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MORPHOLOGICAL AND BIOCHEMICAL (SDS-PAGE) PROFILING OF CHICKPEA CULTIVARS TREATED WITH DIFFERENT CONCENTRATION OF SALT (NaCl)

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Abstract: The research was conducted at the University of Swat, delved into the impact of salt (NaCl) stress on chickpea (Cicer arietinum L.) cultivars including; Karak-1, KC-98, Lawaghar-2000, Sheenghar, Karak-2, Karak-3, Fakhar-e-Thal and Chattan obtained from Agricultural Research Station Ahmad Wala Karak, Khyber Pakhtunkhwa Pakistan with three local varieties; Bittal-2016, Noor-91 and Noor-2013. The study encompassed eight distinct traits evaluated under varying NaCl concentrations, including control, 50, 100, and 200 mM. The average mean of plant height was recorded as 26.75±8.410063cm, number of primary branches 75±1.493039, secondary branches 8.75±2.657536, days of flowering 112.33±1.45, number of flowerings 4.04±2.33, number of pods 1.76±5.66 and plant biomass 33.33±1.76. The obtained data was analyzed with statistical software PAST 4.08 version using Multi-statistical Analysing Tools (MSAT) for significance results. Principle component Analysis (PCA) scattered plot showed positive correlation in a bulk of variance at Component-I in Primary branches (Pr.Br.), secondary branches (S. Br), pods per plants (PPP), while Cluster- II (Number of flower (NOF),100 Seed weight (100 SW) and Cluster-III (Plant height (PH), Days of flowering (DOF) with very little contribution of some scattered genotypes. The first two axis represent 85% of variance in the correlation matrix, while the first 3 axis represent 94% of variance that signifies our results. Principal Component-1, having 75% of variance while PC-2 and 3 with 15% and 12% variance respectively. The SDS-PAGE, dendrogram shows the protein profile relates three distinct Clusters; Cluster- I (Chattan and Sheenghar) Cluster-II (Karak-3, Fakhar-e-Thal and KC-98) while Karak-2 made a separate outline. The tolerable genotypes showed 100% performance in Cluster-II. Noteworthy findings emerged, such as the significant increase in a 43 kDa protein in the salt-tolerant genotype Fakhar-e-Thal compared to Chattan. Furthermore, the investigation unveiled distinct protein profiles and clustering patterns among genotypes based on their origins and salt tolerance. This research underscores the relevance of comprehending salt stress adaptation in chickpea cultivation, particularly in regions prone to salinity stress. The study identified the accessions KC-89, Fakhar-e-Thal and Karak-3 potential against salinity up to 50mM NaCl concentration. These cultivars also found with a similar protein banding pattern and clustered in a same group by analyzing through a reliable biochemical technique, SDS-PAGE.

Keywords: Salt Stress, Chickpea, SDS-PAGE, Multi-Statistical Analyzing Tools (MSAT), Cluster analysis, PCA.

Introduction

The chickpea (Cicer arietinum L.) also known as Bengal gram or garbanzo, is one of the primitive and most popular legume crops in the world, grown in more than 50 countries. It is the most widely grown food legume in South Asia and the 3rd most widely grown food pulses globally, following common bean and field pea (Jukanti et al., 2012). Chickpea (Cicer arietinum L.) is self-pollinated annual grain legume (Asghar et al., 2003). It is diploid (2n = 16) crop. Chickpeas are an excellent source of protein, carbohydrates, fiber, as well as a number of vital vitamins and minerals (Roy et al., 2010). Particularly in desert and low-rainfall settings, chickpea nitrogen fixation is crucial for maintaining soil fertility (Varshney et al., 2014). One of the most important abiotic stresses in agriculture is salinity, which is predicted to have a negative impact on 20% of all land in the globe and nearly half of all irrigated land. In addition to physiological dehydration (water stress), salinity also leads to nutritional ion imbalance in plants (Toker et al., 2007). Despite the fact that chickpeas are sensitive to salinity, especially during the early stages of growth and development, there has been reported to be significant variation among different genotypes, with the most susceptible ones failing to grow in just 25 mM NaCl but tolerant genotypes surviving to a maximum concentration of 100 mM NaCl in hydroponics (Flowers et al., 2009). Also, the drying of the soil at the conclusion of the growing season and higher salt concentrations in the soil due to their accumulation both result in 8 to 10% reduction in yield globally (Flowers et al., 2009). There is no one method for estimating genetic diversity that is better than another; nonetheless, each method has unique consequences for managing germplasm or improving crops. Due to its reliability and simplicity in defining the genetic makeup of crop germplasm, Electrophoresis with Gel (SDS-PAGE) is one of the most popular biochemical procedures.

