Multivariate Analysis for Spring Wheat Genotypes against Various NaCl Treatments

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ABSTRACT An excellent source of nutrients and a staple food crop is wheat (*Triticum aestivum* L.). For most people on the globe, wheat is an essential meal and a source of calories and carbohydrates. Wheat's growth and development are adversely affected by salinity stress, which lowers grain production and quality. The salt tolerance level of forty wheat genotypes was investigated using a complete randomized design (CRD) in 250 mL disposable cups. Diverse genotypes were screened and characterized with regard to their salt tolerance at the seedling stage against four treatment levels (control, 4 dSm^{-1}, 8 dSm^{-1}, and 12 dSm^{-1}). The data of descriptive statistics exhibited the ranges varied from minimum to maximum with grand mean values and standard deviation. A multivariate analysis showed that out of 12 principal components (PCs) only 4 have eigenvalues >1. and showed significant variation in all treatments. The four PCs showed values of 84.24 %, 89.75 %, 90.39 % and 74.87 % for cumulative genetic variation under normal, ST1 (4 dSm^{-1}), ST2 (8 dSm^{-1}), and ST3 (12 dSm^{-1}) conditions, respectively. The genotypes that have been found may offer significant assets for genetic enhancement initiatives aimed at enhancing comprehension of plant resilience to salinity-induced stress.

Keywords: PCA; Salinity; wheat; treatment levels; summary statistics

INTRODUCTION The three most important staple crops in the world, wheat, rice, and maize, account for a significant amount of daily food and protein consumption (Kizilgeci et al. 2021). Recent increases in the frequency and severity of various stresses have a direct impact on agricultural production and quality due to climate change and global warming. Over 20% of soils worldwide are impacted by salinity, and the amount of these soils is constantly growing as a result of human activity and climate change. Abiotic stresses are thought to constitute 50% of the reason for agricultural productivity reductions, which poses a significant risk to the world's food security (Seleiman,et al. 2019). Plants adapt their growth, development, and metabolism to changes in their surroundings. Plants experience rapidly changes in climate as stress. The abiotic factors that have the most effects on crop yield include salt, osmotic stress, drought, and extremely high or low temperatures. Plants that are stressed by water might be caused by several environmental factors.

Pakistan is situated in climatic regions with dry and semi-arid climates. considerable evapotranspiration accumulation was often seen on the soil surface. The minimum winter temperature stays between 2°C and 5°C, and the average summer temperature is around 40°C. In the country, the yearly rainfall ranges from 100 to 700 millimeters. The rate of evaporation is typically much higher than the rate of precipitation. As a result, the transport of salts toward the soil surface is accelerated by limited rainfall, high evaporative demand, and shallow groundwater depth (Song and Wang 2015, Ding et al. 2018). Salt concentrations in the soil which are harmful to crops have mainly been caused by improper watering techniques and poor drainage.

Wheat screening on physio-morphological traits like germination percentage (GP), shoot length (SHL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), germination index (GI), vigor index (VI), chlorophyll content (CC), stomatal conductance (SC), and relative water content (RWC) can be used to study the effect of salinity on wheat seedlings (EL Sabagh et al. 2020). So, these traits can be used as selection criteria for different wheat genotypes against salinity.

This is a beneficial methodology to check the response of plants growing in saline environments at seedling stage. Soil salinity has a significant effect on seedling establishment throughout the early stages of plant growth, which is an indication of good yield (Khayatnezhad et al. 2010). Therefore, wheat plants can be best analyzed against salt stress conditions at the seedling stage. Throughout a plant's life cycle, several, growth stage-specific mechanisms of salt stress response emerge (Walia et al. 2005). Plants show different responses to salt stress based on their physiological and growth characteristics (Ami et al. 2020). Wheat is a moderately salt-tolerant crop; indeed, it has the potential for improvement against salt stress through the selection and breeding of cultivated varieties (Saddiq et al. 2021).

