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# Intra-species profiling of *Cleome viscosa* growing in Swat district (Pakistan)

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Intra-specific genetic variation was studied in 28 genotypes of *Cleome viscosa* L. growing in Swat district, Khyber Pakhtunkhwa, Pakistan. It was found that genotypes showed the utmost allelic variation for leaf upper and lower surface with emerald green (75%), and yellow green (75%) respectively, other leaves lower and upper surfaces were (25%) green and yellow green (26%) respectively. The majority of *C. viscosa* genotypes were (50%) yellow flowers while others were with (29%) white yellow colour and (21%) dull yellow. Most of the seeds were with black (46%). The protein profiling was carried out on 12% gel electrophoresis; seven reproducible bands with molecular weight ranges from 180 to 10 KDa were detected in *C. viscosa*, the locus contribution toward genetic disagreement (LCTGD) of *C. viscosa* was 57%. Notably, L-3, L-4 L-5, was monomorphic in *C. viscosa* and was treated as species specific. L-1, L-2, L-7 were polymorphic. These bands showed 79%, 4%, 14% and 79% variation respectively. In the current investigation the intra-specific variation was observed limited and alone SDS-PAGE did not determine the high level of intra-specific variation; however, diverse germplasm were suggested to be acquired from various sources.

*Keyword*: *Cleome viscosa*; genetic diversity; morphology; SDS-PAGE; Pakistan

## Introduction

*Cleome viscosa* L. is a member of family Capparacae or Cappardaceae. It grows wild in nature and known as wild mustard, dog mustard or locally Jhakhiya (Edeoga et al., 2009) or Jangally Sharsham in Pushto. It is an annual herb, found throughout the world and due to various biological activities it is used as a medicinal plant (Mali, 2010). Different phytochemical compounds have been identified such as tannins, saponins, flavonoids, steroids, alkaloids, phenols, terpenoids, carbohydrate, protein and fixed oil (Niraimathi et al., 2012; Rajani et al., 2014). Shoots and leaves of the plant are used as cooked vegetables when these parts are at the young growing stage (Edeoga et al., 2009). There is a wide variety of clinical constituents in the whole plant and they are used for different pharmacological actions. Phytochemical compounds such as tannins, saponins, flavonoids, steroids, alkaloids, phenols, terpenoids, carbohydrate, protein, fixed oil have been reported in the plant (Niraimathi et al., 2012; Rajani et al., 2014). It is a pantropical weed and mostly distributed in woodland, grassland and wasteland. Both seasonal and humid conditions are suitable for the plant, which grows on sandy soil and also on rocky, calcareous soil and reach up to 1m in height.

Morphological description has a substantial role in the investigation of genetic polymorphism in crop plants but they are severely affected by ecological changes, which thereby complicates the analysis of inherited variation (Nisar et al., 2016). For the estimation of genetic diversity, different molecular methods used such as biochemical assessment at protein level and DNA based techniques have advantages over the classical morphological methods (Ndiaye et al., 2012) but biochemical assessment at protein level is very expensive as compared with the molecular investigation of DNA markers (Win et al., 2011). Amongst biochemical techniques, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a safe, simple, reliable and cheap method (Wadood et al., 2016). The SDS-PAGE method is now extensively used as a biochemical technique to describe the genetic relationship of crop germplasm (Hameed et al., 2009). Enormous attention has been focused on the use of SDS-PAGE over the last two decades for estimation of diversity, reliable judgment and identification of plant varieties. To resolve taxonomic relationships and characterize cultivated varieties in a number of crop plant species, seed storage protein markers have been successfully used; in Lima bean (Lioi et al., 1999; Nisar et al., 2007), in *Lens culinaris* (Wadood et al., 2016), *V. unguiculata* (Win et al., 2011). In the gene expression process proteins are the end product; SDS-PAGE can be used to identify varieties, determine polygenetic relationship in different species, biosystematics analysis and evaluate the passport data (Sammour, 1991).

