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REPORT FOR THE RESPONSE OF GENOTYPES UNDER ABIOTIC STRESS CONDITIONS

DELIVERABLE 1.1

PulpIng

Developing of **Pu**mpkin Pu**lp** Formulation using a Sustainable **In**te**g**rated Strategy

Index

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1. Scope of the document

In this WP, selected pumpkin germplasm will be evaluated under *in vitro* in terms of abiotic stress tolerance aiming to the selection of genotypes with abiotic stress tolerance.

To date, very few studies have focused on pumpkin germplasm evaluation for abiotic stress tolerance and selection of cultivars with improved tolerance and high yield stability under sub-optimal environmental conditions. Breeding for abiotic stress tolerance is seriously hampered by the trait's complex inheritance, the wide environmental variation associated with it as well as the lack of suitable selection methods. The routinely employed screening methodologies are based on estimating yield reduction under stress conditions, usually applied at plant's most critical growth stages. Given that vegetable crops are particularly susceptible to abiotic stresses at early growth stages, the determination of seed germination potential and seedling growth under stress conditions will be employed as a method to select tolerant genotypes. Such shortcut approach bypasses the difficulty encountered in achieving controlled stress in field conditions and further enables the evaluation of a large germplasm collection. Moreover, screening for abiotic stress tolerance at early growth stages has been proven to provide an accurate estimation of yield and growth potential under sub-optimal environmental conditions, while it is less time consuming and allows for testing at controlled environments.

2. Methodology

TN (ISACM) and GR (UTH) have a collection of pumpkin germplasm of local landraces, that needs to be morphologically, molecularly and biochemically characterized for further selection of elite genotypes adapted to the variable environmental conditions of the Mediterranean basin and suitable for organic farming practices and low agrochemical inputs (fertilizers and pesticides). Moreover, pumpkin genotypes will be obtained from the available national gene-banks and official agencies for genetic resources conservation from all the participating countries (e.g. Greek Gene Bank, National GeneBank of Tunisia, National GeneBank of Egypt, Portuguese Bank of Plant Germplasm, National Plant Genetic Resources Centre of Spain) in order to capture a sufficient genetic variability of the species for the Mediterranean basin.
Seeds from commercial pumpkin cultivars (control genotypes) and local landraces (GR and TN) will be collected and used for screening abiotic stress tolerance during germination and early seedling growth. The selected genotypes will be screened for tolerance against various environmental stresses, including drought, high salinity and extreme temperatures (low and high).

2.1 Germplasm evaluation for abiotic stress tolerance

Experiment 1

The experiment aimed at determining the seed germination and seedling growth potential under abiotic stress conditions as a short-cut approach to identify tolerance in pumpkin germplasm at early growth stages. The study was carried out at the Department of Agriculture, Crop production and Rural Environment, University of Thessaly.

The genetic material consisted of commercial pumpkin cultivars (control genotypes, eg. Fytro FS 243 and Big Max) and 5 local landraces whose adaptation to various agroclimatic conditions is not yet established. The selected genotypes were screened for tolerance against various environmental stresses, namely drought, high salinity and extreme temperatures (low and high).

The following genotypes were tested in experiment 1

- Fytro FS 243 (Hybrid) (FS)
- Local Landrace from the region of Trikala, Greece (TRI)
- Big Max: Commercial cultivar (BM)

- Local landrace “Nychaki” from the region of Orestiada, Greece (NYC)
- Local landrace from the region of Lakonia, Greece (cylindrical shaped) (LC)
- Local landrace “Makedonika green” from the region of Karditsa, Greece (MG)
- Local landrace from the region of Lakonia, Greece (spherical shape) (LS)

2.2 Stress treatments and experimental design

Seeds were initially surface-sterilized for 5 min in 20% hypochlorite/H_2O solution supplemented with Tween-20, while gently mixing, and washed 4 times with excess of sterile water.

Sterilized seeds were placed and allowed to germinate in plastic trays containing different solutions as follows:

1. Drought stress. Addition of D-mannitol at different stress levels (0, 100, 200 and 300 mM)
2. Salinity stress. Addition of NaCl at different stress levels (0, 100, 200 and 300 mM)
3. Heat stress
   - Germination and emergence assays: seed incubation at 37-45 °C for a period of 14 days. Germinated or emerged seeds will be counted and expressed as a percentage of the total number of seeds tested.
   - Seedling growth assays: Seedlings will be incubated at 37-45 °C for a period of 4-6 days. Following heat stress, seedlings will be allowed to recover and assessed heat stress tolerance using various growth parameters.
4. Cold stress
   - Germination and emergence assays: seed incubation at 4 °C for a period of 14 days. Germinated or emerged seeds will be counted and expressed as a percentage of the total number of seeds tested.
   - Seedling growth assays: Seedlings will be incubated at 4 °C for a period of 4-6 days. Following cold stress, seedlings will be allowed to recover and assessed for cold stress tolerance using various growth parameters.

Seedlings were grown under controlled conditions (25˚C, 16 h light/8 h dark) for a period of 20 days. In all assays, non-stressed plants were also included as controls. Trays were regularly monitored for the level of containing solution and, when necessary, H_2O was added in order to retain constant concentration of the stress factors applied.

The experimental layout was that of a completely randomised design with four replications, each consisting of 30 seeds. Each experimental plot (tray) consisted of four rows, of which the two middle were used to provide material for the measurements.

Evaluation of tolerance was performed on the basis of the following parameters:

- Germination rate and percentage (%): Estimation at seven time intervals (1^{st} until 7^{th} day). Seeds were considered germinated when the radicle has a length of at least 2 mm.
- Seed water absorbance (WU) (%): Estimation at the 1st and day after start of germination test. WU was expressed as a percentage according to the formula WU (%)= (W2-W1) / W1x 100, where W_1 = initial seed weight and W_2 = seed weight following water absorbance. WU was estimated from 12 seeds (3 per replication) for each cultivar.
- Root and shoot length (cm): Estimation at three time intervals.
- Root and shoot weight (g) Estimation at the 1st and day after start of germination test.
- Seedling water content (WC) (%): Estimation at the 15^{th} day after start of germination test. Seedling water content (WC) was expressed as a percentage according to the formula WC (%) = (fresh weight - dry weight / fresh weight) x 100. For estimation of dry weight, 12 seedlings (3 per replication) of each cultivar was incubated at 70°C for 2 days.

- Seedling vigor index (SVI) (%): Estimation at the 7th day after start of germination test. SVI was calculated following the modified formula SVI = shoot length (cm) × germination percentage.
- Number of seedlings with abnormal phenotype: Estimation throughout the period of observations.

Data were analysed by ANOVA ($p \leq 0.05$), according to the experimental design, combining stress levels and genotypes. Differences between means were compared by the LSD test.

Experiment 2

Based on the results of the first experiment and the availability of seeds, the tolerance of selected genotypes against salinity stress was tested.

The same methodology as in experiment was implemented. The selected genotypes were the following:

- Fytro FS 243 (Hybrid) (FS)
- Local landrace from the region of Trikala, Greece (TRI)
- Local landrace from the region of Meliti, Greece (white pericarp) (MEL)
- Local landrace from the region of Lakonia, Greece (spherical shape) (LS)
- Local landrace from the region of Lakonia, Greece (oval shaped) (LO)
- Local landrace from the region of Lakonia, Greece (cylindrical shaped) (LC)
- Local landrace "Makedonika green" from the region of Karditsa, Greece (MG)

Experiments in Tunis

The studied genetic material consisted of fifteen Tunisian squash landraces, collected from different geographic regions of Tunisia during the period extending from 2018 to 2020 (Table 1). Each landrace was assigned a passport data and an inventory number, according to the National Gene Bank of Tunisia, while full details are available at the Germplasm Resources Information Network - GRIN (http://www.tn-grin.nat.tn/gringlobal/search.aspx).

Table 1. Description of the Tunisian squash landraces employed in this study.

Landrace inventory number	Local name	Origin	Latitude	Longitude	Short description
NGBTUN745 ("745")	Batati Green	Ariana (Kalaat Andalous)	37°033"N	10°11'7"E	globular fruit, light green skin, green flesh
NGBTUN746 ("746")	Batati orange	Siliana (SidiHamada)	35°57'28"N	9°32'57"E	globular fruit, orange skin, light orange flesh
NGBTUN747 ("747")	Galaoui	Ariana (Kalaa Andalous)	37°033"N	10°11'7"E	raised fruit with basal tip, green skin, green flesh
NGBTUN748 ("748")	Karkoubi orange	Sousse (SidiBouali)	35°54'22.21"N	10°32'47.81"E	flattened fruit, dark yellow

					skin, yellow flesh
NGBTUN749 (“749”)	Batati yellow spotted with white	Siliana (SidiHamada)	35°57‘28"N	9°32'57"E	globular fruit, orange skin spotted with white, orange flesh
NGBTUN750 (“750”)	Batati white	Monastir (Sahline)	35°45'05"N	10°42'39"E	globular fruit, white skin, white flesh
NGBTUN751 (“751”)	Bejaoui Green	Siliana (SidiHamada)	35°57‘28"N	9°32'57"E	flattened fruit, dark green skin, light green flesh
NGBTUN752 (“752”)	Batati yellow	Siliana (North)	35°57‘28"N	9°32'57"E	globular fruit, yellow skin, light orange flesh
NGBTUN753 (“753”)	Béjaoui Green	Siliana (South)	35°57‘28"N	9°32'57"E	flattened fruit, dark green skin, light green flesh
NGBTUN1004 (“1004”)	Galaoui large seeds	Ariana (Kalaat Andalous)	37°033"N	10°11'7"E	turbinate interior fruit with basal tip, green skin, white green flesh
NGBTUN1005 (“1005”)	Galaoui smoll seeds	Ariana (Kalaat Andalous)	37°033"N	10°11'7"E	turbinate interior fruit with a big basal tip, green skin, white green flesh
NGBTUN1006 (“1006”)	Karkoubi orange	Monastir (Sahline)	35°45'05"N	10°42'39"E	flattened fruit, dark yellow skin, yellow flesh
NGBTUN1007 (“1007”)	Batati Green	Siliana	35°57‘28"N	9°32'57"E	rounded fruit, green skin, green flesh
NGBTUN1008 (“1008”)	Batati Green	Monastir (Teboulba)	35°45'05"N	10°42'39"E	globular fruit, flat stem end, green skin, light green flesh
NGBTUN1009 (“1009”)	Bejaoui spotted with yellow	Siliana (SidiHamada)	35°57‘28"N	9°32'57"E	globular fruit with flat stem end, spotted with yellow

light green skin,
light green flesh

1. Salinity stress treatments

The experiment was carried out at the Department of Horticulture, High Agronomic Institute of Chott Mariem-Sousse-Tunisia. Following the selection of seeds for size homogeneity, 50 seeds per landrace (five petri dishes with 10 seeds each, n=5) were surface-sterilized for 5 min in 10% H_2O_2 (v/v) and rinsed twice in sterile dH2O. Sterilized seeds were primed via exposure to an eliciting solution of 1.5 mM gibberellic acid (GA_3) for 24 hours, to stimulate germination, and subsequently rinsed in sterile dH_2O. Five to ten seeds, according to size, were placed on sterile petri dishes containing two layers of filter paper moistened daily with 5 mL of appropriate solutions: dH_2O (control), 100, 200 and 300 mM NaCl. Seedlings were grown under controlled conditions for 7 days (25 ± 2 °C, 50 ± 5% relative humidity, 18-h light/6-h dark photoperiod under white fluorescent light (40 µmol m^{-2} s^{-1}).

2. Determination of germination and seedling growth potential under salt stress

Salt tolerance was evaluated on the basis of various parameters related to seed germination and seedling growth potential under salt stress conditions, measured daily until no more germinated seeds were recorded (1st-7th day) (Table 2). Seeds were considered germinated when the protruding radicle was at least 2 mm long. The parameters germination reduction (GR), root length reduction (RLR) and shoot length stress tolerance index (SLSTI) express the decreased values of salt-stressed plants over the control treatment.

Table 2. Description of the evaluation criteria for salinity tolerance employed in this study.

Trait	Unit	Description/Formula
Germination percentage (GP)	%	$GP = \frac{\text{number germinated seeds}}{\text{number of total seeds}} x\ 100$
Shoot length (SL)	mm	At the day of germination
Root length (RL)	mm	At the day of germination
Shoot fresh weight (SFW)	g	Recorded by using a sensitive balance (Sartorius AC 1215, Germany)
Root fresh weight (RFW)	g	Recorded by using a sensitive balance (Sartorius AC 1215, Germany)
Shoot length /Root length Ratio (SRR)	-	Ratio of SL to RL
Germination reduction (GR)	%	GR = GP of controls – GP of stress plants
Shoot length reduction (SLR)	mm	SLR= SL of controls – SL of stress plants
Root length reduction (RLR)	mm	RLR= RL of controls – RL of stress plants
Germination stress tolerance index (GSTI)	%	$GSTI = \frac{\text{GP under salt stress conditions}}{\text{GP under normal conditions}} x\ 100$
Shoot length stress tolerance index (SLSTI)	%	$SLSTI = \frac{\text{SL under salt stress condtions}}{\text{SL under normal conditions}} x100$
Root length stress tolerance index (RLSTI)	%	$RLSTI = \frac{\text{RL under salt stress condtions}}{\text{RL under normal conditions}} x100$

3. Evaluation of salinity tolerance based on biochemical parameters

Based on the obtained data related to the response of the 15 landraces to salinity stress, 4 landraces representing the main types of cultivated squash, were selected for further evaluation. As such, the landraces "748" (Karkoubi), "751" (Bejaoui), "747" (Galaoui) and "746" (Batati) were assessed, employing the content of seedling tissues in MDA (MDA), free proline and chlorophyll a and b as evaluation criteria.

The content of MDA (MDA) was determined using the method applied by Hnilickova et al. (2021) with minor modifications. Briefly, 200 mg of leaf samples were homogenized with liquid nitrogen and, following the addition of 80% ethanol, samples were centrifuged for 5 min at 6000 rpm. Aliquots of 0.7 mL of the supernatant solution were mixed with 0.7 mL of 0.65% thiobarbituric acid (TBA) in 20% TCA (trichloroacetic acid) and 0.01% BHT (butylated hydroxytoluene). A second set of 0.7 mL samples was mixed with 0.7 mL of 20 % TCA and 0.01 % BHT. Following incubation at 95 °C for 25 min and subsequent cooling, samples were centrifuged for 5 min at 6000 rpm. The content of MDA was determined at 532 nm using a UV–Vis spectrophotometer (Evolution 210, Thermo Scientific, Abingdon, GB) and expressed in µmol g^{-1} of fresh weight (FW).

The content of free proline was measured according to the method described by Monneveux and Nemmar (1986). Leaf sample (100 mg) were homogenized in 10 mL of 3% sulfosalicylic acid and, following filtration, the homogenate was heated to 85 °C in a water bath for 60 min. After cooling, 1 mL of ninhydrin reagent was added (ninhydrin reagent consisted of 120 mL distilled water, 300 mL of acetic acid, 80 mL acetic orthophosphoric acid at a density of 1.7, and 25 mg of ninhydrin). The samples were boiled for 30 min and, after cooling, 5 mL of toluene were added and samples were vortexed. The upper phase was recovered and was measured using a UV-Vis spectrophotometer (Evolution 210, Thermo Scientific, Abingdon, GB) at 528 nm. A proline standard curve ranging from 0 to 2.5 mg mL^{-1} of L-proline was used to determine the proline content, expressed in µg mg^{-1} of FW.

For chlorophyll content determination, the extraction of samples was performed as described by Curtis and Shetty (1996.) Briefly, 50 mg of leaf tissue (in triplicate) were extracted into 3 mL of methanol and stored at 23 °C in darkness for 2 h. Absorption of extracts (1.5 mL) was measured at 650 and 665 nm using a spectrophotometer (Evolution 210, Thermo Scientific, Abingdon, GB). Chlorophyll a and chlorophyll b were expressed in mg g^{-1} FW.

Statistical analysis

The experimental layout was completely randomized with three replications. Data were analyzed using ANOVA tests ($P \leq 0.05$), according to the experimental design, combining salt concentrations and genotypes. Differences between means were compared using the Duncan Multiple Range test (DMRT). Statistical analyses were performed using SAS software V9 (SAS Institute, Cary, North Carolina, U.S.).

3. Results

3.1 UTH (Status: finished)

3.1.1 Experiment 1

A) Water stress

Table 3. Seed germination percentage (GP %) of pumpkin genotypes (G) in relation to water stress (mM of Mannitol) throughout the germination test (7 days).

Days	Genotypes (G)	Mannitol (mM) (C)			
		0	100	200	300

1st						Mean (G)
	FS	82.50a	17.50ab	0.00b	0.00b	25.00a
	TRI	2.50c	0.00b	0.00b	0.00b	0.62b
	BM	62.50ab	27.50ab	20.00a	0.00b	27.50a
	NYC	50.00b	25.00ab	20.00a	10.00a	26.25a
	LC	22.50c	0.00b	0.00b	0.00b	5.62b
	MG	5.00c	0.00b	0.00b	0.00b	1.25b
	LS	75.00ab	35.00a	10.00ab	0.00b	30.00a
	Mean (C)	42.85a	15.00b	7.14bc	1.43c	
2nd						Mean (G)
	FS	90.00a	32.50b	0.00c	0.00b	30.62b
	TRI	12.50e	0.00c	0.00c	0.00b	3.12d
	BM	75.00b	42.50b	30.00ab	0.00b	36.87b
	NYC	47.50c	35.00b	27.50b	17.50a	31.87b
	LC	32.50d	0.00c	0.00c	0.00b	8.12c
	MG	25.00d	0.00c	0.00c	0.00b	6.25c
	LS	77.50b	62.50a	35.00a	20.00a	48.75a
	Mean (C)	51.42a	24.64b	13.21bc	5.35d	
3rd						Mean (G)
	FS	92.50a	42.50b	0.00b	0.00b	33.75b
	TRI	27.50d	0.00d	0.00b	0.00b	6.88c
	BM	77.50b	45.00b	30.00a	0.00b	38.12b
	NYC	50.00c	42.50b	35.00a	35.00a	40.00b
	LC	40.00c	0.00d	0.00b	0.00b	10.00c
	MG	47.50c	2.50c	0.00b	0.00b	12.50c
	LS	82.50ab	67.50a	37.50a	35.00a	55.62a
	Mean (C)	59.64a	28.57b	14.28c	10.00c	
4th						Mean (G)
	FS	95.00a	42.50b	2.50c	0.00b	35.00b
	TRI	37.50d	0.00c	0.00d	0.00b	9.37c
	BM	77.50b	45.00b	30.00b	0.00b	38.12b
	NYC	52.50bc	47.50b	32.50b	35.00a	41.87b
	LC	47.50cd	0.00c	0.00d	0.00b	11.87c
	MG	52.50c	0.00c	0.00d	0.00b	13.75c
	LS	85.00ab	77.50a	42.50a	35.00a	60.00a
	Mean (C)	63.92a	30.71b	15.35c	10.00c	
5th						Mean (G)
	FS	95.00a	42.50c	5.00c	0.00d	35.62c
	TRI	47.50d	0.00e	0.00d	0.00d	11.87d
	BM	80.00b	45.00bc	32.50b	2.50c	40.00b
	NYC	52.50d	55.00b	45.00a	32.50b	46.25b
	LC	52.50d	0.00e	0.00d	0.00d	13.12d
	MG	65.00c	2.50d	2.50c	0.00d	17.50d
	LS	87.50ab	85.00a	47.50a	42.50a	65.62a
	Mean (C)	68.57a	32.85b	18.92c	11.07c	
6th						Mean (G)
	FS	95.00a	45.00b	5.00c	0.00d	36.25c
	TRI	47.50c	0.00d	0.00d	0.00d	11.87d
	BM	87.50a	47.50b	32.50b	2.50c	42.50bc

	NYC	52.50c	55.00b	50.00a	32.50b	47.50b
	LC	52.50c	0.00d	0.00d	0.00d	13.12d
	MG	67.50b	2.50c	2.50c	0.00d	18.12d
	LS	87.50a	85.00a	47.50a	45.00a	66.25a
	Mean (C)	70.00a	33.57b	19.64c	11.42c	
7th						Mean (G)
	FS	95.00a	47.50b	5.00c	0.00d	36.87c
	TRI	50.00c	0.00d	0.00d	0.00d	12.50d
	BM	90.00a	47.50b	32.50b	2.50c	43.12bc
	NYC	52.5c	55.00b	55.00a	32.50b	48.75b
	LC	57.50c	0.00d	0.00d	0.00d	14.37d
	MG	72.50b	2.50c	2.50c	0.00d	19.37d
	LS	90.00a	85.00a	47.50a	45.00a	66.87a
	Mean (C)	72.50a	33.92b	20.35bc	11.42d	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 3 shows the germination percentage (GP) for 7 seven consecutive days after germination initiation. In day 1 of the experiment, the best results were recorded for FYTRO FS 243 (FS), followed by local landrace 5 (LS) and BIG MAX (BM) genotypes for the control treatment, while LS showed the highest GP when seeds were subjected to 100 mM of Mannitol. Similarly, local landrace "Nychaki" (NYC) and BM performed better at 200 mM of Mannitol. Finally, BM was the only genotype that seeds germinated at the highest stress level (300 mM of Mannitol).

