Pak. J. Bot., 53(5): 1727-1735, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-5(41)

MORPHOLOGICAL ATTRIBUTES AND TOTAL SEED PROTEIN REVEALED DIVERSITY IN *ZIZIPHUS NUMMULARIA* (BURM. F.) WIGHT & ARN. POPULATIONS FROM MALAKAND DIVISION, PAKISTAN

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Abstract

Ziziphus nummularia (Burm. f.) is a multipurpose wild tree species well adapted to the arid regions of Pakistan. The tree is drought tolerant and may ensure food security due to its sustained fruit production from poor and eroded soils of arid regions. The genetic diversity/evolutionary history of the *Z. nummularia* among the genotypes is still not well understood growing in agro-ecological regions of Malakand Division, KP, Pakistan. This study has evaluated intraspecies morphological as well as total seed protein subunits diversity within 120 genotypes of *Z. nummularia*. It was recognized that except leaf type (alternate), leaf shape (ovate), and leaf color (green) that showed monomorphism; the genotypes exhibited polymorphism of varying degrees in all other morphological traits viz., vigor, leaf margin, tomentose (dense), stem color, spine length, fruit color, and fruit shape. Total seed protein profiling was carried out on 12% slab gel electrophoresis, 12 bands (loci) with molecular weight ranges from 10KDa to 180KDa were detected in *Z. nummularia*. The majority loci represented high polymorphism. The locus contribution towards genetic disagreement (LCTGD) and locus linkage of *Z. nummularia* was 91.66% and 8.333% respectively. Notably, the B-1 locus was monomorphic in *Z. nummularia* and was treated as species-specific whereas, B-2-B-12 was polymorphic. The average genetic disagreement for B1-B-12 was 0.15, 0.24, 0.19, 0.18, 0.15, 0.16, 0.16, 0.13, 0.14, 0.14, 0.14 and 0.31, respectively. Both the UPGMA and PCA analyses revealed the coherence of *Z. nummularia* populations with the agro-ecological regions. To the best of our knowledge, this is the first-ever report documenting intra-specific variation and genetic architecture of the endemic *Z. nummularia* genotypes growing in Districts Dir Lower, Buner, and Swat. The study reveals that the existence of enough diversity within *Z. nummularia* genotypes growing in the region. The collected samples/materials can also be included in the crossing scheme to generate offspring that capture greater genetic diversity for selection again in the future.

Key words: *Z. nummularia*, SDS-PAGE, Genetic diversity, Cluster analysis, PCA.

Introduction

Ziziphus (Mill.) species is an economically important genus of the family Rhamnaceae, consisting of about 100 species found in the tropical and sub-tropical regions of the world (Singh *et al*., 2002; Zhao *et al*., 2014). Six species are found in Pakistan i.e., *Z. jujuba, Z. oxyphylla, Z. mauritiana, Z. spina-christi, Z. rugosa* and *Z. nummularia* (Kaleem *et al*., 2014) *Z. nummularia* is commonly known by the different known names such as Malla or Jher beri, wild bera and karkanda, etc. (Kaleem *et al*., 2014). The species has naturalized to warm and dry climates with sandy or silicic soils and sustains drought and high temperatures (Hammer *et al*., 2001; Pareek, 2001; Warris *et al*., 2015). Further, the deeply growing rooting system supplemented by large contents of sugar and carbohydrates storages in the roots augments the best regeneration ability of this species (Pandey *et al*., 2010). Seeds of Z. *nummularia* due to the tough seed coat do not germinate with ease and grow with aging and weathering in soil from 10 days to 1 month (Pandey *et al*., 2010). All parts of *Z. nummularia* plants are important for their known medicinal value. The fruit is used for food (edible) and used for different ailments/diseases like astringent, stomachic, cures mucous and increase biliousness effects (Oudhia, 2003). Barks having potential nematocidal, anthelminthic, antipyretic, and anti-inflammatory activities (Bachayaa *et al*., 2009). Leaves are boiled and used for treating skin disorders like scabies and rashes etc. (Singh *et al*., 2002).

For genetic diversity different types of DNA-based markers are available and others are rapidly developed, most are expensive and require costly setup (Wang *et al*., 2006). However, DNA markers have the potential to determine genetic diversity at both inter-and intraspecific levels; that may not be observed with other available methods. Seed protein is expressed in the form of DNA makers which can be used as a biomarker for the identification of genetic diversity for different plant species and their germplasm (Muhammad *et al*., 2010). Morpho-biochemical methods are used to screened best genotypes in largely collected germplasm of different crop species (Batos *et al*., 2017; Hasanova *et al*., 2017; Petrovic *et al*., 2017; Saroei *et al*., 2017; Yadegari *et al*., 2017; Jan *et al*., 2017; Arif *et al*., 2015). Among these methods, the SDS-PAGE method is effectively used to determine the taxonomic and evolutionary difficulties of certain plant species (Jan *et al*., 2017). 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 (Ghafoor *et al*., 2003). SDS-PAGE is 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 (Haliem and Al-Huqail, 2013). It provides maximum variability among different crop species and the level of polymorphism depends upon the plant

species (Dhawale *et al*., 2015). Haliem and Huqail (2013) found 168 different polypeptides bands among diverse Fenugreek genotypes of Saudi Arabia and Yemen through the 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*., (2017), evaluated three different ecotypes of *B. rapa* through the SDS-PAGE method, and a high level of variability was noted in protein band 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 (Iqbal *et al*., 2005). Generally, a combination of morphology with measurements of SDS-PAGE diversity of the total seed proteins has been effective, and the genetic diversity of seed storage proteins has been investigated in various plant species of agronomic and commercial importance (Muhammad *et al*., 2018).

