

## EVALUATION AND IDENTIFICATION OF SALT TOLERANT WHEAT THROUGH *IN VITRO* SALINITY INDUCTION IN SEEDS

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### Abstract

Salinity is one of the major growth and yield limiting factor in crop production. Wheat being staple food loses its yield potential when grown in saline soils, however, this problem can be managed by identification and growing the salt tolerant genotypes. Seed is a primitive part which when exposed to salinity gets problem in germination. Current study was planned to investigate seed germination indicators for early selection of salinity tolerant wheat genotypes. Three salinity levels along with one control i.e., 0 mM, 50 mM, 100 mM and 150 mM were used to treat 125 genotypes sown in complete randomized design in replicates. All the genotypes were found significant for germination speed index (GSI), time required for germination of 50% seeds (T50), mean germination time (MGT), mean germination rate (MGR), variance of germination time (VGT), coefficient of variation of germination time (CVt), germination speed coefficient (CVG), and non-significant for final germination percentage (FGP), time required for germination of 90% (T90), germination synchrony (Sync) and uncertainty (Unc). Salinity treatments were found to be significant for all seed germination indicators except uncertainty (Unc). FGP had positive correlation with GSI, MGT, T50, T90, Sync and Unc, and negative correlation with CVG, MGR and CVt. By contrast, MGR showed negative correlation with all indicators except CVt. Moreover, MGT was found positive correlated with FGP, GSI, T50, T90, VGT and Uni, however, negative correlation was observed with CVG, MGR, CVt and Sync. Based on the salt tolerant index (STI), our results categorize wheat genotypes into tolerant (11 genotypes), moderate tolerant (38 genotypes), moderate sensitive (48 genotypes) and sensitive (28 genotypes). Overall, these findings reveal important parameters to select salinity tolerant plants at germination stage which could help in early identification of salt-tolerance genotypes *In vitro*.

**Key words:** Correlation, Germination, Indicators, Parameters, *Triticum aestivum*, Salinity stress.

### Introduction

Wheat is the important grain used by one third population of world and the main source of carbohydrate, protein and minerals in daily food. Recent shifts in climate change has largely affected the crop yields and quality by unpredicted occurrence and extent of various abiotic stresses including drought, salinity and flooding. Abiotic stresses generally affect the cellular homeostasis mechanism and the metabolic processes of the plant cells that ultimately results into reduced plant growth and production (Mickelbart *et al.*, 2015). Salinity is a major culprit that largely compromises crop productivity worldwide, especially grain crops. It badly affects normal plant growth and cause serious threats to development and survival in wheat plant (Houle *et al.*, 2001; Pessaraki, 2015). These threats are often accompanied by abnormalities in biochemical and physiological processes especially in photosynthesis, nutritional uptake and cell metabolism, and cause serious damage to oxidative balance, membrane stability and cell division (Houle *et al.*, 2001; Manjili *et al.*, 2012; Panuccio *et al.*, 2014).

Effects of salinity vary depending upon the plant growth stages and can be assessed by seed germination, plant growth, and plant ability to uptake nutrients with water (Willenborg *et al.*, 2004). Seed germination considered as initial stage of plant life that determines the potential of seed to grow into healthy and productive plant. Salinity restrict seed germination and initial plant growth by creating osmotic pressure around plant root zone and within the root cells causing ion toxicity that hinder water uptake. Seed germination is largely affected by toxicity of sodium and chloride ions (Atak *et al.*,

2006), however, ability to withstand this toxicity vary from species to species and even from plant to plant (Atak *et al.*, 2006). Salt concentration is usually high in areas of low rainfall with high evapotranspiration where soils are insufficient to leach salts down (Neumann, 1995; Saboora *et al.*, 2006). Thus, achieving uniformity in seed germination for a grain crop is a serious issue in regions of water shortage due to salinity (Demir *et al.*, 2003), and significantly reduced plant yields due to low seed germination (Ghoulam and Fares, 2001). Several studies have indicated that the primary reasons for low germination under salinity are the problem in up-taking of water by seeds due to toxicity and high concentration of salts (Khajeh-Hosseini *et al.*, 2003; Atak *et al.*, 2006).

