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Morphological and biochemical characterization of Ziziphus jujube collect from remote areas of Malakand Division, Khyber Pakhtunkhwa, Pakistan

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Abstract

The present work was done in organize to estimate the genetic diversity among the *Ziziphus jujube* genotype. Genetic variation is considered as a basic feature to plants breeding and makes it viable to choose plants with appropriate traits. The methods to study genetic variation are morphological traits and SDS-PAGE. For this purpose, seeds of 100 genotype were collected. Data were recorded of 16 morphological traits in which qualitative are, Frequency, Irrigation, Plant population density, Tree vigorous intermediate (18%), large (71%) and small (11%), Leaf shape, Leaf margin, Leaf colour genotype red (6%), light brown (16%), brown(36%), dark brown(14%) and yellowish (28%), Shoot pubescence, Shoot colour, Seed shape, Shell texture was 99% and soft shell texture are 1%, Shell colour, Shell strength and the remaining traits were show no variation. And quantitative traits are Leaf length (21-52mm), Leaf width (10-29mm), Seed length (14-32mm), Seed width (1-9), Petiole length (2-7mm), 100 Seeds weight (40-250g), and number of branches. In quantitative maximum variation was found for all traits in genotype. SDS-PAGE analyses of seed storage were Which gives significant result, in total 14 bands were observed, the entire band loci are all were polymorphic, the utmost level of variation was found in B18 (0.85%) followed by B1 (0.96%) and B2 (0.94%), B4 (0.85%), B6 (0.75%) and B7 (0.73%). Similarly B12 (0.2%) revealed low level B5 (0.8%), B3 (0.9%), B14 (31%), B13 (0.34%), B11 (0.41%), B9 (0.55%), B8 (0.65%). All the traits show significant variation, for future use in selection of plant.

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Introduction

The family Rhamnaceae contains about 58 genera and 135 species. In Pakistan 6 genera and 21 sepias are present. The family are extensively in distribution and found everywhere on the earth surface. The distribution leads to the tropical, sub-tropical and temperate regions of the world but mostly founded in the Asia and America continent (Ara *et al.*, 2008).The family Rhamnaceae is also known as buckthorn family. The tree size almost medium but may be deviate, generally ranges from 7 to 10 meters at height. The trees were generally shiny deciduous foliage and a fruit are longitude shape through a slight, gloomy red covering and sweet bark, creamy/pale soft tissue adjacent a rock (Li *et al.*, 2007; Zhang *et al.*, 2010). The plants and their fruit have high medicinal value (Plastina *et al.*, 2010; Yu *et al.*, 2012).The fruit is eating and fresh form and dry. The fruit is very nutritious with potassium, phosphorus, calcium and manganese and a rich source of vitamin C and vitamin B complex and anti-oxidant content of fresh fruits is higher than most of fruits (Li *et al.*, 2007; Zhang *et al.*, 2010). The jujube plant has significant product and live in a drought condition. The plant has no resistance towards disease, insect and nematode pests. The fruit of jujube in the market is high, based on their quality andsize long fruit have high price and vice versa. The plant has become more pupilar in recent years the plantation is now occurs in different countries therefore their number is increases day by day (Ecevit *et al.*, 2008).

In any crop genetic diversity is define as the genetic variation within the populations, cultivars and landraces, produced in result of genetic recombination, mutation and introgressions (Hawkes, 1983).In excess of the most recent few decades, the genetic diversity in several plants depends on their morphological characters and through it were evaluated(Nisar *et al.*, 2016). In propagation increases the probabilities for success in developing highly productive new cultivars with moral quality properties over a long period of time due the use of highly diverse germplasm (Bockelman *et al.*, 2010; Horsley *et al.*, 1995).Genetic diversity of interrelated uninhabited species or crop ancestors can also be central to use in breeding to resolve difficulties related to crop disappointment (Geleta, 2007). Heritable variety nside and among crop populaces is resolute using procedures such as morphological depiction, electrophoresis, and DNA (or molecular) marker analysis (Nisar *et al.*, 2008; Ghafoor *et al.*, 2008; Murad *et al.,* 2017). The aim of the study is to explore the genetic divergence among the genotype of ziziphus jujube, and provide stress tolerance specie to a particular area.

Methods and materials

The research work was carried out both in field and in laboratory. The methods and procedures for each experiment are given below.