Although seed storage protein sequences are essentially independent of environmental variation, SDS-PAGE is a realistically reliable approach. But SDS PAGE is the most authentic tool for the diversification of chickpea cultivars. Moreover, SDS-PAGE protein profiling uncovered new data on resistance to salt stress conditions. However, 234 chickpea genotypes cultivated in a Vertisol treatment with an 80 mM NaCl solution were screened by (Serraj et al. 2004). Based on the saline susceptibility index (SSI) and shoot biomass, they found resistant genotypes and reported a 60% drop in biomass 40 days after sowing. The Estimation of genetic assortment based on biochemical exploration using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis or SDS-PAGE (Netra & Prasad, 2007) reported for revealing of diversity in cultivars of chickpea. To investigate protein-based variation among various organisms, scientists employ the SDS-PAGE method. According to Jiang et al. (2016), it is utilised to identify various kinds of protein subunits from various organisms. Phylogenetic relationships, precise genetic variety among genotypes, aid in plant domestication, and usage as a tool for agricultural improvement are just a few of the many benefits of seed protein-based variation (Wadood et al., 2021). The current study had screen out chickpea cultivars responses to different salt concentration levels and to estimate the genetic distances among the indigenous chickpea varieties of District Karak Khyber Pakhtunkhwa based on their biochemical characteristics, amino acids profiling, protein content and band profiling for improvement of Chickpea cultivation in Pakistan.

Materials and methods

Plant material and experiment design

Plant material for this study was sourced from the Agricultural Research Station in Ahmad Wala Karak, Khyber Pakhtunkhwa, Pakistan. It was included eight Chickpea cultivars (Karak-1, KC-98, Lawaghar-2000, Sheenghar, Karak-2, Karak-3, Fakhir-E-Thal, Chattan) and three local varieties Bittal-2016, Noor-91 and Noor-2013 from Swat. The seeds were sterilized and grown in pots with soil and humus. Salt stress, with four doses (Control, S1: 50 mM, S2: 100 mM, S3: 200 mM), were key factors in the experiment.

Total protein extraction, purification and SDS-PAGE

For protein extraction, seeds were finely powdered using a pestle and mortar, and 0.01g of powder per sample was placed in Eppendorf tubes. The extraction buffer contained 0.20M Tris-HCl (pH 6.8), 10% sodium dodecyl sulfate, 20% glycerol, 10mM β-mercaptoethanol, and 0.050% bromophenol blue. After adding the buffer and vortexing for one minute, the solution was centrifuged at 1400 rpm and 40°C for 20 minutes. It was then incubated at 70°C for four hours.

Electrophoresis Solution:

Solution A:

- Contains 3.00M Tris, 0.40% SDS, and pH is adjusted to 8.8 using HCL.
- Prepare by mixing 36.6g Tris, 0.40g SDS, and 70ml purified water in a beaker.
- Vortex the solution for at least a minute and store up to 100ml in the refrigerator.

Solution B:

- Comprises 0.493M Tris, 0.4% SDS, and pH is adjusted to 7.0 using HCL.
- Prepare by mixing 5.98g Tris, 0.40g SDS, and 80ml distilled water in a beaker.
- Vortex the solution, adjust pH to 7.0, and store up to 100ml in the refrigerator.

Solution C:

- Contains distilled water, 30.00% acrylamide, and 0.80% bis-acrylamide.
- Prepare by combining these chemicals in a beaker, stir with a magnetic stirrer, and store up to 100ml in the refrigerator.

APS (Ammonium Persulfate) Solution:

- Create by placing 0.10g of ammonium persulfate in an Eppendorf tube.
- Add distilled water to dissolve the ingredients, resulting in 1ml of 10.00% APS.
- Store this solution in the fridge.

This Electrophoresis Solution is used for protein extraction and gel electrophoresis. For protein extraction, seeds are ground into a fine powder, and 0.01g of the powder is mixed with various solutions containing NaCl. After centrifugation, protein extraction buffer is added, and the mixture is incubated at 70°C for two hours. Finally, 40 microliters of the extracted protein is loaded into each well for electrophoresis.

Gel Preparation:

Glass plates were cleaned with methanol. Gasket-lined plates were clipped together. A 1mm thick vertical gel was formed by pouring separating gel between plates. Stacking gel was added after separating gel polymerization. Plastic combs created wells. Protein samples were loaded into wells. Plates were placed in a gel tank. Gel was transferred to a tray with staining solution and shaken for 20 minutes. After rinsing and de-staining overnight, bands became clear.

Data Analysis:

Collected data statistically analyzed. Quantitative data passed through Multi-Statistical Analyzing Tools (MSAT) for authenticity including; cluster analysis, Principal Component Analysis (PCA), and correlation analysis using PAST 0.48 software. Binary data analyzed with two-way clustering. Genetic dissimilarity estimated using UPGMA in PAST 0.48 for Windows.

