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GERMINATION AND BIOCHEMICAL RESPONSES TO ALKALINITY STRESS IN TWO SESAME CULTIVARS

ABSTRACT

In this study, the effect of different alkaline concentrations (0, 10, 20, 30, 40, 50, 60 mM) on germination and biochemical characteristics of the two sesame (*Sesamum indicum* L.) cultivares (Dashtestan and GL-13) which are registered cultivars of Iran were investigated. The experiment was carried out in a completely randomized design with three replications. Results showed that , germination percentage, germination rate, shoot length and dry weight, root length and dry weight and K^+ content decreased, whereas, malondialdehyde (MDA), proline, total soluble sugars and Na^+ contents increased with increasing alkalinity stress. GL-13 cultivar had the least root and shoot length, proline and K^+ content than Dashtestan.

Key words: alkaline stress, germination, proline, sesame.

INTRODUCTION

Seed germination is the initial and most crucial stage in the plant life cycle. Seed germination is affected by many biotic and abiotic factors, such as temperature, salt, light, water, oxygen concentration, and alkalinity. Successful germination of the seeds under a wide range of environmental conditions (e.g., temperature and moisture) is important for early seedling establishment (Grime and Campbell 1991).

Alkaline stress is caused by the alkaline salts such as Na_2CO_3 or $NaHCO_3$ in the soil (Yang *et al.* 2007). Bicarbonate is the main ion that causes alkalinity and imparts buffer capacity to water, and at concentrations higher than 2 mM it can cause a significant suppression in plant growth of sensitive species due to

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the increase in water pH (Valdez-Aguilar and Reed 2010). Alkaline stress is more destructive to plants than salt stress, suggesting that a high pH environment also causes direct toxicity effects (Shi and Sheng 2005). Alkaline stress is widespread environmental constraints affecting crop productivity (Allakhverdiev *et al.* 2000). Alkalinity is also a limiting factor in seed germination (Shi and Sheng 2005). Yang *et al.* (2007) reported that alkalinity stress can inhibit uptake of inorganic anions such as Cl^-, NO_3^- and $H_2PO_4^-$, largely affect the selective absorption of K^+ — Na^+, and disrupt the ionic balance. However, accumulation of compatible solutes, such as betaine, proline and soluble sugar into the vacuole are considered as the basic strategies for plant re-established cellular homeostasis under alkalinity stress (Li *et al.* 2009).

Sesame (*Sesamum indicum* L.) is considered as an oil seed crop with medicinal properties (Weiss 2000). It is usually cultivated in arid and semi-arid regions (Desphande *et al.* 1996) that salinity and alkalinity are usually one of the most significant factors to limit crop production. So, this experiment was carried out to evaluate response of two cultivars of sesame seed to alkalinity stress in germination and early growth stages.

MATERIAL AND METHODS

The experiment was conducted in Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran, in a completely randomized design in factorial arrangement and at three replications.

Two sesame (*Sesamum indicum* L.) cultivares (Dashtestan and GL-13) grown in Iran were used for the study. Seeds were stored in a cool, dark room prior to the start of the experiment. Seeds were surface sterilized with 0.5% sodium hypochloride for 1 min and washed thoroughly with distilled water. Then 50 seeds of each cultivar were transferred into sterile glass petri dishes of uniform size lined with two layers of Whatman no. 1 filter paper, that wetted with 7 ml of one of the alkalinity solutions (0, 10, 20, 30, 40, 50 and 60 mM $NaHCO_3$). For reduce the risk of infection and evaporation of solution, all of petri dishes were closed with parafilm. All operations were done under laminar flow. Petri dishes were incubated at 25±1 °C. Germinated seeds were scored daily, based on the emergence of the radicle. After eight days, number of total germinated seeds, length and weight of roots and shoots were measured. Remained part of plants was frozen in liquid N_2 and stored under -80°C for biochemical analysis. Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) released using the thiobarbituric acid reaction as described by De Vos *et al.* (1991). Proline content was determined following the method of Bates *et al.* (1973). The content of soluble sugars was measured according to the method of Irigoyen *et al.* (1992). Sodium and potassium concentration were determined by flame photometer (Model PFP7, Germany) (Williams and Twine 1960).

All data were analyzed using SAS software. After analysis of variance (ANOVA) showed significant treatment effects, least significant differences (LSD) test was applied to compare the means at $P<0.05$.

