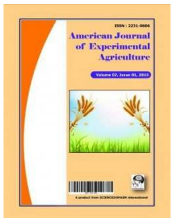

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Gas Exchange Traits and Biplot Analysis Method can be used as Screening Criteria for Salt Tolerance in Maize (*Zea mays* L.) Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Abdul Majeed and MAUH designed the study, wrote the first draft of the manuscript. Authors AN, JA and ZAA managed the analysis of the study. Authors Atif Muhmood, MAA and SSHS managed the review of literature and statistical analysis. All co-authors read and approved the final manuscript.

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ABSTRACT

Water induced salt stress among other abiotic stress is a main problem and decreases crop productions in arid and semiarid areas of the world. However, selection of salt tolerant cultivars by screening method in solution culture at vegetative stage is an easy and economical way to combat with salinity problems. The present hydroponic study was conducted to evaluate the salt tolerance of some newly approved and candidate cultivars of maize on the basis of gas exchange, growth and ionic traits. Three water salinity levels having different electrical conductivity (EC), sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) were developed and fifteen maize cultivars were grown in Hoagland's nutrient solution. The results depicted that the highest reduction

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in plant photosynthetic rate, K^+ ion uptake and K^+: Na^+ under onset of salt stress was recorded in Sadaf, FH-963, FH-722 and FH-793 and the minimal in Sahiwal-2002 and Afgoi. Salt tolerant cultivars uptake less Na^+ which depicted that Na^+ ion exclusion is positively correlated with salt tolerance and plant photosynthetic rate. Biplot analysis method appeared valuable over conventional relative salt tolerance method due to its graphical evaluation of superior cultivars and grouping of cultivars in a particular environment in the biplot graph.

Keywords: Photosynthetic rate; Biplot graph; K^+: Na^+ ratio.

1. INTRODUCTION

Water induced soil salinity is the major threat to worldwide maize productions. Salinity is a main hazard to cultivated land and globally about 800 million hectares of land is salt affected [1]. About 12 billion US dollars annual loss is estimated due to salinity to the world economy and it is still on increase [2]. This huge salt affected area and financial loss is a matter of concern for growers who face a decrease in their output due to this salinity problem. Moreover, increasing food security issues are forcing stake holders and agriculture sector to bring more land under cultivation. Worldwide, about 50% food requirements will be increased by 2050 due to increasing population growth [3,4]. At present, the most suitable land has already under cultivation, and the estimated target can be achieved either by expansion in cultivated areas and developing cultivars that give higher yield on salt affected soil irrigated with poor quality water. By developing salt tolerant cultivars significantly crop yield can be increased in arid and semi-arid regions [5].

Water induced salt stress causes a number of adverse biochemical, ionic and physiological changes in plants that resulted in reduction of plant photosynthetic rate and finally reduced crop yield and quality. However, the decline in plant growth and crop yield generally differ among various species and cultivars [6,7,8]. Of different physiological traits, plant gas exchange traits are the most important one and significant variation in these gas exchange traits happen under salt stress [9]. The plants under salt stress showed a substantial decrease in photosynthetic and transpiration rates [10]. The different responses of plants in relation to various gas exchange traits under salt stress not only occur within species but also within the cultivars of same species [9,6,11]. Plant growth is ultimately reduced under salt stress conditions yet plant species and genotypes differ in their sensitivity or tolerance to salts [12,13]. The development and screening of salt tolerant genotypes is one of the best and economical ways of handling the salinity problems [14]. The field screening results can be misleading because salinity effects intermingle with water logging and drought [15]. In field conditions salinity appears in patches and soil pH, temperature, organic matter, nutrients availability and soil moisture contents differ from one area to other and even within same and between fields. While in hydroponics culture, mineral nutrients are easily available to plants as compared to soil. Therefore this technique is the best one due to less environmental variations [16,17] and this method has been used by a number of scientists for screening of salt tolerant genotypes [18,19,20]. The present study was planned to evaluate the comparative salt tolerance of some newly approved and some candidate maize cultivars at the vegetative stage and to understand the relationships between the salinity induced alterations in different physiological, growth and ionic traits. The different statistical methods such as relative salt tolerance, absolute salt tolerance [21,22] GGE biplot [23] and susceptibility index have been used to distinguish the salt tolerance response of different crop genotypes [24,25]. These methods give confusing results and errors with the exception of GGE biplot [26,17,24]. Furthermore, these methods are also not capable of displaying genotypes average performance across saline treatments and treatment interactions as compared to GGE biplot graphs that depicted better results [24]. Biplot is a statistical method that simultaneously shows the effects of genotypes and the traits. The objective of present study was to evaluate maize genotypes by traits interaction and to study the inter relationships among traits using biplot analysis.

