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Genetic diversity in the natural population of *Ziziphus jujuba* Miil

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Abstract
Genetic diversity is the material basis for the improvement of crops best of the current research work was done for the first time on natural genetic diversity based in total seed protein and phenotypic characteristic in 120 genotypes of *Ziziphus jujube* Mill. The genotypes were presented the utmost allelic variation for vigorous tree; 60 trees were less vigorous whereas 20were high 20 were vigorous and 20 is moderate types. Leaf type; 100% were with alternate, Leaf shape was totally ovate. All were green leaf color. Tomentose; 60 genotypes were dense, 40 were rare, 20 stems were red brown 20 light black, 50 brown, 10 were with purple and 10 were with grey stem with spines. Fruit color; 25,25 genotypes were with yellow red and red brown respectively, 25 were with brown, 25 were with red and 20 were with yelllow. Leaf margin; 80 entire leaf margin and 40 genotypes were with serrate margins. Fruit shape 80 genotypes were round shaped fruit and 40 genotypes were with oval shaped fruits.
Result: We have first time reported on protein profiling seed of *Z. jujuba* was carried out on 12% slab gel electrophoresis. 12 loci with molecular weight ranges from 10KDa to 180KDa were detected in *Z. jujuba*. The locus contribution toward genetic disagreement *(LCTGD)* genetic dissimilarity of *Z. jujuba* was 75.00%, L-6, 9 and L-11, were monomorphic in *Z. jujuba* and was treated as specie specific. L1-2, L-3, L-4, L-5, L-7, L-8, L-10 and L-12 were polymorphic. These alleles showed 33.333%, 50%, 70, 82.50%, 79.17%, 33.333, 70%, 79.17% and 29.166%% respectively. The intra-specific variation was limited and it was observed that SDS-PAGE alone did not exhibit high level of intra-specific variation; therefore, diverse germplasm based on SDS-PAGE is suggested to be acquired from various sources, preferably from center of diversity to build a broad-based gene pool with maximum variability.

Keywords: wild *Z. jujuba* Mill. genetic, phenotypic variation, SDS-PAGE, genetic association, multivariate analysis

1. Introduction
The genus *Ziziphus* Mill. (Jujube) belongs to Buckthorn family (Rhamnaceae). It is a genus of about 100 [1, 2], up to 170[3], species of deciduous or evergreen trees and shrubs. Rhamnaceae family is believed to be originated from China and south Asia and its members are widely distributed in the temperate regions such as Pakistan, China, India, Australia, Syria and Malaysia [4]. The genus *Ziziphus* is the most important genus of family Rhamnaceae economically, sociologically and ecologically.
Jujuba (*Ziziphus jujuba* Mill, 2n = 26=24) is known with different names and commonly referred to as either Jujube or jujuba (with the previous being actually right) the fruits called by a different names i.e. Chinese date, Korean date, Indian date, or Red date and in Pakistan called Markhani in Pashto [5, 6].
It is one of the most important and highly medicinal fruiting plants in the world native to China with about 4000 years history and almost cosmopolitan distribution, though members are dominated in the tropical and subtropical regions of the world between 34°S and 51°N latitude and up to 2800 m above sea level like Australia, Europe, Pakistan and Southern and eastern Asia [7, 8, 9].
It generally understood that phenotypic characters are unstable and influenced by environmental conditions [10].
Due to these defects, biochemical and molecular markers were used for cultivars identification to achieve more exact identification [11]. SDS-PAGE has broadly been used to study genome structure in several crops of agronomic importance like wheat, maize, soybean, sunflower, etc [12, 13]. Seed protein profile has been successfully used to resolve the taxonomic difficulties of *Solanum* and *Capsicum* species [14]. SDS-PAGE is most widely used due to its validity and simplicity for describing genetic structures of group of plants [15], rather trusting on DNA based markers which are expensive and time consuming [16]. However the potentials of DNA markers to resolve inter and intraspecific variations is well known and hence could be used to study variations that could not be revealed by protein markers. Furthermore proteins are expressed form of DNA which can be biomarkers for identification of botanic drugs [17]. The mature seed provides a stable and convenient system for biochemical analysis to establish relationship in parents and hybrids [18].
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 wild plants. Therefore the present study is design to know first time intra specie variation among *Z jujube* genotypes.
Amis of the current investigation, (1) was first time to

extract total seed proteins through SDS PAGE from 120 wild *Z. jujuba* germplasm and (2) To assess genetic diversity based on Phenotypic and total seed protein of 120 germplasm collected from different unexplored region of kp, Pakistan, multivariate analysis.

