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# A STUDY ON GENETIC DIVERSITY OF *CUSCUTA REFLEXA* ROXB. AND FEW MEMBERS OF CONVOLVULACEAE ON THE BASIS OF RAPD AND SDS-PAGE

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**Abstract**

In the research, genetic diversitry of *Cuscuta refelexa* Roxb.and some slected taxa of Convolovulaceae was conducted using DNA markers and SDS-PAGE technique. Four primers were used for the amplification of genomic DNA of twelve samples of 11 species belonging to Convolvulaceae and one species of Cucutaceae. RAPD-PCR was performed by the use of four primers and in total 40 bands were produced and among these 24 bands showed polymorphism. On the basis of pair wise comparison between species, the highest number of polymorphic bands were obtained by primer 4, band size generated by these primers ranged from 200-500bp. Maximum similarity was observed between *Cuscuta reflexa* Roxb. and *Ipomoea pes-tigridis* L. with 100 similarity index at distance level of 1.375. Same plant samples were also evaluated for their total protein variability. Protein was extracted and loaded on polyacrylamide gel for electrophoresis. Observed proteins bands were ranged in size from 20 to 200 kDa. *C. reflexa* has only 20 and 40 kDa protein that is found in every studied member of Convolvulaceae. Maximum similarity was observed between *C. reflexa* and *Ipomoea pes-tigridis* with 100% at distance level of 0.355. A significant result of this study was the identification of close relationship among *C. reflexa* Roxb. and *Ipomoea pes-tigridis* L. confirmed by both SDS and RAPD analysis.

**Key words:** Cuscutaceae; Convolvulaceae; *Ipomoea* species; SDS-OAGE; RAPD; *Cuscuta reflexa* Roxb.

## Introduction

Plants are very important in life of man for coping asic needs of life. The proper identification and their taxonomic demarchation is very important for the proper use of these wild plants. The plants of family Convolvulaceae and Cuscuataceae are very medicinal and have been used in traditional medicienes. There is confusion in the proper identiifcaoton of plants of two families and moreover, these taxa of these families seem to be correlated with each other. The current research was planned to explore the genetic affinity of the plants of two families.

In the genetic makeup of a species genetic diversity comprises of all the numbers of genetic features. Markers are the characters used for identification in plants these are polymorphic that exist in more than one form. There are three types of markers *i.e.* molecular, biochemical and morphological marker. Morphological markers are affected by environment, biochemical includes protein and isozyme they are also affected by the environment so these two are not much reliable the only reliable markers are molecular markers that are stable and not affected by the environment (Chawla, 2004).SDS-PAGE is common because of its ease and value in identifying the genetic organization (Murphy *et al*., 1990; Ogunwenmo, 2003; Miller *et al.*, 2004).This technique has turned out to be a functional means for diversity studies which helps in optimization of variations in germplasm collections (Cwiklimska *et al*., 2010).

Protein polymorphism products of activated genes, are polymorphic and heritable that's why they are employed as genetic markers (Gepts, 1990). The variations in active parts of the genome are reflected in protein profiles observed by polymorphism. There are many techniques for analyzing protein polymorphism, but polyacrylamide gel electrophoresis is best as it provides rapid analysis (Matos *et al*., 2001; Lv *et al*., 2016; Vilhena *et al.*, 2015). To understand genetic diversity among and between genotypes of different plant species this technique has been used vastly (Mei *et al*., 2014; Mukherjee & Datta 2008; Khan *et al*., 2010). At molecular level genetic diversity assessment is more important than at the phenetic level as it includes data on morphological traits, to check the genomic constancy of regenerated plants different molecular markers are used. Molecular markers are proved to be precious tool for the evaluation and characterization of genetic diversity among and between species and populations. Variations are shown by different marker is practically applicable. RAPD markers have more advantages such as no requirement of prior information of any DNA sequence, high frequency of polymorphism, requirement of few nano gram of DNA, technical simplicity, rapidity and feasibility of automation (Amer *et al*., 2018). The aim of this study was to identify position of *Cuscuta* spp. and to determine its association with other Convolvulaceae species by DNA and protein markers.

## Materials and Methods

Fresh tissues of all eleven species for the extraction of DNA and protein were collected carefully from different areas and were placed in cooler with ice, after cleaning every sample was labelled and wrapped with Aluminium Foil and carried to the laboratory and kept frozen until used. Four primers were used from previously reported study (Khalik & Hassan, 2012) for amplification of DNA as shown in Table 1.

