

## INVESTIGATION OF GENETIC DIVERSITY OF *CONVOLVULUS ARVENSIS* L. APPLYING ISSR MOLECULAR MARKERS

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### Abstract

Genetic diversity assessment enables plant breeders and genetic researchers to conserve unique plant genomes. Conservation of sole genetic characteristics enables plants to endure environmental fluctuations in various habitats. The main objective of this research was to investigate the genetic variation in *Convolvulus arvensis* L. (Bindweed) with phenotypic variant flower colors. Genomic DNA was extracted through the CTAB method from five samples each (white and pink). ISSR (Inter Simple Sequence Repeats) molecular markers (UBC-825 and UBC-835) were applied to assess genetic assortment. The in-formativeness of the ISSR markers (polymorphism information content, allelic frequency, and genetic diversity) were analyzed and the dendrogram was constructed by using power markers 3.25. Five pink and two white flower *Convolvulus arvensis* L. samples produce amplification products in ISSR PCR analysis. UBC-835 produces polymorphic bands for both samples. The size of amplicon for pink flower and white flower samples ranges from 200bp-500bp. The in-formativeness of the ISSR marker was 50% polymorphism in samples, the polymorphism information content (PIC) value for markers was 0.1371, the mean value of allelic frequency was 0.9158, and the mean value of genetic diversity in variant samples were 0.1507 observed. Power marker (3.25) and Mega software were used for the construction of the dendrogram. Two different flower colors of bindweed may be due to influences of biotic and abiotic factors. ISSR (UBC-825 and UBC-835) molecular marker indicated limited genetic diversity within bindweed and helped analyze possible environmental fluctuations.

**Key words:** Genetic diversity, *Convolvulus arvensis* L., Molecular Markers, ISSR, Environmental fluctuations

### INTRODUCTION

In every aspect, variations impact the gene pool (Minter *et al.*, 2022; Lopez *et al.*, 2025). Gene variants, also recognized as alleles, offer a collection of possible acclimatization for a species. Variations due to environmental factors and stress are associated with genetic variations starting from DNA to organism level. (Boyce *et al.*, 2020).

In larger populations, more genetic variation may occur due to interspecies competition and alterations in habitat. Variations bring hurdles in species identification for the conservation of specific traits and taxonomic studies of plants. It helps in the

assessment of genetic variations. It will enable a more precise investigation of variable traits and the genetics underlying them. Gene flow, mutation, and genetic drift in genome can have consequences of positive or negative for the survival of species. Assessing genetic diversity at the molecular level rather than the morphological level for the conservation of plants in their natural habitats is need of the time (Govindaraj *et al.*, 2015).

For the evaluation of the genetic diversity between intraspecific and interspecific species, the molecular analysis of plant genomes is essential

(Becher *et al.*, 2021; Fischer *et al.*, 1930). In plants, the phenotype of a particular trait is changed due to variations (Zamudio *et al.*, 2016).

In numerous zones of breeding science investigations genomic diversity, gene mapping, and the use of DNA markers provide significant tools (Amiteye *et al.*, 2021). PCR (polymerase chain reaction), microsatellites, random amplified polymorphic (RAPD), amplified fragment length polymorphism (AFLP), including inter simple sequence repeats (ISSR) markers in addition to more recently the simple sequence repeats (SSRs) (Costa *et al.*, 2016).

For the designing of species-specific primers for SSR polymorphism, understanding of flanking sequences is needed (Vieira *et al.*, 2016; Alves *et al.*, 2022). These procedures play a crucial role in the application of evolutionary biology phylogenetics, genetic diversity, gene tagging, and genome mapping in various crop species. In many cases, the eukaryotic genome contains microsatellites which are also called simple sequence repeats which are variable numbers of tandem repeats (VNTRs) and short tandem repeats (STRs) of 1-4 DNA bases (Remya *et al.*, 2010). They contain many variable numbers of repetitive units that are further distributed across the genome (Qi *et al.*, 2018).