Exploration and collection

Different exploratory trips were arranged to the un-explored agro-filed in district Swat and Dir Khyber Pakhtunkhwa Pakistan. The total 100 samples of *zizipusJujuba,* seeds where collected from two Districts i.e. Dir and swat and some from Malakand Agency Province Khyber Pakhtunkhwa Pakistan, they were used for the evolution the genetic diversity in both agronomical and biochemical markers. The agronomical characters were further categories into qualitative and quantitative traits. The traits were scored in proper time-frame defined in the IPGRI-1994 descriptor.

Morphological characterization

Morphological characterization was carried out for estimating of genetic diversity among the healthy plant of *Ziziphus jujube* tree were divided into two categories qualitative and quantitative traits.

Qualitative traits

A total of eight qualitative traits such as, Frequency, Irrigation, Plant population density, Tree vigorous, Leaf shape, Leaf margin, Leaf colour, Shoot pubescence, Shoot colour, Seed shape, Shell texture, Shell colour, Shell strength.

Quantitative traits

And quantitative traits are Leaf length, Leaf width, fruit length, fruit width, Petiole length, 100 Seeds weight, and number of branches.

Biochemical characterization

The seeds from each sample (from which we have obtained seedlings) were also processed for biochemical characterization. About 5 seeds were grinded into fine powder for SDS-PAGE. After grinding 0.02g of fine flour were taken for crude protein extraction. In each sample 400ul of PEB (0.60g Tris-pure, 0.2g SDS-sigma, 30g Urea-sigma and1ml 2-Mercaptoethanol with pH-4.5) was added. After the addition of PEB the samples were centrifuged at 12000rpm for 20min under 4°C. The crude protein was in the supernatant and the cell-lysate precipitated at the bottom of each E-tube. The E-tube was vortexes and then centrifuged at 14000 rpm for 30 minutes at 25 °C.

There were 12.5% polyacrylamide gels (separation gel) (0.4% SDS, 3M Tris-HCl pH 9) and the staking gel 4.5% (0.4% SDS, 0.4M Tris-HCl pH 7) used for the electrophoretic technique. Then put 10µl of each sample into each well (left to right). Put the gels in the electrode buffer solution (0.125% SDS, 129 M Glycine, 0.025 M Tris) and run it on the 120 V.

Put the gels to stained the protein bands in the staining solution (dissolved 0.2 % in 40% methanol, 10% glacial acetic acid and 50% distal water, with the ratio of 4:1:5) for 30 to 180 minutes. Gels were then de-stain with solution (20% methanol, 5% acetic acid and 75% distal water with ratio of 4:1:15) to de-stained the non-proteinous part at overnight. Data were analyzed using "1" for the presence of protein band and "0" for absent protein band (M.S Excel 2007 sheet) for all genotypes.

These match coefficients were used to find out the relationship among study genotype by cluster analysis using Un-weighted Pair Group Matrix Average strategies (UPGMA) and dendrogram was constructed by software PECORD.

Results and discussion

Qualitative traits

In the current investigation different types of five fruit colour (brown, dark brown, light brown, yellowish and red) were recorded in the 100 genotype red colour with frequency of 6%, light brown with frequency of 16%, were found with brown colour with frequency 36%, dark brown colour with frequency 14% and the yellowish with frequency 28%.

Table 1. Frequency distribution of eight qualitative traits.

Traits	Sub-type	Age %
Irrigation	Rainy	100%
	tube well	0%
fruit color	Brown	36%
	Dark brown	14%
	Light brown	16%
	yellowish	28%
	Red	6%
plant population density	More	0%
	Less	100%
Tree vigorous	Small	11%
	intermediate	18%
	Large	71%
leaf shape	Dentate	100%
leaf margin	flateshape	100%
leaf color	green	100%
shell texture	soft	1%
	hard	99%

It indicates us variability in the fruit colour of zizipus. The tree vigorous divided into three type (intermediate, large, small) the frequency of intermediate is 18%, large with frequency 71% and the small with frequency 11%.In case of leaf margin all the 100 genotype were dentate, which show no variation. The leaflet shape of 100 genotypes of zizipus was all are flat shape which no variation among the genotype.

During the present investigation the leaf colour of all 100 genotype were green which show no variation among the leaf colour of the genotype.