RESULTS

Germination percentages were reduced as alkaline level was increasing. Increasing alkalinity to 60 mM made germination percentage decreasing to 38.65% when compared to control (0 mM) (Table 1). In addition, among studied cultivars, the highest germination percentage was found for Dashtestan cultivar (Table 1). The higher severity of alkaline stresswas, the lower germination rate was found for both cultivars. The highest germination rate was observed in control plants of Dashtestan cultivar (Fig. 1a). Increasing level of alkaline stress to 60 mM, reduced seedling vigor index significantly in GL-13 (94.12%) and Dashtestan (86.99%) in comparison to 0 mM alkalinity (control) (Fig. 1b).

Table 1

Germination parameters means of sesame and statistical groupings for main effect of cultivar and alkaline stress

Treatments	Germination [%]	Shoot dry weight [g]	Root dry weight [g]	K^+ [%]
		Cultivars		
V1	80.4b	0.0485a	0.0151a	3.35a
V2	84.57a	0.0494a	0.0153a	2.74b
		Alkalinity [mM]		
0	97 a	0.065 a	0.018 a	4.42a
10	93.6 ab	0.056 b	0.017 ab	4.1ab
20	90 b	0.052 bc	0.015 bc	3.76b
30	86 c	0.047 dc	0.015 bc	3.5c
40	79.6 d	0.045 d	0.015 bc	2.1d
50	70.6 e	0.043 d	0.014 cd	2.04d
60	59.5 f	0.037 e	0.013 d	1.92d

Means within column followed by the same letter are not significantly different at $P<0.05$ by LSD test.
V1= GL-13, V2= Dashtestan.

The shoot and root length was shortened strongly corresponding to increasing of alkalinity stress. The lowest shoot and root length was observed at 60 mM alkalinity for GL-13 cultivar and the highest values of these traits obtained in control treatment and Dashtestan cultivar (Fig. 1c, d).

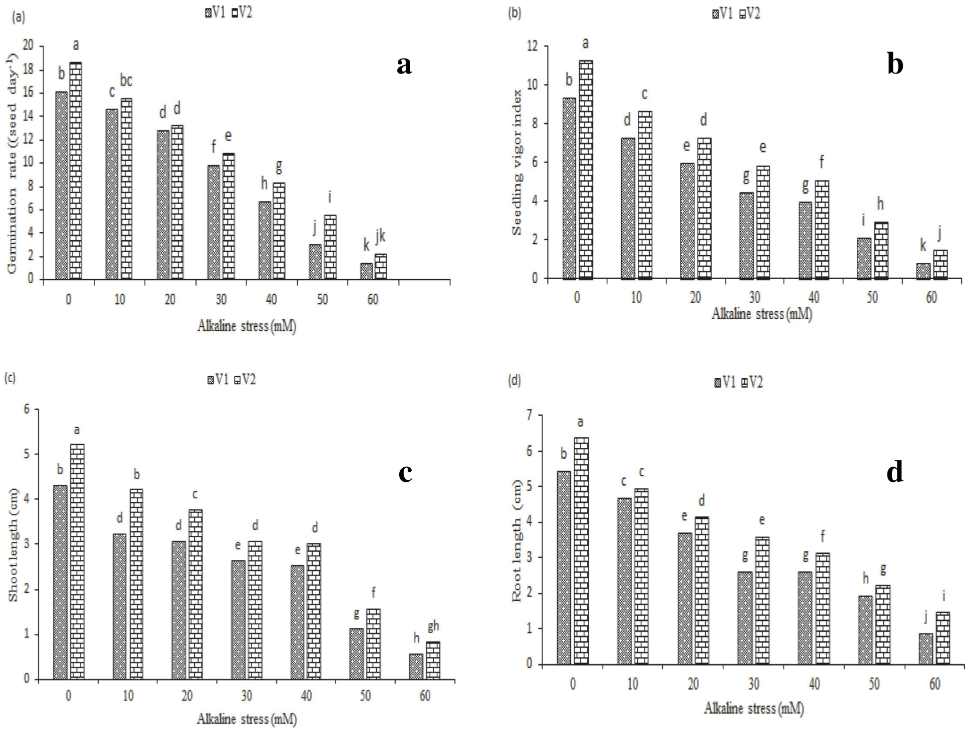

Fig. 1. Effect of cultivar and alkaline stress on germination rate (a), seedling vigor index (b), shoot length (c) and root length (d) of sesame. Means by the same letter are not significantly different at P<0.05 by LSD test. V1= GL-13, V2= Dashtestan.