A DNA segment between two identical microsatellites repeat regions (MSRs) can be amplified by using the PCR based technique of ISSR (Rampersad *et al.*, 2013). Microsatellite repeats help in deriving of primers of two nucleotide groups such as di-, tri-, tetra, or penta nucleotides (Dong *et al.*,

2019). The use of microsatellites helps in the amplification of numerous genomic loci (16–25 bp) in a single PCR cycle. ISSR sequences of genome of several sizes are amplified by this method. Primers are commonly anchored at the location of 3' or 5' end, extending one to four degenerate bases into the adjacent sequences, although they can also be unanchored (Nadeem *et al.*, 2018).

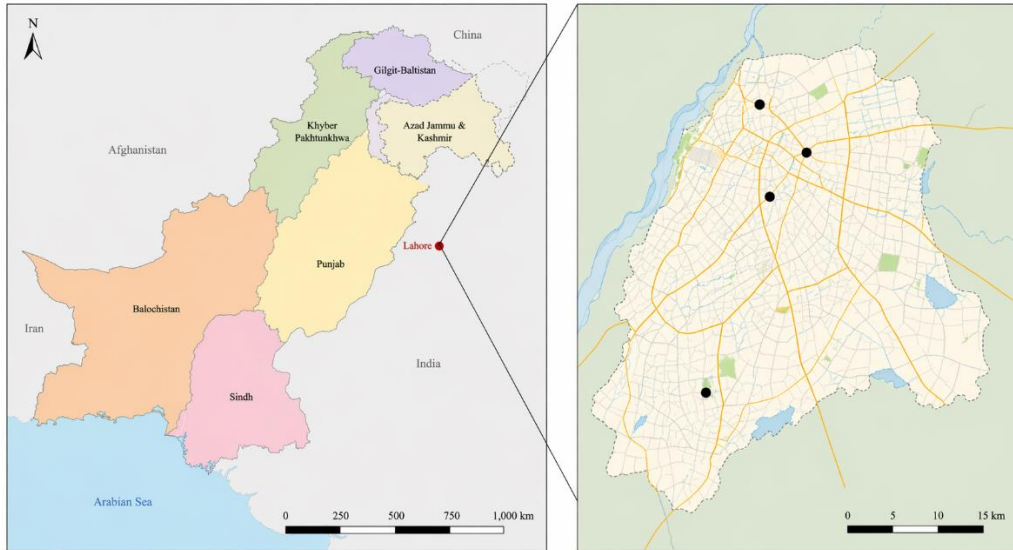
*Convolvulaceae* family is widely distributed in continents of North America and Asia, its species are present all over the world (Klingaman and Oliver *et al.*, 1996). *Convolvulaceae* has 60 genera and almost 2,000 species (Simoes *et al.*, 2022). In Pakistan, both cultivated and native species of *Convolvulaceae* have 13 genera (Ashfaq *et al.*, 2020). *Convolvulus arvensis* L. (field bindweed) is a noxious weed in the world and this has recognized as a weed in almost 54 countries (Frag *et al.*, 2018).

The family *Convolvulaceae* has great medicinal importance, the members of this family are used in the treatment of different diseases such as ulcers, skin problems, pain, inflammation, and edema (Kaur *et al.*, 2012; Khaliq *et al.*, 2022). This research was conducted to assess the genetic diversity between two flower colour variants of *Convolvulus arvensis* L. through comparative analysis. This study provides insight into the genetic variation underlying these distinct variants.

## MATERIALS AND METHODS

### Sample collection

*Convolvulus arvensis* L. (Bindweed) specimens with white and pink flowers were collected from the suburbs of Lahore city in November, 2023.



**Figure 1: Map of study areas**



**Figure 2: Leaves samples collection of white and pink *Convolvulus arvensis* L.**

The leaves were kept in zipper bags and transferred to the Plant Molecular Biology Lab, Department of Botany, Government College University Lahore, Pakistan. Leaves were rinsed by HgCl<sub>2</sub> to sterilize. Afterward, leaves were rinsed with distilled water according to the method describe by the Sharma *et al.* (2005). For further analysis, leaves were stored in -20°C.

**Molecular analysis**

**Genomic dna extraction and gel electrophoresis**

Modified CTAB method was used for the extraction of genomic DNA as described by Yameen *et al.*, (2024) from the leaves of pink and white *Convolvulus arvensis* L. Extracted genomic DNA was

confirmed by 1 % Agarose gel electrophoresis (Lee *et al.*, 2012).

