

A cluster analysis of Ricebean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi) accessions with specified NaCl concentration at seedling stage under controlled conditions

ABSTRACT

*The Ricebean is still seen as a crop that is underappreciated and mistreated. The term salinity describes the buildup of soluble salts in soils, which results in saline soils. The Department of Plant Breeding and Genetics, Faculty of Agriculture, West Bengal, India, used a completely randomized block design (CRBD) and a factorial pattern with three replications to conduct this experiment in a growth chamber. At a salt concentration of 120 mM of NaCl, data were gathered on a variety of seedling growth traits in order to distinguish between genotypes that are salinity tolerant and susceptible. D^2 values should be lower within a cluster than between clusters. The distance between clusters within Cluster III (462.54) is the highest, followed by Cluster II (294.43) and Cluster I (55.88). As a result, these two groups are more diverse, and hybridization between genotypes in these clusters would facilitate gene transfer. The gap between clusters III and II, VI and I, and Clusters VI and IV had the largest **intercluster** distance. The shortest inter-cluster distances were between Clusters IV & I, V & I, and V & II. Except for leaf fresh weight, shoot dry weight, root dry weight, and leaf dry weight and on germination percentage, relative reduction of dry weights, tolerance index (TI), and salinity susceptibility index (SSI) of various seedling traits, genotypes belonging to cluster I were found to have the least relative reduction for most characters. Therefore, it may be assumed that the genotypes KRB-10, KRB-271, KRB-189, KRB-273, KRB-77, KRB-81, KRB-95, and KRB-70, which belong to this cluster I, will be tolerant to salinity. Similar to this, twelve genotypes from cluster IV—KRB-56, KRB-211, Bidhan-1, KRB-73, KRB-102, KRB-66, KRB-272, KRB-104, KRB-274, KRB-44, KRB-39, and Bidhan-2—had the highest relative reduction for the majority of the characters, with the exception of shoot length, total length, shoot dry weight, and tolerance index, and may be insensitive to salinity. The closest association between the genotypes of the cluster's constituents is shown by the inter-cluster distance with the least value. The aforementioned results showed that although the majority of the Ricebean genotypes under investigation had a wide range of individual qualities, when the constellation of traits was considered as a whole, they also belonged to different groups.*

Key words: Ricebean, NaCl, Salinity tolerance, Cluster analysis, Seedling characters

INTRODUCTION

The summer legume known as the Rice bean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi) is adaptable, quick-growing, and underappreciated (Joshi et al., 2008). It is a crop with a high yield and high levels of tryptophan, calcium, phosphorus, and carbohydrates (Chandel, 1984, Singh, 1985. Srivastava, 2001). In India's hilly north-east, it can be grown as a wonderful fodder crop as well as a green manure and cover crop. Over the past few

decades, there has been an increased need for food to sustain a growing population, which has necessitated large-scale research initiatives aimed at uncovering new genetic resources. One of the biggest problems with crop output restrictions is salinity stress, which limits the growth of arable land. According to Shahid et al. (2012), salinity is the buildup of soluble salts in soils, which creates saline soils. Due to the use of underground water in many irrigated areas, this issue also affects many arid and semi-arid regions of the world, when insufficient rainfall causes salt to be leached. Salinity stress damages plant processes and results in a wide range of physiological problems (Munns et al., 2002). The entire plant dies as a result of decreased germination percentage, germination rate, shoot and root length, root and shoot weight, dry root and shoot weight, and seed yield (Jamil, 2006). However, too many reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals, can harm plants since they are produced quickly and frequently build up in their tissues as a result of ion imbalance and hyperosmotic stresses (Chunthaburee et al. 2016). Breeding programs with high yield potential and high salt tolerance genotypes can be strengthened by examining a variety of morphological, physiological, biochemical, enzymatic, and ionic responses at various developmental stages under salt stress as reported in various crops (Azhar and Ahmad 2000; Ali et al., 2002; Khan et al., 2003a; Khan et al., 2003b). The effectiveness of screening crops for salt tolerance in the field is reduced by a number of factors, including climatic variability, changing physio-chemical properties of soil, and the amount of precipitation. A practical and economical method of using salt-affected soils is the selection and breeding of salinity-tolerant cultivars (Munns et al., 2006). However, the presence of genetic variations in the species' gene pool is necessary for this tactic to be effective. Therefore, it is imperative to test current crops for saline tolerance in controlled environments. By removing the aforementioned barriers that restrict the screening technique's efficacy, it will be possible to more easily research a genotype's capacity for salt tolerance. The Mahalanobis distance is a measure of the separation between two points in space caused by at least two correlated variables. The basis for categorising the germplasm collection into several, more or less homogenous groupings is provided by this analysis, which also aids in limiting the amount of germplasm that needs to be examined (Shrimali, et al. 2017). On the other hand, the cluster analysis approach assumes data discontinuities. It exhibits the relatedness of genotypes in accordance with evolutionary relationships or phenotypic performance. It is used to unite related lines or genetic material into one group and to separate it from other groups (Bhandari et al. 2017). Therefore, the aim of this study was to investigate genetic variation and their grouping

pattern for germination attributes in thirty Ricebean accessions during the germination and early seedling stages using a defined and standardized NaCl salt concentration. The salt tolerance intraspecific variation found could be exploited in future breeding programs to improve salt tolerance traits, find the optimal crosses among ricebeans, and choose genotypes that are resistant to salinity stress.