Results also showed considerable decrease in shoot dry weight (43.07%) and root dry weight (27.77%) in comparison with 0 mM alkalinity. In addition, there were no significant differences in shoot dry weight and root dry weight between GL-13 and Dashtestan cultivars (Table 1).

MDA content was positively correlated with the increase of alkaline salt and recorded its highest value at the severest alkalinity ompared with control.The highest stress level (60 mM) increased MDA content 65.36% in GL-13 and 63.18% in Dashtestan comparing to control (Fig. 2a). Total soluble sugars content increased in all alkalinity treatments in comparison to control. The highest total soluble sugars content observed at 60 mM alkalinity stress in Dashtestan cultivar. In 60 mM alkalinity stress, Dashtestan cultivar had 23.86% more total soluble sugars than GL-13 (Fig. 2b). Proline content sharply enhanced with increasing alkalinity stress in both cultivars (Fig-7). Dashtestan variety also showed more proline content than GL-13 at all alkalinity levels. The highest stress level (60 mM) increased proline content 93.47% in GL-13 and 75.78% in Dashtestan, in comparison with control (Fig. 2c)

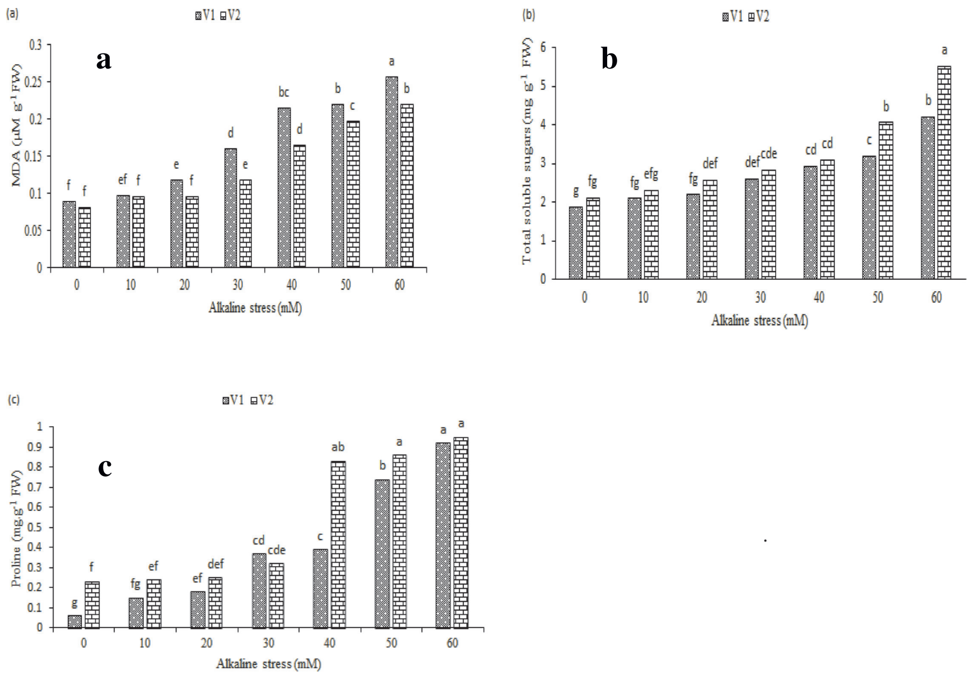

Fig. 2. Effect of cultivar and alkaline stress on MDA (a), Total soluble sugars (b) an proline (c) of sesame. Means by the same letter are not significantly different at P<0.05 by LSD test. V1= GL-13, V2= Dashtestan

Absorption of Na^+ ions was also increased following increasing of alkalinity stress from 0 mM to 60 mM. It was in 90.09% for GL-13 and 75% for Dashtestan. The highest Na^+ ions content was observed at 60 mM alkalinity for GL-13 cultivar, but there was not significant differences at Na^+ content in the case of-plants grown at 50 mM alkalinity. Dashtestan cultivar did not show any reaction for those traits both at 50 and 60 mM alkalinity (Fig. 3a).