2. MATERIALS AND METHODS

2.1 Plant Growth and Description of Treatments

Seeds of 15 maize cultivars, FH 421, FH 810, FH 793, FH 722, FH 985, FH 763, Sahiwal 2002 (SWL -2002), Golden, EV 1098, Afgoi, EV 5098, Sdaf, EV 6089, EV 6098 and Agati 2002 were procured from the maize department, Ayub Agricultural Research Institute Faisalabad and from Maize Millet Research Institute, Yousaf wala, Sahiwal, Pakistan. Healthy seeds of obtained maize cultivars were sown in trays containing pre-washed sand. For the germination of seeds water was sprinkled daily to maintain optimum moisture. When seedling growth reached at 2-3 leaf stage, the seedlings of each maize cultivar were transplanted in holes of thermo pole sheet with the help of foam wrapped at shoot root junction, floating on 200 liters water capacity iron tubs. These iron tubs were lined with polythene sheet and containing half strength Hoagland's nutrient solution [27]. Proper aeration was provided by bubbling air through nutrient solution for 8 hours a day by aeration pump. After one week of transplantation, three levels of water salinity viz. T_1 [Control], T_2 [EC 2.0 dS m^{-1}, SAR 15.0 (m mol $L^{-1})^{1/2}$ and RSC 2.25 m $mol_c L^{-1}$] and T_3 [EC 4.0 dS m^{-1}, SAR 25.0 (m mol $L^{-1})^{1/2}$ and RSC 5.0 m mol_c L^{-1}] were developed. The combinations of four salts ($NaHCO_3$, Na_2SO_4, $CaCl_2.2H_2O$ and $MgSO_4.7H_2O$) were used to prepare these water salinity levels. In control treatment only Hoagland solution was provided and no salt was added. The solution pH was adjusted to 6.5±0.5 throughout the experiment with 1 M NaOH or HCl, as required.

2.2 Measurements of Gas Exchange Attributes

A fully expanded second leaf of maize plant from each repeat was used to record data of different gas exchange traits such as photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs). The above mentioned gas exchange parameters were measured by using an open system LCA-4 ADC portable infrared gas analyzer. All measurements were recorded in noon when light intensity was full (at 10:30 a.m. to 12:15 p.m.) with adjustments stated elsewhere [6,9].

2.3 Plant Harvest and Determination of K^+ and Na^+

The plants were harvested after three weeks onset of salinity stress and plant leaves were washed with distilled water and then dried on filter paper sheet. The plant shoot fresh weight was recorded and then youngest fully expanded second leaf of plants from each repeat was separated and stored at freezing temperature to determine K^+ and Na^+. The rest of the plant samples were dried at 65±2°C for 48 hours in oven to record shoot dry weight of each cultivar. The leaf sap was extracted using the method of [28] and diluted with distilled water as required for the determination of K^+ and Na^+ contents with the help of flame photometer (Janway PFP-7) by using self- prepared standard solutions from reagent grade salts of KCl and NaCl.

2.4 Experimental Design and Biplot Analysis

The experimental plan was CRD (completely randomized design) with factorial arrangements. Data of each trait were subjected to CoStat Computer Program (version 6.303, USA) for calculating ANOVA and means were compared with a least significant difference (LSD) test [29]. The mean data were subjected to principle component analysis and a biplot graph was made to facilitate the visual evaluation of superior cultivars and grouping of cultivars in the biplot graph [23].

3. RESULTS

3.1 Effect of Water Induced Salinity Stress on Plant Gas Exchange Traits

Water induced salt stress significantly reduced the plant photosynthetic rate (A) and stomatal conductance (gs) of all maize cultivars. A significant dissimilarity was found in all maize cultivars on the basis of these traits. The highest salt induced reduction in A and gs under saline (T_2) and saline sodic (T_3) water treatments were recorded in FH-963, FH-722, Sadaf and FH-793 and the minimum being in SWL-2002 and Afgoi (Table 1). However, leaf transpiration rate (E) was also reduced under imposition of salt stress and this reduction was minimal in SWL-2002 followed by Afgoi and maximal in Sadaf and FH-963 (Table 2). The biplot graphs of plant photosynthetic and transpiration rates also showed visual dissimilarity among cultivars under salt stress treatments and depicted those cultivars SWL -2002 and Afgoi made the longest vectors towards salinity treatments and were categorized the salt tolerant cultivars (Figs. 1 and 2). While the cultivars FH-722, Sadaf, FH-963 and FH-793 showed a susceptible response across salinity treatments and was ranked as salt sensitive because these cultivars appeared on the opposite side of salinity treatment vectors in the biplot graph.

