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Comparative effects of PEG_{6000} and NaCl simulation osmatic induced solutions stress on morphological seedling traits in wheat plant under lab conditions

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ABSTRACT

Development of water and salinity stresses tolerant genotypes through screening is one the most important strategy to overcome these problems. In the present study, seeds of 24 local and exotic genotypes of wheat were allowed to germinate at varying levels of polyethylene glycol (PEG_{6000}) as induced osmotic water stress potential (PEG_{6000} 0 (distilled water as a control treatment), 5%, 10%, 15% and 20%) and NaCl as induced osmotic salinity stress potential (0 mM (distilled water as a control treatment), 50mM, 100 mM, 150 mM and 200 mM) for two weeks on three layers of Whatmann paper in Petri dishes in factorial laboratory experiment with three replicates and complete random design. Data were analyzed with analysis of variance ($P<0.01$), mean comparisons of LSD at 0.05 and 0.01 levels, biplot analysis and Euclidean distance. When data was found to be significant ($P<0.01$) the mean comparisons was calculated according to t-test. The results of the study indicated that there was significant decrease on germination parameters (germination percentage) and growth parameters (seedling length, root numbers, seedling fresh and dry weights, tissue water content and vigor index) with subsequent treatments. Biplot analysis classified the tested genotypes in same group as drought and salinity tolerant genotypes. Cluster analysis based on Euclidean distance, classified the genotypes into three groups. These conclusively confirm that the adverse effects of PEG_{6000} on germination and early seedling growth were due to the osmotic effects rather than the specific ion because of seedling growth was reduced by both stresses, but NaCl usually caused less damage than PEG_{6000} to wheat seedlings, suggesting that NaCl and PEG_{6000} acted through different mechanisms. Overall, the genotypes (L (Sids4 X Kasonygiennson-81), Bectora, Canada-462, Benisuaif-6 and CIMMET-113) and (CIMMET-251, CIMMET-270, CIMMET-234, CIMMET-95 and Gimeza-10) showed promising performance against PEG_{6000} and NaCl simulation levels, respectively in compared to other genotypes due to their superior performance in the study.

Keywords: Wheat Plant, Seed Germination, Seedling Morphology Stage, PEG_{6000}, NaCl, Biploit analysis, Euclidean Distance.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the most significant staple food crop. Globally after maize, is the second most produced food among the cereal crops (Datta *et al.,* 2009 and Bhutto *et al.,* 2018).

Seed germination is the most sensitive stage of plant growth under environmental stresses especially salinity and drought conditions (Hassan, 2015; Chachar *et al.,* 2016; Rana *et al.,* 2017; Soare *et al.,* 2018; Zhu *et al.,* 2019 and Ahmed *et al.,* 2020). So, the success in growth and yield production is depending on this stage. Salinity and drought stresses are important environmental factors that affect different developmental stages of crop plants in a similar way by impair cell metabolism and photosynthesis, leading to injury and death of the plant in excessive cases (Jaleel *et al.,* 2007; Nyagah and Musyimi, 2009) acting reduction in plant growth and final crop production (Kausar *et al.,* 2013; El-Hamamsy and Behairy, 2015; Shamaya *et al.,* 2017; Abdallah Hussein and Jin Ho Joo, 2018 and Kayacetin *et al.,* 2018 and Ali *et al.,* 2019). Under these stresses, the seed germination is affected by creating an external osmotic potential that prevents water uptake leading to reduction in water availability due to the toxic effects of Na^+ and Cl^- ions in the cytosol during imbibition and seedling establishment (Sayar *et al.,* 2010).

Polyethylene glycol (PEG) widely used to induce water stress as a non-ionic water polymer which is not expected to penetrate into plant tissue rapidly (Kawasaki *et al.,* 1983). In contrarily, Na^+Cl^- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive genotypes (Genc *et al.,* 2007). Several studies was done in the effects of PEG and NaCl in wheat and other crops, (Datta *et al.,* 2009) found that germination percentage was reduced with a high NaCl concentrations. In addition, Babu *et al.,* (2007) illustrated that the seedling of barley genotypes under salt stress led to decline in the rate of germination percentage, seedling length (root and shoot length) and seedling weight (shoot and root fresh and dry weights). Moreover, (Ali *et al.,* 2019) verified a remarkable variation for genetic materials ability under salinity condition. On the other hand, Gonzalez *et al.,* (2005) reported a reduction in the length of seedling as well as decrease in germination percentage due to the increase intensity of PEG_{6000}. Furthermore, Islam *et al.,* (2018) reported that seedling length and dry weight decreased with the increase PEG_{6000} stress level. Similar results also noticed in other crops such as corn (Ibne Hoque *et al.,* 2015), Cowpea (Murillo Amador *et al.,* 2002) and soybean (Farhoudi *et al.,* 2015).

Biplot analysis is a multidimensional analysis and efficient way to study the trait genotype association and to study multi number of environments, multi genotypes and multi traits together. Therefore, it was used here to identify the principal variables that explain the pattern of correlations to identify the parameters best-describing tolerant genotypes. Furthermore, analysis of genetic relationship (Euclidean) among genotypes is an important component and play a major role in their effective utilization and serve as a platform for the selection of superior genotypes to be used as parents in hybridization programmes in order to identify a desirable segregates for the traits under the concerned study (Salgotra *et al.,* 2015 and Sandhu and Kumar, 2017).

Overall, the present study was carried out with electrolyte (Na^+Cl^-) and nonelectrolyte (PEG_{6000}) osmotic potential agents to determine responsible factors for failure of seed germination of studied bread wheat due to an osmotic barrier due to toxic effects to find out the tolerant and sensitive genotypes to be used in a breeding programme.

MATERIAL AND METHOD

This work was achieved at the laboratory of tissue culture, Genetics Dept., Faculty of Agric., Sohag Univ., Sohag, Egypt in a two-factor factorial in a Completely Randomize Design (CRD) with three replications. The first factor was studied bread wheat genotypes shown in Table 1 collected from the previous mentioned organization and the second factor was solution levels; four levels of NaCl (50, 100, 150 and 200 mM) as a salinity stress factors and four levels of PEG_{6000} (Ψs) {5% (-0.3 Mpa), 10% (-0.6 Mpa), 15% (-0.9 Mpa) and 20% (-1.2 Mpa) w/v} as a drought stress factors, compared to the control level (0 mM / 0 Mpa).

Germination emergence and early seedling growth (15 days) of the studied wheat genotypes were investigated by selecting seeds on the basis of size uniformity. The seeds then disinfected by immersion with a calcium hypochlorite solution, containing 5% active chlorine (NaOCl (bleaching liquid)) for 15 minutes to prevent fungal infection and then washed with sterile distilled water three times to remove NaOCl agent from the seed surface.

The germination tests were carried out by adding 10 ml (uniform amount of desired osmotic solutions) of each level of factors (NaCl and PEG_{6000}) with each genotype (20 seeds sown) on three sterile Whatman's filter paper that had been autoclaved on the bottom of 9 cm diameter glass Petri dishes under laminar flow cabinet to create artificial salinity and drought stress factors to assess the progressive negative effects of NaCl and PEG_{6000}. After that, tightly sealed edges of Petri dishes with an impermeable colorless parafilm in order to avoid water losses.

The cultivated genotypes were incubated in the dark conditions in an incubator at 25±1^{o}C and relative humidity of 90%. Petri dishes were monitored on a daily basis to follow up the germination and infection events, and every three days same concentrations and volume (10 ml) of studied treatments were applied until the two weeks old (when the radicles grew at least 2 mm long) as appeared in Fig. 1. After two weeks old, the effects of $Na^{+}Cl^{-}$ and PEG_{6000} on both seed emergence and early seedling growth stage were considered to evaluate germination percentage, seedling length, root numbers and seedling fresh and dry weights.

Studied Parameters:

1. Germination percentage was estimated after final germination as the following equation (Seed germination= germinated seeds /total seeds x 100) Španić *et al.,* (2017).
2. Seedling Length: was measured by rubber centimeter from the shoot tip to the root tip.
3. Root Numbers: was measured by counting roots every seedling in each petri dish.
4. Seedling fresh and dry weight were measured in weighted in grams with an electronic digital scale (0.0001 g) for fresh weight, then dried on hot air oven at 65^{o}C for 72 hours and weighted again for dry weighted.
5. Tissue Water Content (TWC) was measured and expressed as a percentage according to the Muscoloa *et al.,* (2014) formula: Tissue Water Content = {(seedling fresh weight - seedling dry weight/ seedling fresh weight) x100}.
6. Seedling Vigor Index = Seedling Length x Germination Percentage (Kandil *et al.,* 2012).

All investigated parameters and biplot graphs were performed statistically in one-way ANOVA by XLSTAT software package-2015 to check the variances among treatments group means for studied traits. In addition, the significant differences between the means were compared by LSD values (*P < 0.01*).

Table 1. List of studied wheat genotypes for present research.

Genotype Number	Genotype Name	Genotype Number	Genotype Name
1	CIMMET-224	**13**	Bectora
2	CIMMET-236	**14**	Benisuaif-6
3	CIMMET-270	**15**	Canada-515
4	CIMMET-95	**16**	Sakha-93
5	CIMMET-198	**17**	Gimeza-9
6	CIMMET-108	**18**	Giza-164
7	Baladie	**19**	L (Sids4 X Kasonygiennson-81)
8	Giza-171	**20**	L (Sids4 X Tokwie)
9	Canada-462	**21**	CIMMET-229
10	Sahel-1	**22**	CIMMET-113
11	Sakha-8	**23**	CIMMET-251
12	Gimeza-10	**24**	CIMMET-234

Fig. 1. Seedling performance for the studied wheat genotypes under the levels of PEG_{6000} and NaCl induced solutions.

