



## Monitoring of six grape genotypes in response to salt stress in an arid region in Tunisia: morphological parameters

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### ABSTRACT

**Purpose:** This study aimed to valorize the grape genotypes in the oasis of Tozeur, Tunisia, by exploring their salinity tolerance.

**Research method:** The principal genotypes: Arbi, Chetoui, Guelb Sardouk, Kahla, Sfaxi and Muscat d'Italie were subject to two salt treatments: a gradual stress by adding 25 mM NaCl every week until the final doses of 75, 100 and 150 mM NaCl and a shock by applying 50 and 100 mM NaCl from the treatment onset. The shoot length and the number of leaves were monitored during salt treatments.

**Findings:** The first mortalities were registered at the 3<sup>rd</sup> week for Sfaxi under gradual stress conditions, Guelb Sardouk, and Kahla under salt shock. The effects of 75 mM NaCl gradually added were expressed only by the decline of leaves number for the genotypes Chetoui, Kahla, and Sfaxi, while those of low salt shock (50 mM NaCl) were manifested by plant height and leaves number reductions. Salinity damages were aggravated by increasing the salinity. Another response was observed for the studied genotypes: they kept their vegetative apparatus almost unaffected to preserve the photosynthetic capacity. **Research limitations:** No limitations were founded. **Originality/Value:** The adverse effects of salinity were more relevant at the end of the stress particularly under the high salt dose (150 mM NaCl). The salinity of irrigation water for our grape genotypes shouldn't reach 150 mM NaCl. The genotype Arbi was the most tolerant genotype while Kahla was the most sensitive to salinity.

## INTRODUCTION

Grapevine (*Vitis vinifera*), one of the most widely cultivated fruit crops, is considered as moderately sensitive to salinity (Maas & Hoffman, 1977; Grieve et al., 2012). An electrical conductivity threshold (EC<sub>e</sub> of 1.5 dS m<sup>-1</sup>) of soil saturation paste was proposed beyond which yield were expected to decrease; 9.6% reduction in yield was supposed for every 1 dS m<sup>-1</sup> increase of EC<sub>e</sub> (Maas & Hoffman, 1977; Grieve et al., 2012). In fact, salinity affected negatively shoot, bunch and root number and growth rate (Prior et al., 1992; Walker et al., 1997, 2002; Fisarakis et al., 2001; Ha et al., 2008). Growth rate decline was attributed to either ion toxicity or low external osmotic potential (Garcia & Charbar, 1993).

Upon saline condition, plant could be subjected to ion toxicity, osmotic stress, nutrient balance disturbance and lower rate of photosynthetic assimilation (Flowers, 2004; Munns & Tester, 2008). It has been proposed that plants short exposed to high salinity suffered mainly from water stress. As well as prolonged salt stress, ion toxicity may become more serious. Therefore, preserving a suitable level of tissue hydration was considered as plant ability tightly correlated to salt tolerance (Negrão et al., 2017). Long term exposure to salt stress could also induce ionic stress in plants leading to adult leaves' premature senescence thus decreasing leaf number (López-Aguilar et al., 2003; Ha et al., 2008).

NaCl salinity had drastic effects on photosynthesis and stomatal conductance in grafted 'Sultana' and 'Müşküle' vines (Sivritepe et al., 2010). Sodium was suggested as the responsible of cationic increase leading to growth inhibition (Hong et al., 2009). However, Cl<sup>-</sup> can also be toxic to plants at high concentrations. Several studies have indicated that Cl<sup>-</sup> is potentially more toxic than Na<sup>+</sup> (Teakle et al., 2007; Aydi et al., 2008). While, Fisarakis et al. (2001) considered Na<sup>+</sup> as main responsible for leaf burn even if it occurred at lower level than Cl<sup>-</sup>.

Hamrouni et al. (2011) proved that the main salt tolerance mechanism of the wild grapevine 'Séjnène' were the ability to sustain the photosynthetic activity during salt stress and the capacity of accumulating and storing sodium in the upper part of the plant. Indeed, the wild type 'Séjnène' revealed other mechanism namely chloride exclusion with restriction to its entry and uptake, osmotic adjustment via leaf potassium accumulation, salt stress signalization and cellular protection of components through leaf calcium accumulation (Hamrouni et al., 2011). Therefore, response of *Vitis* scions to salinity can vary according to the comparative exclusion of sodium versus chloride by the genotype of the root system. Numerous factors, such as rootstock–scion combination, irrigation system, soil type and climate, controlled responses to salinity of grapevine.

On the other hand, the salinity affected soils in Tunisia covered about 1.5 million hectares, nearly 10% of the total area of the country. Soil and irrigation water salinity is increasing worryingly at the principal vineyard regions (Hachicha, 2007). Furthermore, climate aridity, unsuitable cultural practices and poor quality of irrigation water amplify the phenomenon of salinization. In the oasis of Tozeur, Tunisia, the salinity of irrigation water varies from 1 g/l for wells with a depth more than 150m to more than 7.5 g/l (Ben Maachia et al., 2014; CRDA Tozeur, 2020). Despite the arid climate and salty irrigation water, these oases are characterized by a very rich fruit genetic heritage. Grapevine, represented with very interesting varieties, is the subject of the present investigation. Our objective was to study salt (NaCl) tolerance of principal grape genotypes from Tozeur's oasis. In fact, the approach counted on the exploration of the variability of the grapevine's salt response aiming the identification of tolerant genotypes and therefore contributing to the improvement of the productivity as well as to better valorization of these grapes and the saline zones.

## MATERIALS AND METHODS

### Plant material and salt treatments conduct

The principal local genotypes in the oasis of Tozeur, Tunisia, were the subject of this study. Five local genotypes were chosen, with the names attributed by farmers in the region indicating their origin (Arbi and Sfaxi), the maturity season (Chetoui), the fruit shape (Guelb Sardouk) or the skin color (Kahla), with an introduced genotype (Muscat d'Italie). These grape genotypes were identified in two previous investigations carried out in the oasis of Tozeur on 2010 and 2012. These oasis grape genotypes showed important adaptation to the area conditions, particularly the high salt load of irrigation water (Ben Maachia et al., 2014; Habib et al., 2019, 2020).

The assay was carried out in the Regional Research Center of Oasian Agriculture, Deguech, Tunisia, under open air. The region of Deguech is characterized by high temperatures (Table 1).

The seasonal pattern of the region of Tozeur is much contrasted since the temperature of the hottest month (July) is above 32°C and that of the coldest month (January) is of the order of 1°C. The average of the minima of the coldest month is between 3°C and 5°C. The average of the maxima of the hottest month exceeds 40°C (Anonymous 6, 2010). In fact, from the plantation date on January to the end of the stress (July), the rain was registered before inducing the salt stress, only on January, February and Mars with respectively 1, 48 and 14 mm (Table 1).

