Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2583-2594. **OPEN ACCESS**

Narrow genetic diversity is a major threat to *Abutilon indicum* L. in Swat valley, Pakistan

Noor Muhammad[1&2*], Muhammad Khalil Ullah Khan[2], Niaz Ali[2] and Syed Fazal Wadood[3]

[1]Department of Pomology, College of Horticulture, Agricultural University of Hebei, Baoding 071001, Hebei, **China**
[2]Department of Botany Hazara University, Mansehra, KP, **Pakistan**
[3]Department of Botany, University of Malakand, KP, **Pakistan**

***Correspondence:** noorpk_1990@yahoo.com **Received 16-08-2020, Revised: 08-10-2020, Accepted: 20-10-00-2020 e-Published: 11-11-2020**

The main objective of this investigation was to explore the ehnomedicinal importance and to examine the declining reasons of *A. indicum* in the flora of Swat valley, for this purpose 200 respondent were interviewed and also the intra species genetic diversity in 40 genotypes of *A. indicum* was examined based on the phenotypic characterization and seed storage protein variability. A significant diversity was found on the basis of morphology. Protein evaluation was conducted on 12 percent gel electrophoresis; 9 bands were noticed in *A. indicum* with molecular weight ranging from 10 to 180 KDa. Intra locus contribution toward the genetic disagreement was 33.334% while intra locus similarity index was 66.67% in genotypes of *A. indicum*. Cluster analysis based on SDSD-PAGE separated the genotypes into two regions' demonstrating low level of genetic diversity and high level of genetic association within genotypes. In this present study the genetic diversity among the genotypes of *A. indicum* was narrow and consequently, it is recommended that SDS-PAGE-based diverse germplasms be acquired from the adjoining regions of Swat to establish a broad gene pool with maximum variability.

Keywords: *Abutilon indicum*, Morphology, SDS-PAGE, Genetic Diversity, Genetic association, Cluster analysis

INTRODUCTION

A. indicum is one of the most important plant with possible pharmaceutical properties (Kaladhar et al. 2014). This plant species is herbaceous weed and found plentifully in wastelands (Rajagopal and Koumara 2015). Different parts of the *A. indicum* plant are used for treatment of numerous human disorders (Rajagopal and Koumara, 2015). This is an Asian medicinal plant and it possesses anti-diabetic and anti- bacterial activities (Partap et al. 2014). It is described that the dried aerial organs are applied for lessening the symptoms of bronchial asthma as it raises pulmonary function in patient (Kumar and Gali, 2011). The leaf fixative is also taken orally to relieve body discomfort and for treatment of piles (Prakshanth et al. 2006). The decoction of fruit mixed with ammonium chloride is applied against haemorrhagic septicaemia (Ali, 1999). According to the flora of Pakistan; the plant is with stout stem, and tall about 1-2m thick 0.3-0.9 cm in diameter. The flowers are yellow in color. The fruits are capsule. The seeds are 3-5 mm in size, reniform, tubercled, black or dark brown (Kirtiar and Basu 1994).

Genetic variability forms the basis for plant existence in nature and crop improvement (Upahyaya et al. 2006; Noor et al. 2018). The presence of genetic variability described in the form of wild species etc. may serve as a source of desirable alleles and may help breeders inbreeding climate resilient species (Hammer et

al. 2003; Upahyaya et al. 2006; Noor et al. 2018). Diversity is also crucial for adapting plant species to diverse habitats with particular reference to climate change (Hammer et al. 2003; Upahyaya et al. 2006; Bhandari et al. 2017).

A population cannot evolve without genetic diversity in response to changing environmental factors and, as a result, can face an increased risk of extinction (Noor et al. 2018). Such as, if a population is exposed to a new disease, whether they occur within the population, selection may act on genes for resistance to the disease (Khan et al. 2020). But if they don't happen if there isn't the right genetic variability the population does not develop and the disease might wipe out (Upahyaya et al.2006; Noor et al. 2018). As a threatened species wanes, genetic diversity is lost and even if the species rebound, the level of genetic variation will not (Noor et al. 2018). Genetic diversity can only be restored gradually by the mutations that occur over many generations. Because of this, an endangered/threatened species with low genetic variation could threaten extinction long after recovery in population size (Muhammad et al., 2018). Evolutionary theory assumes that, for a species' long-term survival, we should to protect not only particular members of a species but also the capacity of a species to adapt in the face of changing ecological factors that means maintaining individuals and genetic diversity (Bhandari et al. 2017; Khan et al. 2020).

The evaluation of the genetic diversity in the threatened plant species is very important for the conservation of these genetic resources, expansion of the genetic base and practical uses in various programs like conservation and breeding (Muhammad et al. 2018; Noor et al. 2018). In order to plan a proper breeding program, it is essential to recognize how much the morphological variation of trait is transmissible (Kearsey and Pooni, 1996; Noor et al.2018; Muhammad et al. 2018), since the competence of a selection program is largely reliant on the amount of genetic variation and heritability of a feature (Falconar et al.1996; Noor et al. 2018; Khan et al.2020). Genetic variance/ diversity of a plant species has been examined by applying either agro-morphological characters or molecular markers (Muhammad et al. 2018; Muhammad et al. 2019; Khan et al. 2020). Genetic diversity is of largely applied interest in any crop/plant development program (Noor et al. 2018). Accurate information on the nature and degree of genetic diversity would help the botanists/scientists in selecting the correct type of parents for conservation programs (Noor et al., 2018; Khan et al. 2020). The knowledge of genetic polymorphism is valuable tool in gene bank management and in planning tests, as it eases well-organized sampling and consumption of germplasm by classifying or eradicating duplicates in the gene stock, and helps in the founding of basic collections (Muhammad et al. 2019).