Previously, a lot of work was carried out in genetic diversity of various plant species. However, a genetic diversity attempt has been carried out to understand the degree of genetic variation in *C. viscosa* growing in the hilly areas of Swat. Therefore, the present study is designed to find out intra species variation and phylogenetic relationship among *C. viscosa*. Today, for this kind of study more reliable techniques such as restriction fragment length polymorphism of both nuclear and organellar DNA, are used (Vijay Kumar et al., 2010). The benefit of using electrophoretic pattern of seed storage is that this method is inexpensive and easy to apply in developing countries where these species is widely cultivated and germplasm is collected and stored (Singh & Ntare, 1985).

The present study is based on morphological and SDS-PAGE analyses of 28 genotypes of *C. viscosa*, which has important local adaptation and widespread use by people for medicinal purposes. The objective of the study is to estimate the intra-specific variation in *C. viscosa* based on morphological and SDS-PAGE characterization.

## Material and methods

**Plant materials.** In the present study, several exploratory trips were arranged to different agro-ecological zones of Swat, Khyber Pakhtunkhwa, Pakistan. A total of 28 genotypes were collected per-zone for morp-

hological characterization and estimation of genetic diversity in seed storage protein profile.

**Morphological characterization.** For morphological data analysis both the qualitative and quantitative characters were taken. With the help of vernier calipers the quantitative characters were measured: petiole length, leaf length, leaf width, seed length, seed thickness, and seed weight, pod length, No. of seeds per pod, No. of pods per plant, flower length, 100 seed weight, No. of branches per plant, plant height and biomass. For each quantitative character 3 different samples (small, medium, large) were measured for mean value. The observed qualitative characters are leaf type (LTY), leaf lower surface colour (LLC), leaf upper colour (LUSC), seed texture (ST), Hilum colour (HC), seed coat colour (SCC), seed shape (SS), leaf pubescent (LP), flower colour (FC).

**Protein profiling.** For the estimation of genetic diversity, sodium dodecyl sulfate poly acryl amid gel electrophoresis SDS-PAGE was performed. Five seeds form each genotype was taken for total seed storage protein profile and ground into fine powder. In fine seed powder (0.01 g) protein extraction buffer (PEB) about 400 µl were added; with composition of 0.5 M Tris-HCL, 0.2% SDS, 5 M urea, 1% B-mercaptoethanol under 8 pH. The E-tube was vertexed to homogenize the PEB and seed fine powder (PEB-FP). To observe the movement of PEB-FP in the separation PAG, the comassive brilliant blue (CBB) was added to the E-tubes as a tracking dye. The E-tubes were centrifuged at room temperature at 13,000 rpm for 10 minutes. Polyacrylamide gel 12% was carried out for electrophoretic process (composition of resolution gel (3.0 M Tris-HCl, pH 9.0, 0.4% SDS) and staking gel (0.4 M Tris-HCl, pH 7.0, 0.4% SDS)). The electrode buffer containing 0.025 M Tris, 129 M glycine and 0.125% SDS was poured in the electrophoresis tank. Similarly, 9 µl PEB-FP was loaded in each well of 12% PAG. At 100 V the electrophoresis was run until the blue line passed to the bottom of gel plates. For the scoring the PAG were stained and de-stained.

**Data analysis.** Descriptive statistical and cluster plotting SPSS and PC-ORD software's were used to analyze the morphological data. For the complementation of cluster analysis information, this multivariate approach was selected, because cluster analysis is more sensitive to closely related individuals.

## Results

**Morphological characterization.** By using the Pearson correlation coefficient, the result for the association coefficient among the various traits for the *C. viscosa* was obtained (Table 2). The petiole length in correlation study of *C. viscosa* was positively correlated with leaf length whereas this was significantly positively correlated with leaf width. Seed length was negatively correlated with flower length and was significantly positively correlated with pod length. No. seed/pod, No. of pod/plant was negatively correlated with plant height. The yield/plant (Y/P) was significantly positively correlated with biomass (BM). Qualitative traits observed on 28 genotypes of *C. viscosa* genotypes were shown in Table 3.