**Table 1.** Monthly distribution of rainfall (mm) in the region of Tozeur (INM: Direction Tozeur)

Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
2015	1	48	14	0	0	0	0	17.5	19.5	0	0	0	100

**Table 2.** Physico-chemical analysis of the used sand (Source: Food quality laboratory, Tunisia)

pH	% MO	ECe (dS m <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup>	P <sub>2</sub> O <sub>5</sub> assim.	K <sub>2</sub> O exch.	Ca exch.	Mg exch.	Na exch.	C/N
8.86	0.08	0.17	0.20	36.57	81.17	4715.02	155.68	85.44	2.33

MO: organic matter; Ece: electric conductivity; N- org+ NO<sub>3</sub><sup>-</sup>: nitrate; P<sub>2</sub>O<sub>5</sub> assim. : assimilable phosphorus oxide; K<sub>2</sub>O exch.: exchangeable potassium oxide; Ca exch.: exchangeable calcium; Mg exch.: exchangeable Magnesium; Na: exchangeable sodium; C/N : Carbone to nitrogen ratio

**Table 3.** Physico-chemical analysis of the peat

pH	% MO	% DM	ECe (dS m <sup>-1</sup> )	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	S	Mg
6	90	25	0.4	210	150	270	150	100

MO: organic matter; DM: dry matter; ECe: electric conductivity; N: nitrogen; P<sub>2</sub>O<sub>5</sub>: phosphorus oxide; K<sub>2</sub>O: potassium oxide; S: sulfur; Mg: Magnesium

**Table 4.** Physico-chemical analysis of the irrigation water (tap water)

pH	Soluble cations					Soluble anions				
	ECe (dS m <sup>-1</sup> )	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	SAR
7.91	2.52	178.7	74.0	302.3	17.0	0.0	124.7	491.7	569.3	10.59

Ece: electric conductivity; Ca<sup>2+</sup>: calcium ion; Mg<sup>2+</sup>: Magnesium ion; Na<sup>+</sup>: sodium ion; K<sup>+</sup>: potassium ion; CO<sub>3</sub><sup>2-</sup>: Carbonate ion; HCO<sub>3</sub><sup>-</sup>: Bicarbonate ion; Cl<sup>-</sup>: chloride ion; SO<sub>4</sub><sup>2-</sup>: sulfate ion; SAR: Sodium adsorption ratio

The cuttings were taken from the field, uniformed on three buds cutting and planted in 3 kg plastic pots containing peat and sand (2:1; w:w). The physio-chemical compositions of the sand and peat used as growing medium are presented in the tables 2 and 3.

The used growing medium (sand + peat) was non-saline, well provided with organic matter and rich in nutrients (Table 2 and 3). The substrate was characterized by a quick warm up at low temperatures which resulted in an early start of root activity (Dhaouadi, 2017). The ratio C/N (Table 2) indicated a characteristic of fast mineralization of the sand (Dhaouadi, 2017).

The cuttings were planted on 1<sup>st</sup> January 2015. Ten replicates were considered for each genotype. The irrigation was applied twice a week with tap water (1.77 g/l of salt with CE=2.52 dS m<sup>-1</sup>) before the beginning of the stress. The physio-chemical composition of the tap water used for the irrigation is presented in the table 4.

Salinity was applied at the end of April 2015, four months after the plantation, once the cuttings achieved a homogenous stage (6-7 leaves). Two salt treatments were applied:

- a gradual salt stress by adding progressively 25 mM NaCl every week until achieving the final doses 75,100 and 150 mM NaCl,
- and a salt shock: the salt doses 50 and 100 mM NaCl were suddenly applied from the beginning of the stress.
- a control treatment: plantlets were irrigated with tap water (28 mM NaCl).

The plantlets were irrigated twice a week, first with salt treatment and secondly with a nutritive solution containing 13% of nitrogen, 40% of P<sub>2</sub>O<sub>5</sub>, 13% K<sub>2</sub>O and 3% of MgO; the nutritive solution was added as a complement since the used growing medium (sand and peat) were well provided with nutrients (Table 2 and 3). A leaching was applied with tap water (ECe=2.52 dS m<sup>-1</sup>) to avoid salt accumulation in the substrate.

### Measured parameters

Regular observations were made to control the morphology of the plants during the salt stress. In fact, the toxicity symptoms appearance on leaves, plantlets death and variation of shoot length and leaves number were mainly assessed during the treatment.

At the end of the salt treatment, plants were taken from pots and washed with distilled water. To evaluate salt stress impact on plant growth, the leaves number was determined, and the length of root and shoot systems were taken. These measures were triplicate. The shoot elongation was measured as described by Shahid et al. (2011) using the following formula:

$$\text{Shoot elongation} = \text{Plant height at the time of harvesting} - \text{Shoot length before salt application} \quad (1)$$

Stress tolerance index (T.I) is a useful tool for determining the high yield and stress tolerance potential of genotypes (Iqbal & Rahmati, 1992). This index was used to evaluate the shoot length and leaves number variations during salt stress.

$$\% \text{T.I.} = \frac{\text{Parameter mean in salt solution}}{\text{Parameter mean in control condition}} \times 100 \quad (2)$$

$$\text{Shoot length stress tolerance index (\%SLSTI)} = \frac{\text{Shoot length in salt solution}}{\text{Shoot length in control condition}} \times 100 \quad (3)$$

$$\text{Leaves number stress tolerance index (\%LNSTI)} = \frac{\text{Leaves number in salt solution}}{\text{Leaves number in control condition}} \times 100 \quad (4)$$

## Statistics

In this work, a complete random device based on the salinity effect was adopted using statistical software STATISTICA. The values of each parameter for the different treatments were compared using analysis of variance (ANOVA) with repeated measures. The Newman-Keul test was used to test the significance of the differences between the data. Means were tested by Newman-Keul's with a significance level of  $P=0.05$ .

## RESULTS

### Plant death

The first cutting death was registered three weeks from the beginning of the gradual salt stress by local genotype Sfaxi (Fig. 1.A). When all salt doses were reached, on the 6<sup>th</sup> week, the death of the local genotypes Guelb Sardouk and Kahla cuttings increased with the increment of salinity from 100 mM to 150 mM NaCl. The local genotype Arbi revealed relatively low death percentage at the highest salt dose (Fig. 1.A). After six weeks of salt stress, the doses 75 and 100 mM NaCl induced the death 40 % and 30 % of the local genotype Sfaxi cuttings, respectively. The genotype Chetoui registered also the death of its cuttings under salt stress of 75 and 100 mM NaCl. The introduced genotype registered the death of its cuttings under control conditions and at high salt dose with low percentage (Fig. 1.A). At the end of the treatment, the gradual salt stress induced the death of low percentage from the local genotype Arbi cuttings. Yet, for the other studied genotypes, the impact of gradual salt stress on their growth was more aggravated at the end of the treatment leading to high death percentages.

Yet, the salt shock caused the death of the genotypes Guelb Sardouk, Kahla and Muscat d'Italie cuttings since the 3<sup>rd</sup> week of the treatment application (Fig. 1.B). After 6 weeks of salt shock, the death rate was aggravated for the genotypes Kahla and Muscat d'Italie while only the shock of 100 mM NaCl induced the death of Chetoui and Sfaxi cuttings (Fig. 1.B). At the end of the salt shock, the death rate was aggravated for the studied grapevine genotypes; the local genotype Arbi revealed the death of its cuttings only under salt shock of 100 mM NaCl with low percentage (Fig. 1.B).

### Variations in shoot length and leaves number

In order to investigate salinity effects on growth parameters, our grape cuttings were monitored during the salt treatment. At control conditions, shoot length increased significantly along the observations for the genotype Muscat d'Italie cuttings; an increase was noted for the local genotypes Arbi and Guelb Sardouk at the end of the experiment (Fig. 2). Indeed, statistically significant rise of leaves number was registered progressively to the end of the treatment, compared to the number registered before the stress onset, for the local grapevines Chetoui, Guelb Sardouk and Sfaxi and after the 6<sup>th</sup> week for Arbi and the introduced grape (Fig. 3). Yet, Kahla, local vine, registered a decline of leaves number at the final samples (Fig. 3).