MATERIALS AND METHODS

2.1 . Plant Materials

Lab studies made up the current investigation. The Officer-in-Charge, All India Coordinated Research Project on Forage Crops of Indian Council of Agricultural Research Kalyani Centre, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India, provided the seeds utilized in the current experiment.

Table 1: List of the genotypes used in the experiment:

Sl. No	Collector No	IC No/ Status	Sl. No	Collector No	IC No/ Status
1	KRB-10	IC 433978	16	KRB-115	IC 552997
2	KRB-44	Local collection.	17	KRB-116	IC 552999
3	KRB-39	Local collection.	18	KRB-126	IC 564832
4	KRB-56	Local collection.	19	KRB-128	IC 564834
5	KRB-66	IC 545609	20	KRB-179	IC 564882
6	KRB-70	IC 545613	21	KRB-189	Local collection.
7	KRB-73	IC 545616	22	KRB-211	Local collection.
8	KRB-77	IC 545620	23	KRB-227	Local collection.
9	KRB-81	IC 552964	24	KRB-263	IC 573526
10	KRB-90	IC 552973	25	KRB-271	Local collection.
11	KRB-95	IC 552978	26	KRB-272	IC 573553
12	KRB-100	IC 552983	27	KRB-273	Local collection.
13	KRB-101	IC 552984	28	KRB-274	Local collection.
14	KRB-102	IC 552985	29	BIDHAN-1	Adopted Variety
15	KRB-104	IC 552987	30	BIDHAN-2	Adopted Variety

IC- Indigenous Collection number given by NBPGR, New Delhi, India

2.2. Screening for salinity tolerance at seedling stage

The experiment was set up in a Completely Randomized Block design (CRBD) with three replications in a growth chamber at the Department of Plant Breeding & Genetics, Faculty of Agriculture, BCKV, Mohanpur, West Bengal, India. In order to determine the ideal salt concentration, the set of 20 Ricebean genotypes were initially tested in three different salt concentrations: 80 mM, 120 mM, and 160 mM. Based on the performance of seedlings and salinity attributes of various genotypes were grown in all three salt concentrations, a suitable dose (120 mM) for screening a larger number of genotypes was found (Nandeshwar et al. 2017). From each of the thirty Ricebean genotypes, 36 viable and healthy seeds were surface sterilized with 0.1 HgCl₂ solutions for two minutes before being completely rinsed in distilled water. The seeds were then arranged in a row for each genotype over a glass plate (20 x 30 cm) lined with blotting paper saturated in saline solution. The

entire set was then placed in a transparent polythene bag. There are three copies of this set. The seeds were then allowed to germinate in the lab with enough light and air in plates with saline solution absorbed filter paper. A salt solution with the specified salinity dosage of 120 mM served as the germination medium in the treatment plates. For the experiment, control sets were preserved in which only pure distilled water was used. The seedlings were allowed to grow for 10 days in a lab environment with enough light, relative humidity (RH) ranging from 70 to 80 percent, and temperatures between 25 and 30 degrees Celsius. Three replications of each treatment and the pertinent control were performed. The final count on the fifth day was used to calculate the germination percentage once the experiment was set up. Data on a number of seedling traits were gathered using a destructive sample technique from seedlings that were 10 days old for all 30 genotypes of rice bean. Data were gathered for the following characters to screen ricebean genotypes at salinity of 120 mM:

- i) Final germination percentage (%).
- ii) Length of roots (cm).
- iii) Length of shoot (cm).
- iv) Total length of seedling (cm).
- v) Fresh weight of Root (mg).
- vi) Fresh weight of shoot (mg).
- vii) Fresh weight of leaf (mg).
- viii) Total fresh weight (mg).
- ix) Dry weight of root (mg).
- x) Dry weight of shoot (mg).
- xi) Dry weight of leaf (mg).
- xii) Total dry weight (mg).
- xiii) Tolerance index (TI).
- xiv) Salinity susceptibility index (SSI).

Dry weight of seedling of a genotype grown in saline condition

$$TI = \frac{\text{Dry weight of seedling of a genotype grown in saline condition}}{\text{Dry weight of seedling of the same genotype grown in non-saline condition (Control condition)}}$$

The Fisher and Maurer (1978) formula was used to calculate the salinity susceptibility index (SSI):

$$SSI = 1 - X_{SS} / X_{NS}$$

Where, in salinity-stressed and non-stressed conditions, X_{SS} and X_{NS} are the mean dry weight of all the seedlings of all the genotypes under study, respectively.

