Evaluation of Wheat (*Triticum aestivum* L.) Accession for Salt Tolerance at Different Growth Stages

Lemma Mamo*, Bethel Nekir, Teshome Bekele, Ashenafi Worku
Ethiopia Institute of Agricultural Research/Werer Agricultural Research Center
**Corresponding author email: lemmamamo2006@gmail.com*

Introduction

Wheat is the major staple food crop worldwide and frequently cultivated on saline soil. Salinity is the serious factor hampering wheat productivity with adverse effect on germination, growth and final economic yield (Feroz *et al.*, 2017). The continuous salinization of arable land is a threat to global food security. Over 800 mha^{-1} of land are affected by salinity, which equates to more than 6% of the world's total land area (FAO 2010) and affects more than 20% of present-day agriculture (Mickelbart *et al.*, 2015). Cereals are grown in almost every region of the world and are exposed to a variety of environmental stresses that severely affect their growth and grain yield (Shahbaz and Ashraf, 2013). As a result, breeding for enhanced salinity tolerance in crop species has received considerable attention as it is an economic and efficient alternative (Ashraf, 2009).

In glycophytes plant species, biochemical, physiological and morphological characteristics are negatively affected, leading to abnormal growth and development and eventual plant death (Nishimura *et al.*, 2011; Beakal *et al.*, 2016; Bethel *et al.*, 2019). Moreover, the highest salinity concentration inhabits percentage of seed germination and emergence of roots due to osmotic effect, which is harmful and prevents the plant in maintaining their appropriate nutritional necessities for their fit development (Kalhoro *et al.*, 2016; Bethel *et al.*, 2019). Most of the crops tolerate salinity to a threshold level and above, where yield decreases as the salinity increases (Khan *et al.*, 2006). Plants growing in saline environments suffer from low osmotic potential of soil solution due to increased concentrations of mineral ions. Eventually, these ions enter into cellular sap, causing ionic imbalance. The negative effects of this disturbance are reflected on plant growth (Perez-Alfocea *et al.*, 1994).

Plant scientists to overcome the salinity have adopted various strategies. Screening is an effective tool to exploit genetic variation among wheat genotypes (Bahmani *et al.*, 2015; Sajid *et al.*, 2017). Characters such as germination, survival and seedling growth or biomass accumulation, have been the most commonly used criteria for identifying salinity tolerance in plants (Khan *et al.*, 2006). The present studies was carried out with the major objective of providing information on the

extent and basis of genetic variation for salinity tolerance in the wheat varieties at the germination, seedling and production stage.

Materials and Methods

Description of study site

The experiment was conduct at Werer Agricultural Research Center, at three growth stages; germination, seedling and reproductive at laboratory, lath house and field condition, respectively through exposing to varying salt stress condition while selecting relatively tolerant at each growth stage and advancing to next growth stage.

Experimental materials and procedures

Germination Test

At germination stage a total of 200 genotypes which are collected from Werer and Kulumsa Agricultural Research Center were studied. Germination stage screenings was conducted through subjecting each wheat genotypes to different salt concentration. Saline solution with an EC value of control (distal water), 15 and 25 dS/m were prepared from NaCl. The experimental design was complete randomized design (CRD) with three replications. Twenty seeds of each genotypes were placed in Petri dish and prepared salt concentration applied. Seeds that produce full radical were considered as germinated. First germination count was made at 7^{th} day after treatment application. Because saline environment often cause delay in seed germination, counting was also done once again at 10^{th} day after application of the treatment to take into account late germinated seeds. Eighteen relatively tolerant genotypes with higher germination percentage were selected and advanced to seedling stage screening.

Seedling stage screening

Seedling stage screening was conducted using eighteen genotypes promoted from seedling stage, under lath house condition. Bulk surface soil (non-saline and alkaline in reaction) was collected and packed into pot. Three salinity levels (control, 10 and 20 dS/m) of saline solutions were prepared from NaCl. Treatments were arranged in CRD with three replications. Accordingly prepared saline solutions were added to each pot maintaining to field capacity and ten seeds of each selected varieties were sown per pot. Emerged seedlings were counted at 7 and 10 days after planting and expressed as percentage of seeds that emerged under control condition. Number of tillers, survived plants, and plant height was recorded. Wheat was maintained up to tillering stage and plant and root length were recorded. All the collected data were statistically analyzed and based on recorded observations ten relatively tolerant materials were selected and advanced to field stage screening.