### Gradual salt stress

During a gradual stress of 75 mM NaCl, the shoot length and leaves number were not significantly affected by salinity for the genotypes Arbi and Muscat d'Italie cuttings (Fig. 2 and 3). However, the shoot length increased significantly at  $p \leq 0.05$ , compared to leaf number registered before the stress onset, for the local genotypes Guelb Sardouk and Kahla cuttings at the end of 75 mM NaCl gradual stress (Fig. 2).

The leaves number decreased significantly for the local genotypes Chetoui, Kahla and Sfaxi cuttings after eight weeks of exposure to 75 mM NaCl stress; an increase of leaves

number was observed for the local genotype Sfaxi at the 3<sup>rd</sup> week of the stress (Fig. 3). Only the local genotype Guelb Sardouk revealed significant increase of leaves number during gradual stress of 75 mM NaCl, compared to leaf number registered before the stress onset (Fig. 3).

Otherwise, long term exposure to 100 mM NaCl added gradually (G.S) caused the decline of shoot length and leaves number for the genotypes Kahla and Muscat d'Italie (Fig. 2 and 3). Indeed, shoot length of the genotype Sfaxi decreased at the end gradual stress of 100 mM NaCl (Fig. 2). Despite the increase at the 6<sup>th</sup> week, decreases of leaves number were registered for the local genotypes Sfaxi and Guelb Sardouk cuttings at the end of gradual stress of 100 mM NaCl (Fig. 3). While, the local genotypes Arbi and Chetoui revealed unaffected shoot length and leaves (Fig. 2 and 3).

The dose 150 mM NaCl generated drastic effects on shoot length and leaves number. In fact, the shoot length decreased after 8 weeks of exposure to 150 mM NaCl gradually added for the genotypes Kahla, Muscat d'Italie and Sfaxi cuttings (Fig. 2). The damage of 150 mM NaCl on shoot length was aggravated along the stress for the local genotype Chetoui cuttings (Fig. 2). Indeed, the leaves number decreased after the 6<sup>th</sup> week of exposure to 150 mM NaCl gradually added for the studied grapevine genotypes to cause the total fall of leaves, except for the local genotype Arbi; for the genotype Kahla, the leaves number decreased from the 6<sup>th</sup> week (Fig. 3).

### Salt shock

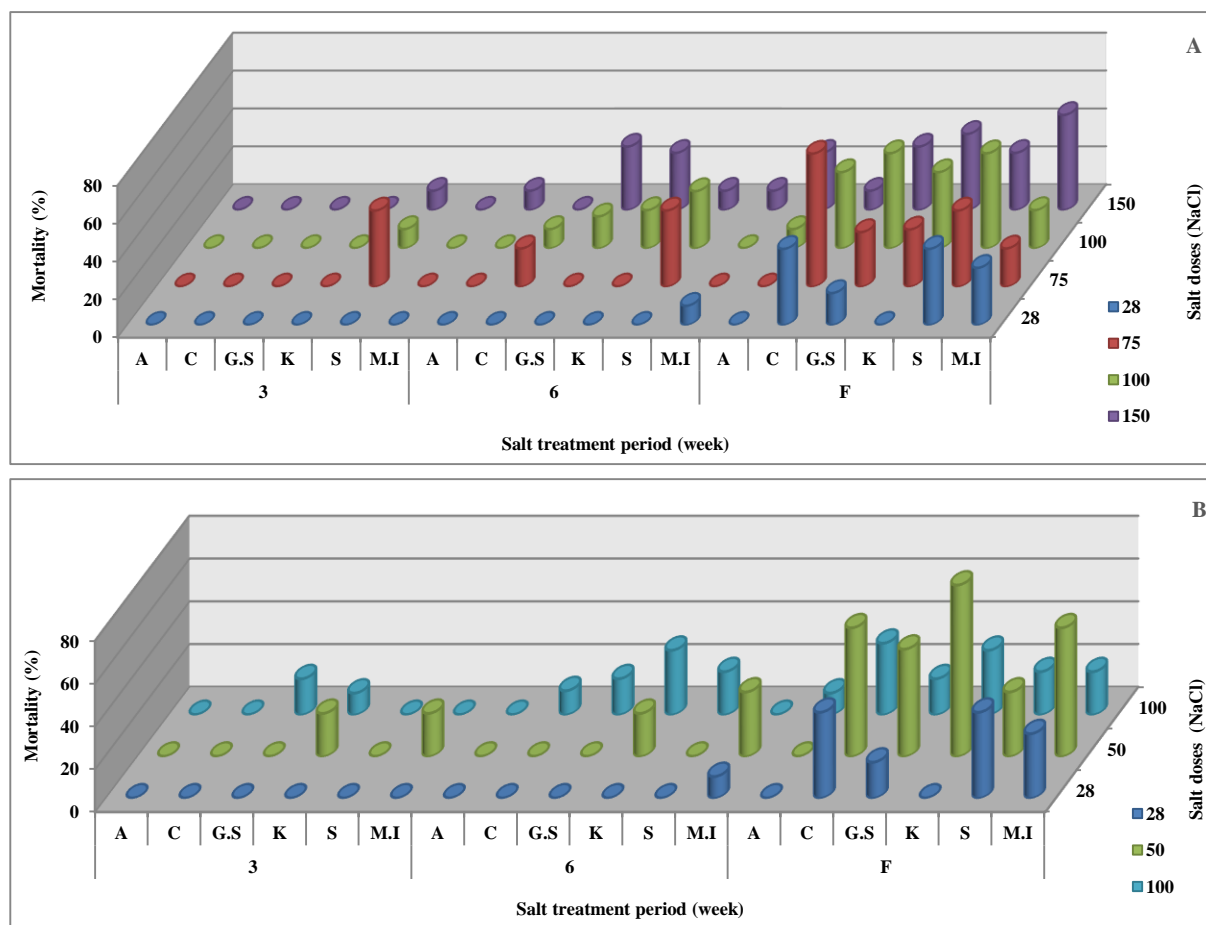
Under a shock of 50 mM NaCl, the shoot length was significantly developed at the end of the stress for the genotype Arbi while it decreased at the end of the stress for the genotype Guelb Sardouk (Fig. 4). The shock of 50 mM NaCl caused a significant decline of shoot length and leaves number from the 6<sup>th</sup> week of the stress for the local genotype Kahla (Fig. 4 and 5). However, the leaves number increased significantly from the 6<sup>th</sup> week of 50 mM NaCl shock for local genotype Arbi cuttings and after eight weeks of exposure to 50 mM NaCl for the genotypes Sfaxi and Muscat d'Italie cuttings (Fig. 5). Despite the decrease of leaves number observed at the end of shock of 50 mM NaCl, the leaves number for the local genotypes Chetoui and Guelb Sardouk were maintained higher than the number registered before the stress application (Fig. 5). Besides, the shock of 100 mM NaCl led to significant decline of shoot length for the local genotypes Guelb Sardouk and Kahla, respectively from the 3<sup>rd</sup> and the 6<sup>th</sup> week. Yet, the local genotype Arbi revealed a significant increase of shoot length from the 6<sup>th</sup> week of 100 mM NaCl shock (Fig. 4).