The research was carried out to check the performance of different wheat genotypes under salinity stress during the germination or seedling stage. Forty genotypes of wheat were subjected to three different salinity levels, 4 dSm-1 (ST1), 8 dSm-1 (ST2), and 12 dSm-1 (ST3), with a control group (N), to check the effect of salinity. The main objective of this study was assessing the effect of salt stress in different wheat cultivars by assessing seedling and germination properties. Wheat breeders can use the results from this experiment to select or screen salt-tolerant cultivars and develop a higher-yielding cultivar in salt stress conditions through a wheat breeding program for sustainable food security.

MATERIAL AND METHODS
In this experiment, 40 distinct wheat genotypes were grown using a completely randomized design to compare salt tolerance at the seedling stage using physio-morphological parameters. Each genotype's five seeds were cultivated in 250 mL plastic cups that were filled with the sand mixture (Fan et al. 2015). The genotypes were watered after planting and then, before applying salt stress, exposed to the initial dose of one of three salinity treatments (ST1, ST2, or ST3), which corresponded to 4 dSm-1, 8 dSm-1, or 12 dSm-1, respectively, along with a control (N).To promote seedling germination, 20 mL of Hoagland solution (Hoagland and Arnon 1950) was added to each treatment. Salt solutions were then created in 1000-mL batches using this computation. To create the correct amount of solution, distilled water was mixed with the appropriate amount of NaCl (Xu et al. 2012). Fifteen days following the date of planting, a 40 mL dosage of saline solution was administered in each cup. Three doses, each comprising 40 mL, were used for each cup, spaced five days apart.

The data were recorded when the plants reached the stage of 3–4 leaves, or seedlings, after 30 days. A ruler was used to measure the shoot length (SL) and root length (RL). Using electrical balance, other seedling characteristics such as SFW, RFW, SDW, and RDW were assessed. A leaf porometer (model SC-1, Decagon Devices, Inc., USA) was used to measure the stomatal conductance (SC) in mmol m-2s-1, and a SPAD meter model CL-01 (Hansatech Instruments, Pentney King's Lynn, United Kingdom) was used to assess the relative chlorophyll concentration (Ellis and Roberts 1981, Ruan et al. 2002): (Karim et al. 1992).

The data recorded in the experiment were summarized and investigated through a principal component analysis (Ahmed et al. 2019) for further study using Minitab (v16) software. For extremely significant effects, the significance threshold was set at 0.01; for substantial effects, it was set at 0.05. Principal components (PCs) were deemed significant if their eigenvalue was greater than 1. The selection criteria for salt stress tolerance were determined using correlation analysis and principal component analysis data to choose salt-tolerant genotypes and advantageous seedling attributes.

RESULTS AND DISCUSSION
Results
Salinity has a serious impact on the physiological and morphological traits of wheat plants at the seedling stage. An experiment was conducted to analyze the seedling attributes of 40 wheat genotypes under various salinity levels ST1, ST2, and ST3, which equated to 4 dSm-1, 8 dSm-1, and 12 dSm-1, respectively. The descriptive data for all studied traits among genotypes are given in Table 1. The table mentioned the minimum, maximum, grand mean and standard deviation values for the studied attributes. Multivariate Analysis
The pattern of variation was examined in saline and normal conditions among 40 wheat genotypes through a multivariate analysis. To determine the significant traits in saline and normal conditions, the principal components (PCs) were also identified. Table 2 shows the eigenvalues, variability, and cumulative % of all studied traits in all stressed conditions. There was a total of 12 principal components (PCs) in the analysis that caused the variability in data, of which four were significant in all treatments except ST2. The first four PCs showed a total of 84.24%, 89.75%, and 74.87% variation under normal, ST1, and ST3 conditions, respectively, while ST2 conditions had 90.39% variation and only three significant PCs. The first component had 46.6% variability in control conditions, 58.44% in ST1, 62.8% in ST2, and 32.08% in ST3. This PC showed a major contribution from the SFW in control conditions, SDW in ST1, SFW in ST2, and VI in ST3 (Table 2). All traits had a positive impact on the variability between treatments, except RWC on ST3 and CC on ST2, which had a negative impact on variability. The second principal component (PC2) had 17.86% variability in control conditions, 14.63% in ST1, 13.19% in ST2, and 19.67% in ST3. The major contribution to variability among all treatments was from GP, while all major traits had a negative impact on the second principal component in salt as well as normal conditions. The third PC was also significant in the control, ST1, ST2, and ST3. PC3 had 10.79% variability in control conditions, 8.8% in ST1, 8.09% in ST2, and 14.3% in ST3. The maximum contribution to variability in PC3 was from SC in control conditions as well as in treatment 1 (ST1) conditions, while in ST3 the major contribution was from SDW while other traits had a negative impact on variability. In normal, ST1, and ST3 conditions, PC4 was also significant. In control conditions, the variability for PC4 was 8.94%, in ST1 it was 7.84%, and in ST3 it was 8.74% (Table 2). Summary statistics for PCA and minimum and maximum values for each trait, along with the mean and standard deviation under normal and salinity stress conditions, are given in Table 3. Factor loading gives the percentage of total variance present in the