Exposure of salt stress and stage of plant are very important and determining factors in selection of best salt tolerant variety. Previous work on screening for salt tolerance in crop plants mostly surrounds evaluating seedlings and mature plants through physiological and morphological characters, however, seed germination criteria could be used as a quick screening approach to identify salt-tolerant genotypes. Various studies indicating salinity based reductions in germination percentage, germination rate and mean germination time (Houle *et al.*, 2001; Sanchez *et al.*, 2014; Hadas 1977; Bayuelo-Jiménez *et al.*, 2002) have lead our attention to use germination characters for selection of salt tolerant genotypes in wheat. Seed indices such as seed vigor, germination rate and index depend upon the number of seed germinated in a specific time frame, but salinity, on the other hand delays/prevents seed germination (Bybord, 2010; Sanchez *et al.*, 2014).

Keeping in view the grain yield losses due to low germination by salinity, it is very crucial to tackle salinity problem at germination level to improve global wheat crop productions by devising quicker and early stage screening approaches against salinity. Current study was designed to establish indicators of salt-tolerance at seed germination stage in wheat. Particularly, the aim was to perform quick and effective screening of wheat genotypes at seed germination stage against salinity for early selection of tolerant genotypes rather than later stages of maturity.

## Materials and Methods

**Parent material and growth conditions:** Parent material (wheat genotypes) were collected from Wheat Research Institute, AARI, Faisalabad, Pakistan. Seeds of one hundred and twenty-five wheat (*Triticum aestivum*) genotypes were subjected to three salinity levels including one control (0 mM (T0), 50 mM (T1), 100 mM (T2) and 150 mM (T3) with three replicates in a CRD manner. The seed surface was sterilized for 3 minutes using 0.5% sodium hypochlorite and then washed with distilled water thrice. 100 seeds per genotype were used for each treatment in each replication. The seeds were placed onto filter papers stacked on top of 3-4 layers of tissue papers in germination trays. Saline solution was prepared using sodium chloride (NaCl) in distilled water and poured onto tissue papers for complete soaking. For the control treatment, distilled water was used for soaking of tissue paper. Germination trays were sealed with parafilm and kept in a growth chamber at  $24\pm 1^\circ\text{C}$  temperature,  $80\pm 3\%$  relative humidity with  $50 \mu\text{mol m}^{-2}\text{s}^{-1}$  light intensity under long day conditions (18-h photoperiod).

**Seed germination data:** To calculate different aspects of seed germination, the germinated seeds were counted from the first day of germination to the seventh day. Germination percentage data was recorded by counting number of seeds germinated each day.

**Salt tolerance index (STI):** Salt tolerance index (STI) based at seed final germination percentage at 150mM was calculated using following formula (Ali *et al.*, 2007).

$$\text{STI} = \frac{\text{Germination percentage under stress condition}}{\text{Germination percentage under controlled condition}} \times 100$$

**Final germination percentage (FGP):** FGP is the percentage of number of seeds germinated out of total seeds used per single experimental unit and calculated by method proposed by (Anon., 2015).

$$\text{FGP} = (n/N) \times 100$$

“ $n$  = seeds germinated counts;  $N$  = total seeds counts”

**Germination speed index (Germination rate index) (GSI):** It is speed at which seed achieve germinated status. It is calculated by observing and recording number

of seeds germinated on each day and calculated using following formula (Maguire, 1962).

$$\text{Germination Index} = \sum_{i=1}^k \left( \frac{ni}{ti} \right)$$

“ $n$  = seeds germinated counts on each day;  $t$  = days counts after the beginning of the test in each count;  $k$  = Last count of the germination test.”

**Time required for germination of 50% of the seeds (T50):** It is the time at which 50% of the seeds within an experimental unit germinate and calculated using formula (Farooq *et al.*, 2005).

$$T_{50} = \frac{ti + \left\{ \left[ \frac{N}{\left( \frac{100}{50} \right)} \right] - ni \right\} (tj - ti)}{(nj - ni)}$$

“ $N$  = Final seeds germinated counts;  $ni$  and  $nj$  = Total seeds germinated counts in adjacent counts in time  $ti$  and  $tj$ , respectively, when  $ni < \frac{N+1}{2} < nj$ ”

**Time required for germination of 90% of the seeds (T90):** It is the time at which 90% of the seeds germinate and calculated using given formula (Farooq *et al.*, 2005).