The seedling growth was expected to be harmed as a result of the saline solution treatment. As a result, the degree of growth reduction compared to the untreated controls showed the

magnitude of influence of a given salt concentration on seedling growth. For statistical analysis, the percentage of relative reduction (RR percent) for distinct seedling characters of all genotypes was examined in this study. A genotype's relative decrease was calculated as –

$$\text{RR\%} = [1 - (\text{Mean performance as measured for a character under salinity} / \text{the same under control})] \times 100.$$

Based on the above-mentioned findings about seedling features, it was established that growing seedlings with 120 mM NaCl salinity as the indicated salt concentration would disclose the genuine image of salinity tolerance of different genotypes. As a result, 30 genotypes of Ricebean were exposed to 120 mM of NaCl salt as an optimum salinity dose for screening on the basis of seedling growth features for salt tolerance in the current study.

2.3. Statistical Analysis

Using SAS version 9.0's Proc GLM techniques, the data gathered on various seeding properties were subjected to an analysis of variance (SAS, 2004). By grouping the germplasm collection into more or less homogeneous groups using the multivariate analysis, the size of the collection that needs to be analysed is reduced. The agglomerative (starting with single elements and aggregating them into clusters) hierarchical clustering based on the Unweighted paired group technique using the arithmetic mean (UPGMA) approach was carried out using the Proc CUSTER tool of SAS (SAS, 2004). The UPGMA technique offers computed conclusions that are more compatible with recognized heterotic groups than the other clusters, as well as more exact grouping information on breeding materials used in pedigrees (Bhandari et al. 2017). To prevent the challenges of distinct scales that could have formed due to measurement scale differences, the data for all seedling attributes were normalized to a mean of zero and a variance of one before to clustering. The generalized (Mahalanobis, 1936) statistics were used to estimate the genetic separation between groups.

$$D_{(ij)}^2 = (\bar{X}_i - \bar{X}_j) S^{-1} (\bar{X}_i - \bar{X}_j)$$

Where X_i and X_j are vectors representing the values for the i th and j th genotypes, respectively; D_{2ij} is the square distance between any two genotypes i and j ; and S^{-1} is the inverse of the pooled variance-covariance matrix within groups. The D^2 values derived from pairs of clusters were taken into account as the computed values of Chi-square (2) and were compared to the tabulated values of 2 at p degrees of freedom, where p is the number of

characters taken into consideration ($p = 13$), for significance at the 1 and 5% probability levels. (Urdan, 2005)

2.3.1. Contribution of Individual Characters

Singh and Chaudhary's method (1985) was used to calculate the character contribution to genetic divergence. Taking into account all potential combinations, each character is ranked using the formula $d_i = y_{ji} - y_{ki}$. y_{ij} is the mean value of the j th genotype for the i th character, y_{ik} is the mean value of the k th genotype for the i th character, and y_{ik} is the mean value of the k th genotype for the i th character, where d_i is the standard deviation. The character with the biggest mean difference should be rated 1 and the one with the least mean difference should be ranked p , where p is the total number of characters/traits.

RESULTS AND DISCUSSION

3.1. Grouping of 30 genotypes in to different clusters

According to Rao (1948), the 'V' statistic, which in turn makes use of Wilk's criterion, was used to conduct the significance test for the correlated variables. 30 genotypes utilized in the current experiment may be divided into 6 groups using Mahalanobis D^2 analysis (Table 2). Following Tocher's method, the clusters were created based on the relative magnitude of the D^2 values (Rao, 1952). The rule that was adhered to was that intra cluster D^2 values ought to be lower than inter cluster D^2 values. It's interesting to note that, according to Table 2, Cluster I included 8 genotypes, Cluster II had 2, Cluster III had 3, Cluster IV had the most genotypes with 12, Cluster V had 4, and Cluster VI was monogenotypic (Table 2). Prior to this, Golabadi et al. (2006) divided 151 genotypes of rice into 6 groups based on their tolerance to salinity. According to the shoot length and root dry weight of rice seedlings growing in salinity, Hosseini et al. (2012) were able to divide 65 genotypes into 5 groups. The present results for cluster formation were consistent with earlier reports by Cha-um et al. (2010) for multivariable cluster analysis used to screen upland rice genotypes for salt tolerance. When compared to genotypes from different clusters, those from the same cluster appear to be more closely related. The majority of the genotypes were grouped into multiple clusters in relation to the attribute under study, which suggested greater genetic difference. Monogenotypic clusters suggest that a particular genotype may have a completely distinct genetic make-up than the other genotypes, which is what caused the cluster to emerge. In the case of safflower genotypes assessed for seed germination and seedling characteristics under salt stress conditions, a similar monogenotypic cluster was previously described by Khodadad (2011). The dendrogram provides an illustration of the cluster analysis of the 30 genotypes

based on the investigated features. The dendrogram, which clearly displayed six groups and the number of genotypes belonging to each cluster (Fig. 1), helped to corroborate the findings of classifying the genotypes into several clusters. The clustering pattern discovered by D^2 statistics was confirmed by grouping these genotypes into 6 groupings. The findings indicate to the effectiveness of the grouping method and suggest that utilizing D^2 statistics to classify germplasm would produce a set of groups from which parents might be chosen for additional breeding programs.

