had the highest LDW (0.088 g), which was significantly different from the LDW of plants exposed to full sun. Statistically significant variations were also indicated by the SLA. A notably elevated average SLA was recorded for the potted dahlia grown in the GM supplemented with LM and CP (25% v/v), with an increment of 14.3% over the SLA measured in control. The plants exposed to 35% and 50% shade displayed an identical LDW but had a substantial increment over control. Compared to plants exposed to full light, the SLA rose in the plants exposed to higher shade levels, increasing by 19.4% and 36.9%, respectively, under 35% and 50% shade.

Flowering characteristics

The DtFBA ranged between 113.8 and 137.2 days. In contrast to plants grown in control, those raised in LM-based GM amended with 50% CP initiated their first bud considerably earlier (24 days) (Table 3). The variation in DtFBA in plants raised in LM-based GM amended with 25% and 50% CP was found to be non-significant. When compared to plants grown in media altered with CP, the plants cultivated in FYM and LM-based GM without CP as an amendment showed a noticeably slower commencement of first bud. The initial bud's emergence was significantly impacted by varying shade levels (Fig. 3). The plants exposed to 50% shade showed a comparatively early (15 days) mean DtFBA. However, the variation was found non-significant in plants that were exposed to sun and at 35% shade.

Table 3 - Effect of growing media on flowering and tuber characteristics of dahlia plants raised in earthen pots for the period 16 November 2022 to 10 May 2023

Growing media	Flowering characteristics					Tuber characteristics*	
	DtBA	DtFBS	DoF	FD	FFW (g)	NoT	TW (g)
GM1	137.22 d	150.89 a	12.16	12.81 a	22.57 a	2.89 b	35.43 b
GM 2	132.44 c	145.33 c	11.62	12.92 a	25.45 c	5.23 a	61.98 a
GM 3	122.56 b	132.33 d	11.81	14.17 bc	25.22 bc	5.42 ab	56.99 a
GM 4	136.67 d	149.22 b	12.39	13.59 ab	23.97 b	4.10 ab	55.14 a
GM 5	115.11 a	125.56 f	11.92	13.98 bc	25.20 bc	5.11 ab	62.93 a
GM 6	113.89 a	123.11 e	11.58	14.50 c	25.39 c	5.08 ab	67.97 a

DtFBA-days to first bud appearance; DtFB- days to full bloom; DoF- duration of flowering; FD-Flower diameter; FFW- flower fresh weight; NoT- number of tubers per plant; Tw- tuber weight; Mean values are representative of observations obtained from nine pots from each of the treatment; *Obtained at the end of the experiment; NoT represents count for healthy intact tubers within a clump without separation; Tw comprises weight of whole clump; Values followed by different letters differ significantly within different growing media treatments, computed following Tukey's mean separation test at p = 0.05 level of significance.

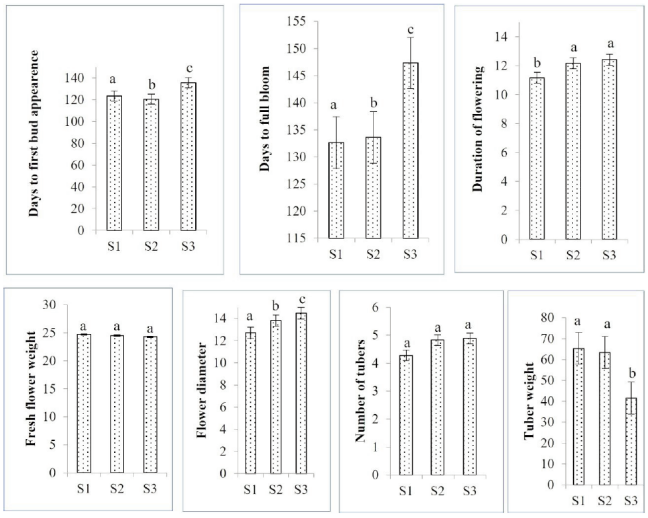

Fig. 3 - Column graphs representing the effect of varying shade levels on flowering and tuber parameters of potted dahlia. Values represent mean values (n=9) ± SEM for different observations. Letters above the error bars represent significant differences computed at P<0.05 following the Tukey's mean separation test. S1: Full sun; S2: 35% shade; S3: 50% shade.

Significant differences were reported in DtFB across plants grown in various media, with LM-based GM supplemented with 25% and 50% CP showing earlier blooming. The plants raised in FYM-based media initiated relatively earlier blooming compared to plants cultivated in control conditions. The DtFBA showed that plants exposed to full light bloomed about two weeks earlier than plants placed under 50% shade. Plants grown in various GM combinations showed a DoF ranging from 11.58 to 12.39 days; nevertheless, the observed variance was deemed non-significant. When placed under varying shade levels, the plants showed noticeably longer DoF than when they were left in full sunlight.

It was discovered that the difference in mean FD across plants grown in various GM was statistically significant. The data showed that, in comparison to the control group, which measured the least diameter, plants grown in CP amended media had a larger FD. The highest diameter of blooms was observed in plants grown in LM-based media supplemented with 50% CP, which showed a 13.1% increase in FD over control.

The amount of shade that plants received also had a substantial impact on FD, which was more pronounced in plants subjected to more intense shadow. When comparing the FD in plants exposed to 50% and 35% shade levels, the diameter of blooms was found to be 13.8% and 8.5%, respectively, larger relative to the control. The difference in FFW between plants grown in different GM was found to be significant, with plants raised in GM altered with CP having a comparatively greater FFW weighed in comparison to those lacking CP. While LM-based media amended with 25% and 50% CP showed non-significant changes, a higher mean FFW was discovered in FYM-based GM amended with 50% CP, which varied substantially from control. The FFW, which ranged from 24.5 to 24.7 g, did not exhibit any significant difference when the shade levels were changed.

Tuber characteristics

When compared to plants grown under control circumstances, there were notable changes in the average NoT per clump. The NoT per clump was nearly two times (1.88) higher in FYM-based media amended with 25% CP (Table 3). Comparing GM2 to other FYM and LM-based media, it was discovered that the NoT per clump obtained in media GM2 was statistically non-significant. The NoT per clump did not significantly differ amongst plants subjected to varying degrees of shade. When compared to tubers collected from plants grown in full sun, the tubers grown in LM-based media supplemented with 50% CP weighed almost twice as much (1.91). While the differences in TW between tubers collected from FYM and LM-based medium were not statistically significant, they were found significant when compared to the control group. When tubers collected from plants exposed to 50% shade were compared to tubers harvested from plants exposed to full sun, the TW reduced to 36.4%. Nevertheless, it was shown that the difference in TW between plants exposed to full light and 35% shade was not statistically significant.

4. Discussion and Conclusions

According to our research, planting dahlias under a green shade net that blocks off 35% of the sun's incoming radiation promotes better-quality flowers and healthier tubers. While dahlia plants need full sun during their growth, these guidelines are appropriate for temperate zones; they do not apply to tropical and subtropical regions that receive higher intensity of sunlight. Dahlias require shade from the afternoon sun, which can scorch their leaves and

cause earlier fading of flowers. While beige or sandstone-colored nets work just as well in blocking out 35% of the sun (Menzel, 2016), the potted dahlia in our study were housed under green shade nets with varied densities of woven nylon. In comparison to plants grown in full sun, those exposed to shade seemed taller. Similar results were found when dahlias were grown under shade nets that captured 35% of the sun's incoming light. These plants typically exhibit a higher level of apical dominance as a means of adaptation for absorbing low light (Yazici and Gunes, 2018)

When plants are subjected to shade, their stem INL increases, which suggests a phototropic reaction to capture available sunlight at higher strata. In addition, plants often exhibit specific structural alterations in their leaves (such as enlarged LL and LW) to optimize leaf surface area and capture enough light for photosynthesis. The outcomes are consistent with several studies that were subjected to partially shaded conditions, including Wang *et al.*, 2009 (in Chrysanthemum); Hlatshwayo and Wahome, 2010 (in Carnation); Mapes and Xu, 2014 (in Salvia). In comparison to the plants cultivated in full sunlight, the damask rose (*Rosa damascena* Mill.) plants planted in 50% shade grew substantially taller and had fewer branches overall (Thakur *et al*., 2019).

A certain light intensity is necessary for plant growth; too much or too little light will damage photosystems and decrease photosynthetic efficiency (Devlin *et al*., 2007). In addition to filtering light, shade nets also regulate the microclimate that surrounds plants (Zhao *et al*., 2012). According to Mathur *et al*. (2018), plants grown in low-light conditions tend to devote more of their energy to vegetative growth for longer periods of time. Other studies also show that shade inhibits the onset of the reproductive phase transition in *Antirrhinum majus*, Lisianthus, and other bedding plants (Faust *et al*., 2005; Lugassi-Ben-Hamo *et al*., 2010).

According to Munir *et al.* (2004), plants exposed to shade exhibited higher fresh and dry biomass weight above ground. However, our research showed that when plants were exposed to a higher shade level, the fresh weight of the tubers decreased. This may be explained by the quantitative effects of light on the growth of tuberous roots (Salisbury and Ross, 1991). The findings are consistent with those of Schulz *et al.* (2019) and Clark and Burge (1999), who reported a decrease in tuber weight in potatoes due to self-shading by leaves as a result of increasing planting density. Because some species are photoperiodically sensitive, the effects of light intensity on blooming are greater in the early phases of development (Adams, 1999).

It was observed that the plants exposed to little shade grow thinner with larger leaves in order to capture as much light as possible (Mathur *et al.*, 2018). Our findings are consistent with those of Kumar *et al.* (2013), who found that plants exposed to up to 50% shade experienced greater levels of LA in sage plants. According to Zervoudakis *et al.* (2012), sage plants exposed to full sunshine had a greater leaf count. However, as the plants were shaded up to 75%, the mean leaf count declined. In plants exposed to shade, the longer stem INL can be the reason for the reduced leaf number that emerged from the node axils.

Research indicates that plants exposed to low light levels had a higher specific light absorption (SLA) (Feng and van Kleunen, 2014). This is thought to be a plant's adaptive reaction to boost photosynthetic efficiency (Gommers *et al*., 2013). It has been demonstrated that species unable to tolerate shade typically have greater light compensation points to maintain their rates of photosynthetic activity. The flexibility and tolerance of leaves in reaction to shade is thus indicated by changing the SLA per unit of dry weight (Valladares and Niinemets, 2008; Liu *et al*., 2016).

In an LM-based CP amended GM, the greater percentage of total porosity combined with air-filled porosity led to an improved aeration status, which is fundamentally necessary for efficient gaseous exchange in a limited pot volume. The study's conclusions are consistent with previous reports by Wazir *et al.* (2009) in Alstroemeria, Awang *et al.* (2009) in *Celosia cristata*, and Dubey *et al*. (2013) in Petunia. *Dahlia hortensis* 'Figaro' has also yielded comparable results (Tariq *et al*., 2012), recommending CP as a preferred amendment for raising potted dahlias. Additionally, research conducted by Riaz *et al*. (2008) supports the use of LM modified with CP for high-quality herbaceous *Zinnia elegans* cv. "Blue Point" production. Several studies [Kiran *et al.*, 2007 (*Dahlia pinnata*); Younis *et al*., 2014 (Dahlia cv. Red Skin); Richardville *et al*., 2022 (Tomato); Singh *et al.*, 2023 (Petunia)] have explored the benefits of amending the GM with LM. These studies have demonstrated LM's potential to improve the physical properties of potting substrate by enriching it with organic matter, in addition to providing crucial micronutrients for

plant uptake. Furthermore, because CP is high in K, flowers have better quality with intense hues. The authors do recommend using washed CP that has a lower salt (NaCl) content, though. To lower the rate of N immobilization by the microorganisms in the substrate mix, the washed CP should ideally be stocked for at least one month (Handreck, 1993; Singh *et al.*, 2022).

The tubers that were taken from plants grown in GM with LM amendments were disease-free and in good health. A well decomposed LM has been shown to have a disease-suppressive effect (Bonanomi *et al*., 2010). Furthermore, dahlia plants grown in GM amended with CP showed greater NoT than control plants (GM1). When compared to soil-based media, the CP tends to retain moisture and controls the media's temperature, which is comparatively lower (1±0.5°C). According to Bethke (2023), the lower temperature in GM has been proven to be favorable for tuber growth and clump formation in potatoes, leading to a larger tuber count.

The current results pave the way for more research into the off-season cultivation of dahlia in nutrient-enriched substrates by adjusting temperature, photoperiod, and light intensity to produce high-quality flowers and robust tubers. Furthermore, shade nets that block at least one-third of the sun's rays can be used to create a favorable environment for cultivars that are sensitive to higher temperatures. This could increase the diversity of dahlia germplasm, particularly in tropical and subtropical regions of the world.

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References

ADAMS S.R., 1999 - *The effects of temperature and light integral on the phases of photoperiod sensitivity in* Petunia hybrida. - Ann. Bot., 83(3): 263-69.

AWANG Y., SHAHAROM S.A., MOHAMAD R.B., SELAMAT A., 2009 - *Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of* Celosia cristata. - Am. J. Agric. Biol. Sci., 4(1): 63-71.

BETHKE P.C., 2023 - *Cool soil increases Potato (*Solanum tuberosum*) tuber number in multiple varieties and alters skin color intensity of 'Red Norland'and 'Adirondack Blue*'. - Am. J. Potato Res., 100(1): 79-86.

BHATTACHARJEE S.K., VINAYANANDA S., DE L.C., 2019 - *Dahlia*, pp. 181-200. - In: BHATTACHARJEE S.K (ed.) *Advances in ornamental horticulture*. Pointer Publishers, Jaipur, Rajasthan, India, pp. 375.

BLAKE G.R., HARTGE K.H., 1986 - *Bulk density*, pp. 363-376. - In: KLUTE A. (ed) *Methods of soil analysis*, Part I. ASA Monograph No 9. Madison, Wisconsin, USA, pp. 1358.

BONANOMI G., ANTIGNANI V., CAPODILUPO M., SCALA F., 2010 - *Identifying the characteristics of organic amendments that suppress soilborne plant diseases*. - Soil Biol. Biochem., 42(2): 136-144.

CLARK G.E., BURGE G.K., 1999 - *Effects of nutrition, planting density, and stem pruning treatments on tuber weight and secondary tuber development in* Sandersonia aurantiaca*.* - New Zealand J. Crop Hort. Sci., 27: 319-324.

DALDA SEKERCI A., GÜLŞEN O., 2016 - *Overview of* dahlia *breeding*. Scientific Papers. Series B, Horticulture. LX, pp. 199-204.

DE HERTOGH A., LE NARD M., 1992 - *The physiology of flower bulbs. A comprehensive treatise on the physiology and utilization of ornamental flowering bulbous and tuberous plants*. - Elsevier, Amsterdam, The Netherlands, pp. 811.

DE L.C., BHATTACHARJEE S.K., 2011- *Ornamental crop breeding*. - Aavishkar Publishers Distributers, Jaipur, Rajasthan, India, pp. 454.

DEVLIN P.F., CHRISTIE J.M., TERRY M.J., 2007 - *Many hands make light work*. - J. Exp. Bot., 58(12): 3071-3077.

DUBEY R.K., SINGH S., KUKAL S.S., KALSI H.S., 2013 - *Evaluation of different organic growing media for growth and flowering of petunia*. - Commun. Soil Sci. Plant Anal., 44(12): 1777-1785.

EVANS E., 1998 - *Dahlias for the home landscape*. - Department of Horticultural Science, Cooperative Extension Service, North Carolina State University College of Agriculture & Life Sciences, North Carolina, USA.

FAUST J.E., HOLCOMBE V., RAJAPAKSE N.C., LAYNE D.R., 2005 - *The effect of daily light integral on bedding plant growth and flowering*. - Hortic. Sci., 40(3): 645-649.

FENG Y., VAN KLEUNEN M., 2014 - *Responses to shading of naturalized and non-naturalized exotic woody species*. - Ann. Bot., 114: 981-989.

GOMMERS C.M.M., VISSER E.J.W., ONGE K.R.S., VOESENEK L.A.C.J., PIERIK R., 2013 - *Shade tolerance: when growing tall is not an option*. - Trends Plant Sci., 18: 65-71.

HAMRICK D., 2003 - *Dahlia*, pp. 329-332. - In: HAMRICK D. (ed.) *Ball redbook. Volume 2. Crop production*. - Ball publishing, Batavia, Illinois, USA, pp. 736.

HANDRECK K.A., 1993 - *Properties of cocopeat dust and its use in the formulation of soilless potting media*. - Commun. Soil Sci. Plant Anal., 24(3-4): 349-363.

HLATSHWAYO M.S., WAHOME P.K., 2010 - *Effects of shading on growth, flowering and cut flower quality in carnation* (Dianthus caryophyllus*).* - J. Agric. Sci., 6(2): 34-38.

KIRAN M., BALOCH J., WASEEM K., JILANI M. S., 2007 - *Effect of different growing media on the growth and development of* dahlia *(*Dahlia pinnata*) under the agro-climatic condition of Dera Ismail Khan*. - Pak. J. Agric. Sci., 10(22): 4140-4143.

KUMAR R., SHARMA S., PATHANIA V., 2013 - *Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea *L.) in north western Himalaya*. - J. Essent. Oil Res., 25(1): 23-32.

LIU Y., DAWSON W., PRATI D., HAEUSER E., FENG Y., VAN KLEUNEN M., 2016 - *Does greater specific leaf area plasticity help plants to maintain a high performance when shaded?* - Ann. Bot., 118(7): 1329-1336.

LUGASSI-BEN-HAMO M., KITRON M., BUSTAN A., ZACCAI M., 2010 - *Effect of shade regime on flower development, yield and quality in Lisianthus.* - Sci. Hort., 124(2): 248-253.

MAPES C., XU Y., 2014 - *Photosynthesis, vegetative habit and culinary properties of sage (*Salvia officinalis*) in response to low-light conditions.* - Can. J. Plant Sci., 94(5): 881-889.

MARINA L.J., 2015 - *Cultivation of* dahlia*:* Review. - Cult. Trop., 36(1): 103-110.

MATHUR S., JAIN L., JAJOO A., 2018 - *Photosynthetic efficiency in sun and shade plants*. - Photosynthetica, 56: 354-365.

McCLAREN B., 2004 - *Encyclopedia of* dahlias. - Timber Press, Portland, Oregon, USA, pp. 212.

MENZEL J., 2016 - *Dahlias in Australia the winkie way*. - Bounty printing, Ringwood Victoria, Australia, pp. 126.

MUNIR M., JAMIL M., BALOCH J., KHATTAK K.R., 2004 - *Impact of light intensity on flowering time and plant quality of* Antirrhinum majus *L. cultivar chimes white*. - J. Zhejiang Univ. Sci., *A* 5: 400-405.

NELSON D.W., SOMMERS L.E., 1982 - *Total carbon, organic carbon and organic matter,* pp. 539-579. - In: PAGE A.L., R.H. MILLER, and D.R. KEENEY (eds.). *Methods of Soil Analysis. Part 2 Chemical and Microbiological Properties Agronomy*, 2nd edition, ASA-SSSA, Madison, Wisconsin, USA.

PAIK I., HUQ E., 2019 - *Plant photoreceptors: multi-functional sensory proteins and their signalling networks.* - Seminars Cell Devel. Biol., 92: 114-121.

REDDY K.M., KUMAR R., KIRAN S. B., 2023 - *Impact of climate change on tuber crops production and mitigation strategies*, pp. 167-184. - In: SOLANKEY S.S., and M. KUMARI (eds.) *Advances in research on vegetable production under a changing clima*te. Volume 2. Springer International Publishing, Cham, Switzerland, pp. 369.

RIAZ A., ARSHAD M., YOUNIS A., RAZA A., HAMEED M., 2008 - *Effect of different growing media on the growth and flowering of* Zinnia elegans cv. Blue Point. - Pak. J. Bot., 40(4):1579-1585.

RICHARDVILLE K., EGEL D., FLACHS A., JAISWAL A., PERKINS D., THOMPSON A., HOAGLAND L., 2022 - *Leaf mould compost reduces waste, improves soil and microbial properties, and increases tomato productivity.* - Urban Agric. Reg. Food Syst., 7(e1): 20022.

ROMER J., 2008 - *Growing dahlias*. -Instructional Technology Center, Iowa State University, Ames, IA, USA.

SALISBURY F.B., ROSS C.W., 1991 - *Plant physiology*. - Wadsworth Publishing Company, Belmont, California, USA, pp. 682.

SCHULZ V.S., MUNZV, STOLZENBURG K., HARTUNG J., WEISENBURGER S., GRAEFF-HÖNNINGERV S., 2019 - *Impact of different shading levels on growth, yield and quality of potato (*Solanum tuberosum *L.)*. - Agron., 9(6): 330.

SINGH A.K., KUMAR R., TOMAR K.S., GUPTA A.K., KUMAR H., PATEL R., 2023 - *Influence of potting media composition on quality flower production of petunia* (Petunia hybrida). - J. Plant Nutr., 46(13): 3101-3108.

SINGH A.K., SINGH R., KUMAR R., GUPTA A. K., KUMAR H., RAI A., KANAWJIA A., TOMAR K.S., PANDEY G., SINGH B., KUMAR S., DWIVEDI S.V., KUMAR S., PATHANIA K., OJHA G., SINGH A., 2022 - *Evaluating sustainable and environment friendly growing media composition for pot mum* (Chrysanthemum morifolium *Ramat.).* - Sustainability, 15(1): 536.

SUBBIAH B.V., ASIJA C.L., 1956 - *A rapid procedure for the estimation of available nitrogen in soils*. - Curr. Sci., 25: 259-260.

