Research Article

In Vitro Evaluation of Oat Cultivars Against Drought and Salinity Stress Tolerance

Sajjad Khan, Fahad Karim Awan*, Zulfiqar Ali Gurmani, Muhammad Usama Hameed, Allah Bakhsh and Kainat Bibi

Crops Sciences Institute, National Agricultural Research Centre (NARC) Islamabad, Pakistan.

Abstract | Agricultural yield in Pakistan is liable to environmental constraints, mainly salt and water stress due to their elevated degree of impression and non-availability of salt and drought tolerant oat cultivars. For the purpose in-vitro study was conducted at NARC fodder laboratory to study the response of two oat cultivars namely NARC and PARC Oat against drought and salinity stress. Salt concentration of 80 mmolL^{-1} showed non-significant effect on germination % and shoot length of both oat cultivars except salt concentration of 120 mmolL^{-1}, but tolerance in NARC, Oat is higher than PARC oat. Salt concentrations positively increased root length, except in PARC oat at 120 mmolL^{-1}. Drought stress (PEG) significantly reduced the shoot length in PARC, Oat cultivar when 20% solution was applied. Where both treatments were applied in combinations to measure the drought and salinity tolerance at initial seeding stages of both oat cultivars, no significant effect was observed on germination %, shoot and root length of both oat cultivars, however PARC, oat performs better at 10 % and 20 % drought stress with salinity level of 80 mmolL^{-1}. However, both cultivars showed significantly stunted performance at 10 % and 20 % drought stress with salinity level of 120 mmolL^{-1} when compared with control treatment. The results of the study present the potential resistance of NARC oat against salinity and PARC oat against drought stress. This study will helps in selection of salt and drought tolerant oat cultivars for the affected areas.

Received | June 22, 2021; **Accepted** | February 01, 2022; **Published** | June 28, 2022
***Correspondence** | Fahad Karim Awan, Crops Sciences Institute, National Agricultural Research Centre (NARC) Islamabad, Pakistan; **Email:** fahad_0207@yahoo.com
Citation | Khan, S., F.K. Awan, Z.A. Gurmani, M.U. Hameed, A. Bakhsh and K. Bibi. 2022. In Vitro evaluation of oat cultivars against drought and salinity stress tolerance. *Pakistan Journal of Agricultural Research,* 35(2): 410-416.
DOI | https://dx.doi.org/10.17582/journal.pjar/2022/35.2.410.416
Keywords | Oat cultivar, In-vitro screening, Drought and salinity stress

Introduction

Oats (*Avena sativa* L.) now a days have received substantial attention for a multiple reasons i.e. high contents of dietary fibres, phyto-chemicals, nutritive value and other health benefits (Prasad *et al.*, 2015). Oat is a dual purpose (cereal and forage) crop, and is widely planted in marginal lands (Hameed *et al.*, 2020). China is one of the largest consumer of forage crop worldwide, with the import of oat hay of 10.39 million tons during 2017 from the United States, Australia etc. After alfalfa oats are the second largest imported forage crop (Sadaqat *et al.*, 2020). Performance of oats under salinity for estimating

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morphological and physiological traits is under observation by a number of researchers, most of the researchers were focused on the grain yield. However, very few studies are available on the response of forage type oats under salinity. (Nevo and Chen, 2010).

Salinity is a major abiotic stress affecting crop production. More than 6% of the world's agriculture land is affected by salinity (Gao *et al*., 2016). Saline affected soils have the potential to be used for growing oat crops. Oat crops are supposed to be moderately tolerant to abiotic stress. (Bai *et al*., 2013). Very few studies are on record regarding the tolerance of oat crops to salinity and alkalinity, to date, only a few have been published (Oraby and Ahmad, 2012). Therefore, there is a need for a better understanding of the genetic variations affecting saline/alkaline tolerance in oat genotypes. Drought and salinity affect the plants in a like manner (Katerji *et al*., 2004). Reduction in water potential is common factor in both salinity and drought (Legocka and Kluk, 2005). Water stress acts by reducing the percentage and rate of germination and seedling growth. NaCl and Polyethylene Glycol (PEG) compounds have been used to simulate osmotic stress effects in Petri dish (in vitro) for plants to maintain uniform water potential (Wafa, 2010).