Our knowledge of the potential of wild plant species and their objective diversity is still narrow and needs improvement (Muhammad *et al*., 2018; Nisar *et al*., 2019). To date, wild plants have received little attention, and thus, the main objective of the current study was to measure intraspecific genetic variation within the population of *Z. nummularia* naturalized in different geographical regions.

To date, little or no information is available about the study and the importance of the *Z. nummularia* genetic diversity. In spite, being widely distributed throughout Pakistan and since the fruits having great potential for food as well as in medicines, yet no varietal development endeavors have been pursued in Pakistan. The present research work has amid to design to know intra-species variation among *Z. nummularia* genotypes based on morphometric collected from different regions of KP, Pakistan, and addition to evaluating the genetic studies based on SDS-PAGE (Total seed protein) of 120 *Z. nummularia* genotypes while the Using of phylogenetic, PCA and multivariate analysis to check relationship among *Z. nummularia* genotypes collected from three different districts of KP, Pakistan.

Method and Material

Exploration and collection: The exploratory trips for the collection of plant genotypes were arranged to 3 different districts of Khyber Pakhtunkhwa, Pakistan, (District Swat, Dir (L), and Buner), during two consecutive years, 2016- 2017, location of the collected samples was presented in (Fig. 1). According to Muhammad *et al.*, 2018, District Swat stretches from 35.2227° North to 72.4258° East longitude and the climatic conditions vary from dry to moist temperate. The climate is influenced by various factors including latitude, altitude, the Indian Ocean Summer, Monsoon, and the Western cyclonic currents, coming from the Mediterranean Sea, in the winter. Dir (L) is in the Dry temperate zone (34.9161° N, 71.8097° E; 4411 ft above sea level) while, District Buner having areas of both Dry and moist temperate regions (34.3943° N, 72.6151° E; 4049 ft above sea level). The current studies use a total of 120 genotypes of *Z. nummularia* were identified for the characterization of total seed protein profiling and morphological.