Table 1. Effect of saline/saline sodic water on plant photosynthetic rate (µmol CO_2 m^{-2} S^{-1}) and stomatal conductance (m mol m^{-2} S^{-1}) of maize genotypes

Maize genotypes	Photosynthetic rate			Stomatal conductance		
	T_1	T_2	T_3	T_1	T_2	T_3
FH 421	15.67±0.88	5.26±0.30	2.19±0.06	165±3.53	57±4 .33	33±8.82
FH 810	16.00±0.58	5.98±0.37	2.77±0.12	171±6.36	64±4 .67	40±5.36
FH 793	16.23±0.68	4.50±0.20	1.97±0.09	167±8.82	53±8 .82	30±5.77
FH 722	15.72±0.47	4.55±0.23	1.88±0.09	160±5.77	47±8 .82	30±2.31
FH 985	16.67±0.88	6.90±0.32	2.92±0.05	169±5.21	70±3 .18	53±3.76
FH 963	15.92±1.00	3.10±0.08	1.93±0.09	170±5.77	44±5 .57	26±2.96
SWL 2002	15.84±0.99	8.64±0.18	5.97±0.15	160±5.77	85 ±2.91	61±4.93
Golden	15.42±0.87	7.08±0.15	3.55±0.25	155±7.64	62±3 .93	44±3.48
EV 1098	14.44±0.84	6.16±0.13	2.91±0.06	165±2.91	63± 8.82	38±3.06
Afgoi	16.26±0.51	7.57±0.23	4.20±0.25	154±4.33	71±6. 66	52±2.33
EV 5098	13.88±1.18	5.01±0.17	2.45±0.16	163±12.02	60 ±5.77	37±3.33
Sadaf	14.90±0.67	4.39±0.19	1.27±0.07	169±4.93	43±5. 13	20±2.03
EV 6089	14.60±0.91	4.97±0.07	2.30±0.11	161±5.93	60± 5.77	40±5.77
EV 6098	15.00±0.72	5.91±0.36	2.98±0.27	160±1.73	53± 8.82	42±3.93
Agati 2002	15.61±0.40	7.57±0.32	3.10±0.09	163±12.02	67±4.81	45±2.91

T_1 (Control), T_2 (EC 2.0, SAR 15 and RSC 2.25), T_3 (EC 4.0, SAR 25 and RSC 5.0)
Each value is an average of 3 replicates ± S.E

Table 2. Effect of saline/saline sodic water on plant leaf transpiration rate (mmol H_2O m^{-2} S^{-1}) and K^+ : Na^+ ratio of maize genotypes

Maize genotypes	Transpiration rate			K^+ : Na^+ ratio		
	T_1	T_2	T_3	T_1	T_2	T_3
FH 421	3.62±0.21	2.34±0.18	1.50±0.12	2.57±0.14	0.57 ±0.012	0.30±0.010
FH 810	3.60±0.16	2.44±0.23	1.92±0.06	2.77±0.11	0.63 ±0.017	0.35±0.005
FH 793	3.48±0.27	2.22±0.21	1.33±0.09	2.25±0.08	0.48 ±0.029	0.22±0.004
FH 722	3.77±0.12	2.00±0.15	1.53±0.12	2.24±0.06	0.45 ±0.043	0.21±0.008
FH 985	4.04±0.13	2.90±0.07	2.14±0.18	2.79±0.15	0.72 ±0.019	0.41±0.009
FH 963	3.72±0.19	1.96±0.18	1.31±0.05	2.23±0.08	0.49 ±0.018	0.19±0.007
SWL 2002	3.75±0.09	3.24±0.15	2.89±0.08	3.07±0.09	1. 24±0.016	0.90±0.021
Golden	3.68±0.23	2.80±0.08	1.97±0.03	2.58±0.08	0.87 ±0.016	0.53±0.011
EV 1098	3.49±0.26	2.56±0.13	1.82±0.16	3.14±0.20	0.8 5±0.018	0.42±0.014
Afgoi	3.80±0.11	3.18±0.09	2.49±0.11	3.28±0.10	1.04± 0.029	0.67±0.025
EV 5098	3.39±0.21	2.82±0.12	1.76±0.18	3.06±0.19	0.8 4±0.014	0.48±0.005
Sadaf	3.77±0.12	1.55±0.19	1.15±0.12	2.40±0.05	0.67± 0.029	0.35±0.0004
EV 6089	3.62±0.25	2.91±0.05	1.80±0.11	2.95±0.14	0.8 2±0.016	0.50±0.002
EV 6098	3.53±0.24	2.85±0.09	1.95±0.03	3.00±0.12	0.8 6±0.038	0.47±0.018
Agati 2002	3.44±0.09	3.03±0.12	1.91±0.16	2.87±0.12	0.84±0.004	0.45±0.020