**Table 1. Primer used for Amplification of DNA.**

| Primer name | Sequence used |
|---|---|
| Oligo1 | 5'-TCCCACGCAA-3' |
| Oligo2 | 5'-GTAGACCCGT-3' |
| Oligo3 | 5'-CAGCACCCA-3' |
| Oligo4 | 5'-ACTTTGGCG-3' |

**RAPD Marker analysis:** Modified CTAB method Doyle (1991) was used for DNA extraction modified mean method was improved to increase the amount and quality of DNA after extraction. First of all, plant material was powdered with the help of mortar and pestle after ice covered in liquid nitrogen. Sample was transferred into falcon tubes (15ml) each falcon tube contained 6ml of CTAB. Then sample was placed in water bath on 60ºCat gentle vacillating for almost 30 minutes. Afterwards cooled to room temperature for 10 minutes, in each tube addition of Chloroform Isoamyl alcohol mixture (24:1) was carried out. For homogenization of mixture phases were mixed and kept at room temperature for 10 minutes. Centrifugation at 4000 rpm was carried out for 15 minutes in order to remove debris and the supernatant clear aqueous phases (containing DNA) were transferred into a new sterile tube. Chloroform: isoamyl alcohol step was repeated again, precipitation of DNA was done with the addition of equal amount of chilled Isopropanol placed the DNA in refrigerator overnight for the settlement. DNA pellet washed two times by 70%ethanol and DNA was dried at room temperature. Afterwards TE buffer was added and stored at 20°C. Amount of TE used related to the quantity of DNA and stored at -20°C for future use. The PCR reactions were carried out in 25 µl PCR tube. The PCR Reaction mixture consisted of 15µl sterile distilled water, 1.5 µl (50 mM) $MgCl_2$, 2.5 µl 10Xbuffer, 0.5 µl (5u/ µl),2 µl (10 pmol/µl) primer, Taq DNA polymerase and 1µl (10 to30 ng/µl) template DNA, for each sample. The PCR profile was automatized at 94°C for 5 min for denaturation, then by 40 cycles of at 94°C for 1min, at (36°C) for 1 min and at 72°C for 1 min, last extension was performed at 72°C for 7 minutes and was hold at 4°C pending samples collection. DNA sample after amplification was loaded in 1% agarose gel. Ethidium Bromide 0.5µl (1mg/100ml used for staining of gel. Electrophoresis was carried out at 80 Volts. When fragments were separated the visualization was done with an ultraviolet (UV) transilluminator and then photographed. In each experiment a sample devoid of template DNA was used as a negative control to check contamination. A standard DNA ladder was used to show the sizes of DNA fragments. Numbers of Polymorphic bands and monomorphic bands were calculated for every primer used. To score genotypes (1) used to show presence of band, and (-0-) for absence of band afterwards data used in data matrix. For the prediction of percentage of polymorphism percentage following equation: (polymorphic bands/total number of bands x 100) was used. After cluster analysis the similarity data was used for the construction of Tree diagram.

**SDS-PAGE analysis:** To study the variations among protein SDS-PAGE analysis was done (Islam *et al*., 2017) to score over the genetic assortment and characterization of the selected plants. The present work was designed in continuation of preceding work by means of dissimilar studies (Shuaib *et al*., 2007). In each eppendrof tube 400µl protein extraction buffer (30% Urea, Tris-HCl 0.05M (pH 8), 1% 2-mercaptoethanol, 0.02% SDS (sodium dodecyl sulfate) was poured. Sample was boiled at 100ºC for 2 min and kept back at 40°C over night and afterward centrifuged for 10 min at 13000 rpm. The supernatant contained dissolved protein set for experiment (Shuaib *et al*., 2007). The resolving gel was prepared by mixing 3 ml 5 ml (5% acrylamide), (1.875M Tris-HCl pH 8.80), 140µl (SDS 10%), 90µl (APS 5%) 6.9 ml distilled water and addition of 14µlof TEMED was added at the end. The stacking gel was prepared by addition of 1 ml (0.6 M Tris-HCl pH 6.8), 7.2 ml distilled water, 1.66 ml (30% acrylamide), 100µl (SDS 10%), 80µl (APS 5%) and 9µl (TEMED)was added at the end, 70% ethanol was used to clean glass plate and seal gasket with clips was applied to fix. Separating gel was placed in the plates cell with extreme care that is no appearance of air bubble at the bottom of the gel and then encrusted with distilled water. Distilled water was removed after 30 min, addition of stacking gel was done and then comb was positioned in the stacking gel. Afterwards sample was loaded and electrophoresis carried out. Glass cabinet was preset by way of electrophoresis apparatus; were crammed with electrode buffer comprising of (25 mM Tris, 0.1% SDS, 192 mM glycine) was used for filling of the electrophoretic trays. Running buffer was used for the cleaning of wells were done with running buffer and the (12µl) of the sample was used for loading of gel and (5µl) of marker10-200kDa (Asbastas#/SM0661) was used afterwards power supply at 120 volts was joined and then remained for three to four hours until the blue line appeared at the base of gel plate (Damania *et al*., 1983). For staining 15% trichloroacetic acid solution was used staining was done in a tray staining solution comprises of ethanol, Coomassie Brilliant Blue, ethanol, 15% trichloroacetic acid solution and glacial acetic acid and shake gently for 40 minutes on an agitator.

