



Research Article

Volume 21 Issue 2 - April 2019  
DOI: 10.19080/ARTOAJ.2019.21.556158

Agri Res & Tech: Open Access J  
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# Ethno-Pharmacognosy and Diversity Encourage Conservation of Wild *Ziziphus* species Collected from KP, Pakistan



Nisar Uddin<sup>1</sup>, Noor Muhammad<sup>1</sup>, Naziat Begum<sup>1</sup>, Mohammad Nisar<sup>2</sup> and Niaz Ali<sup>1\*</sup>

<sup>1</sup>Department of Botany, Hazara University Mansehra, Pakistan

<sup>2</sup>Department of Botany, University of Malakand, Pakistan

Submission: April 05, 2019; Published: April 22, 2019

\*Corresponding author: Niaz Ali, Department of Botany, Hazara University Mansehra, KPK, Pakistan

## Abstract

The current research works to show that the medicinal practices of two different wild *Ziziphus* species (*Ziziphus oxyphylla* Edgew and *Ziziphus mauritina* Lamk), to assess the consensus factor between local communities of different areas of KP, to measure the potential for novel drugs of herbal origin. There was immeasurable promise among the informers concerning medicinal uses of plants with Informants Consensus Factor (ICF) value ranges from 0.992 to 0.124 with an average value as 0.440. The study naked that most of the informants agreed in the application of *Z. oxyphylla* to use and against Jaundice (ICF 0.992) that unveiled the highest fidelity level (100%) respectively.

The present work has tried to genetic relationships among of *Ziziphus* species growing in Swat Valley, KP, Pakistan. The intra and inter genetic diversity among the 50 genotypes of *Ziziphus* species viz., 25 genotypes of *Z. oxyphylla*, 25 of *Z. mauritina*, were tested using morphometric and biochemical profiling. Twenty-four morphological characters were counted for the assessment of intra and inter genetic diversity through traits similarity index and cluster analysis. Total seed protein profiling was carried out on 12% slab gel electrophoresis; 12 reproducible bands were noticed in *Z. muritiana* and 8 *Z. oxyphylla*. Intra locus contribution toward the genetic disagreement was 41.666% in *Z. oxyphylla* and 25% in *Z. muritiana*. In the same way, inter species locus contribution toward genetic diversity was 66.666%. Fascinatingly, locus L-1, 6, 7, 8 was monomorphic in the collected germplasms and may be presented as generic specific locus for *Ziziphus* species.

**Keywords:** Wild *Ziziphus* species; Ethno botanical uses; Quantitative traits; SDS PAGE; Genetic diversity; Multivariate analysis

**Abbreviations:** ICF: Informants Consensus Factor; PRA: Participatory Rural Appraisal; FL: Fidelity Level; BPB: Bromo-Phenol Blue; LCTGD: Locus Contribution Toward Genetic Disagreement

## Introduction

The genus *Ziziphus* consists of almost 100 species has been used in folk and alternative systems of treatment in order to combat different diseases such as; fever, diabetes, skin infections, anti-pyretic, antinociceptive, antioxidant, antilisterial and larvicidal [1-4]. Due to peculiar geographical region, Pakistan exhibits a great diversity of flora as represented by 7 genera along with 13 species for *Ziziphus* [5].

*Ziziphus oxyphylla* Edgew (synonym; *Ziziphus acuminata* Royle) also written as "*Zizyphus oxyphylla* Edgew" belongs to the genus *Ziziphus* and family Rhamnaceae (known as buckthorn family). The plant *Z. oxyphylla*, popular with common names i.e. Mamyanu, Elanai, Tukbari, Phitni, Amlai and Sezen is a small glabrous tree with short, recurved and unequal spine along with edible fruit (oval in shape), belongs to the genus *Ziziphus*. Based on folklore use, the plant is in-use for traditional treatment of diseases i.e. jaundices, diabetes, hypertension as well as in gas trou

bles [6-9], since long, *Z. oxyphylla* distributed in different areas of Pakistan, but mostly in warm temperate and subtropical regions throughout the world, especially in Pakistan and India. In Pakistan it may be found in different regions, particularly the rainy and mountain areas as well as the Himalayan series of mountains. The plants is distributed as; Swat Valley, Northern Pakistan, Chagharzai valley, District Buner, KP, Pakistan [9], Buner, Hazara, Swat, Garhi Habibullah, Dir Kohistan valleys, Pakistan [6,10], Kot Malakand agency, Islamabad, subtropical hills of Darazinda, Takht-e-Suleman range Dera Ismail Khan, Pakistan, Palas Valley, Pakistan, Kotli, Azad Jammu Kashmir, Pakistan [11-13].