Moreover, the local genotype Arbi cuttings showed a significant increase of leaves number from the 6<sup>th</sup> week (Fig. 5). While, for the local genotypes Chetoui and Guelb Sardouk, the leaves number decreased after 8 weeks of 100 mM NaCl shock even with the increases observed respectively, at the 3<sup>rd</sup> and the 6<sup>th</sup> week of the stress. The local genotype Kahla leaves number declined after six weeks of exposure to 100 mM NaCl shock (Fig. 5).

### Shoot elongation

After long term exposure to different salinities gradually applied, the local genotype Arbi revealed the greatest shoot growth; the shoot elongation of the local genotype Arbi cuttings decreased upon the high salt dose (150 mM NaCl) (Fig. 6). This dose affected drastically the shoot elongation of our grape genotypes. In fact, the genotypes Sfaxi and Muscat d'Italie showed the most negatively affected shoot elongation by gradual salt stress (Fig. 6).

On the other hand, after long term exposure to salt shock, the local genotype Arbi cuttings revealed the highest shoot elongation, while the genotypes Kahla, Guelb Sardouk and Muscat d'Italie revealed the most damaged shoot elongation by salt shock (Fig. 6).



**Fig. 1.** The effects of salinity on the studied grapevine genotypes mortality at the 3<sup>rd</sup> week (3), the 6<sup>th</sup> week (6) and at the end of the treatment (F). The genotypes are A: Arbi, C: Chetoui, G.S: Guelb Sardouk, K: Kahla, S: Sfaxi, M.I: Muscat d'Italie. A: variations of mortality in response to gradual salt stress (75, 100 and 150 mM NaCl). B: changes in mortality in response to salt shock stress (50 and 100 mM NaCl); 28 mM NaCl was the control dose.

## Leaves number stress tolerance index (LNSTI)

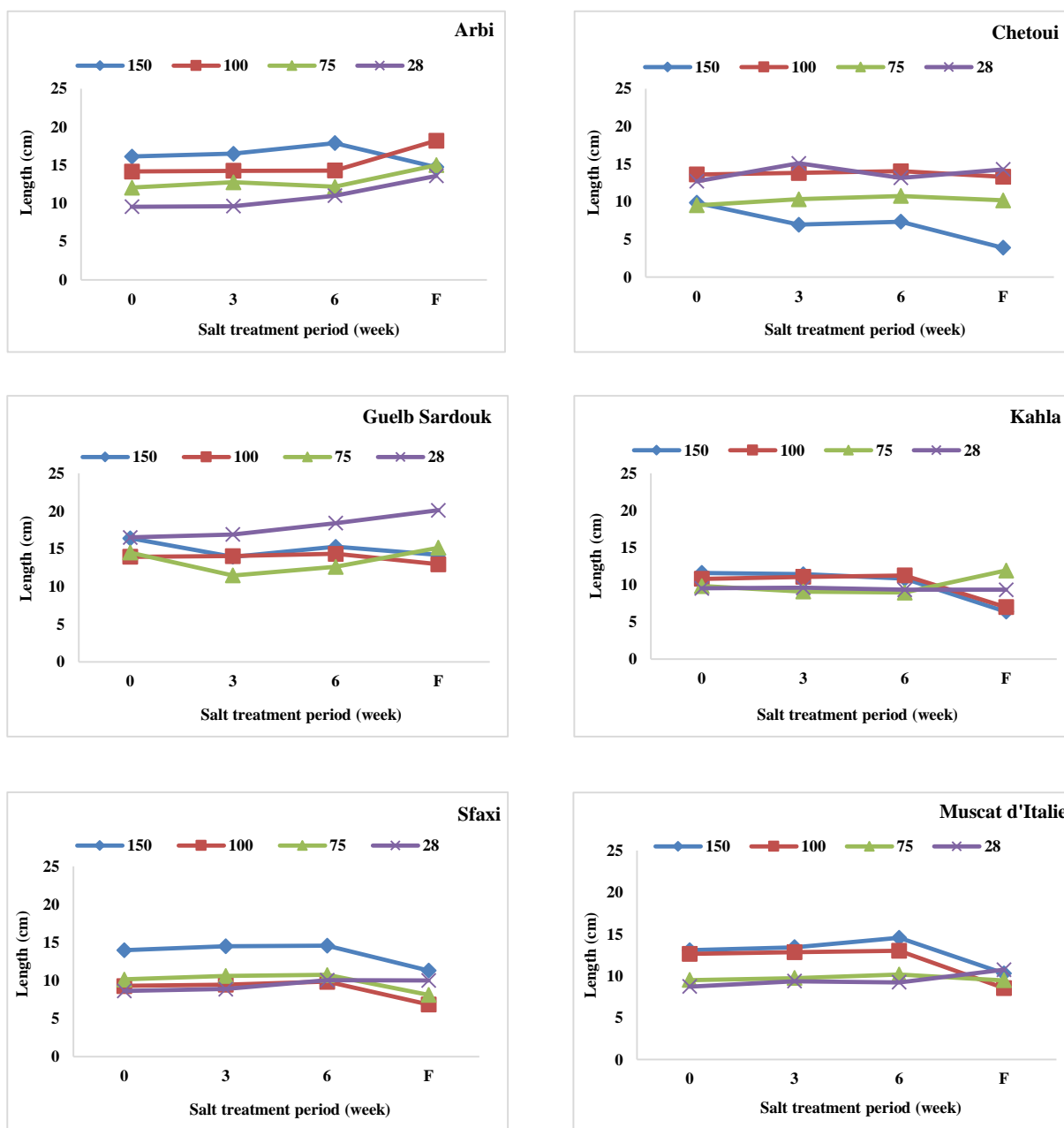
### Gradual salt stress

The leaves number stress tolerance index (LNSTI) decreased with the increment of salinity from 75 to 150 mM NaCl for the local genotypes Chetoui, Guelb Sardouk and Sfaxi during the gradual salt stress. Yet, for the other genotypes the influence of salt stress on LNSTI was relevant due to the long exposure to salinity. In fact, after eight week of gradual salt stress LNSTI decreased; the reduction was correlated with the increase of salinity. The LNSTI became null after 8 weeks of exposure to 150 mM NaCl added gradually, except for the local genotype Arbi which showed the least affected LNSTI during the gradual salt stress (Fig. 7).