TARIQ U., REHMAN S.U., KHAN M.A., YOUNIS A., YASEEN M., AHSAN M., 2012 - *Agricultural and municipal waste as potting media components for the growth and flowering of* dahlia *hortensis* 'Figaro'. - Turk. J. Bot., 36(4): 378-385.

THAKUR M., BHATT V., KUMAR R., 2019 - *Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascene *Mill.) under mid hill conditions of Western Himalayas*. - PLoS ONE, 14(4):e0214672.

VALLADARES F., NIINEMETS Ü., 2008 - *Shade tolerance, a key plant feature of complex nature and consequences*. - Annu. Rev. Ecol. Evol. Syst., 39: 237-257.

WANG Y., GUO Q., JIN M., 2009 - *Effects of light intensity on growth and photosynthetic characteristics of Chrysanthemum morifolium*. - Zhongguo Zhong Yo Za Zhi, 34: 1633-1635.

WAZIR J.S., SHARMA Y.D., DHIMAN S.R., 2009 - *Performance of potted Alstroemeria (*Alstroemeria

hybrida *L.) in different growing media under wet temperate conditions*. - J. Ornam. Hortic., 12(3): 167-174.

YAZICI K., GUNES S., 2018 - *The effects of shading treatments on the plant growth rate of some varieties of aster flowers (*Dahlia *spp.) in the ecologic conditions of Tokat (Turkey).* - Appl. Ecol. Environ. Sci., 16(5): 7191-7202.

YOUNIS A., ANJUM S., RIAZ A., HAMEED M., TARIQ U., AHSAN M., 2014 - *Production of quality* dahlia *(*Dahlia variabilis *cv. Redskin) flowers by efficient nutrients management running title: Plant nutrition impacts on dahlia quality.* - Am.-Eurasian J. Agric. Environ. Sci., 14(2): 137-142.

ZERVOUDAKIS G., SALAHAS G., KASPIRIS G., KONSTANTOPOULOU E., 2012 - *Influence of light intensity on growth and physiological characteristics of common sage (*Salvia officinalis *L.).* - Braz. Arch. Biol. Technol., 55: 89-95.

ZHAO D., HAO Z., TAO J., 2012 - *Effects of shade on plant growth and flower quality in the herbaceous peony* (Paeonia lactiflora *Pall.).* - Plant Physiol. Biochem., 61: 187-196.

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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).

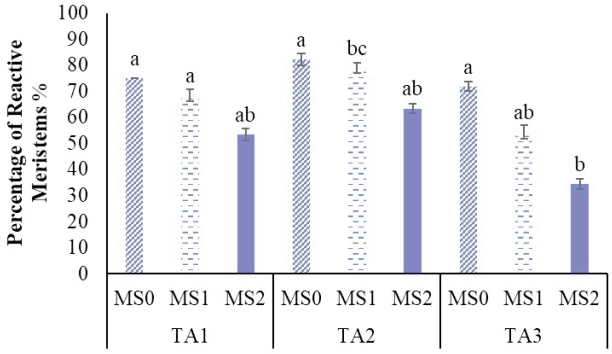

Fig. 1 - Percentage of reactive meristems of the three potato accessions TA1 (Tal Amara1), TA2 (Tal Amara2) and TA3 (Tal Amara3) during culture establishment after 30 days on the different MS media (MS0: 0 hormones, MS1: Kinetin 0.35 mg L^{-1}+ IAA 0.2 mg L^{-1} + GA3 0.1 mg L^{-1} and MS2: BAP: 1 mg 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 ($p<0.05$).

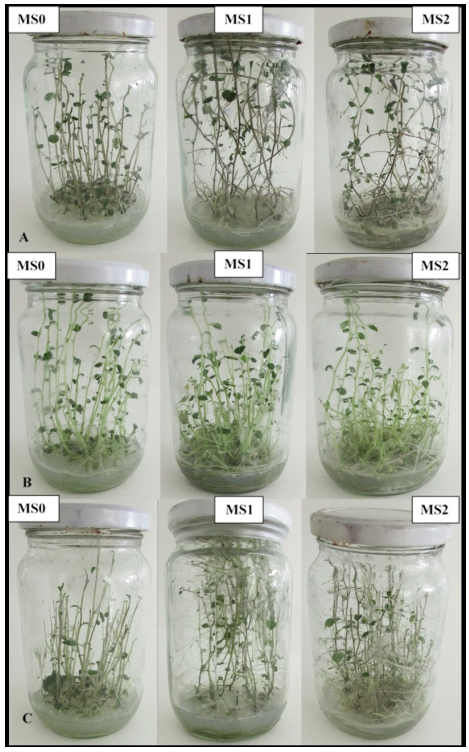

Fig. 2 - Shootlets proliferation in A: Tal Amara1, B: Tal Amara2 and C: Tal Amara3 accessions of potato cultured on different MS media (MS0: 0 hormones, MS1: Kinetin 0.35 mg L^{-1}+IAA 0.2 mg L^{-1}+ GA3 0.1mg L^{-1} and MS2: BAP: 1 mg L^{-1}+ GA3 0.5 mg L^{-1}).

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.

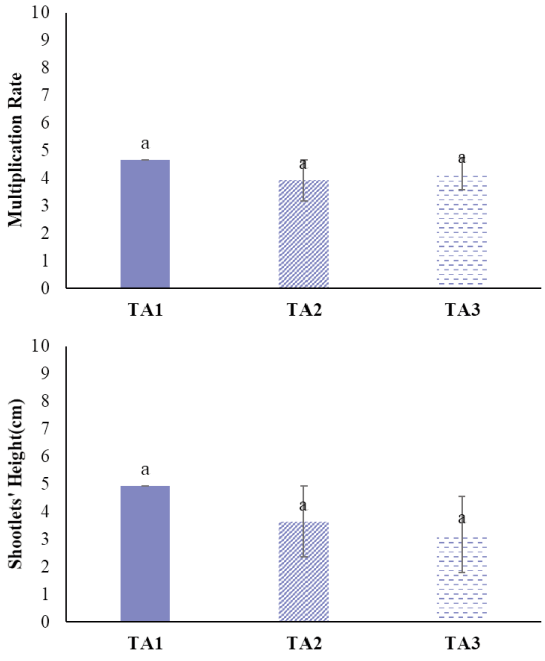

Fig. 3 - Effect of potato accessions (TA1: Tal Amara1, TA2: Tal Amara2 and TA3: Tal Amara3) on the multiplication rate and shootlets height of plants; media and subcultures are combined. same letters indicate not significantly different ($p<0.05$) according to Tukeys' test.

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

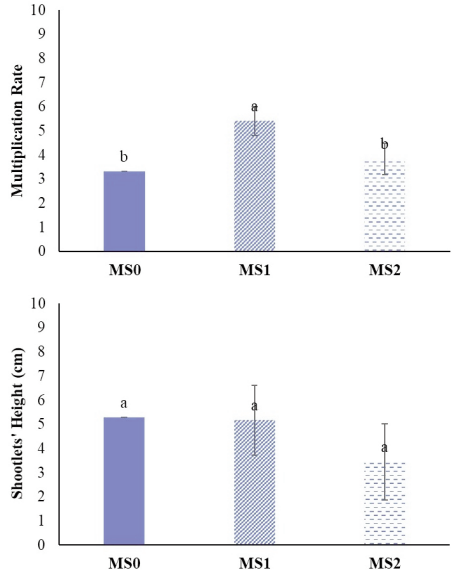

Fig. 4 - Effect of culture media (MS0: Control, MS1: Kinetin 0.35 mg L^{-1} +IAA 0.2 mg L^{-1}+ GA_3 0.1 mg L^{-1} and MS2: BAP: 1 mg L^{-1}+ GA3 0.5 mg L^{-1}) on multiplication rate and shootlets height of potato plants; accessions and subcultures are combined.

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.

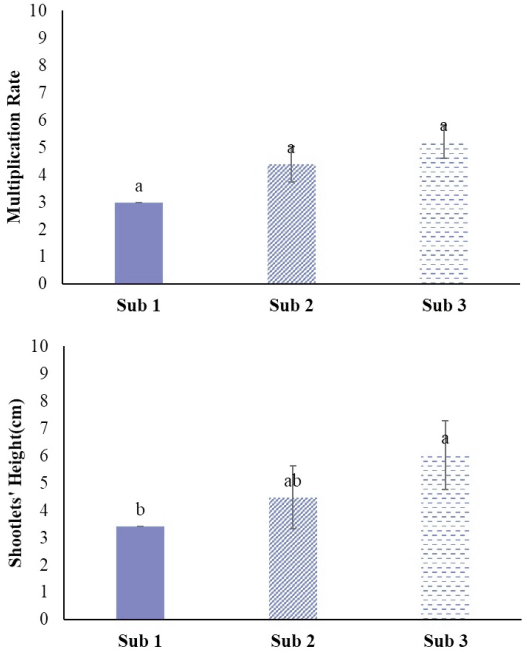

Fig. 5 - Effect of subcultures (sub1, sub2 and sub3) on multiplication rate and shootlets height of potato plants; media and accessions are combined. same letters indicate not significantly different ($p<0.05$) according to Tukeys' test.

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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References

ABDELSALAM Z.K.M., EZZAT A.S., TANTAWY I.A.A., YOUSSEF N.S., GAD EL-HAK S.H., 2021 - *Effect of NaCl salinity stress on potato* (Solanum tuberosum *L.*) *plantlets grown and development under* in vitro *conditions.* - Scientific J. Agric. Sci., 3(2): 1-12.

ADDINSOFT, 2016 - *XLSTAT statistical and data analysis solution for Microsoft excel.* - https://www.xlstat.com/fr/

ALBISKI F., NAJLA S., SANOUBAR R., ALKABANI N., MURSHED R., 2012 - In vitro *screening of potato lines for drought tolerance.* - Physiol. Mol. Biol. Plants, 18(4): 315-321.

ASNAKE D., ALEMAYEHU M., ASREDIE S., 2023 - *Growth and tuber yield responses of potato* (Solanum tuberosum *L.*) *varieties to seed tuber size in northwest highlands of Ethiopia.* - Heliyon, 9(3): e14586.

AZAD M.A.K., KHATUN Z., EATON T.E.-J., HOSSEN M.I., HAQUE M.K., SOREN E.B., 2020 - G*eneration of virus free potato plantlets through meristem culture and their field evaluation.* - Amer. J. Plant Sci., 11(11): 1827-1846.

BAKHSH A., JABRAN K., NAZIK N., ÇALIŞKAN M.E., 2023 - *Conclusions and future prospective in potato production*, pp. 457-470. - In: ÇALIŞKAN M.E., A. BAKHSH, and K. JABRAN (eds.) *Potato production worldwide*. Academic Press, London, UK, pp. 490.

CHEN B.C., WU X.J., GUO H.C., XIAO J.P., 2024 - *Effects of appropriate low-temperature treatment on the yield and quality of pigmented potato (*Solanum tuberosum *L.) tubers*. - BMC Plant Biol., 24(1):274.

CHOUEIRI E., JREIJIRI F., WAKIM S., EL KHOURY M.I., VALENTINI F., DUBLA N., GALLI D., HABCHY R., AKL K., STEFANI E., 2017 - *Surveys of potato-growing areas and surface water in Lebanon for potato brown and ring rot pathogens.* - Phytopathol. Mediterr., 56(1): 87-97.

DALLEH M., BORJAC J., YOUNES G., CHOUEIRI E., CHEHADE A., ELBITAR A., 2023 - In vitro *propagation and microtuberization of potato (*Solanum tuberosum *L.)* Spunta *variety in Lebanon*. - Adv. Hort. Sci., 37(3): 243-253.

DESSOKY S.E., ATTIA O.A., ISMAIL A.I., EL- HALLOUS I.E., 2016 - In vitro *propagation of potato under different hormonal combinations*. - Int. J. Adv. Res., 4(1): 684-689.

EBAD F.A.S., EL-SADEK M.E.A., EL-KAZZAZ A.A, 2015 - *Micropropagation of four potato cultivars* in vitro*.* - Academia J. Agric. Res., 3(9): 184-188.

EMARAA H.A., HAMZA E.M., FEKRY W.A., 2017 - In vitro *propagation and microtuber formation of potato in relation to different concentrations of some growth regulators and sucrose.* - Middle East J. Agric. Res., 6(4): 1029-1037.

GARRAMONE R., COPPOLA G.P., AVERSANO R., DOCIMO T., SEDLÁK P., CARPUTO D., 2023 - In vitro *assessment of salt stress tolerance in wild potato species*. - Agronomy, 13(7): 1784.

GERVAIS T., CREELMAN A., LI X.Q., BIZIMUNGU B., DE KOEYER D., DAHAL K., 2021 - *Potato response to drought stress: physiological and growth basis*. - Front Plant Sci., 12: 698060.

HAJARE S.T., CHAUHAN N.M., KASSA G., 2021 - *Effect of growth regulators on* in vitro *micropropagation of*

potato (Solanum tuberosum *L.*) *Gudiene and Belete varieties from Ethiopia*. - Scientific World J., 2021: 1-8.

HANDAYANI T., WATANABE K.N., 2021 - *Using* in vitro *technique for drought, heat, and combined drought-heat assay in potatoes.* - IOP Conf. Series Earth Environmental Sci., 762(1): 012061.

HUSSEN A., 2021 - *Intra-row spacing on the growth, yield and yield component of potato* (Solanum tuberosum *L.*): A *review*. - Int. J. Curr. Res. Aca. Rev., 9(06): 11-16.

JAFARI F., PANAHANDEH J., AZAR A.M., GIGLOU M.T., 2019 - *The responses of potato cultivars to osmotic and temperature stresses under in vitro conditions.* - Iranian J. Plant Phys., 9(3): 2827-2847.

KHENIFI M.L., BOUDJENIBA M., KAMELI, A., 2011 - *Effects of salt stress on micropropagation of potato* (Solanum tuberosum *L.*). - Afr. J. Biotech., 10(40): 7840-7845.

MARCHIN R.M., BACKES D., OSSOLA A., LEISHMAN M.R., TJOELKER M.G., ELLSWORTH D.S., 2021 - *Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species*. - Global Change Biol., 28(6497): 1133-1146.

MOHAMED F.H., ABD EL-HAMED K.E., ELWAN M.W.M., EL-MAGAWRY N.A., ABD EL-SALAM M.M., 2016 - In vitro *screening of different potato genotypes for heat stress tolerance.* - Catrina, 15(1): 77-93.

MORAIS T.P., ASMAR S.A., SILVA H.F.J., LUZ J.M.Q., MELO B., 2018 - *Application of tissue culture techniques in potato*. - Biosci. J., Uberlândia, 34(4): 952-969.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures*. - Physiol. Plant., 15(3): 473-497.

MUTHONI J., MUCHIRA C., KABIRA J.N., 2014 - *Multiplication rate of selected potato cultivars* in vitro *through single node culture.* - Int. J. Hort., 4(15): 1-4.

MUTHONI J., SHIMELIS H., 2020 - *Heat and drought stress and their implications on potato production under dry African tropics*. - AJCS, 14(09): 1405-1414.

MWAKIDOSHI E.R., GITARI H.H., MAITRA S., MUINDI E.M., 2021 - *Economic importance, ecological requirements and production constraints of potato* (Solanum tuberosum *L.*) *in Kenya*. - IJBS, 08(02): 61-68.

NAHAR L., AYCAN M., HANAMATA S., BASLAM M., MITSUI T., 2022 - *Impact of single and combined salinity and high-temperature stresses on agro-physiological, biochemical, and transcriptional responses in rice and stress-release.* - Plants, 11(4): 501.

OTHMAN M.H.A., ABIDO A.I.A., JABAL A.A.A., 2016 - In vitro *propagation and* ex vitro *acclimatization of potato (*Solanum tuberosum *L.) using nodal cutting explants*. - J. Adv. Agric. Res., 21(1).

PANT N., AGARRWAL R., AGRAWAL S., 2014 - *Mannitol-induced drought stress on calli of* Trigonella foenum-graecum *L. var.* RMt-*303.* - Indian J. Exp. Biol., 52(11): 1128-1137.

PRADHAN S., REGMI T., RANJIT M., PANT B., 2016 - *Production of virus-free orchid* Cymbidium Aloifolium *(L.) Sw. by various tissue culture techniques*. - Heliyon, 2(10): e00176.

RAFIQUE S., ABDIN M.Z., ALAM W., 2019 - *Response of combined abiotic stresses on maize (*Zea mays *L.) inbred lines and interaction among various stresses.* - Maydica, 64(22): 1-8.

REDDY K.R., SEGHAL A., JUMAA S., BHEEMANAHALLI R., KAKAR N., REDOÑA E.D., WIJEWARDANA C., ALSAJRI F.A., CHASTAIN D., GAO W., TADURI S., LONE A.A., 2021 - *Morpho-physiological characterization of diverse rice genotypes for seedling stage high and low-temperature tolerance*. - Agronomy, 11(112): 1-19.

REN R., WANG T., GAO L., SONG P., YANG Y., ZHI H., LI K., 2022 - *Development of comprehensive serological techniques for sensitive, quantitative and rapid detection of soybean mosaic virus*. - Int. J. Mol. Sci., 23(16): 9457.

SALEM J., HASSANEIN A.M., 2017 - In vitro *propagation, microtuberization, and molecular characterization of three potato cultivars*. - Biologia Plantarum, 61(3): 427-437.

SATTAR F.A., HAMOOH B.T., WELLMAN G., ALI M.A., SHAH S.H., ANWAR Y., MOUSA M.A.A., 2021 - *Growth and biochemical responses of potato cultivars under* in vitro *lithium chloride and mannitol simulated salinity and drought stress*. - Plants, 10(5): 924.

SHIWANI K., KUMAR R., RANA A., KUMAR D., SHARMA N., SINGH N., 2021 - *Recent advances in potato propagation,* pp. 255-261*.* - In: BWUE A., A. ROUT, S. NAYAK, K. PRADHAN, and N. KHARE (eds.). *Recent trends in propagation of forest and horticultural crops*. Taran Publication, New Delhi, India, pp. 388.

SINGH P., AGNIHOTRI R.K., BHADAURIA S., VAMIL R., SHARMA R., 2012 - *Comparative study of potato cultivation through micropropagation and conventional farming methods*. - Afr. J. Biotech., 11(48): 10882-10887.

TESSEMA L., MOHAMMED W., ABEBE T., 2020 - *Evaluation of potato (*Solanum tuberosum *L.) varieties for yield and some agronomic traits.* - Open Agric., 1: 63-74.

TICAN A., CIOLOCA M., BĂRĂSCU N., BĂDĂRĂU C. L., HERMEZIU M., 2021 - In vitro *screening of potato varieties for drought stress induced with different osmotic agents.* - Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series), 31(2): 78-84.

VERNER D., ASHWILL M., CHRISTENSEN J., MCDONNELL R., REDWOOD J., JOMAA I., SAADE M., MASSAD R., CHEHADE A., BITAR A., TREGUER D., 2018 - *Droughts and agriculture in Lebanon: causes, consequences, and risk management.* - World Bank, pp. 26.

XHULAJ D.B., GIXHARI B., 2018 - In vitro *micropropagation of potato (*Solanum Tuberosum *L.) cultivars*. - Agric. Forestry, 64(4): 105-112.

ZAKI H.E.M., RADWAN K.S.A., 2022 - *Response of potato* (Solanum tuberosum *L.*) *cultivars to drought stress under* in vitro *and field conditions*. - Chem. Biol. Technol. Agric., 9(1).

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An efficient nutrient medium for asymbiotic seed germination and *in vitro* plant generation of *Vanda tessellata* (Roxb.) Hook. ex G. Don

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Key words: Banana, charcoal, genetic stability, micropropagation, protocorm, RAPD analysis, seedling development.

Abstract: *Vanda tessellata* (Roxb.) Hook. ex G. Don is an epiphytically grown orchid well-known for its excellent floral value and therapeutic qualities. The present investigation deals with a study of asymbiotic seed germination and large-scale *in vitro* plant generation of *Vanda tessellata* by using three different basal media (MS, KC, and VW) and two supplements, charcoal and banana. Of these three media used for seed germination, MS (Murashige and Skoog) gave the best response, followed by KC (Knudson C) and VW (Vacin and Went). MS medium took less time to germinate seeds and maximum protocorm formation was also observed. MS medium with banana powder (15,000 mg/l) showed the best result for developing seedlings from protocorm and maximum growth of leaves and roots of the seedlings. Propagation through secondary protocorm formation was highest in MS media with charcoal (1000 mg/g). *In vitro*-grown plants were successfully acclimatized with an 89.4% survival rate. According to a random amplified polymorphic DNA (RAPD) analysis, the *in vitro* generated plants were clone copies of their parent plant and did not exhibit variations. These findings validated the most trustworthy techniques, which can also be applied for to large-scale medicinal *Vanda tessellata* plant production at the commercial level.

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1. Introduction

Orchids are a unique group of flowering plants belonging to the family Orchidaceae. Orchids are popularly known for their beautiful, attractive

flower, long shelf life, and high purchase prices. Among monocotyledons, the Orchidaceae is a highly evolved family comprising nearly 850 genera (Stewart and Griffith, 1995; Singh *et al*., 2007; Gutierrez, 2010; Madhavi and Shankar, 2019). It has been reported that 28,237 species are distributed in the tropical forests of India, South Asia, Sri Lanka, South and Central America, and Mexico (Willis, 2017). In India, 155 recognized genera with 1256 orchid species are found in different habitats (Singh *et al*., 2019). According to available records, 466 taxa of orchids are found In West Bengal (Mitra, 2021). There are 12 orchid genera recorded in the Purulia district (Paramanik *et al.,* 2020).