*Ziziphus mauritina* Lamk, is most important an well-known species of *Ziziphus* belonged to buckthorn family Rhamnaceae. *Z. mauritiana* genotypes are very important medicine, ethno medicine and traditionally for the control of different diseases cough, sore throat, anti-oxidant, high fevers, jaundice, diabetes and hypertension etc, [14-16s]. well distributed in different region of

tropical and sub-tropical areas of the world and mostly found in all continent of the world and native of Afghanistan, Australia, North Africa, North India, Malaysia, and southern China and Pakistan. *Z. mauritiana* is a dominates species/variety of wild vegetation in different arid and as well as desert while in great examples of drought and hard species of *Ziziphus* [17,18]. In Pakistan, it is widely distributed in three provinces i.e. KPK, (Banuu, Karak and Kohat) while district swat, Buner and Dir is found in only wild from, Punjab (Attock, Chakwal and Mianwali districts) and Sindh province (Karachi, Hyderabad and Nawabshah districts). In Pakistan, jujube is cultivated on an area about 5.425ha with an annual production of 28.000 tones [19].

There is lack of information regarding jujube cultivars in Pakistan. Indigenous cultivars are missing and there is little research work available on their botanical classification [18]. Fluctuation in yield and quality of jujube fruit is greatly affected by the soil properties, climatic factors and cultivar selection [20]. Fruit quality attributes largely depend upon cultivar to be selected. Previously, variations in fruit weight, juice content [21] and seed weight has been recorded in jujube cultivars [22].

Morpho-biochemical methods are used to screened best genotypes in large collected germplasm of different crop species [23-30]. Among these methods the SDS-PAGE method is effectively used to determinate the taxonomic and evolutionary difficulties of certain plant species [28]. The seed storage protein studies help in documentation and description of variability in crop varieties, cultivars and their wild species but also rich genetic variability and phylogeny association of the accessions. It is considered that variability in protein bands intricate the association among the assortment from various geographical regions [31]. SDS PAGE as a powerful tool that has been used in the solution of problems in the field of taxonomy and explains the origin and evolution of cultivated plants, including the fenugreek [32].

It provides maximum variability among different crop species and the level of polymorphism depends upon the plant species [33]. Haliem and Huqail [34] found 168 different polypeptides bands among diverse Fenugreek genotypes of Saudi Arab and Yemen through SDS-PAGE method. They recorded 26 different polymorphic bands characterized Brassica rapa sub-species brown sarson through this method and recorded 83.33% polymorphic protein bands. They also noted four different cluster groups for the twenty studied genotypes. Jan et al. [28], evaluated three different ecotypes of *B. rapa* through SDS-PAGE method and high level of variability were noted in protein bands size. Protein electrophoresis is considered a reliable, practical and reproducible method because seed storage proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations [34,35].

The main objectives of the current research studies on *Ziziphus* species (1) ethno medicinal uses of *Ziziphus* species in local peoples (2) to explore different aspects the genetic relationship of wild *Ziziphus* species (*Z. oxyphylla* and *Z. mauritiana*) genotypes

based on different morphometric collected from different regions of district Swat KP, Pakistan (2) to study genetic diversity based on store seed protein (SDS-PAGE) of wild *Ziziphus* species of their genotypes (3) by the using of multivariate analysis, phylogenetic, PCA, for the checking of their relationship of wild *Ziziphus* species collected from different regions of distract Swat KP, Pakistan.