## Results and Discussion

**RAPD-PCR:** RAPD -PCR was performed by the use of four primers of 40 bands were produced in total, 24 bands showed polymorphism on the basis of pair wise comparison between species as shown in Fig. 1 and Table 2. The highest number of polymorphic bands were obtained by primer 4, size ranged from 200-500bp. Relationship among the considered taxa were shown in a dendrogram prepared on the basis of similarity index. Comparison was done on the basis of 40 bands and for species the number of bands and size of bands were scored. In first group *Ipomoea cairica* (L.) Sweet included with similarity index (100), in 2nd group *Ipomoea carnea* Jacq., *I. hederacia* Jacq., *I. pes-tigridis* L., *I. eriocarpa* R.Br., *Merremia aegyptia* (L.) Choisy, *Convolvulus arvensis* L., *Evolvulus alsinoides* (L.) L., *Ipomoea quamoclit* (L.) included with similarity index with 100.00 similarity index.

**Table 2. RAPD analysis of 12 species.**

| No. | Species | RAPD band size (bp) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 250 | 500 | 750 | 1000 | 1500 | 2000 | ### | 4000 | 5000 | 6000 | 8000 | 10000 |
| 1. | *Cuscut a reflexa* | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. | *Ipomoea hederacia* Jacq | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 3. | *I. carnea* Jacq. | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4. | *I.* pes-*tigrides* L | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. | *Ipomoea cairica* (L.) Sweet | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. | *Ipomoea quamoclit* (L.) Spreng | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 7. | *I. eriocarpa* R.Br.Spreng | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8. | *Ipomoea arachnosperma* Welw., | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9. | *Merremia aegyptia* (L.) Choisy | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10. | *Convolvulus arvensis* L., | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11. | *Evolvulus alsinoides* (L.) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12. | *Convolvulus prostrates* Forssk | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