$$T_{90} = \frac{ti + \left\{ \left[ \frac{N}{\left( \frac{100}{90} \right)} \right] - ni \right\} (tj - ti)}{(nj - ni)}$$

“ $N$  = Final seeds germinated counts;  $ni$  and  $nj$  = Total seeds germinated counts in adjacent counts in time  $ti$  and  $tj$ , respectively, when  $ni < \frac{N+1}{2} < nj$ ”

**Mean germination time (MGT):** MGT is the time taken by number of seeds germinated per day at same time and calculated by following formula (Labouriau, 1983).

$$\text{MGT} = \frac{\sum_{i=1}^k ni ti}{\sum_{i=1}^k ni}$$

“ $Ni$  = seeds germinated counts per day;  $ti$  = Time since the beginning of the germination test up to the  $i$ -th observation”

**Mean germination rate (MGR):** It is the measure of germination time course and is usually expressed as a percentage (Labouriau, 1983).

$$\bar{v} = \frac{CoVg}{100} = 1/\bar{t}$$

“ $\bar{t}$  = Mean germination time;  $CoVg$  = Germination speed coefficient.”

**Variance of germination time (VGT):** Variance of germination time was calculated by method proposed by (Labouriau, 1983).

$$S_t^2 = \frac{\sum_{i=1}^k n_i(t_i - \bar{t})^2}{(\sum_{i=1}^k n_i - 1)}$$

“*t* = Mean germination time; *t<sub>i</sub>* = Time between the beginning of the experiment and the *i*-th observation (day or hour); *n<sub>i</sub>* = seeds germinated counts in time *i*, and *k* is the last count of the germination test.”

**Coefficient of variation of germination time (CVt):** It is the standard deviation of germination time over mean germination time and calculated by following method (Carvalho *et al.*, 2005).

$$CVt = (St/t) 100$$

“*St* = Standard deviation of germination time; *t* = Mean germination time.”

**Germination synchrony (Sync):** The germination synchronization means that synchrony of one seed with another included in the same replication of one treatment and calculated by formula as described below (Primack, 1980).

$$Z = \sum Cni, 2 / N$$

$$Cni, 2 = ni(ni-1)/2 \text{ and } N = \sum ni (\sum ni - 1) / 2$$

“*Cni* = Combination of the seeds germinated in time *i*, two by two; *ni* = seeds germinated counts in time *i*.”

**Uncertainty (Unc):** Uncertainty is associated to the distribution of the relative frequency of germination and calculated by following formula (Labouriau and Valadares, 1976).

$$\bar{E} = \sum_{i=1}^k fi \log 2 fi$$

with *fi* given by,

$$fi = ni / \sum_{i=1}^k ni$$

“*fi* = Relative frequency of germination; *ni* = seeds germinated counts on day *i*.”

**Germination speed coefficient (CVG):** Germination speed coefficient was calculated by method proposed by (Nichols, 1968).

$$CVG = (\sum_{i=1}^k fi / \sum_{i=1}^k (fixi))100$$

“*fi* = germinated seeds counts emerged on day *i*; *xi* = days counts from sowing.”

**Statically data analysis:** The data was statistically analyzed through F-analysis of variance under CRD design and the treatment means were compared by LSD test at *p*<0.05 and *p*<0.01 probability levels. Correlation, principal component analysis and seed germination indicators were analysed through R-programing.

**Results and Discussion**

**Effects of salinity on germination attributes *In vitro*:**

The data recorded on germination related parameters from 125 wheat genotypes showed that salinity causes significant detrimental effects with increasing NaCl concentrations on all characters under study (Table 1, Figs. 1 a, b and 2). In particular, final germination percentage (GFP) was observed to be significant for treatment levels whereas non-significant for genotypes (Table 1). Overall, data indicated that seed germination of genotypes was affected by concentration of salts (Fig. 1a). It was observed that 98% of seeds were germinated under control conditions without any salt treatment (0 mM) compared to the highest level of salt treatment at 150mM where at maximum 59.8% seed germination was recorded (Table 2). As a major indicator of seed germination, the radical length showed growth defects and observed to be largely influenced by induced salinity (Fig. 1b). Germination seed index (GSI), mean germination time (MGT), mean germination rate (MGR), variance of germination time (VGT), coefficient of variation of germination time (CVt) and germination speed coefficient (CVG) were significant for both salt treatments and wheat genotypes. There was continuous decrease in GSI, MGT, VRG of wheat genotypes and continuous increase in mean value of CVt and CVG with increase in level of salinity (Tables 1 and 2). Highest salinity level at 150 mM resulted in significant reduction in time required for germination of 50% (T50) and 90% (T90) of the seeds when compared with the control. The genotypes were significant, upon time required to achieve 50% seed germination, but were non-significant in case of time required to achieve 90% seed germination. Uncertainty (Unc) of germination process was found non-significant (Table 1) and mean value of Unc was nearly unchanged, while Synchrony (Sync) of germination was significant only for treatments (Table 2; Fig. 1).