Vanda tessellata (Roxb.) Hook. ex G.Don is a medicinally important epiphytic orchid of the family Orchidaceae. The pollination mechanism of orchids is highly specialized and seeds are small, thin, and non-endospermic. It is reported that approximately 1,300 to 4 million seeds are present per capsule (Pierik, 1987). Due to the lack of endosperm, a symbiotic association with mycorrhizal fungi is required to provide nutrients to embryos that are required for the seed germination of orchids in their natural habitat (Paramanik *et al.,* 2021). The epiphytic orchid's 30- 60 cm-tall stem is furnished with thickly coriaceous, recurved, plicate, and obtuse-keeled leaves. Flowers are greenish-yellow, with brown specks on the lip's middle lobe (Chauhan, 1999). The petals are shorter than the sepals, yellow with brown lines and white borders. The lip measures 16 mm in length and is bluish with purple specks. Capsules are with acute ribs that are 7.5-9.0 cm long and narrowly clavate-oblong (Fig. 1).

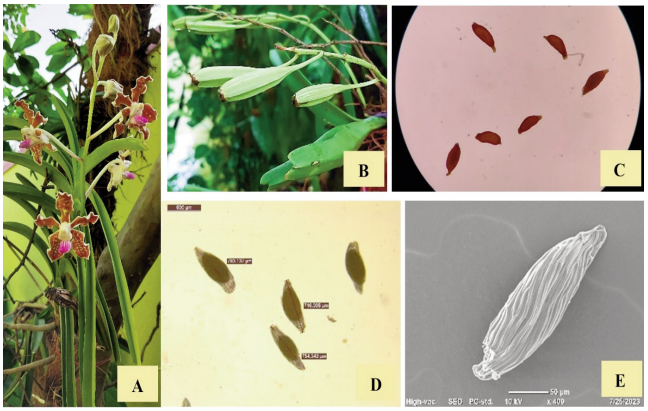

Fig. 1 - Morphological image of *Vanda tessellata*. (A) Plant with flower spike, (B) Mature capsule, (C) Light microscopic image of seeds (10x), (D) Stereo microscope image of seeds, (E) Scanning Electron Microscopy image of seed (400x).

In traditional medicine, *V. tessellata* has been commonly used to treat a variety of ailments, including fever, rheumatism, dysentery, and dyspepsia. The juice of the leaves is applied topically to treat otitis media. The root is used as a bronchitis cure and as an antidote to scorpion stings (Chauhan, 1999).

The value of orchids as a commodity has grown daily. Due to demands from massive collections in the past, the habitat of this medicinally significant orchid is being destroyed, which has caused the species to become rare and limited to minimal areas within its native habitats (Kaur and Bhutani, 2009). In nature, orchids have been propagated vegetatively to solve this problem, but it is a prolonged process. Therefore, plant tissue culture and micropropagation can be extremely effective in preventing the extinction of this orchid and increasing its population (Wochok, 1981). As a result, orchids must depend on external sources of nutrients for germination and large-scale production. The asymbiotic seed germination culture method, which was first commenced by Knudson (1946), is commonly used for seed germination of orchids. Another method for micropropagating orchids is to employ aseptically produced seedlings (Bhadra, 1999).

Molecular markers are crucial for determining the genetic diversity, variation, and resemblance of various plants and their population structure. Genetic variation is responsible for various factors related to *in vitro* culture settings (Pradhan *et al*., 2023). During *in vitro* culture, sometimes somaclonal variation changes the genetic composition of the regenerants (Rawat *et al*., 2013). Several molecular markers have been used to evaluate the genetic fidelity of clones generated *in vitro*. One effective and affordable method for identifying plant genetic variability is random amplified polymorphic DNA (RAPD)(Hussain *et al*., 2008).

This study aimed to develop an efficient nutrient medium for asymbiotic seed germination and large-scale *in vitro* plant generation of *V. tessellata* (Roxb.) Hook. ex G.Don. and also assess the genetic fidelity of *in vitro* regenerants with mother plants through RAPD analysis.

2. Materials and Methods

Establishment of culture

Seeds from 7-month-old, undehisced green pods

were used to establish cultures. Undehisced green pods of *V. tessellata* (Roxb.) Hook. ex G. Don were collected from the trunks of different trees in the Ajodhya hills of Purulia district, West Bengal.

The freshly harvested capsules were first given a five to ten-minute rinse under running tap water. After that, pods were rinsed in 90% ethanol for 20-30 seconds, treated with 0.1% (w/v) mercuric chloride solution for 10 minutes, and then the surface sterilization procedure was completed by washing the material three times in sterile distilled water. After excising the sterilized pods lengthwise, the seeds were scooped out and put in a conical flask with 100 ml of autoclaved distilled water. The mixture was then slowly shaken for five minutes. Culture tubes (25 x 150 mm) with 10 ml of nutrient media were inoculated with 100 µl of the seed suspension. Conical flasks (250 ml, 500 ml) and culture bottles (500 ml) contained 50 to 100 ml of nutrient media were used for plantlet development.

Three basal media, KC (Knudson, 1946), MS (Murashige and Skoog, 1962), and VW (Vacin and Went, 1949), hormone-free, were used for asymbiotic seed germination. Sucrose (3% w/v) was used as the carbon source. The pH of the medium was adjusted to 5.6 before autoclaving. After adding 0.8% (w/v) agar to solidify the media, the media were autoclaved at 125°C (15 psi) for 20 min. The cultures were kept at 24±2°C with a 10-hour photoperiod supplied by 3000-lux white fluorescent Philips lights.

Multiplication and rooting

MS media containing different concentrations of banana powder (15000 mg/l, 30000 mg/l, 60000 mg/l) and activated charcoal (1000 mg/l, 2000 mg/l, 3000 mg/l) added to the medium singly, were used to obtained well-developed seedlings from healthy protocorms. A combination of banana and charcoal in three different concentrations was also used in MS medium for plantlet development. Rooting occurs in the same medium. The cultures were kept at 24±2°C with a 10-hour photoperiod supplied by 3000-lux white fluorescent Philips lights.

Acclimatization of seedlings

Only seedlings with fully grown roots were chosen for the acclimatization phase. The nutrient medium was then completely removed from the entire seedlings by giving them a thorough water wash. The rooted seedlings were transferred to containers filled with potting mix containing small charcoal pieces, coconut husk, sphagnum moss, broken breaks, and dead tree bark (mango). Subsequently, they were kept in the growth chamber to maintain humidity (80%) and temperature (24±2°C) for a few weeks. After that, the plantlets were moved to a moist, shady place in the departmental garden, and water was applied to the plants twice a day.

RAPD fingerprinting analysis

The genetic stability of wild and *in vitro* propagated plantlets was assessed in the current study through the RAPD fingerprinting technique. Genomic DNA was isolated from the leaf tissue of both the control mother plant and five consecutive generations of *in-vitro*-grown plants. Leaf tissue was subjected to whole genomic DNA extraction following the supplied protocol of the DNA extraction kit (DNeasy® Plant Mini Kit-Qiagen, part no. 69104). The quality and quantity of the DNA samples were determined by recording the ratio of absorbance at A260/A280 in a UV-VIS spectrophotometer (UV-1800 SHIMADZU). The integrity of the genomic DNA was confirmed by electrophoresis on a 0.8% agarose gel. PCR amplification was done in 25 µl reaction volume containing 50 ng of genomic DNA, 8 µM primer (RAPD), molecular biology grade water, and 12.5 µl Hi-Chrome PCR Master Mix containing Taq DNA polymerase, dNTPs, $MgCl_2$.

A total of 10 primers from the OPA and OPB series (Integrated DNA Technologies) were used for PCR. Amplification was executed in DNA Thermal Cycler (Eppendorf Mastercycler Nexus X2 Thermal Cyclers). The initial denaturation temperature for the PCR was 94°C, which was succeeded by 35 denaturation cycles for 45 s at 94°C, annealing for 45 s at 27-38°C, and extension at 72°C for 30 s. Following the last cycle, a final extension step was included, lasting 7 minutes at 72°C. The amplified products were electrophoresed in a horizontal gel apparatus (Power PackTM Basic, Bio-Rad) with a "100 bp" DNA ladder (BioLitTM ProxiB) used to visualize and take pictures of the gels. Finally, the Gel Documentation system (Gel DocTM XR+, Bio-Rad, USA) was used to examine and evaluate the stained (0.5 µg/l) gel with ethidium bromide. To verify the reproducibility of each PCR, it was performed three times.

Data collection and statistical analysis

All parameters (germination percentage, protocorm formation percentage, survival, leaf formation, root formation, callus formation,

secondary protocorm formation percentage) were evaluated and analyzed using SPSS and expressed as mean ± standard error (SE). Three replicate cultures were set up for each treatment. One-way analysis of variance (ANOVA) was used to identify significant differences in data of all treatments, and Duncan's multiple range test (p=0.05) was performed to separate the means.

3. Results and Discussion

Asymbiotic seed germination

The shape and colour change of the seed was used to observe the response to seed germination. Most of the seeds were embryonated, and the testa of the seed was ruptured to form a swollen globular structure (Fig. 2 A). The beginning of seed germination and the development of protocorm on three basal media following, MS, KnC, and VW media were periodically recorded from the first inoculation day (Table 1). Among the three basal media, the highest percentage of germination (83.50±0.31%) was recorded in the MS medium in shorter time (45 days). Second-stage protocorm with a slightly elongated apical region was also (70.00±0.06) observed in MS medium after 20 days. In the other media assessed the germination occurred beyond 100 days with lower germination and protocorm formation percentages (Table 1).

Fig. 2 - Asymbiotic seed germination and seedling development of *Vanda tessellata*. (A-B) Early globular stage, (C) Protocorm showing initiation of leaf primordia, (D-E) Protocorm with distinct leaf and developed seedling with leaf and root, (F) Multiple protocorms with many leaf primordia, (G) Axillary shoot formation from multiple protocorms (H) Leaf formation from secondary protocorm in MS with charcoal (1000 mg/l), (I-J) Close view of leaf primordia from secondary protocorm, (K) Protocorm with callus, (L-O) Sequential stage of seedling growth after subculture on medium containing MS with 15000 mg/l banana powder, (P-Q) Stepwise acclimatization.

Seedling development: multiplication and rooting

Healthy protocorms were transferred to MS media supplemented with charcoal or banana powder. The highest percentage of leaf formation (82.40±2.74) and healthy root formation (83.50± 2.00) were found in MS medium-containing banana powder (15000 mg/l). Leaf formation and root formation both were observed in the same medium composition. A little callus development in this medium was also noticed (Fig. 2 K). Secondary protocorms originated from protocorms, were observed and the highest percentage was formed in MS with charcoal (2000 mg/l) (Fig. 2 I and J). A combination of banana and charcoal at different concentrations showed no significant result (Table 2). Only a combination with low concentration

Table 1 - Effect of different media on the seed germination of *V. tessellate*

Media	Time duration required for germination (days)	Germination (%)	Protocorm formation (%)
Murashige and Skoog (MS)	46	83.50±0.31 c	70.00±0.06 c
Knudson C (KnC)	103	28.00±0.05 b	35.00±0.08 b
Vacin and Went (VW)	190	10.00±0.14 a	2.00±0.14 a

The mean of three replicates ± SE (standard error) is displayed in each column. Mean values followed by the same letter do not differ significantly at the 0.05 level (DMRT).

Table 2 - Effect of charcoal and banana and their combination on the morphogenetic responses and growth of *V. tessellata* seedlings after 3 months of *in vitro* culture

Treatments	Survival (%)	Leaf formation (%)	Root formation (%)	Callus formation (%)	Secondary protocorm formation (%)
MS	64.33±2.14 e	37.08±0.93 e	30.30±1.38 b	18.97±1.36 c	10.71±0.82 c
MSC1	63.50±1.79 e	26.70±1.13 d	0.00±0.00 a	0.00±0.00 a	35.50±1.75 e
MSC2	42.00±1.32 d	18.70±0.66 b	0.00±0.00 a	0.00±0.00 a	23.50±1.80 d
MSC3	12.20±1.43 b	10.20±0.70 b	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
MSB1	99.50±0.28 f	82.40±2.74 g	83.50±2.00 d	11.40±0.77 b	5.90±0.94 b
MSB2	42.50±2.34 d	42.50±1.89 f	40.20±1.74 c	0.00±0.00 a	0.00±0.00 a
MSB3	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
MSC1B1	24.40±1.13 c	24.40±1.92 d	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
MSC2B2	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
MSC3B3	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a

The mean of three replicates ± SE (standard error) is displayed in each column. Mean values followed by the same letter do not differ significantly at the 0.05 level (DMRT). C1= Charcoal 1000 mg/l, C2= Charcoal 2000 mg/l, C3= Charcoal 3000 mg/l, B1= Banana 15000 mg/l, B2= Banana 30000 mg/l, B3= Banana 60000 mg/l.

(MSC1B1) showed a slight survival response (24.40±1.13) along with leaf formation as compared to the other two combinations (Table 2). Previously, Aktar *et al*. (2008) and Islam *et al*. (2015) reported that banana homogenate (BH) had beneficial and boosting effects on the regeneration of new PLBs and a healthy shoot system established from PLBs in *Dendrobium* orchids. Mature bananas are fairly rich in vitamin B6 or pyridoxine. However, they have comparatively high concentrations of vitamins A (carotene), C (ascorbic acid), and B-complex (niacin, thiamine, and riboflavin)(Qamar and Shaikh, 2018). Minerals including sodium (Na), iron (Fe), copper (Cu), phosphorus (P), manganese (Mn), zinc (Zn), copper (Fe), and especially potassium (K) can also be found in bananas (Sarma *et al.,* 2021).

Potassium can help to provide resistance to drought, helping orchids to transport water from the roots to the apices, and preventing the orchids from wilting (Xu *et al.,* 2021). According to Minea *et al*. (2004), 10% banana homogenate increased the size of the leaves on *Spathoglottis kimballiana* Hook. f. In *Dendrobium nobile* Lindl. cultures, banana homogenate considerably boosted the formation of leaves (Sudeep *et al*., 1997). Activated Charcoal (AC) can be used in media to reduce phenolic browning. Browning of explant of several plant species has been controlled using the AC (Meziani *et al*., 2016; Mittal *et al*., 2016; Rani and Dantu, 2016; Magrini and Devitis, 2017; Irshad *et al*., 2018). Kim *et al.* (2019) reported that MS medium supplemented with AC had prevented browning in seedling development of *Pecteilis radiata*. The adsorptive qualities of AC are principally noted for both its beneficial and detrimental effects. By adsorbing phenolic molecules and inactivating peroxidase and polyphenol oxidase, the AC stopped browning (Pan and van Staden, 1998), but large concentrations of AC can absorb the PGRs and mineral nutrients in the culture medium, reducing the frequency of seedling conversion. All plants lost their viability after subculturing on the media containing MSB3, MSC2B2, and MSC3B3 (Table 2). Indeed, high concentrations of AC and banana powder drastically affected the survival.

Acclimatization

A vital stage in the micropropagation process is acclimatization. Plantlets (about 5-6 cm) were moved to pots containing charcoal, coconut husk, brick, mango bark, and sphagnum moss in a 2:2:2:1:1 ratio. With a 92% survival rate, the *in vitro*-raised seedlings were acclimated in a plant growth chamber in the laboratory for 2 months. After that, an 89.4% survival rate with more or less similar healthy plants was observed after 10 months of transfer in the poly-house.

Genetic fidelity and assessment of in vivo and in vitro plants by RAPD fingerprinting analysis

According to earlier studies, RAPD is a widely used marker to assess the genetic fidelity of different micropropagated plants (Kawiak and Lojkowska,

2004; Tikendra *et al*., 2019). The genetic make-up of *in vivo* and *in vitro* generated plants was compared using ten randomly selected Random amplified polymorphic DNA (RAPD), a dominant marker. The leaves of the mother plant and the leaves of five successive generations of *in vitro*-grown plants were collected, and the leaf tissues were used to extract the DNA. Seven of the ten randomly chosen RAPD primers produced unique band patterns in the current investigation. These are OPA-03, OPA-10, OPA-11, OPA-15, OPA-18, OPA-19 and OPB-01 (Table 3). *In vitro,* regenerated plants and the mother plant growing in the garden (the plant from which the explants were collected) were both genetically homogeneous, as evidenced by the lack of variance in the banding pattern displayed by any of the primers. The annealing temperature of the seven primers that exhibited scorable monomorphic band patterns is shown in a tabular format. The temperature at which all seven primers were annealed was 27°C. According to Pradhan *et al*. (2023), RAPD is the successful marker to assess the genetic fidelity of *in vitro* grown plants with the mother plant. The current investigation validates the earlier reports.

Six unique monomorphic bands having a size range of 320 bp to 900 bp were generated by OPA 03 (Fig. 3). OPA 18 yielded four unique monomorphic bands having a size range of 450 bp to 1000 bp. Two distinct monomorphic bands were produced from OPA 10, and the size range of the bands was from 800 to 920 bp (Fig. 3). The fact that all of the bands were monomorphic demonstrated the genetic stability of *in vitro* regenerants and the similarity of the genetic makeup of the micropropagated plants to the mother plant, which was the actual objective of the current investigation.

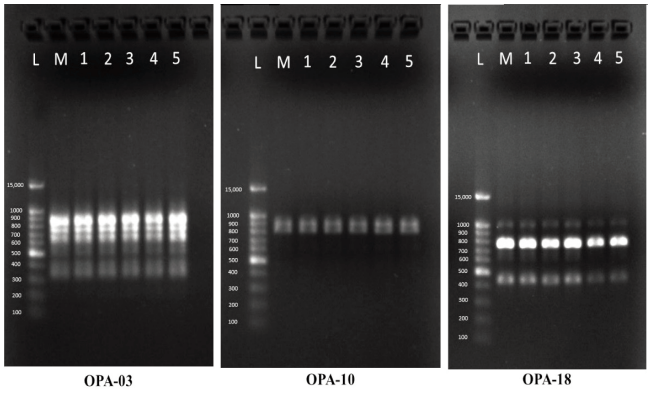

Fig. 3 - Gel electrophoresis of RAPD fragments of *Vanda tessellata* obtained with primer OPA-03, OPA-10, and OPA-18. Lane L 100 bp DNA ladder; lane M mother plant, lanes 1-5 are i*n vitro* regenerated plants of five successive generations.

4. Conclusions

The present research reports a successful nutrient culture medium for asymbiotic seed germination of *V. tessellata*. An efficient, cost-effective nutrient

Table 3 - Effects of sowing date and mulching on marketable root yield (MRY), unmarketable root yield (URY), and weed density (WD)

Primer	Sequence (5'-3')	Tm (°C)	Total bands
OPA-03	AGTCAGCCAC	27	6
OPA-10	GTGATCGCAG	27	2
OPA-11	CAATCGCCGT	27	1
OPA-12	TCGGCGATAG	27	0
OPA-15	TTCCGAACCC	27	2
OPA-18	AGGTGACCGT	27	4
OPA-19	CAAACGTCGG	27	2
OPA-20	GTTGCGATCC	27	0
OPB-01	GTTTCGCTCC	27	2
OPB-12	CCTTGACGCA	27	0
Total			18

Mean values within rows and columns followed by a different letter(s) are significantly different at a 5% probability level.
CV = coefficient of variation. LSD = least significant difference.

media for large-scale *in vitro* plant generation was also achieved by using banana powder supplementation. From the above findings, it may be concluded that MS medium with banana powder is the best medium for overall seedling growth and multiple protocorm formation of *V. tessellata*. Through RAPD analysis, it has been successfully proved that all regenerants were genetically similar to the parent plant. The media reported in the current study does not include the use of plant growth regulators (PGRs) for plant development and multiplication, this condition minimizes the possibility of occurrence of genetic alterations. This study would help the pharmaceutical and floriculture industry and conserve wild populations of orchids in the near future.

References

AKTAR S., NASIRUDDIN K.M., HOSSAIN K., 2008 - *Effects of different media and organic additives interaction on in vitro regeneration of* Dendrobium *orchid.* - J. Agric. Rural Devel., 6: 69-74.

BHADRA S.K., 1999 - *Development of* in vitro *micropropagation techniques in some orchid species of Bangladesh*. - M.Sc. Dissertation, Dept. of Botany, Chittagong University, Bangladesh.

CHAUHAN N.S., 1999 - *Medicinal and aromatic plants of Himachal Pradesh*. - Indus. Publishing Company, New Dehli, India, pp. 632.

GUTIERREZ R.M.P., 2010 - *Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology*. - J. Med. Plants Res., 4(8): 592-638.

HUSSAIN Z., TYAGI R.K., SHARMA R., AGRAWAL A., 2008 - *Genetic diversity in* in vitro-*conserved germplasm of* Curcuma *L. as revealed by RAPD markers*. - Biol. Plant, 52: 627-633.

IRSHAD M., RIZWAN H.M., DEBNATH B., ANWAR M., LI M., LIU S., HE B., QIU D., 2018 - *Ascorbic acid controls lethal browning and pluronic F-68 promotes high-frequency multiple shoot regeneration from cotyledonary node explant of okra (*Abelmoschus esculentus L.*).* - HortSci., 53(2): 183-190.

ISLAM M.O., ISLAM M.S., SALEH M.A., 2015 - *Effect of banana extract on growth and development of protocorm like bodies in* Dendrobium sp*. orchid.* - Scientific J. Krishi Found., 13(1): 101-108.

KAUR S., BHUTANI K.K., 2009 - In vitro *propagation of* Vanda testacea *(Lindl.) Reichb. f. - A rare orchid of high medicinal value.* - Plant Tissue Cult. Biotech., 19(1): 1-7.