Two main factors that are affecting more than 10% of the cultivated areas and limiting plant production are drought and salinity. Desertification and salinity is also increasing in globally causing more than 50% yield losses to important crops (Bartels and Sunkar, 2005). Around 15-20% of cultivated areas and 20-50% of irrigated agricultural areas of the world is damaged by salinity (Pitman and Läuchli, 2002; Tuteja, 2007). Among the cereals, oat is the most sensitive plant to drought stress and it can be cultivated in any kind of soil where water is present. The fact that plants can't benefit from the water in the soil in areas with salinity constitutes important problems for oat. The period when oat (Avena *sativa* L.) is most sensitive to drought is its germination and critical periods. In addition to this; salinity and drought generally effects every phase of growth and development of plants and they are most perceived in germination period (Alam *et al*., 2020). Studies have reported that investigation at plant's germination stage permits good forecast of its response to salinity (Bai *et al*., 2018). No any prominent oat cultivar is available in the country having significant tolerance against salt and drought stress.

The purposes of this study are to identify;

1. Tolerance against salt and drought stress and screening of locally developed oat cultivars during seedling stage.
2. Identify the potential of newly developed cultivars, to tolerate salinity and or drought stress during seedling stage.
3. Study the relationship between tolerance to salinity and drought during seedling stage for both the cultivars.

Material and Methods

In-vitro study was carried out at Fodder and Forage Program, National Agriculture Research Centre Islamabad in December 2020 to evaluate the performance of two Oat varieties namely NARC and PARC oats against salinity and drought stress. The experiment was laid out in completely randomized design (CRD) with three number of replications. To induce drought stress PEG (polyethylene glycol) was used with the concentration of 10% and 20% and for salinity NaCl solution was used in concentration of 80 $mmolL^{-1}$ and 120 $mmolL^{-1}$ with control treatments. Ten seeds were placed in the Petri dishes for each treatment. There were total 18 treatment combinations used in the experiment as follows.

NARC Oat: T1: NACL 0 $mmolL^{-1}$, T2: NACL 0 $mmolL^{-1}$ + PEG 10%, T3: NACL 0 $mmolL^{-1}$ + PEG 20%, T4: NACL 80 $mmolL^{-1}$, T5: NACL 80 $mmolL^{-1}$ + PEG 10%, T6: NACL 80 $mmolL^{-1}$ + PEG 20%, T7: NACL 120 $mmolL^{-1}$, T8: NACL 120 $mmolL^{-1}$ + PEG 10%, T9: NACL 120 $mmolL^{-1}$ + PEG 20%.

PARC Oat: T1: NACL 0 $mmolL^{-1}$, T2: NACL 0 $mmolL^{-1}$ + PEG 10%, T3: NACL 0 $mmolL^{-1}$ + PEG 20%, T4: NACL 80 $mmolL^{-1}$, T5: NACL 80 $mmolL^{-1}$ + PEG 10%, T6: NACL 80 $mmolL^{-1}$ + PEG 20%, T7: NACL 120 $mmolL^{-1}$, T8: NACL 120 $mmolL^{-1}$ + PEG 10%, T9: NACL 120 $mmolL^{-1}$ + PEG 20%.

Drought calculation

To calculate the required PEG amount to obtain a specific value of osmotic potential in the solution, following equation was followed:

$$PEG = \frac{4 - (5{,}16\psi t - 560\psi + 16^{0,5})}{2{,}58t - 280}$$

Where;

Temperature (t) is expressed in Celsius degrees; Osmotic potential (ψ) is expressed in bar (1 bar=0,1 mpa) Polyethylene glycol amount, [PEG], is expressed as g of PEG / g of H_2O; This equation is the result of a model that considers ψ varying quadratically with concentration and linearly with temperature of solution.