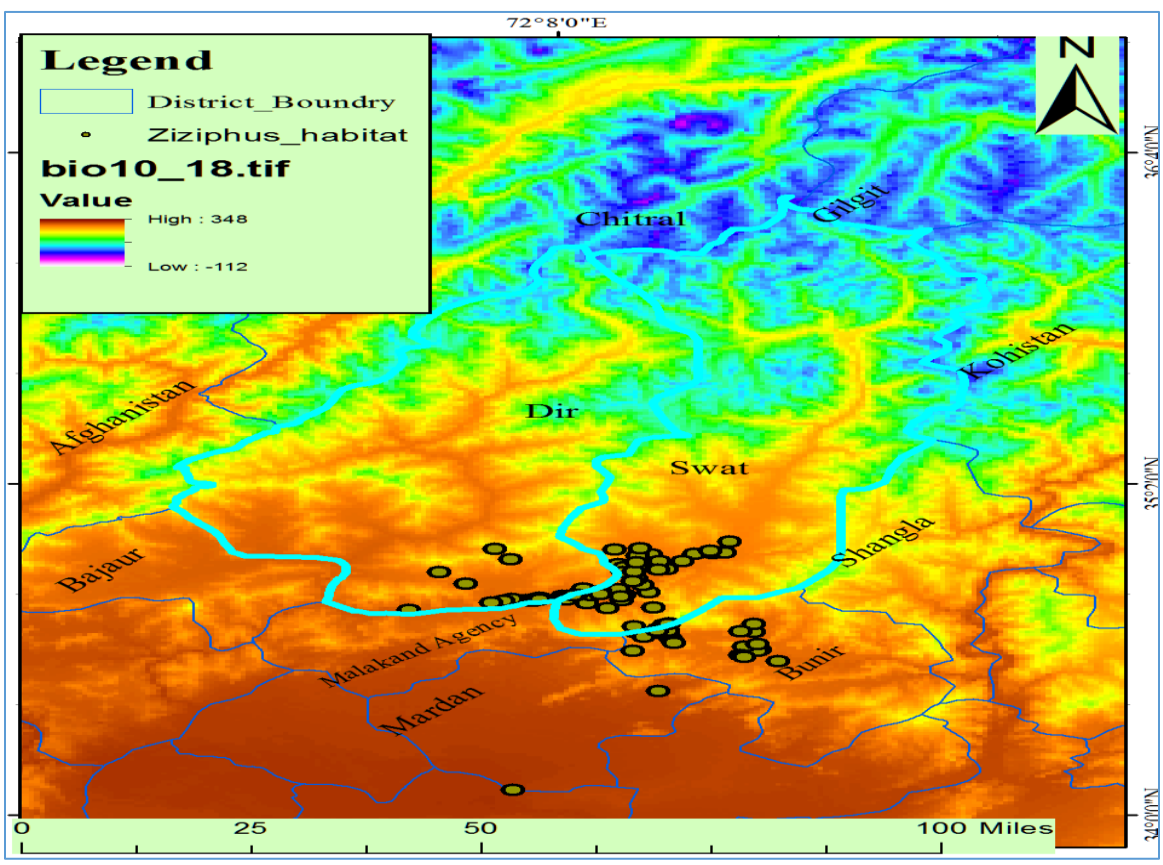

Fig. 1. Represented the collected *Z. nummularia* genotypes from three different districts of KP, Pakistan.

Morphological analysis: 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). Ten qualitative traits i.e. traits i.e. Tree vigorous (TrV), leaf type (LT), leaf shape (LeS), leaf colour (LeC), leaf margin (LeM), stem colure (StC), spines, fruit colure (FtC), fruit shape (FtS) and quantitative characters which were recorded with the help of vernier caliper for the measurement of plant height(feet), leaf width(mm), leaf thickness(mm), branching, petiole length (mm), leaf length(mm), stem diameter(inches), fruit weight, fruit diameter (mm) and fruit length (mm) according to Nisar *et al*., 2019; Muhammad *et al*., 2018; Noor *et al*., 2018).

Protein extraction and their preparation: Total seed protein (SDS-PAGE), a single mature and uncontaminated seed of *Z. nummularia* was selected for the analyses of total protein from each genotype of *Z. nummularia* collected from different unexplored areas of Malakand Division, KP, Pakistan, and the seed were ground through the pestle and mortar for the extraction of total protein using the protocol modified by Nisar *et al*., 2010; Muhammad *et al*., 2018; Noor *et al*., 2018.

Data analysis

The current data was recorded from the design gel (destined) based on 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 (Ghafoor *et al*., 2003; Muhammad *et al*., 2018).

Result

Morphological analysis: In the current morphometric studies of a total of 120 *Z. nummularia* genotypes were collected from different regions of KP, Pakistan. In 120 genotypes some of them shown vigor, high vigor, and less vigor, that as 80% were less vigorous whereas 15% were high vigor and 25% were moderate vigor, in the current work majority of *Z. nummularia* genotypes showed less vigor related to shrubs trees but in few are moderated tress (high and moderate vigors). Some variations are found in leaves of *Z. nummularia* phenotypes such as Leaf type, 100% were with alternate, Leaf shape was ovate, green leaf color and the tomentose were 81 genotypes was noted, dense of the *Z. nummuaeia* were 39% are rare, most of the phenotypic variation was occurs in the stem colores i.e. 50% genotypes stems were red-brown 25% were light black, 28% were brown, 15% were with purple 2% were with grey colored stem and have to consist fine spines for used for the protection. Fruit color; 29%, 29% genotypes were with yellow red and red-brown respectively, 24% were with brown colored fruits, 18% were with red colored fruit and 20% were with yellow-colored fruit. Leaf margin; 40% genotypes were with the entire leaf margin and 60% genotypes were with 80% genotypes were with serrate margins. Fruit shape, 25% genotypes were round-shaped fruit, 45 genotypes were with drupe shaped fruit while 70% genotypes were with oval-shaped fruits the data were represented in (Fig. 2). By using the Pearson correlation coefficient, the result for the association coefficient among the different characters for the *Z. nummularia* was done and a significant positive and negative correlation was found in various traits like the branching of the *Z. nummularia* was negatively correlated with leaf length whereas the leaf length was significantly positively correlated with leaf width and leaf thickness and so on (Table 1).

The CV% (Coefficient of variation) was calculated for these morphological traits and the highest value was noted for the fruit shapes (175.4066) and while the lowest CV was observed in leaf margined (21.9404) and so on (Table 2).