In days 2-7, FS, BM and LS achieved the highest GP at the control treatment (95%, 90% and 90%, respectively), while the highest GP was recorded in day 4 for FS, and in day 7 for BM and LS. In the case of the first drought level (100 mM of Mannitol), LS was the best performing genotype with GP of 85.0% achieved in day 6), while the next best performing genotypes were NYC (55% in day 5), BM (47.5% in day 6) and FS (47.5% in day 7). For the next drought level (200 mM of Mannitol), NYC recorded the highest GP (55.0% in day 7), followed by LS (47.5% in day 6). Finally, the highest drought level (300 mM of Mannitol) severely affected GP for most of the tested genotypes except for LS and NYC which recorded 32.5% (in day 5) and 45.0% (in day 6). Therefore, the results show that the best overall performance was recorded for the genotype LS which had the highest GP under the highest drought level, while at the same time was among the best performing genotypes in the control and mild (100 mM of Mannitol) and moderate (200 mM of Mannitol) stress levels. NYC also showed promising results since it was the second best performing genotype with mean GP of 47.5%, regardless of the stress treatment.

Table 4. Percentage of seed water uptake (WU %) in relation to genotype (G) and water stress (mM of Mannitol) at the 1st day after stress initiation.

Days	**Genotypes (G)**	**Mannitol (mM) (C)**				
		0	100	200	300	
1st						Mean (G)
	FS	150.00a	100.00c	66.67c	83.88c	100.00c
	TRI	150.00a	142.85a	125.00a	117.85a	133.92a
	BM	91.67d	75.00e	66.67c	58.33d	72.91d
	NYC	150.00a	120.00b	110.00b	100.00b	120.00b

LC	75.00e	87.50d	75.00c	100.00b	84.37d
MG	103.12c	103.12c	115.62ab	100.00b	105.46c
LS	118.75b	125.00b	125.00a	112.50ab	120.31b
Mean (C)	119.79a	107.64ab	97.70b	96.00b	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 4 presents the results regarding the water uptake (WU) of seeds 1 day after germination started. High percentage of WU is associated with high tolerance, since this parameter indicates that's seeds are able to absorb water under unfavorable conditions and facilitate germination initiation. Our results showed that under control conditions, FS, TRI and NYC were the best performing genotypes, whereas under drought TRI was the genotype that showed the best overall performance as well as the best performance in each particular drought level. Moreover, LS and NYC recorded high mean GP regardless of the stress level, a finding which is in accordance with the results presented in Table 2. These results also highlight the importance of water absorbance in the first day of germination, although tolerance to drought may involve other parameters since seeds of TRI did not germinate under high drought despite the high WU values.

Table 5. Percentage of seedling water content (WU %) in relation to genotype (G) and waters stress (mM of Mannitol) 15 days after stress initiation.

Days	**Genotypes (G)**	**Mannitol (mM) (C)**				
		0	100	200	300	
15th						Mean (G)
	FS	1625.00a	695.83ab	0.00b	-	580.20a
	TRI	460.00d	0.00c	0.00b	-	115.00c
	BM	830.00c	725.00a	353.75a	-	477.18b
	NYC	1062.50b	760.41a	0.00b	-	455.72b
	LC	0.00e	0.00c	0.00b	-	0.00d
	MG	0.00e	0.00c	0.00b	-	0.00d
	LS	1100.00b	666.66b	0.00b	-	441.66b
	Mean (C)	725.35a	406.84b	50.53c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 5 presents the results of seedling water content (WU) in day 15 where FS performed better under control conditions, while BM and NYC had the best performing under mild stress (100 mM of Mannitol) and BM the best one under moderate stress conditions (200 mM of Mannitol). It also worth's to mention that no seedlings were recorded under the highest stress level (300 mM of Mannitol) in day 15 after germination started.

Table 6. Seedling vigour index (SVI %) in relation to genotype (G) and water stress (mM of Mannitol) 7 days after stress initiation

Days	**Genotypes (G)**	**Mannitol (mM) (C)**

	0	100	200	300	
7th					Mean (G)
FS	127.50c	26.00b	0.00c	-	38.37c
TRI	27.00f	0.00d	0.00c	-	6.75f
BM	184.00a	31.25b	0.00c	-	53.81b
NYC	82.00d	35.50b	14.25b	-	32.93c
LC	83.25bcd	0.00d	0.00c	-	20.81d
MG	53.75e	1.25c	0.00c	-	13.75e
LS	157.50b	80.50a	27.25a	-	66.31a
Mean (C)	102.14a	24.92b	5.92bc	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 6 presents the results regarding seedling vigour index (SVI) which indicates the tolerance of genotypes under stress conditions. According to these results NYC was the best overall performing genotype, while the same genotype was also the most tolerant under mild and moderate stress conditions (SVI values of 35.0% and 14.25, respectively).

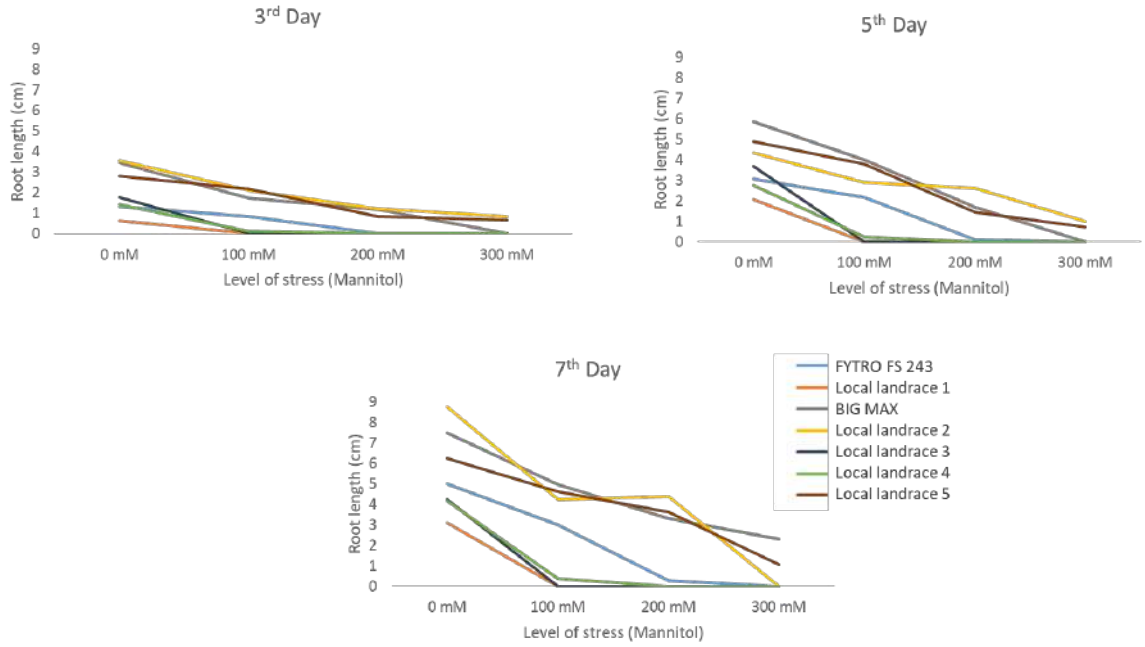


Figure 1. Root length at day 3, 5 and 7 in relation to genotype (G) and water stress level (mM of Mannitol, C).

Decreasing trends in root length were observed with increasing drought with significant differences among the tested genotypes (Figure 1). In particular, TRI, LC and MG were the most susceptible to drought stress since a significant reduction in root length was observed under mild stress (100 mM of Mannitol) even after 3 days from stress initiation, whereas NYC, LS and BM were the most tolerant genotypes with significant decreases after 7 days of moderate and high stress conditions (200 mM and 300 mM of Mannitol).

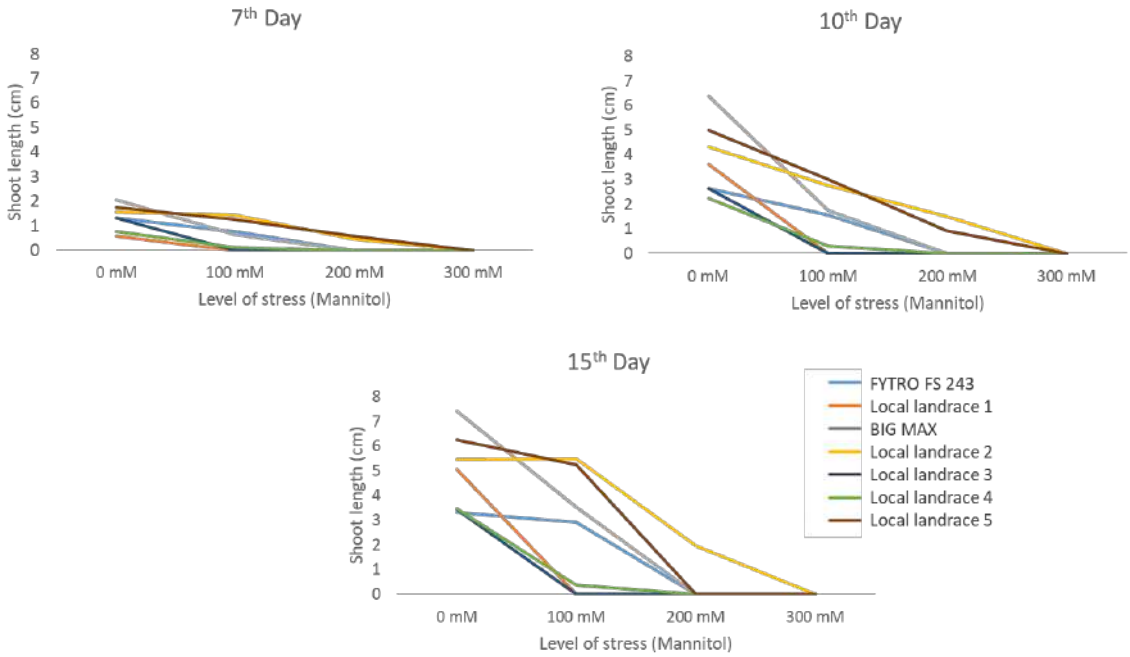


Figure 2. Shoot length at day 7, 10 and 15 in relation to genotype (G) and water stress level (mM of Mannitol, C).

Decreasing trends were also observed in shoot length of seedlings, where TRI, LC and MG were again the most susceptible cultivars to drought stress with significant reduction being observed under 15 days of mild stress (Figure 2). In contrast, NYC showed the highest tolerance since shoot length was reduced after seedlings being subjected to high stress conditions for 15 days, while the shoot length for rest of the genotypes (FS, LS and BM) was reduced when plants were subjected to 200 mM of Mannitol. These findings are also depicted in Photo 1, where NYC is the only genotype with visible roots under the treatment of 300 mM of Mannitol, while LS also had the most developed shoot under moderate drought stress (200 mM of Mannitol).

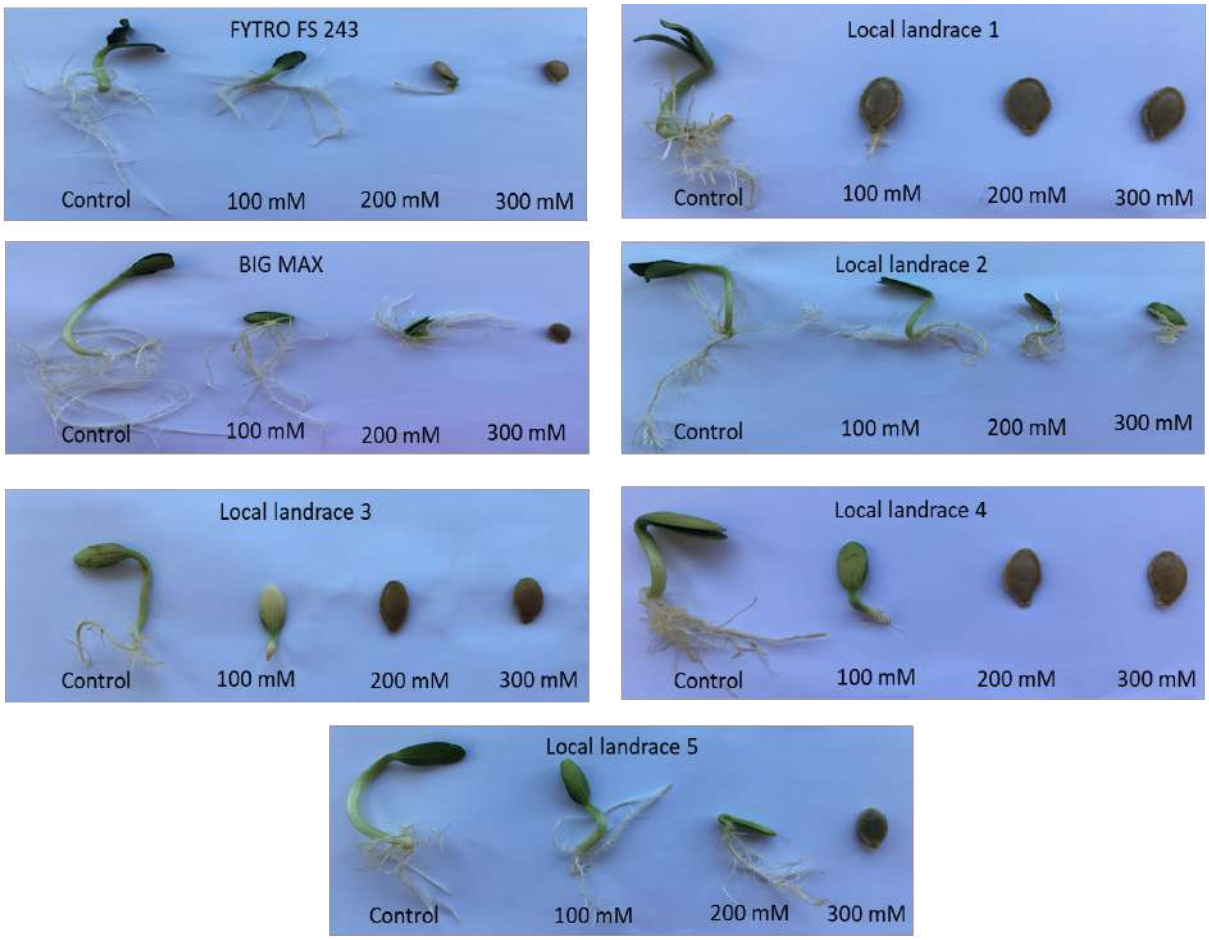


Photo 1. Seed germination of pumpkin genotypes in relation to water stress level (mM of Mannitol) at 10 days after sowing.

B) Salinity stress

Table 7. Seed germination percentage (GP %) of pumpkin genotypes (G) in relation to salinity stress (mM of NaCl (C)) throughout the germination test (7 days).