variable. Each factor was positively and negatively supported by the traits on which the analysis was carried out. The data presented in Table S6 show the factor loading of all treatments. In the given experiment, traits like SFW in normal, SDW in ST1, SFW in ST2, and VI in ST3 conditions showed maximum variance in the first PC. In the second PC, the maximum variance was shown by GP in all treatments. In the first factor or PC, RWC in ST3 and CC in ST2 had a negative impact on the overall variance of the factor. In the second factor or PC, all traits had a negative impact on overall variance except GP, which had a positive impact on PC. A biplot was generated between only two main factors or PCs because the first two components had maximum variability among all principal components. The biplot has four main axes, with the upper right axis having a positive impact on PC1 and PC2, and the genotypes located in that block are best for selection because the varieties in that block have the highest variation among all studied germplasm. The traits in that block can also be used for salinity tolerance selection. Figures 1–5 show the number of varieties present in each block. Both PC1 and PC2 had a negative impact on variability in the lower left block. Among all genotypes, the genotypes present in that block had the lowest performance or were salt-susceptible. The varieties in the upper right block were the best performers in salt stress, as shown in Figures 1–5. These cultivars should be selected for use in future breeding programs.

PCA was used to determine which genotypes perform better in normal and saline conditions. This is a powerful technique for selecting better genotypes for breeding programs. A biplot was constructed between the first two PCs (Figures 1–5) and the results showed that genotypes G7, G10, G17, G35, and G36 were present on the positive axis along with traits like SL, GP, VI, CC, RL, SWF, and RFW, and hence were considered as salt-tolerant, while genotypes G6, G16, G27, and G16 were included in the negative axis (Figure 5) and hence can be considered salt-susceptible genotypes. The remaining genotypes were thought to be neither tolerant nor susceptible since they only showed very little tolerance, but also not enough to qualify as sensitive.

Principal Component Analysis (PCA)

PCA is a data analysis technique that can be used to study and simplify large sets of data. PCA converts complex datasets into smaller ones and correlates the variables (Ahmed et al. 2019, Pour-Aboughadareh et al. 2021). PCA used a variety of primary (principal) components. Every principal component has an eigenvalue that contributes a certain amount to it. Significant principal components are those with eigenvalues larger than 1, while nonsignificant components do not. To find the significant traits in saline and normal conditions, the PCA was executed as indicated in Table 3. The statistical importance of the eigenvalues utilized to choose the statistically significant principal components (PCs) was addressed by (Ahmed et al. 2019).

The PCA results from this study were comparable to those from wheat scientists (Pour-Aboughadareh et al. 2019). The reduction of relative water content (RWC) in the cell may be the cause of the negative correlation between chlorophyll content and other traits, as this condition results in the leakage of electrolytes and the peroxidation of lipids from the chloroplast's thylakoid membrane and a loss of chlorophyll content (Ristic et al. 2007, Djanaguiraman et al. 2010). Both in normal and saline circumstances, all main parameters had a negative effect on the second principal component; these findings are corroborated by prior research in wheat crops utilizing seedling attributes (Marček et al. 2021). Germination % offers an advantage since all other factors grow as germination does. Under normal and ST3 circumstances, VI and SC, respectively, made up most of the variability in PC4. To choose varied parents for hybridization and other plant breeding methods, principal component analysis is also useful. The genotype projection on PC1 and PC2 is helpful in choosing parents. On the two PCs, the predicted genotype pattern revealed the population structure under both normal and drought situations (Marček et al. 2021). Figures 1-5 show that genotypes that appeared in the same square box performed equally well, but genotypes that appeared in other boxes performed differently.