T_1 (Control), T_2 (EC 2.0, SAR 15 and RSC 2.25), T_3 (EC 4.0, SAR 25 and RSC 5.0)
Each value is an average of 3 replicates ± S.E

3.2 Effect of Water Induced Salinity Stress on Plant Ionic Traits

Significant variances were recorded for concentrations of K^+ and Na^+ in the leaf sap of all maize cultivars under salinity stress as compared to control. By increasing salt stress, significant increases in Na^+ concentrations were recorded in each maize cultivar than control. The lowest increase in Na^+ concentrations were recorded in SWL-2002 and Afgoi while the highest in FH-722, FH-963 and FH-793 cultivars at both salinity levels respectively.

The salt tolerant cultivars appeared on the opposite side of salinity vectors in biplot graphs of Na^+ and sensitive cultivars showed longest vectors towards salinity treatment vectors (Fig. 3). The trend in case of K^+ was almost opposite, the salt tolerant maize cultivars uptake higher K^+ and maintained higher K^+: Na^+ as compared to salt sensitive (Table 2). Similarly the PCA biplot graph of K^+ showed visual variation among cultivars under different treatments and the graph showed that cultivars SWL -2002 and Afgoi had the longest vectors with salinity treatments and were ranked as salt tolerant (Fig. 4). Cultivars FH-963 and FH-793 had a susceptible response across salinity treatments and these appeared on the negative side of treatment vectors and were ranked as salt sensitive.

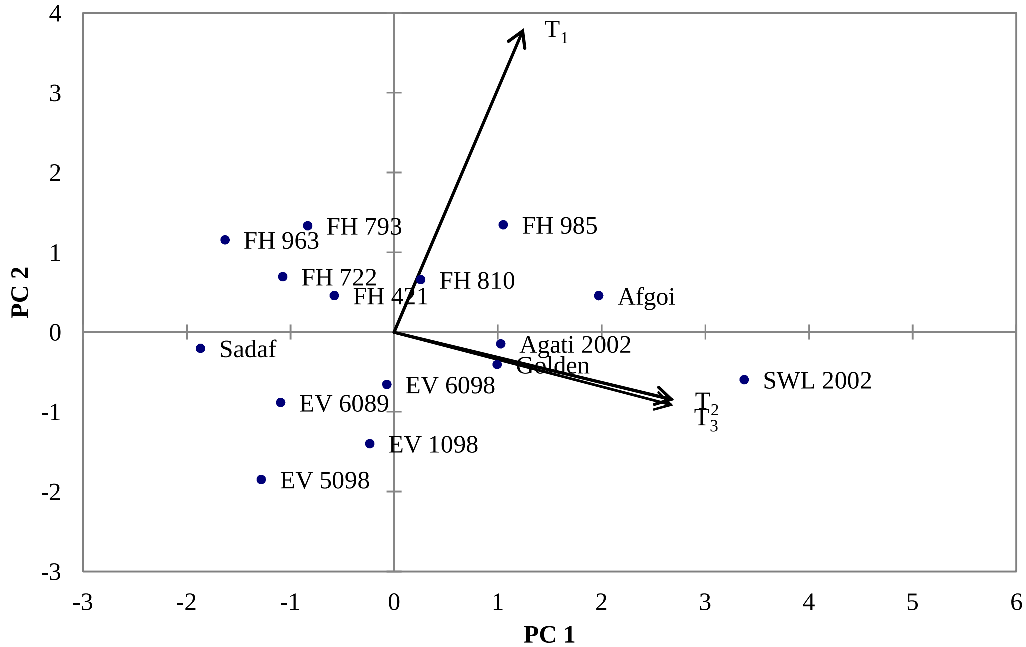

Fig. 1. Biplot graph for plant photosynthetic rate of 15 maize genotypes at three different salinity levels (PC 1, principal component 1, PC 2, principal component 2)