**Correlations among seed germination indicators with respect to salinity:**

Estimated correlation results showed that positive and negative correlation exist among various seed germination indicators. The FGP had positive correlation with GSI, T50 and T90, MGT, VGT, CVt and Unc, while FGP had negative correlation with MGR, Sync and CVG (Table 3). The highest positive correlation was found between MGR and CVG (*r*=0.99) while minimum positive correlation was found between MGT and GSI (*r*=0.02). CVG and MGR were positive correlated with each other, CVt and Sync. Furthermore, both CVG and MGR showed negative correlation to rest of indicators (Table 3). The highest negative correlation was found between MGT with CVG and MGR (*r* = -0.99), while minimum negative correlation was found between MGR and GSI (*r* = -0.01) (Table 3).

**Table 1. Mean square calculated through ANOVA for wheat genotypes and salinity treatments.**

SOV	df	GFP	GSI	T50	T90	MGT	MGR	VRG	Cvt	Sync	Unc	CVG
Treatments	3	39619***	603.36*	18.075***	5.678***	8.5207***	0.598***	1.088***	120.2***	0.0069**	0.0204	5934.3***
Varieties	124	8	0.57**	0.0274*	0.0498	0.0142***	0.00156***	0.0177***	17.43***	0.0016	0.00763	13.9**
Errors	372	8	0.37	0.0198	0.0434	0.0081	0.001	0.0112	9.34	0.0017	0.00816	9.3

\*significant at <0.05, \*\*significant <0.01, \*\*\*significant <0.001

Final germination percentage (FGP), Germination speed index (GSI), Time required for germination of 50% of the seeds (T50), Time required for germination of 90% of the seeds (T90), Mean Germination Time (MGT), Mean germination rate (MGR), Variance of germination time (VGT), Coefficient of Variation of Germination Time (CVt), Germination synchrony (Sync), Uncertainty (Unc), Germination speed coefficient (CVG)

**Table 2. Mean comparison salinity treatment on seed germination indicators of wheat genotypes.**

Treatments	GFP	GSI	T50	T90	MGT	MGR	VRG	Cvt	Sync	Unc	CVG
0	98.5a	13.65b	1.97a	2.79a	2.23 a	0.44 c	0.75 a	39.12 c	0.36 a	1.44 a	44.79 c
50 mM	91.9b	13.06a	1.24b	2.35b	1.73 b	0.57 b	0.54 c	42.66 b	0.35 bc	1.45 a	57.54 b
100 mM	72.2c	13.01c	1.17c	2.36b	1.67 b	0.59 a	0.58 b	45.97 a	0.36 ab	1.42 b	59.84 a
150 mM	59.8d	10.43d	1.12b	2.30b	1.67 b	0.57 b	0.56 b	45.27 a	0.34 c	1.43 ab	57.90 b

Final germination percentage (FGP), Germination speed index (GSI), Time required for germination of 50% of the seeds (T50), Time required for germination of 90% of the seeds (T90), Mean Germination Time (MGT), Mean germination rate (MGR), Variance of germination time (VGT), Coefficient of Variation of Germination Time (CVt), Germination synchrony (Sync), Uncertainty (Unc), Germination speed coefficient (CVG)