Days	Genotypes (G)	NaCl (mM) (C)				
		0	100	200	300	
1st						Mean (G)
	FS	37.50b	2.50c	0.00c	-	10.00b
	TRI	7.50e	0.00d	0.00c	-	1.88d
	BM	40.00ab	0.00d	0.00c	-	10.00b
	NYC	47.50a	45.00a	10.00a	-	25.62a
	LC	12.50d	12.50b	2.50b	-	6.87c
	MG	5.00f	0.00d	0.00c	-	1.25d
	LS	25.00c	10.00b	0.00c	-	8.75bc
	Mean (C)	25.00a	10.00b	1.78c	-	
2nd						Mean (G)
	FS	45.00c	12.50c	0.00c	0.00b	14.38c
	TRI	7.50e	2.50d	0.00c	0.00b	2.50e
	BM	47.50c	45.00b	0.00c	0.00b	23.12b
	NYC	77.50a	77.50a	22.50a	7.50a	46.25a
	LC	25.00d	12.50c	2.50b	0.00b	10.00c
	MG	27.50d	0.00e	0.00c	0.00b	6.87d
	LS	60.00b	45.00b	0.00c	0.00b	26.25b
	Mean (C)	41.43a	27.85b	3.57b	1.07b	
3rd						Mean (G)
	FS	52.50b	22.50c	0.00c	0.00b	18.75d
	TRI	27.50c	7.50d	0.00c	0.00b	8.75e
	BM	72.50a	72.50b	7.50b	0.00b	38.12c
	NYC	85.00a	87.50a	47.50a	7.50a	56.87a
	LC	30.00c	30.00c	7.50b	0.00b	16.87d
	MG	32.50c	7.50d	0.00c	0.00b	10.00e
	LS	82.50a	77.50ab	0.00c	0.00b	40.00b
	Mean (C)	54.64a	43.57a	8.92b	1.07b	
4th						Mean (G)
	FS	62.50c	27.50c	0.00e	0.00b	22.50d
	TRI	27.50e	12.50d	2.50d	0.00b	10.63e
	BM	72.50bc	72.50b	10.00b	0.00b	38.75c
	NYC	87.50ab	87.50a	47.50a	7.50a	57.50a
	LC	37.50d	35.00c	7.50c	0.00b	19.37d
	MG	37.50d	10.00d	0.00e	0.00b	11.87e
	LS	92.50a	85.00a	10.00b	0.00b	46.87b
	Mean (C)	59.28a	47.14b	11.07c	1.07d	
5th						Mean (G)
	FS	67.50b	30.00c	0.00e	0.00b	24.37c
	TRI	35.00d	12.50d	2.50d	0.00b	12.50d
	BM	75.00b	80.00b	12.50b	0.00b	41.87b
	NYC	100.00a	100.00a	60.00a	7.50a	66.87a
	LC	37.50cd	40.00c	7.50c	0.00b	21.25cd

	MG	45.00c	10.00d	0.00e	0.00b	13.75d
	LS	92.50a	87.50b	10.00bc	0.00b	47.50b
	Mean (C)	64.64a	51.42b	13.21c	1.07d	
6th						Mean (G)
	FS	67.50c	30.00d	0.00e	0.00b	24.37c
	TRI	40.00e	22.50e	2.50d	0.00b	16.25d
	BM	85.00b	87.50b	12.50b	0.00b	46.25b
	NYC	100.00a	100.00a	60.00a	7.50a	66.87a
	LC	40.00e	42.50c	7.50c	0.00b	22.50c
	MG	50.00d	10.00f	0.00e	0.00b	15.00d
	LS	92.50ab	87.50b	10.00bc	0.00b	47.50b
	Mean (C)	67.85a	54.28b	13.21c	1.07d	
7th						Mean (G)
	FS	70.00b	32.50d	0.00f	0.00b	25.62c
	TRI	45.00c	30.00d	2.50e	0.00b	19.37cd
	BM	100.00a	95.00ab	12.50c	0.00b	51.87b
	NYC	100.00a	100.00a	62.50a	7.50a	67.50a
	LC	45.00c	42.50c	7.50d	0.00b	23.75c
	MG	50.00c	10.00e	0.00f	0.00b	15.00d
	LS	92.50a	87.50b	17.50b	0.00b	49.37b
	Mean (C)	71.78a	56.78b	14.64c	1.07d	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 7 shows the germination percentage (GP) for 7 seven consecutive days after the germination initiation under different salinity levels. In day 1, NYC showed the highest GP among the tested genotypes, regardless of the salinity level, whereas no seeds germinated for any of the tested genotypes under the highest salinity level tested (300 mM of NaCl). Similarly, in day 2 NYC was again the best performing genotype, while TRI was the only genotype that seeds germinated under the highest salinity level (7.5% of seeds). In days 3 and 4, NYC and LS had the highest GP values (85.0% and 82.5%, respectively) under the control and mild stress (100 mM NaCl) conditions, while when salinity stress became more severe (200 and 300 mM of NaCl) NYC was again the best performing genotype. In days 5 and 6, the highest values of GP under the control conditions were recorded for NYC and LS, while for the salinity levels (100-300 mM of NaCl) NYC was the best performing genotype. It is interesting to mention that LL2 recorded 100.0% of GP in day 5 under the control conditions and the mild (100 mM of NaCl) salinity stress. Finally, after 7 days of germination initiation it was recorded 100.0% GP for NYC and BM and 92.5% GP for LS, while NYC was the best performing genotype under the tested salinity levels. Therefore, the results show that the best overall performance was recorded for the genotype NYC which had the highest GP under the tested salinity level, while it was the genotype with the faster germination rate since all the seeds germinated within 5 days. Moreover, the GP values recorded for the highest salinity level for NYC (7.5%) and the fact than no germination was observed for the rest of the tested genotypes indicates that the species is susceptible to severe stress, while the specific genotype (NYC) showed promising results under moderate stress (200 mM of NaCl)

Table 8. Percentage of seed water uptake (WU %) in relation to genotype (G) and salinity stress (mM of NaCl) at the 1st day after stress initiation.

Day	Genotype (G)	NaCl (mM) (C)				
		0	100	200	300	
1st						Mean (G)
	FS	116.67b	83.33d	75.00c	83.33b	89.58d
	TRI	139.29a	110.72a	107.14a	100.00a	114.28a
	BM	87.50d	50.00e	37.50d	54.17d	57.29e
	NYC	135.00a	95.00ab	70.00c	70.00c	92.50d
	LC	78.12d	50.00e	34.37d	31.25e	48.43f
	MG	100.00c	100.00b	100.00b	87.50b	96.87cd
	LS	112.50b	93.75c	106.25a	100.00a	103.12bc
	Mean (C)	109.87a	83.25b	75.75b	75.17b	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 8 presents the results regarding the water uptake (WU) of seeds 1 day after germination started. TRI was the best performing genotype under the tested salinity levels, followed by NYC under the control and mild (100 mM of NaCl) stress conditions. These results indicate that although seeds of TRI genotype absorbed high amount of waters, this did not result to higher germination percentage in any of the tested conditions. In contrast, NYC seeds which absorbed high amounts of water under the control and mild (100 mM of NaCl) and less amounts under moderate and severe salinity stress were those that presented the highest germination percentage (see results Table 7). This could be due to differences in the activation of enzymes involved in the mobilization of carbohydrate pools that are used for biosynthetic process and the development of roots.

Table 9. Percentage of seedling water uptake (WC %) in relation to genotype (G) and salinity stress (mM of NaCl) 15 days after stress initiation.

Day	Genotype (G)	NaCl (mM) (C)				
		0	100	200	300	
15th						Mean (G)
	FS	1054.16b	1266.66a	0.00b	-	580.20a
	TRI	440.47d	497.50e	0.00b	-	234.49e
	BM	1626.50a	783.33d	300.00a	-	511.45b
	NYC	1055.83b	920.83b	0.00b	-	569.16a
	LC	766.66c	500.00e	0.00b	-	316.66d
	MG	407.14e	333.33f	0.00b	-	185.11f
	LS	762.50c	833.33cd	0.00b	-	398.95c
	Mean (C)	821.32a	733.57b	42.85c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 9 presents the results of seedling water content (WC) in day 15 where a varied response among the tested genotypes was recorded. BM was the best performing genotype under the control and moderate stress, while FS recorded the highest WC values under the mild stress conditions. It also worth's to mention that no seedlings were recorded under the highest stress level (300 mM of NaCl) in day 15 after germination started.

Table 10. Seedling vigour index (SVI %) in relation to genotype (G) and salinity stress (mM of NaCl) 7 days after stress initiation

Day	Genotype (G)	NaCl (mM) (C)				
		0	100	200	300	
7th						Mean (G)
	FS	348.00b	57.00e	0.00b	-	101.25c
	TRI	153.75c	35.50f	0.00b	-	47.31d
	BM	395.00b	114.25c	0.00b	-	127.31b
	NYC	662.50a	430.00a	41.50a	-	283.50a
	LC	96.25d	73.75d	0.00b	-	50.62d
	MG	129.50cd	11.00g	0.00b	-	35.12d
	LS	379.25b	207.50b	0.00b	-	146.68b
	Mean (C)	313.82a	132.71b	5.92c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 10 presents the results regarding seedling vigour index (SVI) which indicates the tolerance of genotypes under stress conditions. According to these results NYC was the best overall performing genotype, while the same genotype was also the most tolerant under mild and moderate stress conditions (SVI values of 430.0% and 41.50%, respectively). As mentioned before, no seedlings were recorded under the highest stress level (300 mM of NaCl) in day 7.

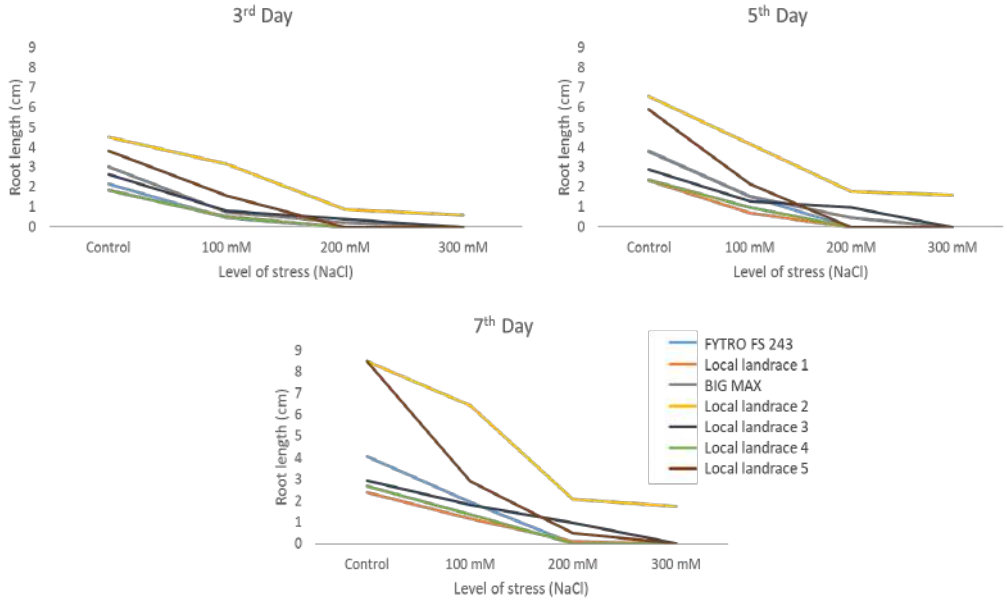


Figure 3. Root length at day 3, 5 and 7 in relation to genotype (G) and salinity stress level (mM of NaCl, C).

Decreasing trends in root length were observed with increasing salinity stress with significant differences among the tested genotypes (Figure 3). In particular, NYC was the most tolerant genotype, especially in day 7 where even under severe stress the roots of the particular genotype had the highest length.

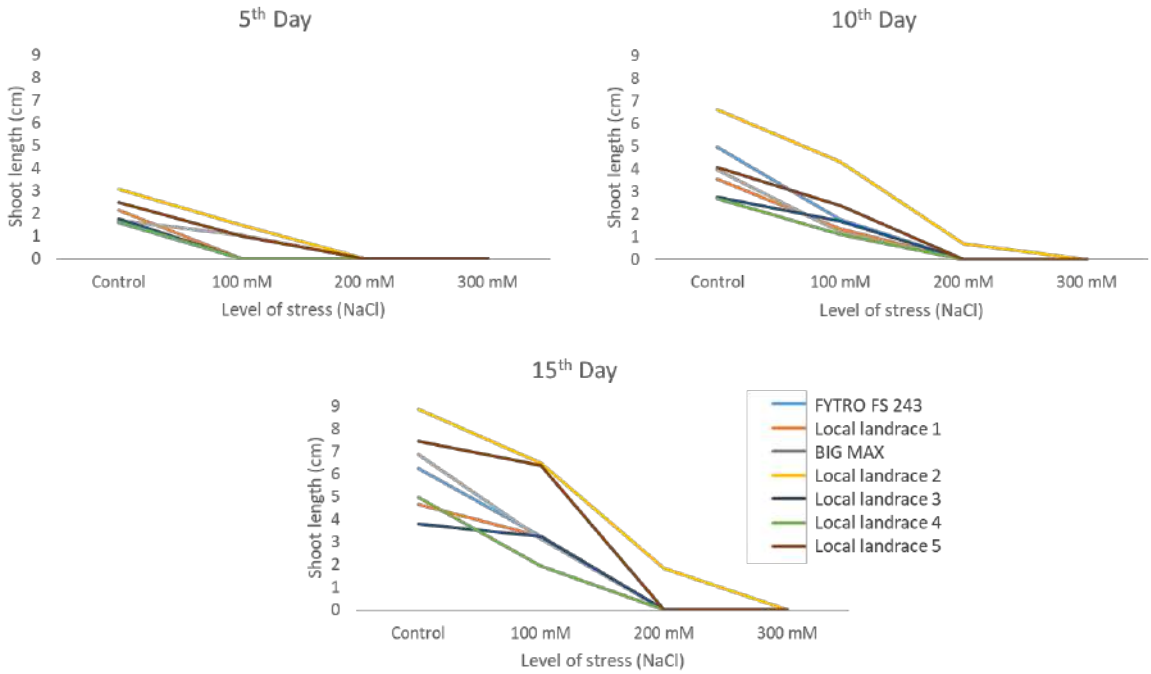


Figure 4. Shoot length at day 5, 10 and 15 in relation to genotype (G) and salinity stress level (mM of NaCl, C).

Decreasing trends were also observed in shoot length of seedlings, where NYC showed the highest tolerance since shoot length was reduced after seedlings being subjected to high stress conditions for 15 days, while the shoot length for rest of the genotypes was reduced when plants were subjected to 200 mM of NaCl (Figure 4). These findings are also depicted in Photo 2, where NYC is the only genotype with visible roots under the treatment of 300 mM of NaCl.

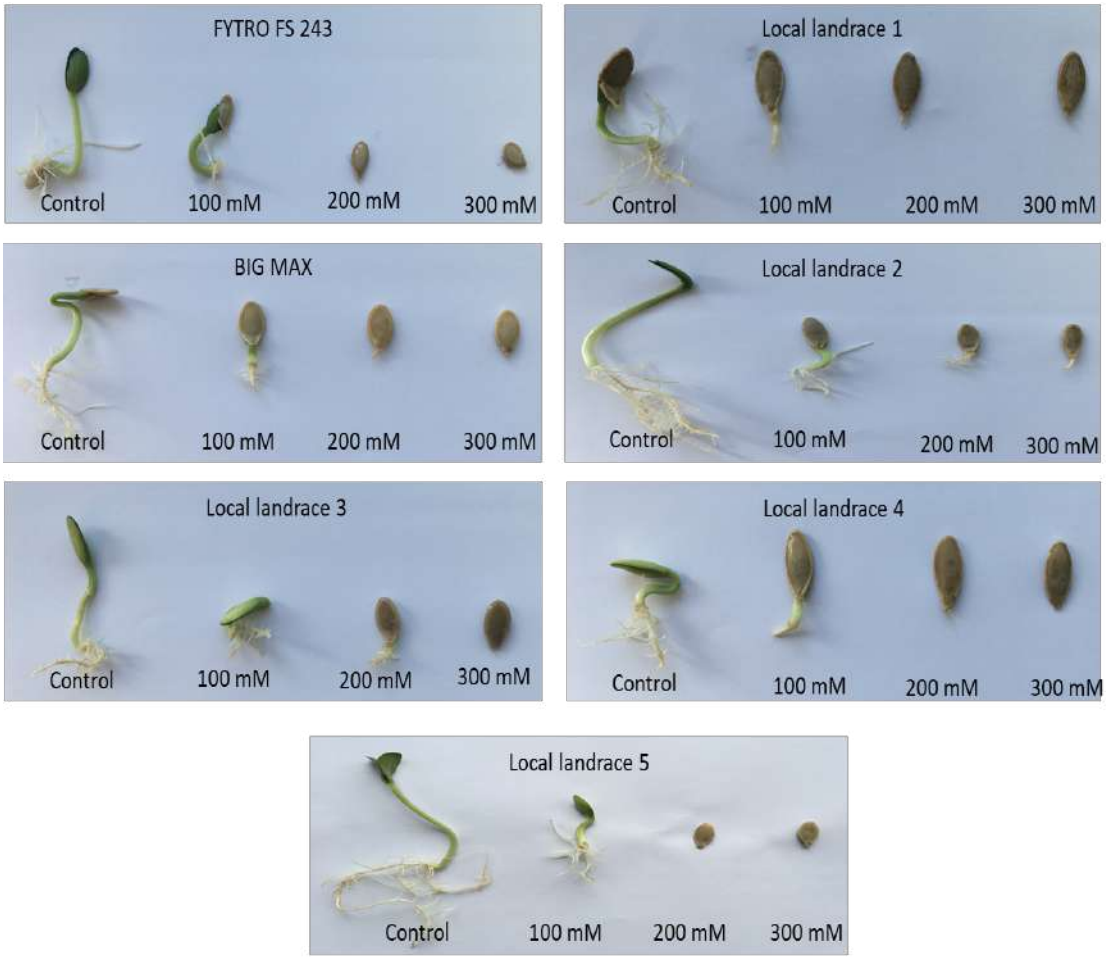


Photo 2. Seed germination of pumpkin genotypes in relation to salinity stress level (mM of NaCl) at 10 days after sowing.

C) Temperature stress

Table 11. Seed germination percentage (GP %) of pumpkin genotypes (G) in relation to temperature conditions (° C) at four sampling dates (1st to 4th day).

Day	Genotype (G)	Temperature (°C) (C)			
		25	37	4	
1st					Mean (G)
	FS	0.00b	0.00b	-	0.00b
	TRI	0.00b	0.00b	-	0.00b
	BM	32.50a	32.50a	-	21.66a
	NYC	0.00b	0.00b	-	0.00b
	LC	0.00b	0.00b	-	0.00b
	MG	0.00b	0.00b	-	0.00b
	Mean (C)	5.41a	5.41a	-	
2nd					
	FS	0.00b	2.50b	-	0.83c
	TRI	0.00b	0.00c	-	0.00d
	BM	77.50a	40.00a	-	39.16a
	NYC	60.00a	2.50b	-	20.83b
	LC	0.00b	0.00c	-	0.00d
	MG	70.00a	0.00c	-	23.33b
	Mean (C)	34.58a	7.50b	-	
3rd					
	FS	15.00b	5.00b	-	6.66c
	TRI	2.50c	2.50c	-	0.83d
	BM	87.50a	50.00a	-	45.83a
	NYC	75.00a	0.00d	-	25.83b
	LC	2.50c	0.00d	-	0.83d
	MG	80.00a	0.00d	-	26.66b
	Mean (C)	43.75a	9.58b	-	
4th			M.O (G)		
	FS	17.50b	5.00b	-	7.50c
	TRI	5.00c	0.00d	-	1.66d
	BM	92.50a	52.50a	-	48.33a
	NYC	82.50a	2.50c	-	28.33b
	LC	2.50d	0.00d	-	0.83e
	MG	82.50a	0.00d	-	27.50b
	Mean (C)	47.08a	10.00b	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 11 presents the results regarding the germination percentage (GP) of seeds of the tested genotypes at different temperatures (e.g. 4 °C, 25 °C and 37 °C) for 4 consecutive days (day 1-4 of the experiment). BM recorded the highest GP in day 1 at 25 °C and 37 °C, while in day 2 and 3 apart from BM, LS also showed high GP values under the same conditions. Moreover, in day 4 LL2 recorded similar GP to LS while BM recorded the highest GP among the tested genotypes.

Finally, as expected none of the seeds of the tested genotypes germinated at low temperatures (4 °C) considering that the species is usually cultivated during the spring-summer period.

Table 12. Seed germination percentage (GP %) of pumpkin genotypes (G) in relation to temperature conditions (° C) at four sampling dates (5th to 8th day) after putting seeds at room temperature.