In order to keep, assess and use germplasm, well and successfully, it is essential to examine the degree of genetic diversity it possesses (Smith & Smith, 1989; Muhammad et al. 2019a). Several of the landraces and wild species are available in the world as genetic resources for plant improvement and conservation, but their application for these programs is still incomplete and we are faced as to how to practice this biodiversity for practical plant development and conservation (Tsujimoto et al. 2000; Noor et al. 2018). The genetic polymorphism within the species generates genetic diversity and is an important material for conservation programs (Muhammad et al. 2018, Muhammad et al. 2019; Khan et al. 2020; Nasir, 2020). Increase in intra locus variation by the improvement of genetic diversity in the existing genetic resources has been a great improvement in the agronomic traits (Noor et al. 2018).

Various tools are available for the examination of genetic diversity in plant species. A knowledge of morphological traits ease the documentation, selection of desired characters, designing new populations, in shifting their desirable genes into broadly grown plants through biotechnological means, resistance to biotic and a biotic stresses that are known to discrete genotypes and increase the importance of the genotypes (Santall et al. 2001; Noor et al., 2018). But these morphometric characterizations some time unstable due to environmental fluctuations (Muhammad et al. 2018; Noor et al.2018; Muhammad et al.2019a; Khan et al.2020). Today various sophisticated tools like DNA based markers are at hand for the evaluation and assessment of genetic polymorphism within and among the species but they are very expensive (Muhammad et al., 2018); assessment of genetic diversity through SDS-PAGE is stable, free of environmental fluctuations, inexpensive and can easily handle in the developing countries like Pakistan (Noor et al.2018; Khan et al.2020)

A lot of work was carried out in economic agronomic and commercially important crops plants but a little is known about the genetic

diversity of the threatened, endangered and ethnomedicinally wild plants. Therefore the present study is design to know intra species variation among the genotypes of *A. indicum*. The current investigation is the first ever report from Pakistan.

MATERIALS AND METHODS

Plant Material

A set of 40 genotypes of *Abutilon indicum* was assessed for the estimation of genetic diversity using morphological and SDS-PAGE characterization (Table 1). The collection sites for each genotype is shown in table 1.

The total numbers of respondents were 200. All the respondents were local inhabitants of district Swat. Each interviewee was questioned in the local language (Pashto) for ethnomedicinal uses and also for the decline reasons of *A. indicum* in the Swat valley. Then this data was statistically evaluated by means of the formula "used value (UV = ∑Ui/N)". where Ui is the number of uses cited by each informants for *A. indicum* and N is the total number of informants. UV reveals the relative significance of locally recognized plants (Muhammad et al. 2019; Muhammad et al.2020).

Table 1: Representation *A. indicum* distribution in District Swat

Genotype	Collection Sites	Longitude	Latitude	Altitude (ft)
At1	Ziarat	34˚49'04.80"N	72˚04'02.66"E	4650
At2	Kohay	34˚43'46.11"N	72˚12'39.91"E	3500
At3	Gari	34˚47'19.72"N	72˚23'42.80"E	3668
At4	Serai	34˚49'16.64"N	72˚20'57.33"E	3899
At5	Gulbanda	34˚46'49.62"N	72˚17'44.40"E	2930
At6	Banjo banda	34˚38'53.10"N	72˚04'28.67"E	2500
At7	Gordand	34˚49'53.75"N	72˚17'08.84"E	3972
At8	Speen Khat	34˚53'03.02"N	72˚21'44.55"E	5178
At9	Tagha	34˚50'12.16"N	72˚17'33.73"E	4147
At10	Kandao	34˚52'37.84"N	72˚53'36.80"E	2092
At11	Swegalai	34˚48'05.95"N	72˚12'09.01"E	5523
At12	Totano Bandai	34˚48'01.47"N	72˚11'18.34"E	6068
At13	Dagha Patay	34˚38'55.88"N	72˚15'26.03"E	3362
At14	Penawrai	34˚47'53.52"N	72˚12'47.69"E	4783
At15	Choor	34˚47'43.02"N	72˚13'10.14"E	4305
At16	Tall	34˚47'33.69"N	72˚10'05.52"E	5163
At17	Dardiyal	34˚33'18.43"N	72˚19'11.86"E	2984
At18	Manrai	34˚44'03.74"N	72˚18'12.09"E	2867
At19	Golden	34˚47'26.96"N	72˚09'44.18"E	3104
At20	Tooth Banrai	34˚47'25.47"N	72˚18'52.82"E	2961
At21	Qalagay	34˚47'25.47"N	72˚18'31.83"E	3116
At22	Manja	34˚49'11.90"N	72˚16'28.47"E	3228
At23	Dadahara	34˚50'07.04"N	72˚17'35.02"E	3093
At24	Sara Shah	34˚46'44.06"N	72˚16'11.02"E	3304
At25	Sabar Shah	34˚49'05.08"N	72˚16'13.00"E	2972
At26	Chegai	34˚50'15.80"N	72˚22'18.96"E	3223
At27	Zawra	34˚53'10.79"N	72˚13'16.44"E	3784
At28	Batoor	34˚53'10.79"N	72˚14'19.05"E	3929
At29	Amlook Garai	34˚53'32.16"N	72˚13.29.21"E	4176
At30	Galagy	34˚44'38.25"N	72˚14'23.77"E	2969
At31	Daam	34˚45'03.56"N	72˚14'59.59"E	3149
At32	Manrogay	34˚44'12.86"N	72˚14'31.46"E	2993
At33	Kalakalay	34˚44'50.67"N	72˚15'20.84"E	3034
At34	Sarkhazano	34˚45'59.70"N	72˚15'25.01"E	2986
At35	Khawri Deer	34˚40'48.56"N	72˚07'53.75"E	2497
At36	Amlook Tangay	34˚39'17.25"E	72˚06'35.41"E	2504
At37	Sarsinai	34˚39'09.29"N	72˚06'35.41"E	2465
At38	Dhero	34˚40'51.59"N	72˚06'36.24"E	2468
At39	Kotlai	34˚40'51.30"N	72˚16'15.23"E	2475
At40	Jabagai	34˚34'46.86"N	72˚07'19.70"E	2737