The double data matrix of 28 genotypes based on morphology was analyzed for the construction of phylogenetic relationship. It represents the similarity of various genotypes and the 28 genotypes of the *C. viscosa* were studied for similarities and the phylogenetic tree was constructed (Fig. 1). The phylogenetic tree divided all the 28 genotypes of *C. viscosa* into four regions (R-1 – R-4). The Region I consisted of 7.1% genotypes (CV1 and CV23). The Region I (R-I) 68 shows 75.0% genetic similarity. The Region II comprised 21.4% CV2, CV7, CV3, CV4, CV5, and CV). Region II was 91.8% similar to genotypes of Region III. The Region R-III has 14.3% genotypes and was 43.8% similar to the genotypes Region IV (R-IV), (CV8, CV9, CV10, CV11, CV12, CV13, CV14, CV15, CV16, CV17, CV18, CV19, CV20, CV21, CV22 and CV24). Whereas R-IV consisted of 14.3% genotypes and has 43.8% similarity morphologically with the genotypes Region III (R-III) (Fig. 1).

**Table 1**
Morphological descriptors used in the characterization of the 28 genotypes of *C. viscosa*

| Morphological descriptors | Abreviatios |
|---|---|
| Petiole | PL |
| Leaf length | LL |
| Leaf width | LW |
| Seed length | SL |
| Pod length | PodL |
| Seeds per pod | S/P |
| Pods per plant | P/P |
| Flower length | FL |
| Flower width | FW |
| Seed weight | SWT |
| Branches per plant | B/P |
| Plant height | PH |
| Biomass | BM |
| Yield per plant | Y/P |
| Leaf shape | LS |
| Leaf colour | LC |
| Flower colour | FC |
| Seed texture | St |
| Hilum colour | HC |
| Seed coat colour | SCc |
| Seed shape | SS |
| Locus | L |
| Cleome | C |
| C. viscosa | CV |
| Genetic disagreement | GD |

The majority of the genotypes have leaf upper and lower surface with emerald green (75.0%), and yellow green (75.0%) respectively; other leaves' lower and upper surfaces were (25.0%) green and yellow green (26.0%) respectively. The majority of *C. viscosa* genotypes were with (50.0%) yellow colour flowers while others were with (28.6%) white yellow colour and (21.4%) dull yellow. Most of the seeds were with black (46.4%). The landraces present seeds with others colours, reddish brown (14.3%), brown (39.3%) and mostly with ovate (78.6%) while other with oblong (17.9%). Mostly, the seeds have coloured hilum. The colour of the hilum can range from yellow (39.3%), white yellow (17.9%), and white (42.9%). Regarding the testa texture, the seeds can be classified in two groups: rough (67.9%) or smooth (32.1%) (Table 3).

**Table 2**
Correlation coefficient among thirteeen quantitative traits of *C. viscosa*

| Descriptors | PL | LL | LW | FL | SL | PodL | S/P | P/P | SWt | B/P | PH | BM | Y/P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PL | 1.00 | | | | | | | | | | | | |
| LL | 0.22 | 1.00 | | | | | | | | | | | |
| LW | 0.44* | 0.12 | 1.00 | | | | | | | | | | |
| FL | –0.11 | –0.42* | –0.16 | 1.00 | | | | | | | | | |
| SL | 0.07 | 0.02 | 0.19 | 0.02 | 1.00 | | | | | | | | |
| PodL | 0.00 | 0.65** | 0.04 | –0.12 | 0.10 | 1.00 | | | | | | | |
| S/P | 0.10 | –0.11 | –0.42* | 0.05 | –0.12 | –0.16 | 1.00 | | | | | | |
| P/P | –0.34 | –0.61** | –0.03 | 0.41* | 0.04 | –0.50** | –0.21 | 1.00 | | | | | |
| SWt | 0.08 | –0.25 | –0.12 | 0.16 | –0.07 | –0.42* | –0.30 | 0.23 | 1.00 | | | | |
| B/P | 0.22 | 0.24 | –0.15 | –0.35 | –0.18 | 0.03 | –0.07 | –0.50** | 0.12 | 1.00 | | | |
| PH | 0.06 | –0.09 | 0.19 | 0.28 | 0.15 | 0.07 | 0.09 | 0.08 | –0.20 | –0.30 | 1.00 | | |
| BM | –0.36 | –0.49** | 0.16 | 0.39* | –0.07 | –0.29 | –0.55** | 0.79** | 0.23 | –0.27 | –0.02 | 1.00 | |
| Y/P | 0.05 | 0.47* | 0.02 | –0.17 | 0.10 | 0.26 | –0.39* | –0.10 | 0.01 | 0.42* | –0.09 | 0.02 | 1.00 |

*Note*: * – correlation is significant at the 0.05 level (two-tailed); ** – correlation is significant at the 0.01 level (two-tailed).