2. Materials and Methods

Exploration and collection

Exploratory trips were arranged to different regions in District Swat Khyber Pakhtunkhwa during two consecutive years, i.e., 2016- 2017. Area, code and there distribution pattern were represented in (Table.1). 120 genotypes were identified and investigated for phenotypic characterization and protein profiling.

Phenotypic Analysis

Both qualitative and quantitative characterizations were carried out. Qualitative traits were noted on the general visualization (phenotypic observations). Seven qualitative traits i.e. Tree vigor, Leaf type, Leaf shape, Leaf colure, Tormentors, stem color, Spines, fruit color, leaf margin, fruit shape.

Quantitative characters which were measured with the help of Venire calipers are: 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).

SDS-PAGE characterization:

For SDS-PAGE analysis single seed of each genotype was ground into a fine powder with the help of mortar and pestle for the extraction of proteins. About 400µl of protein extrication buffer (0.5 M Tris-HCL pH 8.0, 0.2%SDS, 5 M Urea,1%B-mercaptoethanol) was added to 0.01g of seed flour taken in 1.5 ml Eppendorf tube. The E-tube was vortexes thoroughly to homogenize the mixture. Bromo-Phenol Blue (BPB) was added to the protein extraction buffer as tracking dye to monitor the movement of protein in the gel. The homogenate samples were centrifuged at 14,000rpm for 40 minutes at 10ºC.The electrophoretic procedure was carried out using 12% polyacrylamide gel, separation gel (3.0M Tris-HCl pH9.0, 0.4% SDS) and 4.5% stacking gel (0.4M Tris-HCl pH 8.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125% SDS) was poured into the top pool of the apparatus. A total volume of 6.5 µl of the protein extract solution was loaded in each well of the gel with the help of micropipette. The electrophoresis was run at 100V until the blue line passed through the bottom of gel plates. The gels were then stained in 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 containing 5% acetic acid and 20% methanol for 15 minutes [10, 17]

Data analysis

The data were recorded from the destined gel on the basis of presence and absences of protein bands. i.e. '1' for the presence and '0' for the absence of loci and cluster analysis was carried out using software PC-ORD.5.0, SSPS, statistical and MAGA 5.0

3. Result

Phenotypic analysis

In (Fig.1) represented the 60 trees were less vigorous whereas 40 were high and 20 vigorous. Leaf type; 100% were with alternate, Leaf shape was totally ovate. All the genotypes were with green leaf color. Tomentose; 60 genotypes were dense, 40 were rare, 20 genotypes stems were red brown 20 were light black, 50 were brown, 10were with purple 10 were with grey colored stem. All the genotypes were with spines. Fruit color; 20,25 genotypes were with yellow red and red brown respectively, 25 were with brown colored fruits, 15 were with red colored fruit, 10 were with yelllow colored fruit. Leaf margin; 80 genotypes were with entire leaf margin and 40 genotypes were with serrate margins. Fruit shape; 80 genotypes were round shaped fruit, 10 genotypes were with drupe shape fruit while 30 genotypes were with oval shaped fruits.