## Material and Methods

### Samples collection and Ethno-pharmacognosy

Exploratory trips were arranged to different areas of KP, Pakistan years, 2017-2018 and total two *Ziziphus* species (50 genotypes) were identified and investigated for morphological characterization and SDS PAGE protein profiling. The plants (*Ziziphus* species) *Z. oxyphylla* and *Z. mauritiana* specimen were store in the herbarium Department of Botany, Hazara University, Mansehra, KP, Pakistan; the specimens were recognized referring different Floras, viz., Hooker (1872-1897). Ethno medicinal data has been collected through Participatory Rural Appraisal (PRA), which is based on communication with indigenous people and direct communication in the field Martin [36]. The data have been noted through semi-structured interviews with people involved in the plants, organization [37]. A total of 130 residents have been interviewed during the field survey, information on uses of plants to cure various diseases of human being, parts used, of medicine have been collected. Based on the information obtained from the informants in the study area, all the reported diseases have been classified into 12 groups.

The different level of the similarity among information delivered by various informants was calculated by the Informants' Consensus Factor, ICF Trotter and Logan [38] by applying the following formula:

$$ICF = \frac{Nur - Nt}{(Nur - 1)}$$

Where, Nur = number of use reports from informants for a specific plant-use category; Nt = number of taxa or species that are used for that plant use category for all informants.

ICF Values range between 0 and 1, where '1' indicates the highest level of informant agreement. The fidelity level (FL), the percentage of informants claiming the use of a certain plant species for the same major purpose, was calculated for the most frequently reported diseases or ailments as:

$$FL(\%) = \left( \frac{Np}{N} \right) \times 100$$

Where, Np = number of informants that claim a use of a plant species to treat a particular disease; N = number of informants that use the plants as a medicine to treat any given disease [37].

### Morphological analysis

In the current work qualitative and quantitative characterizations were carried out of the collected samples, Qualitative traits were recorded on the general visualization (phenotypic observations). Ten qualitative traits i.e. Tree vigor, Leaf type, Leaf shape, Leaf colure, Tomentose, stem color, Spines, fruit color, leaf

margin, fruit shape and quantitative characters which were measured with the help of vernier caliper for the measurement of plant height(feet), Branching, Leaf length(mm), Leaf width(mm), Leaf thickness(mm), Petiole length(mm), Inter node length(cm), Stem diameter(inches), fruit weight, fruit diameter (mm) and fruit length (mm).

**Protein extraction and their preparation**

Total seed protein (SDS-PAGE), a single mature and uncontaminated seed of *Z. oxyphylla* and *Z. mauritiana* was selected for the analyses of total protein form each genotype collected from different regions of Swat KP, Pakistan. The seed was ground through the pestle and mortar and for the extraction of total protein and were add 400µl protein extraction buffer composition as (0.5M Tris-HCL pH 8.0, 0.2% SDS, 5M Urea, 1% B-mercaptoethanol) to 0.1g of seed powder into 1.5mL eppendr of tube and then vortex for three (3), mints thoroughly to homogenize the powder and solution.

Bromo-Phenol Blue (BPB) solution was added to the protein extraction buffer as tracking dye to monitor the movement of protein in the gel, then samples were centrifuge for 40 mints at 14,000rpm at 10 °C and follow the standard electrophoretic method [4,39]. After centrifugation samples the curd protein were recovered as a clear supernatant on the top of the tube. Then were transferred into a new tube and were store at 120 °C until electrophoresis. After that for the checking of make 12% polyacrylamide gel the separation gel were making form the mixing of (3.0M Tris-HCl pH9.0, 0.4% SDS) and 4.5% stacking gel (0.4M Tris-HCl pH8.0, 0.4% SDS), Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125% SDS) was add into the top of the gel plate and select total volume as 8µl of the protein extraction mixture were loaded into each well of the gel with the help of micropipette and add molecular markers and finally run 100V until the blue color were passed through the bottom of the gel plates, the gel were stained by the staining solution containing 0.2% BPB dissolved in 10% glacial acetic acid,

40% methanol and water in the ratio of 10:40:50. Gels were de-stained in a solution consisting 5% acetic acid and 20% methanol for 15 minutes [40].