The shell texture was divided into two groups (hard, soft) the frequency of hard shell texture was 99% and frequency of soft shell texture are 1% which is SW19.the genotype collect from swat show (table.1).

Table 2. Descriptive statistic of eight quantitative traits.

Parameters	Mean	Standard Error	Standard Deviation	Sample Variance	Range		CV%
					Minimum	Maximum	
LL	38.60	0.69	6.90	47.64	21.00	52.00	0.18
LW	18.96	0.40	3.96	15.68	10.00	29.00	0.21
PL	3.97	0.11	1.14	1.30	2.00	7.00	0.29
IL	15.69	0.32	3.15	9.93	6.00	25.00	0.20
FL	23.61	0.40	3.96	15.72	14.00	32.00	0.17
FW	16.18	0.28	2.82	7.93	11.00	23.00	0.17
100W	138.34	5.45	54.49	2968.89	40.00	250.00	0.39
B	2.46	0.25	2.50	6.27	1.00	9.00	1.02

Note :LL leaf length, LW leaf width, PL pod length, IL inter node length, FL fruit length, FW fruit width, 100W 100 seed weight, B branches per plant.

Quantitative traits

Leaf length

The leaf length was studied in the population showing the following variation. The leaf length from 21-52mm with a mean value 38.60, Standard deviation 6.90, Standard error 0.69, samples variance 47.64, CV% is 0.18 and with maximum value 52 while the minimum value of 21 was found. The frequency distribution of this genotype divided the total studied genotype into three group show in the (Table 2).

Table 3. Total genetic diversity was present in 14 bands of 100 genotypes.

Bands	F	P %	A %	TDG %
B1	4.00	4.00	96.00	0.96
B2	6.00	6.00	94.00	0.94
B3	10.00	10.00	90.00	0.90
B4	15.00	15.00	85.00	0.85
B5	20.00	20.00	80.00	0.80
B6	25.00	25.00	75.00	0.75
B7	27.00	27.00	73.00	0.73
B8	35.00	35.00	65.00	0.65
B9	45.00	45.00	55.00	0.55
B10	36.00	36.00	64.00	0.64
B11	59.00	59.00	41.00	0.41
B12	80.00	80.00	20.00	0.20
B13	66.00	66.00	34.00	0.34
B14	69.00	69.00	31.00	0.31

F=frequency, P%=present percent, A%=absent percent, TDG%=total genetic diversity.

Leaf width

The leaf width was divided into three groups such as, low, medium, and high range from with maximum value 29 while with minimum value as 10 and with mean value 18.96, standard error 0.40, standard deviation 3.96 and sample variance 15.68 (Table 2.

Petiole length

In this case the petiole length divided into three groups, range from 2-7mm. The mean value 3.97 standard error 0.11, standard deviation 1.14 and sample variance 1.30 the maximum value 7 and minimum value 2 (Table 2).