RESULTS

Statistically significant differences were found between the levels of PEG_{6000} and NaCl treatments in all evaluated wheat genotypes as presented in Table 2.

In criteria for screening drought and salt tolerance wheat genotype used PEG_{6000} and NaCl induced simulation, the results presented that exposed to PEG_{6000} and NaCl simulation effects showed significantly depression in the mentioned studied parameters against the increasing of their concentrations (Table 3 & 4).

Seedling length was a dramatic changed between 1.43 - 21.37 cm that were noted at CIMMET-236 in level 5% and L (Sids4 X Kasonygiennson-81) genotypes in 0% of PEG_{6000}. Meanwhile, in the context of NaCl induced the maximum seedling length was 60.60 cm which recorded in level 100 mM and the minimum seedling length was 3.70 cm at 200 mM for the CIMMET-270 and CIMMET-229 genotypes, respectively.

The magnitude root numbers of the studied wheat genotypes varied distinctly under the studied different levels of PEG_{6000} and NaCl. The highest root numbers was noted 7.27 for the Bectora genotype at the level 5% of PEG_{6000}. However the highest root numbers at NaCl was recorded 8.66 for CIMMET-251 genotype in level 0 mM.

In the context of the magnitude seedling fresh and dry weights allocated to root, shoot and leaf (whole plant body), the results revealed significant severely reduction with increasing levels of PEG_{6000} and NaCl, so the studied genotypes was influenced by the different concentrations of them. The heaviest seedling fresh weight in PEG_{6000} was (0.324 g) and obtained from Canada-462 genotype at level 0%. Meanwhile, the maximum seedling fresh weight in NaCl was (0.251 g) and recorded for CIMMET-234 genotype at level 50mM. In addition, the highest seedling dry weight in PEG_{6000} was (0.06 g) and noted in Benisuaif-6 genotype at level 0%, while the largest weight of seedling dry weight in NaCl was (0.164 g) and observed in CIMMET-95 genotype at level 150 mM. Therefore, the pronounced effects for PEG_{6000} and NaCl induced simulation were affected from 5 % PEG_{6000} and 50mM NaCl concentration onwards.

Concerning tissue water content parameter, the results ranged from 89.36 for CIMMET-113 genotype at 0% of PEG_{6000} to 51.54 for CIMMET-234 genotype of 20% PEG_{6000}. While, in NaCl levels the tissue water content was ranged from 91.08 for Gimeza-10 genotype at 50 mM to 25.45 for CIMMET-95 genotype at 150 mM.

There is considerable reduction in the rate of germination percentage for almost all genotypes under the different levels of PEG_{6000} and NaCl. The results presented that the rate of germination percentage was decreased from 5 % to 20% of PEG6000 and from 50 mM to 200 mM NaCl levels onwards for almost all the studied genotypes.

Regarding vigor index parameter the screened results showed that most of the wheat genotypes exerted rapid reduction of vigor index with the increasing of PEG_{6000} and NaCl levels, and observed that the L (Sids4 X Kasonygiennson-81) genotype scored the maximum vigor index in the different levels of PEG_{6000}. While, CIMMET-251 genotype indicated the highest scored vigor index in the context of different levels of NaCl.

Table 2. Analysis of variances for tested traits under consideration treatments for all evaluated wheat genotypes.

S.O.V.	Seedling length				Root Numbers				Seedling Fresh Weight				Seedling Dry Weight			
	PEG_{6000}		NaCl		PEG_{6000}		NaCl		PEG_{6000}		NaCl		PEG_{6000}		NaCl	
	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error
D.F.	23	96	23	96	23	96	23	96	23	96	23	96	23	96	23	96
Mean Square	43.03**	15.13	34.39	42.83	4.46**	1.11	1.59*	0.85	.01**	0.001	.002*	0.001	.000**	0	.001**	0

*, ** Significant at 0.05 and 0.01 levels, respectively.

S.O.V.	Tissue Water Content				Germination Percentage				Vigor Index			
	PEG_{6000}		NaCl		PEG_{6000}		NaCl		PEG_{6000}		NaCl	
	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error	Genotypes	Error
D.F.	23	96	23	96	23	96	23	96	23	96	23	96
Mean Square	178.09**	45.01	248.49**	59.87	646.04**	257.23	258.79**	88.51	577474.39**	175164.74	380597.67	462267.9

*, ** Significant at 0.05 and 0.01 levels, respectively.

Table 3. Effect of PEG_{6000} on germination and seedling growth in exmained wheat genotypes.

No.	Genotype Names	PEG_{6000} Treatments	Seedling Length	Root Numbers	Seedling Fresh Weight	Seedling Dry Weight	Tissue Water Content	Germination Percentage	Vigor Index
1	CIMMET-224	**0 %**	15.47	5.87	0.26	0.03	86.91	100.00	1546.67
		5 %	12.03	5.20	0.18	0.04	78.54	100.00	1203.33
		10 %	14.29	5.93	0.21	0.04	79.38	93.33	1333.33
		15 %	12.86	5.93	0.21	0.05	77.71	93.33	1200.00
		20 %	8.54	5.08	0.15	0.04	73.35	86.67	740.00
2	CIMMET-236	**0 %**	12.80	6.13	0.25	0.05	79.86	100.00	1280.00
		5 %	1.43	1.43	0.12	0.04	62.96	46.67	66.67
		10 %	2.13	1.50	0.13	0.05	63.30	53.33	113.33
		15 %	10.07	4.67	0.17	0.05	72.78	100.00	1006.67
		20 %	7.75	4.50	0.18	0.05	72.06	93.33	723.33
3	CIMMET-270	**0 %**	13.47	5.33	0.24	0.03	85.83	100.00	1346.67
		5 %	12.80	5.73	0.18	0.03	81.69	100.00	1280.00
		10 %	15.20	6.20	0.17	0.03	80.64	100.00	1520.00
		15 %	10.13	5.33	0.15	0.03	80.00	100.00	1013.33
		20 %	11.11	4.86	0.15	0.04	74.88	93.33	1036.67
4	CIMMET-95	**0 %**	15.27	4.20	0.15	0.02	84.85	100.00	1526.67
		5 %	10.97	6.13	0.16	0.03	81.27	100.00	1096.67
		10 %	11.15	6.69	0.15	0.03	78.50	86.67	966.67
		15 %	11.58	6.62	0.16	0.02	86.49	86.67	1003.33
		20 %	11.43	6.40	0.17	0.04	74.44	100.00	1143.33
5	CIMMET-198	**0 %**	14.00	6.00	0.21	0.03	84.01	93.33	1306.67
		5 %	19.60	5.60	0.19	0.03	82.47	100.00	1960.00
		10 %	20.21	5.79	0.21	0.03	83.54	93.33	1886.67
		15 %	15.73	5.60	0.17	0.04	78.74	100.00	1573.33
		20 %	17.43	5.67	0.19	0.04	78.05	100.00	1743.33
6	CIMMET-108	**0 %**	17.40	6.33	0.13	0.02	82.21	100.00	1740.00
		5 %	3.80	2.20	0.10	0.02	77.03	66.67	253.33
		10 %	6.00	2.50	0.10	0.03	68.41	53.33	320.00
		15 %	5.00	2.00	0.12	0.02	81.25	100.00	500.00
		20 %	2.50	2.00	0.11	0.02	81.02	100.00	250.00
7	Baladie	**0 %**	17.50	5.93	0.27	0.03	88.84	100.00	1750.00
		5 %	8.20	6.00	0.12	0.04	63.33	33.33	273.33
		10 %	10.43	1.86	0.14	0.05	67.16	46.67	486.67
		15 %	3.00	2.75	0.13	0.05	59.93	80.00	240.00
		20 %	6.27	4.40	0.11	0.04	61.74	100.00	626.67
8	Giza-171	**0 %**	17.73	5.80	0.29	0.04	87.49	100.00	1773.33
		5 %	11.87	3.40	0.27	0.04	83.75	100.00	1186.67
		10 %	12.75	6.07	0.21	0.05	76.76	93.33	1190.00
		15 %	14.55	6.64	0.25	0.05	81.14	73.33	1066.67