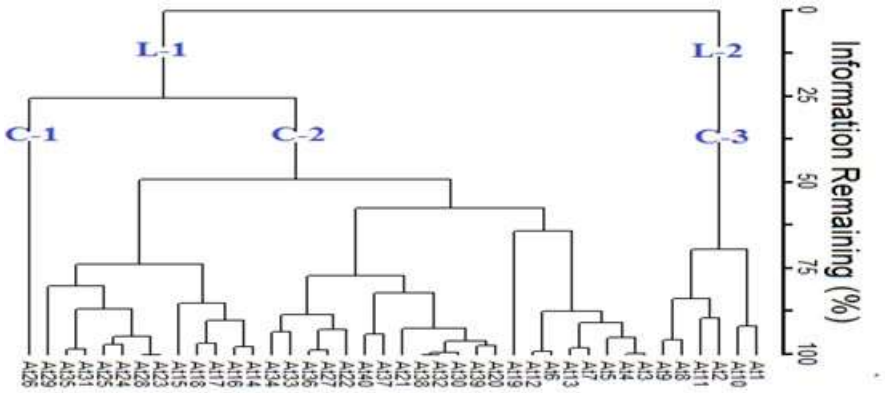

Figure 1: Genetic diversity identified among the genotypes of *A. indicum* through morphological traits analysis. At represents *Abutilon indicum.*

Morphological description

Morphological description; quantitative characters which were measured with the help of vernier calipers are: Petiole length (PL), leaf length (LL), leaf width (LW), seed weight (SWt), Fruit length (FtL), Fruit width (FtW) No. of seed per Fruit (S/F), Flower length (FL), and Flower width (FW). Characters mean was found out after measuring of 3 different samples (small, medium, large) of each quantitative traits. Whereas the qualitative traits were leaf upper surface color (LUC), leaf lower surface color (LLSC), seed texture (ST), Hilum color (HC), seed color (SC), seed shape (SS), flower color (FC). This morphological data was noted on the excel sheet and was subjected to computer software the PC-ord shown in (figure 1).

SDS-PAGE Analysis

For protein extraction, 3 to 5 seeds were crushed to fine flour with mortar and pestle, using the protocol of Laemmli, 1970; modified by Noor et al., 2018.

Data Analysis

The 0, 1 data obtained from gels was noted on the excel sheet and data was analyzed for further interpretation using PC-ord and SPSS computer software (Noor et al. 2018).

RESULTS

Ethnomedicinal Uses

Traditional uses of *A. indicum*

People's responses regarding the medicinal uses of *A. indicum*.

The medicinal uses of *A. indicum* is summed up in table 2. This plant has been used to treat fever, cough, urinary Infection, headache, laxative, aphrodisiac, anthelmintic, ulcer, anti-inflammatory, rheumatism, relief leg pain, toothache, earache, eyewash, mouth wash, gonorrhea, bronchitis, chronic cystitis (Table 2). Around 90.9 percent of people used it to treat Fever, 89.5 percent for Cough, 84.1 percent for urinary Infection, 81.81 percent for headache, 70.4 percent for laxative, 67.7 percent for Aphrodisiac, 64.5 percent for anthelmintic, 62.7 percent for Ulcer, 61.8 percent for Anti-Inflammation, 59.1 percent for rheumatism, 40.9 percent for relief leg pain, 35.4 percent for toothache, 34.5 percent for earache, 32.7 percent for eyewash, 27.7 percent for mouthwash, 26.3 percent for Piles, 25.4 percent for gonorrhea, 24.5 percent for chest problems, 22.7 percent for bronchitis and 21.8 percent used it for chronic cystitis (Table: 3).

Eradication sign of *A. indicum*

In this study, causes and reasons for the serious decline of *A. indicum* in Swat were also studied. The main reason recorded for its decline was soil erosion because it mostly grows on bank of rainy water channels as wild. Other reasons include ease of human access to the plant, over grazing, lack of protection and overuse of its fruits and leaves for medicinal purposes etc. (Table 4). The agreement percentage is 83.5% who believe that its decline is due to its soil erosion, **79**% of people say that reason for its decline is due to its accessibility to humans, **77.5**% of people said that reason for its decline is over grazing, 72.5% of respondents say that reason for its loss is due to the lack of protection while other 65% relate it to the overuse of fruits and leaves as ethnomedine (Table 4).