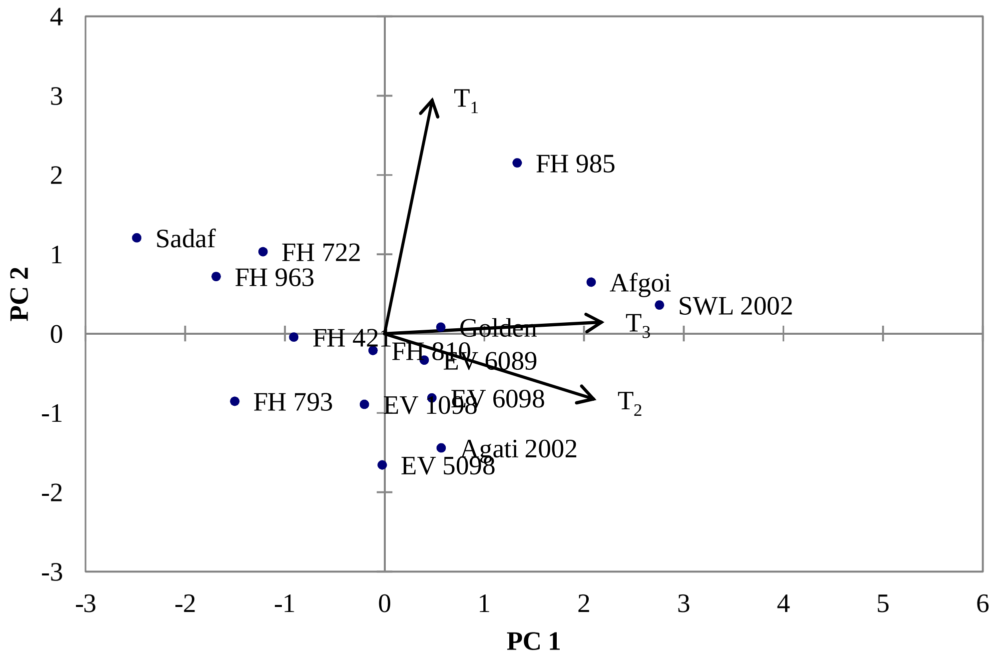

Fig. 2. Biplot graph for plant transpiration rate of 15 maize genotypes at three different salinity levels (PC 1, principal component 1, PC 2, principal component 2)

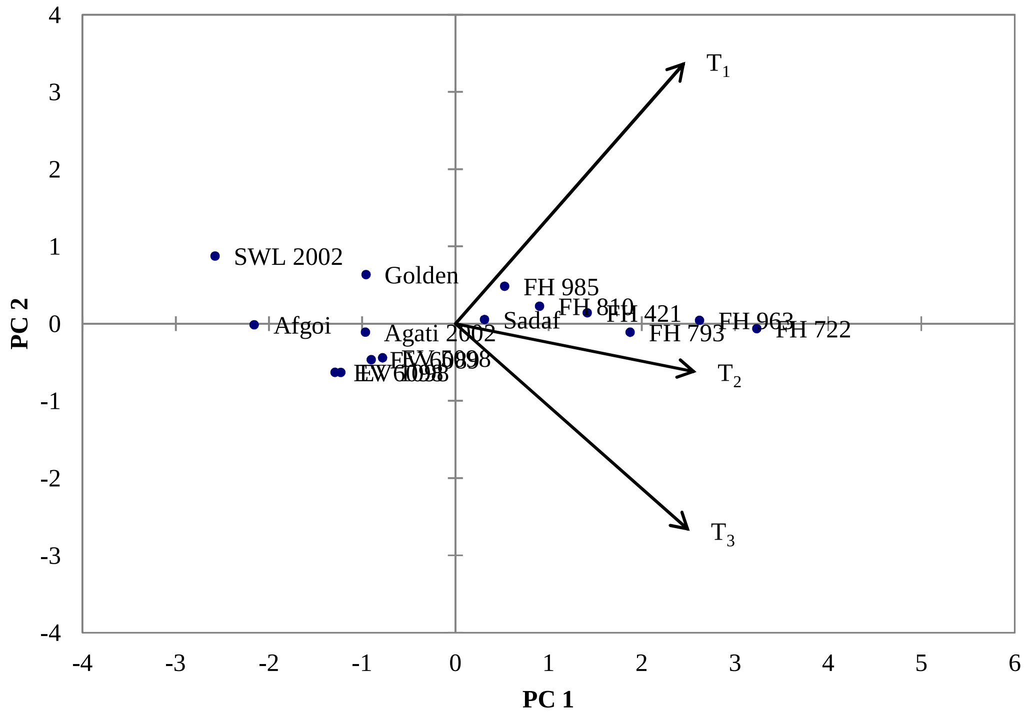

Fig. 3. Biplot graph for Na^+ of 15 maize genotypes at three different salinity levels (PC 1, principal component 1, PC 2, principal component 2)