		20 %	12.04	6.50	0.24	0.05	79.95	80.00	963.33
9	**Canada-462**	**0 %**	11.86	4.79	0.32	0.04	89.05	93.33	1106.67
		5 %	13.60	5.93	0.24	0.04	81.78	100.00	1360.00
		10 %	12.07	5.73	0.18	0.04	78.20	100.00	1206.67
		15 %	11.80	5.87	0.20	0.04	77.67	100.00	1180.00
		20 %	8.43	6.07	0.18	0.06	67.24	93.33	786.67
10	**Sahel-1**	**0 %**	12.46	6.00	0.21	0.04	83.40	100.00	1246.00
		5 %	12.02	5.14	0.21	0.05	78.32	93.33	1122.00
		10 %	12.83	6.07	0.20	0.05	75.84	100.00	1283.33
		15 %	9.34	6.60	0.20	0.05	75.07	100.00	934.00
		20 %	9.85	6.08	0.21	0.05	76.50	80.00	788.00
11	**Sakha-8**	**0 %**	16.93	6.14	0.23	0.03	85.08	93.33	1580.00
		5 %	12.39	5.43	0.21	0.04	81.76	93.33	1156.67
		10 %	9.08	4.54	0.22	0.04	81.27	86.67	786.67
		15 %	6.46	4.36	0.21	0.04	78.80	93.33	603.33
		20 %	6.88	3.75	0.22	0.05	79.13	80.00	550.00
12	**Gimeza-10**	**0 %**	15.30	5.60	0.21	0.04	81.60	100.00	1530.00
		5 %	16.79	4.50	0.20	0.04	82.45	100.00	1678.67
		10 %	13.81	4.00	0.22	0.04	79.72	100.00	1380.67
		15 %	8.63	3.00	0.17	0.04	76.00	100.00	862.67
		20 %	8.04	2.80	0.16	0.05	69.14	93.33	750.67
13	**Bectora**	**0 %**	16.27	6.20	0.21	0.03	83.96	100.00	1626.67
		5 %	12.87	7.27	0.15	0.04	74.87	100.00	1286.67
		10 %	8.29	6.50	0.14	0.04	70.21	93.33	773.33
		15 %	10.93	6.13	0.19	0.04	78.19	100.00	1093.33
		20 %	12.13	5.87	0.14	0.04	67.13	100.00	1213.33
14	**Benisuaif-6**	**0 %**	14.00	5.53	0.16	0.06	60.95	100.00	1400.00
		5 %	10.57	4.80	0.11	0.05	54.42	100.00	1056.67
		10 %	7.00	5.18	0.14	0.05	64.63	73.33	513.33
		15 %	8.42	5.15	0.14	0.05	61.82	86.67	730.00
		20 %	8.43	5.87	0.10	0.05	54.59	100.00	843.33
15	**Canada-515**	**0 %**	16.67	6.07	0.23	0.04	84.64	100.00	1666.67
		5 %	15.17	5.13	0.16	0.04	73.22	100.00	1516.67
		10 %	15.68	5.93	0.17	0.04	75.48	93.33	1463.33
		15 %	10.31	5.15	0.14	0.04	74.86	86.67	893.33
		20 %	11.83	6.27	0.14	0.04	72.25	100.00	1183.33
16	**Sakha-93**	**0 %**	16.32	5.14	0.25	0.03	87.06	93.33	1523.33
		5 %	3.74	5.60	0.11	0.03	68.70	33.33	124.67
		10 %	3.00	5.00	0.20	0.05	74.06	80.00	240.00
		15 %	10.37	5.07	0.16	0.05	69.67	100.00	1036.67
		20 %	10.07	5.87	0.16	0.05	69.88	100.00	1006.67
17	**Gimeza-9**	**0 %**	18.00	5.07	0.27	0.04	85.82	100.00	1800.00

		5 %	18.20	5.33	0.24	0.03	86.87	100.00	1820.00
		10 %	12.93	5.00	0.17	0.04	77.09	100.00	1293.33
		15 %	14.43	5.73	0.23	0.04	81.83	100.00	1443.33
		20 %	11.23	5.47	0.20	0.05	76.29	100.00	1123.33
18	**Giza-164**	**0 %**	17.20	5.87	0.16	0.03	83.70	100.00	1720.00
		5 %	7.13	3.13	0.12	0.04	64.84	53.33	380.00
		10 %	6.83	2.83	0.12	0.05	60.99	40.00	273.33
		15 %	4.20	2.80	0.12	0.05	61.86	33.33	140.00
		20 %	6.47	3.27	0.12	0.05	56.86	100.00	646.67
19	**L (Sids4 X Kasonygie nnson-81)**	**0 %**	21.37	5.80	0.27	0.04	85.03	100.00	2136.67
		5 %	11.60	5.67	0.17	0.04	77.04	100.00	1160.00
		10 %	14.31	6.46	0.20	0.06	70.55	86.67	1240.00
		15 %	15.27	6.07	0.16	0.06	64.81	100.00	1526.67
		20 %	12.47	6.20	0.17	0.05	71.62	100.00	1246.67
20	**L (Sids4 X Tokwie)**	**0 %**	12.30	6.40	0.32	0.03	89.33	100.00	1230.00
		5 %	12.80	5.73	0.25	0.03	87.17	100.00	1280.00
		10 %	15.20	6.20	0.24	0.06	76.03	100.00	1520.00
		15 %	10.13	5.33	0.23	0.05	79.23	100.00	1013.33
		20 %	11.03	5.00	0.18	0.05	71.92	100.00	1103.33
21	**CIMMET-229**	**0 %**	13.73	6.53	0.19	0.04	81.14	100.00	1373.33
		5 %	16.87	5.27	0.20	0.03	83.53	100.00	1686.67
		10 %	15.53	5.33	0.19	0.04	78.95	100.00	1553.33
		15 %	13.87	6.20	0.19	0.04	78.79	100.00	1386.67
		20 %	10.53	5.60	0.17	0.04	76.02	100.00	1053.33
22	**CIMMET-113**	**0 %**	18.60	5.60	0.31	0.03	89.36	100.00	1860.00
		5 %	2.08	2.83	0.17	0.05	71.90	40.00	83.33
		10 %	3.45	1.80	0.15	0.05	65.10	66.67	230.00
		15 %	4.46	3.55	0.16	0.05	65.96	73.33	326.67
		20 %	4.97	5.00	0.17	0.06	67.08	80.00	397.33
23	**CIMMET-251**	**0 %**	19.00	5.27	0.24	0.04	81.77	100.00	1900.00
		5 %	17.25	5.57	0.22	0.04	80.93	93.33	1610.00
		10 %	17.20	5.67	0.21	0.05	75.08	100.00	1720.00
		15 %	10.37	6.07	0.22	0.05	76.14	100.00	1036.67
		20 %	14.00	5.33	0.20	0.06	71.60	100.00	1400.00
24	**CIMMET-234**	**0 %**	14.17	6.27	0.16	0.04	76.37	100.00	1416.67
		5 %	16.00	5.00	0.15	0.03	79.67	13.33	213.33
		10 %	8.31	5.85	0.15	0.05	69.01	86.67	720.00
		15 %	4.00	2.70	0.12	0.05	59.16	66.67	266.67
		20 %	2.61	2.22	0.10	0.05	51.54	60.00	156.67
Mean			**11.56**	**5.12**	**0.18**	**0.041**	**75.74**	**89.83**	**1081.73**
LSD 0.05			**8.89**	**2.86**	**0.13**	**0.00**	**18.08**	**34.45**	**1029.98**
LSD 0.01			**12.67**	**4.07**	**0.18**	**0.00**	**25.75**	**49.05**	**1466.79**
C.V. %			**39.19**	**25.89**	**27.25**	**23.51**	**11.10**	**20.29**	**46.49**

C.V. % (Coefficient of variation), LSD (Least Significant Differences).

Table 4. Effect of NaCl on germination and seedling growth in exmained wheat genotypes.

No.	Genotype Names	NaCl Treatments	Seedling Length	Root Numbers	Seedling Fresh Weight	Seedling Dry Weight	Tissue Water Content	Germination Percentage	Vigor Index
1	CIMMET-224	0 mM	20.37	6.47	0.22	0.03	87.83	100.00	2036.67
		50 mM	19.97	7.07	0.25	0.03	88.46	100.00	1996.67
		100 mM	12.93	6.27	0.21	0.03	88.28	100.00	1293.33
		150 mM	10.17	6.33	0.16	0.03	80.26	100.00	1016.67
		200 mM	7.96	4.85	0.16	0.03	78.71	86.67	690.00
2	CIMMET-236	0 mM	18.00	5.47	0.21	0.03	85.73	100.00	1800.00
		50 mM	19.63	6.10	0.19	0.03	82.71	100.00	1963.33
		100 mM	14.77	6.33	0.18	0.03	80.69	100.00	1476.67
		150 mM	13.60	6.53	0.17	0.03	81.59	100.00	1360.00
		200 mM	6.67	6.27	0.14	0.04	70.15	100.00	666.67
3	CIMMET-270	0 mM	19.40	6.33	0.21	0.02	88.69	100.00	1940.00
		50 mM	18.50	6.64	0.22	0.03	87.65	93.33	1726.67
		100 mM	60.60	6.73	0.19	0.03	84.68	100.00	660.00
		150 mM	9.53	6.67	0.17	0.03	79.60	100.00	953.33
		200 mM	9.67	6.27	0.17	0.03	81.84	100.00	966.67
4	CIMMET-95	0 mM	15.21	6.93	0.14	0.02	85.28	93.33	1420.00
		50 mM	17.79	6.14	0.20	0.03	84.79	93.33	1660.00
		100 mM	13.42	5.25	0.23	0.16	30.00	80.00	1073.33
		150 mM	11.40	6.27	0.22	0.16	25.45	100.00	1140.00
		200 mM	5.73	3.55	0.20	0.14	29.80	73.33	420.00
5	CIMMET-198	0 mM	19.50	6.21	0.19	0.02	88.88	93.33	1820.00
		50 mM	18.57	7.33	0.23	0.03	87.37	100.00	1856.67
		100 mM	18.60	6.93	0.21	0.03	87.30	100.00	1860.00
		150 mM	14.08	6.75	0.19	0.03	86.01	80.00	1126.67
		200 mM	12.20	5.33	0.20	0.04	81.74	100.00	1220.00
6	CIMMET-108	0 mM	17.93	6.13	0.20	0.02	88.30	100.00	1793.33
		50 mM	16.60	6.47	0.22	0.03	88.12	100.00	1660.00
		100 mM	14.40	6.27	0.20	0.03	86.26	100.00	1440.00
		150 mM	12.77	6.33	0.19	0.03	86.01	100.00	1276.67
		200 mM	7.73	5.93	0.17	0.03	82.61	100.00	773.33
7	Baladie	0 mM	16.63	6.93	0.19	0.03	83.47	100.00	1663.33
		50 mM	13.18	6.79	0.17	0.03	83.16	93.33	1230.00
		100 mM	15.46	6.86	0.21	0.03	85.75	93.33	1443.33
		150 mM	8.60	3.60	0.18	0.04	80.00	66.67	573.33
		200 mM	7.88	3.50	0.16	0.04	74.56	80.00	630.00
8	Giza-171	0 mM	19.80	5.73	0.22	0.04	84.40	100.00	1980.00
		50 mM	17.50	6.07	0.23	0.03	84.97	100.00	1750.00
		100 mM	13.36	5.50	0.20	0.04	81.84	93.33	1246.67
		150 mM	13.70	6.13	0.21	0.03	84.45	100.00	1370.00
		200 mM	7.60	2.90	0.17	0.04	75.96	66.67	506.67
9	Canada-462	0 mM	15.89	5.64	0.22	0.03	86.31	93.33	1483.33
		50 mM	17.07	6.29	0.24	0.04	84.12	93.33	1593.33
		100 mM	12.73	5.69	0.21	0.06	73.04	86.67	1103.33
		150 mM	7.00	3.46	0.20	0.04	80.88	73.33	513.33
		200 mM	7.19	5.54	0.18	0.04	78.89	86.67	623.33
10	Sahel-1	0 mM	21.00	6.00	0.22	0.03	85.54	93.33	1960.00
		50 mM	16.93	6.60	0.22	0.03	85.21	100.00	1693.33
		100 mM	14.33	6.13	0.22	0.03	85.28	100.00	1433.33
		150 mM	9.67	5.80	0.17	0.03	79.40	100.00	966.67