Results

The study reveals that salt concentration plays a significant role in shaping various morphological traits in chickpea plants, impacting plant height, branching patterns, flowering, pod formation, and biomass accumulation. The salt stress condition applied after four weeks of germination and data were scored according to the method described by chickpea descriptor (IBPGR, ICRISAT & ICARDA, 1993) with minor modifications.

Plant Height and Salt Concentration: As salt concentration increased, plant height also increased. The tallest plants, at 53cm, were observed at 50mm salt concentration. At 100mM, plant height averaged 48cm, and at 200mM, it decreased to 6cm. Karak-3 accessions exhibited the highest mean height, 37.25cm, while Sheenghar had the lowest height as 27.62cm (Table 1).

Primary Branches and Salt Concentration: The highest number of primary branches were13 occurred at 50mM in Karak-3, Sheenghar, and Chattan genotypes. At 100mM, most genotypes had 9 primary branches, while at 200mM, this number dropped to 9 branches. Karak-3 had the highest mean value for primary branches, 8.5, while Karak-1 had the lowest number scored as 5.75 (Table 1).

Secondary Branches and Salt Concentration: The highest number of secondary branches was recorded at 50mM, with KC-98 having 16 branches. At 100mM, the highest number was 12 branches in KC-98, and at 200mM, only 1 branch was observed. KC-98 had the highest mean value for secondary branches resulted 14, while Karak-1 showed the lowest number of 8.25 (Table 1).

Days of Flowering and Salt Concentration: Salt concentration affected flowering time. The shortest flowering period (95 days) occurred at 100mM in KC-98, while the longest (115 days) was observed. Karak-1 had the highest mean value for days of flowering at 112.33, while Chattan had the lowest value calculated 97.66 (Table 1).

Number of Flowers and Salt Concentration: The highest number of flowers appeared at 50mM, with Karak-3 having 63. At 100mM, Karak-1 had the most (55), while at 200mM, no flowers bloomed due to high salt concentration. Karak-1 had the highest mean value for flowers as 59.33, while Chattan and Sheenghar both had the lowest noted 35 (Table 1).

Flower Color: Dominant flower colors were pink, purple-pink, deep pink, and white (only at 50mM). No flowers were produced at 200mm due to high salt stress.

Number of Pods and Salt Concentration: The highest number of pods (59) was observed at 50mM, while at 100mM, Karak-1 had the most (53). At 200mM, no pods were produced due to high salt concentration. Karak-1 had the highest mean value for pods 55.66, while Chattan had the lowest found as 29.33.

Total Biomass and Salt Concentration: Total plant biomass and 100-seed weight were highest at 50mM, with KC-98 having 48g total biomass. At 100mM, KC-98 had the highest biomass (42g), and at 200mM, no biomass was recorded due to high salt concentration. KC-98 had the highest mean value for biomass, 45.33, while K-3 had the lowest score of 29g (Table 1).

Correlation with Salt Concentration: The data demonstrates how various plant traits correlate with salt concentration, affecting plant growth and development.
These findings emphasized the sensitivity of chickpea to salt stress and its impact on plant morphology, highlighting the need for further research on salt tolerance mechanisms in chickpea cultivars.

Cluster Analysis;
In the classical and two-way clustering analysis, conducted on the mean values obtained from 28 accessions representing seven genotypes, namely Karak-1 (Desi chickpea), Karak-2 (Desi chickpea), Karak-3 (Desi chickpea), Fakhar-e-That (Desi chickpea), Sheenghar (Desi chickpea), KC-98 (Kabuli chickpea), and Chattan (Desi chickpea), three distinct clustered groups emerged.

Group A (KC-92 and Fakher-e-Thal): This group comprises both Kabuli and Desi chickpea plants.
Group B (Karak-2 Desi chickpea and Karak-1 Desi chickpea): Group B consists of Desi chickpea plants, including Karak-2 and Karak-1 (Figure 1).
Group C (Karak-3 Desi chickpea, Sheenghar Desi chickpea, and Chattan Desi chickpea): Group C predominantly represents Desi chickpea plants, including Karak-3, Sheenghar, and Chattan, which are major contributors to chickpea seed production (Figure 1).