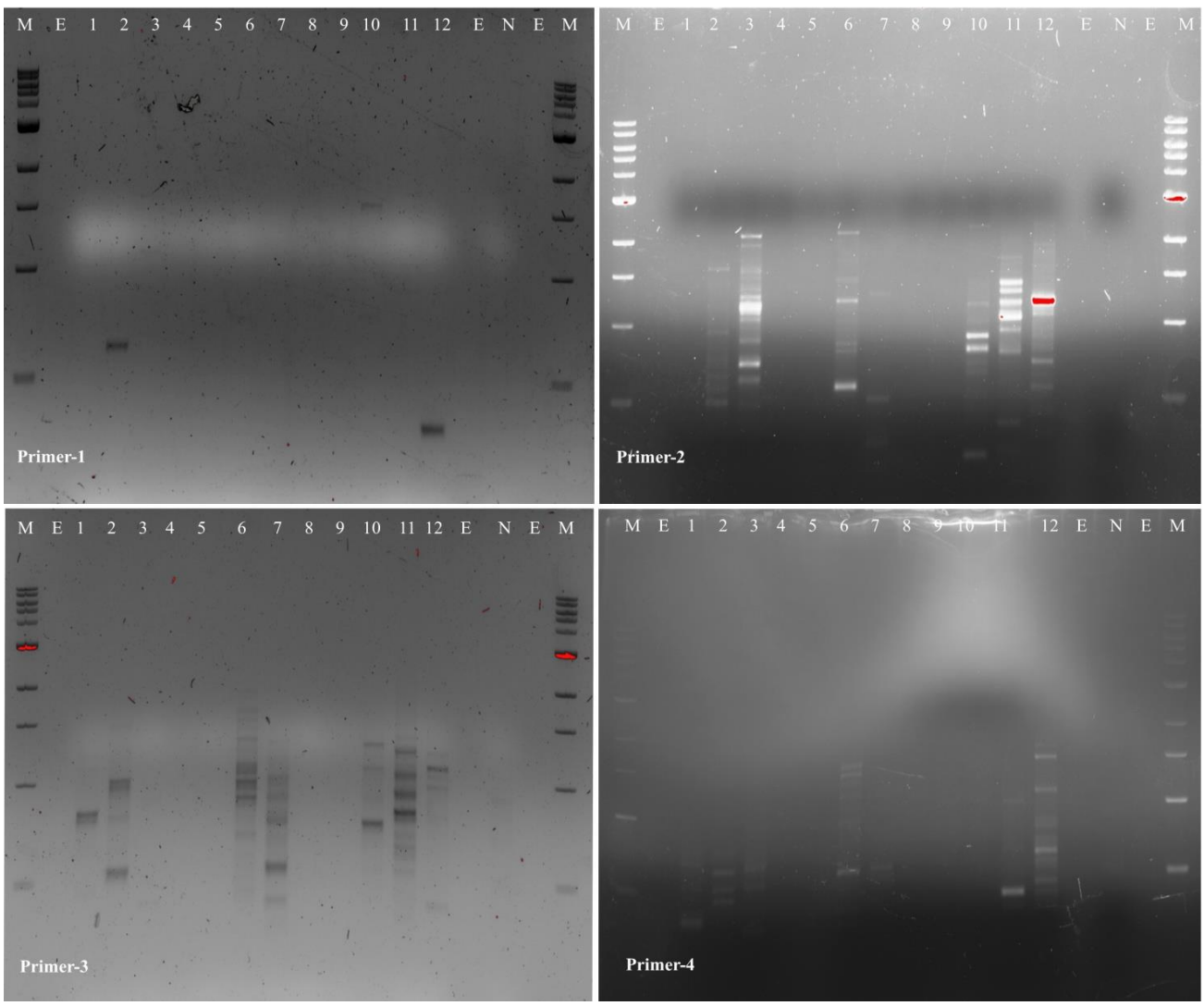


Fig. 1. RAPD analysis from different species of Convolvulaceae and Cuscutaceae. Amplification is shown for primers (oligo1 – 4) where, M: DNA marker, E: Empty. 1-12 are species, N: negative control.

**Cluster analysis:** Scoring of gels were done by visual observation number was assigned to each band 1 was used to show presence and 0 were used to show absence of bands. Genetic similarity was estimated among all species. Dendrogram was built on the basis of similarity to show relationship among the species. The major clusters obtained were two with 77% similarity index as shown in Fig. 2. Cluster one includes *Ipomoea cairica* (L.) Sweet species cluster 2 includes *Ipomoea cairica* (L.) Sweet, *I. carnea* Jacq. *I. hederacia* Jacq., *I. pes-tigridis* L., *I. eriocarpa* R.Br., *Merremia aegyptia* (L.) Choisy, *Convolvulus arvensis* L., *Evolvulus alsinoides* (L.) L., *Ipomoea quamoclit* (L.) and *Cuscuta reflexa* Roxb., of Cuscutacea; Cluster analysis was done on the basis of observed variables, 11 clusters were generated and maximum similarity was observed between 100 similarity index at distance level of 1.375 between *Cuscuta reflexa* Roxb. and *Ipomoea pes-tigridis* L. as shown in Table 3.