KAWIAK A., LOJKOWSKA E., 2004 - *Application of RAPD in the determination of genetic fidelity in micropropagated* Drosera *planlets*. - In Vitro cell. Dev. Biol. Plant., 40: 592-595.

KIM D.H., KANG K.W., ENKHTAIVAN G., JAN U., SIVANESAN L., 2019 - *Impact of activated charcoal, culture medium strength and thidiazuron on non-symbiotic* in vitro *seed germination of* Pecteilis radiata *(Thunb.) Raf.* - South Afr. J. Bot., 124: 144-150.

KUNDSON L., 1946 - *A new nutrient solution for the germination of orchid seeds*. - Amer. Orchid Soc. Bull., 15: 214-217

MADHAVI M., SHANKAR P.C., 2019 - *Effect of different growth additives on seed germination of* Vanda tessellata *(Roxb.) Hook. Ex. G. Don. - A medicinal orchid.* - J. Orchid Soc. India, 33: 105-112.

MAGRINI S., DEVITIS M., 2017 - In vitro *reproduction of three* Limodorum *species (Orchidaceae): impacts of scarification methods and nitrogen sources on mature seed germination and seedling development*. - Plant Biosystems, 151: 419-428.

MEZIANI R., JAITI F., MAZRI M.A., HASSANI A., BEN SALEM S., ANJARNE M., AIT CHITT M., ALEM C., 2016 - *Organogenesis of* Phoenix dactylifera *L. cv. Mejhoul: Influences of natural and synthetic compounds on tissue browning, and analysis of protein concentrations and peroxidase activity in explants.* - Scientia Hortic., 204: 145-152.

MINEA M., PILUEK C., MENAKANI T.A., TANTIWIWAT S., 2004 - *A study on seed germination and seedling development of* Spathoglottis kimballiana. - Agric. Nat. Resources, 38 (2): 141-156.

MITRA S., 2021 - *Diversity of the orchids flora of West Bengal, India.* - Plant archives, 21(2): 740-756.

MITTAL P., DEVI R., GOSAL S.S., 2016 - *Effect of genotypes and activated charcoal on high frequency in vitro plant regeneration in sugarcane.* - Indian J. Biotech., 15: 261-265.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures*. - Physiol. Plant., 15(3): 473-497.

PAN M.J., VAN STADEN J., 1998 - *The use of charcoal in* in vitro *culture-a review.* - Plant Growth Regulation, 26: 155-163.

PARAMANIK M., MAHATO A., RAHA S., 2020 - *Orchids in Purulia District, West Bengal.* - J. Botan. Soc. Bengal, 74(2): 124-131.

PARAMANIK P., KAR D.K., RAHA S., 2021 - *A review on asymbiotic seed germination in orchids through plant tissue culture.* - J. Scientific Enquiry, 1(1): 1-7.

PIERIK R.L.M., 1987 - In vitro *culture of higher plants.* - Martinus Higoff Publ, The Netherlands, pp. 344.

PRADHAN S., PAUDEL Y.P., QIN W., PANT B., 2023 - *Genetic fidelity assessment of wild and tissue cultured regenerants of a threatened orchid,* Cymbidium aloifolium *using molecular markers.* - Plant Gene, 34: 100418.

QAMAR S., SHAIKH A., 2018 - *Therapeutic potentials and compositional changes of valuable compounds from banana. A review*. - Trends Food Sci. Technol., 79: 1-9.

RANI D., DANTU P.K., 2016 - *Sustained shoot multiplication and method for overcoming* in vitro *browning in medicinally important plant,* Piper chaba *Hunt.* - Proc. Nat. Academy Sci., India- Section B: Biological Sciences, 86(2): 407-413.

RAWAT J.M., RAWAT B., AGNIHOTRI R.K., CHANDRA A., NAUTIYAL S., 2013 - In vitro *propagation, genetic and secondary metabolite analysis of* Aconitum violaceum *Jacq.: a threatened medicinal herb*. - Acta Physiol. Plant, 35: 2589-2599.

SARMA P.P., GURUMAYUM N., VERMA A.K., DEVI, R., 2021 - *A pharmacological perspective of banana: Implications relating to therapeutic benefits and molecular docking*. - Food Funct*.,* 12(11): 4749-4767.

SINGH M.K., SHERPA A.R., HALLAN V., ZAIDI A.A., 2007 - *A potyvirus in* Cymbidium *spp. in Northern India*. - Australasian Plant Disease Notes, 2: 11-13.

SINGH S.K., AGRAWALA D.K., JALAL J.S., DASH S.S., MAO A.A., SINGH P., 2019 - *Orchids of India - A pictorial guide.* - Botanical Survey of India, Kolkata, India, pp. 548.

STEWART J., GRIFFITH M., 1995 - *Manual of orchids*. - Timber Press, Portland, Oregon, USA, pp. 370.

SUDEEP R., RAJEEVAN P.K., VALASALAKUMARI P.K., GEETHA C.K., 1997 - *Influence of organic supplements on shoot proliferation in* Dendrobium. - J. Hortic. Navsari, 3: 38-44.

TIKENDRA L., KOIJAM A.S., NONGDAM P., 2019 - *Molecular markers based genetics fidelity assessment of micropropagated* Dendrobium chrysotoxum *Lindl.* - Meta Gene, 20: 100562.

VACIN E.F., WENT F.W., 1949 - *Some pH changes in nutrient solutions*. - Bot. Gaz., 110(4): 605-613.

WILLIS K.J., 2017 - *State of the World's Plants.* - Royal Botanical Gardens, Kew, London, UK, pp.

WOCHOK Z.S., 1981 - *The role of tissue culture in preserving threatened and endangered plant species.* - Biol. Cons., 20(2): 83-89.

XU Q., FU H., ZHU B., HASSAIN H.A., ZHANG K., TIAN X., DUAN M., XIE X., WANG L., 2021 - *Potassium improves drought stress tolerance in plants by affecting root morphology, root exudates, and microbial diversity.* - Metabolites, 11(3): 131.

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Evaluation of salinity tolerance of Yemeni chilli pepper genotypes during germination by using different statistically models

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Key words: Chilli, germination, salinity, tolerance.

Abstract: Evaluating the genotypes of vegetables is a critical component in establishing effective plant breeding programs. In this study, nine genotypes of Yemeni *Capsicum spp.* were collected from various regions in Yemen to assess their germination capabilities under different salinity levels (0, 50, 100, 150, 200, and 250 mM). The experiment was conducted using a factorial completely randomized design (CRD) with three replicates. Results indicated that increasing salinity levels led to a gradual decline in germination percentage (GRP), mean germination rate (MGR), germination time (MGT), and seedling dry matter (DM%). Additionally, variations in the genotypes' responses to salt stress were evaluated using four models: the slope of the regression line (*b*), the integrated evaluation approach (*DV*), Principal components, and the genotypes' salinity susceptibility index (GSSI). All the classified of genotypes was different by analysis models. Based on the integrated value (*DV*), the genotypes were classified into four sensitivity categories: resistant (*A*, *D*, and *G*), moderately resistant (*F* and *V2*), sensitive (*S* and *Z*), and highly sensitive (*H* and *V3*) to salinity stress. The findings demonstrate that the slope of the regression line is a reliable indicator for assessing genotype sensitivity to salinity, aligning consistently with the integrated value model (*DV*). The insights gained from this research are expected to significantly inform breeding strategies aimed at developing salt-tolerant chilli pepper cultivars, which are essential for successful cultivation in challenging environmental conditions.

1. Introduction

Hot peppers (*Capsicum spp*.) are an important vegetable crop cultivated globally in warm and temperate regions for various purposes (Comparini *et al.*, 2021).They are highly valued for their nutritional content, particularly their vitamin C and capsaicin levels, which provide notable health benefits (Taiti *et al.*, 2024) and antimicrobial activity (Serio *et al.*, 2024). This adaptable crop can be consumed fresh, as a spice, or in dried form (Taiti *et al.*, 2015; Arraf and Al-Madhagi, 2025). Over the past

50 years, global production has steadily increased (FAO, 2022). In 2022, Yemen contributed approximately 18,22 tons of hot peppers, cultivated on 3,24 hectares, representing roughly 2.3% of global production (FAO, 2022). Globally, hot peppers were grown on an estimated 689,33 hectares, yielding a remarkable 788,032.04 tons (FAO, 2022).

Salinity poses a significant challenge to agriculture in arid and semi-arid regions due to the accumulation of dissolved salts caused by soil processes, irrigation practices, drainage patterns, and overuse of fertilizers (Khondoker *et al.*, 2023). Urban expansion and competition for water resources further exacerbate the issue (Suarez, 2001; Sahbeni *et al.*, 2023).

Yemen features a range of climates, including semi-humid, semi-arid, and arid tropical types (Alhadi *et al.*, 2023).

Yemen's extensive coastal region, characterized by a warm climate conducive to pepper cultivation, particularly during the autumn and winter seasons, faces significant challenges related to excessive salinity. An estimated 37,100 hectares of non-desert agricultural land are affected by salinity, while an additional 12 million hectares experience erosion. Furthermore, 3.8 million hectares suffer from varying degrees of salinity, with 3-5% of the land at risk of desertification (USAID, 2010; Gregory *et al.*, 2018).

Yemen is home to numerous chilli genotypes (Colonna *et al.*, 2019), distributed across regions with diverse climates, altitudes, and soil properties (Aldobai and Al-shabi, 2010). Salinity significantly impairs plant growth through mechanisms such as cell membrane destabilization (Hasegawa *et al.*, 2000; Mushtaq *et al.*, 2020), disruption of photosynthesis (Momenpour and Imani, 2018; Zhou *et al.*, 2023), nutrient imbalances (Munns, 1993), and cellular damage (Hasanuzzaman *et al.*, 2021; Ahmad *et al.*, 2022).

Salt tolerance varies across species, genotypes, and cultivars (Khoshsokhan *et al.*, 2012), driven by mechanisms such as ion partitioning and proline synthesis (Hasegawa *et al.*, 2000; Farooqi *et al.*, 2021). These adaptations, along with oxidative stress management and regulated growth responses (Binzel *et al.*, 1985; Long *et al.*, 1994; Maggio *et al.*, 2007; Hasanuzzaman *et al.*, 2021), mitigate stress effects but often reduce overall yield, resulting in smaller plants (Greenway and Munns, 1980; Naeem *et al.*, 2020). Furthermore, the response to salt stress is contingent on the growth stage, with certain studies indicating variations in tolerance across different developmental phases (Mangal *et al.*, 2023; Roșca *et al.*, 2023). Notably, vegetable plants, particularly during early life stages, exhibit heightened sensitivity to salt stress, especially during germination and seedling growth (Miceli *et al.*, 2021).

Research on salt stress tolerance in various crops, including pepper (Qiu *et al.*, 2017) and fenugreek (Al-Maqtary *et al.*, 2024), often focuses on specific salt concentrations and exposure durations. Plant responses to salt stress also vary based on climatic conditions and soil characteristics (Läuchli and Epstein, 1990; Munns and Gilliham, 2015). Elevated salinity levels impede seed germination by reducing water absorption due to osmotic stress, followed by ionic stress. Increased salt concentrations in the germination medium negatively affect seed embryo vitality by disrupted ion transport (Zowain, 2014). An negative correlation exists between salinity and germination in various vegetable plants, including cucumbers (Bolton and Simon, 2019), sweet peppers (Chartzoulakis and Klapaki, 2000; Hannachi and Van Labeke, 2018; Karalija *et al.*, 2024), and tomatoes (Singh *et al.*, 2012). For example, chilli pepper and tomato seeds failed to germinate at 200 mM NaCl (Loganayaki *et al.*, 2020).

Increased salinity prolongs germination time and lowers germination rates (Al-swedi *et al.*, 2020; Dawd and Abdulla, 2020). However, Aktas *et al.* (2006) observed genetic variability in salt accumulation and leaf damage in peppers exposed to 150 mM NaCl for 10 days, indicating potential yield discrepancies. Different vegetable genotypes exhibit varying levels of resistance to salt stress, as demonstrated in studies of 26 tomato genotypes (Devi and Arumugam, 2019), 17 chilli pepper genotypes (Howlader *et al.*, 2018), and 13 *Cucurbita* genotypes (Horuz *et al.*, 2022).

The degree of genotype tolerance to salinity depends on inherent resistance mechanisms, including metabolic responses activated during salt stress (Horuz *et al.*, 2022). Chilli peppers are classified as either sensitive (Lycoskoufis *et al.*, 2005; Giorio *et al.*, 2020; Ntanasi *et al.*, 2024) or moderately salt-tolerant (Maas and Hoffman, 1977; Chartzoulakis and Klapaki, 2000; Zamljen *et al.*, 2022). Among the vegetable plants tested by Loganayaki *et al.* (2020), chilli exhibits greater salinity sensitivity compared to tomatoes and cucumbers. Salinity and alkalinity, as critical abiotic stresses, significantly reduce the growth and productivity of pepper plants (Chartzoulakis and Klapaki, 2000;

Demir and Mavi, 2008; Amirinejad *et al.*, 2017).

Ongoing research efforts by institutes and universities aim to develop agricultural techniques to mitigate the adverse effects of salinity on vegetable crop production. These efforts include breeding salt-tolerant plants (Zhu *et al.*, 2000; Singla-Pareek *et al.*, 2003; Yang *et al.*, 2005), employing grafting techniques on vegetables (Santa-Cruz *et al.*, 2002; Edelstein *et al.*, 2005; Estan *et al.*, 2005) or fruit (Momenpour and Imani, 2018), utilizing growth regulators (Sakamoto and Murata, 2001; Abrahám *et al.*, 2003; Hamdia *et al.*, 2004; Amirinejad *et al.*, 2017), and managing soil salinity through excessive irrigation (Semiz *et al.*, 2014; Sahbeni *et al.*, 2023; Tarolli *et al.*, 2024).

Exploring genetic diversity and understanding the physiological traits of various vegetable genotypes will provide a foundation for future research, including selective breeding and grafting. Therefore, the aim of this study is to evaluate the salinity sensitivity stress of Yemeni chilli genotypes. This research could significantly inform breeding strategies for chilli by examining local genotypes based on significant physiological traits.

2. Materials and Methods

Chilli seed collection

Chilli pepper seeds from local genotypes were collected from various regions in Yemen (Fig. 1, Table 1). Additionally, the F1 Shamakh pepper cultivar, designated as the F code, was included in the study.

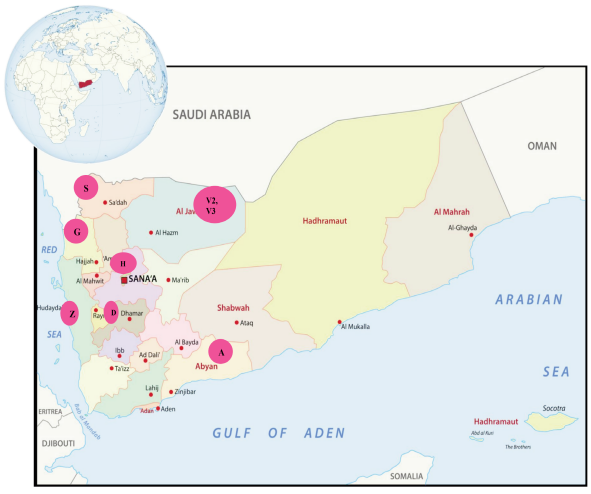

Fig. 1 - The map of Yemen shows the geographic origin of the chili pepper genotypes used in this experiment. The sample names reflect the geographic origin of the samples.

This cultivar, commonly grown in Yemen, was supplied by Agro Star Company, the exclusive agent in Yemen for United Genetics Company (USA) (Fig. 2).

Experimental layout

The study was carried out in the horticultural laboratory using a factorial experimental design based on a completely randomized design (CRD). The experiment included three replicates, with each replicate comprising 10 seeds. Seeds from different genotypes were collected and stored in specially labeled glass containers for future experimental use.

Table 1 - Name and origin of nine chili genotypes used in the study

Research code	Species	Common name	Area of distribution (latitude)	No. fruit per node	Fruit attitude	Spiciness
A	*C. annuum*	Abyani	Abyan (13° 02' 60.00" N) * lahij (13° 02' 60.00" N)	1	hanging	sweet
Z	*C. frutescens*	Zaaitri	Hudaidah (14° 12'00" N)* Taiz (13° 33' 59.99") Ibb (13° 58' 0.01" N)	1	upright	hot
H	*C. frutescens*	Haimi	Sana'a (15.36 N, 44.191006	2	upright	hot
D	*C. annuum*	Dhamari	Dhamar (15° 39' 59.99" N)* Ibb (13° 58' 0.01" N)	1	hanging	hot
V2	*C. chinense*	Jawfi 2	Al-jawf (16° 46' 59.99" N)*	2	Semi upright	hot
V3	*C. chinense*	Jawfi 3	Al-jawf (16° 46' 59.99" N)*	2	hanging	hot
G	*C. annuum*	Hajjai	Hajjai (15° 41' 59.99" N)*	1	hanging	hot
S	*C. frutescens*	Sa'ddi	Sa'dah (16° 56' 5.39" N)*	1	hanging	hot
F	*C. annuum*	Shamakh		1	hanging	

* Main regions where Yemeni chili genotypes were gathered for the research.

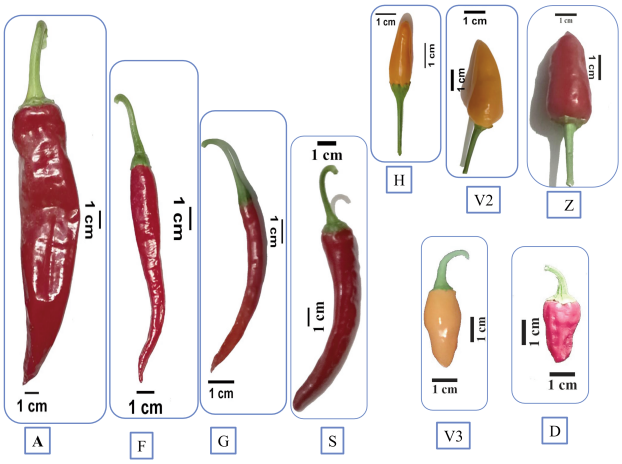

Fig. 2 - Local chilli pepper genotypes utilised in this experiment. The attitude of the peduncle explains the fruit behaviours, with the down peduncle denoted as V2, H, and Z, indicating fruit with upright habits. The length and width of the fruit for different genotypes are shown in cm. The other differences between the fruit of genotypes of chili are clear from colour, size, direction, shape, neck at base of fruit, shape at blossom end, appendage and pedicel with fruit.

Seed sterilization was performed using a solution containing 10% sodium hypochlorite (NaClO), 90% distilled water, and a drop of Tween 20. This process lasted for five minutes. Post-sterilization, the seeds were subjected to a thorough rinsing regime, involving multiple washes with running water, subsequent rinses with distilled water, and finally, a drying phase.

To commence the experimental protocol, 3 milliliters of a sodium chloride (NaCl) solution with different concentrations rate: 0, 50, 100, 200, and 250 mM, were dispensed onto filter paper within Petri dishes. For control treatments, 3 milliliters of distilled water were added to Petri dishes designated for the control group, which did not receive any salinity treatment.

Parameters of study

Data on the seed germination process were carefully recorded daily over a 21-day period, beginning from the start of the experiment. Furthermore, photographic evidence was collected daily for every treatment and replication under examination. The calculated metrics of the germination data is detailed in Table 2.

In the germination equations: N, the total number of seeds in each experimental unit; n_i , the number of seeds germinated in the i^{th} time; k, the last day of germination evaluation; t_i , the period from the commencement of the experiment to the i^{th} observation; G_i, the number of seeds germinated in the i^{th} time; and X_i , the number of days from sowing; SDG denotes the germination standard deviation.

Salinity sensitivity index

The salinity sensitivity index (SSI) values for the each single parameter were calculated separately as (Horuz *et al.*, 2022):

$$SSI = \frac{\text{Salinity level - control}}{\text{Control}} \times 100 \quad (7)$$

Table 2 - The various metrics used to calculate the process of seed germination in the experiment

No.	Measurements	Unit	Equation		References
1	Germination Percentage (GrP)	%	$GrP = \left(\frac{\sum_{i=1}^{k} n_1}{N}\right) \times 100$	1	(Kader, 2005)
2	Mean Germination Time (MGT)	day	$MGT = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$	2	(Ranal and Santana, 2006)
3	Mean Germination Rate (MGR)	day^{-1}	$MGR = \frac{1}{MGT}$	3	(Ranal, 1999)
4	Germination speed coefficient (GSC)	%	$GSC = \left(\frac{\sum_{i=1}^{k} G_i}{\sum_{i=1}^{k} G_i X_i}\right) \times 100$	4	(Ranal, 1999)
5	Coefficient of Velocity of Germination (CVG)	%	$CVG = \left(\frac{SDG}{MGT}\right) \times 100$	5	(Ranal, 1999)
6	Dry Matter (DM)	%	$DM = \frac{Dry\ weight}{Fresh\ weight} \times 100$	6	(Al-Madhagi and Al-Sharagi, 2019)

Evaluation of salt tolerance by an integrated evaluation system

The examination of salt tolerance across all genotypes was comprehensively conducted through the application of subordinate function and standard deviation coefficient techniques, utilizing the Stress Intensity (SI) metric to evaluate the effects of salt stress on NaCl concentrations surpassing 100 mM as described by Xie *et al*. (2021). The value of each evaluation index was calculated by the following equations:

$$SI = \frac{\text{Control - Salinity level}}{\text{Control}} \times 100 \quad (8)$$

$$X(u) \quad \frac{x - x\,min}{x\,max - x\,min} \quad (9)$$

$$X(u)\ 1 - \frac{x - x\,min}{x\,max - x\,min} \quad (10)$$

$$Xj = 1/n \sum_{i=1}^{n} xij \quad (11)$$

$$Vj = \frac{\sqrt{\sum_{i=1}^{n}(Xij - \bar{\bar{Xj}})2}}{xj} \quad (12)$$

$$Wj = \frac{Vj \Sigma Vj\, mj}{\sum_{j=1}^{m} vj} \quad (13)$$

$$DV = \sum_{j=1}^{n}[u(xj) \times Wj] \quad (14)$$

Firstly, standardization of index data was conducted using the subordinate function as defined in [Equations (8) and (9)]. For traits negatively correlated with salinity tolerance (NaCl), the dependency value was determined using the inverse subordinate function (Equation 9). Conversely, for traits positively correlated with salinity tolerance, the dependency value was calculated using Equation (8).