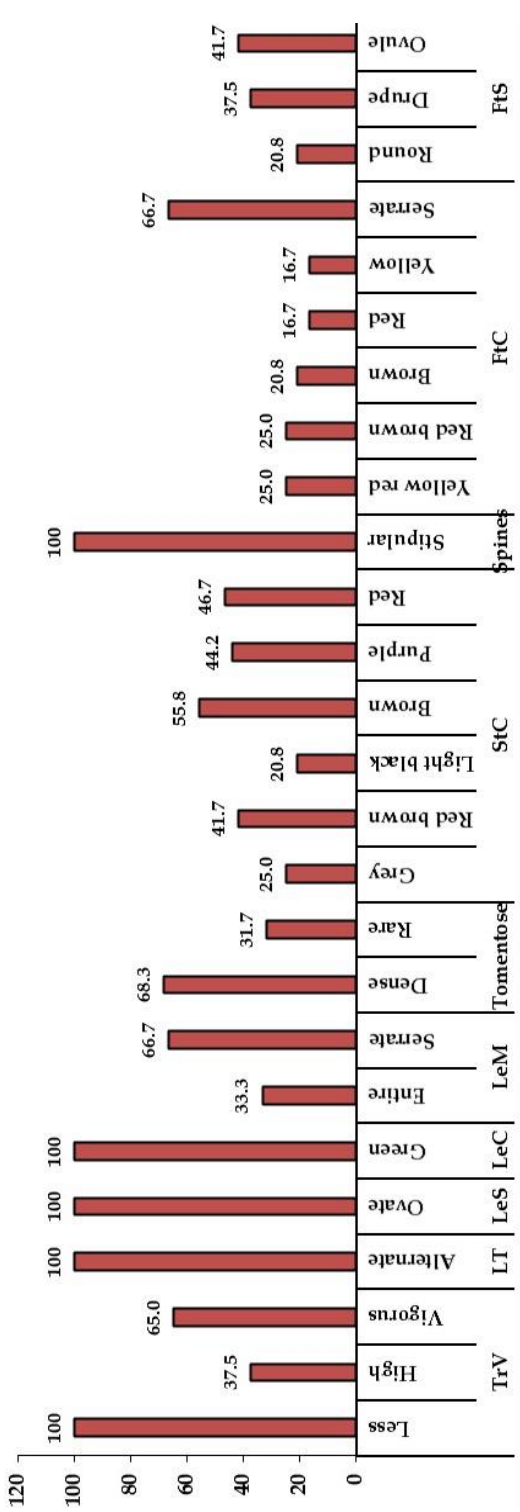

Fig. 2. Frequency distribution of qualitative characters in 120 *Z. nummularia* genotypes reported from three districts of KP, Pakistan. *Note;* TrV were designated for (Tree vigorous), LT (leaf type), LeS (Leaf shape), LeC (Leaf colour), LeM (Leaf margin), StC (Stem colure), Spines, FtC (Fruit colure), FtS (Fruit shape)

Table 2. Descriptive statistics of 10 qualitative traits of *Z. nummularia* were collected from three districts (Swat, Dir (L), and Buner) of KP, Pakistan.

Traits	Minimum	Maximum	Mean	Std. Deviation	CV%
TrV	3	75.2	11.6175	10.41228	89.62582
LT	2	26	6.8	4.03889	59.39544
LeS	11.2	83	27.6538	16.29475	58.92409
LeC	5.8	55.4	19.6525	11.69588	59.51345
Tomentose	0.11	4.45	0.3676	0.40485	110.1333
StC	2.2	34.8	7.3151	4.97924	68.06797
Spines	1.42	65.4	15.555	8.46183	54.39942
FtC	1	21.6	3.655	2.7629	75.59234
LeM	5.02	23.6	1.0802	23.62104	21.9404
FtS	4.2	334.8	17.4867	30.67283	175.4066

Note: CV% = Std. Deviation/mean*100, TrV were designated for (Tree vigorous), LT (leaf type), LeS (Leaf shape), LeC (Leaf colour), LeM (Leaf margin), Spines, StC (Stem colure),, Spines, FtC (Fruit colure), FtS (Fruit shape)

Table 3. Intra locus variations among 120 genotypes of *Z. nummularia* were collected from different regions of KP, Pakistan.

Locus	D-GD	S-GD	B-GD	Avr GD	Status	%D	%S	Status	%B
B1	0	0.46	0.46	0.15	Momo	100	0.00	Momo	0.00
B2	0.27	0.21	0.46	0.24	Poly	41.3	54.35	Poly	0.00
B3	0.23	0.19	0.35	0.19	Poly	50	58.7	Poly	23.91
B4	0.26	0.14	0.29	0.18	Poly	43.48	65.22	Poly	36.96
B5	0.19	0.06	0.25	0.15	Poly	58.7	86.96	Poly	45.65
B6	0.23	0.05	0.24	0.16	Poly	50	89.13	Poly	47.83
B7	0.26	0.05	0.21	0.16	Poly	43.48	89.13	Poly	54.35
B8	0.21	0.04	0.19	0.13	Poly	54.35	91.3	Poly	58.7
B9	0.24	0.09	0.19	0.14	Poly	47.83	80.43	Poly	58.7
B10	0.21	0.09	0.2	0.14	Poly	54.35	80.43	Poly	56.52
B11	0.17	0.15	0.24	0.14	Poly	63.04	67.39	Poly	47.83
B12	0.46	0.02	0.46	0.31	Poly	0.00	95.65	Poly	0.00
GD=91.66 % (GD= Poly loci/Total loci*100)						**GS=8.333% (GS= Mono loci/Total**			**loci*100)**

D = Dir, S= Swat, Avr = Average, % D = % Dissimilarity, % S = % Similarity

The data matrix of 120 genotypes based on morphology/Phenotypes was examined for the construction of phylogenetic tree to represents the similarity and variation among collected *Z. nummularia* genotypes collected from KP, Pakistan 120 total genotypes of the *Z. nummularia* were sightseen for similarities and the dendrogram was constructed (Fig. 3). The dendrogram separated all the 120 genotypes of *Z. nummularia* in 8 regions. Region I was comprised of 5 (Five) genotypes (ZN-D, 17,18,19,24 and ZN-D29) were collected from district Dir whereas Region II included 15 genotypes collected from district Buner. While Region III had 11 genotypes collected from Dir. Region IV comprised 30 genotypes of *Z. nummularia* collected from Swat areas. Region V (Five) in the dendrogram consisted of 21 genotypes collected from the Buner region. Region VI consisted of all genotypes collected from Swat. Region VII was comprised 4 genotypes of *Z. nummularia* collected from Buner, Similarly, Region VIII comprised of the 24 genotypes collected from the region of Dir.