		200 mM	9.73	6.80	0.21	0.04	80.13	100.00	973.33
11	**Sakha-8**	**0 mM**	19.30	6.73	0.19	0.03	83.91	100.00	1930.00
		50 mM	19.36	6.43	0.20	0.03	84.88	93.33	1806.67
		100 mM	12.44	6.56	0.17	0.03	81.38	60.00	746.67
		150 mM	10.83	5.33	0.15	0.03	78.75	40.00	433.33
		200 mM	9.10	4.35	0.13	0.02	84.21	46.67	424.67
12	**Gimeza-10**	**0 mM**	19.25	6.93	0.21	0.02	89.52	93.33	1796.67
		50 mM	19.37	7.47	0.25	0.02	91.08	100.00	1936.67
		100 mM	14.27	7.93	0.19	0.03	81.73	100.00	1426.67
		150 mM	6.73	6.87	0.16	0.03	81.68	100.00	673.33
		200 mM	4.77	6.40	0.13	0.03	76.67	100.00	476.67
13	**Bectora**	**0 mM**	16.30	8.07	0.21	0.03	83.46	100.00	1630.00
		50 mM	14.11	7.64	0.17	0.03	82.23	93.33	1316.67
		100 mM	8.70	6.33	0.15	0.04	74.78	100.00	870.00
		150 mM	7.87	6.60	0.14	0.04	74.20	100.00	786.67
		200 mM	6.46	5.15	0.14	0.04	69.94	86.67	560.00
14	**Benisuaif-6**	**0 mM**	21.53	7.07	0.21	0.03	84.75	100.00	2153.33
		50 mM	17.67	6.33	0.19	0.04	81.82	100.00	1766.67
		100 mM	12.33	6.13	0.18	0.04	79.66	100.00	1233.33
		150 mM	10.07	5.53	0.17	0.04	73.95	100.00	1006.67
		200 mM	5.00	4.67	0.12	0.04	64.59	100.00	500.00
15	**Canada-515**	**0 mM**	16.20	6.47	0.18	0.03	82.57	100.00	1620.00
		50 mM	16.50	7.42	0.19	0.04	79.74	80.00	1320.00
		100 mM	10.29	8.07	0.17	0.04	76.64	93.33	960.00
		150 mM	13.60	6.73	0.21	0.04	78.83	100.00	1360.00
		200 mM	7.68	6.21	0.18	0.04	75.30	93.33	716.67
16	**Sakha-93**	**0 mM**	18.87	5.87	0.23	0.03	86.21	100.00	1886.67
		50 mM	12.73	7.07	0.18	0.03	80.94	100.00	1273.33
		100 mM	11.29	6.29	0.19	0.04	80.42	93.33	1053.33
		150 mM	8.77	6.39	0.17	0.04	75.51	86.67	760.00
		200 mM	5.46	5.21	0.15	0.05	70.60	93.33	510.00
17	**Gimeza-9**	**0 mM**	15.97	5.60	0.18	0.03	83.93	100.00	1596.67
		50 mM	9.87	6.67	0.09	0.03	62.74	100.00	986.67
		100 mM	7.96	5.43	0.20	0.04	79.61	93.33	743.33
		150 mM	7.67	5.80	0.20	0.04	79.00	100.00	766.67
		200 mM	5.73	4.93	0.19	0.04	79.15	100.00	573.33
18	**Giza-164**	**0 mM**	17.60	6.07	0.18	0.03	83.61	100.00	1760.00
		50 mM	14.07	5.80	0.15	0.03	78.27	100.00	1406.67
		100 mM	10.41	5.82	0.15	0.03	78.24	73.33	763.33
		150 mM	9.79	5.93	0.16	0.04	78.11	93.33	913.33
		200 mM	7.26	4.60	0.14	0.03	77.43	66.67	484.00
19	**L (Sids4 X Kasonygiennson-81)**	**0 mM**	19.40	7.20	0.25	0.03	88.79	100.00	1940.00
		50 mM	18.71	7.07	0.23	0.03	88.44	93.33	1746.67
		100 mM	12.31	7.46	0.20	0.03	84.34	86.67	1066.67
		150 mM	11.73	5.33	0.23	0.04	83.48	100.00	1173.33
		200 mM	7.60	4.00	0.17	0.03	81.76	100.00	760.00
20	**L (Sids4 X Tokwie)**	**0 mM**	19.47	5.80	0.20	0.03	84.21	100.00	1946.67
		50 mM	18.60	6.27	0.22	0.04	83.00	100.00	1860.00
		100 mM	16.20	6.93	0.21	0.04	80.69	100.00	1620.00
		150 mM	9.93	7.29	0.20	0.04	78.55	93.33	926.67
		200 mM	7.50	5.10	0.21	0.05	78.08	66.67	500.00
21	**CIMMET-229**	**0 mM**	19.27	5.73	0.18	0.02	88.89	100.00	1926.67
		50 mM	15.63	6.00	0.19	0.02	88.95	100.00	1563.33
		100 mM	12.33	5.73	0.19	0.02	88.72	100.00	1233.33

		150 mM	7.67	4.87	0.18	0.02	88.69	100.00	766.67
		200 mM	3.70	5.67	0.19	0.02	89.89	100.00	370.00
22	**CIMMET-113**	**0 mM**	21.04	6.33	0.23	0.03	85.36	100.00	2104.00
		50 mM	18.33	7.67	0.23	0.04	82.83	100.00	1833.33
		100 mM	15.80	7.67	0.23	0.04	83.39	100.00	1580.00
		150 mM	10.93	7.93	0.18	0.04	79.05	100.00	1093.33
		200 mM	7.87	7.67	0.17	0.04	78.12	100.00	786.67
23	**CIMMET-251**	**0 mM**	6.07	8.66	0.25	0.03	87.43	93.33	566.67
		50 mM	22.80	6.47	0.25	0.03	86.27	100.00	2280.00
		100 mM	12.93	6.13	0.20	0.04	81.22	100.00	1293.33
		150 mM	10.75	6.36	0.21	0.04	79.16	93.33	1003.33
		200 mM	8.69	5.92	0.18	0.04	76.93	86.67	753.33
24	**CIMMET-234**	**0 mM**	18.67	5.87	0.19	0.03	81.71	100.00	1866.67
		50 mM	16.43	7.00	0.22	0.03	86.10	100.00	1643.33
		100 mM	12.64	6.71	0.18	0.04	80.12	93.33	1180.00
		150 mM	12.17	6.87	0.21	0.04	81.33	100.00	1216.67
		200 mM	5.40	5.27	0.16	0.04	74.94	100.00	540.00
Mean			**13.56**	**6.18**	**0.19**	**0.04**	**80.61**	**94.17**	**1294.11**
LSD 0.05			**7.95**	**1.71**	**0.13**	**0.05**	**21.36**	**21.61**	**836.18**
LSD 0.01			**11.32**	**2.44**	**0.19**	**0.07**	**30.42**	**30.77**	**1190.79**
C.V. %			**47.34**	**16.13**	**15.81**	**50.44**	**12.18**	**11.70**	**51.63**

C.V. % (Coefficient of variation), LSD (Least Significant Differences).