Morphological Diversity

Qualitative Traits

The results obtained from the evaluation of genetic diversity in seven qualitative phenotypes of *Abutilon indicum* (Leaf upper surface color, leaf lower surface color, seed color, seed shape, flower color, testa texture, and hilum color) were presented in (Table 5). It was found that three colors (green, grass green and moss green) were found for leaf upper surface; green 60.50%, 16.50% grass green and 23% moss green colors were observed for the leaf upper surface color similarly three colors were observed for leaf lower surface color such as 60.5%, yellow green, 16.5% purplish green and 23% dull yellow color. Flower color was observed to be controlled by three alleles among these 20.0% orange yellow, 70.0% yellow and 10.0% dull yellow. Three colors were found for seed; 36% Black, 29% brown black, 35%gray black. Whereas color were found for hilum i.e. 32.5% yellow, 32.5% white, 35% white yellow. Seed texture was of two types i.e. smooth and rough 62.5% and 37.5% respectively in the present genotypes of *A. indicum*.

Quantitative Traits

A quantitative trait is a measurable phenotype that relies on many genes and the environment's cumulative actions. Data were recorded on seventeen quantitative traits in 40 genotypes of *A. indicum* viz. petiole length, leaf length, leaf width, flower length, flower width No. of seed per fruit, 100 seed weight, branches per plant, plant height.

The data showed that there is extensive morphological variation among the genotypes of *A. indicum*. The utmost variable traits were seeds, weight per plant (SWt), biomass (BM), yield/ plant (Y/P), seed length (SL) number of seeds per fruit (S/F) and plant height (PH). The characters with less variation included pod and seed width these were with 13.0701, 1.59889, 10.46966, 13.25396, 14.91319, 9.81436, 63.70123, 43.07381, 8.71154, 39.78599 CV% (Table 6)

Correlation coefficient was performed among the various traits of *A. indicum* (Table. 7); in correlation study the petiole length in the *A. indicum* was positively correlated with leaf length whereas the leaf width was negatively correlated with leaf length. The flower length was significantly positively correlated with flower width and so on as shown in table 7.

The data of 40 genotypes based on morphology was analyzed for the construction of phylogenetic tree. It represents the similarity of various genotypes and the 40 genotypes of the *A. indicum* were examined for similarities and the phylogenetic tree was constructed (Figure 1). The phylogenetic tree divided all the 40 genotypes of *A. indicum* into two lineages L1 and L2 at a linkage distance 12.5. L1 has only one cluster (C1); consists of only one genotype (At26). While L2 was further separated into two clusters (C2 and C3). The C2 was comprised of 33 genotypes (At3, 14, 15, 7, 13, 6, 12, 19, 20, 39, 30, 32, 38, 21, 37,40, 22, 27, 36, 33, 34, 14, 16, 17, 18, 15, 23, 28, 24, 25, 31, 35, 29 and 26) while C3 has 6 genotypes (At1, 10,2,11,8 and 9).

SDS-PAGE profiling

A total of 9 bands with molecular weight ranging from 10 to 180 KDa were observed in 40 genotypes of *A. indicum*; electrophoregram was presented as figure 2. The genetic association within the genotypes was tested via "Jaccard Similarity Index Two Way Cluster Analysis". Based on seed storage protein analysis, the banding profile dissociates 40 genotypes into 2 regions viz. R-I, and R-II. The R-I contains 12 genotypes (At1,3,4,5,6,7,2,29, 32, 35, 36, and 37) whereas the Region II has 28 genotypes (At18,19,20, 21, 22, 23, 24,25,30,31,33,34,38,16,26,27,28,39 and 40) (Figure-3).

.

Table 2 : Parts used for various diseases

S. No	Medicinal Uses		Part Used	Citation
1	Fever		Roots	
	Dry Cough			Giri et al.2009; Rajagopal and Koumar, 2015
	Urinary Infection			
	Chest Infection			
2	Diuretics		Bark	Singh et al.2002.
	Laxative			
	Aphordisiac			
	Anthelmintic			
3	Ulcer		Leaves	Ganeshan et al.2009; Rajagopal and Koumara 2015

	Inflammation			
	Rheumatism			
	Piles			
	Relief leg Pain			
	Tooth ache			
	Ear ache			
	Eye wash			
	Mouth Wash			
4	Piles		Fruits	Samy et al.,2008
	Cough			
	Gonorrhea			
5	Chest Problems		Seeds	Kumar and Gali 2011; Rajagopal and Koumara 2015
	Bronchitis			
	Piles			
	Chronic Cystitis			

Table 3: Traditional uses of *A. indicum* in district Swat

S. No	Disease category	∑Ui	N	Use value (UV)	%UV
1	Fever	200	220	0.909	90.9
2	Cough	197	220	0.895	89.5
3	Urinary Infection	185	220	0.841	84.1
4	Headache	180	220	0.8181	81.81
5	Laxative	155	220	0.704	70.4
6	Aphordisiac	149	220	0.677	67.7
7	Anthelmintic	142	220	0.645	64.5
8	Ulcer	138	220	0.627	62.7
9	Anti-inflammation	136	220	0.618	61.8
10	Rheumatism	130	220	0.591	59.1
11	Relief leg pain	90	220	0.409	40.9
12	Tooth ache	78	220	0.354	35.4
13	Earache	76	220	0.345	34.5
14	Eye Wash	72	220	0.327	32.7
15	Mouth Wash	60	220	0.277	27.7
16	Piles	58	220	0.263	26.3
17	Gonorrhea	56	220	0.254	25.4
18	Chest Problems	54	220	0.245	24.5
19	Bronchitis	50	220	0.227	22.7
20	Chronic Cystitis	48	220	0.218	21.8