**Table 3. Correlation among seed germination indicators of wheat genotypes under saline condition.**

	CVG	MGR	CVt	Sync	Unc	VGT	T90	MGT	T50	GSI	FGP
CVG	1.00										
MGR	0.99*	1.00									
CVt	0.53*	0.54*	1.00								
Sync	0.19*	0.19*	-0.015	1.00							
Unc	-0.33*	-0.32*	0.021	-0.88*	1.00						
VGT	-0.67*	-0.66*	0.173*	-0.23*	0.32*	1.00					
T90	-0.82*	-0.81*	-0.062	-0.407*	0.53*	0.89*	1.00				
MGT	-0.99*	-0.99*	-0.558*	-0.117*	0.26*	0.65*	0.79*	1.00			
T50	-0.83*	-0.83*	-0.496*	0.153*	-0.001	0.57	0.64*	0.87*	1.00		
GSI	-0.016	-0.01	-0.104*	0.181*	-0.073	-0.025	-0.012	0.02	0.131*	1.00	
FGP	-0.614*	-0.61*	-0.508*	0.082	0.083*	0.304*	0.42*	0.62*	0.63*	0.782*	1.00

\*Significant

Final germination percentage (FGP), Germination speed index (GSI), Time required for germination of 50% of the seeds (T50), Time required for germination of 90% of the seeds (T90), Mean Germination Time (MGT), Mean germination rate (MGR), Variance of germination time (VGT), Coefficient of Variation of Germination Time (CVt), Germination synchrony (Sync), Uncertainty (Unc), Germination speed coefficient (CVG)

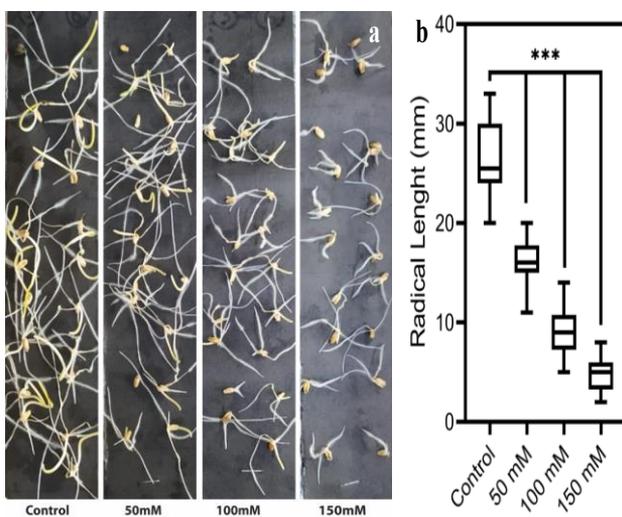


Fig. 1. Seed germination analysis at different treatments of NaCl. a. Representative images of germinated seeds at different induced levels of salt stress. Obvious difference can be seen in radical length under induced salinity. b. Statistical data representing significant differences in radical length at different salt treatments.

**Identification of genotypes based on salt tolerance index (STI):** Salt tolerance index (STI) at germination was calculated at 150 mM salt treatment. The percentage range of seed germination at 150 mM NaCl was observed to be 56 to 67 depending upon the genotype (Fig. 2). Based on STI, 11 varieties were found tolerant, 38 moderately tolerant, 48 moderately sensitive and 28 sensitive (Fig. 3, Table 4). The varieties considered to be tolerant that showed approximately 67% germination at 150mM salt treatment, while varieties germinated less than 60% were categorized as sensitive (Table 4).

**Principal component analysis (PCA):** The two principal components explain 69.2% of the variability, with 50.2% in the first principal component (PC1) and 19.0% in the second component (PC2). All the varieties were grouped clearly in each treatment. MGT, T90 and VGT (VarGer) were found to be on upper side of PC1 while T50 FGP and GSI were located on lower side of PC1. The impact of treatments was significantly higher on all characters as treatments dominantly fall in upper left side of PC1 (Fig. 4).