3.2. Average intra (Diagonal) and inter cluster distance in 30 genotypes of Ricebean

Table 3 lists the typical intra- and inter-cluster distances amongst 30 genotypes of ricebean. The intra cluster distance had a value between 0.00 and 462.54. The intra cluster distance values represent how closely related the genotypes that belong to the same cluster are to one another. On the other hand, large intra cluster D^2 values imply more genetic diversity between genotypes within the same cluster. Clusters with an intra-cluster distance of 0.00 reflect the least genetic diversity between them. It follows that the former should exhibit less heterogeneity. Results depicted in table 3 showed that cluster III had the greatest intra-cluster distance, or 462.54, followed by cluster II (294.43) and cluster I (55.88). Because of their greater diversity, these two clusters would facilitate gene transfer by genotype hybridization between them. No intra cluster distance existed for the remaining three clusters. This suggests that, compared to the other clusters, clusters II and III had more genotypes with diverse phenotypes. This intra cluster heterogeneity among the constituent's genotypes might serve as guideline to choose parents for recombination breeding program. Prior to this, Win et al. (2011) used Ward's clustering technique to divide the 12 Vigna genotypes into two groups for the salt tolerance index. They suggested that these variations in intra and intercluster distances may have resulted from genetic variations on the genotypes. Table 3 shows that the average distance between clusters ranged from 344.04 to 2009.37. Cluster VI and Cluster IV had the greatest intercluster distance, followed by Clusters III and II and Clusters VI and I. According to the features under study, it showed that these cluster pairs were the most divergent, or in other words, their genotypic components were given by their most distantly related parents. Clusters IV and I had the smallest intercluster distances, followed by V and I and V and II. The genotypes that make up the components of these clusters are likely to be closely related if the inter-cluster distance is low. The aforementioned findings show that the majority of the genotypes of Ricebean genotypes under experimental study were extremely variable when taking into account both individual character and collective character constellations. Cha-um et al. (1 c) observed a similar observation. According to the results of

the divergence analysis, genotypes from various clusters that are geographically separated by a large estimated statistical distance may be used in hybridization programs for crop development as well as for examining the salinity resistance in Ricebean's inheritance pattern. Additionally, it was clear that the genotypes from clusters III (KRB-128, KRB-227, and KRB-126), II (KRB-116 & KRB-179), and I (KRB-10, KRB-271, KRB-189, KRB-273, KRB-77, KRB-81, KRB-95, and KRB-70) were the most diverse. Therefore, in a future hybridization programme for genetic enhancement for salinity tolerance, these genotypes could be chosen as parents.

3.3. Cluster mean of 30 genotypes of Ricebean

Since the goal of the current statistical analysis is to identify the clusters of genotypes that exhibit high salinity tolerance on the one hand and low salinity tolerance (susceptibility) on the other, the clusters that would show the lowest relative reduction for the characters under study, with the exception of tolerance index and germination percentage, and the vice versa, would be indicative for identifying tolerant and susceptible clusters, respectively. The results with respect to cluster mean have been presented in the Table 4. The inheritance pattern of salinity resistance in the crop would be revealed by intercrossing the genotypes of such disparate clusters. To find genotypes with the necessary traits, cluster mean analysis would be the most effective method. With the exception of leaf fresh weight, shoot dry weight, root dry weight, and leaf dry weight, it was shown that genotypes belonging to cluster I had the least relative reduction for the most of the traits. The aforementioned characters values, however, were incredibly low. Genotypes KRB-10, KRB-271, KRB-189, KRB-273, KRB-77, KRB-81, KRB-95, and KRB-70 were included in this cluster. It is reasonable to presume that these genotypes will be salt-tolerant. The results also show that when choosing genotypes for salinity resistance, consideration must be given to the features that showed low values for relative reduction, high values for germination percentage, and tolerance index. The aforementioned genotypes were found to yield the least relative reduction when compared to the mean values for relative reduction in each of the seedlings under study's various features (Table 3). The current grouping so supports the earlier observation as well. The results of Table 4 analysis further show that, with the exception of shoot length, total length, shoot dry weight, and tolerance index, the cluster mean for various seedling traits of the 12 genotypes belonging to cluster IV had the biggest relative reduction for the majority of the characters. In this cluster, the genotypes KRB-56, KRB-211, Bidhan-1, KRB-73, KRB-102, KRB-66, KRB-272, KRB-104, KRB-274, KRB-44, KRB-39, and Bidhan-2 were found. It is reasonable to infer that these genotypes will be sensitive to salinity. The findings also