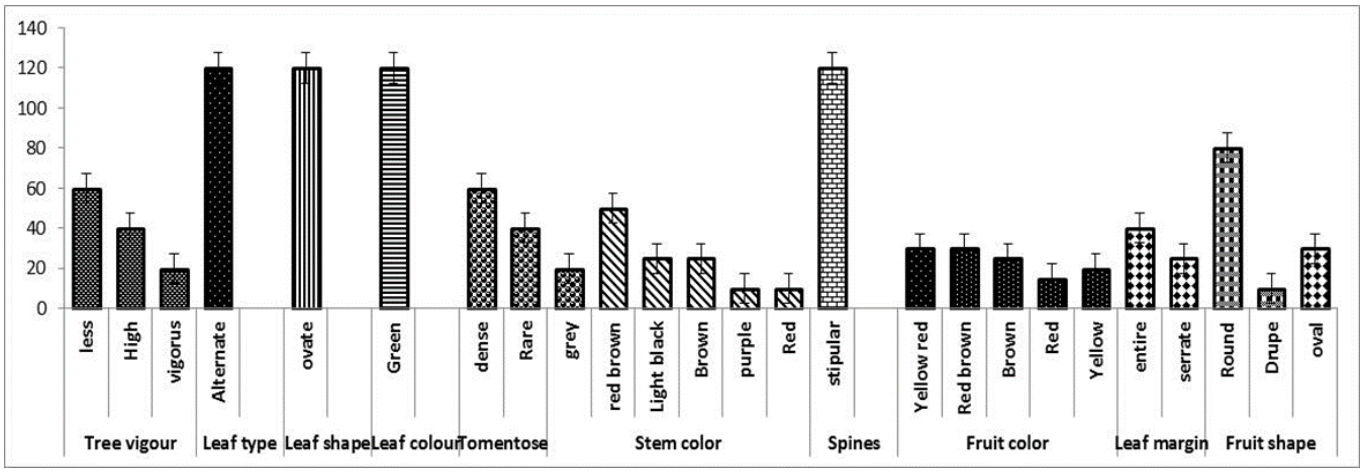

Fig 1: Frequency distribution of qualitative characters in 120 *Z. jujuba* genotypes collected from KP, Pakistan.

Table 1: Represented Area and distribution of *Ziziphus jujuba* genotypes in KP, Pakistan range of Codes, latitude and longitude