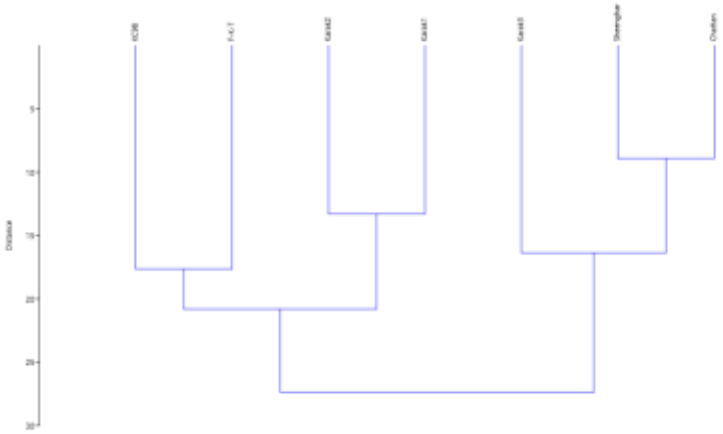

Figure 1. Cluster analysis of morphological traits of seven Chickpea genotypes

Principal Component Analysis (PCA)
Principal Component Analysis (PCA) is a multivariate technique used to analyze a data table with observations characterized by several interconnected quantitative dependent variables. This method helps visualize the patterns of similarity within the data and its variables. The essential information is extracted from the dataset, transformed into a set of new axis data known as principal components, and then presented as points on maps (Table 3, Figure 1)

Eigenvalue and Percentage of Variance
A significant score threshold of 1 and 2 was set for the primary component. The principal component of genotype mean values was considered more significant when it had a score exceeding 252. The first two axes of the correlation matrix explained 85% of the variation, while the first three axes accounted for 94% of the variance, effectively summarizing our findings (Table 2).

Table 2. Principal components (PC) with Eigenvalue and % variance

PC	Eigenvalue	% variance
1	211.026	71.208
2	42.2555	14.259
3	29.2021	9.8539
4	10.749	3.6271
5	2.7484	0.92742
6	0.369281	0.12461

Scatter Plot of Principal Component Analysis with Chickpea genotype
Principal component analysis to categorize seven chickpea accessions (Karak-1, Karak-2, Karak-3, Fakhar-e-That, Sheenghar, KC-98, and Chattan) based on seven morphological traits (plant height, primary branches, secondary branches, days of flowering, number of flowers, flower color, and 100-seed weight).

This analysis revealed that component-1 primarily captured variance contributed by primary branches, secondary branches, and pods per plant. Cluster 2 displayed notable contributions from the number of flowers and 100-seed weight, while cluster 3 was characterized by plant height and days of flowering, with minimal genotype variations (Figure 2).

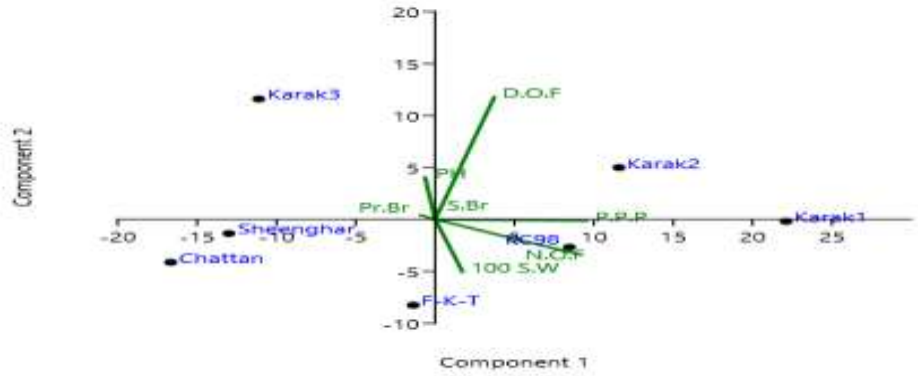

Figure 2. Scatter Plot of Principal Component Analysis with Chickpea genotype

Loading Plot of Principal Component

The PCA loading plot revealed two groups in relation to salt concentration. The first group, with higher mean percentages in days of flowering (D.O.F), the number of flowers (N.O.F), pods per plant (P.P.P), and 100-seed weight (100 S.W), appears to thrive in low-salt conditions. The second group, featuring lower mean percentages in plant height (PH) and primary branches (Pr. Br), seems more adaptable to higher salt concentrations. Secondary branches (S. Br) show a moderate response to varying salt levels. This highlights how these traits correlate with salt concentration in chickpea genotypes and accessions (Table 2, figure 3).

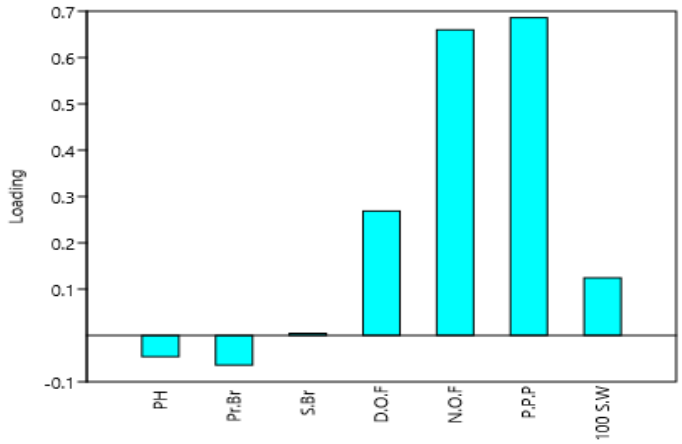

Figure 3. Loading plot for morphological traits of chickpea genotypes

Scree plot; The Scree plot revealed variance distribution across component axes. The first principal component (75%) related to lower salt concentration, while the second (15%) and third (12%) components indicated adaptability to moderately higher salt concentrations. Other principal components had minor impact on salt concentration traits (Figure 4).

