



Analysis of salinity tolerance in two varieties of *Medicago sativa* at the vegetative stage.

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Abstract

In this study, we analyzed salt tolerance in two varieties of *Medicago sativa* at the vegetative stage. Gabès2355 and Californian varieties of *M. sativa* were cultivated under control treatment and under a range of salt concentrations (50 mM, 100 mM, 150 mM, and 200 mM NaCl) in a greenhouse. At harvest, ten quantitative characters related to aerial and root growth were measured for plants of both varieties. Results from ANOVA showed that the variation of the traits measured is explained by the effects of variety, treatment and the interaction of variety x treatment. The treatment factor represents the most pronounced effect. While the Californian variety was more sensitive than the Gabès variety under 50 mM NaCl, it was the most tolerant under higher concentrations of NaCl. Specific correlations between measured traits were noted under control treatment and salt stress. Further work is needed to evaluate the agronomic performances of both varieties in the field under salinity constraint.

1. INTRODUCTION

The United Nations predicts that the world population will be above 9.7 billion by 2050. This implies that the yield of crop plants of economic interest should significantly increase to meet this population growth. However, plants are subject to several environmental constraints which are exacerbated by climate change. Among these constraints, salinity and drought are the main factors limiting productivity of forage crops in the world, particularly in Africa.

Cultivated alfalfa (*Medicago sativa* L.), regarded as the “queen of forage plants”, is one of the forage species most widely used for animal feed. It is an allogamous perennial legume. The *Medicago sativa* complex includes both diploid

($2n = 2x = 16$) and tetraploid ($2n = 2x = 32$) subspecies (Yu et al., 2017). *M. sativa* is cultivated for its high protein content (ranging from 15 to 25%) and its ability to improve soil fertility and structure through biological nitrogen fixation and prevention of soil erosion (Cullen and Hill, 2006). It is also cultivated as an industrial source of protein and carotene, and used in dietetics (Gawel et al., 2017).

Despite the chronic shortage of fodder, especially during dry periods of the year, for livestock feed in the majority of African countries, alfalfa is cultivated in only a few Northern and Southern African countries. In Tunisia, alfalfa is cultivated over an area of approximately 13,000 ha, 77% of which is found in the oases in the South of the country

(Basigalup et al., 2014) while South Africa is the main alfalfa producing country in the South of the continent, with around 300,000 ha under irrigated cultivation (Mauriès, 2003).

Selection of new varieties of *M. sativa* tolerant to salinity could be one of the main solutions to promote its cultivation in marginal areas. In this context, morpho-physiological characterization of varieties under this constraint is a necessary prerequisite for any program aimed at improving agronomic performance in this species.

The present work aims to compare and explore the diversity of responses to salt stress among the local variety Gabès2355 and a Californian variety of *M. sativa*.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Two varieties of *M. sativa* were used. they include the local variety Gabès2355 and the Californian variety kindly provided by Mr. Mohamed Abdelhak Khorchani from the Tunisian Grain Company (COTUGRAIN). The seeds of both varieties were sown in 5 liter pots (diameter of 22.8 cm and height of 18 cm) filled with a mixture of soil and compost (2: 1). Each pot contained two plants. At the 6th leaf stage, the plants were divided into five lots. A first batch was cultivated under control treatment (0 mM NaCl) and the other 4 batches were irrigated with different salt concentrations of 50 mM, 100 mM, 150 mM, and 200 mM NaCl. Twenty (20) replicates per variety and treatment were used, giving 200 plants.

The plants of each variety were organized into 10 blocks. The culture system used was a split plot with total randomization within each block.

2.2. Measured characters

Ten characters were measured for the two studied varieties at harvest. They include the

number of axes (NA), length of stems (LS), number of internodes (NIN), number of leaves (NL), aerial fresh weight (AFW), aerial dry weight (ADW), length of roots (LR), root fresh weight (RFW), root dry weight (RDW), root/shoot ratio (RDW/ADW).

The dry matter was estimated after drying out the aerial and root parts in a Memmert UN55 oven (Memmert GmbH + Co. KG, info@memmert.com) at 60°C for 48 hours.