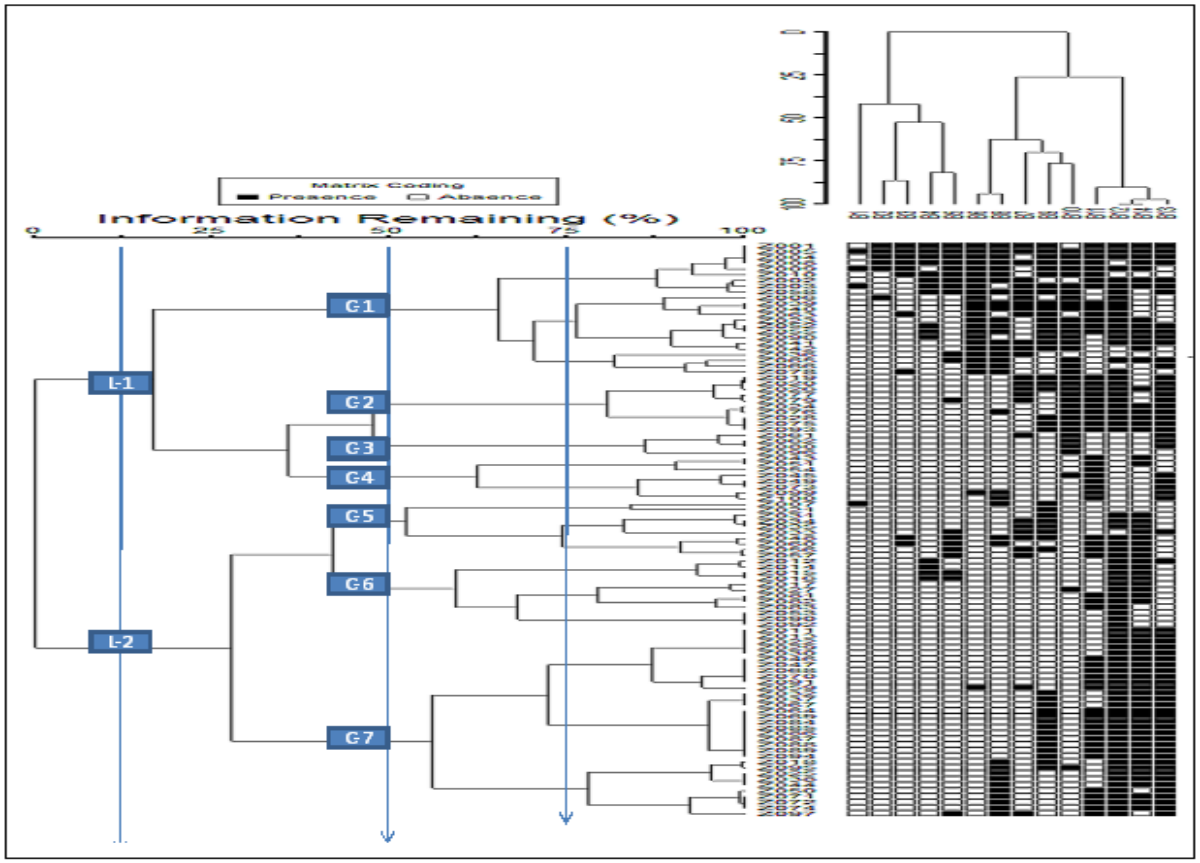

Fig. 1. SDS PAGE dendogram tree for bands of 100 genotypes.

Internodes

The internodes length was studied in the population. The internodes length ranges from 6-25mm with a mean value 15.69, Standard deviation 3.15, Standard error 0.32; sample variance 9.93, CV% for the trait was 0.20(Table2).

The maximum value of 25 while the minimum value was 6mm.The frequency distribution of this genotype divided the total studied genotype into three groups.

Fruit length

The fruit length was studied in the population showing the following variation. The fruit length ranges from 14 to 32mm with a mean value 23.61, Standard deviation 3.96, Standard error 0.40; sample variance 15.72, CV% for the trait was 0.17. The maximum value of was 32mm while the minimum value of 14 (Table 2).

100 seed weight

The frequency distribution of this genotype divided the total studied genotype into three groups. The 100 seed weight was studied in the population. The 100 seed weight from 40-250 with a mean value 138.34, Standard deviation 54.49, Standard error 5.45, sample variance 2968.89, CV% for the trait was 0.39. The maximum value of 250 seed while the minimum value of 40 (Table.2).

Fruit width

The frequency distribution of these divided the total studied genotype into three group. In the present study three types of fruit width were listed. Range from 1-9 with mean value 2.46, standard error 0.25 and standard deviation 2.50 and samples variance 6.27. Series of digit of plant 1 to 8.0 with average value of 3.08 and sample variance 2.13 standard error 0.15, standard deviation 1.46.Maximum range 2.0 while minimum range 1.0 CV% 0.47 (Table2). Numerous previous studies (Kundu *et al.*, 1995; Kundi *et al.*, 1989; Saini *et al.*, 1994) sustain result as they as well create a large sort of variation in fruit and leaf objective and morphological traits in diverse ber cultivars in India.

The current consequences are in conformity with pronouncement of other researchers (Ecevit *et al.*, 2008). Fruit per bearing shoot is an key usually use to assess output of jujube trees, and only 8.5% of cultivars might put extra than one fruit per bearing shoot (Gao *et al.*, 2009; Liu *et al.*, 2009).