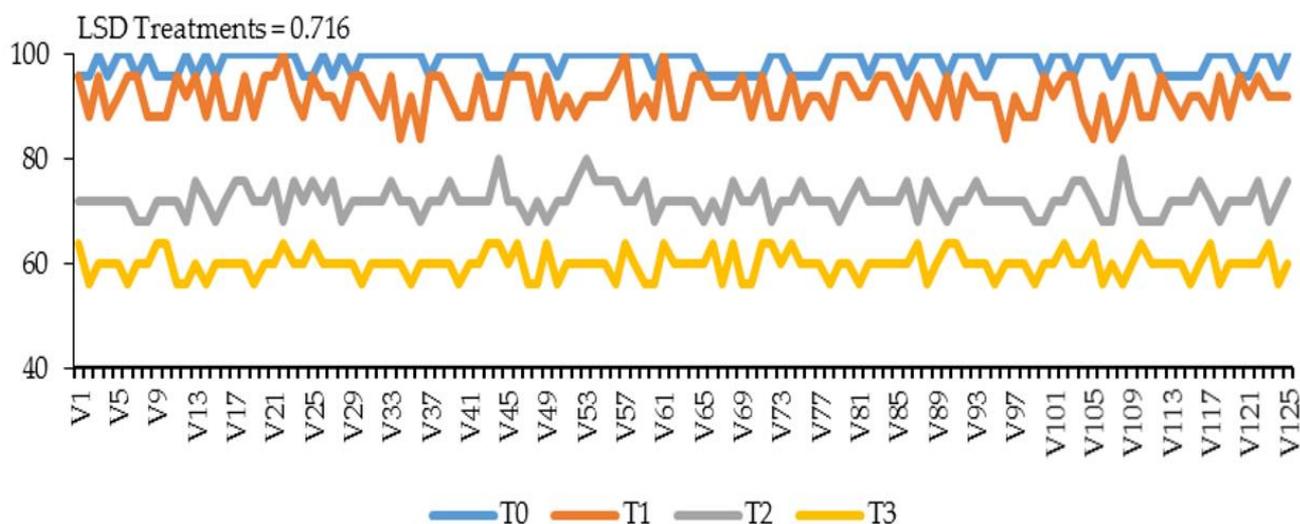


Fig. 2. Final germination percentage (FGP) of varieties under salt treatments i.e. control (T0) 50mM (T1), 100mM (T2) and 150mM (T3).