Area	Code	Longitude	Latitude	Area	Code	Longitude	Latitude	Area	Code	Longitude	Latitude
Matta	ZJ01	34˚47'03.80"N	72˚03'01.66"E	Barikot	ZJ41	34˚40'20.28"N	72˚14'21.43"E	Kuz Shawer	ZJ81	35˚06'08.29"N	72˚11'14.69"E
Madain	ZJ02	34˚41'41.11"N	72˚11'29.91"E	Nagoha	ZJ42	34˚33'45.08"N	72˚12'25.45"E	Bar Shawer	ZJ82	344130.03"N	72˚10'16.71"E
Kalakot	ZJ03	34˚37'17.72"N	72˚13'32.80"E	Parai	ZJ43	34˚31'49.17"N	72˚05'31.43"E	Bagh Deri	ZJ83	344013.75"N	72˚15'17.24"E
Baghdaeri	ZJ04	34˚39'13.64"N	72˚30'47.33"E	Kuza Parai	ZJ44	34˚34'45.62"N	72˚33'29.22"E	Nawakily	ZJ84	345124.40"N	72˚12'14.76"E
Nawakela	ZJ05	34˚41'42.62"N	72˚16'24.40"E	Shamozo	ZJ45	34˚32'42.80"N	72˚45'33.37"E	Ashary	ZJ85	344315.47"N	72˚33'32.92"E
Sheen	ZJ06	34˚34'49.10"N	72˚02'18.67"E	Dadahra	ZJ46	34˚37'44.13"N	72˚43'36.55"E	Pathypur	ZJ86	340744.53"N	72˚19'17.71"E
Pathepore	ZJ07	34˚45'48.75"N	72˚27'18.84"E	Sharf Abad	ZJ47	34˚39'32.35"N	72˚47'28.01"E	Tahsil	ZJ87	341646.77"N	72˚22'26.89"E
Khuzakhela	ZJ08	34˚50'01.02"N	72˚31'24.55"E	Ghadu	ZJ48	34˚33'32.30"N	72˚51'33.11"E	Teghko	ZJ88	344126.93"N	72˚32'39.32"E
Bara Bandai	ZJ09	34˚46'14.16"N	72˚27'23.73"E	Daghy	ZJ49	34˚42'34.73"N	72˚19'11.25"E	Shahderai	ZJ89	345324.43"N	72˚19'13.86"E
Negulai	ZJ10	34˚42'33.84"N	72˚43'26.80"E	Shangla Top	ZJ50	34˚38'20.01"N	72˚08'13.04"E	Kalakla	ZJ90	344230.96"N	72˚16'22.54"E
Kabal	ZJ11	34˚51'07.95"N	72˚22'19.01"E	Rahima Bad	ZJ51	34˚39'16.13"N	72˚04'06.04"E	Malak Khel Kotky	ZJ91	343727.68"N	72˚21'28.38"E
Kanju	ZJ12	34˚44'01.47"N	72˚31'28.34"E	Tandar	ZJ52	34˚39'05.58"N	72˚23'24.84"E	Matta2	ZJ92	342727.58"N	72˚21'28.78"E
Gullabad	ZJ13	34˚39'56.88"N	72˚19'16.03"E	Managoo	ZJ53	34˚40'20.87"N	72˚15'11.20"E	Sambut	ZJ93	343626.94"N	72˚12'36.84"E
Sultan Was	ZJ14	34˚44'43.52"N	72˚15'27.69"E	Manai	ZJ54	34˚41'44.70"N	72˚10'33.12"E	KuzShawer	ZJ94	344214.94"N	72˚11'30.94"E
Kuanju Derai	ZJ15	34˚49'41.02"N	72˚19'20.14"E	Gandawo	ZJ55	34˚41'16.85"N	72˚11'29.25"E	BarShawer	ZJ95	343929.28"N	72˚21'14.91"E
Tanchkai	ZJ16	34˚43'30.69"N	72˚14'04.52"E	Bely Baba	ZJ56	34˚37'11.66"N	72˚03'25.61"E	Bagh Deri	ZJ96	34˚33'17.48"N	72˚31'16.92"E
Tawonship	ZJ17	34˚31'11.43"N	72˚29'18.86"E	Chakot	ZJ57	34˚46'07.13"N	72˚03'18.54"E	Nawakily2	ZJ97	34˚38'35.10"N	72˚16'32.03"E
Aligrama	ZJ18	34˚43'03.74"N	72˚38'22.09"E	Baneer	ZJ58	34˚52'11.79"N	72˚11'14.05"E	Ashary	ZJ98	34˚49'35.10"N	72˚14'22.03"E
Hazara	ZJ19	34˚37'25.96"N	72˚06'34.18"E	Guli	ZJ59	34˚52'51.94"N	72˚38'29.72"E	Pathypur	ZJ99	34˚44'22.93"N	72˚23'39.32"E
Gulljaba	ZJ20	34˚48'27.47"N	72˚13'32.82"E	Olander	ZJ60	34˚48'49.55"N	72˚21'19.13"E	Tahsil	ZJ100	34˚47'11.43"N	72˚24'23.86"E
Kuzabandai	ZJ21	34˚46'26.47"N	72˚12'21.83"E	Kuz Khana	ZJ61	34˚53'43.61"N	72˚44'28.14"E	Khushmuqam	ZJ101	34˚35'22.94"N	72˚18'19.29"E
Bamakhela	ZJ22	34˚47'11.90"N	72˚19'38.47"E	Opal	ZJ62	34˚51'41.36"N	72˚38'14.92"E	Gul muqam	ZJ102	34˚45'26.93"N	72˚10'30.33"E
Chwak	ZJ23	34˚52'09.04"N	72˚57'45.02"E	Bazar Kot	ZJ63	34˚44'37.49"N	72˚33'25.61"E	Khadagzo	ZJ103	35˚06'08.29"N	72˚11'14.69"E
Janu	ZJ24	34˚44'41.06"N	72˚46'41.02"E	Machore	ZJ64	34˚44'43.30"N	72˚14'04.44"E	Chekdar bazar	ZJ104	344130.03"N	72˚10'16.71"E
Barmpatai	ZJ25	34˚40'07.08"N	72˚13'33.00"E	Pagorai	ZJ65	34˚47'13.14"N	72˚07'14.99"E	Gullabad	ZJ105	344013.75"N	72˚15'17.24"E
Seeny	ZJ26	34˚53'18.80"N	72˚21'28.96"E	Neray	ZJ66	34˚41'51.60"N	72˚51'44.76"E	Naspa dir lower	ZJ106	345124.40"N	72˚12'14.76"E
Belibaba	ZJ27	34˚51'11.79"N	72˚15'26.44"E	Larai	ZJ67	34˚43'18.52"N	72˚13'29.55"E	Serai khadagzo	ZJ107	344315.47"N	72˚33'32.92"E
Tobseen	ZJ28	34˚51'13.79"N	72˚19'29.05"E	Kot Kay	ZJ68	34˚49'13.22"N	72˚34'33.08"E	Taronw	ZJ108	340744.53"N	72˚19'17.71"E
Shangla	ZJ29	34˚51'30.16"N	72˚12.19.21"E	Machoor	ZJ69	35˚07'62.53"N	72˚39'09.71"E	Enzaro dir	ZJ109	341646.77"N	72˚22'26.89"E
Poran	ZJ30	34˚41'35.25"N	72˚11'21.77"E	Matta Awan	ZJ70	35˚09'03.32"N	72˚36'07.45"E	Ouch	ZJ110	344126.93"N	72˚32'39.32"E
Chakaser	ZJ31	34˚46'04.56"N	72˚11'39.59"E	Mian Kaly	ZJ71	35˚13'51.77"N	72˚31'04.89"E	Ouch hill	ZJ111	345324.43"N	72˚19'13.86"E
Gulldra	ZJ32	34˚42'15.86"N	72˚11'11.46"E	Alpurai	ZJ72	34˚32'27.39"N	72˚43'21.39"E	Larrum	ZJ112	344230.96"N	72˚16'22.54"E
Shahdra	ZJ33	34˚39'44.67"N	72˚14'10.84"E	Lilaonai	ZJ73	34˚33'01.54"N	72˚20'04.34"E	Darbar chekdara	ZJ113	343727.68"N	72˚21'28.38"E
Manglawr	ZJ34	34˚43'51.70"N	72˚16'15.01"E	Dara	ZJ74	34˚40'11.14"N	72˚17'32.31"E	Matkhanai dir	ZJ114	342727.58"N	72˚21'28.78"E
Charbagh	ZJ35	34˚39'42.56"N	72˚09'43.75"E	Qalay	ZJ75	34˚41'14.72"N	72˚18'12.62"E	Ouch hill 2	ZJ115	343626.94"N	72˚12'36.84"E
Gullibagh	ZJ36	34˚35'11.25"E	72˚04'25.41"E	Peecho	ZJ76	34˚54'24.78"N	72˚18'31.45"E	Tange pain	ZJ116	344214.94"N	72˚11'30.94"E
Sangota	ZJ37	34˚34'07.29"N	72˚05'25.41"E	Shapus	ZJ77	34˚54'31.84"N	72˚14'19.25"E	Gullbad2	ZJ117	343929.28"N	72˚21'14.91"E
Migora	ZJ38	34˚39'45.59"N	72˚03'16.24"E	Malak Khel Kotky	ZJ78	34˚35'18.03"N	72˚14'15.73"E	Gulabad 3	ZJ118	34˚33'17.48"N	72˚31'16.92"E
Amankot	ZJ39	34˚38'48.30"N	72˚12'12.23"E	Matta2	ZJ79	34˚35'22.94"N	72˚18'19.29"E	Katkala	ZJ119	34˚38'35.10"N	72˚16'32.03"E
Tendodag	ZJ40	34˚32'41.86"N	72˚09'14.70"E	Sambut	ZJ80	34˚45'26.93"N	72˚10'30.33"E	Charr	ZJ120	34˚49'35.10"N	72˚14'22.03"E