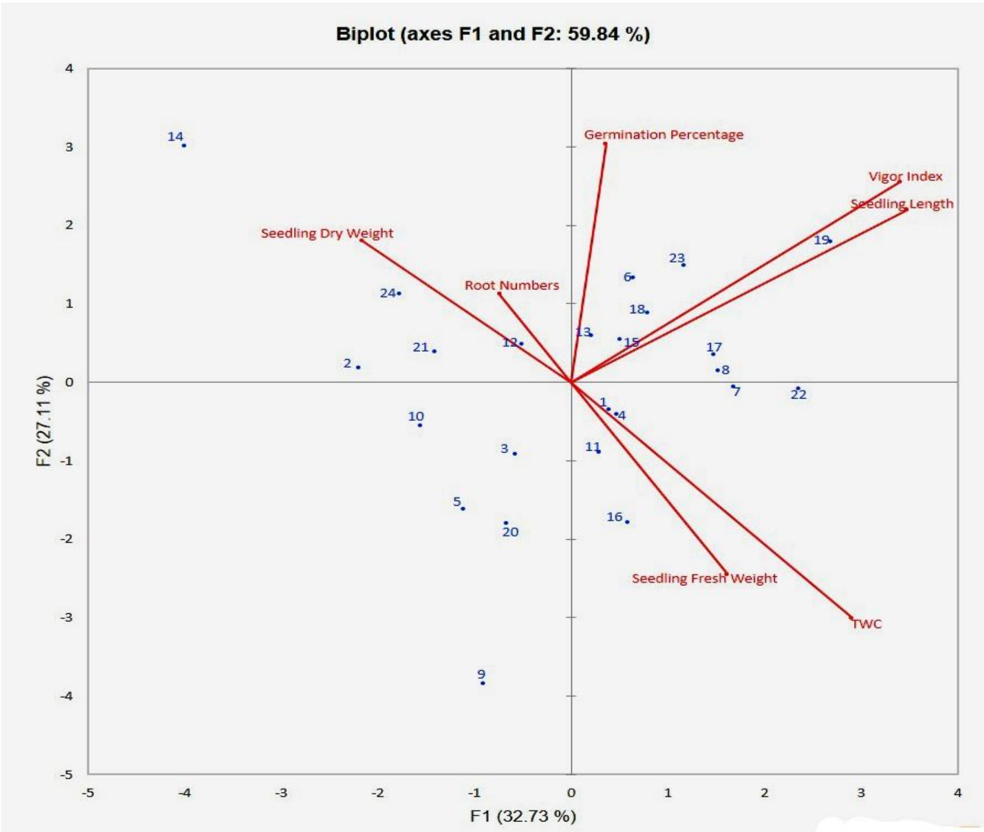

Fig. 2. Biplot analysis under 0% PEG.

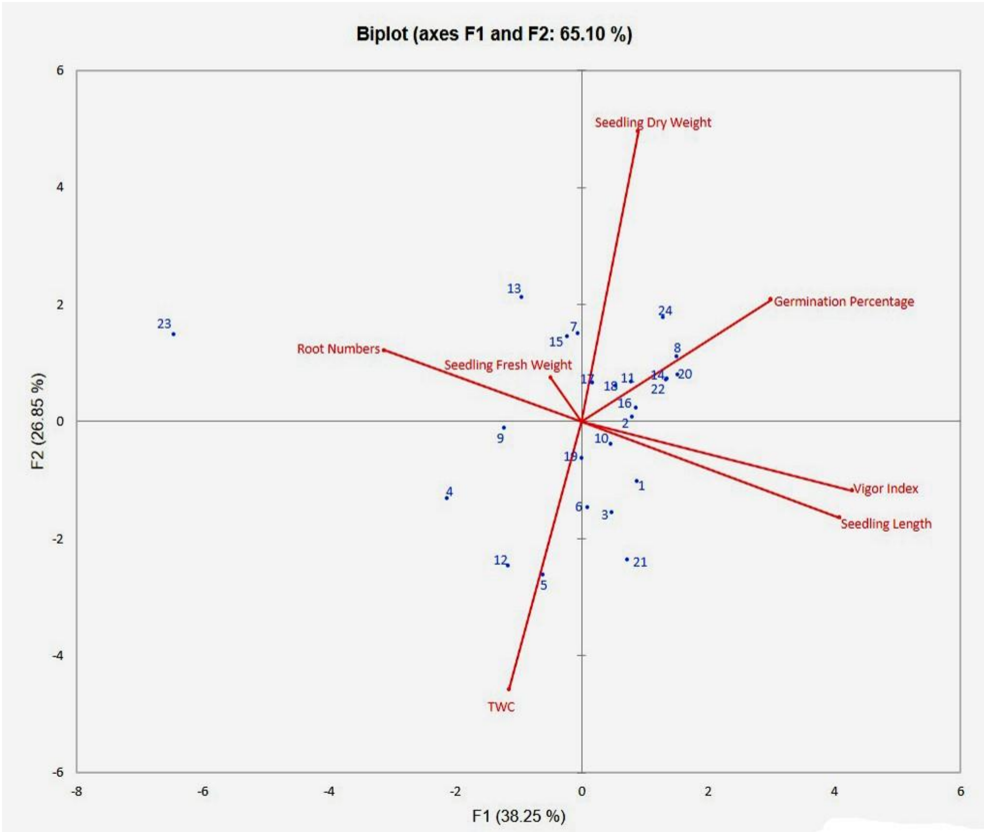

Fig. 3. Biplot analysis under 0 mM NaCl.

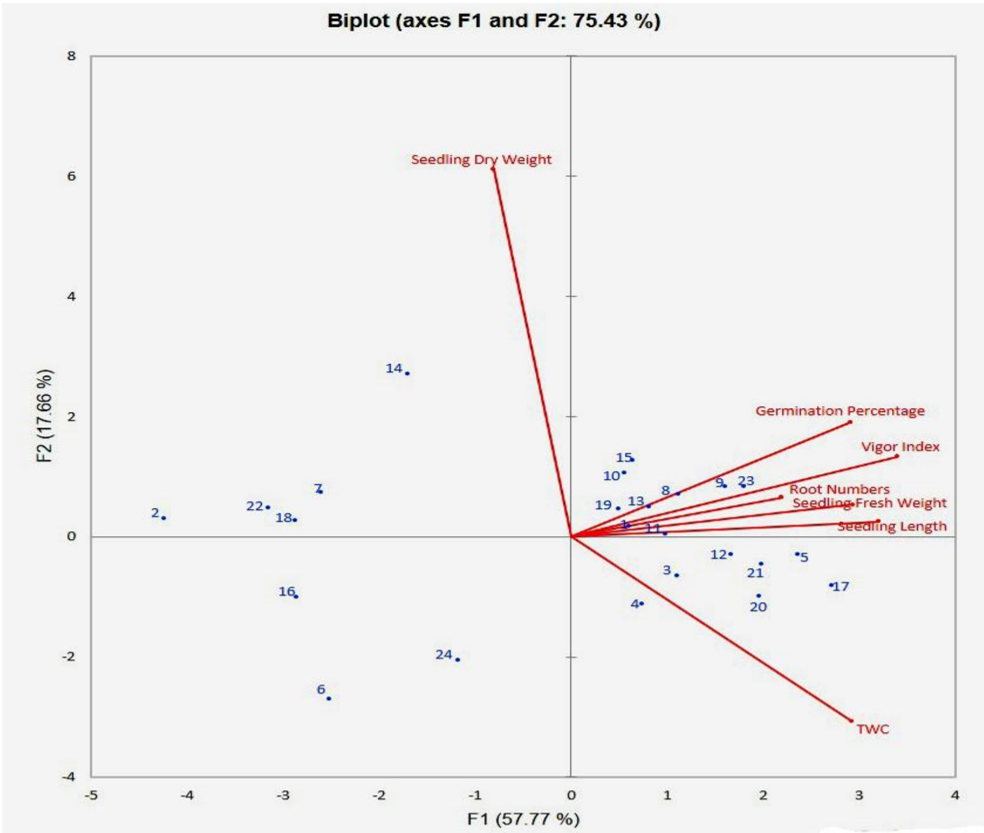

Fig. 4. Biplot analysis under 5% PEG.

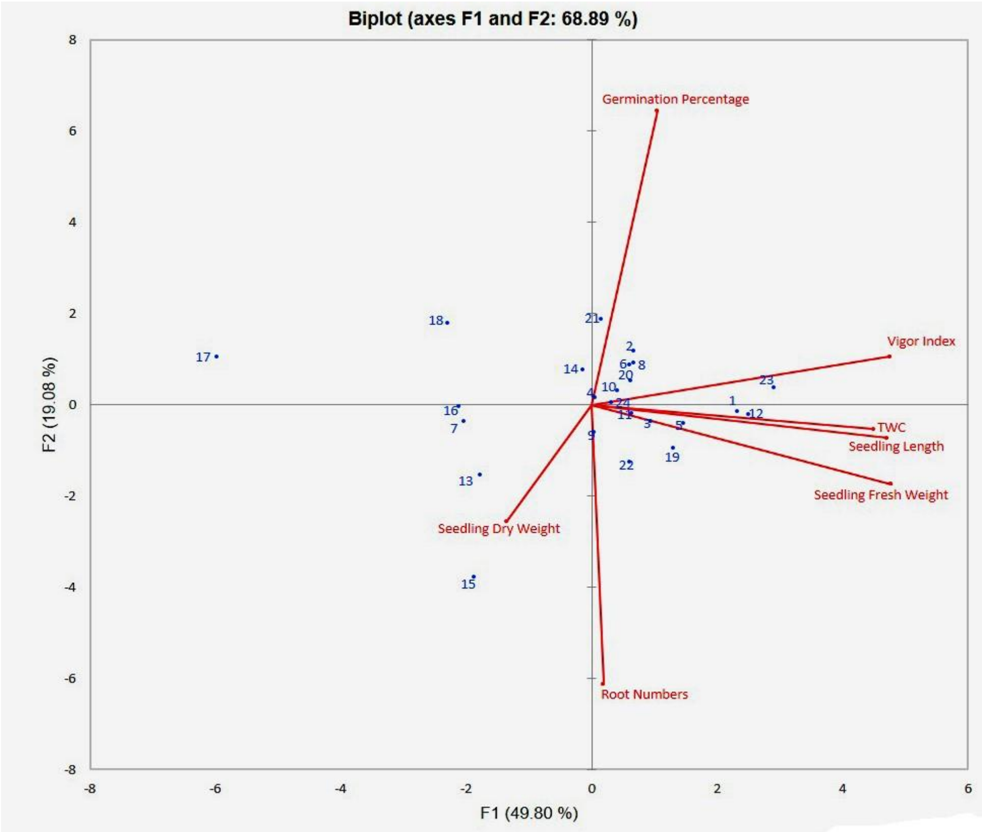

Fig. 5. Biplot analysis under 50 mM NaCl.