Factor loading gives the percentage of total variance present in the variable. Each factor was positively and negatively supported by the traits on which the analysis was carried out by wheat breeders (Yong and Pearce 2013). For the first factor or PC, the negative impact on the overall variance of the factor was RWC in ST3 and CC in ST2. For the second factor or PC, the negative impact on overall variance was given by all traits except GP, which had a positive impact on PC.

A principal component biplot showed that the variables are distributed on the plots as vectors. The division into PC1 and PC2 showed the differences in variables in terms of different characteristics studied in normal and saline conditions. The first two components showed the most variability. They were used to construct the PC biplots. The trait vectors were represented by acute angles, indicating a positive relationship; the genotypes situated on that acute angle are best for selection as these varieties have the maximum variation among the whole available germplasm (Akcura et al. 2011). Figures 1–5 show the number of varieties present in each block. The upper left has a positive impact on PC2 but a negative impact on PC1 for total variability. Similarly, the lower right has a positive impact on PC1 but a negative impact on PC2 on overall variability. The lower left block shows the negative impact of both PC1 and PC2 on variability. The varieties present had the lowest performance and were most salt susceptible among all genotypes. The varieties present in the upper right block were the best-performing varieties in salt tolerance with respect to the traits present in this block. These cultivars should be selected and used in future breeding programs.

PCA was used to determine which genotypes perform better, in normal and saline conditions. It is a powerful technique to select better genotypes for breeding programs. Many scientists (Dadbakhsh et al. 2011, Ahmed et al. 2019) have also reported that genotypes with higher PCA-1 and smaller PCA-2 have more yield potential as compared to smaller PCA-1 and higher PCA-2. The other genotypes demonstrated extremely low levels of

tolerance, but not to the point where they were vulnerable, therefore they were not classified as either tolerant or susceptible.

Table 1. Descriptive statistics for 40 genotypes under control and stress conditions

Trait	eatment	Minimum	Maximum	Mean	SD(n-1)
GP	N	26	93.33	72.19	12.85
	ST1	20	79.84	56.45	12.67
	ST2	19.5	77.57	56.51	12.05
	ST3	17.5	74.06	54.3	11.74
SL(cm)	N	6.5	25.3	17.33	3.36
	ST1	4.8	22.7	14.65	3.24
	ST2	5.1	16.9	12.59	2.82
	ST3	4.5	16	13.46	2.59
RL(cm)	N	6.5	19.7	11.89	2.82
	ST1	5.08	18.13	10.48	2.32
	ST2	4.46	18.25	11.54	2.66
	ST3	3.79	16.27	11.66	2.79
SFW (mg)	N	0.07	0.49	0.281	0.092
	ST1	0.05	0.218	0.14	0.03
	ST2	0.04	0.148	0.11	0.025
	ST3	0.04	0.139	0.122	0.021
RFW (mg)	N	0.03	0.155	0.094	0.026
	ST1	0.028	0.155	0.091	0.023
	ST2	0.024	0.152	0.095	0.024
	ST3	0.022	0.149	0.08	0.027
SDW (mg)	N	0.051	0.357	0.173	0.065
	ST1	0.045	0.172	0.109	0.023
	ST2	0.027	0.118	0.088	0.019
	ST3	0.023	0.109	0.087	0.06
RDW (mg)	N	0.015	0.112	0.067	0.019
	ST1	0.021	0.121	0.071	0.017
	ST2	0.024	0.123	0.079	0.017
	ST3	0.014	0.132	0.061	0.024
SDL (mg)	N	13.4	43.4	29.1	5.5
	ST1	8.21	37.2	25.01	5.43
	ST2	7.05	34.1	24.1	5.48
	ST3	10.3	42.3	26.5	5.98
GI	N	59.15	111.41	98.38	10.48
	ST1	45.5	85.7	75.67	8.06
	ST2	50.39	84.99	75.88	6.83
	ST3	45.65	84.67	72.72	8.5
VI	N	5.23	32.35	21.34	5.69
	ST1	2.65	24.19	14.43	4.5
	ST2	1.18	23.87	13.857	4.64
	ST3	3.08	21.5	13.61	4.14
CC	N	1.26	4.47	2.2	0.601
	ST1	0.55	2.21	1.09	0.366
	ST2	0.45	1.84	1.12	0.315
	ST3	0.39	1.21	0.81	0.221