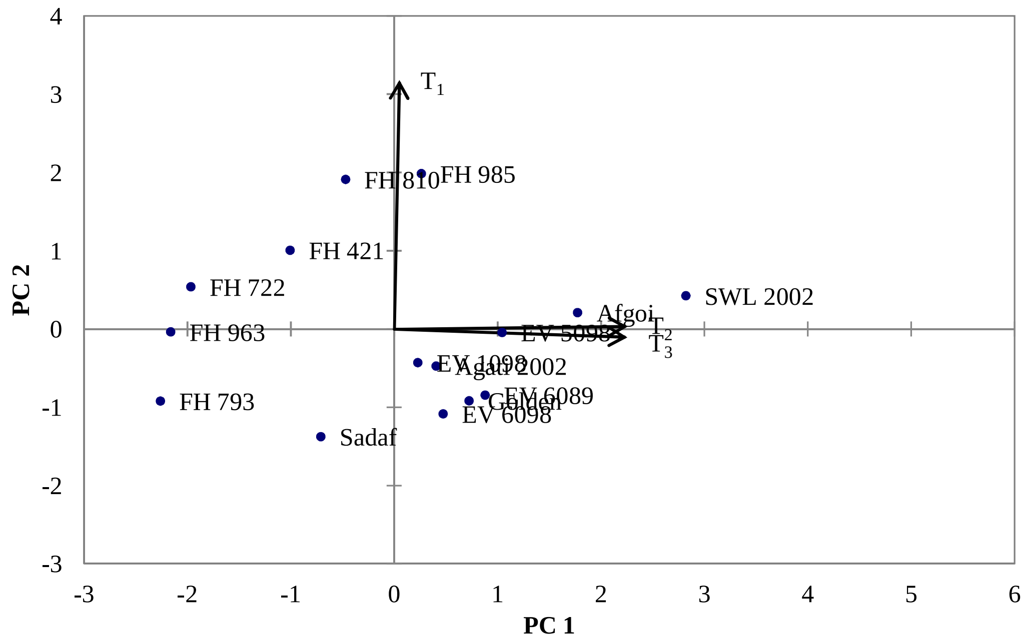

Fig. 4. Biplot graph for K^+ of 15 maize genotypes at three different salinity levels (PC 1, principal component 1, PC 2, principal component 2)

4. DISCUSSION

Selection of cultivars that have capability to grow with poor quality water will help to increase the crop growth and production. Development of salt tolerant variety is very difficult mechanism due to less genetic germplasm resources, lack of proper selection methods and variations in response to salt tolerance at different growth stages. Plant early stage had been shown more sensitive to salt injury than mature stage and it can provide more efficient screening technique [30].

Water induced salt stress causes a number of adverse biochemical, ionic and physiological changes in plants that resulted in reduction of plant biomass. However, the decline in plant growth and crop yield generally differ among various species and cultivars [19,7]. Plant biomass production is related to multiple physiological, biochemical and ionic traits. In present experiment water induced salinity stress caused reduction in gas exchange attributes, such as photosynthetic rate, transpiration rate and in stomatal conductance of maize cultivars that resulted in reduced biomass production. However, salt tolerant maize cultivars showed lower reduction in gas exchange attributes under onset of salt stress as compared to salt sensitive. Similar results were reported by [9] that under salt stress plant biomass and vigor primarily depend on photosynthetic and transpiration rate. In this regard, plant gas exchange traits are the most important that significantly play role in plant biomass production. Under salinity stress plant photosynthetic rate decreased that resulted in lower biomass production [31,10,6]. Similarly in the present study the positive correlation was observed between maize plant photosynthetic rate and shoot fresh weight (Fig. 5). Usually, it is assumed that reduction in plant photosynthetic rate under salt stress occur due to plant stomatal regulations. Similarly, [32,33] reported that stomatal closing is the most important reason which results in lower plant photosynthetic rate under onset of salt stress and it causes reduction of CO_2 partial pressure in leaves [34,35].

The adverse effects of salt stress on plant photosynthetic rate have been observed to occur due to several reasons, such as plant respiration and electron transport system and accumulation of stress metabolites, gene expression and protein synthesis [36,37]. However, different plant genotypes and cultivars give different response in relative to these physiological traits under onset of salt stress. Similarly, [19,6] reported that there is ample evidence of genetic variation for salt tolerance in maize genotypes. Decreased in transpiration and photosynthesis rates might be due to lower shoot growth, unbalanced nutrient uptake and cell membrane damage. The poorer plant fresh and dry biomass productions of maize cultivars were positively correlated with reducing plant photosynthetic and transpiration rate that were also positively associated with lower plant dry biomass production.