Mean germination time (MGT) which was calculated according to the equation of Ellis and Roberts (1981): MGT = ΣDn / Σn.

Where: n= number of seeds which were germinated on day D,

D= number of days counted from the beginning of germination.

Germination Index (GI) which was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as: GI= ∑ (Gt / Tt).

GI= [Number of germinated seeds in first count/ Day of first count] +…+ [Number of germinated seeds in final count/Day of final count)

Germination stress tolerance index = Germination index of stressed treatment/germination index of control treatment * 100.

Field Experiments

Screening for salt tolerance and evaluation for yield performance at field condition was undertaken at Werer Agricultural research center. Top soil (0-30 cm) sample was collected from the experimental site, air dried and sieved through a 2 mm screen. The soil was analyzed in the laboratory for some physical and chemical characteristics by standard methods. Based on initial soil sample analysis, the textural class of the experimental site was found to be silt clay. The soils of study area had 12.45% ESP and soluble salt concentration in the soil was 14.05 dS/m as measured in electrical conductivity (ECe) which indicates that the soils of the study site was saline. The treatments were laid out in RCBD with three replications in size of 3 m x 3m plot. Agronomic data was recorded on plant height, effective tiller number, spike length, root length and grain yield.

Statistical Analysis

All collected data was subjected to analysis of variance using SAS statistical software. Significant difference between and among treatment means were assessed using the least significant difference (LSD) at 0.05 level of probability (Gomez and Gomez, 1984).

Result and Discussion

Germination stage screening

Total Germination Percentage (TGP)

Total germination percentage (TGP) of 200 wheat genotypes were significantly reduced by the increasing levels of saline solution. Total germination percentage of all wheat genotypes showed a decreasing trend with increasing salt stress levels (Figure 1). At 0 dS/m level of salinity, most of wheat genotypes showed more

germination percentage of wh ider different

Seedling stage Screening

Total Germination Percentage

Analysis of variance (ANOVA) for total germination percentage was showed significant ($P \leq 0.05$) difference among tested wheat genotypes at each salt level. The main effect of variety and salt level were highly significant and also the interaction of variety with salt level was significant for total germination percentage. As shown on Table 1 when salt stress increased from 0 to 10 and 20 dS/m germination percentage decreased. Germination percentage did not show consistent trend at germination and seedling stage, because behavior of cultivars varies at both stages. This shows that species /varieties never be selected simply on the basis of higher germination percentage. The same result some varieties appeared to be more sensitive at germination stage. However, it performed quite satisfactorily at seedling stage (Rahman *et al.,* 2008; Homayoun 2011).

Germination mean Time

One of the most common problems in saline soil is seed germination delay. The main effect of application of salt level highly significantly ($P \leq 0.001$) affected germination mean time. However, neither the main effect of variety nor the interaction effect of variety with salt level influenced this parameter (Table 1). In this experiment when the salt stresses increase the time required for wheat germination was increased. Average mean germination time (MGT) at control (0), 10 and 20 ds/m salt concentrations were 8.58, 9.72 and 9.97 days, respectively (Table 2). The highest MGT was observed ec31(d)-27t ntwas whe"(c)4(t)] TJETBT

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tiller numbers were gained by AZAKIA-5 (6.82). However, minimum value (4.98) was recorded at ANGL. Highest spike length was recorded from ANGL (9.09 cm), while lowest spike length (8.16 cm) was obtained from DEBEIRA. From the result root length was not significantly affected by accessions.

Table 1. The main and interaction effect of different level of salt on wheat germination percentage and germination mean time