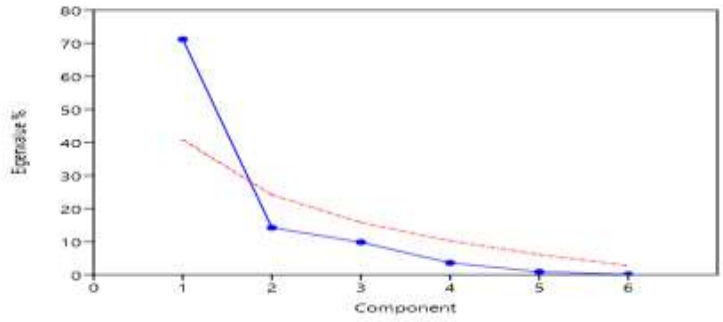

Figure 4. Scree plot for morphological traits of chickpea genotypes

Table 3. Loading chart of PCA for morphological traits

Traits	Principal Components (PC 1-PC 6)					
PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	
PH	-0.045	0.29109	0.41406	0.6429	-0.51678	0.23
Pr. Br	-0.064	0.03	-0.0226	0.078	0.25	
S. Br	0.004	0.07	0.3	0.31	0.79	0.37
D.O. F	0.26	0.84	0.15	-0.25	0.11	-0.3
N.O. F	0.65	-0.2	-0.11	0.5	0.09	-0.5
P.P. P	0.7	-0.01	-0.08	-0.3	-0.14	0.64

3D scatter plot of principal component analysis

In the 3D Scatter Plot, Principal Component 1 absence of vectors, which might not be directly linked to salt concentration. However, Principal Component 3 had two vectors per accession genotype, suggesting a potential connection to salt-related variations. Principal component 2 illustrates these clustered variations, potentially indicative of salt concentration-related traits (Figure 5).

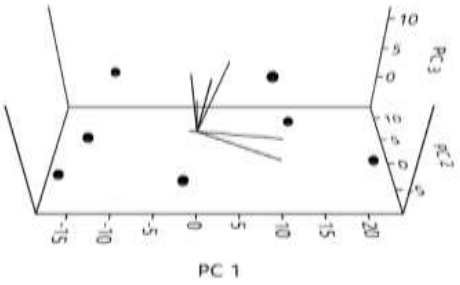

Figure 5. Scattered plot for morphological traits of chickpea genotypes

SDS-PAGE

In our study, SDS-PAGE analysis revealed distinct protein profile differences between tolerant and susceptible genotypes. Notably, at 50 mm salt concentration, Fakhar-e-That displayed significantly higher intensity (4-fold, 1.9-fold, and 0.9-fold) in a 43 kDa protein band compared to Chattan. This indicates that early production of these proteins may influence tolerance. Furthermore, we observed changes in protein expression at 50 mM salt concentration, with a decrease in the tolerant genotype and an increase in the susceptible one. This shift is crucial in understanding how protein patterns evolve in response to salinity-induced stress, suggesting that tolerance, particularly in larger-

molecule proteins, may be linked to rapid synthesis or reduced breakdown of sensitive proteins (Table 4, figure 6).

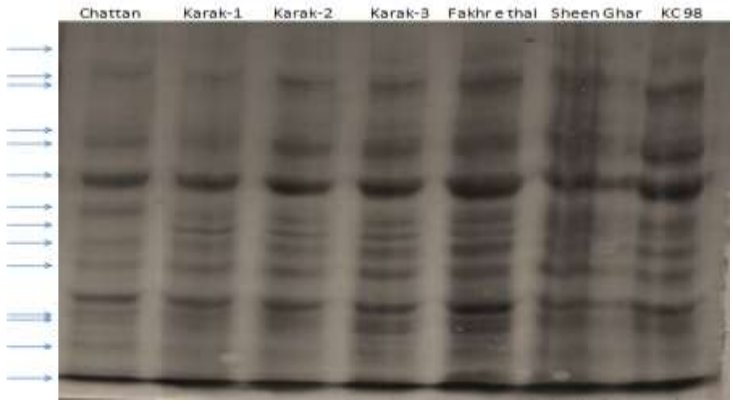

Figure 6. Electrophorogram show the distribution of diverse protein in chickpea genotypes.