### Salt stress shock

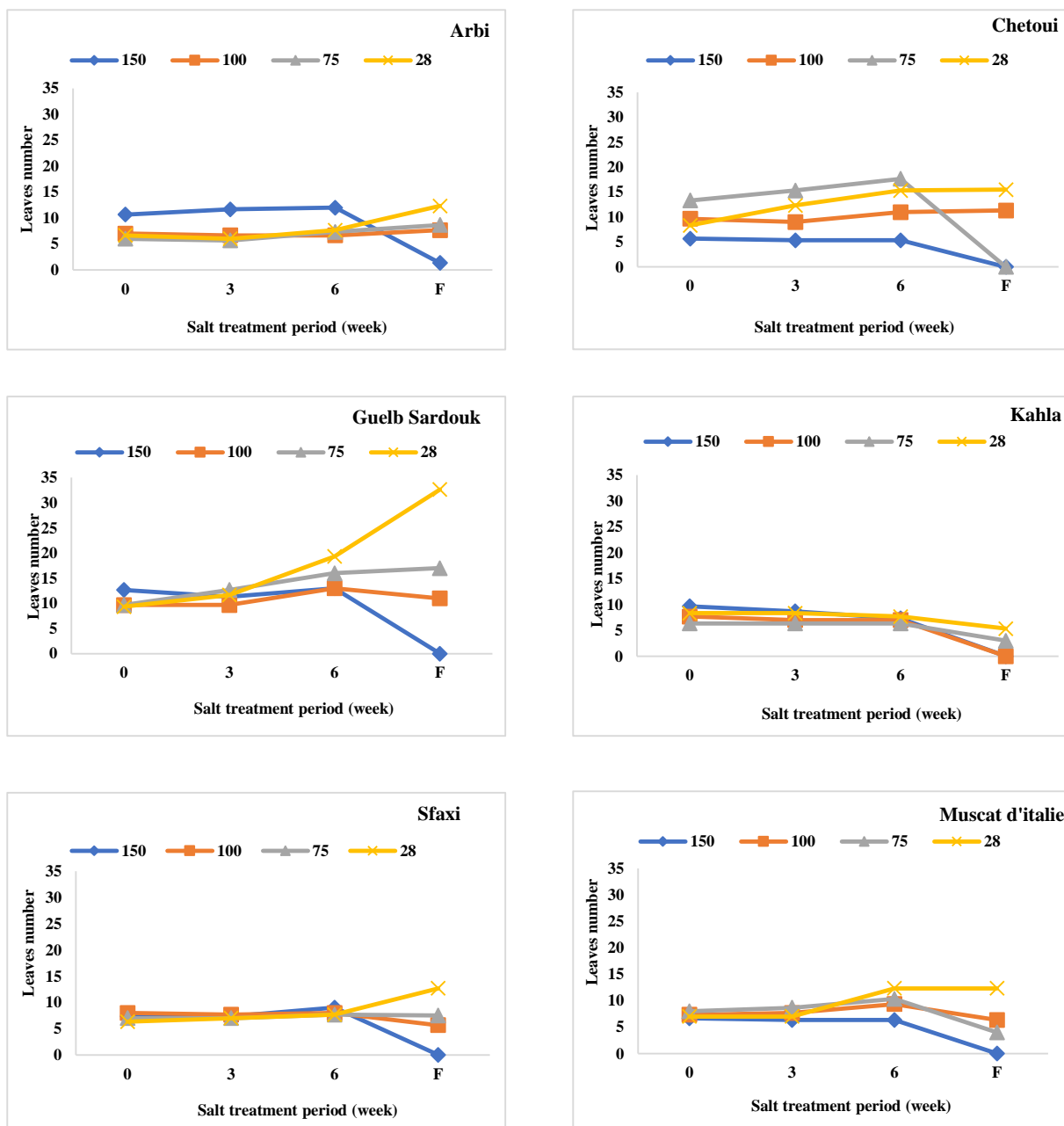
After three weeks of salt shock, the leaves number stress tolerance index (LNSTI) decreased with increasing the shock from 50 to 100 mM NaCl for the genotypes Kahla, Sfaxi and Muscat d'Italie (Fig. 8). However, at the 6<sup>th</sup> week of salt shock, the genotypes Arbi, Guelb Sardouk and Muscat d'Italie showed an increase of LNSTI under 50 mM NaCl (Fig. 8). Indeed, the genotypes Chetoui and Kahla showed higher LNSTI under 100 mM NaCl than that registered under 50 mM NaCl after 8 weeks exposure to salt shock (Figure 8). For the

other studied genotypes, the long-term exposure to salt shock decreased the leaves number stress tolerance index with increasing the salinity (Fig. 8). For the genotypes Arbi and Muscat d'Italie, the LNSTI was maintained higher till the end of 50 mM NaCl shock (Fig. 8).

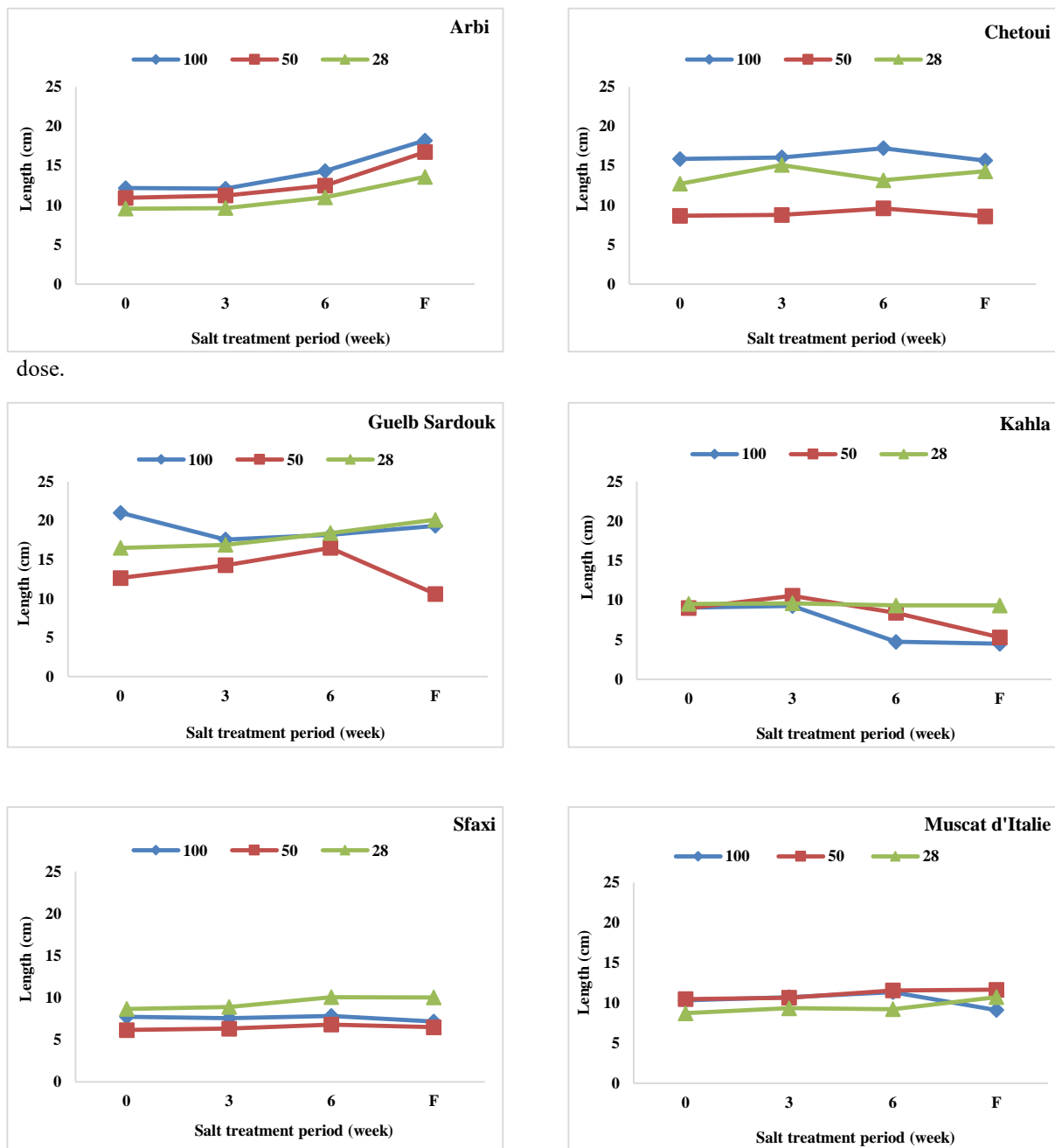


**Fig. 2.** Variations in shoot length during gradual salt stress for the studied grapevine genotypes. The figures present shoot length before salt application (0), at the 3rd week (3), the 6th week (6) and at the end of the treatment (F). 75, 100 and 150 mM NaCl were the applied salt doses of the gradual stress; 28 mM NaCl was the control dose.