Compression of genetic relationship between collected regions based on SDS-PAGE: Biochemical characterization revealed a total of 12 loci, expressed between 10 to 180kDa in genotypes of *Z. nummularia*. Most of the loci (7) were noted in the range of 10 to 80 kDa. It was noted that loci represented high polymorphism in the range of 81 to 180kDa. Locus 1 was monomorphic among all the genotypes collected from 3 districts. This monomorphic band is a species-specific band. Genetic diversity (GD) for loci ranged from 0.13 to 0.31. High GD was noted for L-12 (0.31) followed by L-2 (0.24) and L-3 with 0.19 GD. L-8 calculated a low level of GD (0.13) followed by L-9, 10, and 11, and 12 was calculated 0.14% GD (Table 3). The actual similarity between the different genotypes is based on their loci which are highly similar with a range from 1.00 to 0.53 and 0.43 loci 1 to 6, 7, and 8 loci. While the low similarity was observed in -0.60 and -0.53 in 7 and 12 loci at mean this a new allele are found in these genotypes which show variation the current data result was presented in (Table 2).

The phylogenetic tree of the *Z. nummularia* genotypes represented eleven distinct regions. R(Region)-I, R-V, and R-VII represented genotypes of District Dir, R-II, R-IV, R-VI, R-IX, and R-XI having genotypes of District Buner while R-III, R-XIII, and R-X contained genotypes reported from District Swat. It was found that: R-I and R-II showing similarity in L-12; R-II, R-III, and R-IV in L-1, R-IV, and R-V in L-12 while R-5, R-6, R-7, R-8, R-9, R-10, and R-11 showed similarity in L-9. In R-1, express loci ranged from 2 to 12. The maximum number of loci was expressed in genotypes D001 followed by D014 in which 9 loci were noted. Similarly, a minimum number of loci i.e. 2 was found in genotypes D007 and D019 (Fig. 4).

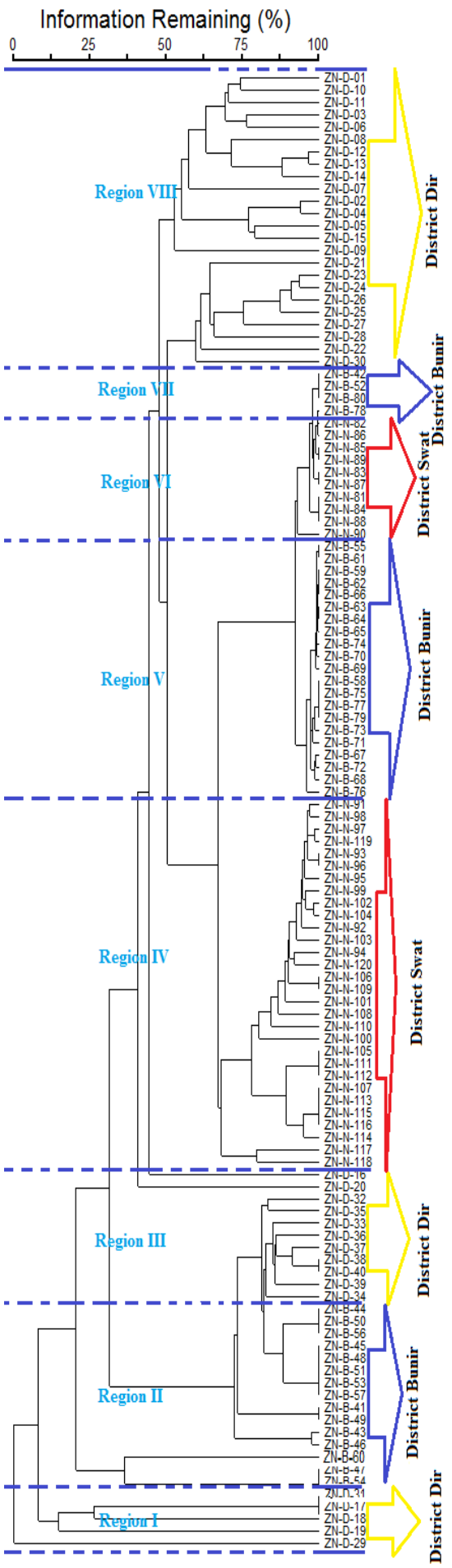

Fig. 3. Intraspecific genetic diversity was identified through morphological traits analysis in 120 different genotypes of *Z. nummularia* collected from three districts of Khyber Pakhtunkhwa, Pakistan. ZN represents *Z. nummularia*, B represents Buner, N shows Swat, D shows Dir.