Dendrogram

Clustering analysis linked protein profiles with time points for each genotype, resulting in three distinct clusters: Chattan and Sheenghar in Cluster I, Karak-3, Fakhar-e-Thal, and KC-98 in Cluster II, and Karak-2 forming a separate group. The tolerant genotype performed exceptionally well in Cluster II. Notably, we observed more protein changes in response to a 50mM NaCl concentration, particularly in the susceptible group. Salinity had the most significant impact on protein accumulation after reaching 50mm, according to the cluster analysis (Table 4, figure 7). This emphasizes the importance of early seedling responses in understanding the tolerance mechanism (Wang et al., 2009).

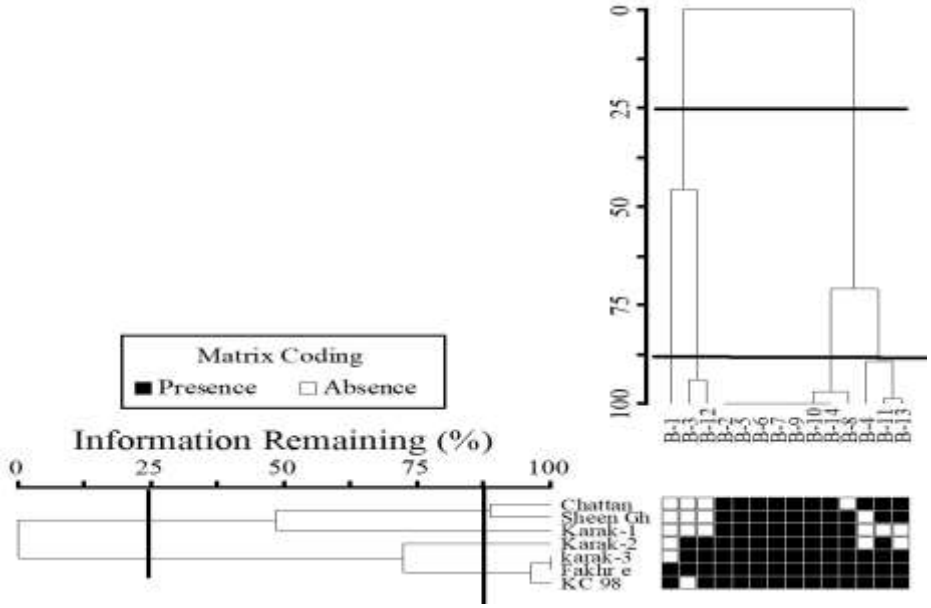

Figure 7. Cluster analysis of chickpea seven genotypes using SDS- PAGE

Scattered diagram SDS-PAGE

We mapped the accessions based on their geographic origin and seed source to explore any potential associations between genetic diversity and origin. Axis 1 primarily represents Chattan, and Axis 2 includes Fakhar-e-Thal, KC-98, and Karak-3, indicating a shared origin. The remaining groups are scattered in between, revealing a distinct group in the upper half. Notably, a consistent pattern

emerges in the lower half of the graph, where accessions tend to cluster based on the geographical source of their quantitative traits (Table 4, figure 8).

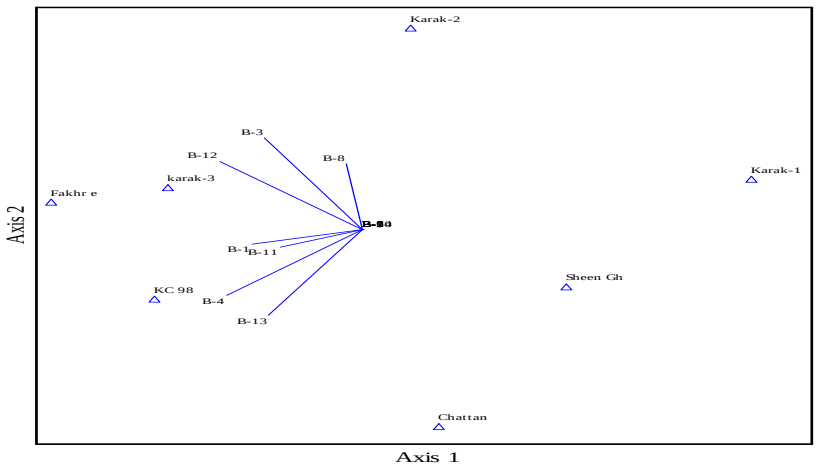

Figure 8. Scattered diagram for SDS-PAGE analysis of chickpea genotypes

Table 4. Cluster analysis using SDS-PAGE in chickpea for seven different genotypes

	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10	B-11	B-12	B-13	B-14
Chattan	0	1	0	1	1	1	1	0	1	1	1	0	1	1
Karak-1	0	1	0	0	1	1	1	1	1	1	0	0	0	1
Karak-2	0	1	1	0	1	1	1	1	1	1	1	1	0	1
karak-3	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Fakhre thal	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sheen Ghar	0	1	0	0	1	1	1	1	1	1	1	0	1	1
KC 98	1	1	0	1	1	1	1	1	1	1	1	1	1	1