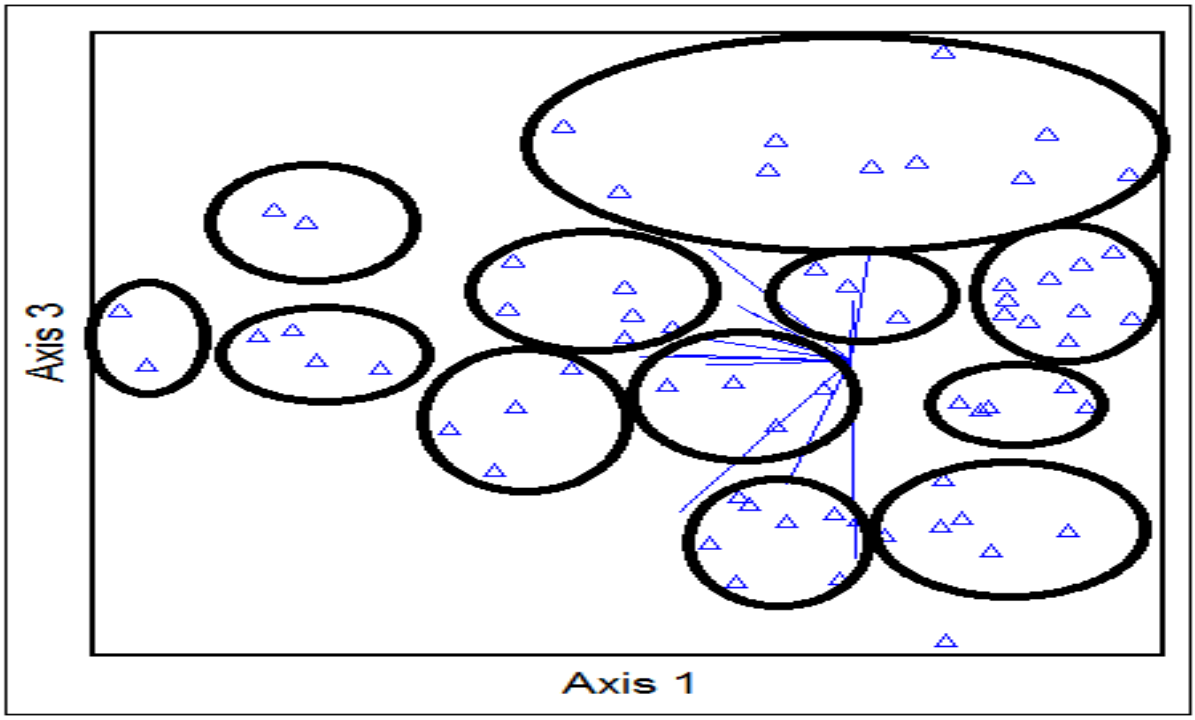

Fig. 2. Scatter plot for 100 genotypes of their SDS PAGE data.

The humidity of jujube fruit ranged from 69.55% to 75.33% which was in agreement with the consequences of other study (Gao *et al.*, 2012).

Protein profiling

On the basis of storage seed protein profile using SDS-PAGE of 100 genotype of zizphusjujuba. The parents / genotypes used in the study were different released varieties, therefore, it was thought justified to determine their differences among themselves on the basis of the profile of their seed storage proteins.

Characterization of genotypes based on seed storage protein/subunits is well documented in different groups of crops such as blackgram(Ghafoor and Ahmad, 2005; Ghafoor *et al.,* 2002), *Capsicum annuum*L. (Anu and Peter, 2003), *Vigna spp* (Sharma, 2012; Chaoudhary, 2013).

In the present investigation all the genotype of zizipus were tested through proteomic assay as similar as overreveal, in order to estimate the genetic diversity in seed storage protein.SDS PAGE was carried out in various combination and was revealed that 15% acryl amide gel concentration and 10 microliter sample gave the best result, for this a dendogram tree was construct show in (Fig. 1) total 14 bands were

observing the band picture were also scan show (fig. 3), all were polymorphic there was no monomorphic bands were found, the utmost level of variation was found in B18 (0.85%) followed by B1 (0.96%) and B2 (0.94%), B4 (0.85%), B6 (0.75%) and B7 (0.73%) polymorphism respectively. Similarly B12 (0.2%) revealed low level of i.e.B5 (0.8%), B3 (0.9%), B14 (31%), B13 (0.34%), B11 (0.41%), B9 (0.55%), B8 (0.65%) (Table.3). the dendogram tree was formed which divided on Linkage distance of 12.5 was taken and genotype divided into two lineages and this lineage further was divided into seven clusters. Cluster 1 had 26 genotype, Cluster 2 had 10 genotype, Cluster 3 had 6 genotype, Cluster 4 had 8 genotype, Cluster 5 had 10 genotype, Cluster 6 had 11 genotype while Cluster 7 had 33 genotype.