### Primers for ISSR markers

ISSR exist among two SSR repeats that are present in an individual's genome (Bardak and Bölek, 2012). PCR amplification of ISSR markers was done by the universal primers. Two primers (UBC 825 & UBC 835) with specific bases were carefully chosen.

UBC 835 5'-AGAGAGAGAGAGAGAGYC-3'

UBC 825 5'-ACACACACACACACACT-3'

### PCR profile

After the genomic DNA isolation, the amplification of ISSR was done by PCR. The profile of PCR was carried out as described by Saran *et al.*, (2015). 25µL reaction mixture was prepared including, DNA: 3µL, Primers: 1µL, dH<sub>2</sub>O: 8µL, and master mix: 13µL. PCR profile was set according to the following conditions, initial denaturation at 94°C for 5 minutes, final denaturation done at 94 °C for 1 minute, annealing temperature at 52°C for 1 minute, primary extension at 72 °C for 1 minute, and elongation at 72 °C for 5 minutes. A total of 30 cycles were performed.

### Agarose gel electrophoresis and binary data matrix

The PCR product was quantified through agarose gel electrophoresis on 1% agarose gel according to the method described by Lee *et al.*, (2012). After PCR amplification of both samples, binary data matrix was created through gel reading on an Excel sheet (Chambon *et al.*, 2020).

### PIC (polymorphism information content) of ISSR primer UBC 835 in PCR

PIC values were calculated for the ISSR primers-UBC 835 for better understanding between

the relationship of white and pink colored *Convolvulus arvensis* L. samples (Godwin *et al.*, 1997).

### Genetic parameters

Genetic parameters such as major allele frequency, number, and sample size, number of samples used, genetic diversity, and PIC, from the samples of the binary data matrix were further studied via Power Marker 3.25 software besides drawn using Mega software (Tamura *et al.*, 2013).

$$PIC = 1 - [f_2 + (1 - f)^2]$$

Frequency of the data set's marker represented by f. The uppermost PIC value for the dominant markers was 0.5. PIC values were equally distributed throughout the population.

### Dendrogram

Power marker 3.25 and Mega were used for the construction of the Dendrogram (Tamura *et al.*, 2013).

## RESULTS

### Genomic DNA extraction

Genomic DNA was extracted from both polymorphic samples from white and pink flowers of bindweed by using the modified CTAB method. Figure 3 represents the gel image. W<sub>1</sub>-W<sub>3</sub> were the genomic DNA of the leaves of white flower bindweed and P<sub>1</sub>-P<sub>3</sub> were the pink flower bindweed.

### ISSR molecular markers pcr and binary data matrix

Five pink flowers (P<sub>1</sub>-P<sub>5</sub>) and two white flowers (W<sub>1</sub>-W<sub>2</sub>) samples of bindweed were amplified with one ISSR primer (UBC-835) No results were obtained with UBC-825. UBC-835 was analyzed

further for the various comparative factors in bindweed samples.

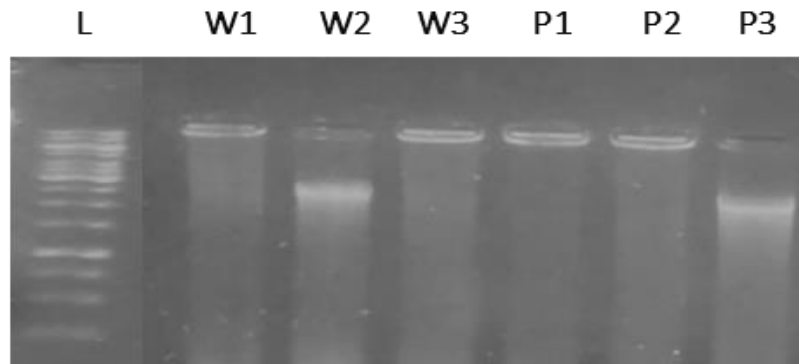


Figure 3: 1% agarose gel showed the genomic DNA bands of leaves of pink and white *Convolvulus arvensis* L.