Salinity (NaCl solution preparation)

One Molar sodium chloride solution was prepared by dissolving 58.44g of NaCl in a final volume of 1 litre. For 0.1m NaCl solution, we put 5.844g of NaCl and dissolve it in one litre of water; similarly, 0.5844g of NaCl was dissolved in 100ml of water.

Seed germination

Seed Germination is one of the initial factors which determines the plant vigour and sets the plant population. Production of any crop in general and specifically in fodder crop purely depends on plant population. Ten seeds were placed in each Petri dish for each treatment. A 5ml solution of each treatment was applied in each petri dish followed by distilled water for irrigation purpose. Numbers of seeds were counted for each treatment to get seed germination percentage.

Shoot and root length

Shoot and root growth is inclined by the ecological factors. Mineral nutrition is a significant factor impelling the root length. It is observed that usually behaviour of root growth towards chemical fertilization is the same to that of shoot growth; however, the extent of the response may differ. Shoot and root lengths of germinated plants of all the treatments were recorded in centimetres.

Statistical analysis

Data were subjected to Anova and LSD using statistical analysis using statistix version 8.0. (Steel *et al.*,1997). Bi-plot was constructed using R statistical software (Yan *et al.*, 2000).

Results and Discussions

Germination (%)

It is obvious that germination percentage of oat cultivars was affected by various salinity and drought levels as presented in table 01. In case of NARC oat cultivar statistical analysis of the data does not shows any significant change in germination percentage with the application of salinity and or drought stress (NaCl and PEG), except where combined application of both treatments was applied @ 120mmolL saline solution with 20% PEG, however increase in germination percentage of NARC oat was recorded i.e. (93.3%) where 10 % PEG solution was applied to assess tolerance against drought compared to control which shows (90.0%) germination. PARC oat also showed declining germination percentage with the introduction of salinity and or drought stress compared to control which shows maximum germination percentage (93.3%), but the difference was non-significant. A reduced germination percentage with significant result was recorded in PARC oat where salinity stress was applied @ 120mmolL^{-1} solely *i.e.* (66.7%) and in combination with drought stress *i.e* PEG @ 10% and 20% which shows reduced germination percentage *i.e.* (70.0% and 50.0%). These results are also illustrated by graphical presentation at Figure 2.

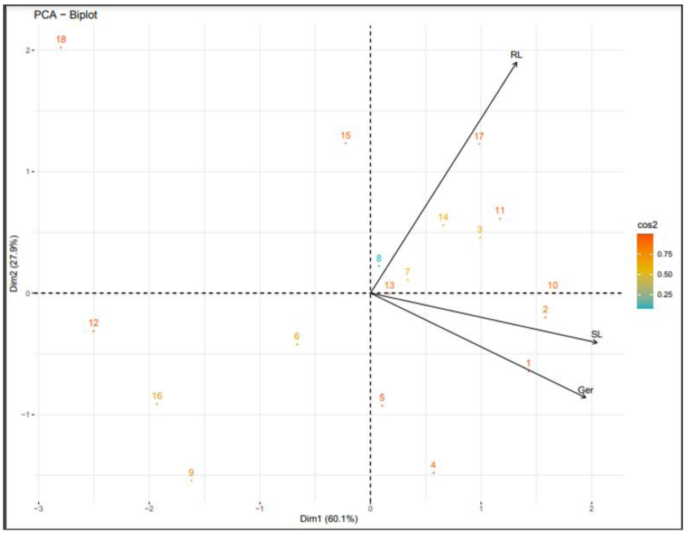

Figure 1: *PCA- Biplot analysis for germination, shoot and root length for different treatments.*