Table 2: Represented correlation coefficient among 120 genotypes of wild *Z. jujuba* collected from different region of PK, 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)	.371**	.236**	1.00								
Leaf width(mm)	.494**	0.10	.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)	.644**	-0.11	.207*	.323**	0.04	.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)	.230*	.379**	.388**	.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).

By using the Pearson correlation coefficient the result for the association coefficient among the different characters for the *Z. jujuba* was performed. Correlation coefficient revealed a significant positive as well as a negative association (p = 0.05 and 0.01) among the studied traits of *Z. jujuba* (Tables 2). Several traits revealed strong interrelationships within phenotype categories, particularly plant height (0.371**), branching (0.236**), leaf length (0.870**), fruit diameter (0.24**) traits etc. contributing traits and a few traits correlating with other categories, such as inherently linked growth and phenology-related traits.

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 86.62581, 69.39543, 56.92408, 58.51344, 120.1332, 78.06796, 53.39941, 74.59233, 31.9402 and 185.406589 (Table 3)

Table 3: Represented descriptive analysis of 10 phenotypic (qualitative) traits of wild *Z. jujuba* collected from unexplored regions of KP, Pakistan

Traits	Minimum	Maximum	Mean	Std. Deviation	CV%
Tree vigor	3	76.2	11.6175	10.41228	86.62581
Leaf type	2	36	6.8	4.03889	69.39543
Leaf shape	11.2	84	27.6538	16.29475	56.92408
Leaf color	5.8	56.4	19.6525	11.69588	58.51344
Tomentose	0.11	5.45	0.3676	0.40485	120.1332
Stem color	2.2	44.8	7.3151	4.97924	78.06796
Spines	1.42	67.4	15.555	8.46183	53.39941
Fruit color	1	31.6	3.655	2.7629	74.59233
leaf margin	58.02	240.6	1.08E+02	23.62104	31.9402
Fruit shape	4.2	344.8	17.4867	30.67283	185.4065

Phylogenetic tree based on morphological data

The data matrix of 120 genotypes on the basis of phenotypic was examined for the construction of phylogenetic tree to represents the similarity of various genotypes, 120 genotypes of the *Z. jujuba* were sightseen for similarities and the dendrogram was constructed (Fig. 2). The dendrogram separated all the 120 genotypes of *Z. jujuba* in 2 regions. The Region I was comprised of 62 *Z. jujuba* genotypes. Whereas the Region II included 58 genotypes *Z. jujuba*.