suggest that when selecting genotypes for salinity resistance, consideration should be given to the characters that showed the greatest values for relative decrease, low values for germination percentage, and tolerance index. The aforesaid genotypes and one genotype from cluster VI, KRB-115, also emerged as producing the highest relative reduction when the mean values for relative reduction in different features of the seedlings under study (Table 4) were taken into account. Thus, the current clustering supports the earlier conclusions much more. However, a separate table (Table 5) was prepared with the top 6 genotypes from each group exhibiting highest and lowest relative reduction respectively for the characters under consideration and their mean values were computed in order to gain a clearer understanding of the aforementioned assumptions and the status of the aforementioned selected genotypes. As a result, the top 6 genotypes with the lowest relative decrease for the characteristics were KRB-10, KRB-271, KRB-273, KRB-77, KRB-81, and KRB-95, while the top 6 genotypes with the highest relative reduction were KRB-56, KRB-211, KRB-73, KRB-66, KRB-44, and KRB-115. Out of these, KRB-115 was from cluster VI, while the first five belonged to cluster IV. Calculating Table 5's mean values for the six genotypes from each group that were previously chosen for the character under study revealed that, with the exception of germination percentage and tolerance index, for which higher values were preferred, there was the least relative reduction across all character under study, as would be expected in the case of genotype identification of the tolerant. The aforementioned statement also held true in the case of genotype selection for vulnerable types..

3.4. Percent contribution of each character towards total divergence

For further selection and the selection of parents for hybridization, it is crucial to consider how different plant characteristics contributed to genetic divergence. The character RR-SDW (20.26%) made the largest contribution to the expression of genetic divergence, followed by RR-LDW (19.77%), RR-SL (13.10%), RR-RFW (11.85%), RR-SFW (9.89%), RR-LFW (6.74%), RR-RDW (7.59%), RR-TDW (3.68), and SSI (3.22%). While the remaining characters barely made a dent in the overall difference (Table 6). Senanayake, et al. (2017) presented a similar finding, stating that under various salt concentrations, SDW and LDW contributed most to overall genetic divergence.

Conclusion

The genotypes were divided into six clusters, with a greater distance between clusters than within clusters. In this experiment, genotypes may be classified as salinity tolerant and susceptible primarily based on smaller and higher relative reductions in total dry weight, tolerance index, and susceptibility index. Based on the aforementioned assertion, it was found

that genotypes in cluster I had the least relative reduction for the majority of characters, with the exception of leaf fresh weight, shoot dry weight, root dry weight, and leaf dry weight, as well as on germination percentage, relative reduction of dry weights, tolerance index (TI), and salinity susceptibility index (SSI) of various seedling traits. Consequently, the genotypes that made up this first cluster were KRB-10, KRB-271, KRB-189, KRB-273, KRB-77, KRB-81, KRB-95, and KRB-70. Consequently, it is reasonable to infer that these genotypes will be salinity tolerant. Tolerance index and shoot length, total length, shoot dry weight, and 12 genotypes from cluster IV showed the biggest relative reduction for the majority of the other parameters. A number of genotypes, including KRB-56, KRB-211, Bidhan-1, KRB-73, KRB-102, KRB-66, KRB-272, KRB-104, KRB-274, KRB-44, KRB-39, and Bidhan-2, belonged to this cluster. So, it stands to reason that these genotypes would be sensitive to salinity. The inheritance pattern of salinity resistance in the crop would be revealed by intercrossing the genotypes of such disparate clusters.

REFERENCES

- Akber, M and Yabuno, T.1977. Breeding for saline resistance varieties of rice IV. Inheritance at delayed type panicle sterility induce by salinity. *Jpn. J. Breeding*.**27**:237-240.
- Ali, Z., Khan, A.S. and Asad, M.A. 2002. Salt tolerance in bread wheat: genetic variation and heritability for growth and ion relation. *Asia J Plant Sci*. **1**:420-422.
- Ashraf, M., 1994. Organic substances responsible for salt tolerance in *Eruca sativa*. *J. Biol .Plant*, **36**:255-259.
- Bhandari HR, Bhanu AN, Srivastava K, Hemantaranjan A. 2017.Assessment of genetic diversity in crop plants: An overview. *Advances in Plants and Agricultural Research*. 2017;7(3):279–286.DOI: 10.15406/apar. 07.00255.
- Chandel, K.P.S. and Singh, B.M.1984. Some of our underutilized plants. *Indian Fmg*. **34**:123-127.
- Cha-um, S., Ashraf, M. and Kirdmanee, C., 2010. Screening upland rice (*Oryza sativa* L. ssp. indica) genotypes for salt-tolerance using multivariate cluster analysis. *Afr. J. Biotechnol*. **9**: 4731–4740.
- Chouhan, R.R., Choudhary, R., Singh, A. and Singh, P.K. 2012. Salt tolerance at Sorghum bicolor cultivars during germination and seedling growth. *Res. J. Recent Sci*. **1(3)**:1-10.
- Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W. and Theerakulpisut, P. 2016. Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Sau. J. Bio Sci*. **23**: 467–477.
- Fisher, R.A. and Maurer, R. 1978. Drought resistance in spring wheat cultivars to grain yield responses. *Aus. J. Agric. Res*.**29**:897-912.