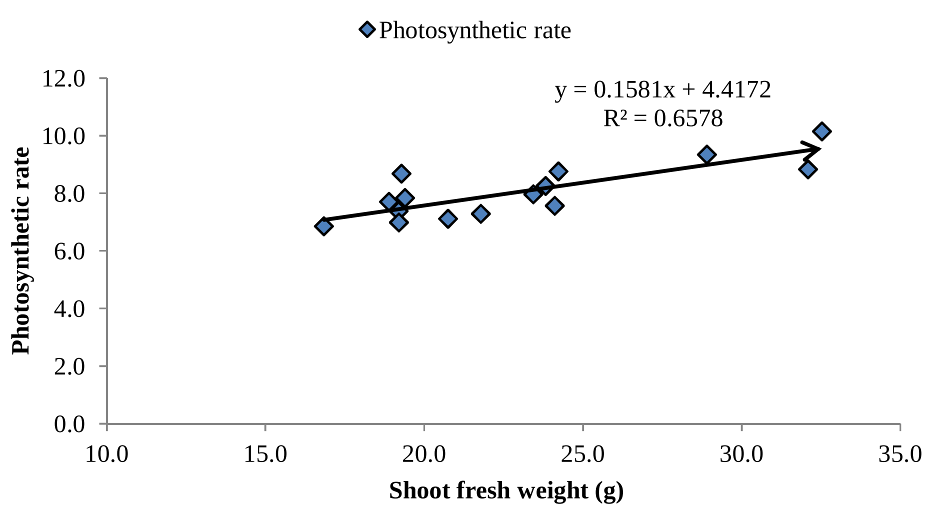

Fig. 5. Correlation of maize plant shoot fresh weight with plant photosynthetic rate

Similarly, positive relationship of plant fresh and dry biomass production under salt stress with gas exchange attributes had already been reported in different crops such as maize [6], cotton [38], barley [39], sorghum [31], and canola [20]. Reduction in stomatal conductance of maize cultivars can be due to less guard cell turgidity and sap flow in response to salinity stress. Similar results were reported in maize [19] and in cotton [38].

When compared within a same treatment and the results of ionic traits showed that response of different maize cultivars were also differed significantly and genotypic differences were also cleared under different poor quality water treatments. Comparison further revealed that cultivars buildup higher Na^+ in their plant leaves indicated poorer growth and photosynthetic rate. This higher concentration of Na^+ in leaves inducing water deficit by disturbing the osmotic balance of leaf cells. Excessive contents of Na^+ in plant leaves also become lethal and lead to plant cells death. The salt tolerant maize cultivars accumulated more K^+ and hence maintained higher K^+: Na^+ ratio than salt sensitive. Increasing uptake of Na^+ concentration and reducing K^+ concentration by enhancing salt stress resulted in a reduction of K^+: Na^+ ratio. It can be due to negative effect of Na^+ on K^+ transport into xylem. Higher K^+: Na^+ ratio in leaf sap of plants under salt stress is considered an important selection criterion for salinity tolerance [40,41,42]. The important mechanism contributing in expression of salt tolerance is ability of plant species or cultivars to keep low levels of Na^+ and higher level of K^+ with in the leaf or tissue. The results depicted a negative relationship between the plant dry biomass production and Na^+ uptake in plant tissue (Fig. 6). Similar results were reported by [6] on maize and [43] on sunflower. The reductions in plant growth under salt stress conditions might be credited to excessive buildup of Na^+ within plant parts [44,45].

The difference of Na^+ accumulation in shoots of different plants might be associated to their abilities to uptake Na^+ by roots and further transfer it to shoots. Similarly, [46] described that plant genotypes differed not only in their rates at which they uptake Na^+ but it also depend on transportation of Na^+ from root to shoots. Similar results were reported by [6] that salt tolerance in maize was correlated with lower shoot Na^+ accumulation. In the present study a negative correlation was observed between plant Na^+ contents and plant dry biomass production, while plant shoot dry weight showed positive relationships with plant K^+: Na^+ ratio and photosynthetic rate (Fig. 6). The better plant growth in control (fit water) could be due to