In this context, $X(u)$ represents the subordinate function value of the μ^{th} indicator, X denotes the observed indicator value, while X_{max} and X_{min} indicate the maximum and minimum values of the indicator, respectively [Equation (10).

$\bar{\bar{X}}j$ signifies the average of the j^{th} assessment index, with n denoting the total number of genotypes, and Xij referring to the j^{th} evaluation index of the i^{th} genotype [Equation (11)].

Vj represents the standard deviation coefficient of the *jth* evaluation index, with Xj depicting the jth evaluation index of genotypes [Equation (12)].

Wj stands for the weighting coefficient of the j^{th} evaluation index [Equation (13)]. $u(xj)$ corresponds to the subordinate function value of the j^{th} evaluation index.

DV denotes the aggregated values for salt tolerance in chilli pepper [Equation (14)]. A lower in the DV value indicates higher salt tolerance.

Genotypes salinity susceptible index (GSSI)

The tolerance genotypes salinity sensitivity index was calculated for germination percentage by the formula (Afzal *et al.*, 2022):

$$GSSI = (1 - \frac{Gs}{Go})/(1 - \frac{AGS}{AGo}) \quad (15)$$

Where *Gs*: an average of certain genotypes under salinity stress conditions, *Go*: an average of genotypes under optimum conditions, *AGs*: an average of all genotypes under salinity stress conditions, and *AGo*: an average of all genotypes under optimum conditions. The criterion for determining the tolerance level to Salinity stress was this: if the GSSI value is 0.5, then the genotype is tolerant (T), if 0.5 <GSSI≤ 1.0, the genotype is moderate (M), and if GSSI> 1.0 then the genotype is sensitive (S) (Pasaribu *et al.*, 2021).

Estimating genotype sensitivity to salinity using slope of the regression line

The sensitivity of each genotype was evaluated using the *R-square* values and slope coefficients calculated for each parameter. The R-square value serves as an indicator of the significance of a trait, with higher values suggesting greater relevance. In this study, the overall *R-square* values for each trait were considered a measure of their importance in assessing genotype sensitivity to salinity stress. According to the established hypothesis, genotypes exhibiting lower slope values in the context of sub-salinity treatments are classified as resistant. This implies that these genotypes maintain their performance despite increasing salinity levels, thereby demonstrating a higher tolerance to salinity stress compared to those with steeper slope values.

Data analysis

The data were analyzed using the statistical analysis program *GeneStat* 12, then the means of single factors (genotypes or salinity) were compared

using the least significant difference test ($LSD_{0.05}$) ($p< 0.05$). The values of the means of the interactions (genotype × salinity) were compared using a multiple range test ($p< 0.05$). SAS 17 was used for correlation analysis and the principal component, while SPSS 21 was used for regression analysis for each genotype.

3. Results

Germination percentage (GrP)

All factors examined, including salinity stress levels, genotype, and their interaction, had highly significant effects on the germination percentage (GrP) of chilli genotypes ($p<0.001$). Among the sources of variation, genotype explained approximately 73% of the total variation, while salinity stress accounted for 27% of the observed changes in GrP (Table 3).

The mean GrP for the genotypes across all genotypes ranged from a low of 53.9±6.77% for the *G* genotype to a high of 97.2±1.35% for the *D* genotype (Table 4). These values were significantly different (*P < 0.05*) from each other except between the *F* and *Z* genotypes.

Increasing salinity levels dramatically reduced GrP, decreasing from 88.89 ± 2.68% at 0 mM to 62.22 ± 4.90% at 250 mM NaCl. The reduction rate was approximately 0.118% for each additional millimole of NaCl, as described by the regression equation: GrP = 94.97 – 0.118 (NaCl), with an R^2 of 0.539. Among the genotypes, the *D* genotype exhibited the lowest salinity sensitivity in terms of GrP, with the lowest slope value (b = -0.013, R^2 = 0.042), ranking first based on the regression slope value. The remaining genotypes were ranked as follows: *A, S, Z, V2, F, V3*, and *G*.

The *H* genotype showed the highest sensitivity to salinity (b = 0.25, R^2 = 0.62) ranking last (order = 9). Notably, certain genotypes maintained higher GrP at higher salinity levels (250 mM), with *S* (83.3%), *A* (73.3%), and *Z* (73.3%) showing no significant difference from the control treatment (0 mM) (Table 4).

The interaction between genotype and salinity stress revealed that the *D* genotype achieved a GrP of 100% under control condition, significantly differing from the *A* and *V2* genotypes. At the 50 mM NaCl, genotype *A* exhibited the lowest GrP, which was significantly different from the other genotypes. Although the *G* genotype maintained a high GrP in the control treatment, its performance declined with salinity levels exceeding 50 mM, with reductions of 30%, 46.67%, and 36.67% at higher salinity concentrations. Similarly, the *F* hybrid cultivar could not maintain a high GrP beyond 200 mM NaCl (Table 4).

Compared to the control, the *D* genotype displayed significantly greater salt tolerance for GrP across all salinity stress levels, with a positive salt sensitive index (SSI) of 33.3% at 0, 50, 150, and 250 mM NaCl. In contrast, the *V2, H, S*, and *A* genotypes showed significant salt resistance up to 150 mM NaCl. The *G, V3*, and *Z* genotypes exhibited the lowest salt resistance (SSI) up to 50 mM NaCl, while the *F* genotype showed reduced resistance up to 100 mM NaCl (Fig. 3).

Mean germination time (MGT)

The mean germination time (MGT) of all chilli genotypes was significantly influenced by salinity stress levels, genotype, and their interaction (genotype × salinity) compared to the control treatment ($p<0.001$). Genotype accounted for approximately 62% of the total effect (100%), while the remaining 38% was attributed to the influence of salinity on MGT (Table 3). Across the genotypes, MGT varied from the shortest time of 5.82 days for the *D* genotype to the longest times of 11.52 days and 11.16 days for the *G* and *V2* genotypes, respectively (Table 5). These differences were statistically significant (*P<0.05*).

Table 3 - The predictive capabilities that explaining the contribution of salinity and genotypes to the variation in the total score (100%) that affected the germination parameters. The chosen model is a forward stepwise

Factors	GrP	MGT	MGR	GSC	CVG	DM%
Genotypes	73.0	62.0	70.0	70.2	68.0	100
NaCl	27.0	38.0	30.0	29.8	32.0	0

GrP= Germination percentage; MGT= Mean germination time; MGR= Means germination rate; DM%= Dry matter, GSC= Germination speed coefficient; CVG= Coefficient of velocity of germination.

Table 4 - Interaction effects of Yemeni chili genotypes and NaCl levels on the germination percentage (GrP) after 21 days

Genotypes	NaCl mM						Mean genotypes	R2	Coefficient		Order**
	0	50	100	150	200	250			c	b	
A	76.67 ± 14.53 d-g	73.33 ± 6.67 e-h	73.33 ± 6.67 e-h	76.67 ± 6.67 d-g	56.67 ± 8.82 h-j	73.33 ± 8.82 e-h	71.67 ± 3.55 e	0.045	76.15	-0.036	2
D	96.67 ± 3.33 a-c	100 ± 0a	100 ± 0a	100 ± 0 a	86.67 ± 3.33 a-e	100 ± 0a	97.22 ± 1.35 a	0.042	98.88	-0.013	1
F	100 ± 0 a	100 ± 0a	100 ± 0a	83.33 ± 6.67 a-f	90 ± 10 a-e	56.67 ± 3.33 h-j	88.33 ± 4.14 b	0.56	107.14	-0.150	6
G	83.33 ± 6.67 a-f	90 ± 5.77a-e	36.67 ± 18.56 kl	30 ± 0 l	46.67 ± 3.33 j-l	36.67 ± 14.53 kl	53.89 ± 6.77 f	0.41	80.31	-0.21	8
H	93.33 ± 3.33 a-d	96.67 ± 3.33 a-c	96.67 ± 3.33 a-c	93.33 ± 3.33 a-d	50 ± 5.77 i-k	36.67 ± 13.33 kl	77.78 ± 6.39 de	0.62	108.30	-0.25	9
S	93.33 ± 6.67 a-d	96.67 ± 3.33 a-c	93.33 ± 3.33 a-d	100 ± 0 a	76.67 ± 3.33 b-g	83.33 ± 8.82 a-f	90.57 ± 2.62 b	0.21	97.93	-0.059	3
V2	66.66 ± 3.33 f-i	86.67 ± 8.82 a-e	86.67 ± 8.82 a-e	86.67 ± 6.67 a-e	60 ± 20.82 g-j	50 ± 11.55 i-k	72.78 ± 5.29 e	0. 13	84.44	-0.093	5
V3	96.67 ± 3.33 a-c	96.67 ± 3.33 ab	90 ± 0 a-e	93.33 ± 3.33 a-d	63.33 ± 18.56 g-j	50 ± 15.28 i-k	81.68 ± 5.62 cd	0.48	105.23	-0.188	7
Z	93.33 ± 3.33 a-d	100 ± 0 a	83.33 ± 12.02 a-f	83.33 ± 3.33 a-f	93.33 ± 3.33 a-d	73.33 ± 3.33 e-h	87.79 ± 2.87 bc	0.24	96.34	-0.069	4
Mean NaCl	88.89 ± 2.68 ab	93.33± 2.06 a	84.44± 4.34bc	82.96± 4.13c	69.26±4.43d	62.22±4.90e	GrP = 94.97 – 0.118 (NaCl), (R2 = 0.539) *				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).

Table 5 - Interaction effects of Yemeni chili genotypes and NaCl levels on the mean germination time (MGT) after 21 days

Genotypes	NaCl mM						Mean Genotypes	*R2*	Coefficient		Order **
	0	50	100	150	200	250			*c*	*b*	
A	9.28 ± 0.7 l-r	7.64 ± 1.27 r-w	7.47 ± 0.83 s-w	10.93 ± 0.15 f-l	11.71 ± 1.4 d-i	14.10± 0.05 ab	10.191 ± 0.65	0.53	7.34	0.023	7
D	4.32 ± 0.63 A	4.23 ± 0.33 A	6.57 ± 1.11 v-y	5.6 ± 0.38 x-A	8.14± 1.16 q-v	6.1± 2.01 w-z	5.83 ± 0.49 g	0.21	4.42	0.011	2
F	8.47 ± 0.33 p-u	8.53 ± 0.18 o-u	9.77 ± 0.23 j-q	10.78 ± 0.57 g-m	11.76± 0.48 c-i	14.16± 0.32 ab	10.58 ± 0.49 bc	0.86	7.78	0.022	5
G	9.85 ± 0.39 j-p	9.61 ± 0.44 k-q	12.32 ± 0.88 c-g	14.33 ± 2.19 a	11.37± 0.64 e-j	11.67 ± 0.33 d-i	11.52 ± 0.55 a	0.137	10.35	0.009	1
H	5.58 ± 0.33 x-A	6.56 ± 0.87 v-y	8.82 ± 0.45 n-t	10.88 ± 0.33 g-l	13.11± 1.34 a-d	12.93 ± 1.16 a-e	9.65± 0.77 de	0.82	5.46	0.033	9
S	4.44 ± 0.16 zA	4.46 ± 0.29 zA	4.5± 0.10 zA	5.43 ± 0.53 y-A	7.91 ± 0.37 r-v	9.75 ± 0.98 j-q	6.08 ± 0.52 g	0.73	3.38	0.022	5
V2	9.14 ± 0.92 m-s	10.52 ± 1.23 h-n	10.22 ± 0.62 i-o	11.08 ± 0.28 f-k	12.61 ± 1.56 b-f	13.41 ± 0.9 a-c	11.16 ± 0.49 ab	0.46	9.12	0.016	3
V3	5.73 ± 0.08 x-A	6.94 ± 0.58 u-y	6.93 ± 0.55 u-y	10.41 ± 0.9 i-n	12.28± 1.02 c-g	12.14 ± 0.77 c-h	9.07 ± 0.67 e	0.791	5.39	0.029	8
Z	4.74 ± 0.39 zA	5.53 ± 0.35 y-A	8.12 ± 0.61 q-v	7.89 ± 0.88 r-v	7.25 ± 0.35 t-x	11.25± 0.41 e-k	7.47 ± 0.54 f	0.677	4.79	0.021	4
Mean NaCl	6.84±0.45 e	7.14±0.45 e	8.30±0.46 d	9.704±0.59 c	10.68±0.50 b	11.72±0.53 a	MGT= 6.45 + 0.0208 (NaCl) (*R2*= 0.775) *				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).
** The genotypes are listed in descending order according on the values of the regression slope line (*b*).

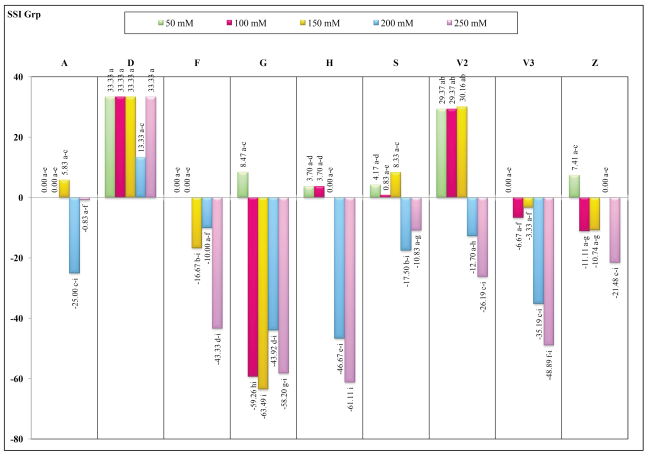

Fig. 3 - Salinity sensitivity index (SSI) for Germination Percentage (GrP) in chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT).

Furthermore, the MGT for all genotypes significantly increased ($p < 0.05$) with rising salt levels compared to the control, escalating from 6.84 ± 0.45 days at 0 mM to 11.72 ± 0.53 days at 250 mM. Regression analysis indicated an increase of approximately 0.0202 days for every 1 mM NaCl addition to the control level (MGT = 6.45 + 0.0208 NaCl) ($R^2 = 0.775$). The genotypes were arranged in descending order of MGT response as follows: *G, D, V2, Z, F, S, A, V3*, and *H*. Genotype *G* exhibited the least change in MGT ($b = 0.009$, $R^2 = 0.137$), while the *H* genotype showed the most substantial change ($b = 0.033$, $R^2 = 0.815$).

The interaction between genotypes and salinity stress showed a variation of results. MGT of the *G* genotype was significantly higher (9.85 ± 0.39 days) than that of the other genotypes in the control treatment (0 mM). At salinity levels ranging from 50 to 250 mM, the MGT for the *G* and *V2* genotypes was significantly greater than that of the other genotypes ($p<0.05$). Notably, the *D* genotype consistently exhibited the lowest MGT across all salinity levels. While several genotypes demonstrated increased MGT at the highest salinity levels, genotypes *F*, *H*, and *S* maintained their MGT up to 150 mM NaCl, whereas *G*, *V3*, and *Z* maintained their MGT up to 100 mM NaCl (Table 5).

Salinity sensitivity index (SSI) compared to the control indicated that the *A* genotype exhibited a negative sensitivity to salinity up to 100 mM. MGT values for both 50 mM and 100 mM NaCl were lower than those of the control, the *G* genotype showed negative sensitivity at 50 mM NaCl. In contrast, all other genotypes displayed positive SSI across all salinity levels. The SSI for the *H, V3, S*, and *F* genotypes increased linearly with rising salinity levels. The highest SSI for MGT was recorded at the 250 mM salinity level (139.2%) for the *Z* genotype, whereas the lowest SSI was at 100 mM NaCl (-19.8%) for the *A* genotype (Fig. 4).

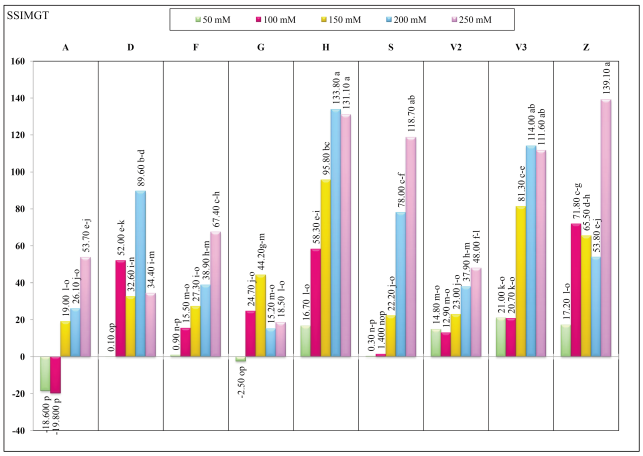

Fig. 4 - Salinity sensitivity index (SSI) for Mean Germination Time (MGT) in chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT) at p<0.05.

Germination speed coefficient (GSC)

Germination speed coefficient (GSC) of chilli genotypes was significantly influenced by salinity stress, genotype, and their interaction (genotype × salinity) compared to the control treatment ($p < 0.001$). As shown in Table 3, genotype accounted for approximately 70.2% of the total variation, with the remaining 29.8% attributed to the effect of salinity on GSC.

GSC values between genotypes ranged from the lowest (8.94 ± 0.35%) in genotype *G* to the highest (19.146 ± 1.42%) in genotype *D* (Table 6). These values were statistically different from other genotypes, except for genotype *S*, where no significant differences were observed between *D* and *S*, or between *H* and *V3*.Salinity stress led to a significant reduction in GSC across all genotypes as salt concentrations increased beyond 50 mM NaCl ($p < 0.05$). GSC decreased from 16.4 ± 1.09% at 0 mM to 9.5 ± 0.88% at 250 mM NaCl.

Regression analysis indicated that for every 1 mM increase in NaCl concentration, GSC declined by approximately 0.030% (GSC = 16.518 - 0.030 × NaCl,

Table 6 - Interaction effects of Yemeni chili genotypes and NaCl levels on Germination Speed Coefficient (GSC) after 21 days

Genotypes	NaCl mM					Mean	*R2*	Coefficient		Order **
	0	50	100	150	200	250	Genotypes		*c*	*b*	
A	10.91±0.89 k-s	13.81±2.21 g-j	13.71±1.47 g-j	9.15±0.13 p-x	8.76±0.94 q-x	7.09±0.03 x	10.57±0.74 d	0.39	13.34	-0.022	4
D	24.07±3.15 a	23.93±1.97 ab	16.32±3.23 e-g	18.03±1.26 d-f	12.88±2.08 h-l	19.65±4.98 cd	19.146±1.42 a	0.2	22.97	-0.031	5
F	11.85±0.45 i-o	11.73±0.25 i-p	10.25±0.24 l-u	9.33±0.52 o-x	8.53±0.35 r-x	7.07±0.16 x	9.79±0.43 de	0.9	12.25	-0.02	3
G	10.18±0.4 n-v	10.45±0.49 k-t	8.24±0.59 t-x	7.38±1.32 wx	8.85±0.51 q-x	8.59±0.25 r-x	8.94±0.35 e	0.21	9.924	-0.008	1
H	18.05±1.04 d-f	15.74±1.85 fg	11.4±0.59 i-q	9.21±0.29 o-x	7.78±0.73 u-x	7.86±0.7 t-x	11.67±1.01 c	0.81	17.17	-0.044	8
S	22.57±0.83 ab	22.61±1.56 ab	22.24±0.48 a-c	18.73±1.67 de	12.71±0.58 h-m	10.47±1.09 k-t	18.22±1.25 a	0.78	24.92	-0.054	9
V2	11.16±1.09 j-r	9.77±1.15 o-w	9.86±0.63 n-w	9.04±0.23 q-x	8.22±1.16 t-x	7.53±0.54 v-x	9.26±0.41 e	0.46	10.95	-0.014	2
V3	17.46±0.26 d-f	14.59±1.12 gh	14.62±1.15 gh	9.75±0.84 o-w	8.25±0.66 t-x	8.3±0.49 s-x	12.16±0.91 c	0.83	17.14	-0.04	6
Z	21.39±1.87 bc	18.22±1.13 d-f	12.45±0.89 i-n	13.04±1.64 h-k	13.85±0.67 g-i	8.91±0.34 q-x	14.64±1.07 b	0.69	19.99	-0.043	7
Mean NaCl	16.4±1.09 a	15.65±1.02 a	13.23±0.86 b	11.52±0.82 c	9.98±0.52 d	9.5±0.88 d	*GSC = 16.517917 - 0.030433 (NaCl) (*R2* = 0.758)				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).
** The genotypes are listed in descending order according on the values of the regression slope line (b).

$R^2 = 0.758$). Genotype *S* exhibited the greatest reduction in GSC ($b = -0.05$, $R^2 = 0.78$), ranking 9th in the rate of change, whereas genotype *G* exhibited the smallest change ($b = -0.01$, $R^2 = 0.216$), ranking 1st. Genotypes *V2, F, A, D, V3, Z*, and *H* ranked 2nd through 8th, respectively, in the table 6 are reported the values of the genotypes according to their variation rate.