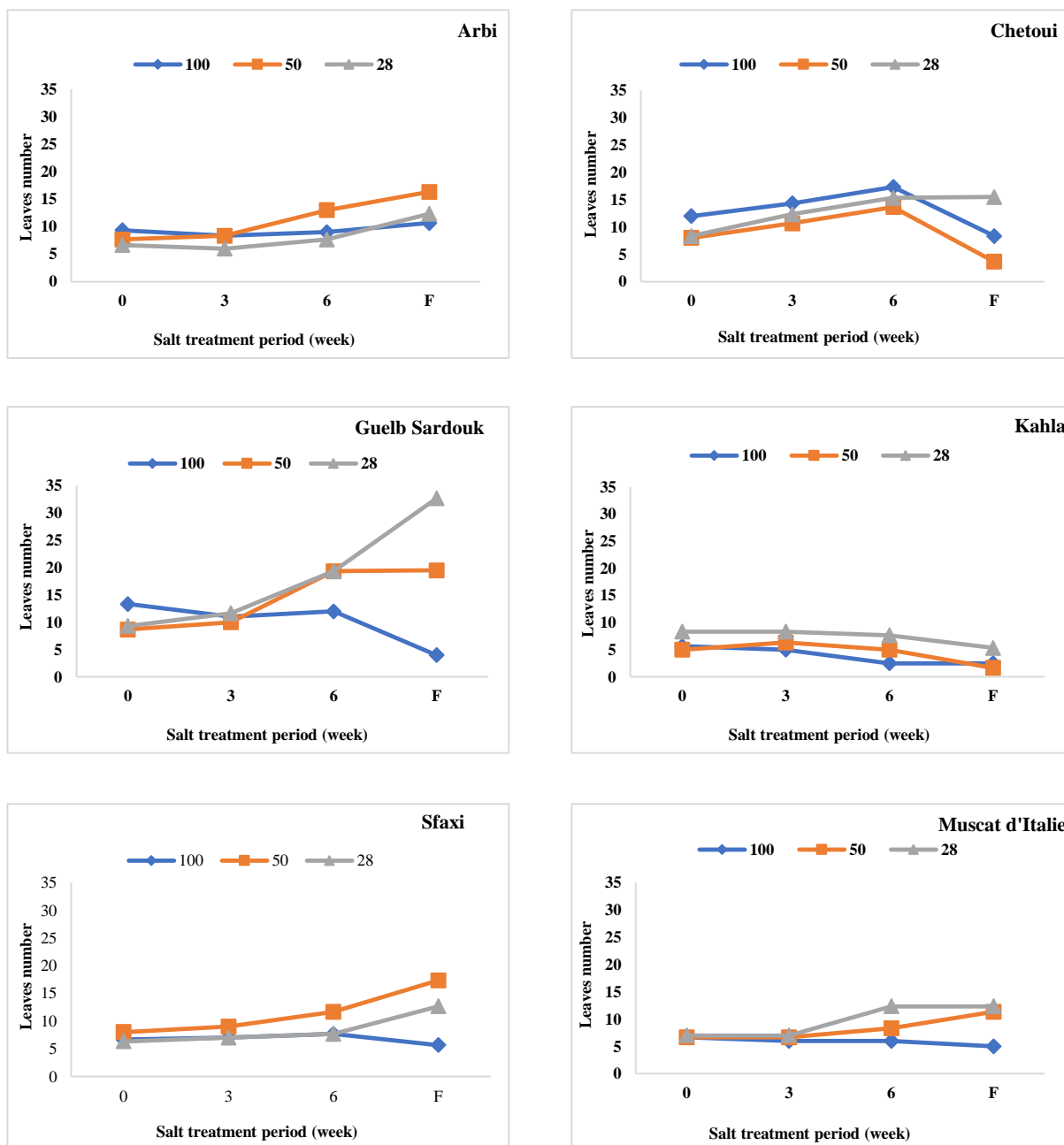




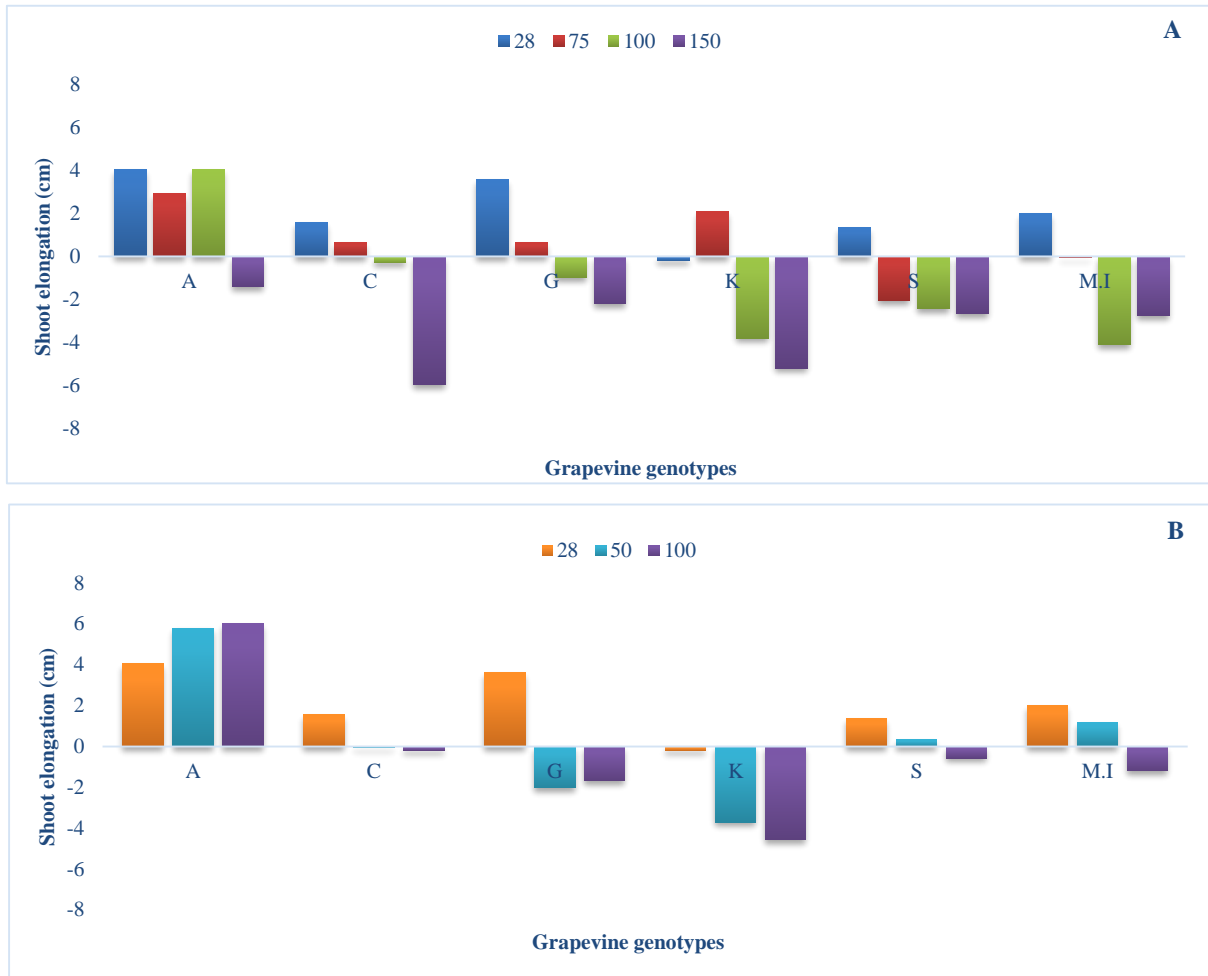
**Fig. 3.** Variations in leaves number during gradual salt stress for the studied grapevine genotypes. The figures present leaves number before salt application (0), at the 3<sup>rd</sup> week (3), the 6<sup>th</sup> week (6) and at the end of the treatment (F). 75, 100 and 150 mM NaCl were the applied salt doses of the gradual stress; 28 mM NaCl was the control dose.