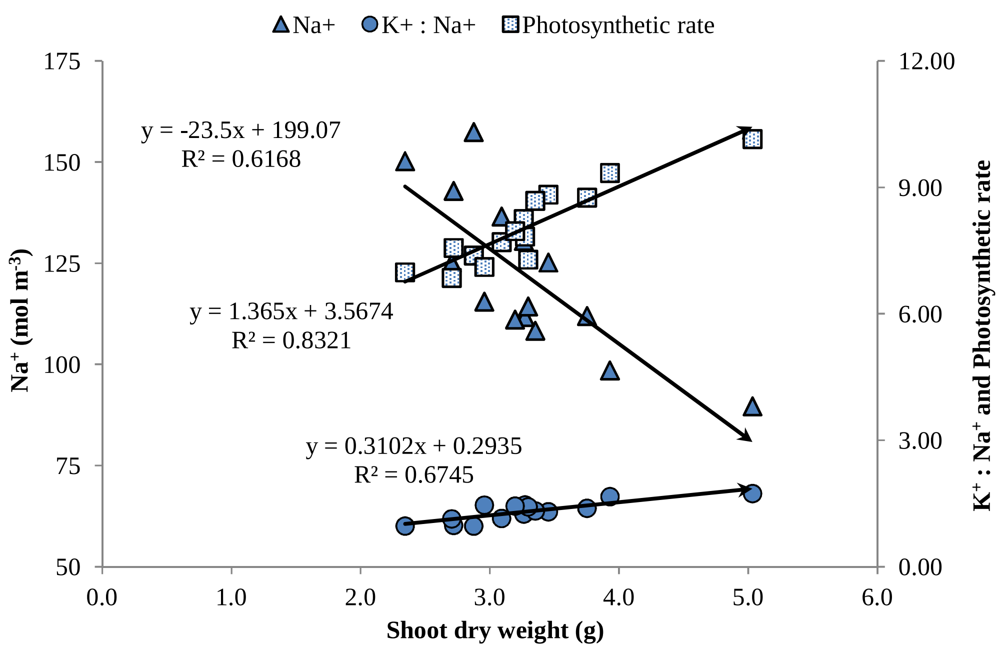

Fig. 6. Correlation of maize plant shoot dry weight with plant photosynthetic rate, Na^+ and K^+ : Na^+

higher uptake of K^+, resultant in high K^+: Na^+ [19]. Salt tolerant genotypes maintained higher growth rate, protect metabolic system and is normally associated with limited Na^+ transportation into shoot [47]. Additionally, K^+: Na^+ ratio in plant leaves is also a good technique to define plant resistance against salt stress. Reduction in K^+: Na^+ of maize genotypes grown in poor quality water (saline-sodic water) can be due to antagonism of Na^+ with K^+ uptake (Table 2). Wide dissimilarities among maize genotypes for K^+: Na^+ can be due to their limited capability for the accumulation of Na^+ by plant root cells and also the transportation of Na^+ to leaves by monitoring their entry into root xylem from plant root cells. Actually, it was reported that higher K^+: Na^+ was more significant for plants than simply retaining a lower Na^+ concentration [48,49]. Previously many researchers reported that higher K^+: Na^+ in plant tissue was a suitable parameter for selection of salt tolerance in various crops [12,6]. Therefore, in the present study the salt tolerant maize cultivars SWL-2002 and Afgoi accumulated less shoot Na^+, depicted higher plant photosynthetic rate and retained greater K^+: Na^+ ratio under water induced salt stress.

The principle component analysis of gas exchange and ionic traits showed visual variation among cultivars under different poor quality water treatments. Cultivars SWL-2002 and Afgoi were ranked as salt tolerant because these appeared towards salinity treatments in the biplot graphs. While cultivars Sadaf, FH-963, FH-722 and FH-793 were ranked as highly salt sensitive because these cultivars appeared on opposite to salinity treatments. Similarly, [24,50] reported that salt tolerant wheat cultivars made longest vectors towards salinity treatments and sensitive appeared on the negative side of treatment vectors in the biplot graph.

5. CONCLUSION

It can be concluded that biplot analysis method appeared to be the best statistical methodology for screening of salt tolerance in maize cultivars due to its graphical display and depiction of average performance of cultivars across different treatments and in a particular environment. Screening for salinity tolerance under controlled conditions at early growth stages on the basis of different gas exchange and ionic traits in solution culture is a simple and quick method for determining genotypic differences. Leaf sap ionic traits like K^+, K^+: Na^+ and photosynthesis system related attributes like stomatal conductance, transpiration and photosynthetic rates affected significantly at different levels of salt stress, hence can be used as screening criteria. The present results also provided guidelines to plant breeders interested to develop salt tolerant varieties that they can use these salt tolerant cultivars as parental material for further breeding program.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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