- Garg, N. and Singla, R. 2004. Growth, photosynthesis, nodule nitrogen and carbon fixation in chickpea cultivars under salt stress. *Braz. J. Plant Physiol.* **16**:137-146.
- Ghoielam, C. and Farses, K. 2001. Effect of salinity on seed germination and early seedling growth of sugar beet. *Seed Sci & Technol.* **29**:357-364.
- Golabdi, M., Arzani, A. and Mismohammadi, S.A.M. 2006. Assessment of drought tolerance in segregating population of durum wheat. *Afric. J. Agric. Res.* **1** (5):162-171.
- Hosseini, S. J., Sarvestani, Z. T. and Pirdashti, H. 2012, Analysis of tolerance indices in some Rice (*Oryza sativa* L.) genotypes at salt stress condition, *Intl. Res. J. Appl. Basic. Sci.*, **3**(1):1-10
- Jamil, M., Lee, D.B., Jung, K.Y., Ashraf, M., Lee, S.C. and Rha, E.S. 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *J. Cen. Europ. Agri.*, **7**(2): 273-282.
- Joshi, 2008. In: Smartt, J. Haq, N. New crops and uses: their role in a rapidly changing world. *CUC*, UK pp: 234-248
- Khan, A.A., Rao, S.A. and McNilly.T.M. 2003 a. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica*, **131**: 81-89.
- Khan, A.S., Asad, M.A. and Ali. Z. 2003 b. Assessment of genetic variability for NaCl tolerance in wheat. *Pak. J. Agri. Sci.*, **40**: 33-36.
- Khodadad, M. 2011. Evaluation of safflower genotypes (*Cathamus tinctorius* L.) seed germination and seedling characters in salt stress condition. *Afric. J. Agric. Res.* **69**(7):1667-1672.
- Mahalanobis, P.C.I. 1936. On the generalized distance in statistics. *Proc. Nat. Inst. Sci. India*, **12**: 49-55.
- Marscher, H. 1995. Mineral nutrition of higher plants. Academic press. London. U.K.P.889.
- Mass, E. V. and Grieve C. M., 1991. Tiller development in salt stressed wheat. *Crop Sci.*, **34**: 1594-1603
- Misra, N. and Dwivedi, U.N. 2004. Genotypic differences in salinity tolerance of green gram cultivars. *Plant Sci.* **166**:1135-1142.
- Munns, R., James, R.A. and Lauchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* **57**: 1025–1043.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* **25**:239-250.
- Nandeshwar B. C., Karande P.T., Bhoite A.G., Shinde S.R, Shinde, S.B. and De. D. K. 2017. Effect of NaCl concentrations on germination and growth attributes at early seedling stages in ricebean (*Vigna umbellate* (Owhi) and (Ohashi)) accessions. *Intr J of Tropical Agric.* **(35)3**:653-656.
- Orak, A. and Ates, E. 2005. Resistance to salinity stress and available water levels at seedling stage of common vetch (*V. sativa* L.). *Plant Soil and Environ.* **51**:51-56.