Discussion

The investigation focused on chickpea plant responses to differing salt concentrations, systematically evaluating key morphological and physiological traits. These traits encompassed plant height, primary and secondary branch numbers, flowering duration, floral counts, pod production, and 100-seed weight. Notably, plant height displayed a noteworthy variation, with the tallest plants observed in control conditions for Kabuli chickpea. The introduction of increasing salt concentrations resulted in a diminishing trend in plant height, primary and secondary branches. Further analyses revealed that lower salt concentrations corresponded to earlier flowering, increased flower and pod numbers, implying a direct impact of salinity on plant developmental dynamics. Additionally, a distinct reduction in 100-seed weight was observed with elevated salt concentrations, ultimately leading to the absence of seeds at the highest salt concentration (200mM). The application of cluster analysis effectively delineated the chickpea accessions into distinct groups, illuminating the genetic diversity and relationships among these varieties. The results are in accordance with the previous research who described that salinity having negative impact on chickpea growth and production because of reducing the efficiency of irrigated land throughout the word (Silva & Geros 2009). Similarly, Toker et al., 2007 and Molassiotis et al., 2006 reported that high salt concentration in a soil causing oxidative damage to biomolecules and cell death. In our results it has also been concluded that chickpea is sensitive to salinity. In this context, the identified tolerant chickpea cultivars; KC-98, Fakhare -Thal and Karak-3 treated with different salt concentration revealed maximum growth and production at 50mM and even at 100mM. These results are agreed with the experimental work of Flower et al., 2009 and Arefian, 2014.

The Eigenvalue and % variance of the first two axis showed 85% of variance in correlation matrix, while the first 3 axis showed 94% of variance which signified our results. The results showed a bulk

of variance at component-1 (Primary branches (Pr. Br.) secondary branches (S. Br) pods per plants (P.P.P) while Cluster 2nd (Number of flower (N.O.F) and 100 Seed weight (100 S.W) and group 3rd (plant height (PH), Days of flowering (D.O.F) with very little contribution of some scattered genotypes. The results are in accordance with the previous research who described that salinity having negative impact on chickpea growth and production because of reducing the efficiency of irrigated land throughout the word (Silva & Geros, 2009). Similarly, Toker et al., 2007 and Molassiotis et al., 2006 reported that high salt concentration in a soil causing oxidative damage to biomolecules and cell death

Moreover, an in-depth exploration of protein profiles was conducted through SDS-PAGE analysis, uncovering significant differences in protein expression patterns between tolerant and susceptible genotypes. These variations, more pronounced at 50mM salt concentration, might be indicative of underlying mechanisms that contribute to salt tolerance in certain genotypes. The majority of the additions were grouped based on where their morphological characteristics are originated. The obtained results from the biochemical analysis of the selected chickpea accessions from Karak in the current study are highly supported by Ghafoor et al., 2004; Nisar et al., 2007 and Ahmad et al., 2012 and 2015. Nevertheless, the previous researchers worked on different local and exotic chickpea accessions through SDS-PAGE analysis. These findings are aligned with previous research underscoring the detrimental effects of salinity on chickpea growth and production. They underscore the pressing need for the identification and cultivation of salt-tolerant chickpea cultivars to ensure robust yields in salinity-affected agricultural landscapes.

Conclusion

The study revealed a critical turning point in protein patterns, with tolerant genotypes experiencing a decline at 50mM salt while susceptible ones showed an increase. This suggests that salt tolerance, especially in larger proteins, may involve rapid synthesis or reduced degradation. Salt significantly affected chickpea traits, with optimal growth and yield at 50mM and decreased production at 100mM and 200mM. PAST analysis confirmed these relationships, and PCA dendrograms indicated three clusters with a stronger hierarchy at 50mM salt. SDS-PAGE showed substantial protein changes primarily after 50mM salt, with specific genotypes sharing similar protein constituents.

Recommendations

Chickpea accessions, including eight cultivars and three local varieties from Swat, were assessed for salt tolerance. Salinity adversely affects chickpea growth beyond 100Mm or 200mM salt levels. SDS-PAGE analysis highlighted KC-98, Fakhar-e-Thal, and Karak-3 as salt-tolerant genotypes with intact protein profiles at 50mM salt concentration. These cultivars are recommended for improving chickpea yield in Pakistan.