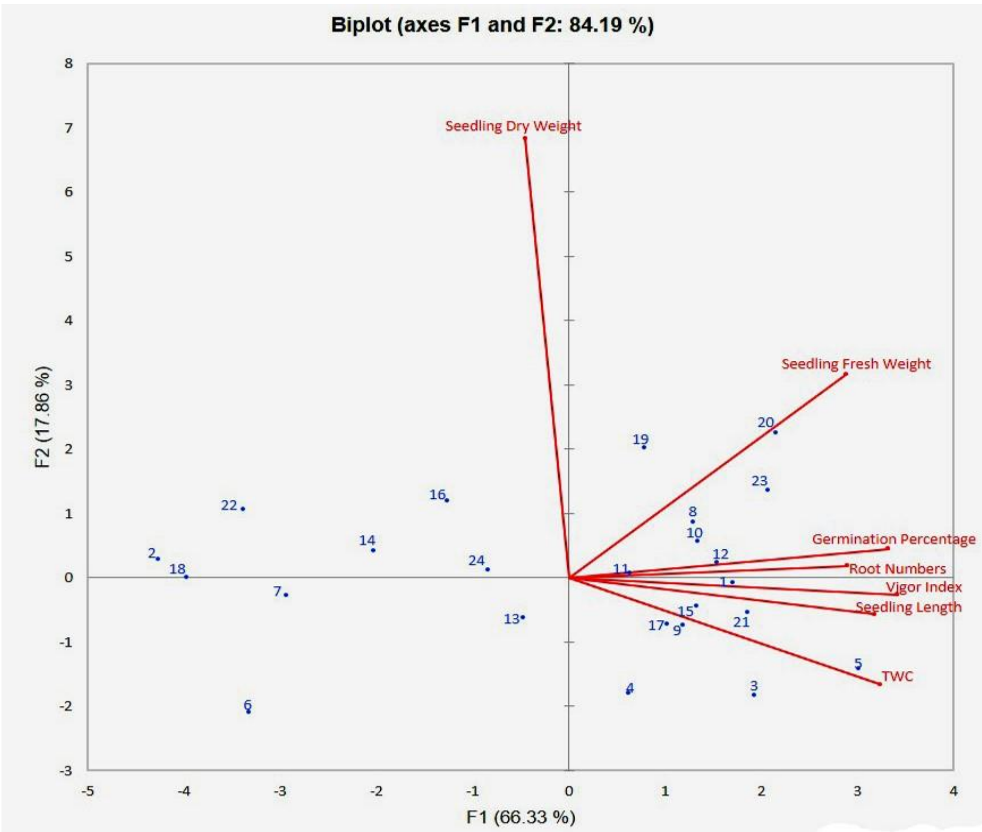

Fig. 6. Biplot analysis under 10% PEG.

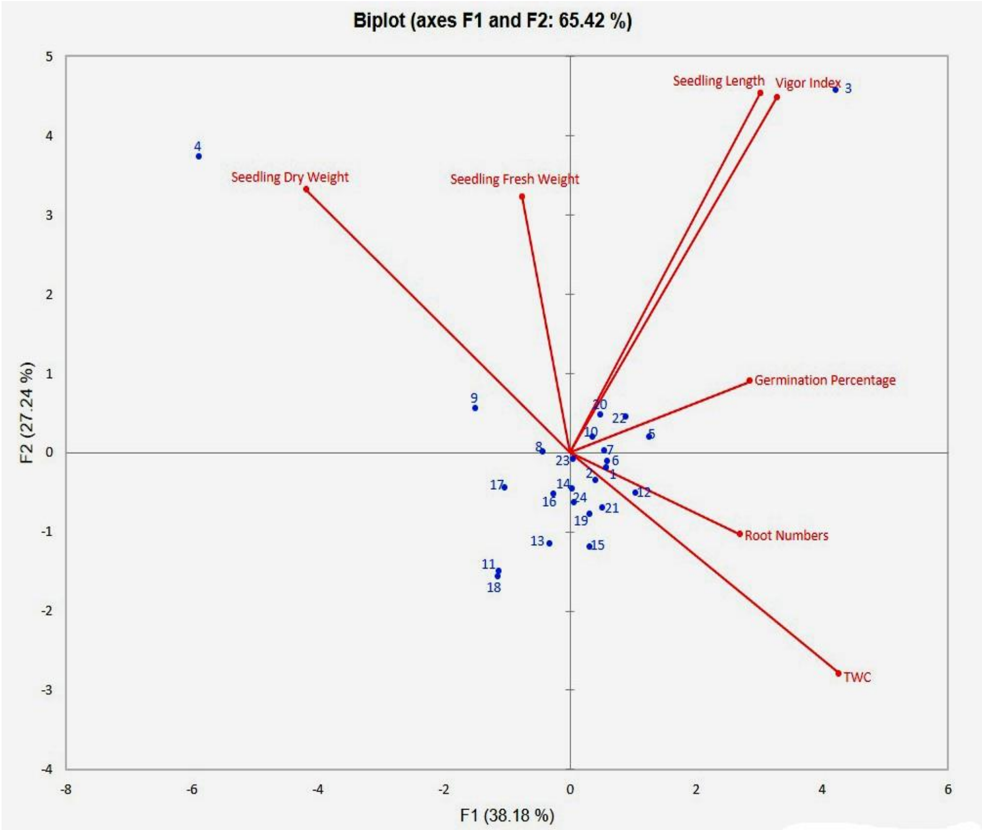

Fig. 7. Biplot analysis under 100 mM NaCl.

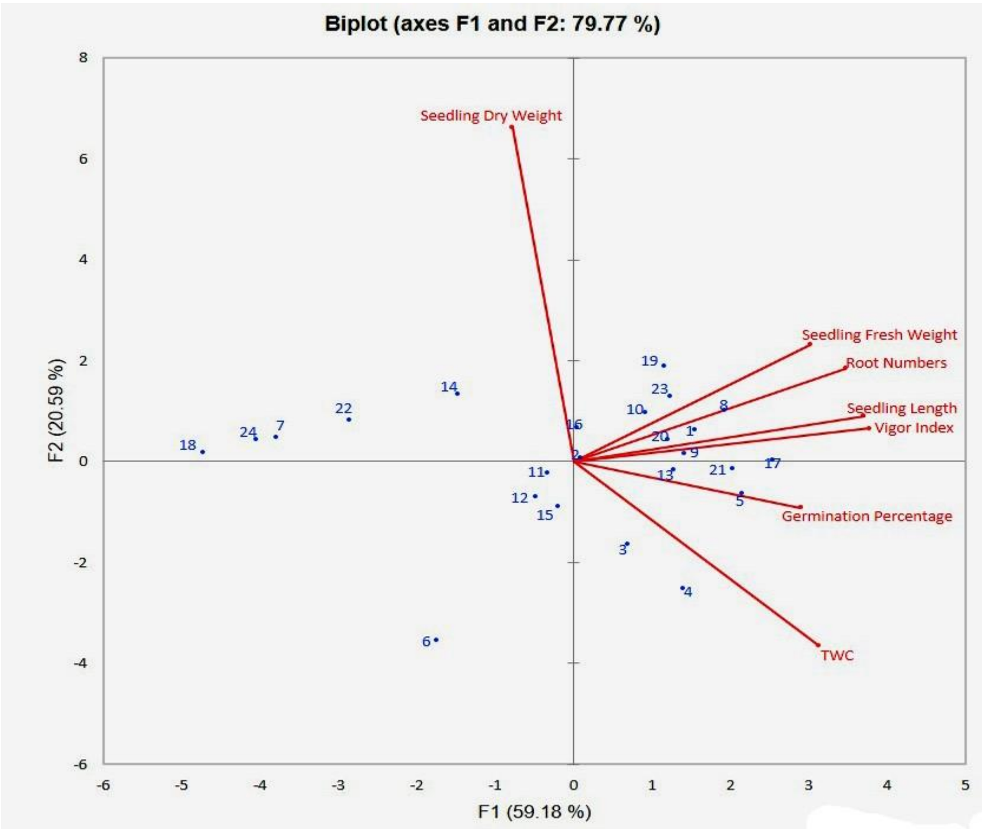

Fig. 8. Biplot analysis under 15% PEG.