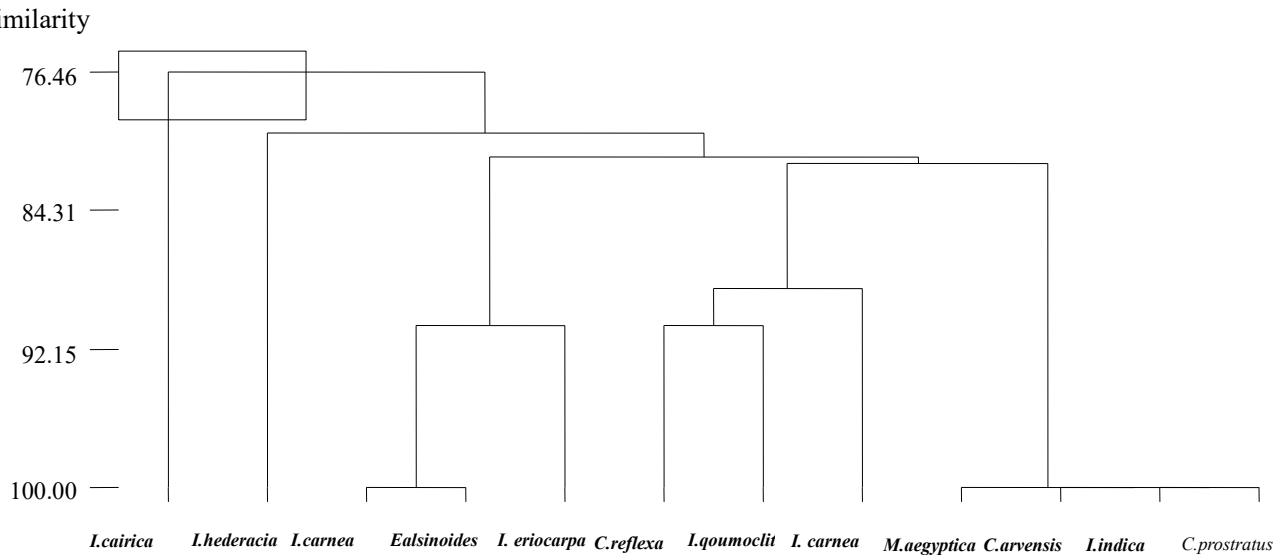


Fig. 2. Cluster analysis of the plant species on the basis of RAPD analysis.

**Table 3. Hierarchical cluster analysis of variables based on RAPD data of these species.**

| Steps | No of clusters | Similarity level | Distance level | Cluster joined | | New cluster | No of observation in new cluster |
|---|---|---|---|---|---|---|---|
| 1 | 11 | 100.00 | 0.000 | 11 | 12 | 11 | 2 |
| 2 | 10 | 100.00 | 0.000 | 10 | 11 | 10 | 3 |
| 3 | 9 | 100.00 | 0.000 | 9 | 10 | 9 | 4 |
| *4* | 8 | 100.00 | 0.000 | 4 | 5 | 4 | 2 |
| 5 | 7 | 91.08 | 0.178 | 1 | 8 | 1 | 2 |
| 6 | 6 | 91.08 | 0.178 | 4 | 7 | 4 | 3 |
| 7 | 5 | 88.92 | 0.222 | 1 | 3 | 1 | 3 |
| 8 | 4 | 81.98 | 0.360 | 1 | 9 | 1 | 7 |
| 9 | 3 | 81.94 | 0.361 | 1 | 4 | 1 | 10 |
| 10 | 2 | 80.86 | 0.383 | 1 | 6 | 1 | 11 |
| 11 | 1 | 79.58 | 0.408 | 1 | 2 | 1 | 12 |

**SDS PAGE analysis:** Protein analysis of 12 species, 11 from Convolvulaceae and one from Cuscutaceae were performed. Protein was extracted and loaded on polyacrylamide gel for gel electrophoresis as shown in Fig. 3. A standard marker was also loaded to measure the size of protein. Scoring of gel was done by comparing with marker. Few bands were unique many were shared by many species. The number of band changes from species to species as 5 were recorded in *Ipomoea* spp. and 3 were in *Convolvulus*. +Sign was used to show the presence of bands and -ve was used to show the absence of band data and further analyzed to find similarity index as shown in Table 4.

**Cluster analysis:** Cluster analysis was done with the help of MINITAB software on the basis of observed variables. Two major clusters were generated. Cluster 1 includes *Ipomoea carnea* Jacq. and cluster 2 includes *Ipomoea cairica* (L.) Sweet, Ipomoea *hederacia* Jacq., *Ipomoea pes-tigridis* L., *I. eriocarpa* R.Br., *Merremia aegyptia* (L.) Choisy, *Convolvulus arvensis* L., *C. prostrates* Forssk, *Evolvulus alsinoides* (L.) L., *Ipomoea quamoclit* (L.) Spreng *Cuscuta reflexa* Roxb., Maximum similarity was observed between species 100 similarity index at distance level of 0.355 between *Cuscuta* and *Ipomoea* spp. as shown in Fig. 4. The dendrogram represents that major two groups of species were generated in separate way. In one group 10 species are included and group two includes only one species. Further these 10 plant species were sub-divided in four sub-groups and showed a common ancestor as shown in Table 5