Table 4: Different reasons for decline of *Abutilon indicum* in Swat Valley

Reason for Decline	∑Ui	N	Use value (UV)	%UV
Soil erosion	167	200	0.835	83.5
Accessibility of Human	158	200	0.79	79
Over grazing	155	200	0.775	77.5
Lack of Protection	145	200	0.725	72.5
Overuse of Fruits and leaves	130	200	0.65	65

Table 5: Qualitative traits observed on 40 genotypes of *A. indicum*

Traits	Color	%age
	Green	60.50%
Leaf Upper Color Surface	Grass green	16.50%
	Moss Green	23%
	Yellow green	60.5%
Leaf lower Color Surface	Purplish green	16.5%
	Dull yellow	23%
	orange Yellow	20.0%
Flower color	Yellow	70. 0%
	Dull yellow	10.0%
	Black	36%
Seed color	Brown Black	29%
	Grey Black	35%
Seed Shape	Reniform	100%
	Yellow	32.50%
Hilum color	White	32.50%
	white Yellow	35%
Testa Texture	Rough	37.50%
	Smooth	62.50%

Table 6: Mean ranges and coefficient of variance for the descriptors observed on *A. indicum*

Traits	Minimum	Maximum	Mean	Std.Deviation	CV%
Petiole length	3.5	6	4.5308	0.59218	13.0701
Leaf length	12.67	20	15.5658	1.59889	1.59889
Leaf width	7.1	11.57	8.7275	0.91374	10.46966
Flower length	4.5	7.83	6.1817	0.81932	13.25396
Flower width	3.53	5.67	4.4525	0.66401	14.91319
Fruit length	2.6	3.97	3.4583	0.33941	9.81436
Seed/Fruit	3.33	18	5.9083	3.76366	63.70123
Seed weight	9.87	44.3	12.2008	5.25535	43.07381
Branches/Plant	22	32.67	28.3167	2.46682	8.71154
Plant Height	3.4	15.53	4.5792	1.82188	39.78599

Table 7: Correlation coefficient among 10 quantitative traits of *A. indicum*

Traits	Petiole Length	Leaf length	Leaf width	Flower length	Flower width	Fruit length	Seed/ Fruit	Seed Weight	Branches/ Plant	Plant Height
Petiole Length	1.00									
Leaf length	-0.07	1.00								
Leaf width	-0.14	0.516**	1.00							
Flower length	0.16	0.363*	0.491**	1.00						
Flower width	-0.17	0.441**	0.493**	0.503**	1.00					
Fruit length	0.01	-0.01	0.23	-0.06	0.12	1.00				
Seed/ Fruit	-0.373*	0.473**	0.19	0.07	0.21	0.20	1.00			
Seed Weight	0.14	-0.30	-0.12	-0.12	-0.19	0.08	-0.07	1.00		
Branches /Plant	0.07	0.13	-0.06	-0.13	0.27	-0.19	-0.03	-0.26	1.00	
Plant Height	0.19	0.03	0.03	0.23	0.13	0.10	-0.01	-0.10	0.18	1.00
*. Correlation is significant at the 0.05 level (2-tailed).										
**. Correlation is significant at the 0.01 level (2-tailed).										

Table 8: Intra-locus variation among *A. indicum*

Band/Locus	Present (%)	Absent (%)	Variation (%)	status	GD
B/L-1	6(15%)	34(85%)	85%	poly	0.15
B/L-2	12(30%)	28(70%)	70%	poly	0.12
B/L-3	34(85%)	6(15%)	15%	poly	0.85
B/L-4	40(100)	0.00	Nil	mono	1.00
B/L-5	40(100)	0.00	Nil	mono	1.00
B/L-6	40(100)	0.00	Nil	mono	1.00
B/L-7	40(100)	0.00	Nil	mono	1.00
B/L-8	40(100)	1.00	Nil	mono	1.00
B/L-9	40(100)	1.00	Nil	mono	1.00
GD= 33.334 (GD= Poly loci/Total loci)*100					
GS= 66.67 (GS= Mono loci/Total loci)*100					

GD= Genetic Disagreement, GS= Genetic Similarity, B/L= Band/ Locus

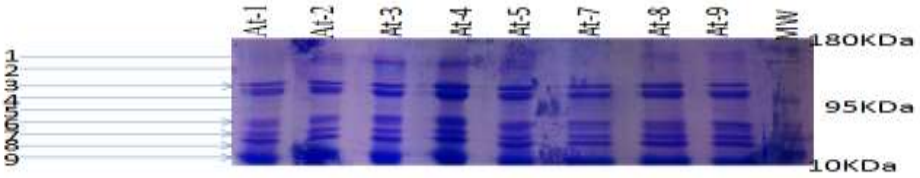

Figure 2: Protein banding pattern displaying diversity in total seeds storage protein of 9 genotypes of *A. indicum*. Arrow indicates the location of protein bands. MW=Molecular weight marker, At= *A. indicum*.