**Data analysis**

The current data was recorded from the design gel (destined) on the basis of absences and presences of total seed protein gel bands, 1 is denoted for the presence and 0, for the absence of the loci were arranged in Microsoft excel 2010, and this 0, 1 data were analyzed for cluster analysis and PCA (Principles Component Analysis) was performed by PCord 5.0, SSPS and Statistics.

**Results**

**Ethno-pharmacognosy**

The current research work on two wild *Ziziphus* species *Z. oxyphylla* and *Z. mauritiana* collected from different regions of KP, Pakistan, we have been in listed for control of diseases categories as12 and for each *Ziziphus* species , scientific name, family , local name, illnesses to be treated, and parts used for the different diseases were noted in (Table 1). Consumption of plant parts as medicine among the informants shows disparities. Fruits are mostly used part for majority, followed by roots, leaves and bark, this was the first time of the study area threat to the species is marginal as seeds are the leading plant part used for medicinal purposes. It was supposed that the collection of part of plant as medicinal part from the wild were not manageable. According to residents, this type of activity is done by the collectors related to illegal activity of medicinal plants. *Ziziphus* species mostly *Z. oxyphylla* is vulnerable to this type of activity in the study region. ICF values were established to know the settlement among the informants of Swat valley for usage of plants to cure certain illness groups. The ICF values ranges from 0.992 to 0.124 with an average value of 0.440. Jaundice has the highest ICF value 0.992 with 130 use-reports for 2 plant species.

**Table 1:** Showed chemical constituents, botanical name, Common name, parts used and their habit of wild *Ziziphus* species collected from KP, Pakistan.

S.NO	Scientific Name	Family	Common Name	Chemical Constituent/Compounds	Habit	Use Parts
1	<i>Ziziphus oxyphylla</i> Edgew	Rhamnaceae	Enalai	Alkaloid, O desmethylnummularine-R N-oxide, Oxyphylline-E, Ramosine-A, Hemsine-A N-oxide, Kaempferol-3-O-galactoside (Trifolin), Flavonoids Kaempferol-3-O-rhamnosyl (1-6)-(4"-trans-p-coumaroyl)-, galactoside,,Quercetin-3-O-glucoside (Isoquercetrin), Oxyphylline-A 14-membered cyclopeptide, Kushecarpin-A	Shrub tree	Leaves, Root, Fruit, Pericarp
2	<i>Ziziphus mauritiana</i> Lamk,	Rhamnaceae	Wild Ber	Betulinic aldehyde, betulinic acid, ceanothic acid, frangulofoline, spinosin, daucosterol, sucrose, docosanoic acid, stearic acid and Palmitic acid	Tree	Fruit, Leaves, Tuber

The specie liable for this high consensus was *Z. oxyphylla* with 130 of the defined events, linked by Blood purification values for ICF 0.8140, 107 use reports and 2 species respectively, for the use of Digestive infection ICF values 0.682, 90 and for 2 species etc (Table 2). Medicinal plants thought to be effective in treating specific illness have high ICF values. The high ICF value for Urinary

infection possibly unveiled that this ailment is common in the study area High ICF values also designate that the specie predictably used to treat these illnesses are worth searching for bioactive compounds. The least agreement (ICF=0.124) between the informers was detected for plants used to cure Lever protection. The low ICF value as noted in our study could be due to a lack of com-

munication among people in various areas. To discover conventionally significant medicinal species in the society, Fidelity Level (FL) of plants has been predicted based on use reports which have been cited by 50 or more informants for being used against a given disorder and the examination demonstrated that the highest FL value found in *Z. oxyphylla* followed by *Z. mauritina* respectively. The least FL value was found in the case of *Z. oxyphylla*. FIC and

FL studies presented that the most commonly used species in the study area are *Z. oxyphylla* (ICF = 0.992) with 130 use-reports and FL value (100%). When choosing the most ideal plant species for each ailment category, we took the high-fidelity Level (%) in each category of ailment due their high biological compounds which were used for the control of various numbers of diseases (Table 2).

**Table 2:** Categories of diseases and \*ICF (Informants consensus factor) for each genotype for two wild *Ziziphus* species and different diseases groups based on highest fidelity level (\*FL %) in each category TF% (Total informants=130).