2.3. Data analysis

The data obtained were subjected to a two-way analysis of variance (variety and treatment). Only the characters which showed a significant variety x treatment interaction were retained for further statistical analyzes. The comparison of means was made using Duncan's test at the 5% level. The correlations between the measured parameters were carried out by estimating the Pearson correlation coefficient. All these analyzes were performed using SPSS software (version 20.0 SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Our results showed that the variability of the traits measured is explained by the effects of variety, treatment and the interaction of variety x treatment (Table 1). The treatment factor represents the most pronounced effect. Five of the ten traits measured showed a significant difference between the two varieties, while only three traits whose variation is explained by the interaction of variety x treatment were observed. Accordingly, numerous studies reported that the abiotic stress treatment was the main factor responsible of the variation of characters measured among lines of *M. truncatula* (Arraouadi et al., 2011; Hdira et al., 2021), *Medicago* species (Badri et al., 2016a,b), and *Hordeum marinum* (Saoudi et al., 2019).

The number of internodes (NIN) revealed significant differences among both varieties with

Table 1. Effects of variety, treatment and the interaction of variety x treatment on measured traits for the two varieties of *M. sativa*.

| | | NA | LS | NIN | NL | AFW | ADW | LR | RFW | RDW | RDW/ADW |
|---------------------|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| Variety | F | 2.33 | 2.83 | 42.61 | 2.06 | 2.39 | 0.86 | 13.51 | 11.27 | 15.75 | 39.97 |
| | P | 0.129 | 0.095 | 0.000 | 0.154 | 0.125 | 0.356 | 0.000 | 0.001 | 0.000 | 0.000 |
| Treatment | F | 4.71 | 48.55 | 25.91 | 56.85 | 32.23 | 32.96 | 4.37 | 18.92 | 21.54 | 11.43 |
| | P | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 |
| Variety x treatment | F | 1.41 | 1.96 | 4.06 | 0.54 | 2.45 | 2.19 | 0.70 | 4.98 | 0.33 | 0.47 |
| | P | 0.235 | 0.105 | 0.004 | 0.704 | 0.050 | 0.075 | 0.594 | 0.001 | 0.857 | 0.759 |

F: Coefficient of Snedecor-Fisher. Significant ($P \leq 0.05$). The number of axes (NA), length of stems (LS), number of internodes (NIN), number of leaves (NL), aerial fresh weight (AFW), aerial dry weight (ADW), length of roots (LR), root fresh weight (RFW), root dry weight (RDW), and the root/shoot ratio (RDW/ADW).

highest values noted for the Californian variety under control treatment and salinity constraint (Fig. 1). While there is no significant difference for NIN in each variety under control treatment and 50 mM NaCl, a significant decrease was noted under 100 mM, 150 mM and 200 mM NaCl. Each studied variety showed similar means of NIN under 150 mM and 200 mM (Fig. 1). In addition, there was no significant difference of NIN among the two varieties under these concentrations.

The decrease in NIN ranged from 2.21 to 22.06% and from 0.88 to 30.68% under salt stress for the Gabès and Californian varieties, respectively (Fig. 2.). Whereas a slightly higher decrease in NIN was found for Gabès under 50 mM and 100 mM NaCl, the highest reductions were registered for the Californian variety under 150 mM and 200 mM NaCl.

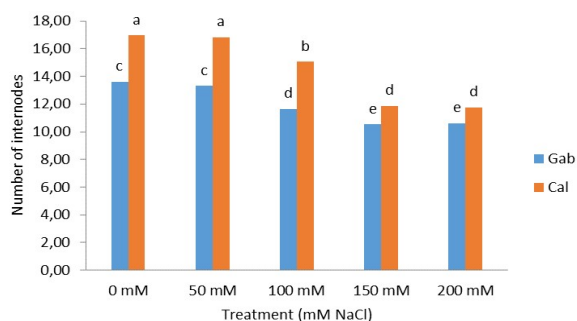


Fig. 1. Means of the number of internodes (NIN) measured for both varieties of *M. sativa* under control treatment and 50 mM, 100 mM, 150 mM and 200 mM NaCl. Means followed by the same or common letter(s) are not significantly different among studied varieties based on the Duncan test at 5%.

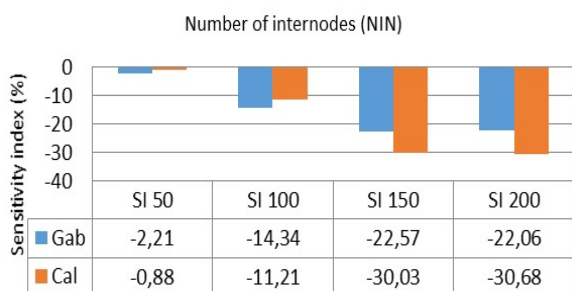


Fig. 2. Sensitivity index (SI) of the number of internodes (NIN) measured for both varieties of *M. sativa* under 50 mM, 100 mM, 150 mM and 200 mM NaCl.