Day	Genotype (G)	Temperature (°C) (C)			
		25	37	4	
5th					Mean (G)
	FS	17.50c	5.00b	-	7.50c
	TRI	5.00d	0.00d	-	1.66d
	BM	100.00a	52.50a	-	50.83a
	NYC	87.50b	2.50c	-	30.00b
	LC	2.50e	0.00d	-	0.83e
	MG	82.50b	5.00b	-	29.16b
	Mean (C)	49.16a	10.83b	-	
6th					
	FS	22.50c	12.50b	7.50c	14.16c
	TRI	12.50d	0.00e	0.00d	4.16d
	BM	100.00a	95.00a	62.50a	85.83a
	NYC	92.50ab	2.50d	7.50c	34.16b
	LC	2.50e	0.00e	0.00d	0.83e
	MG	85.00b	5.00c	20.00b	36.66b
	Mean (C)	52.50a	19.16b	16.25b	
7th					
	FS	22.50c	15.00c	15.00c	17.50d
	TRI	12.50d	0.00e	0.00d	4.16e
	BM	100.00a	95.00a	77.50a	90.83a
	NYC	92.50ab	2.50d	12.50c	35.83c
	LC	2.50e	0.00e	0.00d	0.83f
	MG	85.00b	25.00b	42.50b	50.83b
	Mean (C)	52.50a	22.91b	24.58b	
8th					
	FS	27.50c	17.50c	40.00b	28.33d
	TRI	15.00d	0.00e	5.00c	6.66e
	BM	100.00a	95.00a	90.00a	95.00a
	NYC	97.50a	2.50d	35.00b	45.00c
	LC	5.00e	0.00e	0.00d	1.60f
	MG	87.50b	45.00b	95.00a	75.83b
	Mean (C)	55.41a	26.66c	44.16b	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

When seeds were put at 25 °C in days 5-8 of the experiment, the GP of BM reached the maximum values in day 5 for those seeds that were germinated at 25 °C, while a rapid increase was also

observed in GP values for the seeds that were initially subjected to 37 °C and 4 °C in day 5 and 6, respectively (Table 12). NYC and LS also recorded high GP values but only in the case of seeds that were initially germinated at 25 °C, while a significant increase of GP was also observed in day 8 for seeds that were initially germinated at 4 °C. It seems that pumpkin seeds of most of the genotypes were more susceptible to high temperature than low temperature stress which indicates that sowing at high temperatures should be avoids since it significantly affects GP even when the temperatures are restored at optimum levels.

Table 13. Percentage of seedlings water content (WC %) in relation to genotype (G) and temperature conditions (°C) at 15 days after stress initiation.

Day	Genotype (G)	Temperature (°C) (C)			
		25	37	4	
15th					Mean (G)
	FS	291.66b	400.00b	745.83a	479.16a
	TRI	206.66c	0.00d	100.00e	102.22e
	BM	375.55a	287.50c	275.50c	306.85c
	NYC	382.14a	0.00d	235.41d	205.85d
	LC	42.85d	0.00d	0.00f	14.28f
	MG	285.11b	512.50a	325.00b	374.20b
	Mean (C)	264.00a	200.00b	277.29a	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

The percentages of seedling water content (WC) in day 15 of the experiment are presented in Table 13. BM and NYC recorded the highest values at 25 °C, while at 37°C and 4 °C the highest values were recorded for LS and FS, respectively. Moreover, the highest overall values were recorded for FS seeds that were put at 4 °C.

Table 14. Seedling vigour index (SVI %) in relation to genotype (G) and temperature conditions (°C) at 12th day after stress initiation.

Day	Genotype (G)	Temperature (°C) (C)			
		25	37	4	
12th					Mean (G)
	FS	82.50c	78.75c	160.00b	107.08d
	TRI	70.25c	0.00e	17.50d	29.25e
	BM	685.00a	260.00a	437.50a	460.83a
	NYC	480.00b	5.00d	143.25b	209.41c
	LC	5.00d	0.00e	0.00e	1.66f
	MG	489.00b	212.00b	96.75c	265.91b
	Mean (C)	301.95a	92.62c	142.50b	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Seedling vigour indices (SVI) in relation to genotype and temperature conditions are presented in Table 14. Seedlings of BM were the best performing compared to the rest of the tested genotypes, regardless of the temperature conditions, followed by NYC and LS at 25 °C, LS at 37 °C and FS and NYC at 4 °C.

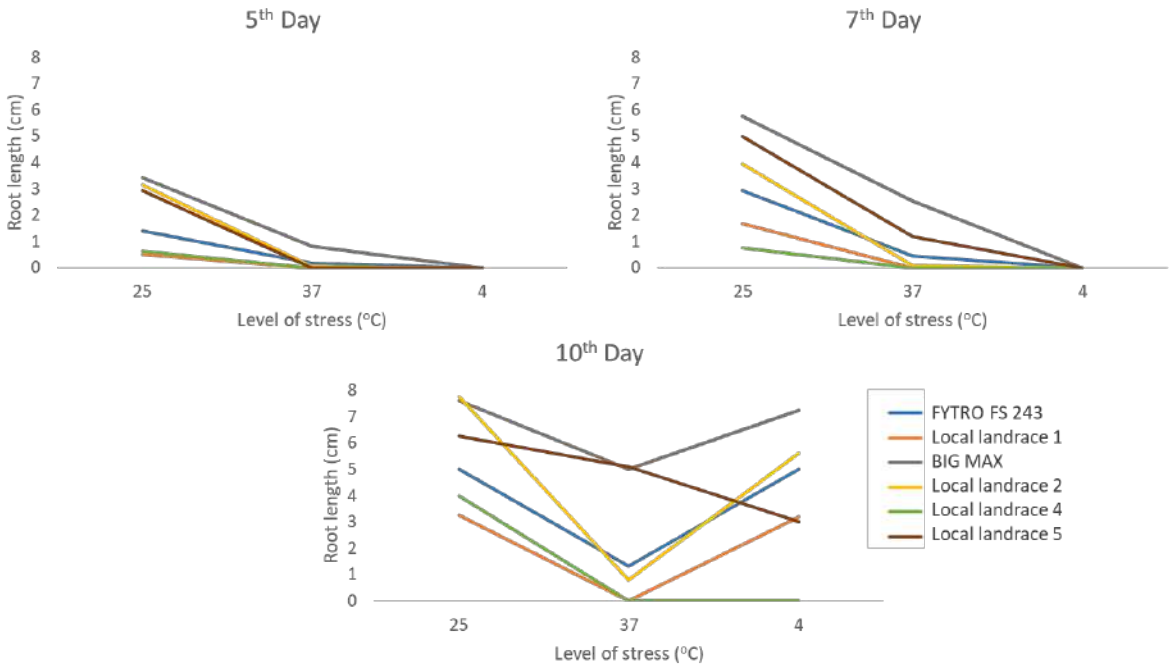


Figure 5. Root length at day 5, 7 and 10 in relation to genotype (G) and temperature stress (°C).

Root length decreased at 37 °C and 4 °C when compared to 25 °C for all the tested genotypes in day 5 and 7 of the experiment, while in day 10 root length of genotypes FS and TRI did not differ between the 25 °C and 4 °C (Figure 5).

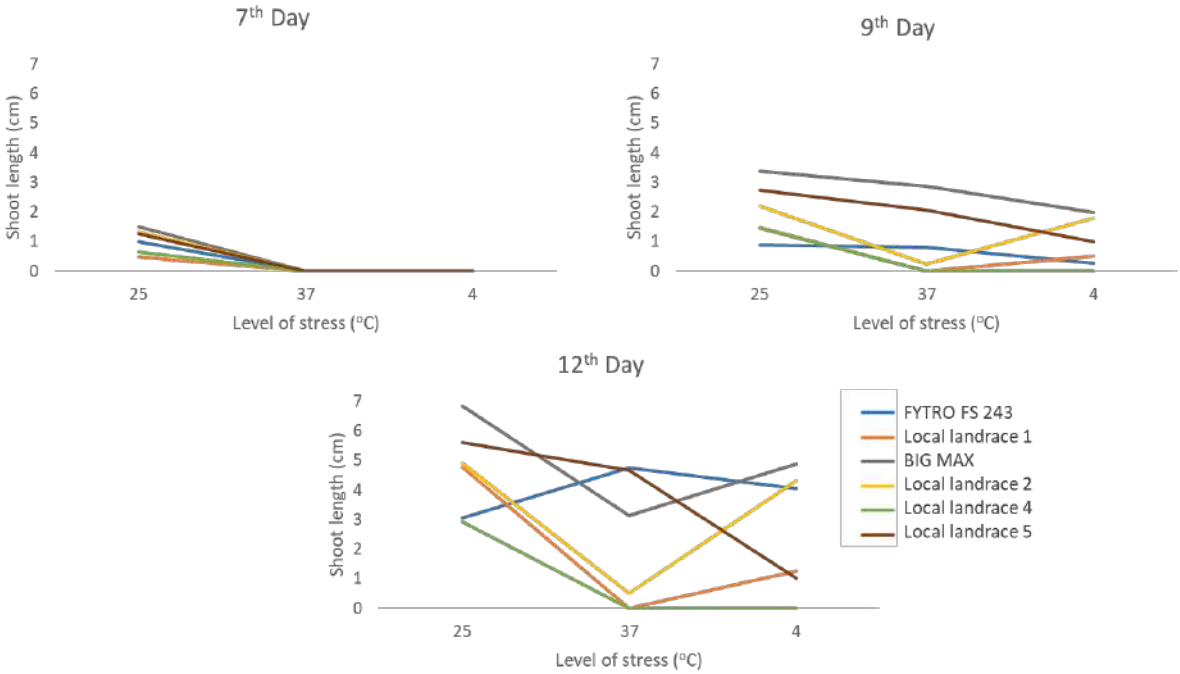


Figure 6. Shoot length at day 7, 9 and 12 in relation to genotype (G) and temperature stress (°C).

Shoots started to develop after day 9 of the experiment for seeds subjected to 37 °C and 4 °C, except for the case of TRI and MG where shoots did not develop up to day 12 at 37 °C and 4 °C (only MG) indicating that these genotypes are very susceptible to non-optimal temperatures (Figure 6). In contrast, shoot length of FS was higher at 37 °C compared to 25 °C and 4 °C, while shoot length of TRI at 4 °C was similar to 37 °C.

3.1.2 Experiment 2

Table 15. Germination percentage (GP %) of pumpkin genotypes (G) in relation to salinity stress (mM of NaCl (C)) throughout the germination test (7 days).

Days	Genotypes (G)	NaCl (mM) (C)				
		0	100	200	300	
1st						Mean(G)
	FS	12.50b	17.50a	0.00a	0.00a	7.50b
	TRI	0.00e	0.00b	0.00a	0.00a	0.00d
	MEL	35.00a	0.00b	0.00a	0.00a	8.75a
	LS	2.50d	0.00b	0.00a	0.00a	0.625c
	LO	35.00a	0.00b	0.00a	0.00a	8.75a
	LC	0.00e	0.00b	0.00a	0.00a	0.00d
	MG	5.00c	0.00b	0.00a	0.00a	1.25b
	Mean(C)	12.85a	2.50b	0.00c	0.00c	
2nd						Mean(G)
	FS	37.50b	45.00a	0.00a	0.00a	20.625a
	TRI	0.00e	0.00c	0.00a	0.00a	0.00d
	MEL	62.50a	0.00c	0.00a	0.00a	15.625b
	LS	2.50d	0.00c	0.00a	0.00a	0.625b
	LO	67.50a	12.50b	0.00a	0.00a	20.00a
	LC	0.00e	0.00c	0.00a	0.00a	0.00d
	MG	10.00c	0.00c	0.00a	0.00a	2.50c
	Mean(C)	25.71a	8.21b	0.00c	0.00c	
3rd						Mean(G)
	FS	62.50b	80.00a	7.50a	0.00a	37.50a
	TRI	0.00e	0.00e	0.00b	0.00a	0.00g
	MEL	77.50a	0.00e	0.00b	0.00a	19.375c
	LS	10.00d	0.00e	0.00b	0.00a	2.50e
	LO	80.00a	47.50b	7.50a	0.00a	33.75b
	LC	0.00e	2.50d	0.00b	0.00a	0.625f
	MG	22.50c	10.00c	0.00b	0.00a	8.125d

	Mean(C)	36.07a	20.00b	2.14c	0.00d	
4th						**Mean(G)**
	FS	75.00b	85.00a	10.00a	0.00a	42.50a
	TRI	2.50e	0.00e	0.00c	0.00a	0.625f
	MEL	82.50a	0.00e	0.00c	0.00a	20.625b
	LS	12.50d	0.00e	0.00c	0.00a	3.125d
	LO	82.50a	70.00b	7.50b	0.00a	40.00a
	LC	0.00f	5.00d	0.00c	0.00a	1.25e
	MG	22.50c	10.00c	0.00c	0.00a	8.125c
	Mean(C)	39.64a	24.28b	2.50c	0.00d	
5th						**Mean(G)**
	FS	82.50a	85.00a	10.00b	0.00a	44.375a
	TRI	2.50c	0.00e	0.00c	0.00a	0.625f
	MEL	82.50a	0.00e	0.00c	0.00a	20.625b
	LS	12.50bc	0.00e	0.00c	0.00a	3.125d
	LO	82.50a	75.00b	17.50a	0.00a	43.75a
	LC	0.00d	5.00d	0.00c	0.00a	1.25e
	MG	25.00b	12.50c	0.00c	0.00a	9.375c
	Mean(C)	41.07a	25.35b	3.92c	0.00d	
6th						**Mean(G)**
	FS	87.50a	90.00a	10.00ab	0.00b	46.875a
	TRI	2.50c	0.00c	0.00b	0.00b	0.625c
	MEL	82.50a	0.00c	0.00b	0.00b	20.625b
	LS	15.00bc	0.00c	0.00b	0.00b	3.75bc
	LO	85.00a	77.50a	20.00a	12.50a	48.75a
	LC	5.00bc	7.50bc	0.00b	0.00b	3.125bc
	MG	25.00b	15.00b	2.50b	0.00b	10.625bc
	Mean(C)	43.21a	27.14b	4.64c	1.78c	
7th						**Mean(G)**
	FS	87.50a	90.00a	10.00b	0.00c	46.875b
	TRI	2.50e	2.50e	0.00d	0.00c	1.25g
	MEL	82.50a	0.00f	0.00d	0.00c	20.625c
	LS	15.00c	0.00f	0.00d	0.00c	3.75f
	LO	85.00a	80.00b	30.00a	15.00a	52.50a
	LC	5.00d	12.50d	0.00d	2.50b	5.00e
	MG	25.00b	15.00c	2.50c	2.50b	11.25d
	Mean(C)	43.21a	28.57a	6.07b	2.85b	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 15 shows the germination percentage (GP) for 7 seven consecutive days after the germination initiation under different salinity levels. In day 1, MEL and LO showed the highest GP among the tested genotypes, regardless of the salinity level, whereas no seeds germinated for any of the tested genotypes under the highest salinity level tested (300 mM of NaCl). The highest GP was recorded for the control treatment, especially for MEL and LO genotypes, while FS genotype showed the highest GP values for the treatment of 100 Mm OF NaCl. In day 2, FS and LO were the best performing genotypes, regardless of salinity level, while no genotypes germinated under the highest salinity level. The highest GP was recorded for the control treatment, especially for MEL and LO genotypes, while FS genotype showed the highest GP values for the treatment of 100 Mm OF NaCl. Similar results were recorded for days 3 to 7. It is interesting to highlights that only LO, LC and MG genotypes germinated under the highest salinity level (300 Mm NaCl) with very low percentages, while FS and LO where the best performing genotypes under the low salinity level (100 mM NaCl) with very high GP values (90% and 80%, respectively). Therefore, the results show that the best overall performance was recorded for the genotypes FS and LO which had the highest GP under the tested salinity levels, while FS, MEL and LO were the genotypes with the faster germination rate since a high percentage of seeds (>77.5%) germinated within 3 days. Moreover, the GP values recorded for the highest salinity level for LO (15.0%) and the fact than no germination was observed for most of the tested genotypes indicates that the species is susceptible to severe stress, while specific genotypes (FS and LO) showed promising results under the low level of stress (100 mM of NaCl).

Table 16. Percentage of water uptake (WU %) in relation to genotype (G) and salinity stress (mM of NaCl) at the 1st and 4th day after stress initiation.

Days	Genotypes (G)	NaCl (mM) (C)				
		0	**100**	**200**	**300**	
1st						**Mean(G)**
	FS	86.17ef	82.97d	64.89e	62.76f	74.20e
	TRI	161.65a	127.81a	134.58a	137.59a	140.41a
	MEL	138.18b	103.93b	121.51b	123.33b	121.74b
	LS	91.84de	104.96b	69.14e	115.95c	95.47c
	LO	80.32f	81.96d	87.70d	74.59e	81.14d
	LC	105.18c	96.69c	80.66d	112.26c	98.70c
	MG	99.15cd	101.68bc	95.37c	90.75d	96.74c
	Mean(C)	108.93a	100.00b	93.41c	102.46b	
4th						**Mean(G)**
	FS	122.34c	105.31c	86.17d	111.70c	106.38cd
	TRI	178.19a	176.31a	161.65a	137.59a	163.43a
	MEL	146.96b	110.90c	134.54b	128.18ab	130.15b
	LS	107.44d	125.53b	107.09c	100.00d	110.01c
	LO	121.31c	127.86b	106.55c	83.60e	109.83c
	LC	101.88d	89.62d	83.49d	126.88b	100.47d
	MG	108.82d	83.61e	89.07d	116.38c	99.47e
	Mean(C)	126.71a	117.02b	109.79c	114.90bc	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 16 presents the results regarding the water uptake (WU) of seeds at the 1st and 4th day after germination started. TRI was the best performing genotype under the tested salinity levels at both measurements, followed by MEL genotype in day 1, while in day 4 the second best perfoming genotypes were MEL (at 0, 200 and 300 mm of NaCl) and LS and LO under the 100 mM of NaCl. Some of the genotypes recorded the highest WU values for the control treatment, while LS and LO recorded the highest values for the level of 100 mM NaCl (day 4) and LC and MG for the level of 300 mM of NaCl (day 4). These findings could be due to differences between the genotypes in the activation of enzymes involved in the mobilization of carbohydrate pools that are used for biosynthetic process and the development of roots.

Table 17. Seedling vigour index (SVI %) in relation to genotype (G) and salinity stress (mM of NaCl) 5 days after stress initiation

Day	Genotypes (G)	NaCl (mM) (C)				
		0	100	200	300	
5th						Mean(G)
	FS	51.00b	33.25a	0.00a	0.00a	21.06a
	TRI	0.00d	0.00c	0.00a	0.00a	0.00d
	MEL	51.75b	0.00c	0.00a	0.00a	12.93b
	LS	0.00d	0.00c	0.00a	0.00a	0.00d
	LO	70.25a	21.75b	0.00a	0.00a	23.00a
	LC	0.00d	0.00c	0.00a	0.00a	0.00d
	MG	10.75c	0.00c	0.00a	0.00a	2.68c
	Mean(C)	26.25a	7.85b	0.00c	0.00c	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Table 17 presents the results regarding seedling vigour index (SVI) 5 days after stress initiation which indicates the tolerance of genotypes under stress conditions. According to these results LO was the best overall performing genotype, while the only genotypes that were able to germinate under the low salinity level (100 mM of NaCl) were FS and LO. None of the genotypes germinated under the moderate (200 mM of NaCl) and the high salinity levels (300 mM of NaCl).

Table 18. Content of Chlorophyll a (µg/mL) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Chla (µg/mL)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	20.54c	26.15a	-	-	11.67a
TRI	-	-	-	-	-
MEL	45.16a	-	-	-	11.29a
LS	26.18b	-		-	6.54b
LO	14.03d	21.25b	7.46	-	10.68a
LC	-	5.75c	-	-	1.4c
MG	15.38d	6.81c	-	-	5.55b
Mean(C)	17.33a	8.56b	1.06c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

The highest overall chlorophyll a content (µg/mL) was recorded for MEL genotype under the control treatment 0 (mM of NaCl) (Table 18). Moreover, FS genotype had the highest chlorophyll a content under the low salinity level (100 mM of NaCl), followed by the LO genotype (26.15 and 21.25 µg/mL, respectively), whereas the same genotype (Lo) was the only genotype where chlorophyll a was recorded under the moderate salinity level (200 mM of NaCl). Chlorophyll a was not detected in any of the tested genotypes under the highest salinity level studied (300 mM of NaCl).