No.		Germination Percentage			Germination Mean Time		
	Treatments (Genotypes)	0 dS/m	10 dS/m	20 dS/m	0 dS/m	10 dS/m	20 dS/m
1	PAUON 76	80.00^{abcd}	43.33^{abcdef}	23.33^{ab}	8.53	9.78	9.50
2	AZAKIA-5	80.00^{abcd}	26.67^{def}	10.00^{b}	8.61	10.00	10.00
3	ANGL	83.33^{abcd}	26.67^{def}	20.00^{ab}	8.56	9.80	10.00
4	ETBW 5954	100.00^{a}	40.00^{abcdef}	10.00^{b}	8.50	10.00	10.00
5	ETBW 5510	83.33^{abcd}	56.67^{abc}	13.33^{b}	8.57	9.26	10.00
6	DEBEIRA	96.67^{ab}	23.67^{def}	10.00^{b}	8.53	9.80	10.00
7	SANDALL-3	63.33^{de}	23.33^{def}	10.00^{b}	8.55	10.00	10.00
8	PBW343	76.67^{bcd}	20.00^{ef}	10.00^{b}	8.75	10.00	10.00
9	VEE7	53.33^{e}	33.33^{bcdef}	10.00^{b}	8.50	10.00	10.00
10	NQALAB 91*2	86.67^{abc}	30.00^{cdef}	46.67^{a}	8.54	9.22	10.00
11	JNRB.5/PIFED	70.00^{cde}	63.33^{a}	20.00^{ab}	8.62	9.34	10.00
12	HUBARA-3*2	96.67^{ab}	43.33^{abcdef}	10.00^{b}	8.61	9.64	10.00
13	WBLL1	86.67^{abc}	60.00^{ab}	46.67^{a}	8.55	9.80	10.00
14	DEBEIRA//TEUEE	86.67^{abc}	40.00^{abcdef}	13.33^{b}	8.67	9.41	10.00
15	DEBEIR/	73.33^{cd}	50.00^{abcd}	46.67^{a}	8.78	9.75	10.00
16	FLORKWA-1	83.33^{abcd}	46.67^{abcde}	23.33^{ab}	8.60	9.80	10.00
17	KAUZ/RAYON//3//	63.33^{de}	30.00^{cdef}	20.00^{ab}	8.58	9.55	10.00
18	KAUZ'/SERI/4/CHEN	90.00^{abc}	16.67^{f}	10.00^{b}	8.53	9.75	10.00
	LSD(0.05)	22.44	28.61	29.36	NS	NS	NS
	CV (%)	16.75	46.09	90.12	1.56	3.39	2.05
	Salt		6.20**			0.10**	
	Variety		15.19**			NS	
	Salt x Variety		26.91*			NS	

The same letter within each column for each salt concentration are not significantly different at α= 0.05, based on LSD test. Where: NS (Non-significant); LSD (Least Significant Difference); CV (coefficient of Variance); ** (Significant at α= 0.001) and * (Significant at α= 0.05)

Table 3: The response of wheat varieties for plant height, effective tiller number, spike length, root length and grain yield under saline soil condition.

No.	Wheat Genotypes	Plant height (cm)	Effective tiller (#)	Spike length (cm)	Root length (cm)	Grain Yield (kg/ha)
1	PAVON76	78.18^{abcd}	5.07^{bc}	8.51^{ab}	10.18	3112.6^{abc}
2	AZAKIA-5	73.09^{cd}	6.82^{a}	8.09^{b}	9.78	3104.2^{abc}
3	ANGL	81.04^{ab}	4.98^{c}	9.09^{a}	10.22	2728.3^{c}
4	ETBW 5954	83.51^{a}	5.11^{bc}	8.97^{a}	11.04	3466.5^{ab}
5	DEBEIRA	76.39^{bcd}	5.04^{bc}	8.16^{b}	10.08	3097.1^{abc}
6	SANDALL-3	81.78^{ab}	5.47^{bc}	8.54^{ab}	10.51	3709.2^{a}
7	PBW-343	71.92^{d}	6.02^{abc}	8.45^{ab}	11.15	3017.2^{bc}
8	VEE7	76.59^{bcd}	6.20^{ab}	8.24^{b}	11.5	3327.6^{abc}
9	INRB	76.51^{bcd}	5.91^{abc}	8.99^{a}	9.81	3075.9^{abc}
10	GAMBOO	78.82^{abc}	6.00^{abc}	8.29^{b}	9.4	3230.4^{abc}
	LSD(0.05)	6.55	1.19	0.68	NS	691.28
	CV (%)	8.96	22.3	8.45	24.49	23.02

Grain yield

Grain yield showed significant difference ($P \leq 0.05$) among tested wheat genotypes, indicating existence of genetic variability among tested genotypes in response to salinity stress tolerance. Highest grain yield of 3709.2 kg/ha was recorded from SANDALL-3 genotype while minimum grain yield of 2728.3 kg/ha was obtained from ANGL genotype. The result clearly suggest genetic variability for salinity response and grain yield significantly affected by salt. High concentrations of sodium (Na+), chloride (Cl−), magnesium (Mg2+), calcium (Ca2+), sulphate (SO4 2−) and bicarbonate (HCO3 −) in soil disturb plant growth and development, ultimately leading to loss in yield (Shahbaz and Ashraf, 2007; Shahbaz *et al.,* 2011; *et al.,* 2012; Perveen Shahbaz *et al.,* 2012; bethel *et al.,* 2019).