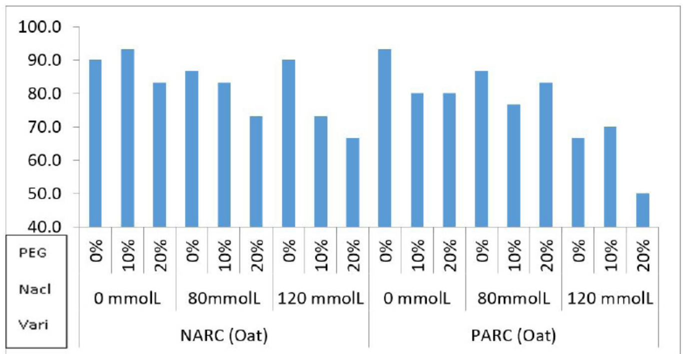

Figure 2: *Seed germination (%) against salinity and drought stress in two oat cultivars.*

These results depict that germination percentage of both oat cultivars is inversely proportion to salinity

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and drought stress *i.e.* with the increase in salinity and drought stress germination percentage decreases, but in most of the treatments the data of decline in germination percentage was non-significant which is sufficient to say that both oat cultivars are tolerant against salinity and drought, however tolerance in NARC oat is higher as compared to PARC oat. These results are in conformity with (Khoshsokhan *et al*., 2012) who revealed that response of seed germination percentage to induced salt stress through sodium chloride may vary depending on concentrations. At low concentration of salt stress no remarkable effect on germination percentage was observed, but at higher concentration of salt stress, germination rate and percentage was significantly reduced compared to the control. Drought stress showed significant effect on germination percentage and seed vigour (Rabiei *et al*., 2009). Combined application of NACL and PEG significantly affect the germination of seed. These results are in line with (Murillo-Amador *et al*., 2002).

Table 1: *Mean comparison for all the treatments.*

Variety	Salt (NACL)	Drought (PEG)	Germination (%)	Root Length (cm)	Shoot Length (cm)
PARC (Oat)	0 mmolL	0%	93.3 ^{a}	12.2 ^{a}	9.0 ^{a}
		10%	80.0 ^{abc}	11.8 ^{abcd}	8.3 ^{abc}
		20%	80.0 ^{abc}	7.8 ^{ef}	5.7 ^{c}
	80 mmolL	0%	86.7 ^{abc}	12.9 ^{a}	8.6 ^{a}
		10%	76.7 ^{abc}	12.0 ^{abc}	9.0 ^{a}
		20%	83.3 ^{abc}	13.9 ^{a}	8.2 ^{abc}
	120 mmolL	0%	66.7 ^{cd}	8.0 ^{def}	6.0 ^{bc}
		10%	70.0 ^{bcd}	12.7 ^{a}	8.0 ^{abc}
		20%	50.0 ^{d}	11.9 ^{abc}	5.7 ^{c}
NARC (Oat)	0 mmolL	0%	90.0 ^{ab}	11.7 ^{abcde}	9.5 ^{a}
		10%	93.3 ^{a}	8.6 ^{cdef}	8.4 ^{ab}
		20%	83.3 ^{abc}	11.2 ^{abcd}	8.1 ^{abc}
	80mmolL	0%	86.7 ^{abc}	11.5 ^{abc}	9.9 ^{a}
		10%	83.3 ^{abc}	9.0 ^{bcdef}	8.5 ^{ab}
		20%	73.3 ^{abc}	11.0 ^{abcde}	8.7 ^{a}
	120 mmolL	0%	90.0 ^{ab}	12.5 ^{a}	8.0 ^{abc}
		10%	73.3 ^{abc}	9.2 ^{bcdef}	8.1 ^{abc}
		20%	66.7 ^{cd}	6.1 ^{f}	8.1 ^{abc}
			LSD value 21.645	LSD value 3.318	LSD value 2.628

Shoot length (cm)

Shoot length was affected by salt and drought stress but difference was significant in PEG (Table 1). In PARC oat longest shoot length was recorded with salinity level 80 mmolL^{-1} *i.e.* 8.6 cm and 9.9 cm in NARC respectively, longest shoot length with combined application of salt and drought stress was measured in PARC oat *i.e.* 9.0cm with application of 80 mmolL^{-1} salt and 10% PEG. However, at higher concentration of salt and drought stress a significantly reduced shoot length was recorded in PARC oat. This difference of growth at same level of NACL and PEG stress, might be due to specific cultivar response against treatment. By studying the relationship among the variables it can be concluded that both oat cultivars are tolerant to Nacl and PEG, but PARC oat performs better than NARC oat in case of root length (Figure 1). These results are also presented in bar chart graph at Figure 3.