**Table 3**
Qualitative traits observed on 28 genotypes of *C. viscosa*

| Genotype | Leaf color upper surface colour | Leaf lower surface colour | Flower colour | Seed colour | Seed shape | Hilum colour | Testa texture |
|---|---|---|---|---|---|---|---|
| CV1 | emerald green | brown green | white yellow | black | ovate | yellow | rough |
| CV2 | emerald green | brown green | yellow | black | ovate | white yellow | rough |
| CV3 | emerald green | brown green | yellow | black | ovate | white | rough |
| CV4 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV5 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV6 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV7 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV8 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV9 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV10 | emerald green | brown green | white yellow | black | ovate | white | rough |
| CV11 | emerald green | brown green | yellow | black | ovate | white | smooth |
| CV12 | emerald green | brown green | yellow | black | ovate | white | smooth |
| CV13 | emerald green | brown green | yellow | black | ovate | white | smooth |
| CV14 | emerald green | brown green | yellow | reddish brown | ovate | white | smooth |
| CV15 | emerald green | brown green | yellow | reddish brown | ovate | yellow | smooth |
| CV16 | emerald green | brown green | yellow | reddish brown | ovate | yellow | smooth |
| CV17 | emerald green | brown green | yellow | reddish brown | ovate | yellow | smooth |
| CV18 | emerald green | brown green | yellow | brown | ovate | yellow | smooth |
| CV19 | emerald green | brown green | yellow | brown | ovate | yellow | smooth |
| CV20 | emerald green | brown green | yellow | brown | ovate | yellow | rough |
| CV21 | emerald green | brown green | yellow | brown | ovate | yellow | rough |
| CV22 | green | yellow green | yellow | brown | ovate | yellow | rough |
| CV23 | green | yellow green | dull yellow | brown | ovate | yellow | rough |
| CV24 | green | yellow green | dull yellow | brown | oblong | yellow | rough |
| CV25 | green | yellow green | dull yellow | brown | oblong | yellow white | rough |
| CV26 | green | yellow green | dull yellow | brown | oblong | yellow white | rough |
| CV27 | green | yellow green | dull yellow | brown | oblong | yellow white | rough |
| CV28 | green | yellow green | dull yellow | brown | oblong | yellow white | rough |

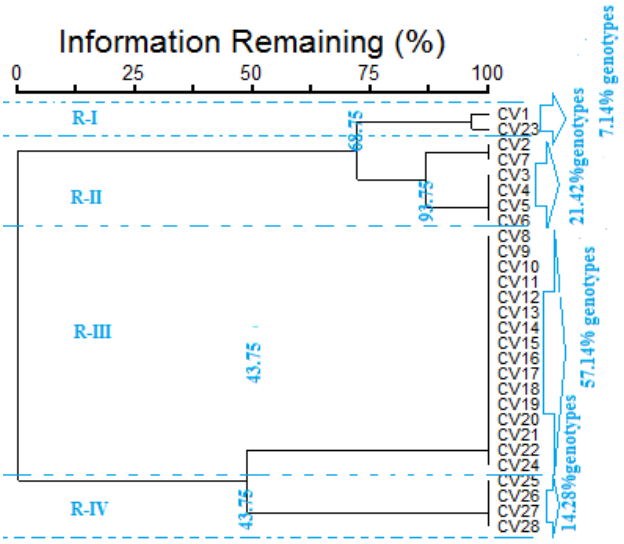


**Fig. 1.** Intra-species variation identified through morphological analysis in 28 different genotypes of *C. viscosa* collected from Swat, Khyber Pakhtunkhwa, Pakistan: CV indicates genotypes of *C. viscosa*