Conclusion and Recommendation

During laboratory screening out of 200 wheat genotypes having higher total germination percentage at different salinity stress levels 18 were selected and promoted to seedling stage screening for further evaluation. Germination percentage was showed significant ($P \leq 0.05$) difference among tested wheat genotypes under all salt level. At seedling stage when the salt stress increase the time required for wheat germination was increased. Based on yield data from tested genotypes SANDALL-3, ETBW 5954 and VEE7 showed superior grain yield and the inferior was from ANGL. Ultimately, the results of this research will be a helpful and starting point for working on the validation and registration on future for salt tolerant wheat varieties.

References

Amor NB, Hamed KB, Debez A, Grignon C and Crabbedly. 2005. Physiological and antioxidant responses of perennial halophyte Crithmum maritimum to salinity. Plant Science 4: 889-899.

AOSA. 1983. Seed Vigor Hand Testing Book, Contribution No. 32 to the Handbook on Seed Testing. Association of Official Seed Analysis. Springfield, USA. 122-128 p.

Ashraf M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, 27: 84-93.

Ashraf MY, Akhtar K, Sarwar G and Ashraf M. 2002. Evaluation of arid and semi-arid ecotypes of guar (*Cyamopsis tetragonoloba* L.) for salinity (NaCl) tolerance. J. of Arid Environ. 52: 473-482.

Ashraf MY, Akhtar K, Hussain F and Iqbal J. 2006. Screening of different accessions of three potential grass species fron Cholistan desert for salt tolerance. Pak. J. Bot., 38: 1589-1597.

Attri H, Dey T, Sinha BK and Bawa V. 2018. Effect of Salinity Treatments of NaCl on Germination and Early Seedling Stage in Wheat (*Triticum aestivum* L.) Cultivars. Int. J. Pure App. Biosci. 6 (5): 1227-1232.

Bahmani K, Noori SAS, Darbandi AI and Akbari A. 2015. Molecular mechanisms of plant salinity tolerance: a review. Australia journal of crop science, 9(4):321-336.

Beakal Tadesse, Hussein Mohammed, Alemayehu Assefa. 2016. The effect of salinity on germination, vegetative and final growth stage of different rice (*Oryza sativa* L.) genotypes. *Journal of Animal and Plant Sciences*, 29(3):4651-4664.

Bethel Nekir, Lemma Mamo, Ashenafi Worku and Teshome Bekele. 2019a. Evaluation of Wheat Varieties/Lines for Salt Tolerance at Different Growth Stages. Greener Journal of Soil Science and Plant Nutrition, Vol. 6(1), pp. 1-7, 201

Datta JK, Nag S, Banerjee A and Mondal NK. 2009. Impact of salt stress on five varieties of Wheat (*Triticum aestivum* L.) cultivars under laboratory condition. J. Appl. Sci. Environ. Manage. 13(3): 93 – 97.

Ellis RA and Roberts EH. 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci Technol 9: 373-409.

FAO. 2010. FAO land and plant nutrition management service. www.fao.org [last accessed 10 December 2010].

Feroz A, Ali Z, Usman B, Niaz M, Saeed W, Abbas M, Khalid U, Manzoor N, and Hassan W. 2017. Evaluation of Saline Tolerant Wheat (*Triticum aestivum* L.) in F2 Segregating Populations International Journal of Scientific and Research Publications, 7 (4):2250-3153.

Gomez and Gomez H. 1984. *Statistical analysis for agricultural research*. .John Willy and Sons Inc. pp.120-155.

Hassen A, Maher S and Cherif H. 2014. Effect of Salt Stress (NaCl) on Germination and Early Seedling Parameters of Three Pepper Cultivars (*Capsicum annuum* L.) Journal of Stress Physiology & Biochemistry, 10(1) :14-25.

Homayoun H. 2011. Effect of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars at germination stage. Advances in Environmental Biology 5(7):1716-1720.