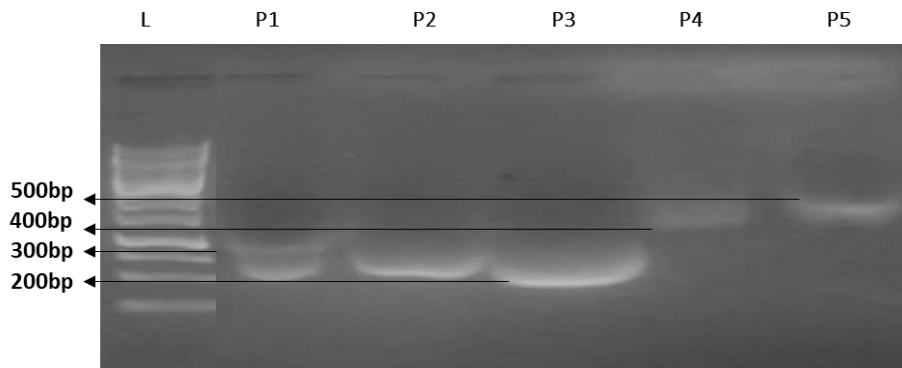


Figure 4: 1% agarose gel showed the ISSR-PCR (with primer UBC-835) amplification of pink flowering *Convolvulus arvensis* L.

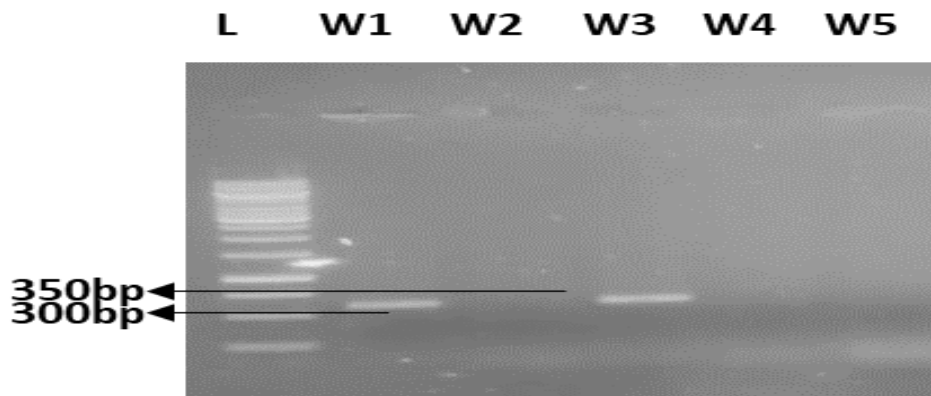


Figure 5: 1% agarose gel showed the ISSR-PCR (with primer UBC-835) amplification of sample of white flowering *Convolvulus arvensis* L.

The samples of pink flowers produce more polymorphic bands than samples of white flowers. On gel electrophoresis PCR products of ISSR showed several bands ranging from 200bp to 2000 bp via gel. Samples of five pink flowers labeled as P1 to P5 (200bp, 300bp, 400bp, and 500bp) respectively, and five samples of white flowers W1-W5 were utilized. There were only two amplified products, each measuring 300bp and 350bp, in samples W1 and W3, respectively. Binary data matrix is made through ISSR

PCR gel images. Samples of pink flowers yield excess polymorphic bands in comparison with samples of white flowers. The incidence of bands (polymorphic) over gel images denoted 1 and 0 indicated no band as in the Excel sheet. Samples of white flowers were designated as ‘W’. Samples of pink flowers were named ‘P’.