**Table 4. Salt tolerance categories of wheat genotypes, based on salt tolerance index (STI) at 150 mM salt stress.**

Salt tolerance category	STI range	Name of accessions
Tolerant	67%	FAREED06 (V1), HOOSAM3(V9), SAAR(V10), Pak2013 (V25), SUNCO//TNMU/TUI (V42) SHARP/3/PRL/SARA//TSI/VEE#5/5/VEE/LIRA//BOW/3/BCN/4/KAUZ (V44), TRCH// PRINIA/PASTOR (V66), LYP-73 (V68), NR-371(V71), 99108(V74), V-13005(V90)
Moderately tolerant	63%	SA-42 (V4), NACUZARIF-76(V7), FRONTANA(V13), PARULA=PRL(V15), 122526(V24), Long grain(V27), V06018(V37), DOLLARBIRD(V45), FRET2/KUKUNA// FRET2/3/PARUS/ 5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ (V65), V-11365(V75), 11B2049(V76), 11BT004(V77), V-11001(V82),PFAU/SERI.1B//AMAD/3/(V86), V-13241(V94), 10FJ21(V100), NR-429(V103), TWS-12464(V107), morroco(V112), 10821(V113), 11526(V114), Chakwal-86 (V116), 11380 (V120), 11287 (V121), CROC-1/AE.SQ(224)//OPATA/3/FLAG-7 (V22), KIRITATI (V46), NINGMAI-50 (V49), WBLLI*2/VIVITSI/3/T.DICOCCOMP194624/AE.SQ(409)//BCN/4/WBLL1*2/ (V56), V-11186 (V61), NR-403 (V72), V-12253 (V87), V-13016 (V91), MSW(V102), Triticumpyrum (V-2) (V105), NIBGE GANDUM N (V110), LU26S (V117), 10813(V123)
Moderately sensitive	60%	PASINA 90 (V3), SATLUJ-86 (V5), WH-542 (V8), NING-8319 (V16), HARRIER 17.B (V17), PB81//F3.71/TRM/3/BULBUL (V18), WL 711/CROW ‘S’//ALD #1 / CMH77A.917/3/HI 666/PVN ‘S’(V20), KANZ*4/KS85-8-4/5/2*FRET2*2/4/SNI (V21), SERI.1B*2/3/KAUZ*2/ (V23), 13248 (V26), NSW-14 (V28), V-04181 (V31), V-04048 (V32), V05115 (V33), V06129 (V34), TW69019 (V36), KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ (V38), WHEAR/VIVITSI//WHEAR (V39), INQALAB91*2/KUKUNA// (V41), SUNCO//TNMU/TUI (V43), HD 2169/C591//PBW343 (V51), AS2002/WL711//SHAFaq (V52), INQ91/YR-31 (V53), V-04179/T7 T.sphaerococcum (V54), V-04179/T7 (T.sphaerococcum)-drought (V55), TOBA97/PASTOR*2//(V58), MUNAL#1 (V62), TACUPETO F2001/BRAMBLING//(V63), ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/ PRL/SARA// TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA (V64), 76377 (V73), NW-10-1111-7 (V79), V-11046 (V80), 12292 (V83), D67.2/PARANA 66.270//AE.SQ (320)/3/CUNNINGHAM/4/ VORB (V84), ATTILA*2/PBW65*2// (V85), V-12130 (V92), V-12057 (V93), V-13270 (V96), 122557 (V97), 12BT012 (V98), TW/424 (V101), NR-449 (V104), 14C036 (V109), 14170 (V111), Kohistan-97 (V119), 11386 (V122), 11464 (V125)
Sensitive	< 60%	SHAHKAR 95 (V6), CHILERO=CHIL'S' (V12), HARTOG=HTG (PAVON) (V14), PB-96/87094//MH-97 (V19) V-02192 (V30), V-056132 (V35), WHEAR/CHAPIO//WHEAR (V40), PFAU/ WEAVER*2// (V47), PGO/SERI//BAV92 (V48), WBLLI*2/VIVITSI/3/T (V56), DICOCCOMP194624/AE.SQ(409)// BCN/4/ WBLL1*2/T (V57), SPELTA P1348764//INQ.91*2/TUKORU/3/WBLL1*2/TUKURU (V59), V-12284 (V78), NR 411 (V81), V-11160 (V88), V-12066 (V95), 12C027 (V99), 088200 (mono tiller early maturity with less lodging) (V106), NR-487 (V108), 10849 (V118), MIRAJ-08 (V2), CHAM-4 (V11), CROC_1/AE.SQUARROSA (205)//FCT/3/PASTOR (V50), V-11179 (V60), SOKOLL*2/ TROST (V67), YECORA-70 Sr2 (V69), NR 388 (V70), Pasban-90 (V115),Chakwal-97 (V124)

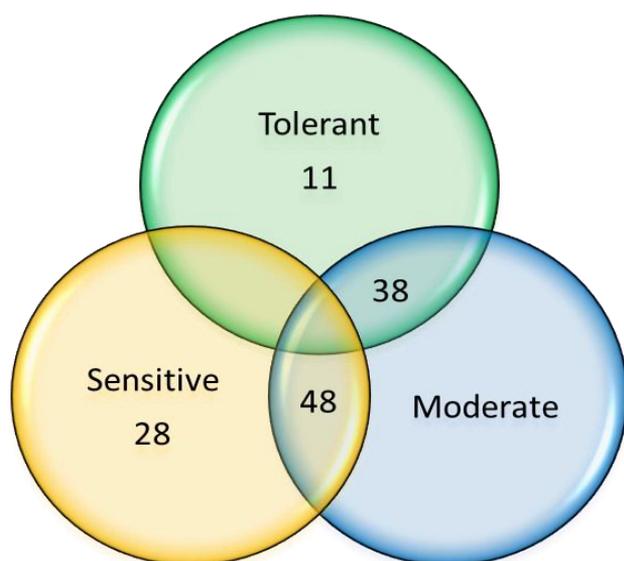


Fig. 3. Distribution of varieties on the basis of tolerance and sensitivity against salinity.