SC	N	6.2	21.2	13.5	3.75
	ST1	5.3	20.2	11.6	3.19
	ST2	4.2	18.6	10.8	3.45
	ST3	1.7	14.8	7.64	2.79
RWC	N	12.22	82.24	56.35	13.83
	ST1	19.14	51.99	30.39	6.69
	ST2	18.5	50.15	27.13	6.73
	ST3	1.73	14.83	7.64	2.78

GP= germination percentage, SL= shoot length, RL= root length, SFW= shoot fresh weight, RFW= root fresh weight, SDW= shoot dry weight, RDW= root dry weight, CC= chlorophyll content, SC= stomatal conductance, VI= vigor index, GI= germination index, RWC= relative water content, N= control, ST1= 4dS/m salt treatment, ST2= 8dS/m salt treatment ST3= 12dS/m salt treatment

Table 2. Eigenvalues, variability %, and cumulative % of traits in control and saline condition.

	ironments	PC1	PC2	PC3	PC4
Eigenvalue	N	5.59	2.14	1.29	1.07
	ST1	7.01	1.75	1.06	1.00
	ST2	7.54	1.58	1.00	0.74
	ST3	3.85	2.36	1.72	1.04
Variability (%)	N	46.64	17.86	10.79	8.94
	ST1	58.44	14.63	8.83	7.84
	ST2	62.89	13.19	8.09	6.21
	ST3	32.08	19.67	14.37	8.74
Cumulative %	N	46.64	64.50	75.30	84.24
	ST1	58.44	73.08	81.91	89.75
	ST2	62.89	76.08	84.18	90.39
	ST3	32.08	51.75	66.12	74.87

Table 3. Summery statistics for PCA and genotypes minimum, maximum value along with mean and standard deviation

Variable		Minimum	Maximum	Mean	Std. deviation
GP	N	26.000	93.333	72.190	12.851
	ST1	21.500	79.840	56.504	12.556
	ST2	19.560	77.573	56.428	12.192
	ST3	17.510	74.067	54.170	11.963
SL	N	6.533	25.300	17.338	3.346
	ST1	4.833	22.700	14.663	3.239
	ST2	4.650	16.867	12.575	2.846
	ST3	4.467	15.967	13.453	2.595
RL	N	6.500	19.700	11.896	2.828
	ST1	5.075	18.133	10.481	2.324
	ST2	4.458	18.247	11.548	2.665
	ST3	3.700	16.267	11.661	2.792
SFW	N	0.069	0.491	0.281	0.093
	ST1	0.045	0.218	0.140	0.031
	ST2	0.043	0.148	0.113	0.025
	ST3	0.041	0.139	0.122	0.021
RFW	N	0.030	0.155	0.095	0.026
	ST1	0.028	0.155	0.092	0.023
	ST2	0.024	0.152	0.095	0.024
	ST3	0.022	0.149	0.080	0.027
SDW	N	0.051	0.357	0.174	0.066
	ST1	0.045	0.172	0.109	0.024

	ST2	0.027	0.118	0.089	0.019
	ST3	0.023	0.109	0.086	0.018
RDW	N	0.022	0.112	0.067	0.019
	ST1	0.020	0.107	0.070	0.018
	ST2	0.017	0.094	0.071	0.017
	ST3	0.014	0.092	0.057	0.019
GI	N	59.153	111.416	98.382	10.483
	ST1	45.502	85.705	75.678	8.064
	ST2	50.399	84.992	75.887	6.832
	ST3	45.656	84.671	72.727	8.507
VI	N	5.239	32.359	21.344	5.696
	ST1	2.650	24.195	14.431	4.507
	ST2	1.188	23.874	13.857	4.644
	ST3	3.088	21.501	13.616	4.149
RWC	N	12.222	82.241	56.350	13.830
	ST1	19.147	51.993	30.399	6.690
	ST2	18.511	50.151	27.134	6.735
	ST3	6.825	49.997	27.042	11.058
CC	N	1.257	4.467	2.276	0.601
	ST1	0.554	2.214	1.101	0.367
	ST2	0.450	1.843	1.073	0.315
	ST3	0.390	1.210	0.816	0.222
SC	N	6.200	21.200	13.573	3.754
	ST1	5.333	20.200	11.609	3.195
	ST2	4.200	18.633	10.880	3.458
	ST3	1.733	14.833	7.641	2.786