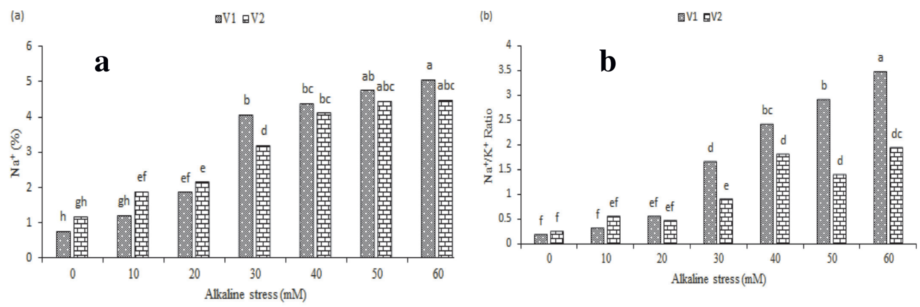

Fig. 3. Effect of cultivar and alkaline stress on Na^+ (a) and Na^{+}/K^+ ratio (b) content of sesame. Means by the same letter are not significantly different at P<0.05 by LSD test. V1= GL-13, V2= Dashtestan

Basing on results shown in Table 3 K^+ ions absorption for GL-13 surpassed 18.48% this one found for Dashtestan. Besides, it was shown that 56.56% decreasing of K^+ ions absorption was made by increasing alkalinity stress from 0 to 60 mM (Table 1). With increasing alkalinity stress Na^+/K^+ ratio increased in both cultivars and the highest Na^+/K^+ ratio obtained at 60 mM alkalinity in GL-13 cultivar (Fig. 3b).

DISCUSSION

All studied traits (germination percentage, germination rate, seedling vigor index, shoot and root length, shoot and root dry weight) were shown to be decreased under different levels of alkalinity stress (see Table 1 and Fig. 1). The highest values of germination rate, seedling vigor index, shoot and root length were found for the control treatment and Dashtestan cultivar (Fig. 1). Shi *et al.* (1998) reported that alkalinity could be a limiting factor for seed germination. Importantly, alkaline and neutral salts have severe effects on seedling growth, and plants may respond differently to them (Shi and Sheng 2005). Also Guo *et al.* (2009) and Zhang and Chun-Sheng (2009) found that alkaline stress is a limiting factor for seed germination.

Increasing alkalinity stress, resulting higher amount of MDA, total soluble sugars and proline in both cultivars seemed to play an important role. Accumulation of betaine, proline, soluble sugar and compartmentalize of Na^+ ions into the vacuole are considered as the basic strategies for plant re-established cellular homeostasis under alkaline stress (Li *et al.* 2009; Yang *et al.* 2009). MDA is one of the end-products of oxidation unsaturated fatty acids in cellular membranes. In terms of stress, production of reactive oxygen species (ROS) in cell membrane will enhance MDA (Munns and James 2003). Our results confirm the findings of Zhang and Chun-Sheng (2009) who reported increasing rate of MDA under alkalinity stress. Accumulation of soluble sugars and proline in terms of stress protects cell membranes against high concentrations of inorganic ions and reactive oxygen species (Ahmad and Sharam 2010, Bohnert *et al.* 1995). Yang *et al.* (2009) reported that alkalinity stress increased soluble sugars content in barley. Also, Liu *et al.* (2010) reported significant increase soluble sugar content in alkalinity stress condition in sunflower. In plants, proline plays an important role in protecting membrane stability (Ashraf and Haris 2004). Increasing proline content with increasing alkaline stress was reported in different studies on wheat (Guo *et al.* 2009) and barley (Yang *et al.* 2009).

Our result showed that increasing of alkaline stress reduced K^+ ions content and increased Na^+ ions content influenced Na^+/K^+ ratio. This result is confirmed by the study of Abdel Latef and Tran (2016), who found similar changes in Na^+ and K^+ ions balance for maize plants under increasing of alkaline stress level. The lower K^+ uptake under alkaline stress maybe due to the repressive effect of the stress on the absorption of this cation and competition between Na^+ and K^+

ions for binding sites needful for various cellular functions (Azooz *et al.* 2015). Also, Yang *et al.* (2009) found that alkali stress inhibits absorption of inorganic anions such as Cl^-, NO_3^- and $H_2PO_4^-$, greatly affect the selective absorption of K^+, Na^+ and break the ionic balance. Thus, plants in alkaline soil must cope with physiological drought and ion toxicity, and also maintain intracellular ion balance and regulate pH outside the roots.

CONCLUSIONS

Results show that with increasing alkalinity stress germination traits and K^+ content increased whereas, MDA, total soluble sugars, proline, Na^+ and Na^+/K^+ ratio decreased. Dashtestan cultivar was superior to GL-13 in alkalinity stress. Dashtestan cultivar had better performance by more germination percentage, seedling vigor index, root and shoot length, total soluble sugars content and less Na^+ and Na^+/K^+ concentration.