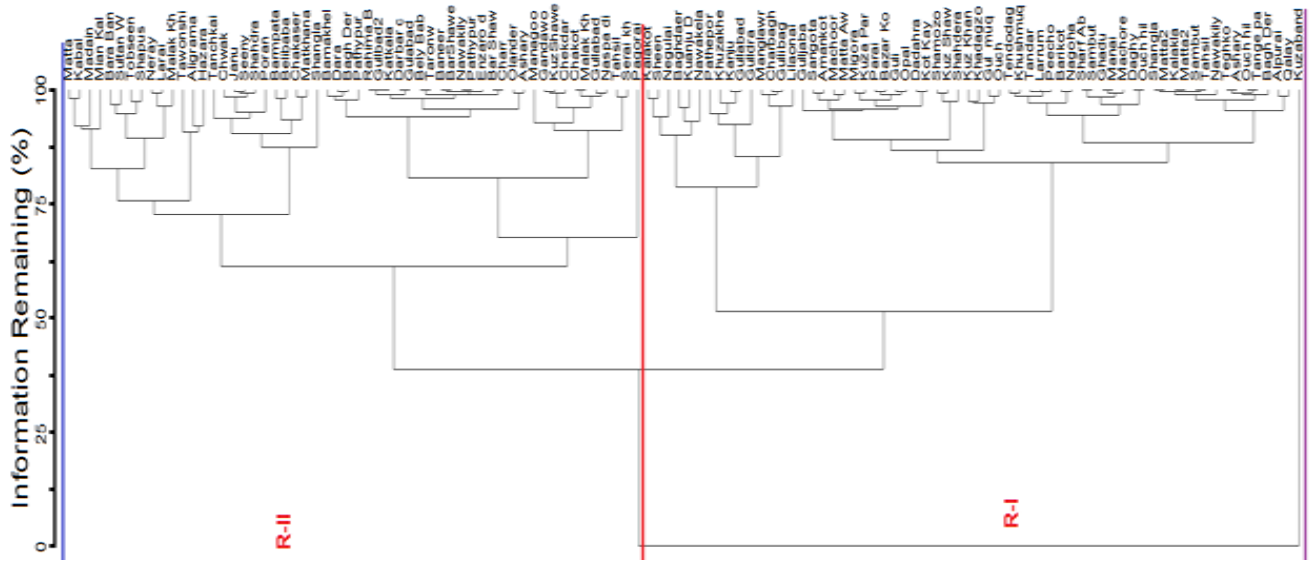

Fig 2: Intra -specific genetic diversity identified through Phenotypic traits analysis in 120 different genotypes of *Z. jujuba* genotypes collected from different districts of KP, Pakistan.