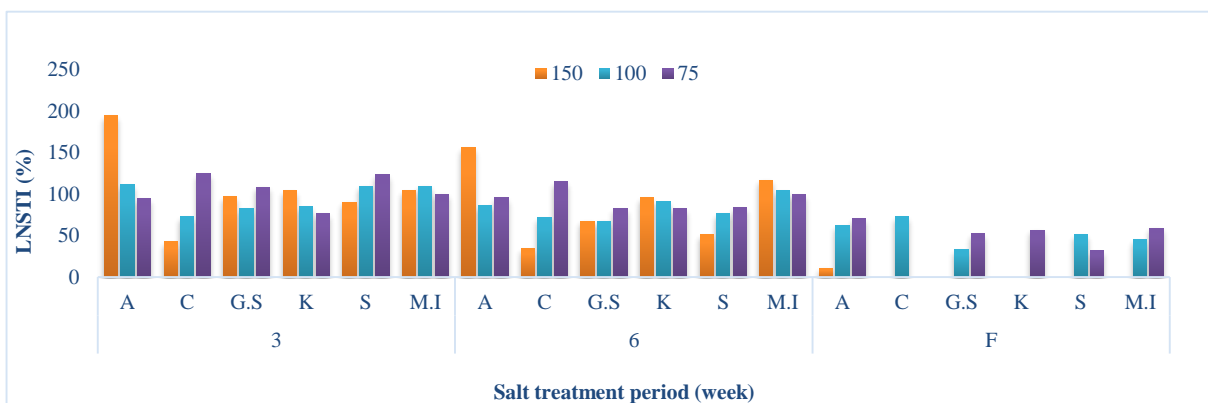
**Fig. 4.** Variations in shoot length during salt shock stress for the studied grapevine genotypes. The figures present shoot length before salt application (0), at the 3<sup>rd</sup> week (3), the 6<sup>th</sup> week (6) and at the end of the treatment (F). 50 and 100 mM NaCl were the applied salt doses of the salt shock; 28 mM NaCl was the control dose.



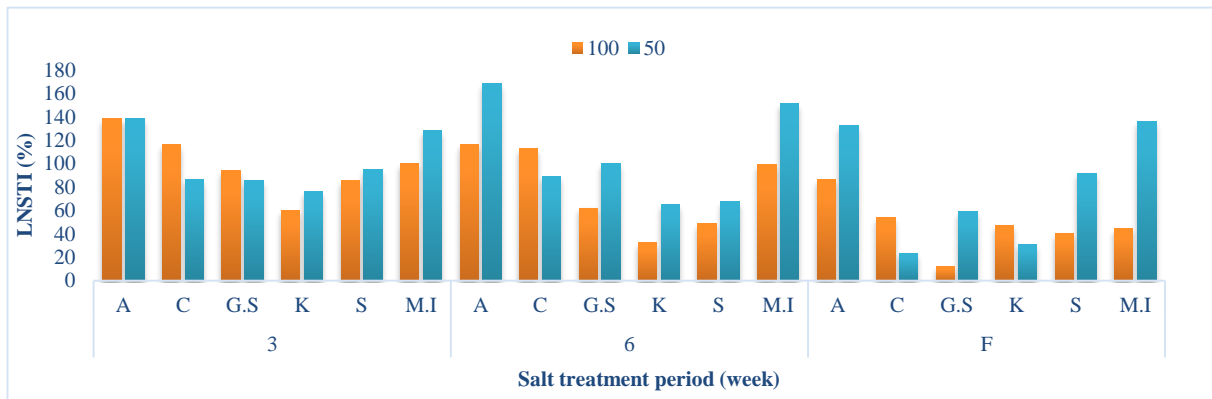
**Fig. 5.** Variations in leaves number during salt shock stress for the studied grapevine genotypes. The figures present leaves number before salt application (0), at the 3<sup>rd</sup> week (3), the 6<sup>th</sup> week (6) and at the end of the treatment (F). 50 and 100 mM NaCl were the applied salt doses of the salt shock; 28 mM NaCl was the control dose.



**Fig. 6.** The effects of salinity on shoot elongation for the studied grapevine genotypes, A: Arbi, C: Chetoui, G.S: Guelb Sardouk, K: Kahla, S: Sfaxi, M.I: Muscat d'Italie. A: changes in shoot elongation during gradual saline stress (75, 100 and 150 mM NaCl). B: variations in the elongation of the shoot during a salt shock stress (50 and 100 mM NaCl); 28 mM NaCl was the control dose.



**Fig. 7.** Leaves number stress tolerance index of the studied grapevine genotypes during gradual salt stress. The capital letters present the genotypes A: Arbi, C: Chetoui, G.S: Guelb Sardouk, K: Kahla, S: Sfaxi, M.I: Muscat d'Italie. 75, 100 and 150 mM NaCl were the applied salt doses of the gradual stress



**Fig. 8.** Leaves number stress tolerance index of the studied grapevine genotypes during salt shock. The capital letters present the genotypes A: Arbi, C: Chetoui, G.S: Guelb Sardouk, K: Kahla, S: Sfaxi, M.I: Muscat d'Italie. 50 and 100 mM NaCl were the applied salt doses of the gradual stress.

## DISCUSSION

Salinity actions on grapevines were manifested by the reduction of shoot, bunch and root number, decreased vigor, necrosis of leaf margin, adult leaves' premature senescence due to ionic stress thus leaf number decline and the death of the vine (Fisarakis et al., 2001; López-Aguilar et al., 2003; Ha et al., 2008). In fact, in the present study, the first cutting death was registered from the 3<sup>rd</sup> week of salt stresses application only for the genotype Sfaxi under gradual salt stress while under salt shock, it was registered for Guelb Sardouk, Kahla and Muscat d'Italie. The lowest death rate was registered by the genotype Arbi under both salt stress conditions. Yet, for the other studied genotypes, the death rate increased during the long-term exposure to both salt treatments (gradual and shock). Besides, the decrease of vegetative growth expressed either by the reduction in leaves' number of or in leaf area was considered as the first responses of glycophytes to salty stress (Munns, 2005). Some authors suggested that the adverse effects of salts occurred at the whole plant and were more noticeable on the leaves (Murkute et al., 2005; Rochdi et al., 2005; El-Hendawy et al., 2005). The depressive action of salt is also manifested by the reduction of the height of the plants (Shani & Ben-Gal, 2005; Chookhampaeng et al., 2011). In fact, growth decline depends essentially on the duration of treatment and the water salinity (El-Hendawy et al., 2005; Rochdi et al., 2005) which was confirmed by our results.