Counts	Diseases category	Reports	Taxa Used	*ICF
1	Jaundice	130	2	0.992
2	Blood Purification	107	2	0.814
3	Digestive Infection	90	2	0.682
4	Anti-diabetic	90	2	0.682
5	Hypertension	77	1	0.589
6	Gas Troubles	50	2	0.372
7	urinary Diseases	40	2	0.295
8	Fungal Disease	30	2	0.217
9	Obesity	28	2	0.202
10	Constipation	24	2	0.171
11	Wounds of Healing	20	2	0.14
12	Lever Protection	17	1	0.124
Fidelity Level (FL %)				
	Botanical name	Diseases category	Reported	Fidelity Level (FL %)
1	<i>Ziziphus oxyphylla</i> Edgew	Jaundice	130	100
2	<i>Ziziphus mauritina</i> Lamk,	Blood Purification	107	82.307

**Morphological characterization**

In the current work qualitative and quantitative characterizations were carried out of the collected samples, Qualitative traits were noted on the general visualization (phenotypic observations). qualitative traits i.e. Leaf pubescent, Leaf shape, Leaf col-

or; fruit color; Seed shape and quantitative characters which were measured with the help of vernier caliper for the measurement of plant height (feet), Branching, Leaf length (mm), Leaf width (mm), Leaf thickness (mm), Petiole length (mm), Inter node length (cm), Stem diameter (inches), fruit weight, fruit diameter (mm) and fruit length (mm).

**Table 3:** Intra and interspecific genetic diversity in 17 morphological characters studied in *Z. muritiana*.

	PH	BR	LL	LW	LT	PL	InL	StD	FtW	FtD	FtL
PH	1										
BR	-0.26	1									
LL	0.12	0.23	1								
LW	0.14	0.12	0.860**	1							
LT	-.464*	0.02	0.16	0.26	1						
PL	0.28	-0.17	-0.39	-.496*	-.586**	1					
InL	.574**	-0.11	-.418*	-.511**	-.571**	.512**	1				
StD	.681**	-0.34	-0.27	-0.24	-0.24	.533**	.526**	1			
FtW	-0.29	0.14	-0.17	-0.32	0.01	0.15	0.05	-0.02	1		
FtD	0.01	-0.14	0.2	0.1	-0.13	0	-0.08	0.05	.492*	1	
FtL	0.11	0.04	0.25	0.11	0.04	-0.2	0.15	0.15	.515**	.774**	1
*. Correlation is significant at the 0.05 level (2-tailed).											
**. Correlation is significant at the 0.01 level (2-tailed).											

By using the Pearson correlation coefficient, the result for the association coefficient among the various traits for the two species

of *Ziziphus* (*Z. oxyphylla* and *Z. muritiana*) was performed (Table 3 and 4). In correlation study the petiole length in the *Z. oxyphylla*, is



negatively correlated with leaf length in the while positively correlated with leaf length in *Z. muritiana*. Leaf width is negatively correlated with the leaf length in *Z. oxyphylla* while positively cor-