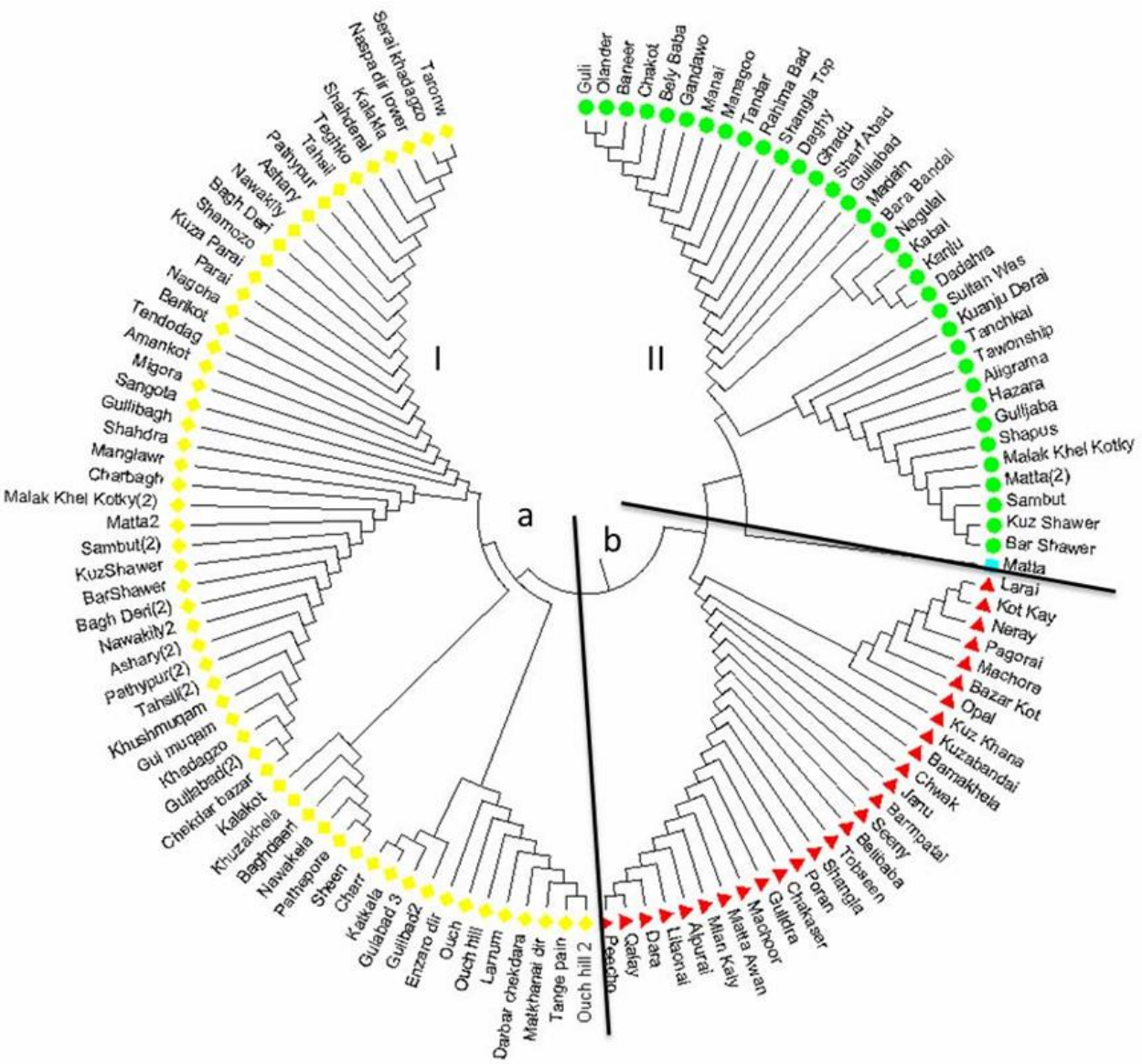

Fig 3: Evolutionary tree show genetic association in 120 genotypes of (*Z. jujuba*) based on biochemical screening (SDS-PAGE).

SDS-PAGE characterization

Total 12 alleles ware recorded within molecular weight ranging from 10 to 180kDa were detected in 120 *Z. jujuba* genotypes. The UPGMA phylogenetic tree was constructed by from 120 genotypes of *Z. jujuba* collected from different region of KP, Pakistan. MAGA. 5 based on the of total protein alleles. The cluster was divided into two clusters (I and II) and cluster I are again divided into sub clusters (a and b) presented into (Fig.3).The overall intraspecific locus disparity among 120 genotypes of *Z. jujuba* is represented in (Table. 4) and Notably, L- 6, 9 and L-11, were monomorphic in *Z. jujuba* and was treated as specie specific. L1-2, L-3, L-4, L-5, L-7, L-8, L-10 and L-12 were polymorphic. These bands showed 33.333%, 50%, 70, 82.50%, 79.17%, 33.333, 70%, 79.17% and 29.166%% respectively. The locus contribution toward genetic disagreement (LCTGD) of *Z. jujuba* was 58.33% (Table 4).

Table 4: Intra locus variation toward genetic disagreement among collected genotypes of wild *Z. jujuba*

Bands	Present	Absent	Variation	Status	GD
L-1	80(66.66%)	40	33.333	Poly	0.666
L-2	60(50%)	60	50%	Poly	0.5
L-3	20(30%)	100(70%)	70%	Poly	0.3
L-4	21(17.5%)	99(82.5%)	82.50%	poly	0.175
L-5	15(12.5%)	95(79.166%)	79.17%	poly	0.125
L-6	120(100%)	0.00	Nil	mono	1.00
L-7	80(66.66%)	40	33.333	poly	0.666
L-8	20(30%)	100(70%)	70%	poly	0.3
L-9	120(100%)	0.00	Nil	mono	1.00
L-10	15(12.5%)	95(79.166%)	79.17%	poly	0.125
L-11	120(100%)	0.00	Nil	mono	1.00
L-12	65(54.1666%)	55(29.166%)	29.166%	poly	0.35

GD= 75.00% (GD= Poly loci/Total loci*100)

4. Discussion

The aim of this work was to identify the genetic diversity and a core collection of *Z. jujuba* genotypes. In current study an effort were made with respect to genetic diversity and the causes of the genetic redundancy. The current status of genetic structure is briefly discussed. Furthermore, 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.