The drastic effects of salty water irrigation were more relevant after long term exposure to high salinity. The damages of gradual stress of 75 mM NaCl were expressed only by leaves number reduction at the end of the stress for the genotypes Chetoui, Kahla and Sfaxi. The damages of gradual salt stress were aggravated with increasing salinity with reductions of plant height upon 100 mM NaCl for the genotypes Kahla, Sfaxi and Muscat d'Italie. Moreover, the gradual stress of 100 mM NaCl decreased the leaves number for the genotypes Guelb Sardouk, Kahla, Sfaxi and Muscat d'Italie. These results agreed with leaves number reduction shown on four rootstocks (1103 Paulsen, 110 Richter, 140 Ruggeri and SO4) after long term exposure to 100 mM NaCl added progressively (Hanana et al., 2015). Going to 150 mM NaCl, the decline of shoot length was more relevant for Guelb Sardouk, Kahla, Sfaxi and Muscat d'Italie associated with leaves senescence for the studied genotypes, except for Arbi.

Otherwise, the adverse effects of long-term salt shock (50 and 100 mM NaCl) were expressed by shoot growth and leaves number reduction for the local genotype Kahla cuttings

since the 6<sup>th</sup> week of stress application. The shoot length decreased also for the local genotype Guelb Sardouk cuttings at the end of 50 mM NaCl shock. For the genotypes Chetoui and Guelb Sardouk, foliar damages were not pertinent; their leaves number were maintained higher than the number registered before the stress application. Going to 100 mM NaCl, the drastic effect of the salt shock was manifested by shoot length and leaves number reduction for Guelb Sardouk. After 8 weeks exposure to 100 mM NaCl shock, the leaves number decreased also for the genotypes Chetoui and Muscat d'Italie. Our results corroborated with several studies on salt stress. The plant growth reduction expressed by plant height and leaves number decreases observed for our grape genotypes were also registered by two grapes Seedless Red and Ghezel Uzum upon salt stress of 50, 100 and 150 mM NaCl (Karimi & Yusef-Zadeh, 2013). Indeed, shoot length and leaves number were significantly reduced at all salinity levels (5, 25, 50 and 100 mM of NaCl) by one-year-old Sultana (*Vitis vinifera* L.) vines, own-rooted and grafted on 41B, 110R, 140Ru, 1103P and SO4 (Fisarakis et al., 2001). The decrease of leaves number after long term exposure to the low salinities (75 and 50 mM NaCl) of both gradual and shock stress could be confirmed by foliar damages registered by *clementine Hernandina* after 16 weeks of 35 and 70 mM NaCl stress (Askri et al., 2017). Long term exposure to 100 mM NaCl induced also leaves number decline for five grapes, Superior Seedless, Syrah, Muscat d'Italie, Razegui, and Asli (Hanana et al., 2014).

In fact, growth reduction, most common effect of abiotic stress on plant physiology, was considered as salt adaptation mechanism involved by plants to avoid salinity damages (Zhu, 2002, Ben Mahioul et al., 2009). Growth reduction, adaptive capacity necessary for the survival of a plant exposed to abiotic stress, allowed the plant to accumulate energy and resources to combat stress before the increase of the imbalance between the inside and the outside of the organ to a threshold where the damage won't be irreversible. Thus, the growth is inversely correlated with salt stress resistance of a species or genotype (Zhu, 2002).

Otherwise, 8 weeks exposure to 75 mM NaCl increased the leaves number for local genotype Guelb Sardouk while the genotypes Arbi and Muscat d'Italie kept their vegetative apparatus intact. Indeed, the local genotypes Arbi and Chetoui revealed unaffected vegetative apparatus under gradual stress of 100 mM NaCl. The growth parameters weren't also significantly affected by long term exposure to salt shock of 100 mM NaCl for local genotype Sfaxi. These responses were shown in previous studies by tolerant varieties. The tolerant genotype Cardinal didn't show significant reduction of leaves number after 75 days of 100 mM NaCl stress (Hanana et al., 2014). Also, for the wild grapevine 'Séjnène' the total leaves number per plant didn't vary significantly after long term exposure to gradual stress of 100 mM NaCl (Hamrouni et al., 2011). Hamrouni et al. (2011) suggested that the wild grapevine 'Séjnène' resisted salinity by keeping its vegetative apparatus almost intact, which preserves its photosynthetic capacity.

Otherwise, our grapevine genotypes maintained their vegetative growth under low salinities. In fact, the leaves number increased after 8 weeks exposure to 75 mM NaCl for local genotype Guelb Sardouk; the dose 75 mM NaCl gradually added induced an increase of shoot length for the local genotypes Guelb Sardouk and Kahla. The genotypes Arbi, Sfaxi and Muscat d'Italie showed increases of leaves number under 50 mM NaCl shock. Moreover, the local genotype Arbi revealed developed plant height and leaves since the 6<sup>th</sup> week of salt shock application.

## CONCLUSION

According to our data, we could also suggest the local genotype Arbi as the tolerant genotype among the tested grapevine genotypes. In fact, the local grape genotype Arbi showed two

mechanism to tolerate salinity, a resistance capacity to 75 and 100 mM NaCl ( $EC_e = 6.26$  and  $8.34 \text{ dS m}^{-1}$ ), by maintaining its vegetative apparatus almost intact and, as response to salt shock, preserving the vegetative growth. Moreover, cuttings death was registered at the end of the salt treatments upon the high salt doses with low percentages. Indeed, the genotype Arbi cuttings showed the higher leaves number stress tolerance index. The negative influence of the high salt dose 150 mM NaCl on the local genotype Arbi cuttings was pertinent at the end of the treatment.

The local genotype Sfaxi showed tolerance capacity to salt shock by preserving its vegetative development (50 mM NaCl or  $EC_e = 4.17 \text{ dS m}^{-1}$ ) or by keeping its vegetative apparatus almost unaffected (100 mM NaCl).

The other grapevine genotypes involved the capacity of growth reduction to avoid the drastic effects of salinity; this capacity was more relevant at 100 mM NaCl of both gradual and salt shock particularly for Chetoui, Guelb Sardouk, Kahla and the introduced grapevine Muscat d'Italie. Furthermore, we could classify the local genotype Kahla as the most salt sensitive genotype especially to the salt shock; the salt damages on the vegetative apparatus were noticed since the 6<sup>th</sup> week of stress.

To conclude, we could suggest the high salt dose 150 mM NaCl ( $EC_e 12.5 \text{ dS m}^{-1}$ ) as the tolerance's threshold for the tested genotypes. It induced severe decline of leaves number and shoot length till the total fall of leaves. Therefore, compared to the electrical conductivity threshold ( $EC_e$  of  $1.5 \text{ dS m}^{-1}$ ) suggested by Maas and Hoffman (1977), the principal grape genotypes, from the oasis of Tozeur, revealed tolerance capacity to high salinity. These capacities could be due to the particularity of the microclimate characterizing Tozeur's oases. Therefore, a more thorough study was needed to better evaluate the idea of using these genotypes as rootstocks.

### Conflict of interest

The authors have no conflict of interest.

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