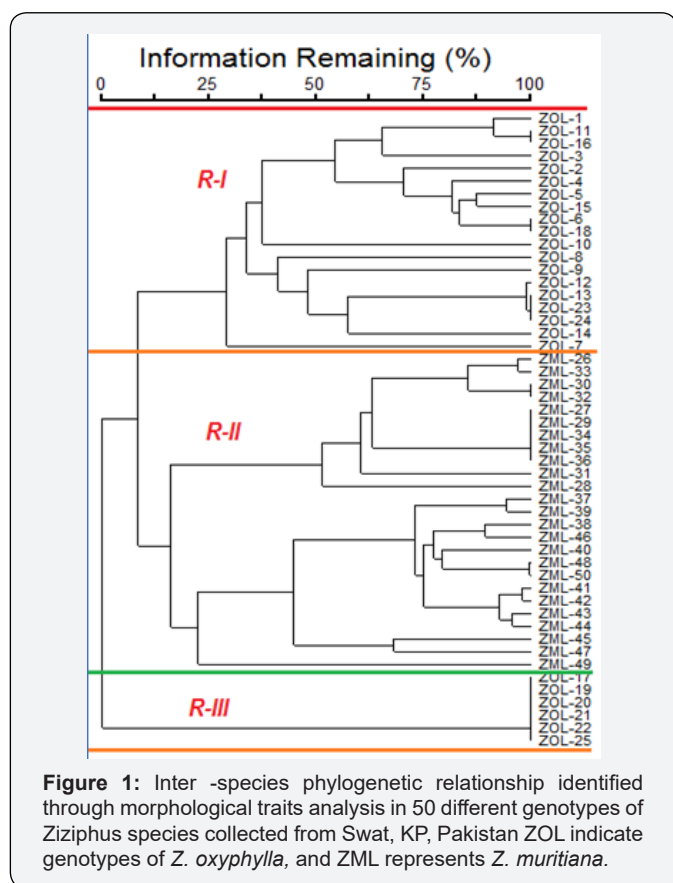
related in the *Z. muritiana*. The flower length in *Z. muritiana* and *Z. oxyphylla* is negatively correlated with the leaf length and leaf width and so on.

**Table 4:** Intra and interspecific genetic diversity in 17 morphological characters studied in *Z. oxyphylla*.

	PH	BR	LL	LW	LT	PL	InL	StD	FtW	FtD	FtL
PH	1										
BR	0.05	1									
LL	-0.01	.446*	1								
LW	.527**	0.33	.396*	1							
LT	0.05	0.14	0.03	0.21	1						
PL	.444*	-0.1	0.25	.489*	.401*	1					
InL	0.35	-0.15	-0.23	0.04	-0.1	-0.06	1				
StD	.761**	-0.17	-0.09	.499*	0.13	.487*	.439*	1			
FtW	0.09	-0.24	-.658**	-0.17	0.29	-0.01	-0.06	0.01	1		
FtD	-0.07	.534**	-0.01	0.17	0.21	-0.11	0.09	-0.23	0.28	1	
FtL	-0.11	.476*	-0.08	0.08	0.12	-0.21	0.06	-0.31	0.36	.963**	1

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\*. Correlation is significant at the 0.05 level (2-tailed).



**Figure 1:** Inter -species phylogenetic relationship identified through morphological traits analysis in 50 different genotypes of *Ziziphus* species collected from Swat, KP, Pakistan ZOL indicate genotypes of *Z. oxyphylla*, and ZML represents *Z. muritiana*.

The double data matrix of 50 genotypes based on morphology was analyzed for the construction of phylogenetic tree to represents the similarity of various species or genera and the two species of the *Ziziphus* were investigated for similarities and the phylogenetic tree was constructed. The phylogenetic tree divided the two species in three groups R1. RII and RII (Figure 1). R1 and RII consisted of total genotypes of *Z. oxyphylla*. While the RII was

into composed of all genotypes of *Z. muritiana*. The similarity indexes were performed for all the genotype of 2 species that was 23.529% for *Z. muritiana* and *Z. oxyphylla* (Table 5).

**Table 5:** Intra and interspecific genetic diversity in 17 morphological characters studied in *Z. oxyphylla* and *Z. muritiana*.

Traits	<i>Z. oxyphylla</i>	<i>Z. muritiana</i>	Trait Similarity Index Z.O & Z. M
PH	9.366	12.08	NA
BR	8.5	9	NA
LL	7.5	4.555	NA
LW	*5.0866	*5.952	*5.519
LT	5.8	4.065	NA
PL	*3.8889	*2.453	*3.171
InL	8.333	4.8	NA
StD	*3.9	*3.866	*3.883
FtW	24.5	16.4	NA
FtD	5.8966	3.666	NA
FtL	4.5666	3.06666	NA
LS	Ovate	Oblique	NA
LC	*Green	*Green	*Green
LP	Absent	Present	NA
FC	Purple White	White Blue	NA
St	Rough/Smooth	Granular	NA
SS	Flattened Shaped	Plano-convex	NA

Total TSI = 23.529% ((homologous trait/total traits) \*100).