Under control treatment, the highest value of aerial fresh weight (AFW) was observed for Gabès (Fig. 3.). There were significant differences in AFW values for Gabès under the different salt treatments while the Californian

variety showed similar behavior under 50 mM and 100 mM NaCl. Under salt stress (150 mM NaCl), the Californian variety exhibited the highest aerial vigor.

On the other hand, there is a decrease in AFW with the augmentation of salt stress level for both varieties (Fig. 4.). The percentage of reduction of AFW ranged from 18.99 to 73.84% and from 27.46 to 59.07% for Gabès and the Californian variety, respectively. While the Californian variety was more sensitive than Gabès under 50 mM NaCl, it was the most tolerant for the remaining concentrations of NaCl.

As mentioned below, there is a proportionally opposite relationship between aboveground biomass and salt concentration in the two varieties studied (Fig. 5.). Our results showed that the two concentrations (127.11 mM and 160.49 mM NaCl) induced a reduction of 50% of AFW for Gabès and the Californian variety, respectively. This result suggests that the Californian variety is more tolerant than Gabès to the high concentrations of salt.

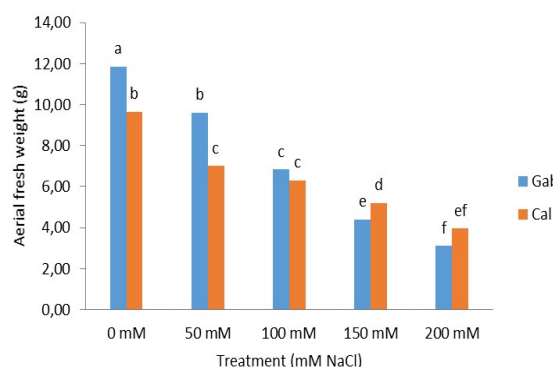


Fig. 3. Means of the aerial fresh weight (AFW) measured for both varieties of *M. sativa* under control treatment and 50 mM, 100 mM, 150 mM and 200 mM NaCl. Means followed by the same or common letter(s) are not significantly different among studied varieties based on the Duncan test at 5%.

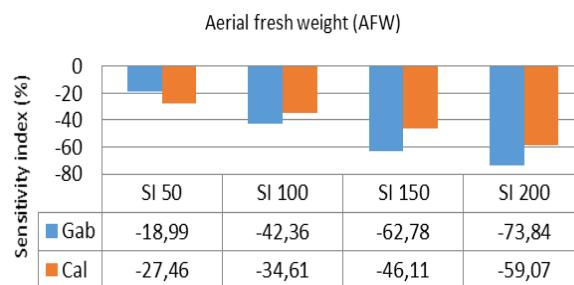


Fig. 4. Sensitivity index (SI) of the aerial fresh weight (AFW) measured for both varieties of *M. sativa* under 50 mM, 100 mM, 150 mM and 200 mM NaCl.

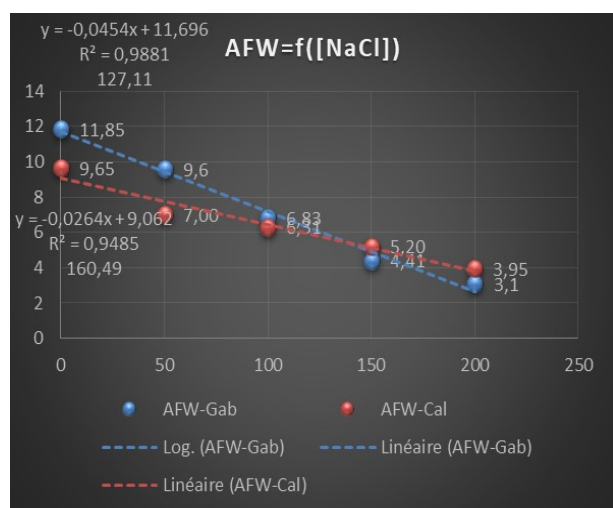


Fig. 5. Linear regression of the aerial fresh weight (AFW) as function of the salt concentrations (50 mM, 100 mM, 150 mM and 200 mM NaCl) in Gabès (Gab) and Californian (Cal) varieties of *M. sativa*.

Under control treatment, Gabès showed higher RFW than the Californian variety (Fig. 6.). While there was no significant difference in RFW for Gabès under the control treatment and 50 mM NaCl, a significant decrease was registered under the remaining concentrations of salt. Furthermore, the Californian variety exhibited similar values of RFW under salt stress.