GP= germination percentage, SL= shoot length, RL= root length, SFW= shoot fresh weight, RFW= root fresh weight, SDW= shoot dry weight, RDW= root dry weight, CC= chlorophyll content, SC= stomatal conductance, VI= vigor index, GI= germination index, RWC= relative water content
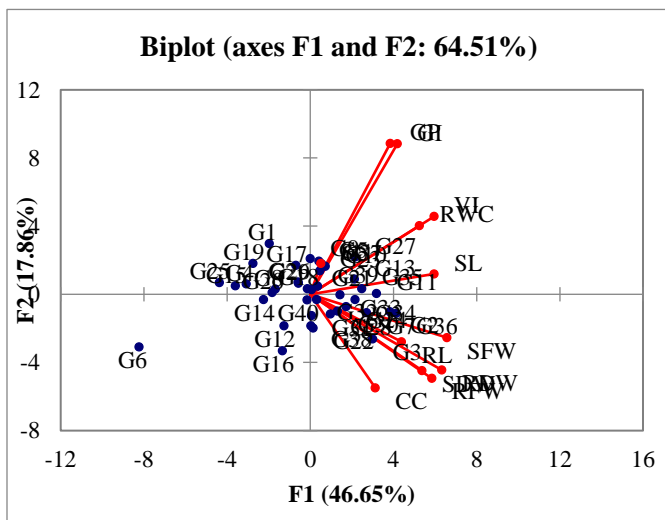

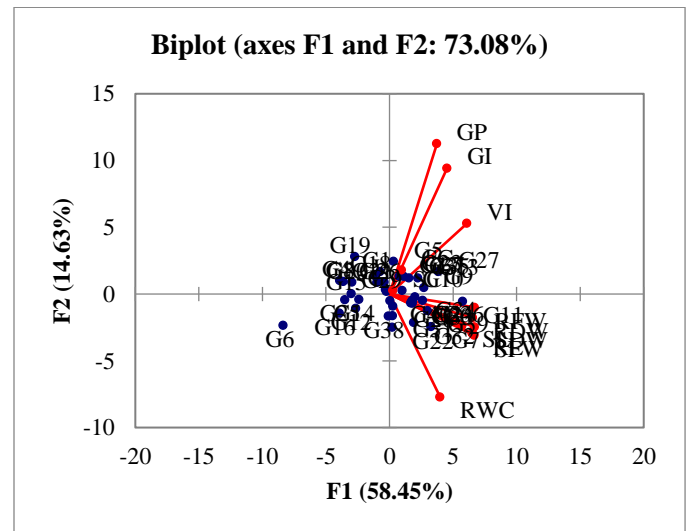**Figure 1.** Biplot analysis graph for normal conditions.

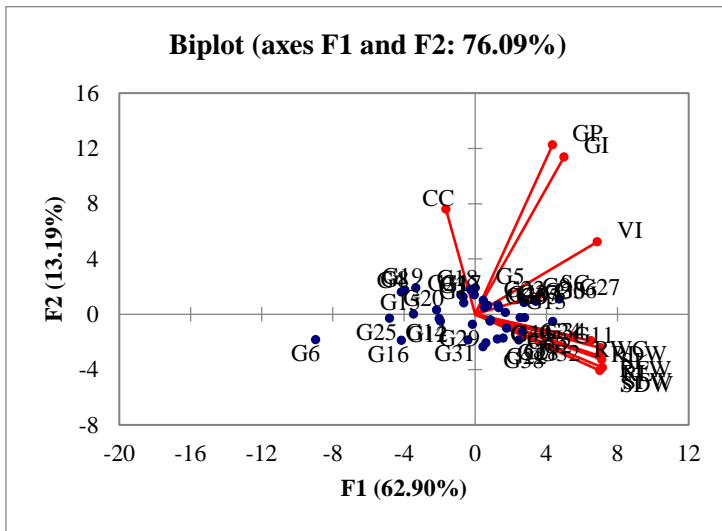**Figure 2.** Biplot analysis graph for ST1 conditions.