Table 19. Content of Chlorophyll a (mg/g fresh weight) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Chla (mg/g fresh weight)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	0.78c	0.95a	-	-	0.43a
TRI	-	-	-	-	-
MEL	1.58a	-	-	-	0.39ab
LS	1.05b	-	-	-	0.26c
LO	0.47d	0.69b	0.28	-	0.36b
LC	-	0.24c	-	-	0.06e
MG	0.50d	0.21c	-	-	0.18d
Mean(C)	0.63a	0.30b	0.04c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Similar trends were recorded for chlorophyll content on a fresh weight basis (mg/g fresh weight) (Table 19).

Table 20. Content of Chlorophyll b (µg / ml) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Chlb (µg/ ml)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	32.13a	17.11a	-	-	12.31a
TRI	-	-	-	-	-
MEL	14.40b	-	-	-	3.60c
LS	8.30c	-	-	-	2.07d
LO	20.30b	13.26ab	11.79	-	11.34a
LC	-	6.17bc	-	-	1.54d
MG	19.77b	3.14c	-	-	5.72b
Mean(C)	13.56a	5.67b	1.68bc	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Regarding chlorophyll b content (µg/mL) presented in Table 6, FS genotype recorded the highest values for the control and the low salinity level (0 and 100 mM of NaCl, respectively), while LO genotype was the second best genotype and the only one where chlorophyll b was detected under

the moderate salinity level (200 mM of NaCl) (Table 20). Chlorophyll b was not detected in any of the tested genotypes under the highest salinity level studied (300 mM of NaCl).

Table 21. Content of Chlorophyll b (µg / g fresh weight) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Chlb (mg/ g fresh weight)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	1.22a	0.63a	-	-	0.46a
TRI	-	-	-	-	-
MEL	0.50cd	-	-	-	0.12d
LS	0.33d	-	-	-	0.08e
LO	0.69b	0.43ab	0.44	-	0.39b
LC	-	0.25bc	-	-	0.06e
MG	0.65bc	0.09c	-	-	0.18c
Mean(C)	0.48a	0.20b	0.06c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Similar trends were recorded for chlorophyll content on a fresh weight basis (mg/g fresh weight) (Table 21).

Table 22. Total Chlorophyll (Tchl) content (µg / ml) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Tchl (µg/ ml)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	52.67b	43.27a	-	-	23.98a
TRI	-	-	-	-	-
MEL	59.56a	-	-	-	14.89b
LS	34.49c	-	-	-	8.62d
LO	34.34c	34.52b	19.25	-	22.03a
LC	-	11.93c	-	-	2.98e
MG	35.16c	9.95d		-	11.28c
Mean(C)	30.89a	14.24b	2.75c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

The highest overall total chlorophyll content (µg/mL) was recorded for the MEL genotype under the control treatment, followed by the FS genotype (Table 22). Moreover, the latter genotype was the best performing under the low salinity level (100 mM of NaCl), while LO genotype was the only genotype where chlorophyll was detected under the moderate salinity level (200 mM of NaCl). Finally, no chlorophyll was detected in any of the tested genotypes under the highest salinity level (300 mM of NaCl) due to lack of germination.

Table 23. Total Chlorophyll (Tchl) content (mg/g fresh weight) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Tchl (mg/g fresh weight)

Genotypes (G)	Control	100 mM	200 mM	300 mM	Mean(G)
FS	2.00a	1.58a	-	-	0.89a
TRI	-	-	-	-	-
MEL	2.08a	-	-	-	0.52c
LS	1.39b	-	-	-	0.34d
LO	1.17c	1.12b	0.72	-	0.75b
LC	-	0.50c	-	-	0.12e
MG	1.15c	0.31d	-	-	0.36d
Mean(C)	1.11a	0.50b	0.10c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Similar trends were recorded for chlorophyll content on a fresh weight basis (mg/g fresh weight) (Table 23).

Table 24. The ratio of Chlorophyll α to Chlorophyll b in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Chla/Chlb				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	0.64c	1.49d	-	-	0.531b
TRI	-	-	-	-	-
MEL	3.14a	-	-	-	0.785a
LS	3.13a	-	-	-	0.783a
LO	0.69c	1.60c	0.63	-	0.731a
LC	-	1.80b	-	-	0.450c
MG	0.78b	2.31a	-	-	0.773a
Mean(C)	1.19a	1.03b	0.09c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

The ratio of Chlorophyll a to Chlorophyll b is presented in Table 24. The highest overall values were recorded for MEL and LS genotypes under the control treatment, MG genotype recorded the highest value under the low salinity level (100 mM of NaCl).

Table 25. Content of total carotenoids (μg/mL) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Car (μg/mL)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	5.65c	2.38a	-	-	0.81c
TRI	-	-	-	-	-
MEL	11.49a	-	-	-	2.87a
LS	6.08b	-		-	1.52b
LO	2.47d	2.49a	1.36	-	0.33d
LC	-	0.30c	-	-	0.07f
MG	2.23d	1.34b	-	-	0.22e
Mean(C)	1.03a	0.84b	0.19c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

The highest overall total carotenoids content was recorded for MEL genotype under the control treatment (Table 25). Moreover, FS and LO genotypes had the highest total carotenoids content under the low salinity level (100 mM of NaCl), while LO genotype was the only one where total carotenoids were detected under the moderate salinity level (200 mM of NaCl). Finally, no carotenoids were detected under the highest salinity level (300 mM of NaCl) for any of the tested genotypes due to lack of germination.

Table 26. Content of carotenoids (mg/g fresh weight) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	***C*ar (mg/ g fresh weight)**				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	0.012b	0.013a	-	-	0.006a
TRI	-	-	-	-	-
MEL	0.022a	-	-	-	0.005ab
LS	0.014b	-	-	-	0.003cd
LO	0.008c	0.010b	0.008	-	0.006a
LC	-	0.008c	-	-	0.002d
MG	0.008c	0.011ab	-	-	0.004bc
Mean(C)	0.009a	0.006b	0.001c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

Similar trends were recorded for chlorophyll content on a fresh weight basis (mg/g fresh weight) (Table 26).

Table 27. Concentration of proline (mM) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

	Proline Concentration (mM)				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	8.74a	7.08a	-	-	3.95b
TRI	-	-	-	-	-
MEL	7.25b	-	-	-	1.81d
LS	5.75d	-	-	-	1.43e
LO	6.6c	6.32b	9.99	-	5.73a
LC	-	7.05a	-	-	1.76d
MG	5.62d	4.90c	-	-	2.63c
Mean(C)	4.85a	3.62b	1.42c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

FS genotype recorded the highest overall values of proline content under the control and the low salinity level treatments (0 and 100 mM of NaCl, respectively) (Table 27). LO genotype was the

only one where proline was detected under to moderate salinity level (200 mM of NaCl), while no proline was detected in any of the tested genotypes under the highest salinity level (300 mM of NaCl) due to lack of germination.

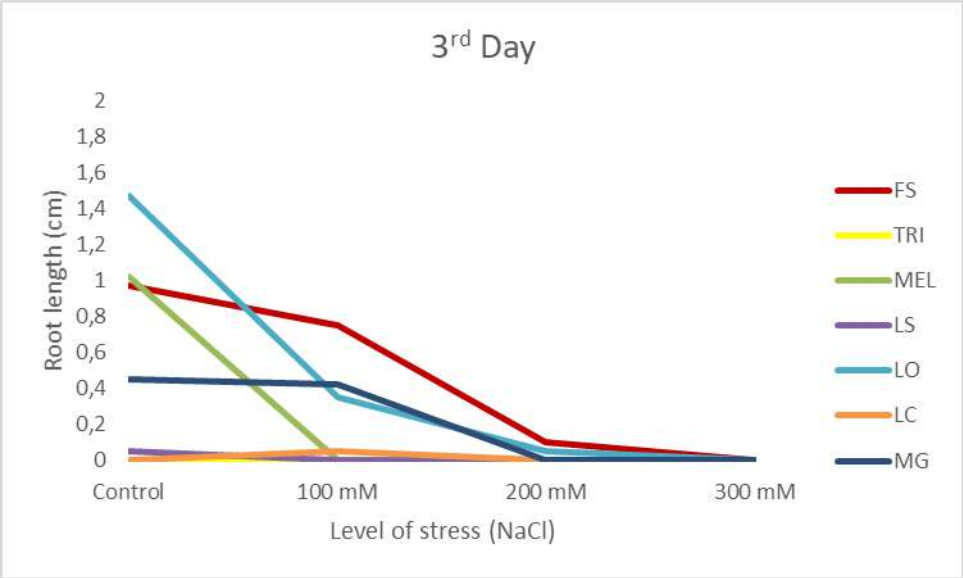
Table 28. Concentration of proline (mg/g fresh weight) in relation to genotype (G), salinity stress (mM of NaCl) and their interaction.

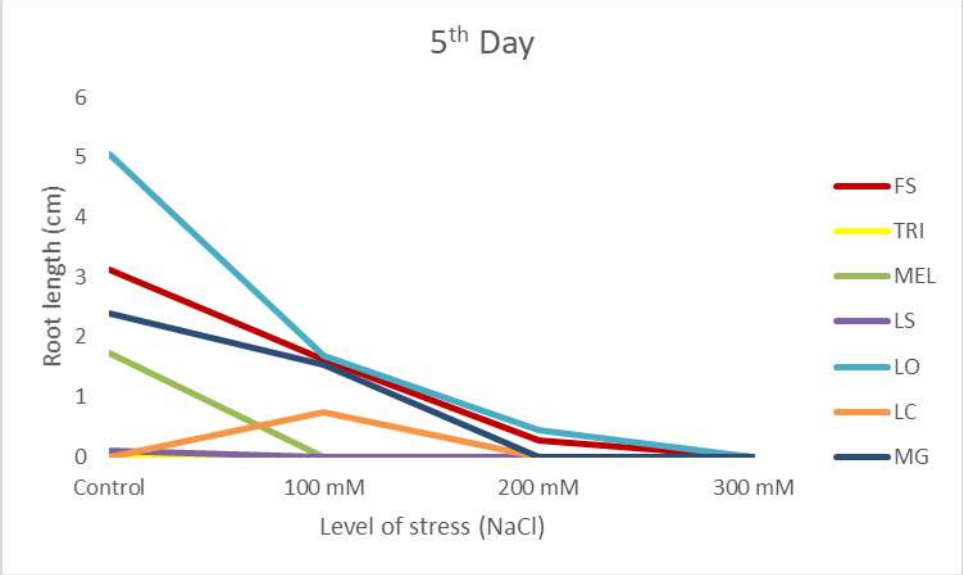
	Proline (mg/g) fresh weight				
Genotypes (G)	**Control**	**100 mM**	**200 mM**	**300 mM**	**Mean(G)**
FS	5.79a	4.83b	-	-	2.65b
TRI	-	-	-	-	-
MEL	4.41b	-	-	-	1.10e
LS	4.23b	-	-	-	1.05e
LO	4.43b	4.39c	6.82	-	3.91a
LC	-	5.42a	-	-	1.35d
MG	3.55c	3.35d	-	-	1.72c
Mean(C)	3.20a	2.57b	0.97c	-	

Means on the same line followed by the same Latin letter are not significantly different according to Least Significant Difference (LSD) test (p=0.05). General means (C and G) are compared within the same line and column, respectively, according to Least Significant Difference (LSD) test (p=0.05).

FS genotype recorded the highest overall values of proline content under the control, while LC genotype had the highest content under the low salinity level (100 mM of NaCl) (Table 28). LO genotype was the only one where proline was detected under to moderate salinity level (200 mM of NaCl), while no proline was detected in any of the tested genotypes under the highest salinity level (300 mM of NaCl) due to lack of germination.

Seedling growth is presented in the following figures (Figure 7 and 8).


3rd Day
Root length (cm)
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Control
100 mM
200 mM
300 mM
Level of stress (NaCl)
FS
TRI
MEL
LS
LO
LC
MG


5th Day
Root length (cm)
6
5
4
3
2
1
0
Control
100 mM
200 mM
300 mM
Level of stress (NaCl)
FS
TRI
MEL
LS
LO
LC
MG

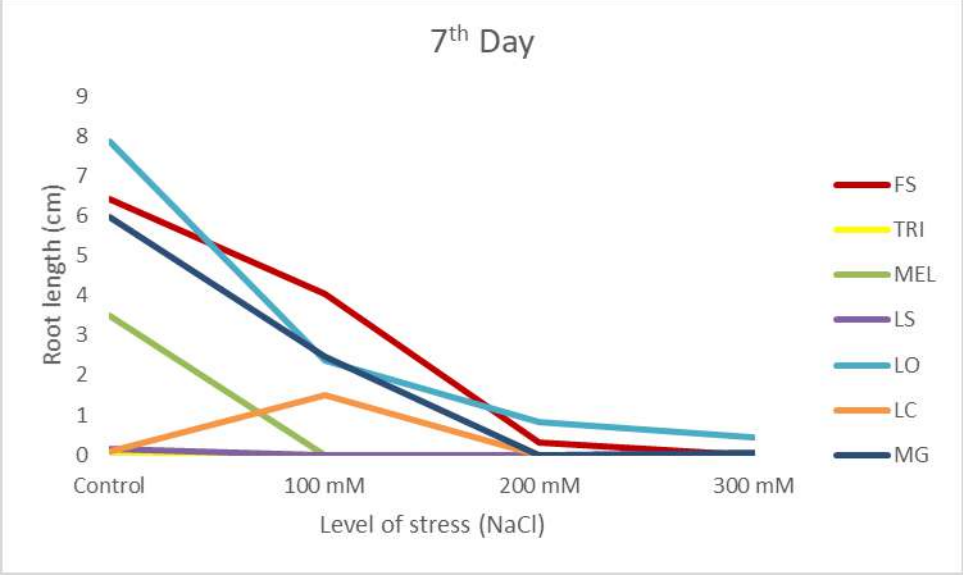
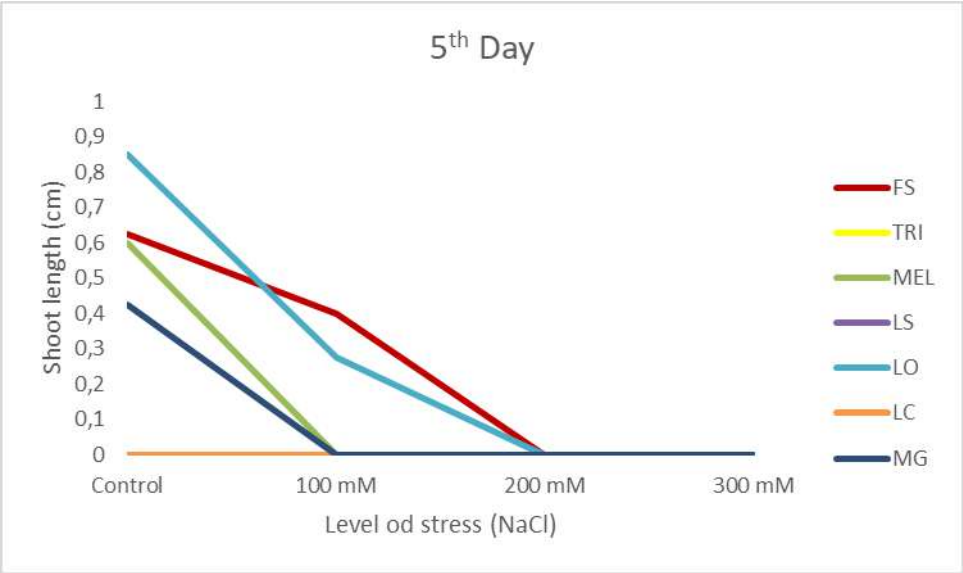

7th Day
Root length (cm)
9
8
7
6
5
4
3
2
1
0
Control
100 mM
200 mM
300 mM
Level of stress (NaCl)
FS
TRI
MEL
LS
LO
LC
MG

Figure 7. Root length at day 3, 5 and 7 in relation to genotype (G) and salinity stress level (mM of NaCl. C).

Decreasing trends in root length were observed with increasing salinity levels with significant differences among the tested genotypes, especially under the low and moderate salinity (100 and 200 mM of NaCl), while under the high salinity level none of the tested genotypes showed root growth (Figure 7). In particular, FS was the most tolerant genotype, especially in day 7 and the low salinity level, while LO genotype was more tolerant under moderate salinity (200 mM of NaCl).



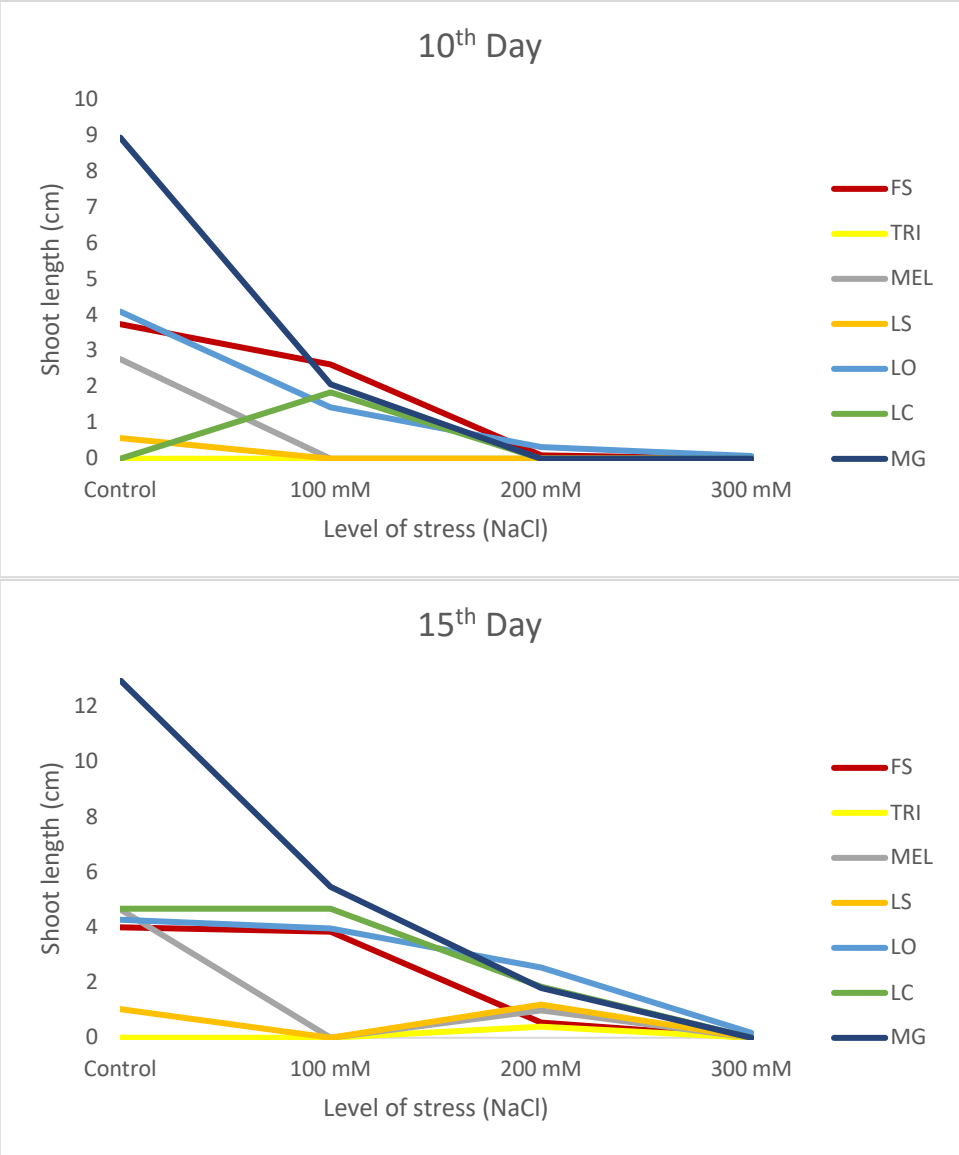


Figure 8. Shoot length at day 5. 10 and 15 in relation to genotype (G) and salinity stress level (mM of NaCl. C).