**Table 1: Showed the binary data matrix made through the ISSR-PCR gel images**

Sample	M1	M2	M3	M4	M5
P1	0	0	1	1	0
P2	0	0	0	1	0
P3	0	0	0	1	0
P4	0	1	0	0	0
P5	1	0	0	0	0
W1	0	0	0	0	1
W2	0	0	0	0	0
W3	0	0	0	0	1
W4	0	0	0	0	0
W5	0	0	0	0	0

**Table 2: Showed the in formativeness parameters (No. of total bands, No. of polymorphic bands, and % age of Polymorphism and PIC values) of ISSR primer UBC-835 in PCR**

Sr. No.	Primer	No. of Total Bands	No. of Polymorphic Bands	% Polymorphism	PIC Value
1.	UBC 835	8	4	50	0.1371

**PIC of ISSR primer UBC 835 in PCR**

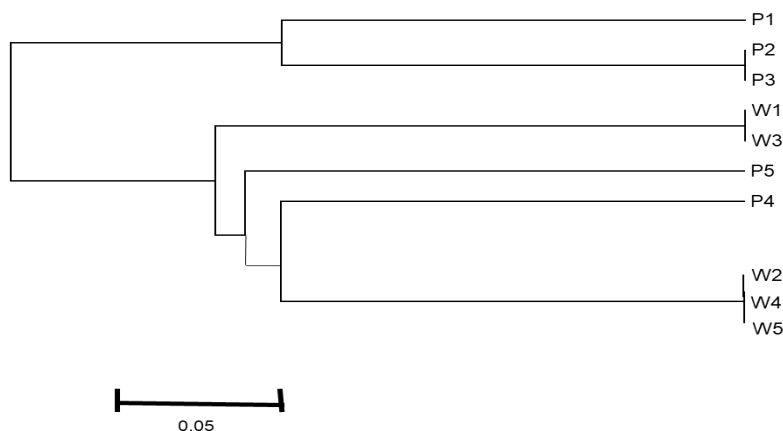
Several metrics, including the percentage of polymorphic bands with polymorphism and the polymorphism information content (PIC), were used to assess the in-formativeness of the ISSR marker UBC-835. Overall, 50% polymorphism was found in both samples, and the ISSR marker's PIC value was 0.1371.

**Genetic parameters**

The higher the PIC value corresponds to a marker that is evenly spread in the population. Major markets had a maximum value of 1, so the highest PIC for them was also 0.5. In the PCR reactions of bindweed genomic DNA samples, only one ISSR primer UBC-835 yielded amplified products (Table 3).

**Table 3. Showed the genetic parameters (Allele Frequency, Sample Size, No. of observation, allele no., availability, gene diversity and PIC)**

Marker	Allele Frequency	Sample Size	No. of Obs.	Allele no.	Availability	Gene Diversity	PIC
M1	0.9474	19.00	19.00	2.00	1.00	0.0997	0.094
M2	0.9474	19.00	19.00	2.00	1.00	0.0997	0.094
M3	0.9474	19.00	19.00	2.00	1.00	0.0997	0.094
M4	0.8421	19.00	19.00	2.00	1.00	0.2659	0.230
M5	0.8947	19.00	19.00	2.00	1.00	0.1884	0.170
<b>Mean</b>	0.9158	19.00	19.00	2.00	1.00	0.1507	0.137



**Figure 6: Showed the phylogenetic association among the selected *Convolvulus arvensis* L. (white & pink) samples.**

### **Dendrogram, UPGMA tree using Euclidean distance**

A dendrogram based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) calculated by the Euclidean distance explained phylogenetic association among bindweed polymorphic samples. Two main clusters within the ten bindweed samples were divided by the dendrogram. The first is related to a genetic similarity between three pink flower samples P1, p2, and p3. Other cluster showed all white flower samples of bindweed and their genetic similarity, as well as the pink flower forms (P4 and P5). Their evolutionary relationships are also depicted in a phylogenetic tree according to UPGMA which agrees with results obtained from dendrogram.

### **DISCUSSION**

Bindweed traits vary in flower colors which is due to its genetic diversity (Surmacz *et al.*, 2023a). ISSR marker has ability to assess the genetic variation that causes alteration in the phenotype traits of flowers of *Convolvulus arvensis* L. (Rasul *et al.*, 2025). Leaf area & size, and seed production in their growing season and alteration in colour of flower were to the variation in the genomic traits (Surmacz, 2023b). FCP (flower color polymorphism) present in *Convolvulus arvensis* L. observed morphologically in Lahore areas. *Convolvulus arvensis* L. has flowers of colour pink & white in different areas due to the presence of genetic diversity within a species (Mehrafarin *et al.*, 2009). In both selected samples of *Convolvulus arvensis* L. genetic diversity was shown in the variations in ISSR sequence in specific trait. It showed the most prominent change in both selected samples of *Convolvulus arvensis* L. In selected samples of pink-flower the range of genetic diversity was from values

of 0.0997 to 0.2659. But on the other hand, the values for the sample white flower was 0.1884. The mean genetic diversity values which were calculated in all samples were about 0.1507.