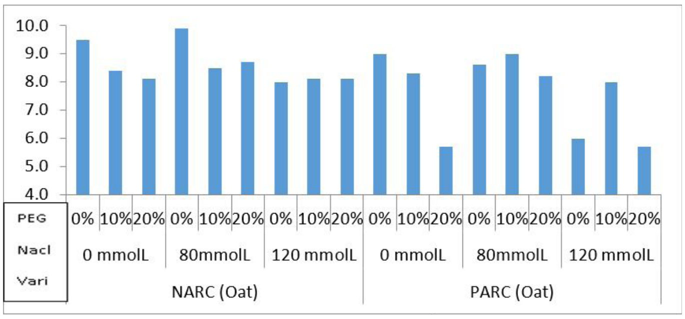

Figure 3: *Shoot length (cm) against salinity and drought stress in two oat cultivars.*

Seedling growth was reduced with the application of salt (Nacl) and or drought (PEF) stress, that might be these results are in agreement with (Hosseini *et al*., 2003), who reported the effects of salt stress on growth and fixation of nitrogen by soybean plants. Declining trend is observed in root and shoot length, and seedling fresh and dry weight by increasing NaCl and PEG. It is noted that with increase in drought and salt stress shoot length decreases but the preference of both varieties is different as NARC performed better than PARC oat (Benlioglu and Ozkan, 2015) specified that a considerable reduction in shoot development was observed as the stress factor increased, which is one of the key parameters determining the stress tolerance of genotypes.

Root length (cm)

Data regarding root length (cm) of oat varieties as affected by various salinity and drought levels is presented in Table 1. Increase in the root length of both oat cultivars was observed with the application of salt and drought stress. Maximum increase in root length was recorded in PARC oat where salinity level of 80 mmolL^{-1} was applied in combination with 20% PEG. *i.e.* 12.9cm as compared to control treatment *i.e.* 12.2 cm, while NARC oat shows maximum root length of 12.5 cm with salt concentration of 120 mmolL^{-1} comparing to control *i.e.* 11.7 cm, however in case of PARC oat sole application of Nacl @ 120 mmolL^{-1} root length was significantly reduced *i.e.* 8.0 cm. In NARC oat longest root length *i.e.* 12.5 cm was noted with salinity level of 80 mmolL^{-1}, Combined application of salt and drought stress PARC oat is found highly tolerant by obtaining maximum root length of 13.9 cm with 80mmolL salt level and 20% PEG. On the basis of mean values of data and by studying the relationship among the variables it can be concluded that both oat cultivars are tolerant to Nacl and PEG, but PARC oat performs better than NARC oat in case of root length (Figure 1). Results illustrated in bar chart also shows that both cultivars respond unlikely against different treatments of salt and or drought stress. (Figure 4).

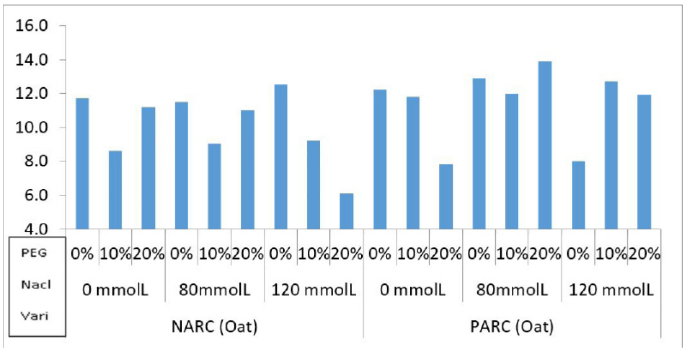

Figure 4: *Root length (cm) against salinity and drought stress in two oat cultivars.*