In terms of the genotype × salinity interaction, genotype *D* displayed the highest GSC (24.07 ± 3.15%) at 0 mM NaCl ($p<0.05$), with no significant difference from genotype *S*. At salinity levels between 50 and 250 mM, genotypes *D* and *S* exhibited significantly higher GSC than other genotypes ($p<0.05$), with genotype *D* maintaining the highest GSC at 250 mM NaCl.

The salinity sensitivity index (SSI) analysis for GSC revealed that genotype *A* exhibited a positive SSI up to 100 mM NaCl, exceeding the control values. Genotypes *D* and *G* also demonstrated positive SSI at 50 mM NaCl. Conversely, genotypes *Z, V3, V2, H*, and *F* exhibited negative SSI across all salinity levels, with SSI values for genotypes *F, H, S, V3*, and *V2* decreasing linearly as salinity levels increased. The highest SSI for GSC was observed at 100 mM NaCl (25.3%) in genotype *A*, while the lowest was recorded at 200 mM NaCl (-57.97%) in genotype *H* (Fig. 5).

Mean germination rate (MGR)

The mean germination rate (MGR) of chilli

Fig. 5 - Salinity sensitivity index (SSI) for Germination Speed Coefficient (GSC) in chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT) at p<0.05. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

genotypes was significantly influenced by salinity stress levels, genotype variations, and their interaction (genotype × salinity) compared to control conditions (*p <0.001*). The genotype effect accounted for approximately 70% of the total variation (100%), while salinity contributed 30% to the observed changes in MGR (Table 3). Across the examined genotypes, MGR varied significantly, with the lowest rate recorded for genotypes *F, G*, and *V2* (0.09 seeds/day) and the highest for genotype *D* (0.19 seeds/day) (Table 7). These differences were statistically significant (*p<0.05*); however, no significant difference in MGR was observed between genotypes *D* and *S*.

A marked decline in MGR was observed across all genotypes when salinity levels exceeded 50 mM compared to the control treatment (*p<0.05*). Specifically, MGR decreased from 0.16 ± 0.01 seeds/day at 0 mM NaCl to 0.09 ± 0.008 seeds/day at 250 mM NaCl. Regression analysis indicated that for every 1 mM increase in NaCl, MGR decreased by approximately 0.0003 seeds/day, represented by the equation: MGR = 0.165153 - 0.000304 (NaCl) (R^2 *= 0.758*).The genotypic ranking based on the slope (*b*) of MGR responses to salinity is presented in Table 7. Genotype *S* demonstrated the greatest decline (*b* = -0.00053, R^2 = 0.78), (order 9), whereas genotype *G* exhibited the least decline (b = -0.00008, R^2 = 0.216), while remaining genotypes *V2, F, A, D, V3, Z*, and *H* were ranked in between of them, respectively.

In terms of the genotype × salinity interaction, MGR for genotype *D* was significantly higher (*p < 0.05*) than that of other genotypes under control conditions (0 mM NaCl), except for genotypes *S* and *Z*. At salinity levels ranging from 50 to 250 mM, MGR for genotypes *D* and *S* was significantly higher than that of the remaining genotypes, with genotype *D* achieving the highest MGR (0.19 ± 0.05) at 250 mM (Table 7).

The salinity sensitivity index (SSI) for MGR was positive for genotypes *D, G*, and *S* at 50 mM NaCl, while genotype *A* maintained a positive SSI up to 100 mM NaCl. In contrast, genotypes *Z, V3, V2, H*, and *F* exhibited negative SSI values for MGR across all salinity levels. The decline in SSI was linear for genotypes *F, H, S, V2*, and *V3* with increasing salinity. The highest SSI value for MGR (24.9%) was recorded at 100 mM NaCl for genotype *A*, while the lowest SSI (-58.08%) was observed at 250 mM NaCl for genotype *Z* (Fig. 6).

Table 7 - Interaction effects of Yemeni chili genotypes and NaCl levels on the mean germination rate (MGR) after 21 days

Genotypes	NaCl mM						Mean Genotypes	*R2*	Coefficient		Order **
	0	50	100	150	200	250			*c*	*b*	
A	0.11 ± 0.01 j-q	0.14 ± 0.02 g-j	0.14 ± 0.01 g-j	0.09± 0.001 n-s	0.09 ± 0.01 n-s	0.07± 0.00 s	0.11 ± 0.007 d	0.391	0.134	-0.00022	4
D	0.24± 0.03 a	0.24± 0.02 a	0.16± 0.03 f-g	0.18± 0.01 de	0.13 ± 0.02 h-l	0.19± 0.05 b-d	0.19± 0.014 a	0.199	0.230	-0.00030	5
F	0.12 ± 0.004 i-n	0.12± 0.002 i-n	0.10± 0.002 k-s	0.09± 0.01 m-s	0.09 ± 0.003 n-s	0.07± 0.001 s	0.09± 0.004 de	0.895	0.122	-0.00019	3
G	0.10± 0.004 k-s	0.11 ± 0.004 k-r	0.08± 0.01 o-s	0.07± 0.01 rs	0.09 ± 0.005 n-s	0.09 ± 0.002 n-s	0.09 ± 0.004 e	0.216	0.99	-0.00008	1
H	0.18±0.010 de	0.16 ± 0.02 e-h	0.11± 0.01 j-o	0.09± 0.003 n-s	0.08 ±0.01 q-s	0.08±0.01 p-s	0.12 ± 0.01 c	0.805	0.172	-0.00044	8
S	0.23±0.01 a	0.23 ± 0.02 a	0.22± 0.005 ab	0.19 ± 0.02 c-e	0.13±0.01 h-l	0.11 ±0.01 k-r	0.18± 0.013 a	0.781	0.249	-0.00053	9
V2	0.11±0.01 j-p	0.09± 0.01 l-s	0.09± 0.01 k-s	0.09± 0.002 n-s	0.08±0.01 o-s	0.08 ±0.01 rs	0.09± 0.004 e	0.459	0.110	-0.00013	2
V3	0.18 ± 0.002 d-f	0.15± 0.01 f-i	0.15 ± 0.01 f-i	0.09± 0.01 l-s	0.08±0.01 o-s	0.08±0.01 o-s	0.12± 0.009 c	0.822	0.172	-0.00039	6
Z	0.21± 0.02 a-c	0.18± 0.01 de	0.12± 0.01 i-m	0.13± 0.02 h-k	0.14 ±0.01 g-j	0.09± 0.003 n-s	0.15 ± 0.011 b	0.686	0.200	-0.00043	7
Mean NaCl	0.16±0.01 a	0.16±0.01 a	0.13±0.008 b	0.12±0.008 c	0.09±0.005 d	0.09±0.008 d	MGR= 0.165153 - 0.000304 (NaCl), ($R2$=0.758)				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).
** The genotypes are listed in descending order according on the values of the regression slope line (b).

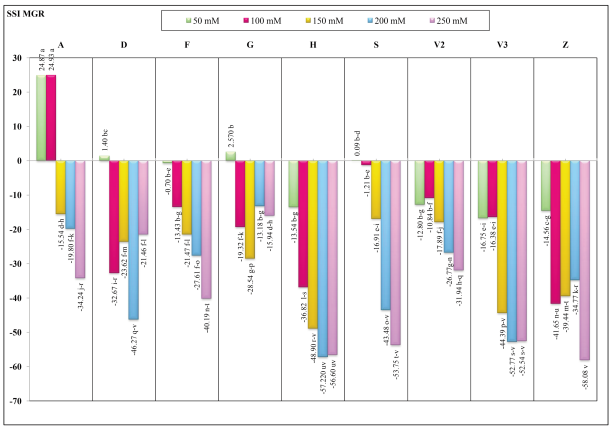

Fig. 6 - Salinity sensitivity index (SSI) for Mean Germination Rate (MGR) for chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT) at p<0.05. Genotypes: A= Abyani; Z= Zaaitri ; H= Haimi; D= Dhamari; V2= Jawfi 2; V3= Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

Coefficient of velocity of germination (CVG)

The coefficient of velocity of germination (CVG) for all chilli genotypes was significantly influenced by salinity stress levels, genotype differences, and their interaction (genotype × salinity) compared to the control treatment (*p<0.001*). Genotype alone accounted for approximately 68% of the total variation (100%), while salinity contributed an additional 32% to the CVG (Table 3). Among the genotypes, CVG ranged from 18.75±1.45 for genotype *F* to 37.37±3.61 for genotype *V3* (Table 8), with significant differences observed (*p < 0.05*). However, no significant differences were noted among genotypes *V3, A, D, H, S*, and *Z*. Furthermore, increasing salinity levels led to a dramatic decrease in CVG compared to the control (0 mM), with values dropping from 38.48±3.65 (at 0 mM) to 25.69 ± 2.10 (at 250 mM). No significant differences were observed between the 50, 100, and 150 mM NaCl treatments, or between the 200 and 250 mM NaCl treatments (Table 8).

The regression analysis indicated that for every 1 mM increase in NaCl, CVG decreased by approximately 0.0485, as described by the equation: CVG = 37.725 - 0.0485 (NaCl) (R^2 = 0.166). The genotypes were ranked according to their CVG response, with *S, H, F, D, A, Z, G, V3*, and *V2* arranged from 1 to 9, respectively. The *S* genotype exhibited the least impact from salinity (*b* = -0.010, R^2 = 0.004), ranking first, while the *V2* genotype showed the greatest impact (*b* = -0.106, R^2 = 0.53), placing last

Table 8 - Interaction effects of Yemeni chili genotypes and NaCl levels on the Coefficient of velocity of germination (CVG) after 21 days

Genotypes	NaCl mM					Mean	*R2*	Coefficient		Order **
	0	50	100	150	200	250	Genotypes		c	b	
A	45.72± 12.31 a-f	31.36± 8.77 c-o	24.7± 3.03 i-o	36.81± 5.76 a-k	31.03± 8.73 d-o	31.12± 1.39 c-o	33.46± 3.06 ab	0.057	37.87	-0.035	5
D	40.89± 9.68 a-j	29.92± 4.13 e-o	52.68± 17.34 a	23.77± 6.19 j-o	40.96± 5.74 a-j	28.48± 12.21 f-o	36.12± 4.24 a	0.026	40.25	-0.033	4
F	14.17± 4.75 o	20.02± 1.05 k-o	15.96± 4.87 no	19.77± 4.85 k-o	21.66± 1.51 k-o	20.95± 3.49 k-o	18.75± 1.45 d	0.121	15.70	-0.024	3
G	43.07± 7.24 a-h	27.61± 4.35 g-o	30.55± 0.15 e-o	18.09± 9.09 l-o	14.79± 2.26 o	21.82± 0.43 k-o	25.99± 3.21 c	0.42	37.21	-0.087	7
H	31.36± 9.37 c-o	34.19± 11.77 b-l	50.39± 3.52 ab	43.69± 4.06 a-g	32.72± 3.12 c-n	25.64± 7.38 h-o	36.33± 3.33 a	0.021	39.16	-0.023	2
S	33.74± 4.92 b-m	32.49± 6.24 c-n	19.72± 4.31 k-o	42.03± 15.31 a-i	29.23± 3.27 f-o	34.79± 10.11 b-l	32± 3.32a b	0.004	30.72	0.010	1
V2	40.53± 6 a-j	36.37± 6.3 a-k	33.98± 5.3 b-m	24.84± 6.31 i-o	18.4l± 5.53 m-o	16.01± 5.66 no	28.35± 3.004 bc	0.535	41.61	-0.106	9
V3	48.33± 11.17 a-d	52.59± 7.09 a	30.79± 2.22 e-o	36.53± 5.6 a-k	33.2± 11.54 b-n	22.77± 2.72 k-o	37.37± 3.61 a	0.349	50.24	-0.103	8
Z	48.48± 22.35 a-c	35.32± 12.02 b-l	41.35± 1.2 a-i	47.3± 13.64 a-e	17.2± 1.79 m-o	29.66± 4.84f -o	36.55± 4.87 a	0.12	46.73	-0.081	6
Mean NaCl	38.48±3.65a	33.32±2.66 b	33.35±2.99 b	32.54±3.16 b	26.58±2.30 c	25.69±2.10 c	* CVG= 37.725 - 0.0485(NaCl), ($R2$=0.166)				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).
** The genotypes are listed in descending order according on the values of the regression slope line (b).

(Table 8).

Interaction between genotype and salinity stress, under control conditions (0 mM), genotype *Z* showed a CVG of (48.48 ± 22.35) higher than the lower values observed for genotypes *F* (14.17 ± 4.75) and *H* (31.36 ± 9.37) (Table 8). At salinity levels ranging from 50 to 250 mM, the CVG for genotype *F* was statistically lower than that of the other genotypes. The genotype *F* showed a positive salinity sensitivity index (SSI) for CVG across all salinity levels. In contrast, genotypes *V2, A*, and *G* exhibited negative SSI values for CVG at all salinity levels, with genotype *V3* showing negative values above 50 mM NaCl. The *H* genotype maintained a positive SSI up to 200 mM NaCl, while genotype *Z* exhibited positive SSI values at the initial two salinity levels. The highest SSI value for CVG was observed at the 200 mM NaCl (98.5%) for genotype *F*, whereas the lowest was recorded at 200 mM NaCl (-65.46%) for genotype *G* (Fig. 7).

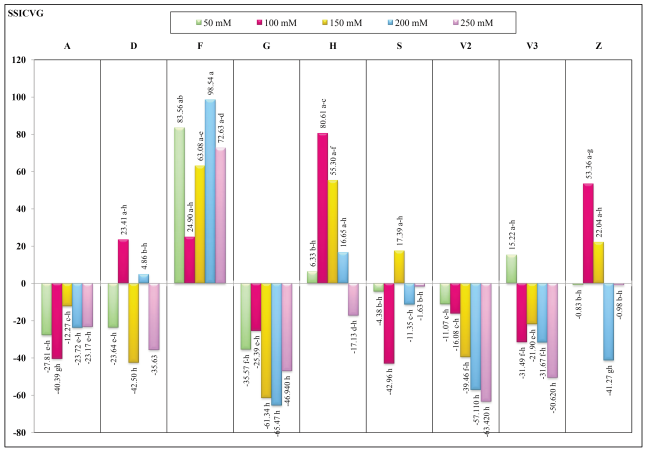

Fig. 7 - Salinity sensitivity index (SSI) of Coefficient of Velocity of Germination (CVG) in chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT) at p < 0.05. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

Dry matter of germinated seed (DM%)

The dry matter percentage (DM%) of all chilli genotypes was significantly influenced by salinity stress levels, genotype differences, and the interaction between genotype and salinity stress when compared to the control treatment (*p < 0.05*).

Across the genotypes, DM% ranged from 7.77 ± 0.74% for genotype *G* to 17.95 ± 1.38% for genotype *Z*. No significant differences were observed among genotypes *S, H, F*, and *A* (Table 9). Furthermore,

Table 9 - Interaction effects of Yemeni chili genotypes and NaCl levels on the percentage of dry matter (DM%) after 21 days

Genotypes	NaCl mM						Mean	*R2*	Coefficient		Order **
	0	50	100	150	200	250	Genotypes		*c*	*b*	
A	10.95± 0.25 g-o	10.33± 1.33 h-o	10.1± 0.89 h-o	10.19± 0.6 h-o	13.01± 0.2 e-l	10.24± 0 h-o	10.8± 0.45 cd	0.40	9.18	0.014	2
D	14.86± 1.72 d-i	16.67± 1.84 b-f	15.01± 5.07 d-h	20.31± 0.62 ab	13.48± 0.87 e-k	16.9± 1.18 b-e	16.2± 1.04 b	0.004	15.72	0.003	3
F	11.95± 1.83 e-m	13.87± 0.92 e-k	9.49± 1.96 k-q	15.55± 1.75 c-g	6.79± 2.84 n-r	5± 0 p-r	10.44± 1.06 d	0.27	13.94	-0.026	8
G	9.98± 0.98 h-o	10.34± 0.51 h-o	9.39± 1.57 k-q	4.5± 0.71 r	4.06± 0.04 r	8.33± 2.15 l-r	7.77± 0.74 e	0.258	10.05	-0.018	5
H	12.28± 1.51 e-m	13.05± 1.96 e-l	11.74± 0.7 f-n	13.37± 1.03 e-l	7.7± 0.3 m-r	4.83± 0 qr	10.5± 0.86 d	0.51	14.19	-0.030	9
S	11.45± 1.9 g-o	10.98± 1.16 g-o	10± 2.13 h-o	14.82± 3.12 d-i	6.53± 2.93 o-r	4.67± 0.6 r	9.74± 1.1 d	0.21	12.77	-0.024	7
V2	9.7± 1.25 j-p	10.02± 0.61 h-o	11.1± 0.23 g-o	11.55± 1.7 g-o	14.71± 1.08 d-j	15.98± 0.35 b-g	12.18± 0.67 c	0.66	8.89	0.026	1
V3	12.23± 1.52 e-m	13.37± 3.28 e-l	14.38± 0.79 d-k	9.86± 2.85 i-o	10.44± 4.5 h-o	13.42± 0 e-k	12.28± 0.99 c	0.008	12.80	-0.004	4
Z	19.81± 6.08 a-c	22.17± 4.23 a	16.71± 3.39 b-f	14.18± 0.78 d-k	19.06± 2.4 a-d	15.78± 1.85 b-g	17.95± 1.38 a	0.074	20.23	-0.018	5
Mean NaCl	12.58±0.93 ab	13.42±0.93 a	11.99±0.82 bc	12.7±0.96 ab	10.64±1.08 cd	10.57±1.07 d	*DM%= 13.253599 -0.010085(NaCl) , (*R2* =0.12)				

Means containing the same Latin letters are not considered significant, as determined by LSD 0.05 for single factors (genotypes or salinity) or by the multiple range Duncan test (MRDT) for the interaction (genotypes × salinity).
Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.
The values for R^2 and the regression coefficients, where c = the intercept and b = slope of the line, are provided for the simple regression analysis of each genotype.
* Simple regression equation was performed for the mean of all genotypes (df = 18).
** The genotypes are listed in descending order according on the values of the regression slope line (b).

increasing salinity levels led to a significant decline in DM%, with values decreasing from 12.58 ± 0.93% at 0 mM to 10.57 ± 1.07% at 250 mM. No significant differences were detected between salinity levels from 0 mM to 150 mM (Table 9).The regression analysis indicated the effect of salinity on dry matter, with the order of genotypes ranked as *V2, A, D, V3, G, Z, S, F*, and *H* from 1 to 9, respectively.

According to the R^2 values, salinity had a minimal influence on genotypes *D* (b = -0.003, R^2 = 0.004) and *V3* (b = -0.004, R^2 = 0.008), while it exerted the most significant effect on genotype *H* (b = -0.03, R^2 = 0.51) (Table 9). Regarding the interaction between genotype and salinity stress, the DM% for genotype *Z* was significantly higher than the one of the other genotypes across all salinity levels tested (0, 50, 100, 200, and 250 mM). At 150 mM NaCl, genotype *D* exhibited a significantly higher DM% compared to the other genotypes (Table 9).

In terms of the salinity sensitivity index (SSI) for DM%, genotypes *V2* and *D* displayed positive SSI values across all salinity levels. Genotype *A* showed a positive SSI value under salinity levels up to 150 mM. In contrast, genotype *G* exhibited negative SSI values under salinity levels up to 50 mM. The lowest SSI value for DM% was recorded at the 150 mM salinity level (-71%) for genotype *G*, while the highest SSI value was observed at the 250 mM salinity level (72.7%) for genotype *D* (Fig. 8).

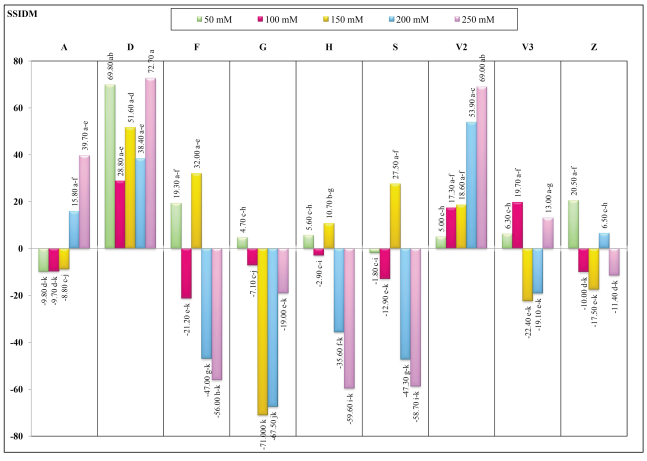

Fig. 8 - Salinity sensitivity index for Dry matter of seedling (DM%) in chili genotypes. Values represent the mean of three replicates. Different letters denote significant differences according to the Duncan Multiple Range Test (DMRT) at p<0.05. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

Pearson correlation and principal component analysis

The Principal component analysis (PCA) conducted in this study (Table 10) classified the variables into two primary components have eigenvalues greater than 1, which together explained 76.2% of the total variance observed. The first principal component (PC1) exhibited an eigenvalue of 2.75, accounting for 55.11% of the overall variance (Table 10). The coefficients associated with PC1 indicate higher correlations with: DM% (0.713), GrP (0.808), MGT (-0.715), MGR (0.881), and CVG (0.552) (Table 11). The second principal component (PC2) demonstrated an eigenvalue of 1.079, explaining 21.585% of the variance in the data (Table 11). The biplot diagram (Fig. 9) displays both the first and second principal component (PC) scores of the various parameters. Strong positive correlation of PC1 with both CVG and DM%, as confirmed by the Pearson correlation coefficient (r= 0.44, p> *0.01*) (Fig. 10). Additionally, MGR and GrP exhibited a significant positive correlation (r= 0.58, p> *0.01*), indicating their

Table 10 - The Eigenvalue, variability (%) and the cumulative percentage of the principal component for first, second, third, fourth and fifth components

Number of principal component	Cumulative Percentage	Eigenvalue	Percentage
1	55.11	2.755.500	55.11
2	76.69	1.079.260	21.58
3	87.12	0.521455	10.42
4	96.24	0.455748	9.11
5	100.00	0.188037	3.76

Table 11 - The coefficients of the principal component score (Prin) for first to fifth components

Parameters	Prin1	Prin2	Prin3	Prin4	Prin5
DM%	0.71321	0.37635	0.57006	-0.15021	-0.0464
grp	0.80819	-0.01046	-0.00483	0.58824	0.02586
mgt	-0.71563	0.62246	0.07473	0.13852	0.27505
mgr	0.88102	-0.23142	-0.13593	-0.23594	0.31001
cvg	0.55258	0.70463	-0.41521	-0.1109	-0.11599

The principal components are based strongly correlated of parameters with each component. Number with light color means no correlation.