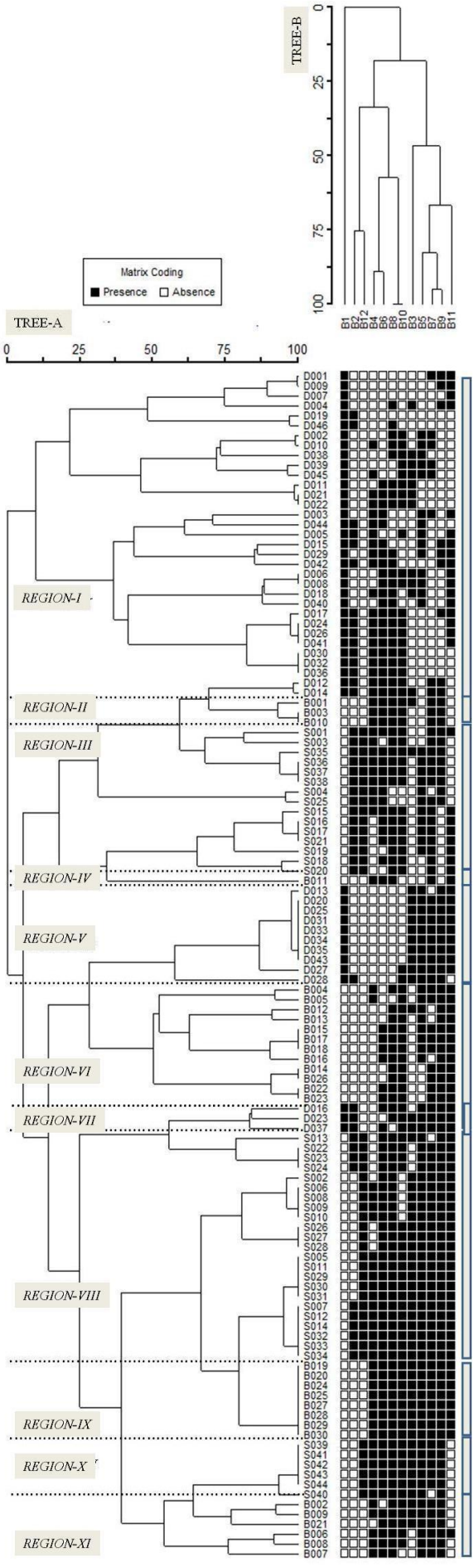

Fig. 4. Phylogenetic analysis shows genetic association in 120 genotypes of *Z. nummularia* based on biochemical screening (SDS-PAGE).

Table 1. Correlation coefficient among 120 genotypes of *Z. nummularia* collected from different regions of KP, Pakistan.

Plant height(feet)	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)	Fruit length (mm)
Branching	-0.07	1.00									
Leaf length(mm)	0.371**	0.236**	1.00								
Leaf width(mm)	0.494**	0.10	0.870**	1.00							
Leaf thickness(mm)	0.02	0.13	0.11	0.16	1.00						
Petiole length(mm)	0.06	0.01	0.16	0.15	0.09	1.00					
Inter node length(cm)	0.16	0.11	-0.17	-0.16	-0.09	0.05	1.00				
Stem diameter(inches)	0.644**	-0.11	0.207*	0.323**	0.04	0.209*	0.09	1.00			
Fruit weight	-0.09	-0.04	-0.09	-0.07	0.07	0.14	0.04	-0.12	1.00		
Fruit diameter (mm)	0.04	0.12	0.09	0.14	0.00	-0.03	-0.14	0.04	-0.10	1.00	
Fruit length (mm)	0.230*	0.379**	0.388**	0.449**	0.11	0.08	0.07	0.18	0.18	.245**	1.00

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

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

Table 4. Locus similarity among the genotypes collected from 3 districts (Dir, Swat and Buner).

	R-1 (Dir)	R-2 (Buner)	R-3 (Swat)	R-4 (Buner)	R-5 (Dir)	R-6 (Buner)	R-7 (Dir)	R-8 (Swat)	R-9 (Buner)	R-10 (Swat)	R-11 (Buner)
Locus-1**	*0.00*	***1.00***	***1.00***	***1.00***	*0.00*	*1.00*	*0.00*	*1.00*	*1.00*	*1.00*	*1.00*
Locus-12**	***1.00***	***1.00***	*0.00*	***1.00***	***1.00***	*1.00*	0.67	0.04	0.88	0.17	*1.00*
Locus-9**	0.73	0.50	0.47	*1.00*	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***
Locus-2	0.55	*1.00*	*0.00*	*1.00*	0.90	*1.00*	*0.00*	0.57	*1.00*	*1.00*	*1.00*
Locus-3	0.67	*1.00*	0.93	*1.00*	*0.00*	0.92	0.33	0.13	*0.00*	*0.00*	0.50
Locus-4	0.39	0.50	0.40	*0.00*	*1.00*	0.83	*1.00*	0.35	*0.00*	*0.00*	0.17
Locus-5	0.58	*1.00*	0.27	*1.00*	*0.00*	0.33	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
Locus-6	0.36	0.50	0.20	*0.00*	*1.00*	0.50	0.33	*0.00*	*0.00*	*0.00*	0.17
Locus-7	0.76	0.50	0.07	*0.00*	0.10	0.25	*0.00*	0.04	*0.00*	0.17	*0.00*
Locus-8	0.30	0.50	0.13	*0.00*	*1.00*	0.08	0.33	*0.00*	*0.00*	*0.00*	*0.00*
Locus-10	0.39	0.50	0.13	*1.00*	0.80	*0.00*	*0.00*	0.22	*0.00*	*0.00*	0.17
Locus-11	0.48	*1.00*	0.47	*0.00*	0.10	*0.00*	*0.00*	*0.00*	*0.00*	*1.00*	0.83
Regions	*R-I & R-II*	*R-II & R-III*	*R-III & R-IV*	*R-IV & R-V*	*R-V & R-VI*	*R-VI & R-VII*	*R-VII & R-VIII*	*R-VIII & R-IX*	*R-IX & R-X*	*R-X & R-XI*	*R-XI & R-X*
Locus similarity	Locus12	Loucus1	Loucus1	Locus12	Locus9	Locus9	Locus9	Locus9	Locus9	Locus9	Locus9