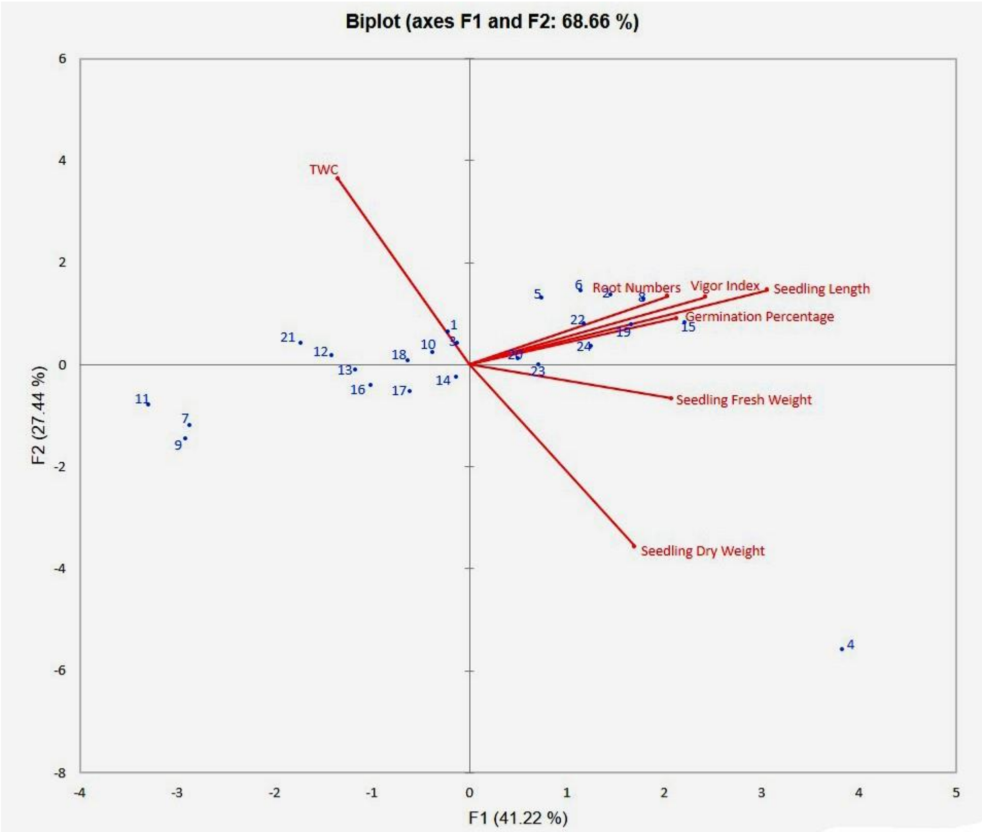

Fig. 9. Biplot analysis under 150 mM NaCl.

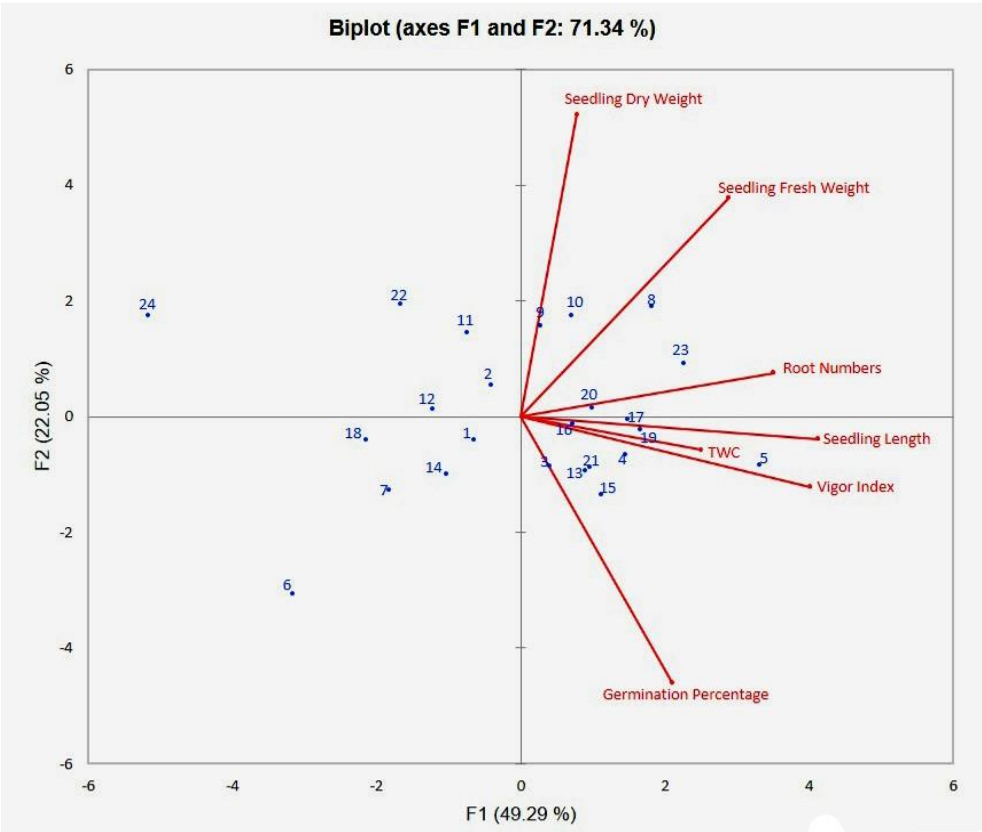

Fig. 10. Biplot analysis under 20% PEG.

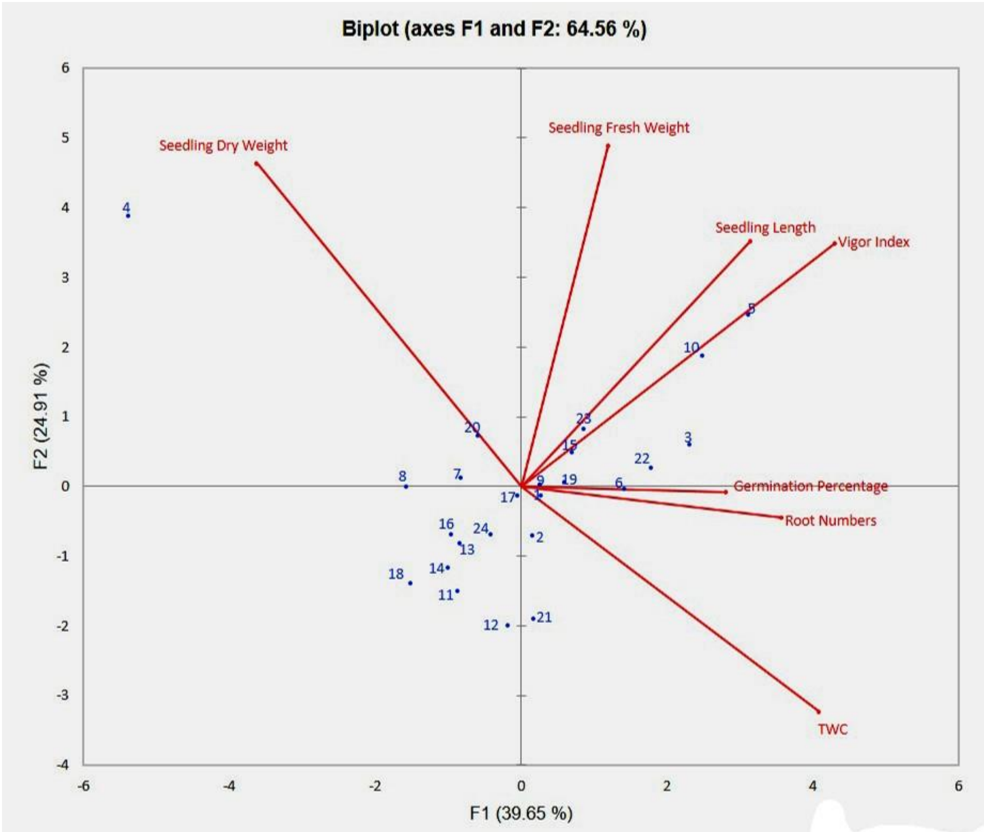

Fig. 11. Biplot analysis under 200 mM NaCl.

The distribution of studied genotypes and traits was performed on biplot analysis formed by two axes, one is the vector length of the traits and the other is the cosine angle among the traits. These axes presented 59.84, 75.43, 84.19, 79.77 and 71.34% of the total variability for PEG_{6000} condition and 65.10, 68.89, 65.42, 68.66 and 64.56% of the total variability for the NaCl condition, respectively (Figs. (2-11)). The longer of the vector length revealed the more discriminatory of the trait and vice versa. In case of the cosine angle the smaller angle between the two traits is the more linked of both traits and vice versa. Thus, the genotypes which are presented away from the origin in the positive direction of a discriminatory trait are performing best with respect to that trait. Whereas, the genotypes which lie away from the origin in the opposite direction of a discriminatory trait are performing low.

Euclidean similarity index was performed to assess the level of similarity and dissimilarity among studied genotypes against induced solutions of PEG_{6000} and NaCl levels (Fig. 12). The results exhibited two groups of PEG_{6000} and three groups of NaCl concentrations. These groups screened a stable performance in all PEG_{6000} and NaCl levels that presented variable response in performance during the time of the study. The first group of PEG_{6000} including 15 genotypes which are CIMMET-224, Bectora, CIMMET-270, Gimeza-10, Giza-171, L (Sids4 X Tokwie), CIMMET-95, Canada-462, Sahel-1, CIMMET-198, Canada-515, CIMMET-229, Gimeza-9, L (Sids4 X Kasonygiennson-81) and CIMMET-251. While, the the second group viewd nine genotypes which are CIMMET-236, Giza-164, CIMMET-108, Baladie, CIMMET-113, CIMMET-234, Sakha-8, Benisuaif-6 and Sakha-93. On the other hand there are 12 genotypes were allocate in the primary group of NaCl which are CIMMET-224, Sahel-1, CIMMET-108, Giza-171, L (Sids4 X Tokwie), Benisuaif-6, L (Sids4 X Kasonygiennson-81), CIMMET-236, CIMMET-113, Gimeza-10, CIMMET-234 and CIMMET-198. Moreover, the genotypes CIMMET-95, Canada-515, CIMMET-229, CIMMET-251, Baladie, Sakha-93, Canada-462, Giza-164, Sakha-8, Bectora and Gimeza-9 were appeared in the second group. In addition, the genotype CIMMET-270 was observed in the third group.