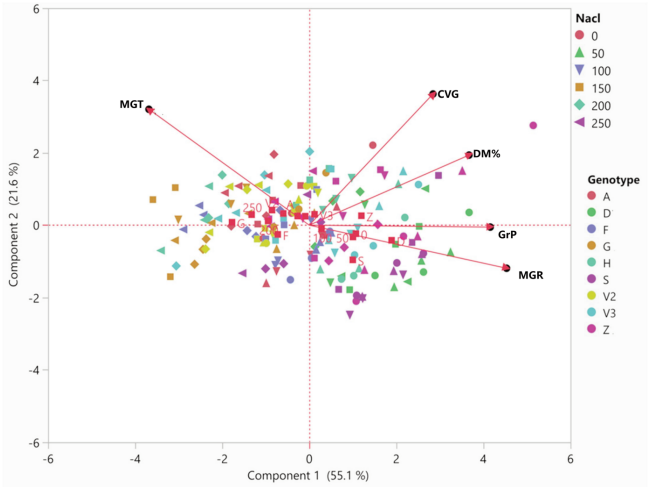

Fig. 9 - Biplot Principal Component Analysis (PCA) of various parameters contributing of MGT, CVG, DM% and GrP to salinity and genotypes. Genotypes: A= Abyani; Z= Zaaitri H= Haimi; D= Dhamari; V2= Jawfi 2; V3= Jawfi 3; G=Hajjai; S = Sa'ddi; F= Shamakh.

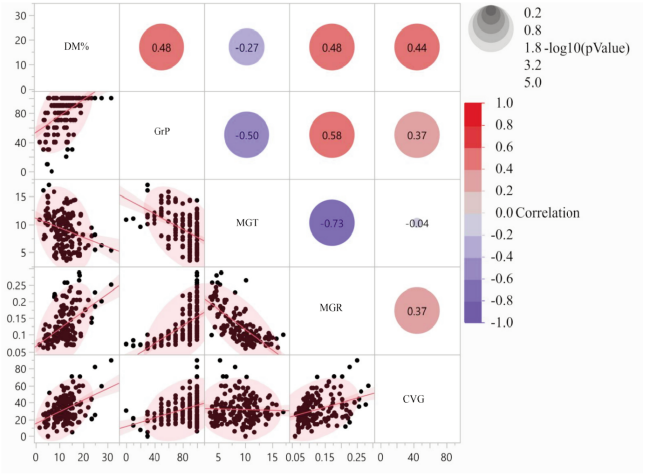

Fig. 10 - The correlation matrix (Pearson) displaying the relationships among parameters investigated in the current study. Significant correlations are detailed below the diagonal, whereas above the diagonal, correlations between parameters under various treatments. The degree of correlations between these parameters under treatment is shown by varying sizes of circles and shades of color that correspond to different correlation values.

effectiveness as prominent indicators of salinity stress resilience.

Conversely, PC1 exhibited a negative correlation with MGT. The analysis revealed a significant negative correlation between MGT and MGR (r= -0.73, $p>0.01$), suggesting that genotypes characterized by shorter MGTs tend to display higher MGR under saline conditions. A similar negative correlation was observed between MGT and GrP (r = -0.50, $p > 0.01$), indicating that genotypes with lower MGTs achieve higher GrP in response to salinity stress.

The PCA biplot (Fig. 9) and data from Table 12 show that the genotypes were distributed across all

Table 12 - The coefficients of the principal component score (Prin) for first to fifth components for genotypes and salinity, with ranking of Yemeni chili genotypes for salinity tolerance, determined by the cumulative coefficients of the principal component score (Prin) for first and second components values

Factors		Prin1	Prin2	Prin3	Prin4	Prin5	Order	Grope
Genotypes	A	-0.59566	0.32256	-0.09868	-0.08734	-0.09748	6	3
	D	189.235	-0.41852	0.10463	-0.20911	0.10728	1	1
	F	-0.72275	-0.25615	0.43255	0.80284	0.18231	8	4
	G	-177.313	0.0807	-0.2378	-0.34424	0.1064	9	4
	H	-0.26658	0.25372	-0.35926	0.03307	-0.0791	5	4
	S	10.002	-0.95791	-0.67273	-0.0669	0.21997	4	1
	V2	-0.85458	0.41669	0.34601	0.11202	-0.01167	7	3
	V3	0.12219	0.29816	-0.15171	0.02071	-0.17465	3	2
	Z	119.796	0.26074	0.637	-0.26106	-0.25305	2	2
	0	107.166	-0.23484	-0.36013	-0.16135	-0.05502		
Salinity	50	100.443	-0.32537	-0.01125	0.09954	-0.01775		
	100	0.28284	-0.0954	-0.11151	0.06181	-0.10089		
	150	-0.09113	0.24656	0.10626	0.19838	-0.05322		
	200	-0.94522	0.11829	0.15719	-0.02344	0.04212		
	250	-132.258	0.29076	0.21944	-0.17494	0.18476		

The principal components are based strongly correlated of parameters with each component. Number with light color means no correlation. Order = the rank of genotypes according to summation of values the prin1 and Prin 2. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

four quadrants, highlighting significant genetic variation among the tested genotypes. Genotypes D and S appeared in the quadrant with the highest PC1 and lowest PC2 values, indicating tolerance and a strong correlation with MGR and GrP. Genotypes Z and V3 were positioned in the quadrant with the highest PC1 and highest PC2 values, displaying a strong correlation with CVG and DM, suggesting moderate resistance. In contrast, genotypes A and V2 were located in the quadrant with the lowest PC1 and highest PC2 values, indicating sensitivity and a close correlation with MGT. Genotypes F and G were placed in the quadrant with the lowest PC1 and lowest PC2 values, reflecting very high sensitivity and showing no correlation with germination parameters. Based on the cumulative PC1 and PC2 scores, the chilli genotypes were ranked from 1 to 9, with genotype D ranked the highest (Order 1) and genotype G ranked the lowest (Order 9), indicating its heightened sensitivity to salinity stress (Table 12).

PC1 of NaCl showed a positive correlation at concentrations ranging from 0 to 100 mM. However, it was negatively correlated, with no significant effect at 150 mM, and significantly negatively correlated at concentrations of 200 to 250 mM NaCl.

Evaluation of salinity tolerance in Yemeni chilli genotypes by integrated value (DV)

The salt tolerance levels among the chilli genotypes in this experiment were assessed using the integrated value (*DV*), as presented in Table 12. The weighted coefficients indicate the significance of various parameters in measuring the sensitivity of the genotypes to salinity. Notably, germination rate percentage (GrP), mean germination time (MGT), mean germination rate (MGR), and germination speed coefficient (GSC) collectively accounted for over 70% of the overall weight in this study.

The arrangement of integrated values (*DV*) ranked the genotypes according to their salt tolerance, with genotype A occupying the top position (rank 1), indicating higher resistance to salinity. In contrast, genotype *H* ranked last (order 9), reflecting greater sensitivity to salinity. Based on the integrated value (*DV*), the genotypes were categorized into four groups: resistant (*D*, *A*, and *G*), moderately resistant (*F* and *V2*), sensitive (*S* and *Z*), and highly sensitive (*H* and *V3*) to salinity (Fig. 11).

Additionally, the Genotypes Salinity Susceptibility

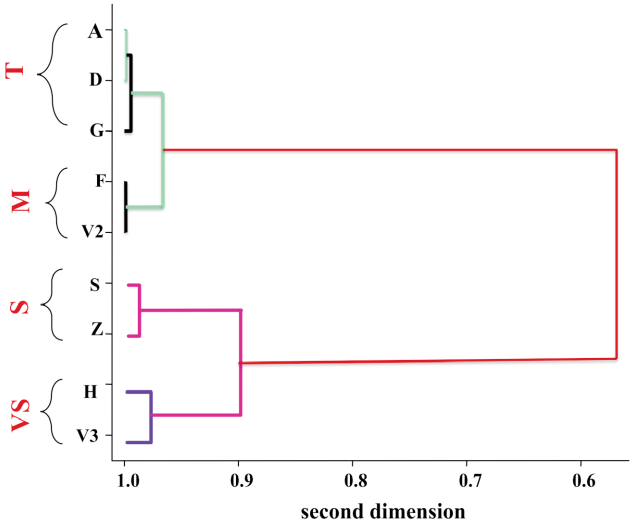

Fig. 11 - Cluster analysis of 9 chilli genotypes using integrated value (VD). The first group contains salinity-resistant (T) genotypes. The second group contains genotypes that are moderately sensitive (M) to salinity; the third group contains genotypes that are sensitive (S) to salinity; and the fourth group contains genotypes that are very sensitive to salinity (VS). Genotypes: A= Abyani; Z =Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

Index (GSSI) was calculated based on GrP (Table 13). The results indicated that genotypes *A* and *D* were tolerant to salinity, while genotypes *F*, *G*, *H*, and *V3* were sensitive. Genotypes *V2* and *Z* are exhibited moderate sensitivity to salinity. Interestingly, the ranking of genotypes according to the subordinate function of GrP (O GrP) slightly differed from that of the GSSI, as genotype *A* ranked fifth in O GrP but was classified as tolerant in the GSSI assessment.

Evaluation of salinity tolerance in Yemeni chilli genotypes by regression slop

In this study, the significance of various traits was assessed through the R-square (R^2) values derived from total regression analyses for each genotype across all measured characteristics. Higher R^2 values indicate a greater significance of the trait, while lower values suggest diminished relevance. The R^2 values for germination percentage (GrP), mean germination time (MGT), mean germination rate (MGR), and germination speed coefficient (GSC) were all above 50%, with values of 53, 77, 76, and 76 %, respectively (Table 13). These findings demonstrate that salinity significantly affects these traits, providing a reliable measure of the sensitivity of the

genotypes employed in this experiment. Conversely, the R^2 values for germination velocity coefficient (CVG) and dry matter (DM) were markedly lower, indicating that these traits are less significant, with R^2 values below 0.25.

Table 14 summarizes the slope values obtained from the regression analyses for each genotype across all parameters. Genotypes with higher slope values (*b*) are regarded as being more a*dv*ersely affected by salinity and thus exhibit lower resistance to salt stress. Based on the summation of slope values for each genotype across all parameters, the genotypes were ranked from 1 to 9, with genotype *A* achieving the highest rank, followed by genotype *D*.

Table 13 - The values of the subordinate function, integrated value (DV), and order of each chili genotypes under salt stress. GrP means germination percentage, MGT means germination time, MGR means germination rate, MD% means dry matter, GSC means germination speed coefficient, CVG germination velocity coefficient on the 21th day, GSSI Genotypes Salinity Susceptible Index and Wj is the weighted coefficient

Genotypes	GrP	MGT	MGR	DM%	GSC	CVG	D	Order	O GrP	Grope (GSSI) *
A	0.074	0.238	0.059	0.093	0.060	0.099	0.622	1	5	T
D	0.036	0.261	0.086	0.091	0.079	0.080	0.634	2	1	T
F	0.096	0.251	0.082	0.098	0.085	0.082	0.695	4	6	S
G	0.099	0.245	0.071	0.084	0.073	0.098	0.671	3	7	S
H	0.125	0.325	0.157	0.086	0.162	0.083	0.937	9	9	S
S	0.053	0.314	0.148	0.088	0.153	0.072	0.828	6	3	T
V2	0.072	0.264	0.101	0.091	0.104	0.101	0.733	*5*	4	M
V3	0.103	0.328	0.157	0.079	0.162	0.098	0.926	8	8	S
Z	0.049	0.340	0.163	0.076	0.168	0.080	0.876	7	2	M
WJ	0.18	0.20	0.20	0.11	0.21	0.11				

The principal components are based strongly correlated of parameters with each component. Number with light color means no correlation. Order = the rank of genotypes according to summation of values the prin1 and Prin 2. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

Table 14 - The ranking of Yemeni chili genotypes for salinity tolerance, determined by the cumulative regression line slope values of seed germination parameters. The R^2 values represent the strength of association for each parameter

Genotypes	GrP	MGT	MGR	CVG	DM%	GSC	∑	Order
A	-0.036	0.023	-0.00022	-0.035	0.014	-0.022	-0.0342	1
D	-0.013	0.011	-0.0003	-0.035	0.003	-0.031	-0.0343	2
F	-0.15	0.022	-0.00019	-0.035	-0.026	-0.02	-0.1892	6
G	-0.21	0.009	-0.00008	-0.035	-0.018	-0.008	-0.2541	8
H	-0.25	0.033	-0.00044	-0.035	-0.03	-0.044	-0.2824	9
S	-0.059	0.022	-0.00053	-0.035	-0.024	-0.054	-0.0965	4
V2	-0.093	0.016	-0.00013	-0.035	0.026	-0.014	-0.0861	3
V3	-0.188	0.029	-0.00039	-0.035	-0.004	-0.04	-0.1984	7
Z	-0.069	0.021	-0.00043	-0.035	-0.018	-0.043	-0.1014	5
R2	0.539	0.775	0.758	0.166	0.12	0.758		

The principal components are based strongly correlated of parameters with each component. Number with light color means no correlation. Order = the rank of genotypes according to summation of values the prin1 and Prin 2. Genotypes: A = Abyani; Z = Zaaitri ; H= Haimi; D=Dhamari; V2 = Jawfi 2; V3 = Jawfi 3; G= Hajjai; S = Sa'ddi; F = Shamakh.

In contrast, genotype *H* was ranked last (order 9), indicating its greater susceptibility to salinity stress.

4. Discussion and Conclusions

Natural hybridization in peppers plays a vital role in the development of numerous species and varieties; however, it also complicates their classification due to overlapping traits (Comparini *et al.*, 2021). Of approximately 35 *Capsicum* species found in nature, only five have been domesticated for human use (Comparini *et al.*, 2021; Swamy, 2023).This study focuses on three Yemeni chilli species: *C. frutescens, C. annuum, and C. chinense*, as presented in Table 1. Seed germination marks the beginning of the plant's life cycle and requires specific conditions to ensure successful germination. Salinity hinders seed germination by inducing osmotic stress and ionic toxicity (Hasanuzzaman *et al.*, 2021; Fu and Yang, 2023). Salinity stress disrupts essential physiological processes in plants, , leading to a reduced K^+/Na^+ ratio and imbalances in ascorbate/dehydroascorbic acid and glutathione/oxidized glutathione levels (Kaya *et al.*, 2020). Furthermore, it reduces sugar content, alters organic acid metabolism, and promotes the accumulation of phenolic compounds (Zamljen *et al.*, 2022). These physiological disruptions limit germination percentage, delay germination time, and reduce both germination rate and biomass production (Gupta and Huang, 2014).

The results showed a decrease in germination and biomass of hot chilli genotypes under salt stress, with a more pronounced impact observed in salt-sensitive genotypes compared to moderate and salt-tolerant ones. These findings align with the findings of Sarkar *et al.* (2023). This decline in germination parameters can be attributed to disruptions in nutrient uptake and the accumulation of sodium ions, which lead to ion-specific toxicity and increased osmotic pressure, and nutrient imbalances(Munns, 1993), as well as damage to plant cells and tissues (Hasanuzzaman *et al.*, 2021; Ahmad *et al.*, 2022).

A lower MGT value indicates faster seed germination (Kader, 2005), while a higher GSC value reflects quicker seed germination. In contrast, mean germination rate (MGR), calculated as the inverse of MGT, and represents the rate of seed germination per unit of time. The coefficient of velocity of germination (CVG) is another metric used to assess germination speed; it typically increases with a higher number of germinated seeds and a shorter germination period (Talská *et al.*, 2020).

The gradual reduction in GrP, MGR, CVG, and GSC, but increasing the MGT, is due to salinity's influence and is inevitable, given the limited tolerance of plants to salt. This reduction in salt tolerance is influenced by the plant's capacity to absorb salt concentrations and its response to salt stress, whether by enhancing osmosis through the production of organic compounds like proteins, proline, and sugars or by excluding salt via selective ion permeability (Wien and Stützel, 2020). These response mechanisms vary depending on plant species, varieties, and genotypes (Loganayaki *et al.*, 2020; Ali *et al.*, 2022). The most significant CVG values were observed in the tolerant genotypes examined in this study. Elevated salt concentrations can impede water absorption due to intracellular osmotic pressure, disrupting cell division and elongation, thereby more effectively inhibiting water absorption than reducing seed germination (Meyer and Boyer, 1981; Munns, 1993; Hasegawa *et al.*, 2000; Mushtaq *et al.*, 2020; Hasanuzzaman *et al.*, 2021; Ahmad *et al.*, 2022; Zhou *et al.*, 2023).

Simple correlation analyses are commonly used because they are easy to calculate. Yet, for complex traits, a basic analysis may not be sufficient. In such cases, principal component analysis, or non-linear PCA, can be utilized.

The accumulation of dry matter in seedlings indicates the absorption of NaCl ions and the genotypes' response mechanisms to salt stress. The dry matter percentage decreases with increasing salinity levels; a negative correlation was found between DM% and MGT (r= -0.26, $p>0.01$), the genotypes that content a higher dry matter percentage at high salinity levels are considered resistant, with a positive correlation with GrP (r=0.49, $p>0.01$) and with MGR (r = 0.48, $p>0.01$) (Fig. 10).

Genotype *D* exhibited higher DM% across all salt concentrations compared to the control, while genotype *A* maintained stable DM% levels. Although variations in DM% were observed among genotypes, indicating points of peak resistance, stepwise regression analysis revealed that 100% of the observed effects were attributed to genetic differences (Table 3). This suggests that, while DM% can be indicative of salinity tolerance, its overall significance as a trait was relatively limited in this

study. These findings highlight the potential of DM% as a useful physiological marker for evaluating salinity tolerance, especially during the germination stage.

Numerous researchers have also highlighted the presence of genetic variances in salt tolerance among various vegetable crops, such as in tomato (Devi and Arumugam, 2019) and in pepper (Howlader *et al.*, 2018). Salt stress exerts a*dv*erse effects on seed germination percentage, plant length, root length, root/plant length ratio, as well as fresh and dry weights of seedlings, along with the seedling vigor index (Kayacetin, 2022). Seed germination and seedling growth represent the plant growth stages most susceptible to salt stress (Miceli *et al.*, 2021). In this investigation, the germination rate percentage of most genotypes significantly decreased, but they were able to maintain up to 50% germination even at very high NaCl concentrations (250 mM), indicating that the threshold for poor germination among most Yemeni hot pepper genotypes was 200 to 250 mM NaCl. Similarly, the PCS revealed a negative effect at 200 and 250 mM NaCl concentrations (Table 12).

In this study, distinct variations were observed among hot pepper genotypes regarding their salinity tolerance index. Genotypes *A* and *D* genotypes exhibited superior resistance in terms in both germination percentage and dry matter accumulation. In contrast, these genotypes, showed heightened resistance specifically in terms of germination speed and rate. Consequently, the most salt-tolerant genotype was identified based on the slope of the regression line, the integrated value (*DV*), principal component analysis, and the Genotype Salinity Susceptibility Index (GSSI) indicators.

PCA helps identify key traits impacting salinity tolerance (Negrão *et al.*, 2016; Mubushar *et al.*, 2022). This study used principal component analysis (PCA) to evaluate variables, with the first and second PCs explaining the majority of the variation (76.7%). The distribution of genotypes across the four quadrants highlighted distinct groupings. Salt-tolerant genotypes showed high GrP and MGR values and low MGT. Among all genotypes, D consistently ranked as the most resistant, achieving the first position across all analytical methods. However, the classification of other genotypes differed depending on the analysis model.

The cumulative value of the weighted coefficients (*Wj*) for GrP, MGT, MGR, and GSC exceeded 76% of the overall weights in this study. And the Cumulative Percentage of the first and second PCS (Table 10) was about 79.69% in which similar to *Wj*. Correspondingly, the R-square values for GrP, MGT, MGR, and GSC were all greater than 50%, indicating their reliability in assessing the sensitivity of the genotypes to salinity stress. These findings suggest that these four characteristics could serve as fundamental parameters in a framework designed to evaluate the tolerance of chilli pepper germination to salinity stress. Additionally, the R-square value may be considered a viable alternative to the weighted coefficient (*Wj*) in this assessment.

Despite minor discrepancies between the integrated value (*DV*) and the slope of the regression line in the arrangement of genotypes (Tables 12 and 13), we propose that the slope is a more effective metric for evaluating salinity sensitivity. This is primarily because the slope quantitatively represents the extent of decline in each characteristic as salinity levels increase across all genotypes. In contrast, the *DV* calculation depends on higher salinity levels, which may not fully capture the nuanced responses of genotypes.

Principal components (PCs) effectively highlight the relationship between variables and their respective impacts, while summated regression slope values provide a comprehensive measure of the overall influence across all traits.