The loci expressions in R-II, in both genotypes shown in the same number of loci, were express. The genotype B003 express loci are 6 was observed while genotype B010 in which 6 loci were recorded. The loci in expression as an R-III ranged from 10 to 6. The maximum number of loci present or express in genotypes S035 in which 10 loci are expressly followed by S031, S036, S037, and S038 in which 9 loci were noted. In the same way, a minimum number of loci that are genotypes number S004 and S020 in which 6 loci are expressed. R-IV, only one genotype is expressed in which 5 number of is expressed in B011 genotype. In R-V, express loci ranged from 5 to 7. Maximum numbers of loci were expressed in genotypes D027 and D028 followed by D020, D025, D031, D033, D034, D035, and D043 in which 6 loci were noted. Similarly, the minimum number of loci i.e. 5 was found in genotype number D013. In R-VI, express loci ranged from 5 to 7 in which different numbers of loci were noted such as the maximum number of were recorded in genotype B004, B015, B017, and B018, in which 7 loci are expressed. While the minimum 5 numbers of loci express in genotypes were noted in B013, B014, B022, B023, and B026 followed by B006 and B016 in which 6 loci were noted. In R-VII, the express loci ranged from 9 to 10 in which a maximum number of loci were expressed in genotype D037 in which 10loci were expressed. A minimum number of loci is noted in genotypes D016 and D023 in which 9 numbers of loci were recorded. While In R-VIII, express loci ranged from 8 to 11. The maximum number of loci was expressed in genotypes S007, S012, S032 and S033, 11 loci were noted. Followed by S005, S011, S029, S030, S003, and S034 in which 10 loci were recorded. Similarly, the minimum number of loci i.e. 9 was found in genotypes S002. Expression of loci in R-XI ranged from 9 to 10. The maximum number of loci was recorded in genotype B030 in which 10 loci were noted. Similarly, the minimum loci are expressed in loci B019, B020, B024, B025, B027, B028, and B029 in which mention 9 loci in these genotypes. In R-X, Express loci ranged from 7 to 9. Maximum numbers of loci were present in genotypes S039, S041, S042, S043, and S044, recorded 9 loci. Similarly, a minimum number of loci were noted in loci S040, in which 7 loci are noted. In R-IX, express loci ranged from 6 to 8. The maximum number of loci was expressed in genotypes B006 and B009, 8 loci were noted. Followed by B002, B006, and B021 in which 7 loci were recorded. Similarly, the minimum number of loci i.e., 6 was found in genotypes B007 (Fig. 4).

The relationship between the genotypes collected from three different districts which show linkage based on their loci, genotypes S-014, B-015, and D-016 are closely linked with each other while the D-031, S- 018, and B-001 are very far away from each other date was presented in the (Fig. 1).

Characterizations of biochemical markers (SDS-PAGE) of wild *Z. nummularia*: For an effective breeding program, information regarding the extent and nature of genetic diversity within a crop species is essential. It is particularly useful for characterizing individual accession and as a guide in the selection of parents for hybridization. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm. Total seed protein was analyzed through the slab type SDS-PAGE using 12.00 % polyacrylamide gel. Electropherogram showing seed protein banding pattern in 120 samples of *Z, nummularia* is presented. We investigated the variation based on the electrophoretic pattern of the molecular weight range higher than 180 KD. While the genetic diversity based on total seed storage protein banding pattern was determined SDS-PAGE, though, its magnitude was low, a total of 12 loci/ bands were used for the analysis for genetic diversity was determined on the different number of protein-peptide b/w the two compared. The gel is divided into two regions in R-I loci from 180kD to 80kD were recorded while from 80kD to 20kD loci were placed into R-II. The R-I was comprised of five loci (B-1 to B-5) and R-II started from B-6 to B-12 (Fig. 2).