Decreasing trends were also observed in shoot length of seedlings, where FS showed the highest tolerance at low salinity (100 mMof NaCl) until day 10, while for prolonged stress coditions MG and LO genotypes were the most tolerant under the low (100 mM of NaCl) and moderate (200 mMof NaCl) salinity levels, respectively (Figure 8). None of the tested genotypes showed shoot growth under the high salinity level (300 mM of NaCl).

3.2 CBBC/ISACM (Status: Finished)

Considering that the development of abiotic stress-tolerant germplasm is one of the most effective means for enhancing squash production in abiotic stress conditions. Tunisian team (ISA CM) take care of the first part of the Germplasm evaluation for abiotic stress tolerance aimed at investigating the response of squash germplasm to salt stress and water stress at germination stage and examining the potential of early selecting salt-tolerant and water stress-tolerant genotypes at laboratory level. As such, 15 Tunisian squash landraces were subjected to NaCl-induced salinity stress. at varying stress levels (0, 100, 200 and 300 of NaCl). and their response was assessed on the basis of traits related to seed germination and seedling growth potential.

Effect of salt stress level on traits related to seed germination and seedling growth potential

The obtained data revealed a significant effect of the salt stress level (P<0.001) on the germination and seedling growth potential of the tested squash landraces. with the stress effects being in most cases analogous to the stress level applied. therefore leading to most drastic effects at high stress levels for all traits under study. The data underline the significant effect of landrace on germination and seedling growth potential of squash under salinity stress conditions. The differential response of the landraces to salt stress was evidenced for all the traits under study. All traits related to germination and seedling growth potential under salt stress conditions were differentially affected by the landrace and the salt level applied.

Table 29. Analysis of variance on mean of squares for traits related to seed germination and seeding growth in squash germplasm under salt stress conditions.

S.O.V.	DF	GP (%)	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SRR	GR (%)	SLR (%)	RRL (%)	GSTI (%)	SLSTI (%)	RLSTI (%)
Acc	14	4066.84	340.99	152.93	2.59	0.05	54.80	3801.80	487.89	115.30	0.39	26109.49	18310.15
Trt	3	17405.85	1103.65	697.33	8.37	0.25	139.28	7553.02	22.90	227.89	0.98	1484.58	44510.66
Acc*Trt	42	620.42	46.74	14.07	0.16	0.004	18.93	455.40	9.14	6.58	0.05	669.14	1321.40
CV(%)		13.35	8.96	11.32	16.98	24.92	20.63	10.23	20.69	21.74	29.69	15.39	16.62

Upon stress. the germination of all landraces was severely affected, with the effects of stress being in general analogous to its level (Table 29). At 100 mM NaCl. Batati green proved as the most capable landrace retaining a high germination rate (50%), while Batati orange, Galaoui and Batati yellow also showed relatively high germination rate (> 40%). In contrast, Batati green from Seliana, Batati green from Monastir and Bejaoui spotted with yellow landraces which were of medium-high innate germinability, were incapable of germination at all stress levels. Moreover, at 200 mM NaCl, Batati orange and Galaoui landraces presented the highest germination rate (35%), whereas Galaoui large seeds and Karkoubi orange ranked as the landraces with the lowest germination rate values. Finally, at 300 mM NaCl, Galaoui, followed by Batati orange, proved as the best performing landraces (25% and 20%, respectively). Interestingly, Batati green, although of superior performance at 100 mM NaCl, suffered great losses at both 200 mM and 300 mM NaCl.

Table 30. Mean effect of the salt stress level (0, 100, 200 and 300 mM NaCl) on squash germplasm. consisting of 15 Tunisian landraces.

NaCl concentration (mM)	GP (%)	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SRR	GR (%)	SLR (%)	RRL (%)	GSTI (%)	SLSTI (%)	RLSTI (%)
Control	86.35 a	12.27 a	7.36 a	0.90 a	0.14 a	1.28c	-	-	-	-	-	-
100	23.33 b	7.06 b	4.66 b	0.61 b	0.08 b	1.71b	63.01c	5.20b	2.71c	0.27 a	60.13a	63.79a
200	15.00 c	6.37 c	3.30 c	0.47 c	0.06 c	1.80 b	71.35b	5.89a	4.07b	0.17 b	54.20b	44.04b
300	8.40 d	6.32 c	2.05 d	0.31 d	0.04 d	3.58 a	77.94a	5.94a	5.31a	0.09 c	54.58b	27.52c
F Value	8816**	2141.20**	2879.11**	815.17**	649.38**	745.38**	362.56**	143.95**	791.03**	296.89**	19.75**	16.58**

Note 1: Means in the same column followed by the same letter are not significantly different at P<0.05, according to Duncan's multiple range test.
Note 2: Parameters from GR to RLSTI were evaluated for all landraces compared to the control (see material and methods).

The data underline the significant effect of landrace on germination and seedling growth potential of squash under salinity stress conditions (Table 30). The differential response of the landraces to

salt stress was evidenced for all the traits under study. All traits related to germination and seedling growth potential under salt stress conditions were differentially affected by the landrace and the salt level applied (Table 31).

Table 31. Mean effect of the landrace on traits related to seed germination and seedling growth.

Accession	GP (%)	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SRR	GR (%)	SLR (%)	RRL (%)	GSTI (%)	SLSTI (%)	RLSTI (%)
NGBTUN745	41.97 bc	8.03 gh	4.72 de	0.60 de	0.07 e	1.78 h	74.56 c	5.96 d	2.00 h	0.238 e	56.67 e	68.87 b
NGBTUN746	48.42 a	8.32 g	4.48 e	0.60 de	0.02 h	2.38 e	67.00 de	2.12 h	3.65 e	0.321 ab	80.85 a	49.80 e
NGBTUN747	49.33 a	7.85 h	2.97 g	0.59 de	0.03 fg	3.62 b	64.00 ef	1.64 h	2.54 fh	0.343 a	82.25 a	44.33 ef
NGBTUN748	41.53 cd	9.35 d	4.66 de	0.83 b	0.10 cd	2.07 f	64.63 ef	4.78 e	2.04 gh	0.286 cd	64.20 d	67.57 b
NGBTUN749	40.00 cd	9.28 de	4.01 f	0.56 ef	0.10 cd	2.90 d	60.00 g	4.10 f	4.13 d	0.296 bc	72.54 b	42.95 gh
NGBTUN750	39.50 d	5.35 i	2.98 g	0.76 c	0.10 cd	3.32 c	71.33 c	1.97 h	2.83 f	0.234 e	73.00 b	44.66 fg
NGBTUN751	31.25 e	9.79 c	8.04 b	0.87 b	0.09 d	1.20 i	65.00 ef	10.64 c	5.28 c	0.190 f	40.54 f	57.38 c
NGBTUN752	42.75 b	8.77 f	5.82 c	1.13 a	0.15 a	1.54 h	71.00 cd	3.85 f	2.33 gh	0.260 de	67.43 cd	70.50 b
NGBTUN753	28.75 f	8.96 ef	3.04 g	0.62 d	0.11 b	4.54 a	61.67 fg	1.98 h	3.37 e	0.179 f	81.11 a	32.62 h
NGBTUN1004	23.25 gh	10.86 b	4.73 d	0.52 f	0.10 cd	3.15 c	66.33 e	3.59 f	4.15 d	0.095 h	73.98 b	50.82 de
NGBTUN1005	22.00 gh	10.73 b	5.95 c	0.58 de	0.10 cd	1.91 fg	54.67 h	4.20 f	4.20 d	0.131 g	69.99 bc	54.60 cd
NGBTUN1006	28.08 f	13.44 a	8.28 a	0.41 g	0.10 cd	1.74 gh	92.33 a	2.80 g	2.14 gh	0.151 i	81.98 a	81.65 a
NGBTUN1007	21.50 h	2.69 k	1.84 i	0.21 h	0.04 f	0.36 j	86.00 b	10.75 c	7.39 b	0.03 i	0.00 g	0.00 i
NGBTUN1008	16.75 i	2.90 k	1.33 j	0.13 i	0.03 h	0.55 j	67.00 de	11.60 b	5.30 c	0.04 i	0.00 g	0.00 i
NGBTUN1009	24.00 g	3.78 j	2.28 h	0.18 h	0.03 h	0.41 j	96.00 a	15.15 a	9.14 a	0.03 i	0.00 g	0.00 i
CV %	13.54	8.96	11.32	16.98	23.92	20.63	10.23	20.69	21.74	26.69	15.39	16.62

Note: Means in the same column followed by the same letter are not significantly different at P<0.05, according to Duncan's multiple range test.

Effect of landrace and the salt stress level on traits related to seed germination and seedling growth potential

Based on analysis of variance on individual data, all traits related to germination and seedling growth potential under salt stress conditions were differentially affected by the landrace and the

salt stress level applied (Table 32). In the absence of stress, germination was considerably affected by the landrace, thus substantiating a variable germination rate, which could be mainly attributed to the medium longevity of seed whose fruit were harvested at different periods.

Table 32. Response of squash landraces to varying salt stress levels (0, 100, 200 and 300 mM NaCl) in relation to traits related to seed germination and seedling growth.

NaCl concentration (mM)	Accession	GP (%)	SL (mm)	RL (mm)	SFW (g)	RFW (g)	SRR
Control	NGBTUN745	97.88* ± 1.53	12.5 ± 1.65	6.22 ± 0.79	0.90 ± 0.17	0.15 ± 0.02	2.04 ± 0.40
	NGBTUN746	98.66 ± 1.50	9.91 ± 1.77	7.22 ± 0.75	0.77 ± 0.12	0.04 ± 0.01	1.38 ± 0.2
	NGBTUN747	97.33 ± 2.29	9.08 ± 0.68	4.88 ± 0.48	0.70 ± 0.37	0.07 ± 0.03	1.87 ± 0.23
	NGBTUN748	90.00 ± 8.66	12.94 ± 1.81	6.18 ± 0.60	1.09 ± 0.28	0.15 ± 0.02	2.11 ± 0.37
	NGBTUN749	85.00 ± 4.33	12.36 ± 1.57	7.00 ± 1.16	0.76 ± 0.06	0.15 ± 0.01	1.78 ± 0.40
	NGBTUN750	93.00 ± 2.59	6.83 ± 0.74	5.10 ± 0.33	1.15 ± 0.12	0.16 ± 0.02	1.34 ± 0.17
	NGBTUN751	80.00 ± 4.33	17.77 ± 1.82	12.00 ± 2.00	1.28 ± 0.16	0.14 ± 0.02	1.50 ± 0.15
	NGBTUN752	96.00 ± 2.59	11.66 ± 1.11	7.50 ± 0.61	1.71 ± 0.17	0.19 ± 0.05	1.45 ± 0.18
	NGBTUN753	75.00 ± 4.33	10.45 ± 0.37	5.57 ± 0.32	0.83 ± 0.03	0.18 ± 0.02	1.88 ± 0.14
	NGBTUN1004	73.00 ± 5.66	13.55 ± 1.10	7.69 ± 1.45	0.63 ± 0.05	0.15 ± 0.01	1.81 ± 0.34
	NGBTUN1005	63.00 ± 6.67	13.88 ± 0.96	9.11 ± 1.10	0.85 ± 0.14	0.15 ± 0.03	1.54 ± 0.23
	NGBTUN1006	97.33 ± 1.87	15.55 ± 0.52	9.89 ± 0.42	0.69 ± 0.07	0.16 ± 0.02	1.57 ± 0.09
	NGBTUN1007	86.00 ± 5.19	10.75 ± 0.36	7.39 ± 0.36	0.86 ± 0.03	0.17 ± 0.02	1.45 ± 0.10
	NGBTUN1008	67.00 ± 5.66	11.60 ± 0.76	5.30 ± 0.28	0.52 ± 0.03	0.16 ± 0.02	2.19 ± 0.17
	NGBTUN1009	96.00 ± 2.59	15.15 ± 0.77	9.14 ± 0.30	0.73 ± 0.09	0.10 ± 0.01	1.66 ± 0.09
100	NGBTUN745	50.00 ± 5.66	7.72 ± 0.56	5.05 ± 0.46	0.67 ± 0.04	0.08 ± 0.01	1.54 ± 0.20
	NGBTUN746	40.00 ± 4.33	8.97 ± 0.61	5.70 ± 0.47	0.65 ± 0.22	0.03 ± 0.05	1.58 ± 0.18
	NGBTUN747	45.00 ± 4.33	8.03 ± 0.40	3.85 ± 0.41	0.68 ± 0.04	0.04 ± 0.01	2.10 ± 0.20
	NGBTUN748	35.00 ± 3.32	8.63 ± 0.54	5.28 ± 0.22	0.93 ± 0.25	0.10 ± 0.03	1.64 ± 0.15
	NGBTUN749	40.00 ± 4.85	9.00 ± 0.86	4.40 ± 0.30	0.64 ± 0.03	0.10 ± 0.01	2.05 ± 0.26
	NGBTUN750	30.00 ± 5.33	4.83 ± 0.93	4.49 ± 0.28	0.92 ± 0.06	0.11 ± 0.01	1.07 ± 0.19
	NGBTUN751	30.00 ± 5.33	8.53 ± 0.50	9.32 ± 0.32	1.00 ± 0.04	0.11 ± 0.01	0.92 ± 0.06
	NGBTUN752	35.00 ± 4.33	8.94 ± 0.55	6.33 ± 0.29	1.29 ± 0.08	0.17 ± 0.01	1.41 ± 0.10
	NGBTUN753	25.00 ± 3.33	7.63 ± 0.37	3.47 ± 0.28	0.71 ± 0.04	0.12 ± 0.01	2.21 ± 0.22
	NGBTUN1004	25.00 ± 3.33	10.36 ± 0.72	6.24 ± 0.26	0.58 ± 0.02	0.11 ± 0.01	1.66 ± 0.11
	NGBTUN1005	15.00 ± 2.59	11.08 ± 0.81	6.49 ± 0.20	0.69 ± 0.02	0.10 ± 0.01	1.71 ± 0.16
	NGBTUN1006	10.00 ± 4.39	12.23 ± 0.49	9.36 ± 0.33	0.44 ± 0.03	0.10 ± 0.01	1.30 ± 0.07
	NGBTUN1007	0	0	0	0	0	0
	NGBTUN1008	0	0	0	0	0	0
	NGBTUN1009	0	0	0	0	0	0
200	NGBTUN745	15.00 ± 4.33	6.61 ± 0.39	4.12 ± 0.20	0.53 ± 0.03	0.04 ± 0.00	1.60 ± 0.13
	NGBTUN746	35.00 ± 4.33	7.72 ± 0.56	3.41 ± 0.27	0.50 ± 0.04	0.01 ± 0.00	2.28 ± 0.32
	NGBTUN747	35.00 ± 4.33	7.53 ± 0.44	2.18 ± 0.32	0.55 ± 0.03	0.01 ± 0.00	3.51 ± 0.51
	NGBTUN748	25.00 ± 3.23	8.16 ± 0.28	4.15 ± 0.25	0.74 ± 0.03	0.08 ± 0.03	1.97 ± 0.15
	NGBTUN749	25.00 ± 3.25	8.22 ± 0.75	2.98 ± 0.32	0.49 ± 0.14	0.08 ± 0.01	2.78 ± 0.30
	NGBTUN750	25.00 ± 3.25	3.69 ± 0.46	1.61 ± 0.18	0.64 ± 0.02	0.08 ± 0.01	2.31 ± 0.34
	NGBTUN751	10.00 ± 4.23	7.25 ± 0.43	6.38 ± 0.28	0.85 ± 0.04	0.08 ± 0.01	1.14 ± 0.10
	NGBTUN752	25.00 ± 3.33	7.63 ± 0.45	5.87 ± 0.30	0.88 ± 0.03	0.13 ± 0.01	1.30 ± 0.10
	NGBTUN753	10.00 ± 3.33	8.35 ± 0.29	2.17 ± 0.20	0.57 ± 0.03	0.09 ± 0.03	3.86 ± 0.35
	NGBTUN1004	5.00 ± 1.73	9.77 ± 0.70	3.45 ± 0.28	0.53 ± 0.02	0.08 ± 0.01	2.86 ± 0.39
	NGBTUN1005	10.00 ± 4.23	9.82 ± 0.32	4.67 ± 0.13	0.51 ± 0.03	0.08 ± 0.01	2.12 ± 0.10
	NGBTUN1006	5.00 ± 1.73	10.81 ± 0.47	8.49 ± 0.38	0.33 ± 0.02	0.08 ± 0.01	1.27 ± 0.07
	NGBTUN1007	0	0	0	0	0	0
	NGBTUN1008	0	0	0	0	0	0
	NGBTUN1009	0	0	0	0	0	0
	NGBTUN745	5.00 ± 1.73	5.28 ± 0.56	3.48 ± 0.29	0.33 ± 0.03	0.02 ± 0.00	1.53 ± 0.25

300	NGBTUN746	20.00 ± 3.33	6.67 ± 0.67	1.59 ± 0.26	0.50 ± 0.01	0.01 ± 0.00	4.0 ± 0.93
	NGBTUN747	25.00 ± 3.33	6.75 ± 0.43	0.99 ± 0.19	0.47 ± 0.03	0.01 ± 0.00	7.02 ± 1.41
	NGBTUN748	17.00 ± 4.13	7.66 ± 0.33	3.02 ± 0.31	0.54 ± 0.10	0.07 ± 0.01	2.56 ± 0.21
	NGBTUN749	10.00 ± 3.23	7.55 ± 0.48	1.56 ± 0.24	0.35 ± 0.03	0.05 ± 0.01	4.99 ± 1.04
	NGBTUN750	10.00 ± 3.23	6.05 ± 1.01	0.72 ± 0.12	0.32 ± 0.04	0.06 ± 0.02	8.55 ± 1.33
	NGBTUN751	5.00 ± 1.73	5.63 ± 0.59	4.43 ± 0.29	0.35 ± 0.03	0.04 ± 0.01	1.27 ± 0.14
	NGBTUN752	15.00 ± 4.33	6.86 ± 0.40	3.59 ± 0.20	0.64 ± 0.03	0.12 ± 0.01	1.91 ± 0.11
	NGBTUN753	5.00 ± 1.73	9.42 ± 0.32	0.94 ± 0.15	0.38 ± 0.02	0.05 ± 0.02	10.19 ± 1.51
	NGBTUN1004	5.00 ± 1.73	9.77 ± 0.70	1.58 ± 0.18	0.35 ± 0.02	0.05 ± 0.01	6.25 ± 0.85
	NGBTUN1005	5.00 ± 1.73	8.07 ± 0.46	3.55 ± 0.17	0.26 ± 0.10	0.05 ± 0.03	2.27 ± 0.13
	NGBTUN1006	5.00 ± 1.73	15.16 ± 0.51	5.38 ± 0.26	0.21 ± 0.02	0.06 ± 0.02	2.82 ± 0.15
	NGBTUN1007	0	0	0	0	0	0
	NGBTUN1008	0	0	0	0	0	0
	NGBTUN1009	0	0	0	0	0	0

**Means ± SD*

Effect of the salt stress level on the content of MDA, proline and chlorophyll a and b

The results indicate that the different stress levels significantly affected the content of MDA, free proline and chlorophyll of squash seedlings (Table 33). Regarding MDA content. a significantly increased content was noted at 100 mM NaCl, while the respective value for 200 and 300 mM NaCl did not differ significantly.