Numerous devices are now accessible for records of required differences in the genotypes, including Phenotypic, biochemical and molecular markers [19]. However phenotypic description is the principal step in the description and alliance of crops genotypes but these are highly subjective by the environment16. In the present Phenotypic study both qualitative and phenotypic study was performed. Qualitatively, 60 trees were less vigorous whereas 40 were high 20 were vigorous. Leaf type; 100% were with alternate, Leaf shape was totally ovate. All the genotypes were with green leaf color. Tomentose; 60 genotypes wer dense, 40 were rare, 20 genotypes stems were red brown 20 were light black, 50 were brown, 10were with purple 10 were with grey colored stem. All the genotypes were with spines. Fruit color; 20, 25 genotypes were with yellow red and red brown respectively, 25 were with brown colored fruits, 15 were with red colored fruit, 10 were with yelllow colored fruit. Leaf margin; 80 genotypes were with entire leaf margin and 40 genotypes were with with serrate margins. Fruit shape; 80 genotypes were round shaped fruit, 10 genotypes were with drupe shape fruit while 30 genotypes were with oval shaped fruits. Similarly, quantatively, branching in the *Z. jujuba* was negatively correlated with leaf length whereas the leaf length was significantly positively correlated with leaf width and leaf thickness. The petiole length was positively correlated with internode length. Stem diameter was negatively correlated with fruit weight. The fruit diameter was positively correlated plant fruit length. The coefficient of variation for qualitative traits (Tree vigor, Leaf type, Leaf shape, Leaf color, Tomatoes, Stem color, Spines, Fruit color, leaf margin, Fruit shape) were 86.62581, 69.39543, 56.92408, 58.51344, 120.1332, 78.06796, 53.39941, 74.59233, 31.9402. 185.406589.

On the other side practice of genetic indications such RAPD, SSR, RFLP etc. are more consistent approaches for explanation of variation/diversity among different crops species but they are highly affluent 20, 3. Now the most easy, straight forward, cost effective and reliable methods to identify genetic variations in germplasm may be study of seeds storage protein by (SDS -PAGE) that consist of 60% of the total protein contents21, 10.These proteins are broken down to provide the basic nourishment for seed germination and seedling growth. Moreover seed proteins in nucleotide sequence are largely independent of environmental conditions22. Keeping this in view the present study was first time investigated seed protein on the basis of SDS-PAGE on *Z. jujuba* plant. Genetic diversity in black gram at biochemical level by SDS-PAGE to find out the unique and most important characteristics of specific germplasm and to document Phenotypic in seed due to High intra-specie locus contribution toward genetic disagreement SDS-PAGE could be a consistent procedure for characterization of this specie and intra-specie locus toward genetic disagreement (LCTGD) genetic dissimilarity of *Z. jujuba* was 58.33%. Notably, L-6, 9 and L-11, were monomorphic in *Z. jujuba* and was treated as specie specific. L1-2, L-3, L-4, L-5, L-7, L-8, L-10 and L-12 were polymorphic. These bands showed 33.333%, 50%, 70, 82.50%, 79.17%, 33.333, 70%, 79.17% and 29.166%% respectively. 12 loci with molecular weight ranges from 10KDa to 180KDa were detected in *Z. jujuba*. The similar result was found in the published article17, 10. Furthermore, morphology and biochemical investigation are very helpful but not enough but may be complemented with

additional based molecular markers for more accurate evaluation of genetic diversity.

5. Conclusion

In conclusion, the current investigation that the total seed protein was first time study of Z. jujuba genotypes. The genetic pool of dissimilarity within genotypes of this specie is the basis for selection as well as for plant improvement and conservation. A better knowledge of genetic diversity and its distribution in the genotypes of the studied plant is essential for its conservation and use. It will help greatly in describing what to conserve as well as where to conserve, and will enhance our information and understanding of the taxonomy, origin and evolution of Z. jujuba.

Significance Statement

There is a great potential in *Ziziphus jujuba* as a cash crop/fruit crop particularly in the arid area/ regions especially north and south areas of KP, Pakistan and requires national policy to support its cultivation

Author's contributions

NU carried out collection and experimental work. NU and NM carried out data collection, and literature search and manuscript preparation. NA, ML and MN refined the manuscript for publication. All authors have read and approved the manuscript.

6. References

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