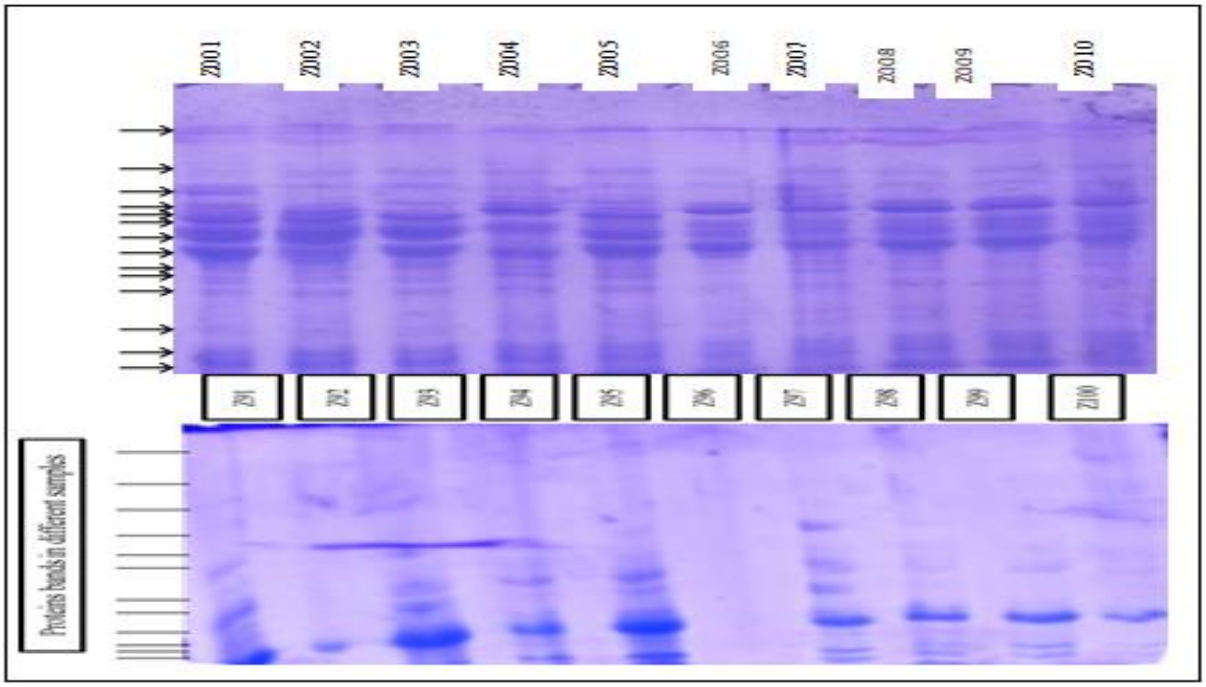

Fig. 3. Gel picture of *ziziphus jujube*.

The genetic association within landraces, found through TWCA was reconfirmed through Scatter Plots and Principal Component Analysis (PCA). Similar pattern of genetic linkage among landraces were found that indicated the coordination of both these numerical techniques (i.e. TWCA and PCA) for exploring the genetic association and genetic diversity among 100 genotypes of zizipus (Fig.2).

Conclusion

It was concluded that to investigate genetic diversity on the bases of agro-morphological characters and protein profiling, a total 18 morphological traits were record.On the basis of frequency distribution high level of genetic diversity was found in qualitative characters except Leaf shape, Leaf margin, Leaf color. In case of quantitative characters, maximum variation was found for all traits in genotype. SDS-PAGE analyses of seed storage were Which gives significant result, in total 14 bands were observed, the entire band loci are all were polymorphic, there were no monomorphic bands found, the utmost level of variation was found in B18 (0.85%) followed by B1 (0.96%) and B2 (0.94%), B4 (0.85%) polymorphism respectively. Similarly B12 (0.2%) revealed low level B5 (0.8%), B3 (0.9%).

In each clusters the genotype were from different climatic regions of Malakand Division, Khyber Pakhtunkhwa**.** These genotype shown some distinctiveness and interestingly. It makes them important for further investigation.

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