\*- Traits similarity within two species.

**SDS- PAGE analysis**

Total 12 bands were observed in the both of species, the phylogenetic relationship among the 2 species through phylogenetic tree has been shown in the (Figure 2). The phylogenetic tree

was divided into two regions. R-I comprised of only genotype of *Z. oxyphylla* collected from (KP, Pakistan), while Region II consists of genotypes of *Z. muritiana* collected from (KP, Pakistan) respectively.

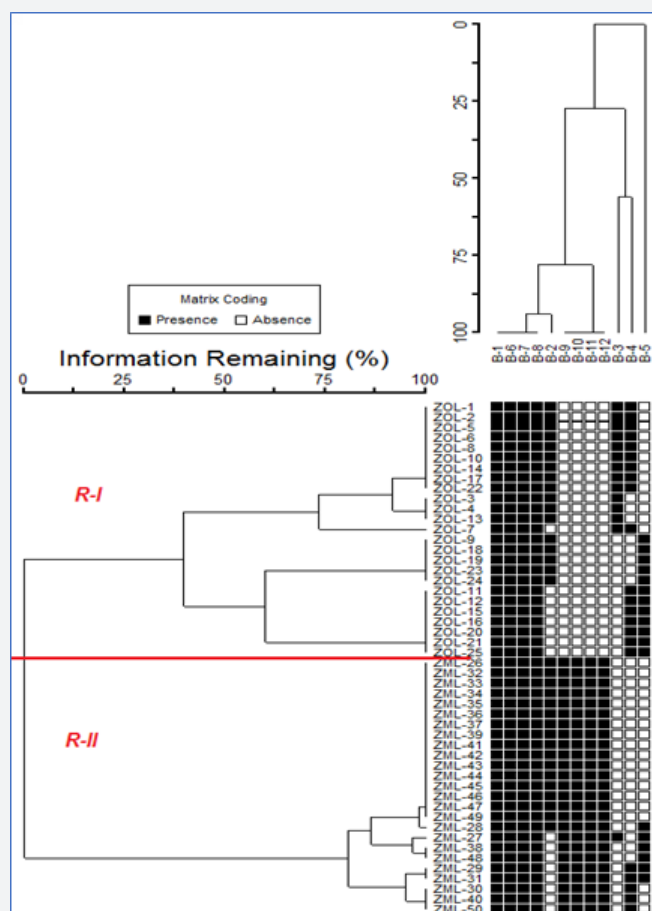


Figure 2: Intra and interspecific genetic diversity in 50 genotypes studied through SDS-PAGE in *Z. oxyphylla* and *Z. muritiana*.

### Locus variation

Table 6 show interspecific variation among 50 genotypes of the *Ziziphus* (*Z. oxyphylla* and *Z. muritiana*) species. Among all the genotypes, 12 loci (L1-L12) were noted out of these L1, 6, 7 and 8 was monomorphic and were marked as generic specific which is used to discriminate the *Ziziphus* species. Moreover, the loci L-2, L-4, L-5, L-9, L-10, L-11 and L-12 marked as polymorphic with 34, 70, 68, 70, 50, 50, 50 and 50 percent genetic diversity, respectively. The inter species comparative locus contribution toward genetic disagreement (CLCTGD) was 66.66% in the two species of 50 *Ziziphus* genotypes (Table 6 and Figure 2). Intraspecific locus variation among 25 genotypes of *Z. oxyphylla* is represented in Table 7, Notably, L-9, 10, 11, 12 were absent in *Z. oxyphylla*. L-1, L-6, L-8 was monomorphic in *Z. oxyphylla*. While L-2, 3, 4 and L-5 was polymorphic and the locus contribution toward genetic disagreement (LCTGD) of *Z. oxyphylla* was 41.66% (Table 7).

The Table 7 represents the intraspecific variation among the 25 genotypes of *Z. muritiana*, exhibited high intra-specific locus variation. Among 12 loci, out of which L-1, 7, 8, 9, 10, 11 and 12 was monomorphic, while L-2, L-4, L-5 and L-6 were polymorphic.