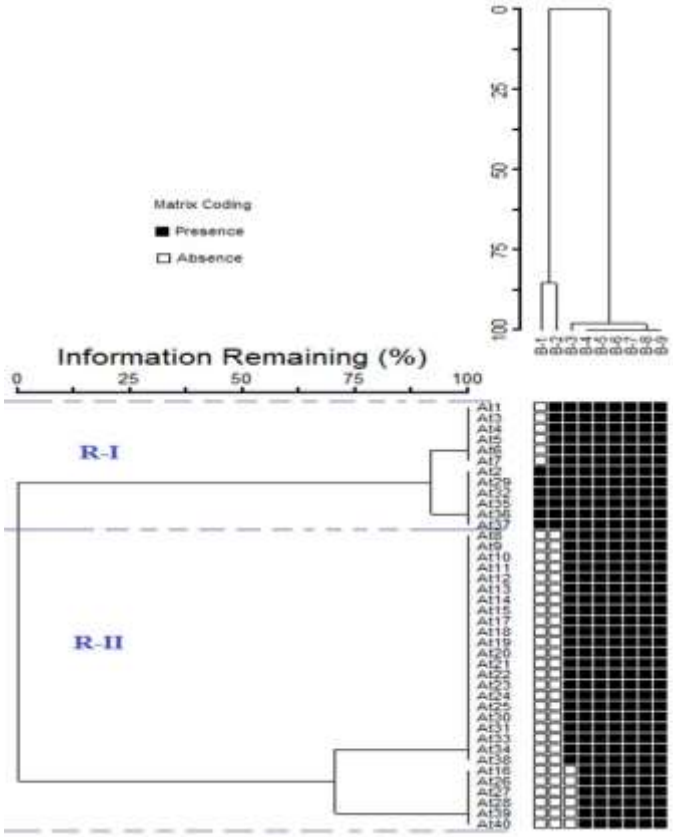

Figure 3: Cluster analysis shows the banding pattern from B-01 to B-09 exhibiting genetic relationship of 40 *A. indicum* genotypes, At= *A. indicum*.

Locus variation

Intraspecific locus variation among 40 genotypes of *A. indicum* is denoted in Table 8 Notably, B/L-4, B/L-5, B/L-6, B/L8 and B/L-9 were monomorphic in *A. indicum*. B/L-1, B/L-2 and B/L-3 were polymorphic and showed 85%, 70%, and 15% percent variation respectively and the locus contribution toward genetic diversity of *A. indicum was* 33.334% and locus contribution toward the genetic similarity was 66.67% (Table: 8)

DISCUSSION

Genetic diversity determines a population's potential fitness, and ultimately its long-term survival, because genes encode phenotypic information (Noor et al., 2018). Genetic diversity in population genetics, plays a significant role in assessing a population's probability of surviving, environmental change, novel pathogens not previously observed, as well as a population's average health over successive generations (Kleinhans and Willows-Munro, 2019). Through population genetics theory, heterozygosity is also strongly related to population size (which is evidently of vital significance to conservation itself). All things being equal, small populations-across their entire genomes would be less heterozygous than similar, albeit larger, populations. This reduced i.e. low genetic diversity makes small populations more susceptible to the above challenges (Khan et al., 2020).

Populations, genetically reduced may be less able to compete with the introduced exotic plants (Bhandari et al., 2017; Muhammad et al., 2018). Generally speaking, genetic degradation may have a cascading impact through the ecosystem (Khan et al., 2020). Under natural circumstances some reduction of genetic diversity is possible like the conditions resulting from natural, genetic selection etc. (Bhandari et al., 2017; Noor et al., 2018; Muhammad et al., 2018; Khan et al., 2010). Such losses, however, are generally not severe, are often offset by mutation and gene flow, and usually do not occur in conjunction throughout the species (Noor et al., 2018). In general, the loss of genetic diversity presents a much more serious threat to previously more common species that have recently lost their habitat and abundance than to species that are naturally limited in their occurrence (Bhandari et al., 2017; Noor et al., 2018; Khan et al., 2010).

Globally, gene banks retain a huge number of significant plant germplasm (about 6 million) of which, the breeders use much less than 1 percent (Upahyaya et al., 2006; Bhandari et al., 2017). This is due to the lopsided strategy of plant breeding targeted only at a few key aspects which contribute to yield at the cost of other aspects (Khan et al., 2020). Several other genotypes accessions have different characteristics and remain unused (Hammer et al., 2003; Noor et al., 2018). This results in a narrow genetic base of plant species leading to genetic instability that could be catastrophic in the sense of changing climate. Increased agricultural mechanization has laid the foundation for monoculture over a wide area of land (Upahyaya et al., 2006; Noor et al., 2018). This has substituted several farmers' field landraces and local varieties which are the genetic reservoirs of several desirable traits (Bhandari et al., 2017; Muhammad et al., 2018). Besides this, in the name of urbanization and development, the degradation of natural environments has limited the potential of generating natural variety in the form of wild types and wild relatives of plants (Khan et al., 2020). With improvement in agricultural production, few lines were extensively used to breed new varieties / hybrids, almost to the detriment of others. It has resulted in plateau yield and vulnerability to various biotic and abiotic stresses of these varieties (Hammer et al., 2003; Upahyaya et al., 2006). Genetic diversity in the form of various landraces and germplasms is the source of valuable genes, such as biotic and abiotic stress (Upahyaya et al., 2006; Noor et al.,2018).