The results shown in Table 1, NaCl stress had no significant effect on the growth of roots. Different plant cultivars have different responses on plant growth and physiological changes against salt stress. Plants usually accumulate the salt ion in plant roots or store them in the stems, thereby reduced the transportation and harmful effects to other plant parts. In addition, plants could improve salt tolerance by excreting the unnecessary salt ions out of the body through special ion channels. During drought stress, roots continue to elongate, these findings are also confirmed by (Brunner *et al*., 2015), who explained continued root elongation by the plant's during drought stress is a result in need to reach groundwater.

Conclusions and Recommendations

Significant differences were observed among PARC and NARC oats for the traits studied, showing that there is sufficient variability to have an effective selection. Both cultivars respond positively at medium range of sat and salinity stress i.e 80 mmolL^{-1} NACL and 20% PEG, however, higher concentration of salt and drought stress has significantly affected the germination percentage, shoot and root length of both oat cultivars. On the basis of results of initial growth stages, it is summarized from the study that PARC oat is more tolerant against salinity and drought stress compared to NARC oat cultivar. On the basis of tolerance these two cultivars can be included in oats breeding program for further research on salinity and drought tolerance.

Novelty Statement

Research work will be helpful for fodder production in climate change scenario, specially in salt and drought affected areas. Research will improve fodder availability in the country.

Author's Contribution

Sajjad Khan: Main researcher, wrote introduction.
Fahad Karim Awan: Co-researcher, wrote the manuscript, analysed the data and correspondence.
Zulfiqar Ali Gurmani: Supervised the research and wrote the results.
Muhammad Usama Hameed: Varietal selection and prepared treatments for the experiment.
Allah Bakhsh: Shared the ideas regarding fodder research.
Kainat Bibi: Recorded data, Equipment and chemical handling, take care of the overall expermint.

Conflict of interest

The authors have declared no conflict of interest.

References

Alam, H., Khattak, J.Z., Ksiksi, T.S., Saleem, M.H., Fahad, S., Sohail, H. and Saud, S. 2020. Negative impact of long-term exposure of salinity and drought stress on native *Tetraena mandavillei* L. Physiol. Plant., https://doi.org/10.1111/ppl.13273

Bai, J.H., Liu, J.H., Zhang, N., Yang, J.H., Sa, R.l. and Lan, W. 2013. Effect of alkali stress on soluble sugar, antioxidant enzymes and yield of oat. J. Integrat. Agric., 12(8): 1441-1449. https://

doi.org/10.1016/S2095-3119(13)60556-0

Bai, J., Yan, W., Wang, Y., Yin, Q., Liu, J., Wight, C. and Ma, B. 2018. Screening oat genotypes for tolerance to salinity and alkalinity. Front. Plant Sci., 9: 1302. https://doi.org/10.3389/fpls.2018.01302

Katerji, N., Van Hoorn, J.W., Hamdy, A. and Mastrorilli, M. 2004. Comparison of corn yield response to plant water stress caused by salinity and by drought. Agric. Water Manage., 65(2): 95-101. https://doi.org/10.1016/j.agwat.2003.08.001

Legocka, J. and Kluk, A. 2005. Effect of salt and osmotic stress on changes in polyamine content and arginine decarboxylase activity in Lupinus luteus seedlings. J. plant Physiol., 162(6): 662-668. https://doi.org/10.1016/j.jplph.2004.08.009

Wafa'a, A. 2010. Comparative Effects of Drought and Salt Stress on Germination and Seedling Growth of Pennisetum divisum (Gmel.) Henr. Am. J. Appl. Sci., 7(5): 640-646. https://doi.org/10.3844/ajassp.2010.640.646

Bartels, D. and Sunkar, R. 2005. Drought and salt tolerance in plants. Crit. Rev. Plant Sci., 24(1): 23-58. https://doi.org/10.1080/07352680590910410

Benlioğlu, B. and Özkan, U. 2015. Determination of responses of some barley cultivars (*Hordeum vulgare* L.) to salt stress in different doses at the germination period. J. Central Res. Inst. Field Crops.