REFERENCES

Abdel Latef, A. A. and Tran, L. S. P. 2016. Impacts of priming with silicon on the growth and tolerance of maize plants to alkaline stress. Front. Plant Sci. 7: 1-10.

Ahmad, P. and Sharma, S. 2010. Physio-biochemical attributes in two cultivars of mulberry (*Morus alba* L.) under $NaHCO_3$ stress. Int J Plant Prod, 4: 1735-1743.

Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., Inaba, M. and Murata, N. 2000. Ionic and osmotic effects of NaCl induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol. 123: 1047_1056.

Ashraf, M. and Harris, P. J. C. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Azooz, M. M., Metwally, A. and Abou-Elhamd, M. F. 2015. Jasmonate-induced tolerance of *Hassawi okra* seedlings to salinity in brackish water. Acta Physiol. Plant. 37: 1–13.

Bates, L. S., Waldern, R. P. and Teave, I. D. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Bohnert, K. H., Nelson, D. E. and Jensen, R. G. 1995. Adaptations to environment stresses. Plant Cell Physiol. 7: 1099-1111.

De Vos C., Schat H. M., De Waal M. A., Vooijs R. and Ernst W. 1991. Increased to copper-induced damage of the root plasma membrane in copper tolerant *Silene cucubalus*. Plant Physiol. 82: 523-528.

Desphande, S. S., Deckhands, U.S. and Salunkhe D.K. 1996. Sesame oil. In Hui, Y.H. (ed.). Bailey's Industrial Oil and Fat Products. Interscience Publishers, New York, pp. 457-497.

Grime, J. P. and Campbell, B. D., 1991. Growth rate, habitat productivity, and plant strategy as predictors of stress response. In: Mooney, H. A., Winner, W. E., Pell, E. J. and Chu, E. (Eds.), Response of Plants to Multiple Stresses. Academic Press, Inc., San Diego, London, pp. 143–159.

Guo, R., Shi, L. and Yang, Y. 2009. Germination, growth, osmotic adjustment and ionic balance of wheat in response to saline and alkaline stresses. Soil Sci. Plant Nutr. 55: 667–679.

Irigoyen, J. J., Einerich, D. W. and Sánchez-Díaz, M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativd* L.) plants. Physiol Plant. 84(1): 55 -60.

Li, X. Y., Liu, J. J., Zhang, Y. T., Lin, J. X. and Mu, C. S. 2009. Physiological responses and adaptive strategies of wheat seedlings to salt and alkali stresses. Soil Sci. Plant Nutr. 55: 680-684.

Liu, J., Guo, W. Q. and Shi, D. C. 2010. Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. Photosynthetica. 48 (2): 278-286.

Munns, R. and James, R. A. 2003. Screening methods for alkalinity tolerance: a case study with tetraploid wheat. Plant Soil. 253: 201-218.

Shi, D. and Sheng, Y. 2005. Effect of various salt–alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. Environmental and Experimental Botany, 54: 8–21

Shi, D. C., Sheng, Y. M. and Zhao, K. F., 1998. Stress effects of mixed salts with various salinities on the seedlings of *Aneurolepidium chinense*. Acta Botanica Sinica. 40: 1136–1142.

Valdez-Aguilar, L. A. and Reed, D. W. 2010: Growth and nutrition of young bean plants under high alkalinity as affected by mixtures of ammonium, potassium, and sodium. J. Plant Nutr. 33: 1472-1488.

Weiss, E. A., 2000. Oil seed Crops. 2nd ed. Blackwell Science, Oxford.

Williams V. and Twine S. 1 960. Flame photometric method for sodium, potassium and calcium. In: Peach K. and Tracey M. V. Modern Methods of Plant Analysis. Springer-Verlag Berlin, 56: 6-1 2.

Yang, C., Chong, J., Kim, C., Li, C., Shi, D. and Wang, D. 2007. Osmotic adjustment and ion balance traits of an alkaline resistant halophyte *Kochia sieversiana* during adaptation to saline and alkaline conditions. Plant Soil. 294: 263_276.

Yang, C., Xu, H., Wang, L., Liu, J., Shi, D. and Wang, D. 2009. Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. Photosynthetica. 47: 79–86

Zhang, J. T. and Chun-Sheng, M. U. 2009. Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. Soil Science and Plant Nutrition, 55: 685–697