Table 1. Basic statistics of chickpea accessions data with coefficient of variation age (%)

Origin	Trait	Control	50mm	100mm	200mm	MIN	MIX	Mean	Std. Dev	Std. E	C.V%
K1	Plant height	29	46	27	5	5	46	26.75	15.84523	8.410063	59.23452
K2	Plant height	40	45	38	8	8	45	32.75	17.65543	8.380284	53.90972
K3	Plant height	50	53	40	6	6	53	37.25	21.12843	10.78096	56.72063
KC-98	Plant height	53	48	35	4	4	53	35	21.21952	11.00757	60.62719
F-K-T	Plant height	40	43	38	5	5	43	31.5	17.30194	8.892881	54.9268
SH.GH	Plant height	40	42	26	2.5	2.5	42	27.625	16.0099	9.099851	57.9544
Chattan	Plant height	46	48	25	3	3	48	30.5	18.566	10.53961	60.87214
K1	Primary branches	5	9	7	2	2	9	5.75	4.301163	1.493039	74.80283
K2	Primary branches	8	10	9	2	2	10	7.25	4.220133	1.796988	58.20873

K3	Primary branches	12	13	7	2	2	13	8.5	4.755949	2.533114	55.95234
KC-98	Primary branches	8	10	6	1	1	10	6.25	5.094348	1.931105	81.50957
F-K-T	Primary branches	9	12	8	3	3	12	8	4.270608	1.870829	53.3826
SH.GH	Primary branches	11	13	8	2	2	13	8.5	5.080307	2.397916	59.76832
Chattan	Primary branches	12	13	7	1	1	13	8.25	47.97519	2.75	581.5175
K1	Secondary branches	11	13	10	1	1	13	8.75	55.51405	2.657536	634.4463
K2	Secondary branches	13	15	12		12	15	13.33333	54.43896	0.881917	408.2922
K3	Secondary branches	15	15	9		9	15	13	55.06148	2	423.5499
KC-98	Secondary branches	14	16	12		12	16	14	51.71621	1.154701	369.4015
F-K-T	Secondary branches	16	14	10		10	16	13.33333	46.43131	1.763834	348.2348
SH.GH	Secondary branches	16	15	6		6	16	12.33333	49.97366	3.179797	405.1918
Chattan	Secondary branches	12	13	7	1	1	13	8.25	47.97519	2.75	581.5175
K1	Daysof flowering	110	112	115		110	115	112.3333	29.18504	1.452966	25.98075
K2	Daysof flowering	115	113	110		110	115	112.6667	32.94035	1.452966	29.237
K3	Daysof flowering	118	110	112		110	118	113.3333	42.5002	2.403701	37.50017
KC-98	Daysof flowering	110	110	105		105	110	108.3333	32.39753	1.666667	29.90541
F-K-T	Daysof flowering	98	100	96		96	100	98	26.92706	1.154701	27.47659
SH.GH	Daysof flowering	105	102	103		102	105	103.3333	38.12305	0.881917	36.89327
Chattan	Daysof flowering	100	98	95		95	100	97.66667	35.10366	1.452966	35.94231
K1	No.of flower	60	63	55		55	63	59.33333	4.041452	2.333333	6.811436
K2	No.of flower	53	55	50		50	55	52.66667	2.516611	1.452966	4.778376
K3	No.of flower	35	40	33		33	40	36	3.605551	2.081666	10.01542
KC-98	Noof flower	52	54	43		43	54	49.66667	5.859465	3.382964	11.79758
F-K-T	No.of flower	54	51	43		43	54	49.33333	5.686241	3.282953	11.52616
SH.GH	No.of flower	40	43	22		22	43	35	11.35782	6.557439	32.4509
Chattan	No.of flower	40	43	22		22	43	35	11.35782	6.557439	32.4509
K1	Pods per plant	55	59	53		53	59	55.66667	12.53395	1.763834	22.51608
K2	Pods per plant	48	50	45		45	50	47.66667	10.07968	1.452966	21.14619
K3	Pods per plant	30	35	28		28	35	31	4.335897	2.081666	13.98676
KC-98	Pods per plant	45	50	40		40	50	45	3.710346	2.886751	8.245213
F-K-T	Pods per plant	36	45	30		30	45	37	5.180894	4.358899	14.00242
SH.GH	Pods per plant	30.5	34	30.2		30.2	34	31.56667	2.333809	1.219745	7.393272
Chattan	Pods per plant	35	38	15		15	38	29.33333	8.595348	7.218803	29.30232
K1	100 seed weight	34	36	30		30	36	33.33333	3.05505	1.763834	9.165151
K2	100 seed weight	32	34	25		25	34	30.33333	4.725816	2.728451	15.57961
K3	100 seed weight	28	35	24		24	35	29	5.567764	3.21455	19.19919
KC-98	100 seed weight	46	48	42		42	48	45.33333	3.05505	1.763834	6.739082

F-K-T	100 seed weight	34.5	36	32		32	36	34.16667	2.020726	1.166667	5.91432
SH.GH	100 seed weight	34.5	36	32		32	36	34.16667	2.020726	1.166667	5.91432
Chattan	100 seed weight	32.5	33.7	24		24	33.7	30.06667	5.288037	3.053049	17.58771

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