- Pal, M., Singh, D.K., Rao, L.S. and Singh, K.P. 2004. Photosynthetic characteristics and activity of antioxidant enzymes in salinity tolerant and sensitive rice cultivars. *Ind. J. Plant. Physiol.* **9**:407-412.
- Parida, A.K. and Das, A.B. 2005. Salt tolerance and salinity effects on plants: *A Rev. Ecotoxicol. Environ. Safety.* **60**: 324-349.
- Patel, P.K., Kajal, S.S., Patel, V.R., Patel, V.J., and Khristi, S.M. 2010. Impact at saline water stress on nutrient uptake and growth of Cowpea. *Braz. J. Plant Physiol.* **22(1)**:43-48.
- Poonia, S.R. and Jhonsen, J. B. 1976. Effect of different concentrations of Ca⁺⁺ and Na⁺ in growth medium on the yield and chemical composition at wheat and dhaincha. *Ind. J. Agric. Sci.* **44**:871-874.
- Rahman, M.M. and Ahsan, M. 2001. Salinity constraints and agricultural productivity in coastal saline area of Bangladesh. *Soil resources in Bangladesh: Assessment and Utilization.*
- Rao, C.R. 1948. The utilization of multiple measurements in problem of biological classification. *J. Roy. Stat. Soc.* **10(B)**:159-203.
- Rao, C.R. 1952. Advance statistical methods in biometric research. *John Wiley and Sons.*, New York.
- Rehman, M.S., Miyake, H. and Takeoka, Y. 2001. Effect of sodium chloride on seed germination and early seedling growth of rice. *Pak. J. Bio. Sci.* **4(3)**:351-355.
- Rohlf, F.J. 2005. NTSYS pc (Numerical Taxonomy and Multivariate Analysis System). Version 2.2. *Exeter Software Appl. Biostat Inc.*, New York.
- SAS 2014. (System Analysis Software). SAS Statistical Software Version NC, USA. 9.1;
- Senanayake, R.M.N.H., Herath H.M.V.G., Wickramesinghe, I.P. Udawela, U.A.K.S. and Sirisena D.N. 2017. Phenotypic Screening of Rice Varieties for Tolerant to Salt Stress at Seed Germination, Seedling and Maturity Stages. *Tropical Agricultural Research.* 29 (1): 90 – 100.
- Shahid, M. A., Pervez, M. A., Balal, R. M., Abbas, T., Ayyub, C. M., Mattson, N. S., Riaz, A. and Iqbal, Z. 2012. Screening of pea (*Pisum sativum* L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. *AJCS* **6(9)**:1324-1331
- Shrimali J, Shekhawat AS, and Kumari S. 2017. Genetic divergence in Barley (*Hordeum vulgare* L.) under normal and limited moisture stress conditions. *International Journal of Current Microbiology and Applied Science*, **6(8)**:2220-2226.
- Singh RK and Chaudhry BD, 1985. Biometrical methods of quantitative genetic analysis. Kalyani Publishers, Ludhiana, India. 30-34.
- Singh, S.P., Misra, B.K., Sikka, K.C., Chandel, K.P.S. and Plant, K.C. 1985. Studies on some nutritional aspect of ricebean (*Vigna umbellata*). *J. Food. Sci. Technol.* **22**:180-185.
- Srivastva, R.P., Srivastava, G.K. and Gupta, P.K. 2001. Nutritional quality of ricebean (*Vigna umbellata*). *Ind. J. Agril. Biochem.* **14**:55-56.

Stepien, P and Klobus, G. 2006. Water relation and photosynthesis in *Cucumis sativa* L. leaves under salt tolerance of durum wheat. *Aust. J. Agric. Res.* **51**:69-74.

Tehran Natural Resources Bureau. 2003. The final report at the Tehran rangeland, Tehran, Iran, Iran, pp-128.

Urdan TC. 2005. Statistics in plain English. 2nd Ed. Lawrence Erlbaum Associates, Mahwah, New Jersey, USA.

Verma, S.K. 1981. Specific ion effect on easily growth of wheat (*Triticum aestivum*). *Ind. J. Phy.* **24**:291-294.

Waisel, Y.1972. Biology at Halophytes. Academic press Inc. pp.395.

Win, K.T., Zaw, A.O., Tadashii, H., Taiichiro, O. and Hirata, Y. 2011. Genetic analysis of Myanmar *Vigna* species in responses to salt stress at the seedling stage. *Afr. J. Biotechnol.* **10(9)**: 1615-1624.

Table 2: Grouping of 30 genotypes of Ricebean into different clusters.

Sl. No.	Clusters	Number of genotypes	Genotypes
1	I	8	KRB-10, KRB-271, KRB-189, KRB-273, KRB-77, KRB-81, KRB-95, KRB-70
2	II	2	KRB-116, KRB-179
3	III	3	KRB-128, KRB-227, KRB-126
4	IV	12	KRB-56, KRB-211, BIDHAN-1, KRB-73, KRB-102, KRB-66, KRB-272, KRB-104, KRB-274, KRB-44, KRB-39, BIDHAN-2
5	V	4	KRB-90, KRB-100, KRB-101, KRB-263
6	VI	1	KRB-115

Table 3: Intra (Diagonal) and inter cluster distance in 30 genotypes of Ricebean

	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster
1 Cluster	55.88					
2 Cluster	694.42	294.43				
3 Cluster	1271.41	1643.71	462.54			
4 Cluster	344.04	801.06	999.03	580.12		
5 Cluster	444.72	660.95	1319.71	894.57	60.17	
6 Cluster	1465.15	1405.47	1451.97	2009.37	810.92	0.00

Table 4: Cluster mean for different seedling characters in 30 genotypes of Ricebean.