DNA extraction and PCR were done with the help of the universal ISSR primers. Each selected primer of ISSR has ability to use as reverse and forward primer. The selected primer of ISSR can amplify the sequence in 3' to 5' and 5' to 3' direction in a sequence. In this experiment, UBC-825 and UBC-835 universal primers of ISSR were used to assess genetic diversity in both selected samples. Due to limitations in results only few ISSR primers can amplified the genomic DNA and showed polymorphic bands same results were got by the experiments performed by (Reddy *et al.*, 2002). Only one ISSR primer (UBC-835) can be amplified the DNA of both samples of *Convolvulus arvensis* L. Different polymorphic bands on gel were observed. Amplification of products depends upon the availability of ISSR in a genome of samples. ISSR primer amplifies the nucleotide sequence between two SSRs (simple sequence repeats). Polymorphism information content (PIC) value for ISSR primer (UBC-835) in both polymorphic bindweed samples were calculated. In the pink flower samples, its value was 0.0948 to 0.2306 and in the white flower sample, it was 0.1706. Mean PIC value for ISSR marker in bindweed was 0.1371. This PIC value is considered low for ISSR markers in bindweed samples. The polymorphism information content (PIC) values are higher for genetic markers that are equally distributed throughout the population (Serrote *et al.*, 2020).

ISSR marker of UBC-835 informativeness was analyzed by using different parameters, polymorphism information content (PIC) of

polymorphic samples and % age polymorphism of polymorphic bands (Mignouna *et al.*, 2003). Overall, about 50% polymorphism in both selected samples were present and the PIC value of the ISSR marker was 0.1371 obtained. The dendrogram further explains the phylogenetic relationship between bindweed polymorphic samples that were drawn by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Euclidean Distance. Ten binding-weed samples are divided into two main groups through dendrogram. The first group represented the genetic similarity between three pink flower samples (P1, P2, P3). The second group indicated the genetic similarity between five white flower bindweed samples from W1 to W5. The second group of dendrograms represented the evolutionary relationship of pink flower samples P4 and P5 to other white flower samples. Genetic diversity was also indicated between two polymorphic samples of bindweed in a dendrogram, and it also represented their evolutionary relationship in phylogeny.

## CONCLUSION

*Convolvulus arvensis* L., known as Bindweed is a clambering vine that grows wild in fields. In bindweed, morphological variation is caused by polymorphic alleles for the same characteristic. The genetic diversity of bindweed was examined in the current study utilizing the ISSR (inter simple sequence repeats) molecular marker. The location and repetition of the ISSR sequence in a genome is so unique two individuals are not alike. Here, ISSR polymorphic bands representing variations in sequences in particular loci are produced by marker amplification in PCR reactions of genomic DNA samples. Different parameters like Major allele frequency, PIC value for markers, and genetic diversity in both samples

facilitated to distinction of two varieties of bindweed. The evolutionary association was analyzed by making Dendrogram which indicates phylogeography. Flower color polymorphisms also known as FLP were found in bindweed, which grows in two different habitats and has two different colors white and pink. Genetic variation was the cause of bindweed's range of bloom types. The physical variance in bindweed flower hues was caused by this genetic diversity. In this study, the ISSR markers UBC – 825 and UBC – 835 yielded limited results. Therefore, incorporating a broader range of markers would be necessary to obtain a comprehensive profile of genetic variations.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

## Authors' contributions statement

MNA and AH collected the samples. WA helped in arranging data in tables. AH, AA, RG, AA, and MI contributed in molecular studies and in writing the Research paper. AH, IA, SR analyzed the research paper and identified some mistakes and solved out. This research work was done under the supervision of MUFA. All authors read and approved of the final manuscript.

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## DATA AVAILABILITY

The datasets produced and examined in the present investigation are available in this manuscript.

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