Brunner, I., Herzog, C., Dawes, M.A., Arend, M. and Sperisen, C. 2015. How tree roots respond to drought. Front. Plant Sci., 6: 547. https://doi.org/10.3389/fpls.2015.00547

Gao, Y., Lu, Y., Wu, M., Liang, E., Li, Y., Zhang, D. and Deng, D. 2016. Ability to remove Na+ and retain K+ correlates with salt tolerance in two maize inbred lines seedlings. Front. Plant Sci., 7: 1716. https://doi.org/10.3389/fpls.2016.01716

Hameed, M., Z. Ali, G.S. Khan and A. Bakhsh. 2020. PARC oat: A new lodging-resistant, late-maturing and high-yielding variety of oats (*Avena sativa* L.) for Pakistan. Pak. J. Agric. Res., 33(3): 601-608. https://doi.org/10.17582/journal.pjar/2020/33.3.601.608

Hosseini, K.M., Powell, A. and Bingham, I. 2003. The interaction between salinity stress and seed vigour during germination of soyabean seeds. Seed Sci., Technol., 31(3): 715-725. https://doi.org/10.15258/sst.2003.31.3.20

Khoshsokhan, F., Babalar, M., Chaghazardi, H. and Moghadam, M. 2012. Effect of salinity and drought stress on germination indices of two thymus species. Cercetari Agronomice in Moldova, 45(1): 27-35. https://doi.org/10.2478/v10298-012-0003-z

Murillo-Amador, B., López-Aguilar, R., Kaya, C., Larrinaga-Mayoral, J. and Flores-Hernández, A. 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. J. Agron. Crop Sci., 188(4): 235-247. https://doi.org/10.1046/j.1439-037X.2002.00563.x

Nevo, E. and Chen, G. 2010. Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant Cell Environ., 33(4): 670-685. https://doi.org/10.1111/j.1365-3040.2009.02107.x

Oraby, H. and Ahmad, R. 2012. Physiological and biochemical changes of CBF3 transgenic oat in response to salinity stress. Plant Sci., 185: 331-339. https://doi.org/10.1016/j.plantsci.2012.01.003

Pitman, M.G. and Läuchli, A. 2002. Global impact of salinity and agricultural ecosystems Salinity: environment-plants-molecules (pp. 3-20): Springer. https://doi.org/10.1007/0-306-48155-3_1

Rabiei, E., Khodambashi, M., Pirbalouti, A.G. and Shahraki, A.D. 2009. Effect of drought stress on seed traits of oat (Avena sativa). Res. Crops, 10(1): 12-15.

Parasad, R., Jha, A., Sabikhi, L., Kumar, A. and Unnikrishnan, V. 2015. Nutritional advantages of oats and opportunities for its processing as value added foods-a review. J. food Sci. Technol., 52(2): 662-675. https://doi.org/10.1007/s13197-013-1072-1

Sadaqat Shah, S., Li, Z., Yan, H., Shi, L. and Zhou, B. 2020. Comparative Study of the Effects of Salinity on Growth, Gas Exchange, N Accumulation and Stable Isotope Signatures of Forage Oat (*Avena sativa* L.) Genotypes. Plants, 9(8): 1025. https://doi.org/10.3390/plants9081025

Steel, R., Torrie, J. and Dickey, D. 1997. Principles and procedures of Statistics. A Biometrical Approach. New York: The MeGraw-Hill Companies: Inc.

Tuteja, N. 2007. Mechanisms of high salinity tolerance in plants. Methods Enzymol., 428: 419-438. https://doi.org/10.1016/S0076-6879(07)28024-3

Yan, W., Hunt, L.A., Sheng, Q. and Szlavnics, Z. 2000. Cultivar evaluation and mega-environment investigation. https://doi.org/10.2135/cropsci2000.403597x