The double data matrix of 28 genotypes based on SDS-PAGE was investigated for the construction of a phylogenetic tree (Fig. 2). It represents the similarity of various genotypes and the 28 genotypes of the *C. viscosa* were studied for similarities and the phylogenetic tree was constructed (Fig. 1). The phylogenetic tree divided all the 28 genotypes of *C. viscosa* into four regions (R-1 – R-4). Region I was consisted of 7.1% genotypes (CV1 (Khwazakhela) and CV23 (Mangultan)). Region I (R-I) showed 68.8% genetic similarity with R-II. Region II comprised 21.4% genotypes CV2 Derai, CV7 Kanju, CV3 Kandak, CV4 Cheno Baba, CV5 Nasrat, and CV) Dughalo.

Region II was 91.8% similar to genotypes of Region III. Region R-III has 14.3% genotypes and was 43.8% similar to the genotypes Region IV (R-IV); (CV8, CV9, CV10 Tooth Banrai, CV11 Kabal, CV12 Bezobanar, CV13 Jawaro, CV14 Jangir, CV15 NawagaiSar, CV16 Hazara, CV17 GulJaba, CV18 Kalakalay, CV19 Sirsinsi, CV20 Mahak, CV21 AkhunKalay, CV22 Bandai and CV24 Totanobandai).

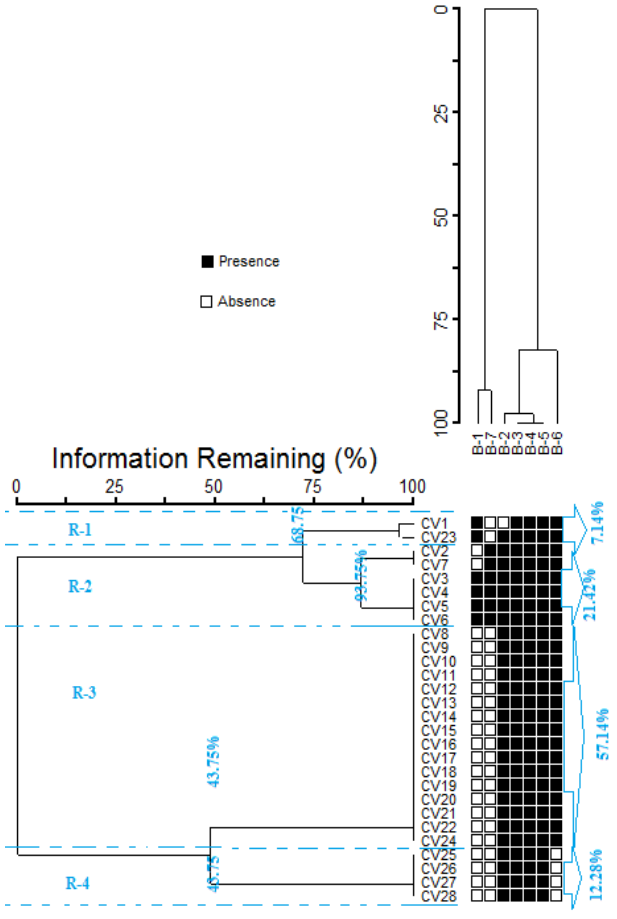


**Fig. 2.** Intra-species variation identified through SDS-PAGE analysis in 28 different genotypes of *C. viscosa* collected from Swat, Khyber Pakhtunkhwa, Pakistan: CV indicates genotypes of *C. viscosa*

Whereas R-IV consisted of 14.3% genotypes (CV25 Shah Derai, CV26 Langr and CV28 Ziarat) and has 43.8% similarity genetically with the genotypes of Region III (R-III).

The overall intraspecific locus variation among 28 genotypes of *C. viscosa* is represented in Table 7 and notably, L-3, L-4 L-5, was monomorphic in *C. viscosa* and was treated as species specific. L-1, L-2, L-7 were polymorphic. These bands showed 78.6%, 3.6%, 14.3% and 78.6% respectively. The locus contribution toward genetic disagreement (LCTGD) of *C. viscosa* was 57.2% (Table 4).