## Discussion

The current investigation of genetic diversity was based on variation of polymorphic characters biochemical and molecular markers. To check the genetic similarity different approaches including conventional were used as reported by Noshad *et al*., (2020). The reason for the usage of such procedures is not only the comparison of methods but to also find how much polymorphic amplified products are formed. Genetic similarity can be different on the basis of generation of polymorphic bands obtained from RAPD analysis. This was in accordance with work of Matos *et al*., (2001) less similarity was obtained between the objects more polymorphic products were generated. It has been founded that as much as polymorphic product generated lesser is the similarity between objects. Mainly data generated by RAPD

analysis, recommended groups and to verify sectional classification of *Ipomoea* Austin (1997). A relative RAPD and SDS-PAGE analysis of different species of Convolvulaceae and Cuscutaceae was achieved in this work.

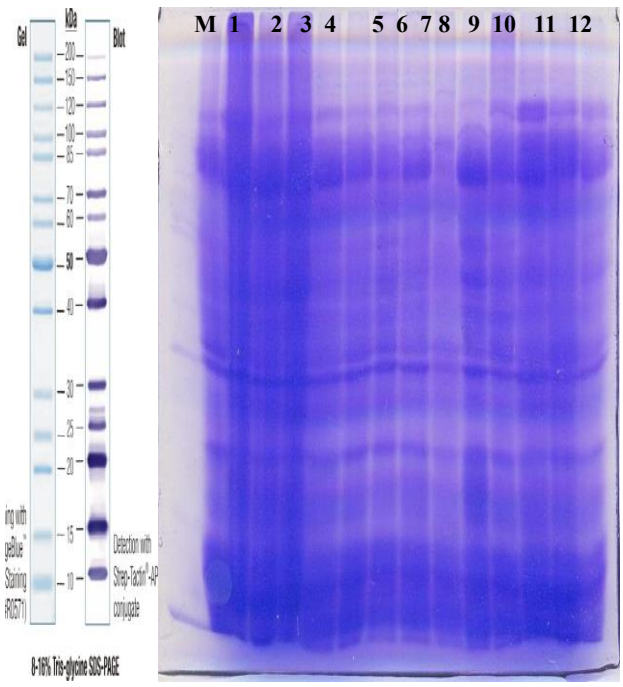


Fig. 3. SDS-PAGE of different species of Convolvulaceae and *Cuscuta reflexa* from Cuscutaceae. In this Fig. M represents marker, 1. represents *Ipomoea carnea* Jacq. 2. represents *Ipomoea cairica* (L.) Sweet, 3. show Ipomoea *hederacia* Jacq., 4. shows *Ipomoea pes-tigridis* L., 5. represents *Merremia aegyptia* (L.) Choisy, 6. *Convolvulus arvensis* L., 7. *C. prostratus* Forssk, 7. *Evolvulus alsinoides* (L.) L., 8. *Ipomoea quamoclit* (L.) Spreng 9. *Cuscuta reflexa* Roxb., 10. *Ipomoea eriocarpa* R.Br 11. *Ipomoea quamoclit* (L.) 12. *Ipomoea indica* (Burm. f.) Merr.

RAPD technology could be efficiently used to demonstrate genetic relationships among different populations of *C. pluricaulis.* By analysing the genetic and chemical profiling, it is possible to identify the elite population. DNA analysis by RAPD -PCR provides more accurate information about association among all species. A more significant results from this study was the identification of relationship between *Ipomoea* species and *Cuscuta reflexa* in the Group I. On the basis of cluster analysis there was found very close association among *Ipomoea hederacia* and *Ipomoea quamoclit*, *Ipomoea indica*. *Ipomoea ereocarpa* and *Convulvulus arvensis*. These species are morphologically similar this is in accordance with work of Khalik (2008). In our study group II that includes *Ipomoea carica* it is considered as separate group with very low genetic similarity based on RAPD analysis. *I. cairica* has unique characters such as leaf-blade palmate divided into 5-7 lobes; seed surface with micro reticulate periclinal cells. This was in accordance with work of Miller *et al*., (2004). Sub-group II includes *Cuscuta reflexa, Ipomoea pes-tigridis, Ipomoea hederacia* and *Cuscuta reflexa* that has maximum similarity with *Ipomoea pes-tigridis* and this finding is in accordance with the work of Khalik (2008). At species level, SDS-PAGE is most significant and important technique used for the recognition of entire cell proteins. To determine genetic diversity, one of the most valuable method because of practically simple, easy and quick in action it resolve most of technical obstacle William *et al*. (1990) therefore RAPD has been extensively utilized to fine genetic association among various accession of different plant species. For the recognition of proteins this method entails huge amount of statistics for enclose of all well-known mark species (Leisner *et al*., 1994). To flourish breeding techniques of crop plants, absolute indulgent of the genetic diversity is one of the pre-requirements. For the present and future genetic improvement evaluation of genetic diversity, systematic study could be great important (Mayuri *et al.,* 2019). SDS-PAGE was used for the analysis of molecular weights of proteins. For the comparison of morphological and biochemical traits molecular markers are of great importance. They are more comprehensive in number and are secured to be used in Molecular biology. (Barbosa *et al*., 2013) Environmental factors found no effect on these markers. For the assessment of genetic diversity, the application of markers with diverse applications in plant in breeding, cultivar identification, germplasm and linkage mapping molecular are well reported (Jahufer *et al*., 2003).