When evaluating the impact of salinity on genotypes, the R-squared value is a critical metric for assessing the significance of the parameters. Moreover, our hypothesis regarding the efficacy of the regression line slope (*b*) has been validated. In contrast, the Genotype Salinity Susceptibility Index (GSSI) model is not recommended, as it evaluates parameters independently rather than offering a comprehensive understanding of the genotypes' responses to salinity stress.

Previous scholarly investigations, alongside our findings, indicate that the *DV* value is a superior metric for assessing salt tolerance (Fang *et al.*, 2017; Xie *et al.*, 2021). Cluster analysis based on the *DV* value enabled a comparative evaluation of salt tolerance across different genotypes. The results of this study clearly categorized the genotypes into four clusters: the first cluster, comprising *A* and *D*, exhibited salt tolerance and thus represents a valuable set of materials suitable for cultivation in

saline environments. The analysis effectively delineated varying degrees of salinity sensitivity among the genotypes, classifying them as resistant (*D*, *A*, and *G*), moderately resistant (*F* and *V2*), sensitive (*S* and *Z*), and highly sensitive (*H* and *V3*) to salinity.

Yemeni chilli genotypes exhibit considerable variation in salinity tolerance. Results have identified genotypes A and D as promising candidates for cultivation in saline environments, designating them as elite genotypes. These genotypes offer valuable prospects for hybridization with those exhibiting moderate to low salt tolerance, aiming to enhance resilience and productivity. The study underscores the efficacy of using the regression line slope as a robust method for assessing genotypic sensitivity to salinity. These findings are pivotal for a*dv*ancing the development of salt-tolerant chilli cultivars, optimizing breeding strategies, and promoting sustainable agricultural practices in saline-affected regions.

References

ABRAHÁM E., RIGÓ G., SZEKELY G., NAGY R., KONCZ C., SZABADOS L., 2003 - *Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in* Arabidopsis. - Plant Mol. Biol., 3(51): 363-372.

AFZAL M., ALGHAMDI S.S., MIGDADI H.H., EL-HARTY E., AL-FAIFI S.A., 2022 - *Agronomical and physiological responses of faba bean genotypes to salt stress.* - Agriculture, 2(12): 235.

AHMAD A., BLASCO B., MARTOS V., 2022 - *Combating salinity through natural plant extracts based biostimulants: A review.* - Front. Plant Sci., 13: 862034.

AKTAS H., ABAK K., CAKMAK I., 2006 - *Genotypic variation in the response of pepper to salinity.* - Scientia Horticulturae, 110(3): 260-266.

AL-MADHAGI I., AL-SHARAGI H., 2019 - *Schinus molle leaves compost improves the growth, quality and productivity of strawberry (*Fragaria × ananassa *Duch) in potting culture.* - J. Hort. Plant Res., 7: 26-39.

AL-MAQTARY E., AL-MADHAGI I., AL-MUREISH K., 2024 - *Salicylic acid alleviates the adverse of salinity stress in fenugreek (*Trigonella foenum-graecum*).* - Asian J. Biol., 20(4): 30-58.

AL-SWEDI F., ALSHAMARI M., AL ZAIDI I., RIHAN H.Z., 2020 - *Impact of salinity stress on seed germination in lettuce (*Lactuca Sativa*).* - J. Res. Lepidoptera, 51: 374-385.

ALDOBAI H., AL-SHABI J., 2010 - *Estimation of the Morphological variation and yield components of some hot peppers genotypes in Yemen.* - Egypt. J. Appl.Sci. 25(6A): 402-421.

ALHADI F., IBRAHIM H., ALKADASY A.K., 2023 - *Evaluation of some growth parameters of millet (*Pennisetum glaucum *(L.) R. Br.) landraces cultivated in Al-Mawaset District, Taiz Governorate, Yemen* - Sana'a University J. Appl. Sci. Techn., 4(1): 400-410.

ALI L., SHAHEEN M.R., IHSAN M.Z., MASOOD S., ZUBAIR M., SHEHZAD F., 2022 - *Growth, photosynthesis and antioxidant enzyme modulations in broccoli (*Brassica oleracea *L. var.* italica*) under salinity stress.* - South African J. Bot., 148: 104-111.

AMIRINEJAD A.A., SAYYARI M., GHANBARI F., KORDI S., 2017 - *Salicylic acid improves salinity-alkalinity tolerance in pepper (*Capsicum annuum *L.).* - Adv. Hort. Sci., 31(3): 157-163.

ARRAF E.A., AL-MADHAGI I.A., 2025 - *Comparing effects of priming chili pepper seed with different plant biostimulants, with balancing effects on vegetative and root growths and seedling quality* - Int. J. Hortic. Sci. Techn. , 12(4):: 1173-1196.

BINZEL M.L., HASEGAWA P.M., HANDA A.K., BRESSAN R.A., 1985 - *Adaptation of tobacco cells to NaCl.* - Plant Physiol., 79(1): 118-125.

BOLTON A., SIMON P., 2019 - *Variation for salinity tolerance during seed germination in diverse carrot [*Daucus carota *(L.)] germplasm.* - HortSci., 54(1): 38-44.

CHARTZOULAKIS K., KLAPAKI G., 2000 - *Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages.* - Scientia Horti., 86(3): 247-260.

COLONNA V., D'AGOSTINO N., GARRISON E., ALBRECHTSEN A., MEISNER J., FACCHIANO A., CARDI T., TRIPODI P., 2019 - *Genomic diversity and novel genome-wide association with fruit morphology in* Capsicum*, from 746k polymorphic sites.* - Scientific Reports, 9: 10067, pp. 1-14.

COMPARINI D., TAITI C., LANZA M., VITA F., PANDOLFI C., LUTI S., SPINELLI F., PAZZAGLI L., MANCUSO S., 2021 - *Comparison of wild and domesticated hot peppers fruit: volatile emissions, pungency and protein profiles.* - Adv. Hort. Sci., 35(3): 305-327.

DAWD S.M., ABDULLA S.S., 2020 - *Effect of different salt concentrations on ratio, speed, growth and development of seedlings of some vegetable crops.* - Int. J. Agricult. Stat. Sci., 16(1): 1755-1759.

DEMIR I., MAVI K., 2008 - *Effect of salt and osmotic stresses on the germination of pepper seeds of different maturation stages.* - Brazilian Arch. Biol. Technol., 51: 897-902.

DEVI N.D., ARUMUGAM T., 2019 - *Screening of tomato genotypes at various levels of salinity.* - J. Pharmacognosy Phytochem., 8(3): 3199-3201.

EDELSTEIN M., BEN-HUR M., COHEN R., BURGER Y., RAVINA I., 2005 - *Boron and salinity effects on grafted and non-grafted melon plants.* - Plant Soil, 269(1): 273-284.

ESTAN M.T., MARTINEZ-RODRIGUEZ M.M., PEREZ-ALFOCEA F., FLOWERS T.J., BOLARIN M.C., 2005 - *Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot.* - J. Exp. Bot., 56(412): 703-712.

FANG Z., HU Z., ZHAO H., YANG L., DING C., LOU L., CAI Q., 2017 - *Screening for cadmium tolerance of 21 cultivars from Italian ryegrass (*Lolium multiflorum *Lam) during germination* - Grassland Sci., 63(1): 36-45.

FAO, 2022 - *United National food and agricultural statistical database*. - http://www.fao.org.

FAROOQI M.Q.U., ZAHRA Z., AFZAL M., GHANI M.I., 2021 - *Recent advances in plant adaptation to climate change. An introduction to compatible solutes*, pp. 1-9. - In: WANI S.H., M.P. GANGOLA, and B.R. RAMADOSS (eds.) *Compatible solutes engineering for crop plants facing climate change*. Springer, Cham, Switzerland, pp. 266.

FU H., YANG Y., 2023 - *How plants tolerate salt stress.* - Curr. Issues Mol. Biol., 45(7): 5914-5934.

GIORIO P., CIRILLO V., CARAMANTE M., OLIVA M., GUIDA G., VENEZIA A., GRILLO S., MAGGIO A., ALBRIZIO R., 2020 - *Physiological basis of salt stress tolerance in a landrace and a commercial variety of sweet pepper (*Capsicum annuum *L.).* - Plants, 9(6): 795.

GREENWAY H., MUNNS R., 1980 - *Mechanisms of salt tolerance in non halophytes.* - Ann. Rev. Plant Physiol., 31(1): 149-190.

GREGORY P.J., ISMAIL S., RAZAQ I.B., WAHBI A., 2018 - *Soil Salinity: Current status and in depth analyses for custainable use chapter 2*, pp. 4-11. - In: *Challenges and opportunities for crop production in dry and saline environments in Arasia Member States*. FAO-IAEA, Wien, Austria, pp. 124.

GUPTA B., HUANG B., 2014 - *Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization.* - Int. J. Genomics, 2014: 701596.

HAMDIA M., SHADDAD M., DOAA M., 2004 - *Mechanisms of salt tolerance and interactive effects of Azospirillum brasilense inoculation on maize cultivars grown under salt stress conditions.* - Plant Growth Reg., 44(2): 165-174.

HANNACHI S., VAN LABEKE M.-C., 2018 - *Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena *L.).* - Scientia Hort., 228: 56-65.

HASANUZZAMAN M., RAIHAN M.R.H., MASUD A.A.C., RAHMAN K., NOWROZ F., RAHMAN M., NAHAR K., FUJITA M., 2021 - *Regulation of reactive oxygen species and antioxidant defense in plants under salinity.* - Int. J. Mol. Sci., 17 22: 9326.

HASEGAWA P.M., BRESSAN R.A., ZHU J.-K., BOHNERT H.J., 2000 - *Plant cellular and molecular responses to high salinity.* - Ann. Rev. Plant Biol., 51(1): 463-499.

HORUZ A., BALKAYA A., YıLDıZ S., SARıBAŞ Ş., UYGUR V., 2022 - *Comparison of the salt stress tolerance of promising turkish winter squash (*Cucurbita maxima *Duch.) and pumpkin (*Cucurbita moschata *Duch.) lines and interspecific hybrids.* - Gesunde Pflanzen, 74(1): 69-86.

HOWLADER M.H.K., ISLAM M.N., BISWAS S., UDDIN M.E., SHILA A., HAQUE M.Z., MAHMUD N., 2018 - *Salt tolerance of chili genotypes during germination and seedling growth.* - Malays. J. Halal Res., 1(2): 1-7.

KADER M.A., 2005 - *A comparison of seed germination calculation formulae and the associated interpretation of resulting data.* - J. Proc. Royal Soc. New South Wales, 138(3-4): 65-75.

KARALIJA E., LOŠIĆ A., DEMIR A., ŠAMEC D., 2024 - *Effects of seed priming on mitigating the negative effects of increased salinity in two varieties of sweet pepper (*Capsicum annuum *L.).* - Soil Syst., 8(1): 35.

KAYA C., ASHRAF M., ALYEMENI M.N., AHMAD P., 2020 - *The role of endogenous nitric oxide in salicylic acid-induced up-regulation of ascorbate-glutathione cycle involved in salinity tolerance of pepper (*Capsicum annuum *L.) plants.* - Plant Phys. Biochem., 147: 10-20.

KAYACETIN F., 2022 - *Assessment of safflower genotypes for individual and combined effects of drought and salinity stress at early seedling growth stages* - Turkish J. Agric. For., 46(5): 601-612.

KHONDOKER M., MANDAL S., GURAV R., HWANG S., 2023 - *Freshwater shortage, salinity increase, and global food production: A need for sustainable irrigation water desalination. - A scoping review.* - Earth, 4(2): 223-240.

KHOSHSOKHAN F., BABALAR M., CHAGHAZARDI H., MOGHADAM M., 2012 - *Effect of salinity and drought stress on germination indices of two thymus species.* - Cercetări Agronomice în Moldova, 45 (1): 27-35.

LÄUCHLI A., EPSTEIN E., 1990 - *Plant responses to saline and sodic conditions* - Agric. Salinity Assessment Manag., 71: 113-137.

LOGANAYAKI K., TAMIZHMATHI S., BRINDA D., GAYATHRI S., MARY M.C., MOHANLAL V., 2020 - In vitro *evaluation of tomato (*Lycopersicon esculentum *Mill.), chilli (*Capsicum annum *L.), cucumber (*Cucumis sativus *L.) and Bhendi (*Abelmoschus esculentus *L.) for salinity stress.* - Inter. J. Chem. Studies, 8(2): 2364-2367.

LONG S.P., HUMPHRIES S., FALKOWSKI P.G., 1994 - *Photoinhibition of photosynthesis in nature.* - Ann. Rev. Plant Biol., 45(1): 633-662.

LYCOSKOUFIS I., SAVVAS D., MAVROGIANOPOULOS G., 2005 - *Growth, gas exchange, and nutrient status in pepper (*Capsicum annuum *L.) grown in recirculating nutrient solution as affected by salinity imposed to half*

of the root system. - Scientia Hort., 106(2): 147-161.

MAAS E.V., HOFFMAN G.J., 1977 - *Crop salt tolerance. - Current assessment.* - J. Irrig. Drain. Eng., 103(2): 115-134.

MAGGIO A., RAIMONDI G., MARTINO A., DE PASCALE S., 2007 - *Salt stress response in tomato beyond the salinity tolerance threshold.* - Environ. Exp. Bot., 59(3): 276-282.

MANGAL V., LAL M.K., TIWARI R.K., ALTAF M.A., SOOD S., KUMAR D., BHARADWAJ V., SINGH B., SINGH R.K., AFTAB T., 2023 - *Molecular insights into the role of reactive oxygen, nitrogen and sulphur species in conferring salinity stress tolerance in plants.* - J. Plant Growth Reg., 42(2): 554-574.

MEYER R., BOYER J., 1981 - *Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials.* - Planta, 151: 482-489.

MICELI A., MONCADA A., VETRANO F., 2021 - *Use of microbial biostimulants to increase the salinity tolerance of vegetable transplants.* - Agronomy, 11(6): 1143.

MOMENPOUR A., IMANI A., 2018 - *Evaluation of salinity tolerance in fourteen selected pistachio (*Pistacia vera *L.) cultivars.* - Adv. Hort. Sci., 32(2): 249-264.

MUBUSHAR M., EL-HENDAWY S., TAHIR M.U., ALOTAIBI M., MOHAMMED N., REFAY Y., TOLA E., 2022 - *Assessing the suitability of multivariate analysis for stress tolerance indices, biomass, and grain yield for detecting salt tolerance in advanced spring wheat lines irrigated with saline water under field conditions.* - Agronomy, 12: 3084.

MUNNS R., 1993 - *Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses.* - Plant, Cell Environ., 16(1): 15-24.

MUNNS R., GILLIHAM M., 2015 - *Salinity tolerance of crops - What is the cost?* - New Phytologist, 208(3): 668-673.

MUSHTAQ Z., FAIZAN S., GULZAR B., 2020 - *Salt stress, its impacts on plants and the strategies plants are employing against it: A review.* - J. Appl. Biol. Biotechn., 8(3): 81-91.

NAEEM M., BASIT A., AHMAD I., MOHAMED H.I., WASILA H., 2020 - *Effect of salicylic acid and salinity stress on the performance of tomato plants.* - Gesunde Pflanzen, 72: 393-402.

NEGRÃO S., SCHMÖCKEL S.M., TESTER M.A., 2016 - *Evaluating physiological responses of plants to salinity stress.* - Ann. Bot., 119(1): 1-11.

NTANASI T., SAVVAS D., KARAVIDAS I., PAPADOPOULOU E.A., MAZAHRIRH N., FOTOPOULOS V., ALIFERIS K.A., SABATINO L., NTATSI G., 2024 - *Assessing salinity tolerance and fruit quality of pepper landraces*. - Agronomy, 14(2): 309.

PASARIBU S.A., BASYUNI M., PURBA E., HASANAH Y., 2021 - *Drought tolerance selection of GT1 rubber seedlings with the addition of polyethylene glycol (PEG) 6000.* - Biodiversitas. J. Biol. Div., 22(1): 394-400.

QIU R., JING Y., LIU C., YANG Z., WANG Z., 2017 - *Response of hot pepper yield, fruit quality, and fruit ion content to irrigation water salinity and leaching fractions.* - HortScience, 52(7): 979-985.

RANAL M.A., 1999 - *Effects of temperature on spore germination in some fern species from semideciduous mesophytic forest.* - Am. Fern J., 89(2): 149-158.

RANAL M.A., SANTANA D.G.D., 2006 - *How and why to measure the germination process.* - Brazilian J. Bot., 29: 1-11.

ROȘCA M., MIHALACHE G., STOLERU V., 2023 - *Tomato responses to salinity stress: From morphological traits to genetic changes.* - Front. Plant Sci., 14: 1118383.

SAHBENI G., NGABIRE M., MUSYIMI P.K., SZEKELY B., 2023 - *Challenges and opportunities in remote sensing for soil salinization mapping and monitoring: A review.* - Remote Sensing, 15(10): 2540.

SAKAMOTO A., MURATA N., 2001 - *The use of bacterial choline oxidase, a glycinebetaine-synthesizing enzyme, to create stress-resistant transgenic plants.* - Plant Physiol., 125(1): 180-188.

SANTA-CRUZ A., MARTINEZ-RODRIGUEZ M.M., PEREZ-ALFOCEA F., ROMERO-ARANDA R., BOLARIN M.C., 2002 - *The rootstock effect on the tomato salinity response depends on the shoot genotype.* - Plant Sci., 162(5): 825-831.

SARKAR A.K., ORAON S., MONDAL S., SADHUKHAN S., 2023 - *Effect of salinity on seed germination and seedling growth of bullet cultivar of chilli (*Capsicum annuum *L.).* - Brazilian J. Bot., 46(3): 513-525.

SEMIZ G.D., SUAREZ D.L., ÜNLUKARA A., YURTSEVEN E., 2014 - *Interactive effects of salinity and N on pepper (*Capsicum annuum *L.) yield, water use efficiency and root zone and drainage salinity.* - J. Plant Nutr., 37(4): 595-610.

SERIO A., MAGGIO F., BEN HSOUNA A., BEN SAAD R., TAITI C., GARZOLI S., 2024 - *Exploring the metabolome and antimicrobial properties of* Capsicum annuum *L. (Baklouti and Paprika) dried powders from Tunisia* - Molecules, 29(22): 5236.

SINGH J., SASTRY E.D., SINGH V., 2012 - *Effect of salinity on tomato (*Lycopersicon esculentum *Mill.) during seed germination stage.* - Physiol. Mol. Biol. Plants, 18: 45-50.

SINGLA-PAREEK S., REDDY M., SOPORY S., 2003 - *Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance*. - Proceedings Nat. Academy Sci., 100(25): 14672-14677.

SUAREZ D.L., 2001 - *Sodic soil reclamation: Modelling and field study.* - Soil Res., 39(6): 1225-1246.

SWAMY K., 2023 - *Origin, distribution, taxonomy, botanical description, genetic diversity and breeding of capsicum (*Capsicum annuum *L.).* - Int. J. Dev. Res., 13: 61956-61977.

TAITI C., COMPARINI D., MOSCOVINI L., VIOLINO S., COSTA C., MANCUSO S., 2024 - *Influence of the drying process on the volatile profile of different* Capsicum *species.* - Plants, 13(8): 1131.

TAITI C., COSTA C., MENESATTI P., COMPARINI D., BAZIHIZINA N., AZZARELLO E., MASI E., MANCUSO S., 2015 - *Class-modeling approach to PTR-TOFMS data: A peppers case study.* - J. Sci. Food Agric., 95(8): 1757-1763.

TALSKÁ R., MACHALOVÁ J., SMÝKAL P., HRON K., 2020 - *A comparison of seed germination coefficients using functional regression.* - Appl. Plant Sci., 8(8): e11366.

TAROLLI P., LUO J., PARK E., BARCACCIA G., MASIN R., 2024 - *Soil salinization in agriculture: Mitigation and adaptation strategies combining nature-based solutions and bioengineering.* - iScience, 27(2): 108830.

USAID, 2010 -*Yemen-propery rights and resource governance profile.* - https://www.land-links.org/country-profile/yemen/

WIEN H.C., STUTZEL H., 2020 - *The physiology of vegetable crops.* - CABI, Wallingford, UK, pp. 497.

XIE Y., LIU X., AMEE M., YU H., HUANG Y., LI X., CHEN L., FU J., SUN X., 2021 - *Evaluation of salt tolerance in Italian ryegrass at different developmental stages.* - Agronomy, 11(8): 1487.

YANG A., DUAN X., GU X., GAO F., ZHANG J., 2005 - *Efficient transformation of beet (*Beta vulgaris*) and production of plants with improved salt-tolerance* - Plant Cell, Tissue Organ Cult., 83(3): 259-270.

ZAMLJEN T., MEDIC A., HUDINA M., VEBERIC R., SLATNAR A., 2022 - *Salt stress differentially affects the primary and secondary metabolism of peppers (*Capsicum annuum *L.) according to the genotype, fruit part, and salinity level.* - Plants, 11(7): 853.

ZHOU X.-J., HUANG H.-X., ZHANG J.-X., 2023 - *Effects of salt stress on photosynthetic characteristics of Gymnocarpos przewalskii seedlings* - Acta Prataculturae Sinica, 32(2): 75.

ZHU G., KINET J.M., BERTIN P., BOUHARMONT J., LUTT S., 2000 - *Crosses between cultivars and tissue culture-selected plants for salt resistance improvement in rice,* Oryza sativa. - Plant Breeding, 119(6): 497-504.

ZOWAIN A., 2014 - *Effect of salt stress on germination attributes in maize* - Iraqi J. Agric. Sci., 45(7): 738-745.