Table 33. Mean effect of the salt stress level (0, 100, 200 and 300 mM NaCl) on the content of MDA. free proline and chlorophyll a and b.

NaCl concentration (mM)	MDA	Proline	Chl a	Chl b
Control	1.16 c	0.78 b	30.09 b	60.64 b
100	1.75 a	0.53 c	35.47 a	69.88 a
200	1.44 b	0.82 a	25.70 d	52.44 d
300	1.35 b	0.92 a	28.91 c	56.34 c
CV (%)	57.25	4.96	3.45	2.76
F Value	33.6**	471.2**	552.3**	737.5**

Note: Means in the same column followed by the same letter are not significantly different at P<0.05, according to Duncan's multiple range test.

Effect of landrace and salt stress level on the content of MDA, free proline and chlorophyll a and b

Our findings indicate that the content of MDA, proline and chlorophyll were differentially affected by the landrace and the salt stress level applied (Table 34). In relation to MDA, "748" landrace showed a decreasing trend as NaCl increased. Furthermore, all the other landraces exhibited a decreased content upon stress. yet they showed a notable increase either at 100 mM NaCl ("747" and "746") or 300 mM NaCl ("751").

Table 34. Mean effect of the landrace on the content of MDA, free proline and chlorophyll a and b.

Accession	MDA	Proline	Chl a	Chl b
NGBTUN 746 (Batati)	1.60 a	1.00 a	12.49 d	25.69 d
NGBTUN 747 (Galaoui)	0.78 b	0.79 b	14.81 c	27.89 c
NGBTUN 748 (Kerkoubi)	0.80 b	0.48 d	41.79 b	76.98 b

NGBTUN 751 (Bejaoui)	0.92 b	0.60 c	51.07 a	108.74 a
F Value	15.5**	1466.3**	12473.9**	21337.9**

Note: Means in the same column followed by the same letter are not significantly different at P<0.05, according to Duncan's multiple range test.

Response of squash landraces to varying salt stress levels (0, 100, 200 and 300 mM NaCl) in relation to the content of MDA, free proline and chlorophyll a and b

The results indicated that the content of MDA, free proline and chlorophyll of squash seedlings were differentially affected by the landrace and the salt stress level applied (Table 35). In relation to MDA, Karkoubi orange landrace showed a decreasing trend as NaCl increased. Furthermore, all the other landraces exhibited a decrease content upon stress, yet they showed a notable increase either at 100 mM NaCl (Galaoui and Batati orange) or 300 mM (Bejaoui grenn). Accordingly, the response of these four landraces to the different stress levels varied considerably in terms of the free proline content. Chlorophyll a and b were generally reduced in stressed plants, as compared to the control treatment, yet deviations from such decreasing trend were observed depending on the landrace and stress intensity.

Table 35. Response of squash landraces to varying salt stress levels (0, 100, 200 and 300 mM NaCl) in relation to the content of MDA, free proline and chlorophyll a and b.

Accession	NaCl concentration (mM)	MDA	Proline	Chl a	Chl b
NGBTUN 746 (Batati)	Control	0.88 ± 0.03	1.42 ± 0.03	14.74 ± 0.21	31.06 ± 0.66
	100	4.24 ± 0.03	0.74 ± 0.007	11.64 ± 0.16	24.36 ± 0.50
	200	0.58 ± 0.08	1.13 ± 0.05	9.91 ± 0.16	20.74 ± 0.34
	300	0.68 ± 0.09	0.71 ± 0.076	13.68 ± 0.14	26.59 ± 0.79
NGBTUN 747 (Galaoui)	Control	0.92 ± 0.12	0.59 ± 0.02	13.69 ± 0.15	26.04 ± 0.29
	100	1.86 ± 0.65	0.44 ± 0.003	13.23 ± 0.20	25.85 ± 0.22
	200	0.36 ± 0.06	0.56 ± 0.02	15.57 ± 0.41	29.49 ± 0.50
	300	0.55 ± 0.21	0.8 ± 0.009	16.82 ± 0.36	30.19 ± 1.11
NGBTUN 748 (Karkoubi)	Control	1.79 ± 0.40	0.47 ± 0.05	37.46 ± 0.57	72.18 ± 0.95
	100	0.65 ± 0.03	0.48 ± 0.04	57.03 ± 0.78	103.58 ± 1.55
	200	0.60 ± 0.04	0.55 ± 0.05	30.21 ± 0.56	55.23 ± 0.60
	300	0.20 ± 0.07	0.40 ± 0.01	42.46 ± 0.83	76.92 ± 0.89
NGBTUN 751 (Bejaoui)	Control	1.10 ± 0.22	0.63 ± 0.09	54.50 ± 0.55	113.28 ± 1.70
	100	0.25 ± 0.10	0.46 ± 0.004	59.99 ± 3.80	125.72 ± 3.95
	200	0.20 ± 0.03	1.06 ± 0.05	47.06 ± 0.40	104.30 ± 3.52
	300	1.56 ± 0.20	0.98 ± 0.03	42.69 ± 0.31	91.68 ± 2.38

**Means ± SD*

Based on the results related to salt stress response, 4 landraces were selected representing the main types of cultivated squash (Karkoubi, Galaoui, Bejaoui and Batati) to evaluate D-Mannitol-induced water stress. at varying stress levels (0, 100, 200 and 300 mM D-Mannitol).

Effect of water stress and landraces on traits related to seed germination and seedling growth potential

Biometric analysis of four local accessions squash, growing from grains germinated on Petri's dishes with different concentrations of mannitol (0, 100, 200, 300 mM) during growth, showed statistically significant differences in the length of the tested organs (roots and shoots) (Table 36). Plants of NGBTUN748 landrace watered with mannitol solution at a concentration of 300 mM

during growth had the shortest roots (2.55 cm), a decrease of 32.69% in comparison with control group (7.80 cm). The roots of the NGBTUN747 landrace treated with 300 mM D-Mannitol wilted after treatment. Statistically significant differences in shoot length were observed in different concentrations of mannitol (100, 200 and 300 mM) in relation to the shoots of seedlings growing on the Petri's dishes with distilled water (control). In the case of high concentration of mannitol (300 mM), shoots of Karkoubi pink landrace (NGBTUN748) reached about 5.14 cm, a decrease of 35.58% in comparison with length shoots of control seedlings. In low concentration (100 mM), the length of shoots of the seedlings was about 32.83% less than the control length shoots of the seedlings (Table 36). For Bejaoui green landrace (NGBTUN751), in the case of high concentration, a decrease of 73.74% was observed. In low concentration, the length of shoots was about 19.74% less than the length of shoots of the control seedlings. For Galaoui landrace (NGBTUN747), plants watered with mannitol solution at a concentration of 200 mM during germination phase had the shortest shoots (4.40 cm) in comparison with control group (10.60 cm), a decrease of 58.49%. However, shoots of Galaoui landrace (NGBTUN747) treated with 300 mM D-Mannitol wilted after treatment. For Batati orange landrace (NGBTUN746), significant differences in the length of shoots was found between squashplants which had grown from grains germinating on dishes with 100, 200 and 300 mM of D-Mannitol and plants watered with distilled water (Table 36).

Table 36. Effect of Mannitol levels and landraces on traits related to seed germination and seedling growth potential.

Accessions	Treatment							
	Control		100 mM		200 mM		300 mM	
	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)
NGBTUN748	7.800 ± 1.783	7.988 ± 1.124	5.566 ± 1.011	5.366 ± 0.976	3.000 ±0.968	5.960 ±1.194	2.555 ± 1.102	5.146 ± 0.900
NGBTUN751	6.766 ± 1.837	10.133 ± 1.151	4.655 ± 1.191	8.133 ± 1.096	4.600 ± 1.310	6.233 ± 1.829	3.000 ± 0.870	2.266 ± 1.014
NGBTUN747	7.850 ± 0.852	10.600 ± 1.224	6.933 ± 0.960	5.866 ± 1.165	5.816 ± 1.058	4.400 ± 0.952	0.000 ± 0.000	0.000 ± 0.000
NGBTUN746	4.566 ± 1.075	10.022 ± 0.523	3.944 ± 1.077	5.633 ± 1.162	4.250 ± 0.892	5.550 ± 1.194	3.383 ± 1.172	5.533 ± 1.607

Generally, watering with increasing concentrations of mannitol in germination phase showed inhibitory effect on the length of squash shoots for all landraces (Table 37). Additionally, statistical analysis showed their inhibitory effects on the growth of squash roots. The highest concentrations of mannitol (300 mM) completely inhibited the development of shoots and roots of Galaoui landrace (NGBTUN747). Plants watered with mannitol during germination phase, had the shortest remainder of shoot and root in comparison with control.

Table 37. Effect of Mannitol levels and landraces on fresh weight of shoot and root.

Treatment	Accessions	FWR (g)	FWS (g)	SL/RL (SSR)
Control	NGBTUN748	0.678 ± 0.087	0.593 ± 0.197	1.087 ± 0.319
	NGBTUN751	0.037 ± 0.017	0.325 ± 0.090	1.629 ± 0.571
	NGBTUN747	0.023 ± 0.017	0.328 ± 0.100	1.356 ± 0.143
	NGBTUN746	0.070 ± 0.023	0.573 ± 0.112	2.307 ± 0.580
100 mM	NGBTUN748	0.597 ± 0.197	0.327 ± 0.087	0.978 ± 0.177
	NGBTUN751	0.021 ± 0.011	0.262 ± 0.092	1.852 ± 0.519
	NGBTUN747	0.014 ± 0.008	0.199 ± 0.086	0.839 ± 0.127
	NGBTUN746	0.053 ± 0.021	0.450 ± 0.136	1.462 ± 0.229
200 mM	NGBTUN748	0.187 ± 0.099	0.272 ± 0.146	2.149 ± 0.777
	NGBTUN751	0.018 ± 0.033	0.180 ± 0.104	1.469 ± 0.586
	NGBTUN747	0.013 ± 0.008	0.172 ± 0.087	0.748 ± 0.074
	NGBTUN746	0.047 ± 0.009	0.404 ± 0.135	1.311 ± 0.147
300 mM	NGBTUN748	0.063 ± 0.019	0.226 ± 0.087	2.365 ± 1.187
	NGBTUN751	0.004 ± 0.001	0.176 ± 0.090	0.731 ± 0.206

	NGBTUN747	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
	NGBTUN746	0.021 ± 0.008	0.288 ± 0.099	1.957 ± 1.281

According to the results of fresh matter of roots and shoots of four landraces of squash seedlings, it was indicated that mannitol-induced reduction of fresh matter of shoots and roots for all landraces (Table 37). The shoot/root ratio (SSR) ranged from 1.629 (control) to 1.852 (100 mM D-mannitol) for Bejaoui green landrace (NGBTUN751) and ranged from 1.087 (control) to 2.149 (200 mM D-Mannitol) and 2.365 (300 mM D-Mannitol) for Karkoubi pink landrace (NGBTUN748), suggesting that water stress is more pronounced on root elongation than on shoot.
The reduction of germination percent (RG %), was more important for seeds treated with 100 mM of D-Mannitol for the landrace Galaoui (NGBTUN747) to reach a value of 16.66%. For seeds treated with 200 mM of D-Mannitol, the reduction of germination percent was more important for the landrace Galaoui (NGBTUN747) and less important for the landrace Batati orange (NGBTUN746) and ranged from 3.33% (Batati orange) to 30% (Galaoui).
The reduction of germination percent was important with increasing D-Mannitol levels (300 mM) and the landrace Bejaoui green (NGBTUN751) has the highest value (50%) and the local accession Batati orange (NGBTUN746) has the lowest value (6.66%).

Table 38. Effect of Mannitol levels and landraces on root length reduction, shoot length reduction and germination reduction.

Treatment	Accessions	RRL (%)	RSL (%)	RG (%)
100 mM	NGBTUN748	2.233 ± 1.423	2.038 ± 0.850	11.666 ± 2.134
	NGBTUN751	2.211 ± 1.332	2.000 ± 1.574	0.000 ± 0.000
	NGBTUN747	0.916 ± 0.327	4.783 ± 1.041	16.667 ± 2.285
	NGBTUN746	0.622 ± 0.389	4.388 ± 1.041	3.337 ± 1.855
200 mM	NGBTUN748	4.800 ± 1.814	2.622 ± 0.720	16.888 ± 4.164
	NGBTUN751	2.166 ± 0.868	3.900 ± 0.851	14.444 ± 5.299
	NGBTUN747	1.983 ± 0.392	6.200 ± 1.257	30.000 ± 3.611
	NGBTUN746	0.316 ±0.146	4.472 ± 1.038	3.337 ± 1.732
300 mM	NGBTUN748	5.244 ± 0.724	2.842 ± 0.978	35.777 ± 4.155
	NGBTUN751	3.766 ± 0.513	7.866 ± 1.695	50.000 ± 1.689
	NGBTUN747	7.850 ± 0.892	10.600 ± 1.224	30.000 ± 5.031
	NGBTUN746	1.183 ± 0.420	4.488 ± 1.609	6.666 ± 2.279

The physiological indices such as reduction root length (RRL), reduction shoot length (RSL), root length stress index (RLSI) and shoot length stress index (SLSI) were calculated, and the significant differences were recorded (Table 38). The most important reduction of the root length (RRL) was recorded for landraces Karkoubi pink (NGBTUN748) and Bejaoui green (NGBTUN751) treated with 100 mM of D-Mannitol (2.23 and 2.21% respectively). The lowest value of reduction root length was recorded to landraces Galaoui (NGBTUN747) and Batati orange (NGBTUN746) treated with 100 mM of D-Mannitol (0.91 and 0.62% respectively). For 200 mM of D-Mannitol, the most important reduction of the root length (RRL) was recorded to the landrace Karkoubi pink (NGBTUN748) (4.80%), whereas the lowest value was recorded to Batati orange landrace (NGBTUN746) (0.31%). Galaoui landrace (NGBTUN747) treated with 300 mM of D-Mannitol recorded the highest value for the reduction root length (RRL) (7.85%) and Batati orange landrace recorded the lowest value (1.18%) (Table 38).
The reduction of shoot length (RSL) was more important for Galaoui landrace (NGBTUN747) with increasing D-Mannitol levels and ranged from 4.78% (100 mM D-Mannitol) to 10.60% (300 mM D-Mannitol) (Table 39). The lowest values of RLSI were noted on stressed seedlings of Bejaoui green landrace (NGBTUN751) with 100 mM D-Mannitol (70.52%), on stressed seedlings of Karkoubi pink landrace (NGBTUN748) with 200 mM D-Mannitol (39.60%) and on stressed seedlings of Galaoui landrace (NGBTUN 747) with 300 mM D-Mannitol (0.00%).

Table 39. Effect of Mannitol levels and landraces on root length stress index, shoot length stress index and drought stress index.

Treatment	Accessions	RLSI	SLSI	DTI
100 mM	NGBTUN748	73.084 ± 4.183	67.120 ± 2.484	86.122 ± 2.644
	NGBTUN751	70.524 ± 5.792	81.092 ± 4.332	100.000 ± 0.000
	NGBTUN747	88.173 ± 4.471	54.442 ± 1.741	70.138 ± 4.127
	NGBTUN746	86.089 ± 2.781	56.106 ± 3.465	90.167 ± 1.786
200 mM	NGBTUN748	39.602 ± 4.254	75.480 ± 5.488	81.053 ± 4.297
	NGBTUN751	71.955 ± 8.249	62.901 ± 6.903	85.555 ± 5.298
	NGBTUN747	74.312 ± 2.157	41.677 ± 2.848	80.972 ± 3.546
	NGBTUN746	93.910 ± 2.800	55.283 ± 3.586	83.604 ± 1.520
300 mM	NGBTUN748	34.897 ± 6.116	64.827 ± 3.259	58.388 ±4.579
	NGBTUN751	45.310 ± 3.698	22.849 ± 3.618	17.777 ± 1.689
	NGBTUN747	0.000 ± 0.000	0.000 ± 0.000	33.888 ± 5.127
	NGBTUN746	76.812 ± 9.122	55.246 ± 5.180	72.719 ± 1.864

Physiological and biochemical contents on the studied landraces submitted to water stress

During the germplasm evaluation and in addition to the germination and early seedling traits, physiological parameters such as chlorophyll fluorescence, Photosynthetically Active Radiation (PAR) and Evapotranspiration (ETR) were assessed at growth stage under greenhouse while biochemical traits such as malondialdehyde (MDA), free proline, chlorophyll a and b, carotenoids, phenols, flavonoids and 2,2 diphenyl-1-picrylhydrazyl (DPPH) activity were evaluated on both roots and true leaves at growth plant stage .

Chlorophyll fluorescence was considerably affected by the landrace, as evidenced by the mean F_v/F_m of landraces under study (Table 40), which ranged from 0.76 to 0.82 in landraces "748" and "747", respectively. Although such values suggest a high water stress tolerance ability and sensitivity for "748" and "747" landraces, respectively. Moreover, the landraces "751" and "746", were characterized by a mean F_v/F_m of 0.81 and 0.80 respectively located in the range (0.79- 0.82) which indicates the absence of stress state for these two accessions.

Table 40. Mean effect of the squash germplasm landraces on chlorophyll fluorescence parameters for different landraces of squash at plant stage

Landrace	F_0	F_m	F_v/F_m	F_v
"748"	499.38^{d}	1738.54^{c}	0.76^{c}	3.57^{c}
"751"	474.22^{c}	1569.54^{d}	0.81^{ab}	5.02^{a}
"747"	443.10^{b}	2420.15^{a}	0.82^{a}	4.97^{a}
"746"	418.94^{a}	2294.63^{b}	0.80^{b}	4.53^{b}
F-Value	39.97**	164.51**	90.77**	229.10**

** Means in the same column followed by the same letter are not significantly different at $p < 0.05$, according to Duncan's Multiple Range test; F_0: Minimal chlorophyll fluorescence intensity; F_m : Maximal chlorophyll fluorescence intensity ; F_v/F_m : Maximum quantum efficiency of PSII photochemistry ; F_v : Variable chlorophyll fluorescence.