Assessment was made on the basis of banding pattern obtained thorough SDS-PAGE protein analysis with common bands between all the species. The bands with low molecular weight and with high molecular weight were present and recorded in each studied species. The degree on with which association occurs depends occurs is dependent upon the molecular weight of the bands. Though inconsistency did not occur in the banding patterns of major bands except variations occur between the minor bands. Investigation has been carried out on the leaf protein of the *Ipomoea* by SDS-PAGE. Electrophoretic examination of proteins exposed a total of 41 protein bands of the 11 species under investigation. Investigation showed that few bands were unique and constant shown by their elctrophorogram. The Fig. 3 of bands changed from one species to other, through the largest number (16) in *Ipomoea carnea*, and the lowest number (4) in *Ipomoea cairica*. To compare, easily the 41 protein bands were combined jointly with molecular masses and the number of bands from each molecular mass were scored for each species. Cluster analysis was done with the help of MINITAB software on the basis of observed variables. Two major clusters were generated clusters one include *Ipomoea carnea* Jacq. and cluster includes cluster 1 *Cuscuta reflexa, Ipomoea caraica, Ipomoea qoumoclit, Ipomoea pes-tigrides, Merremia aegyptica, Convolvulus arvensis* and *Convolvulus prostrates* and cluster 2 *Ipomoea carnea.* The dendrogram represents that major two groups of species were generated in separate way. In one group 10 species are included and group two includes only one species. Further these 10 plant species were divided in four groups and showed a common ancestor. Our SDS-PAGE of seed proteins investigation outcome provides valuable information on associations between strongly related taxa. Though, the associations among the sections and subgenera of the genus *Ipomoea* remain unclear from SDS-PAGE analysis. For the determination of association among species RAPD analysis were performed this was in accordance with the work of Khalik (2008).