Biplot (axes F1 and F2: 76.09%)

Figure 3. Biplot analysis graph for ST2 conditions.

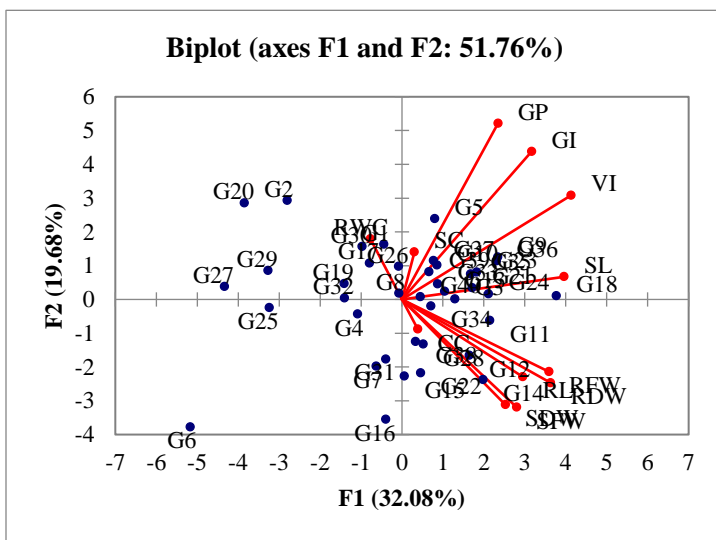**Biplot (axes F1 and F2: 51.76%)**

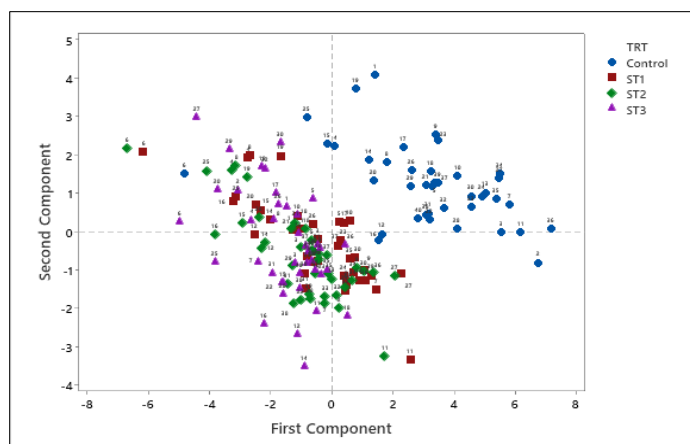**Figure 4.** Biplot analysis graph for ST3 conditions.

Figure 5. Combined biplot analysis graph for N, ST2, ST2, and ST3.

CONCLUSION

In this Research, a complete randomized design was used to evaluate forty different genotypes of wheat against salt stress. The most widely used and consumed grain crop worldwide is wheat. Salinity stress, however, poses serious threats to the world's food, nutritional security, and wheat production. Because salt causes oxidative stress as well as ionic and hormonal imbalances, it has a deleterious impact on a variety of biological processes, including seed germination, plant growth, photosynthesis, ATP generation, water relationships, nutrient uptake, and yield. According to the multivariate analysis, the first four PCs were significant and their biplot showed a difference between genotypes under control conditions and different salinity stress levels. The genotypes G7, G10, G17, G18, G36, and G35 were considered salt tolerant due to their performance under saline conditions. Three genotypes were considered susceptible to salinity stress (G6, G16, and G27) due to their having the worst performance. The genotypes and selection criteria for desired attributes were clearly distinguished in the current investigation. Future efforts in wheat breeding can utilize the best-performing genotypes to create cultivars that are resistant to salt stress, which will help fulfill the world's need for wheat and provide food security for future generations.

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