Molecular markers have been applied to examine the degree of genetic divergence (Muhammad et al., 2019). The protein profiling of genotypes and practice of genetic markers have been usually and efficiently applied to find out the taxonomic and evolutionary features of numerous plants (Muhammad et al., 2018)

Amongst biochemical procedures, SDS-PAGE is commonly used due to its strength and ease for examining genetic assembly of plants genotypes (Javid et al., 2004). SDS-PAGE is mainly a persistent technique because seed storage proteins are mainly sovereign of ecological variation (Khan et al., 2020).

Abutilon indicum has been investigated for medicinal compound. Although the present study based on morphometric and SDS-PAGE is a first documented attempt to find out intra- specific genetic polymorphism in *Abutilon indicum* from Pakistan.

In the present examination, 40 genotypes of *A. indicum* naked a significant level of Intra-

genotypic genetic similarity and low genetic diversity, tested through phenotypic profiling and SDS-PAGE characterization. The genotypes showed wonderful intra-allelic variation for leaf upper surface color. Genotypes having green 60.50%, 16.50% grass green and 23% moss green leaf upper surface color while three colors were also found for leaf lower surface such as 60.50% yellow green, 16.50% purplish green and 23% dull yellow color. Flower color was observed to have 20.0% orange yellow, 70.0% yellow and 10.0% dull yellow. Three colors were found for seeds; 36% Black, 29% brown black, 35%gray black. Whereas 3 colors were also found for hilum color i.e. 32.5% yellow, 32.5% white, 35% white yellow. Moreover seed texture was of two types i.e. smooth and rough, 62.5% and 37.5% respectively in the present genotypes of *A. indicum*. Similarly the CV% calculated for petiole length, leaf length, leaf width, flower length, flower width seed length, seed width and seed thickness, pod length, No. of seed per pod, No. of pods per plant, 100 seed weight, branches per plant, plant height was 13.0701, 1.59889, 10.46966, 13.25396, 14.91319, 9.81436, 63.70123, 43.07381, 8.71154, 39.78599 respectively.

The protein profiling of 40 genotypes of *A. indicum* was tested through 12% slab gel electrophoresis. The phylogenetic tree based on SDS-PAGE divided all the species into 2 regions. R-I and R-II enclosed 30% and 70% genotypes respectively.

The examined genetic diversity (33.334%) was very low. Extinction risk has associated with low genetic diversity, and decreased fitness in populations, with low genetic diversity was reported by several investigators (Kleinhans and Willows-Munro, 2019). Low genetic diversity, is mainly associated with low juvenile survival, decreased population growth, low body size and decreased adult lifespan (Noor et al., 2018; Muhammad et al., 2019b). Due to High intra-species genetic similarity SDS-PAGE could be a consistent procedure for characterization of the genotypes of *A. indicum* and Notably, B/L-4, B/L-5, B/L-6, B/L8 and B/L-9 was monomorphic in *A. indicum* genotypes. B/L-1, B/L-2 and B/L-3 shows 85%, 70%, and 15% percent variation respectively and locus contribution toward the genetic similarity was 66.67%.

CONCLUSION

In this study the ehnomedicinal significance of *A. indicum* in the flora of Swat valley, it's declining reasons and genetic diversity was investigated using phenotypic and seed storage protein profiling. Morphologically, significant variation was observed among the genotypes *A. indicum* but genetic diversity examined through SDS-PAGE profiling was very low, it is perceived that morphological characterization is mostly affected by environmental fluctuations. The protein profiling was carried out on 12% gel electrophoresis; 9 bands were observed. Intra species genetic disagreement was 33.334% while Intra species genetic similarity index was 66.67% in the genotypes of *A. indicum*. Cluster analysis based on SDSD-PAGE separated the genotypes into two regions' respectively demonstrating low level of genetic diversity and high level of genetic association within genotypes. The genetic diversity among the genotypes of *A. indicum* was narrow and therefore, diverse germplasms based on SDS-PAGE is suggested to be acquired from the adjoining regions of Swat, to build a broad based gene pool with maximum genetic variability.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Authors are thankful to the residents of the study area for sharing their information's about ethnomedicinal uses and reasons for declination of *A. indicum* species in the Swat valley.

AUTHOR CONTRIBUTIONS

NM did experimental work and wrote the paper, MKUK helped in experiment, NA and SFW helped in analyzing the data**.**

REFERENCES

Ali, Z.A. 1990. Folk veterinary medicine in Moradabad District (Uttar Pradesh), India. Fitoterapia. 70:340-347.

Bhandari, H.R., Bhanu, A.N., Srivastava, K.,

Singh M.N., Shreya., Hemantaranjan, A. (2017).Assessment of genetic diversity in crop plants – an overview. Advances in Plants & Agriculture Research. 7(3):279–286.

Falconer, D.S., Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*, Ed 4. Longmans Green, Harlow, Essex, UK.

Ganesan, S., Pandi, N.R., Banumathy, N. 2009. Ethnomedicinal Survey of Alagarkoil Hills (Reserved forest), Tamil Nadu, India.

Giri R.K., Kanungo, S.K., Patro, V.J., Das, S., Sahoo, D.C. 2009. *Journal of pharmaceutical research*. 2(11): 1725-7

Hammer, K., Arrowsmith, N., Gldis, T. (2003). Agrobiodiversity with emphasis on plant genetic resources. Naturwissenschaften. 90(6):241–250.

Javid, A., Ghafoor, A., Anwar, R. 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pakistan Journal of Botany. 36:87-96.