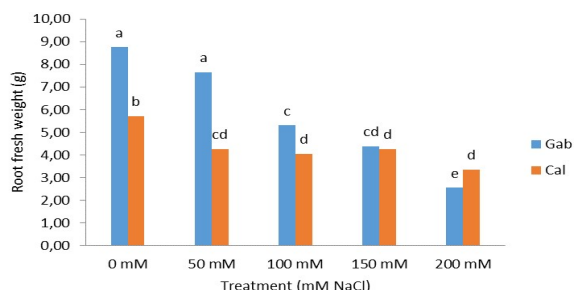


Fig. 6. Means of the root fresh weight (RFW) measured for both varieties of *M. sativa* under control treatment and 50 mM, 100 mM, 150 mM and 200 mM NaCl. Means followed by the same or common letter(s) are not significantly different among studied varieties based on the Duncan test at 5%.

On the other hand, significant reductions of RFW ranging from 12.57 to 70.86% and from 25.44 to 41.23% were noted for Gabès and Californian variety, respectively (Fig. 7.). As for AFW, the Californian variety was more affected for RFW than Gabès under 50 mM NaCl, while it was the most tolerant under the remaining levels of salt. Accordingly, Jabri et al. (2021) showed that the Californian variety was less affected than an

ElHamma variety for root length and fresh weight under salt stress.

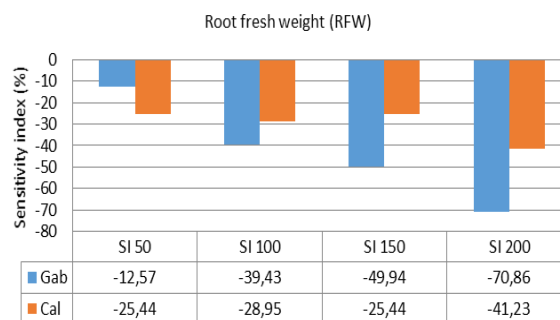


Fig. 7. Sensitivity index (SI) of the root fresh weight (RFW) measured for both varieties of *M. sativa* under 50 mM, 100 mM, 150 mM and 200 mM NaCl.

Among the three possible correlations, one significant correlation was found between AFW and RFW under the control treatment, 50 mM and 100 mM NaCl while three correlations were registered under 200 mM NaCl (Table 2). Accordingly, numerous studies reported that the relationship between analyzed traits is affected by abiotic stress treatment. Arraouadi et al. (2011b, 2012) showed that most correlations between measured characters were noted under salt stress. Moreover, Badri et al. (2016a) reported that relationship between measured traits for natural populations of *M. truncatula* were dependent on the treatment factor. Saoudi et al. (2019) showed that there are specific correlations between measured characters for lines of *H. marinum* under control treatment and salt stress.

Table 2. Correlations between measured characters for the two varieties of *M. sativa* under control treatment and salinity conditions.

| | Control treatment | | | 50 mM NaCl | | |
|-----|-------------------|--------|------|-------------|--------|------|
| | NIN | AFW | RFW | NIN | AFW | RFW |
| NIN | 1.00 | | | 1.00 | | |
| AFW | -0.09 | 1.00 | | 0.23 | 1.00 | |
| RFW | -0.30 | 0.76** | 1.00 | -0.09 | 0.76** | 1.00 |
| | 100 mM NaCl | | | 150 mM NaCl | | |
| | NIN | AFW | RFW | NIN | AFW | RFW |
| NIN | 1.00 | | | 1.00 | | |
| AFW | -0.03 | 1.00 | | 0.26 | 1.00 | |
| RFW | 0.19 | 0.81** | 1.00 | -0.11 | 0.39 | 1.00 |
| | 200 mM NaCl | | | | | |
| | NIN | AFW | RFW | | | |
| NIN | 1.00 | | | | | |
| AFW | 0.59** | 1.00 | | | | |
| RFW | 0.54** | 0.82** | 1.00 | | | |

**Correlation is significant at the 0.01 level (2-tailed).

4. CONCLUSION

Overall, all measured traits for both varieties were affected by salt stress while only three characters (NIN, AFW and RFW) were explained by the interaction of variety x treatment. The Californian variety was less affected by salinity than the Gabès variety. The two concentrations (127.11 mM and 160.49 mM NaCl) lead to a reduction of 50% of the aerial fresh weight for Gabès and the Californian variety, respectively. Further work is needed to assess the productivity of both varieties in the field under salinity constraint.

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