The locus contribution toward genetic disagreement (LCTGD) of *Z. muritiana* was 25% (Table 7).

### Discussion

Many of the local communities in the regions depends on the consumption of medicinal plants to use for the control/use against different number of diseases, However, the vanishing of these plant species is steadily reported chiefly due to fluctuations in the environment, land degradation and unsustainable use of these plants; moreover, the expansion of invasive species has donated a lot to their disappearance [41]. Conservation of medicinal plant genetic diversity has freshly created a lot of attention in the tropics as a result of many years of misconduct, adverse environment as well as socio-economic changes. Population genetic theory expects that the reduction in the genetic diversity limits a species ability to keep pace with the changing selection pressure [42]. Plant species mainly the medicinal plants rely on the existing genetic diversity for constancy and survival under the ever-fluctuating environments [43]. Understanding medicinal plants species population genetic structure is vital for their conservation, planning and justifiable organization [44].

Therefore, a common goal line of conservation is to preserve genetic diversity in “red listed” species, which is crucial for long-term survival and evolutionary response to the altering environment [45]. One main implication of this method, from the viewpoint of conservation genetics, is that it could help us set sampling intervals of areas within populations to optimize the genetic diversity in collections from local populations of rare, endangered, or endemic plant species [46]. For the purpose of conservation of plant species, most of the investigations deal only with determination of genetic diversity in individual populations [40].

The purpose of this study was to recognize the phylogenetic relationship, genetic diversity, genetic structure, and a core collection of *Ziziphus* species. Now, we explain our consequences with respect to genetic diversity and the causes of the genetic idleness. The current position of genetic structure is briefly debated. Moreover, we further clarify the competence of the plan used to build the core collection. Genetic redundancy is a significant issue in plant genetic resource management. The identification of duplicates is important in germplasm repositories, particularly when considering the construction of core collections [40].

Various apparatuses are now presented for documentation of required differences in the genotypes, including morphological / phenotypic, biochemical and molecular markers [40]. Though morphological description is the principal step in the description and alliance of crops genotypes, but these are highly subjective by the environment [40]. The double data matrix of 50 genotypes based on morphology was analyzed for the construction of phylogenetic tree to represents the similarity of various species or genera and the two species of the *Ziziphus* were investigated for similarities and the phylogenetic tree was constructed (Figure 1). The phylogenetic tree divided the two species in three groups R1, RII and RII (Figure 2). R1 and RII consisted of total genotypes of *Z. oxyphylla*. While the RII was into composed of all genotypes of *Z. muritiana*. The similarity indexes for all the genotype of 2 species was 23.529% for *Z. muritiana* and *Z. oxyphylla* [47].

The two plant species under the genus *Ziziphus* study exposed that no two plants have similar protein banding patterns which demonstrates the presence of genetic diversity between these species. The presence of common bands/locus (L-1, 6, 7, 8) among these two *Ziziphus* species suggests their close genetic similarity and common ancestry [40]. Also, [40] accredited the appearance of a common locus/band in all individual in a population to the fact that the gene coding for the enzyme or protein does not differ. Due to High inter-species locus contribution toward genetic disagreement SDS-PAGE could be a reliable technique for identification of these two species, while intra-specie locus contribution toward genetic diversity was high in genotypes of *Ziziphus oxyphylla* (41.66%) as compare to *Z. muritiana* (25%).

## Conclusion

Best of our current study was first time to investigated that the seed protein and ethno medicinal uses of wild *Ziziphus* species

collected from different regions of KP, Pakistan, the genetic pool of dissimilarity within genotypes and *Z. oxyphylla* and *Z. mauritiana* as well, is due to selection as well as for the crops/plants species improvement and their conservation for the better studies of genetic diversity and its distribution in the wild *Ziziphus* species of the studies needed duet to their conservation and will be help greatly to labeling what to conserve as well as where to conserve and will be and will enhance our information and understanding of the taxonomy, origin and evolution of wild *Ziziphus* species (*Z. oxyphylla* and *Z. mauritiana*) respectively

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DOI: [10.19080/ARTOAJ.2019.21.556158](https://doi.org/10.19080/ARTOAJ.2019.21.556158)

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