Kaladhar, D.S.V.G.K., Saranya, S.K., Vadlapudi, V., Yarla N.S. 2014. Evaluation of Anti-inflammatory and Anti-proliferative Activity of Abutilon indicum L. Plant Ethanolic Leaf Extract on Lung Cancer Cell Line A549 for System Network Studies. Cancer Science and Therapy. 6(6): 188-94.

Kearsey, M.J., Pooni, H.S. 1996. The genetical analysis of quantitative traits. 1st edition. Chapman and Hall, London p. 381.

Khan, M.K.U., Muhammad, N., Uddin, N., Ali, N., Umer, M., Ullah, S. 2020. Genetic diversity in threatened plant species *Alnus nitida* (Spach.) Endel. Plant Science Today. 7(3):314-318. https://doi.org/10.14719/pst.2020.7.3.759

Kirtiar, KR, Basu, B.D. 1994. Indian Medicinal Plants, Edn 2, Vol. I, Dehradun, 314-317

Kleinhans, C., Willows-Munro, S. 2019. Low genetic diversity and shallow population structure in the endangered vulture, *Gyps coprotheres*. Scientific reports. **9,** 5536. https://doi.org/10.1038/s41598-019-41755-4

Kumar, G.G., Gali, V. 2011. Phytochemical screening of *Abutilon indicum* and Celosia argentea Linn. International Journal of Pharma and Bio Sciences 2(3):463-467

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. – Nature. 227: 680-685.

Muhammad, N., Uddin, N., Ali, N. 2019. Ethnomedicinal uses and inter specific diversity encourage conservation of Rhynchosia species growing in hilly areas of Swat. Agricultural Resources & Techechnology: Open Access Journal. 20(2): ARTOAJ.MS.ID.556124

Muhammad, N., Uddin, N., Khan, M.K.U., Ali, N. 2019a. Inter Specific Genetic diversity within the ethnomedicinally important Ipomoea L. species through morphological and biochemical profiling. International Journal of Environmental Science Natural Resources. 19(3):556012. https://doi.org/10.19080/IJESNR.2019.19.556012

Muhammad, N., Uddin, N., Khan, M.K.U., Mengjun, L., Xuan, Z,, Ali, N., Liu, Z. 2020. Ethnomedicinal and cultural uses of *Ziziphus* species in flora of Malakand Division KP, Pakistan. Singapore Journal of Scientific Research. 10:1-7. https://scialert.net/abstract/?doi=sjsres.2020.1.7

Muhammad, N., Uddin, N., Liu, M., Ali, N. 2019b. Ethno medicine, antioxidant potential and inter-specific variations of *Punica* L. species growing in Swat District, KP, Pakistan. International Journal of Botany Studies. 4(4):36-46.

Muhammad, N., Wadood, S.F., Khan, W, Ali, N., Nisar, M. 2018 Intra-species profiling of *Cleome viscosa* growing in Swat District (Pakistan). Biosystems Diversity. 26(1):52-55. http://doi.org/10.15421/011808

Nasir, O. 2020. Exploring the Physiological and Molecular Mechanism of the Gum arabic (Acacia senegal) Based on Gene Expression Microarray. Bioscience Research. 17(1): 327-346.

Noor, M., Ali, N., Nisar, M., Abd Allah, E.F., Hashem, A., et al., 2018. Genetic diversity within natural populations of the medicinal plant *Rhynchosia minima* (L.) DC. Applied Ecology and Environmental Research. 16(5):5633-51. http://dx.doi.org/10.15666/aeer/1605_56335651.

Prakshanth, V., Neelam, S., Padh, H., Rajani, M. (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology. 107:182-188

Prathap, M., Alagesan, A., Ranjitha Kumari, B.D. 2014 Anti-bacterial activities of silver nanoparticles synthesized from plant leaf extract of *Abutilon indicum* (L.) Sweet. Journal of Nanostructure in Chemistry. 4,

106. https://doi.org/10.1007/s40097-014-0106-1

Rajagopal R.R., Koumara, V.K. 2015. *Abutilon indicum* L (Malvaceae)-Medicinal Potential Review. Pharmacognosy Journal. 7(6):330-332. DOI: 10.5530/pj.2015.6.2

Samy R.P., Thwin M.M. 2008. Gopalakrishnakone P, Ignacimuthu S. Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India. Journal of Ethnopharmacology. 115(2):302-312. doi:10.1016/j.jep.2007.10.006

Santalla, M., Amurrio, J.M., De Ron, A.M. 2001. Food and feed potential breeding of green dry and vegetable pea germplasm. Canadian Journal Plant Science. 81: 601-610.

Singh, A.K., Raghubanshi, A.S., Singh, J.S. 2002. Medical Ethnobotany of the tribals of Sonaghati of Sonbhadra district, Uttar Pradesh, India. Journal of Ethnopharmacology. 81:31-41.

Smith, J.S., Smith, O.S. 1989. The description and assessment of distance between inbreed lines of maize. The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbreed lines. Maydica. 34: 151-161.

Tsujimoto, M., Koyama, K., Tsuboi, Y., Goto, M., Kobayashi, N. 2002, ApJ, 566, 974

Upahyaya, H.D., Furman, B.J., Dwivedi, S.L., et al., 2006. Development of a composite collection for mining germplasm possessing allelic variation for beneficial traits in chickpea. Plant Genetic Resources. 4(1):13–19.