### Discussion

Salinity is one of the major yield limiting factor in crop plants that often linked with low seed germination in salt affected areas. Our data on various seed germination related parameters in wheat genotypes are in agreement with the notion of salinity involvement in reduced seed germination in wheat. Particularly, it is important to note that FGP was considerably reduced with raise in salt concentration (Tables 1, 2 and Fig. 1a,b) with exception of some varieties which perform better than other varieties under high salt treatments (Fig. 2). In comparison, no obvious differences were observed for germination under normal conditions without any salts indicating highest seed germination in control. These findings are in related with previous reports in wheat where germination percentage was reduced at 7dS/m salinity level compared to 3.5dS/m (Aflaki *et al.*, 2017). These adverse effects in germination are might be due to reduction in water absorption by seeds due to high salinity which decreased osmotic pressure and create abnormalities in normal water uptake mechanisms (Bayuelo-Jiménez *et al.*, 2002). Our correlation results in line with previous studies also showed that germination percentage, seed vigor and daily germination mean are positively correlated to each other, while negative correlation was found in mean of germination time with germination rate, coefficient and germination time mean (Aflaki *et al.*, 2017).

Seeds tend to absorb water more slowly under saline conditions than in normal conditions. It has been noticed that increased salt concentrations causes accumulation of phenolic compounds in seeds that disturb normal metabolic processes (Ayaz *et al.*, 2000). These accumulated inhibitors and limited water movement, in turn, decreases germination rate (Hadas 1977). In addition, excessive toxic cations and anions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) are produced in seeds due to saline condition, decreasing germination rate and percentage of seed germination

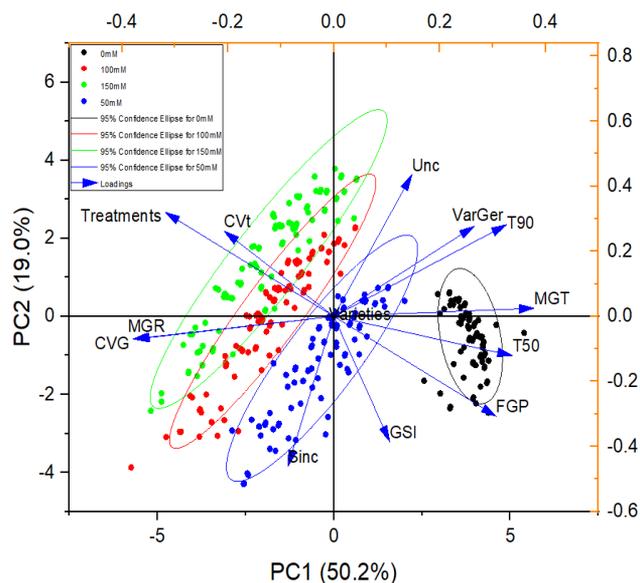


Fig. 4. PCA analysis among different salinity levels and seed growth attributes in wheat.

(Houle *et al.*, 2001; Sanchez *et al.*, 2014). Moreover, lower concentration of  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  and  $\text{KCl}$  also affect germination rate, but non-significantly affect germination percentage (Panuccio *et al.*, 2014). It is suggested that the salt tolerant plants have relatively higher germination due to accelerated absorbing of sodium ions (Zhang *et al.*, 2010). Furthermore, salt tolerant varieties are found to have higher membrane stability, production of higher amount of peroxidases, rubisco, proline and high stomatal conductance compared to salt sensitive varieties (Manjili *et al.*, 2012; Pazuki *et al.*, 2013).

A recent report on screening of wheat for salinity tolerance demonstrates that the effect of genotype was not significant for germination rate coefficient, seed vigor, average of daily germination compared to the effect of salinity which was significant on genotypes. In addition, germination stress index, mean of seed vigor and germination was observed to be higher in control than salinity treatments (Aflaki *et al.*, 2017). These reports are in line with our findings of effects of salinity on various germination related parameters in wheat (Figs. 1 and 2, Tables 1 and 2) suggesting that screening of salt tolerant genotypes at germination can be a useful and quicker approach for crop breeding against salinity.

### Conclusions

Salinity, not only affects plant morphology, but also disturbs biochemical and physiological processes in plants. During sowing under saline conditions, seed is directly exposed to salts and largely compromises germination. It is found that excess amount of sodium ions inhibit seed imbibition mechanics and delay or prevent seed to germinate. As a result, seed germination index, germination time, and germination percentage become abnormal causing significant damage to plant yields per unit area. To overcome this issue, salt tolerant genotypes with higher survival rate at germination should be identified. In this study, we identified better

performing salt genotypes at higher level of salinity through a much quicker approach which is a useful resource for wheat breeding program against salinity. Overall, this simple approach will lead to the much quicker screening of salt tolerant genotypes at early stages of seed germination.

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