Clusters	Ger %	RR-SL	RR-RL	RR-TL	RR-SFW	RR-RFW	RR-LFW	RR-TFW	RR-SDW	RR-RDW	RR-LDW	RR-TDW	TI	SSI
1 Cluster	87.82	21.21	54.99	42.65	21.87	23.57	60.44	28.78	18.95	21.04	28.41	22.02	78.00	0.47
2 Cluster	74.89	14.20	75.03	46.47	34.17	26.06	18.40	30.87	24.76	33.04	45.09	47.98	51.39	0.93
3 Cluster	76.81	37.20	66.10	54.14	37.40	44.34	77.55	49.32	15.08	54.78	62.36	40.69	59.26	0.83
4 Cluster	72.89	68.41	75.31	72.22	73.30	76.10	89.00	79.37	70.11	63.93	83.65	68.73	31.26	1.43
5 Cluster	63.89	76.16	72.38	73.22	72.10	65.33	83.11	75.90	46.00	20.58	74.70	48.56	52.37	0.92
6 Cluster	80.55	31.38	61.96	44.19	52.34	20.51	33.48	30.91	88.03	16.67	24.09	77.75	22.25	1.26

Table 5: Derived table for cluster means of only different selected 6 tolerant and 6 susceptible genotypes in three different clusters mainly 1, 4 and 6 clusters out of six clusters

Clusters	Ger %	RR-SL	RR-RL	RR-TL	RR-SFW	RR-RFW	RR-LFW	RR-TFW	RR-SDW	RR-RDW	RR-LDW	RR-TDW	TI	SSI
1 Cluster	89.793	20.036	51.302	40.193	22.763	20.460	58.549	28.102	19.584	19.686	27.920	21.551	78.463	0.457
4 Cluster	62.730	69.213	76.013	72.293	78.819	72.805	91.744	78.762	74.179	63.678	85.015	72.699	27.000	1.545
6 Cluster	80.551	31.382	61.961	44.192	52.340	20.511	33.483	30.910	88.031	16.670	24.091	77.752	22.251	1.262

Table 6: Contribution of individual characters towards total genotypic divergence in 30 genotypes of Ricebean.

Sl. No.	Characters	Number of times appearing as first in rank	Per cent contribution towards divergence D ² statistics
1	Germination %	0	0.00
2	RR-SL	57	13.10
3	RR-RL	6	1.38
4	RR-TL	0	0.00
5	RR-SFW	43	9.89
6	RR-RFW	52	11.95
7	RR-LFW	38	8.74
8	RR-TFW	1	0.23
9	RR-SDW	88	20.23
10	RR-LDW	86	19.77
11	RR-RDW	33	7.59
12	RR-TDW	16	3.68
13	TE	1	0.23
14	SSI	14	3.22

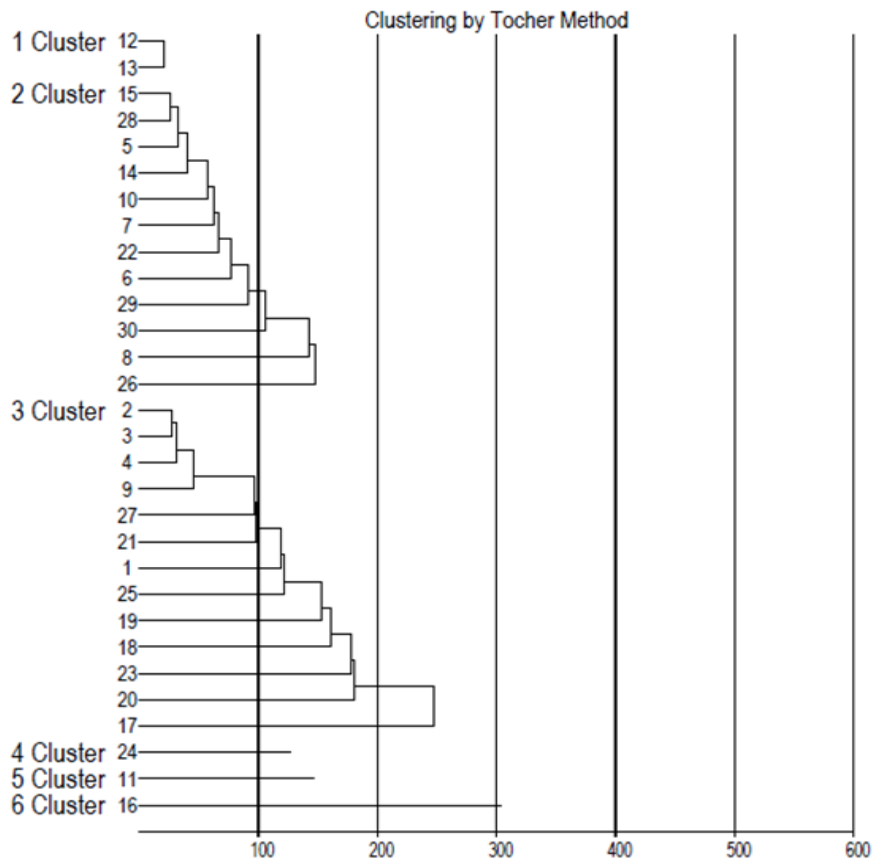


Figure 1: Dendrogram showing the clustering of 30 Ricebean genotypes into groups