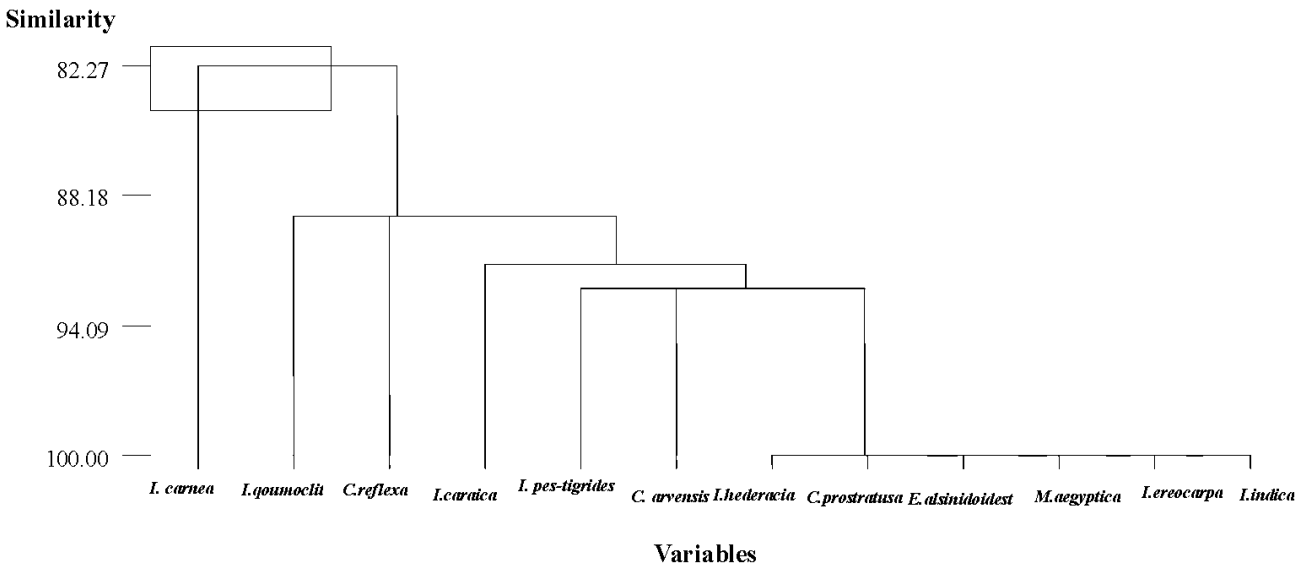


Fig. 4. Cluster analysis of 11 species on the basis of SDS-PAGE analysis.

**Table 4. SDS-PAGE analysisof different species of Convolvulaceae and Cuscutaceae.**

| Species | Protein Band Size (kDa) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **200** | **150** | **120** | **100** | **85** | **70** | **60** | **50** | **40** | **30** | **25** | **20** | **15** | **10** |
| *Ipomoea cairica* (L.) Sweet, | - | - | - | - | - | - | - | - | + | - | - | + | - | - |
| *Ipomoea carnea* Jacq. | + | + | - | - | - | - | - | - | _ | - | - | _ | - | - |
| *Cuscuta reflexa* Roxb. | - | - | - | - | - | - | - | - | + | - | - | + | - | - |
| *Ipomoea hederacia* Jacq. | + | + | - | - | - | - | - | - | + | - | - | + | - | - |
| *Evolvulus alsinoides* (L.) | + | + | - | - | - | - | - | - | + | - | - | + | - | - |
| *I.* pes-*tigrides* L. | + | + | - | - | - | - | - | - | + | - | - | + | - | - |
| *Ipomoea eriocarpa* R.Br. | + | - | - | - | - | - | - | - | + | - | - | + | - | - |
| *Ipomoea arachnosperma* Welw. | + | + | - | - | - | - | + | - | + | - | - | + | - | - |
| *Ipomoea quamoclit* (L.) Spreng | - | + | - | - | - | - | + | | + | - | | + | - | - |
| *Merremia aegyptia* (L.) –Choisy | + | + | - | - | - | - | _ | - | + | - | | + | - | - |
| *Convolvulus arvensis* L. | + | + | - | - | - | - | _ | | + | - | - | + | - | - |
| *Convolvulus prostratus* Forssk | + | + | - | - | - | - | _ | | + | - | - | + | - | - |

**Table 5. Cluster analysis of 11 plant species on the basis of SDS-PAGE analysis.**

| Steps | No of clusters | Similarity level | Distance level | Cluster joined | | New cluster | No of observation in new cluster |
|---|---|---|---|---|---|---|---|
| 1 | 11 | 100.00 | 0.000 | 11 | 12 | 11 | 2 |
| 2 | 10 | 100.00 | 0.000 | 10 | 11 | 10 | 3 |
| 3 | 9 | 100.00 | 0.000 | 6 | 10 | 6 | 4 |
| *4* | 8 | 100.00 | 0.000 | 5 | 6 | 5 | 5 |
| 5 | 7 | 100.00 | 0.000 | 4 | 5 | 4 | 6 |
| 6 | 6 | 92.43 | 0.151 | 8 | 9 | 8 | 2 |
| 7 | 5 | 92.43 | 0.151 | 4 | 8 | 4 | 8 |
| 8 | 4 | 91.29 | 0.174 | 4 | 7 | 4 | 9 |
| 9 | 3 | 89.09 | 0.218 | 3 | 4 | 3 | 10 |
| 10 | 2 | 89.09 | 0.218 | 1 | 3 | 1 | 11 |
| 11 | 1 | 82.27 | 0.355 | 1 | 2 | 1 | 12 |

## Conclusion

On the basis of SDS-PAGE and RAPD analysis it has been founded that *Cuscuta* has close resemblance with *Ipomoea* species. On the basis of our study it is concluded that genus *Cuscuta* should be placed in Convolvulaceae due to most of similarities with other genera.

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