**Table 4**
Intra-locus variation among *C. viscosa*

| Locus | Present (%) | Absent (%) | Variation (%) | Status | GD |
|---|---|---|---|---|---|
| L-1 | 6 (21.4) | 22 (78.6) | 78.6 | poly | 0.2142 |
| L-2 | 27 (96.4) | 1 (3.6) | 3.6 | poly | 0.9642 |
| L-3* (specie specific locus) | 28 (100.0) | 0.0 | Nil | mono | 1.0000 |
| L-4* (specie specific locus) | 28 (100.0) | 0.0 | Nil | mono | 1.0000 |
| L-5* (specie specific locus) | 28 (100.0) | 0.0 | Nil | mono | 1.0000 |
| L-6 | 24 (85.7) | 4 (14.3) | 14.3 | poly | 0.8571 |
| L-7 | 6 (21.4) | 22 (78.6) | 78.6 | poly | 0.2142 |

*Note*: locus contribution toward genetic disagreement GD = 57.1 (poly loci/total loci*100).

## Discussion

Exploration of genetic variability among the genotypes is of the utmost significance to crop improvement studies (Win et al., 2011; Simon et al., 2007). SDS-PAGE of seed total storage protein is a standard procedure of studying the genetic variability and relationship of diverse taxa (Nisar et al., 2007). Genetic disagreement in different plant species have been carried out by using electrophoretic patterns of total seed proteins as revealed by SDS-PAGE of seed storage protein (Ladizinsky & Hymowitz, 1979; Potokina et al., 2000). In cultivated plant species many studies have been carried out based on SDS-PAGE. However, this is the first attempt to find out intra-specific relationship among the *C. viscosa* genotypes using SDS-PAGE.

In this study, 28 genotypes of Pakistani *C. viscosa* genotypes disclosed a considerable level of intra-genotypic genetic diversity tested through morphological and biochemical characterization. The greatest allelic variation among the *C. viscosa* genotypes was for leaf upper and lower surface with emerald green (75.0%), and yellow green (75.0%) respectively; other leaves' lower and upper surfaces were with (25.0%) green and yellow green (26.0%) respectively. The majority of *C. viscosa* genotypes were with (50.0%) yellow flowers while others were with (28.6%) white yellow and (21.4%) dull yellow flowers. Most of the seeds were with black (46.4%). The landraces are represented by seeds with others colours, reddish brown (14.3%), brown (39.3%) and mostly with ovate (78.6%) while other with oblong (17.9%). Mostly the seeds have coloured hilum. The colour of the hilum can range from yellow (39.3%), white yellow (17.9%), and white (42.9%). Regarding the testa texture, the seeds can be classified in two groups: rough (67.9%) or smooth (32.1%). The phylogenetic tree based on SDS-PAGE divided all the species into three regions. R-1 and R-II has 68.8% similarity whereas the genotypes of R-II and R-III are 91.8% genetically similar and the genotypes R-III and R-IV 43.8% similarity.

Due to high intra-species locus contribution toward genetic disagreement, SDS-PAGE could be a consistent procedure for characterization of this species and intra-species locus contribution toward genetic disagreement in genotypes of *C. viscose* was 57.1%. Particularly, L-3, L-4 L-5, were monomorphic in *C. viscosa* and were treated as species specific.

## Conclusion

Seed storage protein electrophoresis is a powerful procedure for evaluation of phylogenetic relationship and genetic diversity and this technology is particularly believed to be a consistent method because seed storage proteins are largely autonomous of environmental fluxes. A better amount of variability as regards the soluble seed protein content could be evidently observed in the selected genotypes of *C. viscosa*. In the present study, efforts have been made to determine the genetic diversity and phylogenetic relationship among the selected genotypes of *C. viscosa*, which may prove important in improving the economically important legume crops by manipulating their wild relatives. The genotypes of *C. viscosa* selected for the analysis by SDS-PAGE revealed a considerable genetic variance in the analyses of total germplasm and hence the results achieved by this study could be of broader range. Today there is still a need to assess genetic variability and conserve genetic resources, particularly of wild species and pulses for prospective benefits in plant breeding. There is a general realization that expansion of the genetic base is a real need if genetic vulnerability is to be reduced and further improvement to be made.

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