Jalali V, Asadi-Kapourchal S and Homaee M. 2017. Evaluating performance of macroscopic water uptake models at productive growth stages of durum wheat under saline conditions. *Agricultural Water Management*, 180:13–21.

Kalhoro NZ, Rajpar I, Kalhoro S, Amjad Ali, Sajjad Raza, Muneer Ahmed, Fahad Ali Kalhoro, Muhammad Ramzan and Fazli Wahid. 2016. Effect of Salts Stress on the Growth and Yield of Wheat (Triticum aestivum L.). American Journal of Plant Sciences, 7: 2257-2271.

Khan MA, Shirazi MU, Ali M, Mumtaz S, Sherin A and Ashraf MY. 2006. Comparative performance of some wheat genotypes growing under saline water. Pak. J. Bot., 38: 1633-1639.

Mickelbart MV, Hasegawa PM and Bailey-Serres J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat. Rev. Genet. 16, 237–251.

Mirza RA and Mahmood K. 1986. Comparative effect of sodium chloride and sodium bicarbonate on germination, growth and ion accumulation in Phaseolus aureus, Roxb, c.v. 6601. Biologia, 32: 257-268.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell and Environment 25, 239-250.

Nishimura T, Chaum S, Takagaki M, Ohyama K. 2011. Survival percentage, photosynthetic abilities and growth characters of two indica rice (*Oryza sativa L.*) cultivars in response to isosmotic stress. *Spain Journal of Agricultural Research*, 9: 262-270.

Oyiga BC, Sharma RC, Shen J, Baum M, Ogbonnaya FC, Leon J and Ballvora A. 2016. Identification and Characterization of Salt Tolerance of Wheat Germplasm Using a Multivariable Screening Approach. J Agro Crop Sci, 1-14.

Perez-Alfocea F, Santa-Cruz A, Guerrier G and Bolarin MC. 1994. NaCl stress-induced organic solute changes on leaves and calli of Lycopersicon esculentum, L. pennellii and their

Qadir M, Oster J, Schubert S, Noble A and Sahrawat K. 2007. Phytoremediation of sodic and saline-sodic soils. *Advances in Agronomy*, 96: 197-247.

Rafiq S, Iqbal T, Hameed A, Rafiqi ZA, and Rafiq N. 2006. Morphobiochemical analysis of salinity stress response of wheat. Pakistan Journal of Botany 38(5), 1759-1767.

Rahman M, Kayani SA and Gul S. 2000. Combined effects of temperature and salinity stress on corn cv. Sunahry, Pak. J. Biological Sci., 3(9): 1459-1463.

Rahman M, Soomro UA, Zahoor-ul-Haq M and Gul S. 2008. Rahman, Effects of NaCl Salinity on Wheat (*Triticum aestivum* L.) Cultivars. World Journal of Agricultural Sciences 4 (3): 398-403.

Redondo-Gomez S, Mateos Naranjo E, Garzon O, Castillo JM, Luque T, and Figueroa ME. 2008. Effects of salinity on germination and seedling establishment of endangered Limonium emarginatum (Willd.) O. Kuntze. J. Coastal Res., 24, 201-205.

Sajid Z, Liaqat S, Anum W, Ammar A, Kanwal N, Zafar A, Ahmad RA, Ahmed G, Karim A. 2017 Screening of wheat (*Triticum aestivum* L.) genotypes for salinity tolerance at seedling stage. International Journal of Biosciences 10 (4):275-283.

Shahbaz M and Ashraf M. 2013. Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences,* 32: 237–249.

Shahbaz M and Ashraf M. 2007. Influence of exogenous application of brassinosteroid on growth and mineral nutrients of wheat (*Triticum aestivum* L.) under saline conditions. Pak. J. Bot. 39: 513–522.

Shahbaz M, Ashraf M, Akram NA, Hanif A, Hameed S, Joham S, and Rehman R. 2011. Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). Acta Physiol. Plant. 33: 1113–1122.

Shahbaz M, Ashraf M, Al-Qurainy F, and Harris PJC. 2012. Salt tolerance in selected vegetable crops. Crit. Rev. Plant Sci. 31: 303–320.

Shirokova YI, Forkutsa and Sharafutdinova N. 2000. Use of electrical conductivity instead of soluble salts for soil salinity monitoring in central Asia. *Irrigation and Drainage Systems,* 14(3): 199-206.