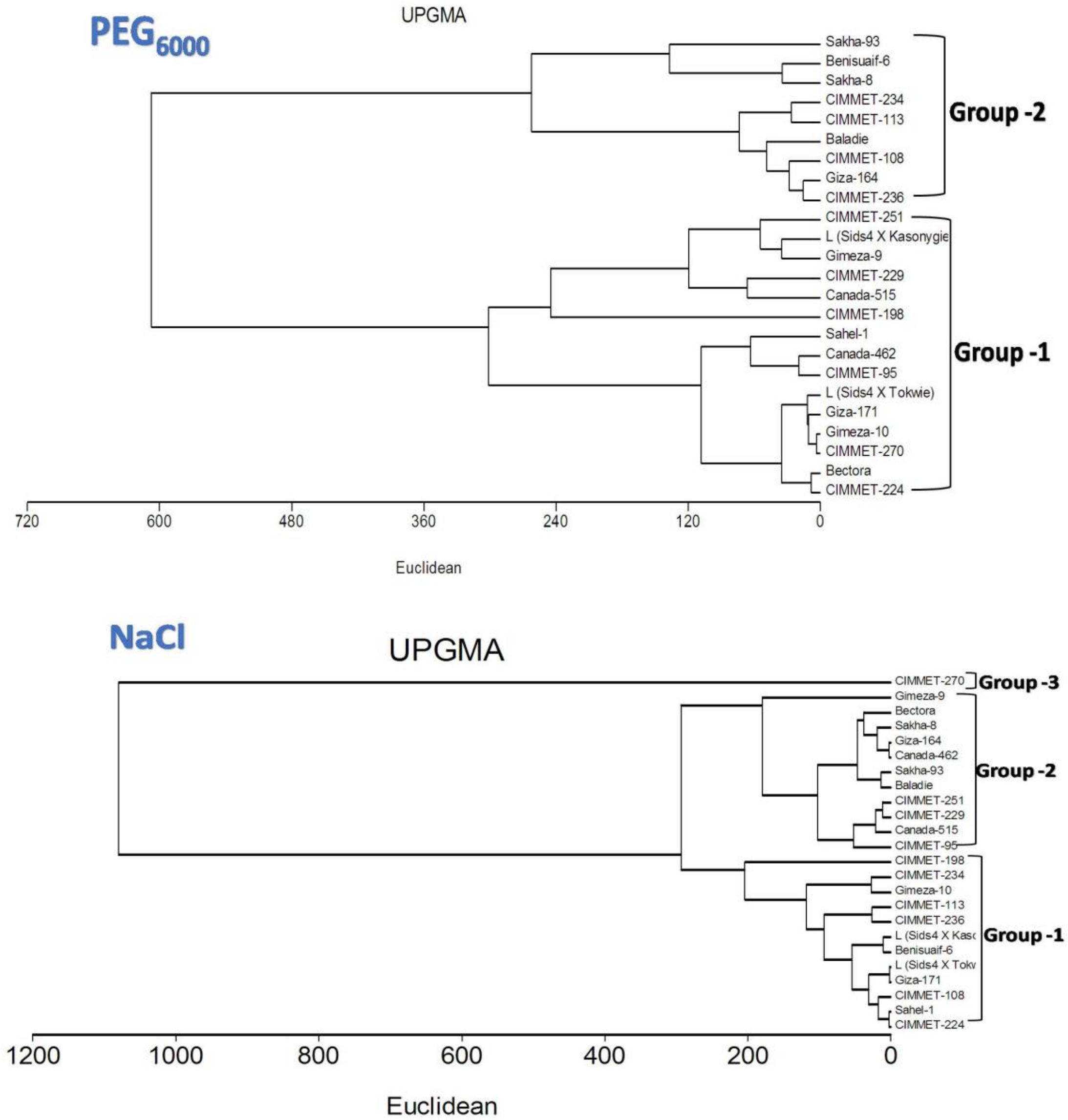

Fig. 12. Paired group (UPGMA), similarity index (Euclidean) of 24 wheat genotypes under the different levels of PEG_{6000} and NaCL.

DISCUSSION

PEG and NaCl treatments had a significant effects on most investigated traits (*P<0.01*) showing high level of variability. It indicated that these sets of genotypes exhibited much variation for these traits, which referred that the treatments have influenced the genotypes for almost all the traits.

Germination parameters were decreased with increasing osmotic potential of PEG_{6000} and NaCl due to some metabolically disorders related to reduction trend in water absorption including general reduction in hydrolysis and utilization of the seed reserve, which takes place during germination (Khakwani *et al.,* 2011 & Ali *et al.,* 2014 & Slamani and Aleksander 2015 & Hassan, 2015 & Chachar *et al.,* 2016 & Valentina *et al.,* 2017 & Sheshaiah *et al.,* 2017 & Chaniago *et al.,* 2017 & Hussein and Jin 2018) because of Seedling dry weight is the consequence of plant physiological and biological activity.

Therefore, our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination with respect to PEG_{6000}. This might be explained by more rapid water uptake in NaCl solutions and achievement of a moisture content that allowed germination. These results are in line with the findings of Kayacetin *et al.,* 2018 who reported that both NaCl and PEG treatments decreased final germination percentage and seedling growth properties in wild mustard, but the effects of NaCl compared to PEG were less on germination when evaluate the effect of NaCl and peg-induced osmotic stress on the germination and early seedling growth of wild mustard. Ouhaddach *et al.,* 2017 showed that, the reduction in the plants growth can be attributed to a combination of effects osmotic and specific to the ions Na^+ and Cl^-. This, therefore, leads to a reduction of the growth which is the result of a decline in the number of cell divisions. Datta *et al.,* 2009 and Shamim *et al.,* 2014 explained that Increasing PEG and NaCl concentrations in the growth medium caused a consistent decrease in seed germination percentage and seedling growth. Thus, the variation in genotypes over the osmatic environments could provide scope for breeding drought tolerant genotypes. (Ahmad *et al.,* 2017 & Rana *et al.,* 2107) reported that seedling length, seedling fresh and dry weight, germination percentage and vigor index of wheat plants reduced and delayed with the increment of water stress induced by PEG. In addition, Hellal *et al.,* 2018 was done the same work but in barely crop. Bhutto *et al.,* 2018 evaluated Salinity tolerance during germination in completely randomized design with three replications. The results showed that different salt stress (NaCl) levels had significant effects on germination (%), seedling length (cm), seedling fresh and dry weights (g) of wheat varieties. Alom *et al.,* 2016 estimated wheat genotypes as salt tolerance through seed germination. The results revealed that germination percentage, germination vigor index, shoot length, root length, and seedling dry weight were found to be affected due to salinity. (Verma *et al.,* 2019) studied genetic variation of rice germplasm for improvements of abiotic stress tolerance. The results presented that the considerable variation across genotypes for root, shoot and drought tolerance traits. Furthermore, the principal component analysis (PCA) revealed the fresh shoot weight, root volume, dry shoot weight, fresh root weight and drought score as a major contributor to diversity. Hasan *et al.,* 2017 studied 33 wheat genotypes under 5 different salt concentrations (0, 5, 10, 15 and 20 dSm-1) with a complete randomize design (CRD) with 5 replications and the results showed that marked reduction of germination percentage, shoot and root length, shoot and root dry weight and vigor index was observed with the increasing of salt concentration for most of the wheat genotypes. Bilkis *et al.,* (2016) affirm that seedling length was significantly higher at normal saline conditions as compared to 12 dS m-1 salinity level and there were 6.45 to 36.80% shoot length and 19.67 to 62.93% root length reduction. Soare *et al.,* 2018 reported that 25% PEG concentration played the main role for the significant decrease of the germination percentage (GP) and vigor index (VI). Baque *et al.,* 2018 treated Pre-sowing seed wheat genotypes with Polyethylene Glycol (PEG) under salt stress condition to find out the effect of PEG on the germination, seedling growth and water relation behavior of wheat under different salt levels. Findings showed that seed priming with 10% PEG solution for 9 h had significant effect on germination, seedling growth and water relation behavior of wheat genotypes under salt stress condition. In addition, priming helped to reduce the magnitude of reduction. These results suggest that seed priming had significant effect to trigger the germination, seedling growth and water relation behavior of wheat genotypes under salt stress condition.

So, it may be concluded that among the 24 wheat genotypes, the genotypes (L (Sids4 X Kasonygiennson-81), Bectora, Canada-462, Benisuaif-6 and CIMMET-113) and (CIMMET-251, CIMMET-270, CIMMET-234, CIMMET-95 and Gimeza-10) showed promising performance against PEG_{6000} and NaCl simulation levels, respectively in compared to other genotypes due to their superior performance in the tested parameters.

CONCLUSION

The results verified a remarkable variation for genetic materials under PEG_{6000} and NaCl conditions. Therefore, from the foregoing present piece of work the increased of PEG_{6000} and NaCl concentrations leads to inhibited germination of wheat. Genotypes (L (Sids4 X Kasonygiennson-81), Bectora, Canada-462, Benisuaif -6 and CIMMET-113) and genotypes (CIMMET-251, CIMMET-270, CIMMET-234, CIMMET-95 and Gimeza-10) may be excellent genotypes to grow in regions where water deficiency and salinity stresses may be common during the germination and early seedling growth stages. These also may serve as excellent parents to initiate a breeding program using recurrent selection to develop even better water stress tolerant lines. It has also emerged that the PEG_{6000} and NaCl techniques would be suitable for screening drought tolerance in large populations prior to yield testing trials which could significantly reduce the over all cost and manpower. Using this method maximum germplasm can be tested for drought tolerance under limited space and resources.

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Author contributions
All authors contributed to the study conception and design. Material preparation, data collection, analysis, manuscript writing equally, as well all authors reviewed and approved the manuscript. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.

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