Effect of the landrace and the D-mannitol level on chlorophyll fluorescence parameters

Figure 9 indicated that all parameters related to chlorophyll fluorescence under water stress conditions were differentially affected by the landrace and the D-Mannitol level applied. Under the absence of stress, F_0, F_m, F_v and F_v/F_m were considerably affected by the landrace. This indicates that these landraces have differential abilities to withstand water stress. Among landraces, "748" (Karkoubi orange) and "746" (Batati orange) presented the lowest and the highest F_0 under normal conditions (357.72 and 651.55, respectively). For maximal chlorophyll fluorescence intensity (F_m), those later landraces

presented the highest and the lowest F_m under normal conditions (2520.87 and 2089.11, respectively). Upon stress, the chlorophyll fluorescence parameters of all landraces were severely affected, with the effects of stress being in general analogous to its level. At 100 mM D-Mannitol, "751" (Bejeoui green) and "747" (Galaoui) proved to be the most landraces affected representing a quantum ratio (F_v/F_m) of 0.85 and 0.75, respectively which is outside the range of 0.79 to 0.82, indicator of the sensitivity to abiotic stress. Moreover, at 200 and 300 mM D-Mannitol, the four landraces (Karkoubi orange, Bejaoui green, Galaoui and Batati orange) show the similar trends by presenting a quantum ratio (F_v/F_m) out of the mentioned range (Fig.2). Although, "Karkoubi orange" and "Batati orange", in spite of their superior performance at 100 mM D-Mannitol, suffered at both 200 mM and 300 mM D-Mannitol. Furthermore, the response of most landraces to water stress involved a drastic reduction in F_m. In agreement with these findings, the F_v followed an increasing trend as D-Mannitol concentration increased for Bejaoui green landrace. The highest values for F_v parameters were recorded in "Batati orange" both in control and 100 mM D-Mannitol. The F_v was more profoundly affected at 300 mM D-Mannitol, with the landraces showing varying values ranging from 3.48 to 5.42 (Fig. 9).

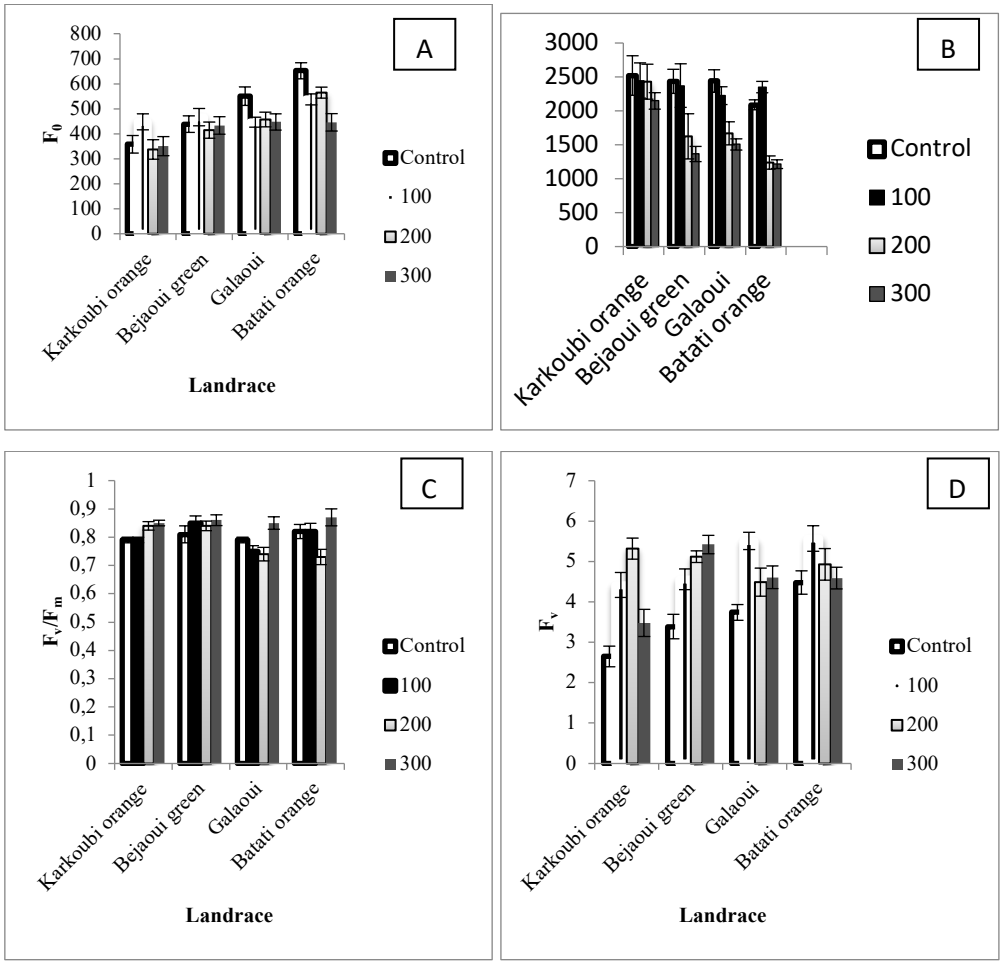


Figure 9. Response of squash germplasm landraces to varying levels of D-Mannitol on chlorophyll fluorescence parameters (F_0 (A), F_m (B), F_v/F_m (C) and F_v (D)) measured after 45 days in squash landraces at plant stage.

Regarding the response of the landraces, our results revealed a significant effect of these landraces on the content of chlorophyll a, chlorophyll b and carotenoids in squash

plants (Table 41). In relation to chlorophyll a (Ch a), the highest content was recorded in landrace "746" (11.24 mg.gFW^{-1}), while the lowest value was recorded in "748" (7.48 mg.gFW^{-1}) . The landrace "746" was further distinguished by the highest content of chlorophyll b (Ch b) (4.94 mg .gFW^{-1}), while landrace "748" showed the lowest content of Ch b (2.67 mg g^{-1} FM). Regarding carotenoids content, the highest content was recorded in landrace "751" (0.69 mg.gFW^{-1}) vs. "748" landrace showing the lowest value (0.53 mg.gFW^{-1}).

Table 41. Mean effect of the squash germplasm landraces on chlorophyll a, chlorophyll b and carotenoids for different landraces of squash at plant stage

Landrace	Ch a	Ch b	Car.
"748"	7.48^{d}	2.67^{b}	0.53^{d}
"751"	9.03^{c}	3.94^{ab}	0.69^{a}
"747"	11.06^{b}	3.20^{b}	0.62^{c}
"746"	11.24^{a}	4.94^{a}	0.65^{b}
F-Value	1505.76**	3.06*	96.85**

** Means in the same column followed by the same letter are not significantly different at $p < 0.05$, according to Duncan's Multiple Range test; Cha: chlorophyll a; Chb: chlorophyll b; Car.: carotenoids.

Results in Table 42 display the evapotranspiration (ETR) and photosynthetically active radiation (PAR), which were affected by landraces. The highest value of ETR was recorded in "746" landrace (179.44 mm/day), while the lowest value was attributed to "751" and "748" landraces (104.08 and 108.94 mm/day, respectively). Thus, indicate the differential responses of these landraces under drought stress by adjusting stomatal closure. The effect of drought stress on photosynthetisis was very similar to that on transpiration in all the landraces, with a differential ability between them. Regarding photosynthetically active radiation (PAR), the highest value was recorded in "746" landrace (1790.80 µmol m^{-2} s^{-1}) and the lowest value was recorded in "751" landrace (957.83 µmol m^{-2} s^{-1}). The evapotranspiration values ranged from104.08 to 179.43 mm/day, while the photosynthetically active radiation values ranged from 957.83 to 1790.84 µmol m^{-2} s^{-1}.

Table 42. Mean effect of the squash germplasm landraces on evapotranspiration and photosynthetically active radiation for different landraces of squash at growth stage

Landrace	ETR (mm/day)	PAR (µmol m^{-2} s^{-1})
"748"	108.944^{c}	1327.375^{c}
"751"	104.084^{c}	957.833^{d}
"747"	137.986^{b}	1341.305^{b}
"746"	179.439^{a}	1790.840^{a}
F-Value	370.74**	5923.84**

** Means in the same column followed by the same letter are not significantly different at $p < 0.05$, according to Duncan's Multiple Range test; ETR: evapotranspiration; PAR: photosynthetically active radiation.

Regarding the effects of landraces and D-Mannitol levels on the evapotranspiration and the Photosynthetically Active Radiation, our findings indicate the differentially responses of these four squash landraces submitted to different water stress applied (Table 43). In general transpiration and photosynthesis activities decreased in all landraces submitted to increased stress levels as one of the first response of squash plants to drought stress since stomatal closure adjusts the photosynthesis by restring gas exchange between the atmosphere and the inside of the leaves. The photosynthetically active radiation (PAR) value's decrease became more pronounced as D-Mannitol level intensified, especially under 200 and 300 mM, for "751" (Bejaoui) landrace, thus showing its more sensitivity compared to another landraces. Whereas all landraces showed an

elevated values of PAR at 100mM. Interestingly, "748" and "746"landraces maintained high photosynthesis activity at 100mM and 200mM compared to another two landraces. Furthermore, all the other landraces exhibited a decreased content upon stress; at 300mM of water stress evapotranspiration and photosynthesis activity were more reduced in "751" landrace and "747" thus showing the lowest ability to overcome the negative effect of water stress.

Table 43. Response of squash landraces to varying water stress levels (0,100,200, and 300mM D-Mannitol) in relation to the evapotranspiration and photosynthesis activities

Landrace	D-Mannitol concentration (mM)	ETR (mm/day)	PAR ($\mu mol\ m^{-2}\ s^{-1}$)
"748"	Control	135.67 ± 3.00	1551.05 ±22.86
	100	104.60 ± 2.37	1328.61 ±19.08
	200	101.55 ± 0.97	1269.66 ±26.67
	300	93.94 ±2.85	1160.16 ±30.77
"751"	Control	120.17 ±40.16	1471.88 ±30.60
	100	125.00 ±3.15	1328.51 ±19.08
	200	115.23 ± 2.98	954.33 ±27.01
	300	55.92 ± 2.95	358.11 ±25.80
"747"	Control	189.99 ±8.69	1935.49 ±34.63
	100	155.00 ± 3.09	1838.33 ±29.74
	200	131.42 ± 3.85	1256.72 ±25.27
	300	75.52 ± 2.82	934.66 ±25.20
"746"	Control	194.52 ±6.51	1846.16 ±30.49
	100	192.55 ± 3.05	1659.49 ±26.84
	200	176.61 ± 3.24	1160.16 ±30.77
	300	154.06± 2.39	1080.17 ±24.94

The results indicate that the contents of osmoprotectants were differentially affected by the landrace and the D-Mannitol level applied (Table 44). In relation to MDA, "751", "747" and "746" landraces showed an increasing trend as D-Mannitol level increased. Furthermore, "748", landrace exhibited a decreased content upon 300 mM D-Mannitol, yet it showed a notable increase either at 100 and 200 mM D-Mannitol. Accordingly, the response of these four landraces ("747", "746", "748" and "751") to the different D-Mannitol levels varied considerably in terms of the free proline content. The water stress response of all landraces involved an increased content of free proline. In relation to the D-Mannitol level effects, the application of 300 mM D-Mannitol induced proline accumulation in "751" and "747", whereas "748" "746" and presented a peak at 300 mM D-Mannitol. Similarly, the content of TP and TF was differentially affected both by the landrace and the D-Mannitol level. TP and TF were generally increased in stressed plants, as compared to the control treatment. Specifically, "748" landrace showed the highest content of TP and TF at 300 mM D-Mannitol.

Table 44. Response of squash landraces to varying D-Mannitol concentration in the content of MDA, FP, TP, TF and DPPH activity (means ± SD)

Landrace	D-Mannitol concentration (mM)	MDA ($\mu mol\ g^{-1}$ FW)	Free proline ($\mu g\ mg^{-1}$ FW)	TP (mg GA/100 mg DW)	TF (mg QE/ 100 mg DW)	DPPH (%)
"748"	Control	11.00 ± 0.48	0.64 ± 0.14	30.97 ± 0.17	40.15 ± 0.43	22.90 ± 1.04
	100	12.23 ± 0.34	1.45 ± 0.29	41.59 ± 1.22	54.29 ± 0.94	32.48 ± 1.73

	200	13.04 ± 1.49	1.79 ± 0.19	43.18 ± 0.53	55.96 ± 0.57	34.62 ± 1.30
	300	10.17 ± 1.00	2.29 ± 0.31	95.31 ± 2.13	56.71 ± 0.56	35.52 ± 1.31
"751"	Control	9.83 ± 0.71	0.66 ± 0.07	28.09 ± 1.88	39.22 ± 0.82	21.17 ± 1.94
	100	12.54 ± 0.93	1.77 ± 0.16	33.03 ± 2.59	42.15 ± 0.89	26.89 ± 1.63
	200	13.96 ± 1.45	1.82 ± 0.14	34.90 ± 3.53	44.26 ± 0.99	28.85 ± 1.51
	300	17.11 ± 1.05	1.46 ± 0.17	37.10 ± 3.25	44.83 ± 0.93	29.64 ± 1.54
"747"	Control	10.78 ± 0.14	1.15 ± 0.42	32.33 ± 1.03	39.19 ± 1.45	24.21 ± 0.98
	100	14.42 ± 1.37	1.67 ± 0.25	35.24 ± 1.15	45.89 ± 1.69	27.30 ± 1.04
	200	15.27 ± 1.16	1.48 ± 0.10	41.48 ± 1.07	50.84 ± 1.51	30.35 ± 0.85
	300	15.86 ± 1.84	1.57 ± 0.13	42.03 ± 1.01	51.41 ± 1.45	30.84 ± 0.60
"746"	Control	10.83 ± 0.09	1.15 ± 0.12	33.29 ± 1.26	41.38 ± 1.26	25.55 ± 1.41
	100	12.41 ± 1.12	1.59 ± 0.27	34.91 ± 1.73	43.21 ± 1.41	28.03 ± 1.68
	200	15.65 ± 1.42	1.81 ± 0.11	36.43 ± 1.74	50.02 ± 1.62	29.36 ± 1.16
	300	16.16 ± 1.88	2.87 ± 0.28	39.39 ± 1.05	50.62 ± 1.33	31.18 ± 1.36

The variation in the contents of MDA, FP, TP, TF and DPPH activity in the shoots and the roots of squash landraces subjected to the water stress revealed a significant difference of root and shoot content of osmoprotectants was recorded (Table 30). As displayed in Table 15, the contents of MDA, free proline, TF and DPPH were higher in roots (13.78 µmol g^{-1} FW, 2.10 µg mg^{-1} FW, 47.20 mg QE/ 100 mg DW and 28.98 %, respectively) than in shoots (12.71 µmol g^{-1} FW, 0.95 µg mg^{-1} FW, 46.56 mg QE/ 100 mg DW and 28.38 %, respectively), whereas, content of TP was higher in shoots (37.57 mg GA/100 mg DW) than in roots (36.22 mg GA/100 mg DW).

Table 45. Mean effect of shoot ad root on the content of MDA, FP, TP, TF and DPPH activity

Organ	MDA (µmol g^{-1} FW)	Free proline (µg mg^{-1} FW)	TP (mg GA/100 mg DW)	TF (mg QE/ 100 mg DW)	DPPH (%)
Shoot	12.71[b]	0.95[b]	37.57[a]	46.56[b]	28.38[b]
Root	13.78[a]	2.10[a]	36.22[b]	47.20[a]	28.98[a]

** Means in the same column followed by the same letter are not significantly different at $p < 0.05$, according to Duncan's Multiple Range test; MDA: Malondialdhehyde; TP: Total phenols; TF: Total flavonoids; DPPH: 2,2-diphényl 1-picrylhydrazyle.

ISA-CM has presented the results of the project in the following journal and conferences:

1. Neji Tarchoun, Wassim Saadaoui, Najla Mezghani, Ourania I. Pavli, Hanen Falleh and Spyridon A. Petropoulos. 2022. The Effects of Salt Stress on Germination, Seedling Growth and Biochemical Responses of Tunisian Squash (Cucurbita maxima Duchesne) Germplasm. Plants 2022, 11, 800. https://doi.org/10.3390/ plants11060800.

2. **Oral presentations with abstract**

- Hamdi, K.; Saadaoui, W.; Tarchoun, N. Valorization of squash (Cucurbita maxima Duch) Biodiversity: approaches and main results. 4th International Congress of Biochemistry and Microbiology Applied Technologies BMAT. 2021. Hammamet Sud, 05-06 November.
- Tarchoun, N. ; Hamdi, K. ; Saadaoui, W. 2021. Characterization and Valorization of local squash (*Cucurbita maxima* Duch) landraces: approaches and main results. 1st International congress SUSTAINABLE AGRICULTURE : TOOLS AND INNOVATIONS (AgriNov2021), held in Faculty of Sciences and Technics-USMS, Béni Mellal, Morocco, on 27-30th Octobre 2021.
- Saadaoui, W. ; Hamdi, K. ; Tarchoun, N. Genetic relationships among local germplasm collection of *Cucurbita maxima* Duchesne through molecular markers and antioxidant properties analysis. The Fourth International Congress of Biochemistry & Microbiology Applied Technologies, BMAT, 28 - 30 May 2021 Hammamet- Tunisie.

-Neji TARCHOUN[1], Wasim SAADAOUI[1], Khawla HAMDI[1], Hanen FALLEH[2] and Riadh KSOURI. Effect of seed priming on squash (*Cucurbita maxima* Duch.) seed germination and seedling characteristics under salt stress. 5th International Congress of Biochemistry & Microbiology Applied Technologies BMAT'2022 (4-6 November 2022)

3. **Oral presentations with paper**

- Hamdi, K. ; Saadaoui, W. ; Sestili,S. ; Palatani, C. ; Tarchoun, N. Genetic relationship between Tunisian and Italian *Cucurbita maxima* Duch. Germplasm. 10th International Molecular Biology and Biotechnology Congress (04-08 Oct. 2021 online, Turkey, University of Cukurova).

- Tarchoun N.; Wassim SAADAOUI , Khawla HAMDI , Hanen FALLEH, Lillian BARROS, Riadh KSOURI .Assessment of salt and water stress tolerance of Tunisian Squash (*Cucurbita maxima* Duchesne) germplasm during the germination and early seedling growth. IV. BALKAN AGRICULTURAL CONGRESS AGRIBALKAN 2022/ 31 August - 2 September, 2022, Edirne, Turkey

-Neji TARCHOUN[1], Wasim SAADAOUI[1], Khawla HAMDI[1], Hanen FALLEH[2] and Riadh KSOURI. The Physiological and Biochemical Responses of four Local Squash Landraces under Drought stress. 11th International Molecular Biology and Biotechnology Congress (10-12 Oct. 2021, online, Turkey, University of Cukurova).

4. **Poster**

Insaf Msetra, Wassim Saadaoui and Neji Tarchoun. Assessment of water stress tolerance of squash local landraces by application of D-Mannitol during germination and growth stage. 4th Euro-Mediterranean Conference for Environmental Integration. 20-23 October 2022. Sousse-Tunisia

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Hnilickova, H.; Kraus, K.; Vachova, P.; Hnilicka, F. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. Plants 2021, 10, 845, doi:10.3390/plants10050845.

Monneveaux, P.; Nemmar, M. Contribution à l'étude de la résistance à la sécheresse chez le blé tendre (*Triticum aestivum* L.) et chez le blé dur (*Triticum durum* Desf.): étude de l'accumulation de la proline au cours du cycle de développement. Agronomie 1986, 6, 583–590, doi:10.1051/agro:19860611.