Bands contribution toward genetic disagreement: In the present data based on seed storage protein, 11 loci were polymorphic, and 1 band was monomorphic. The genetic disagreement was 91.66% while genetic similarity was 8.333%. The average genetic disagreement for B1-b12 was 0.15, 0.24, 0.19, 0.18, 0.15, 0.16, 0.16, 0.13, 0.14, 0.14, 0.14, and 0.31 respectively. The data table indicated and divided into regions. The Regions R-I and R-II consisted of the genotypes collected from Dir and Buner respectively, the Locus 12 was found to be similar in these two regions. Whereas R-II and R-III included the genotypes collected from Buner and Swat regions respectively, the common locus between these two (Buner and Swat), was Locus 1. R-III and R-IV enclosed the genotypes of Swat and Buner respectively have common locus 12. Similarly, the regions R-IV, V, VI, VII, VIII, IX, X, and XI enclosed the genotypes collected from Buner, Dir, Swat, Buner, and Swat, Buner represented and locus 9 as a common locus respectively, (Table 4).

Discussion

The purpose of this study was to recognize the genetic diversity, genetic structure, and a core collection of *Z. nummularia* genotypes. Now, we explain our consequences concerning genetic diversity and the causes of 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 (Hjalmarsson & Ortiz, 2000).

Various apparatuses are now presented for documentation of required differences in the genotypes, including morphological/phenotypic, biochemical, and molecular markers (Potokina, 2000; Kamel, 2005; Jannatabad *et al*., 2014). Though the morphological description is the principal step in the description and alliance of crop genotypes these are highly subjective by the environment (Nisar *et al*., 2016; Muhammad *et al*., 2018). In the present morphological study both qualitative and morphological study was carried; a significant

variation was found at both morphological and seed storage protein profile. Based on morphological characterization a high variation was found as morphology is severely affected by environmental factors (Nisar *et al*., 2016; Muhmmad *et al*., 2018) The coefficient of variation for qualitative traits (Tree vigor, Leaf type, Leaf shape, Leaf color, Tomentose, Stem color, Spines, Fruit color, leaf margin, Fruit shape) were 89.62582, 59.39544, 58.92409, 59.51345, 110.1333, 68.06797, 54.39942, 75.59234, 21.9404, 175.4066 the current result was similar to online cited as (Azam-Ali *et al*., 2001; Khadivi-Khub *et al*., 2014).

The reason for the differences in the morphological and SDS-PAGE may be due to the geographical, climatic, and altitudinal isolation (Muhammad *et al*., 2018)

On the other side practice of genetic indications such as RAPD, SSR, RFLP, etc. are more consistent approaches for the explanation of variation/diversity among different crop species but they are highly affluent (Zecevic, 2000). Now the easiest, straightforward, cost-effective, and reliable methods to identify genetic variations in germplasm may be the study of seed storage protein by (SDS -PAGE) that consists of 60% of the total protein contents (Nisar *et al*., 2019). These proteins are broken down to provide the basic nourishment for seed germination and seedling growth. Moreover, seed proteins in nucleotide sequences are largely independent of environmental conditions (Iqbal, 2005). Keeping this in view the present study was the first-time investigated seed protein based on SDS-PAGE on *Z. nummularia* plant. Genetic diversity in black gram at the biochemical level by SDS-PAGE to find out the unique and most important characteristics of specific germplasm and to document morph-metric in seed due to High intra-species locus contribution toward genetic disagreement SDS-PAGE could be a consistent procedure for the characterization of this species and Intra-species locus The locus contribution toward genetic disagreement *(LCTGD)* genetic dissimilarity of *Z. nummularia* was 91.66% and 8.333%% respectively. Notably, B-1 band was monomorphic in *Z. nummularia* and was treated as species-specific. B-2-B-12 was polymorphic. The average genetic disagreement for B1-B-12 was 0.15, 0.24, 0.19, 0.18, 0.15, 0.16, 0.16, 0.13, 0.14, 0.14, 0.14, and 0.31 respectively.

Conclusion

The current investigation of genetic diversity in *Z. nummularia* using morphometric and the total seed protein is the first-time study from Pakistan. Based on the morphological as well as total seed protein subunits intraspecies diversity was evaluated within 120 genotypes of *Z. nummularia*. It was recognized that except leaf type (alternate), leaf shape (ovate), and leaf color (green) that showed monomorphism; the genotypes exhibited polymorphism of varying degrees in all other morphological traits viz., vigor, leaf margin, tomentose (dense), stem color, spine length, fruit color, and fruit shape. Total seed protein profiling was carried out on 12% slab gel electrophoresis, 12 bands (loci) with molecular weight ranges from 10 to 180KDa were detected in *Z. nummularia*. The locus contribution towards genetic disagreement (LCTGD) and locus linkage of *Z. nummularia* was 91.66% and 8.333% respectively. Notably, the B-1 locus was monomorphic in *Z. nummularia* and was treated as species-specific whereas, B-2-B-12 was polymorphic. The average genetic disagreement for B1-B-12 was 0.15, 0.24, 0.19, 0.18, 0.15, 0.16, 0.16, 0.13, 0.14, 0.14, 0.14 and 0.31 respectively.

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(Received for publication 25 May 2019)