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Assessment of morphological and biochemical diversity of *Berberis lycium* in three districts of Azad Jammu and Kashmir

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Abstract

This research study was carried out during 2012-2014 in the Department of Plant Breeding and Molecular Genetics for the estimation of morphological and biochemical diversity of *Berberis lycium* in three districts of Azad Jammu and Kashmir. The study was important for the detection of morphological and biochemical diversity of berberis in selected areas. Nine morphological and two biochemical characters were used to elucidate morphological and biochemical diversity. Morphological studies showed that ecotypes Hurnamaira and Tolipeer from district Poonch are more diverse and showed most of the variation. Correlation studies, factor loadings and cluster analysis revealed that morphological traits plant height, fruit diameter, 100-fruit weight and days to fruit maturity are intended for most of the variation. Biochemical analysis showed that ecotypes Pallandri and Morifurman Shah were more diverse amongst the others on the basis of antioxidant activity and total phenolic contents respectively. On the basis of present study it was concluded that ecotypes Hurnamaira, Tolipeer, Pullandri, and Morifurman Shah are more diverse and resposible for most of the variation this may be due to environmental factors.

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Introduction

Herbs and medicinal plants make up a large part of the ingredients used by people throughout the ages to help combat disease and illness. Scientific experiments on the antimicrobial properties of plant components were first acknowledged in the late 19th century (Stiffness and Douros 1982). *Berberis lycium* belongs to family Berberidaceae, which contains approximately 15 genera and 650 species. It has chromosome number of 2n=28. It is found in temperate regions of the northern hemisphere (Bottini *et al.,* 2002). It is locally known as "rasaut" and commonly as "barberry".

Berberis shows extremely high morpho-pathological and phytochemical variation making taxonomic identification difficult (Rao *et al.,* 1998; Khan *et al.,* 2014). Overlapping characters, especially in leaves, stem, and flower and berry size make field identification often challenging. Leaf texture and serrations vary from season to season and with the age of the plant in some of the species (Rao *et al.,* 1998; Tiwari and Adhikari, 2011; Lucas *et al.,* 2012). Such a variation may be due to environmental and hybridization effects.

In Pakistan, there are 29 different Berberis species (20 species, 6 subspecies, 2 varieties and 1 forma). These species are mostly distributed in the Northern mountainous ranges (Khan *et al.,* 2014a; eFlora, 2014). Stewart and Stewart (1935) were the first to report Berberis from Gilgit-Baltistan (Khan *et al.,* 2014a).

At high altitude berberis species are dwarf and less productive, this morphological diversity in Berberis may be due to environmental factors like errosion, deforestation and overexploitation (Malik *et al.,* 1994). In field, more than one species of Berberis usually occur together. The nature of the Berberis species varies with place charactres like pollen morphology, flower colour, bark thickness, stem girth and leaf shape show great variations (Rao *et al.,* 1999). For distinguishing one specie to the other conventional techniques based on macro-morphological characters may be effective which may share similar histological characteristics, making microscopic examination not so accurate (Yan *et al.,* 2007). Different species of the same genus may have totally different or weaker biochemical action like barberry as compaired with the authentic counterpart (Yip *et al.,* 2007).

In Pakistan, from ancient times, various parts of medicinal plants have been used to treat specific diseases. Today, there is widespread interest in drugs derived from plants (Zaika, 1975). The short comings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (Gordon and David, 2001). To resolve the possible and valuable use of herbal medicine, it is necessary to improve the study of medicinal plants that get place in tradition (Nair *et al.,* 2005).

Therefore, more objective and definitive methods like DPPH assay, anti-proliferative and anti-migratory activity, antimicrobial activity and phenolics are necessary for identification and authentication of diversity in herbs. In Berberisberberine is consider to be an active alkaloid having pharmacological effects (Saied *et al.,* 2007). Existing gene pool can be characterized for morphological biochemical and molecular diversity to develop a reliable identification key which would lead to characterization, selection and approval of better germplasm for further cultivation. Multivariate techniques are widely used in genetics to study genetic variability in the various germplasm of plants (Phundan and Narayanam, 2009).

Multivariate techniques include principal component and cluster analysis which have analogous efficacy to establish the most appropriate cross combinations. For determining family relationships cluster analysis is a suitable method (Mellingers, 1972). The common aim of cluster analysis is to increase the homogeneity within clusters and increasing heterogeneity between clusters (Hair *et al.,* 2006). The major benefit of using principal component analysis over cluster analysis is

that each genotype can be assigned to one group only (Mohammadi, 2002).

This study was aimed at assessment of geographic distribution, morphological variations, biochemical fluctuations and taxonomic anomalies of *Berberis lycium* found and reported from Poonch, Bagh and Sudhnoti in Azad Jammu and Kashmir. Study will help to field surveyors, taxonomists and several other researchers equally in their future concerned projects. Moreover, study calculates geographic distribution of Berberis across these geographical areas for the first time.

Materials and methods

The research study was carried out in 2012-214 in the department of plant breeding and molecular genetics at The University of Poonch Rawalakot to elucidate the biodiversity of *Berberis lycium*in three districts viz; Poonch, Bagh and Sudhnoti of Azad Jammu and Kashmir the plant populations were visited three times in selected locations Hurnamaira, Datot, Tolipeer, Topa, Mori Furman Shah, Hullar, Trarkhal, Baloch and Pullandri of Azad Jammu and Kashmir during flowering, fruiting and mature fruit harvesting and comprehensive data were collected. These locations were selected because of difference in altitude and diverse agro-climatic conditions.

Morphological Studies

Ten plants were randomly selected from each selected location of three districts of Azad Jammu and Kashmir for morphological analysis. Morphological data was collected from the selected plants only. The morphological studies were conducted for the following parameters.

Plant height (cm), Plant canopy (cm), Number of branches per plant, Number of thorns on main branch, Main stem diameter (cm), Days from flower initiation to 50% flowering, Days from fruit initiation to 90% fruit maturity, Fruit diameter (cm), 100-fruit weight (g).

Biochemical studies

Barberry provides an excellent source of vitamins and minerals for human consumption. The biochemical studies were one of the important parts of this research. Following biochemical constituents in barberry were estimated using the methods described by AOAC 2005.

Antioxidant activity

The radical scavenging activity of the extract was tested against the scavenging of DPPH radicals. The extract with high antioxidant activity exhibits strong scavenging activity against the generation of OH radical. The antioxidant activity of *Bereris lycium* (fruits) was measured using the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical as described by Hatano *et al.,* (1988). The activity was expressed as percentage scavenging of DPPH by the extracts calculated as;

$$\text{DPPH radical scavenging activity} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

Displayed in graph of scavenging effect percentage against the extract concentration.

Total phenolic contents

The total phenolic contents were analyzed using the Folin-ciocalteau method with some modification (Ghafoor and Choi, 2009).

The absorbance was read at 765 nm on a spectrophotometer. Measurements were recorded in triplicates. Gallic acid of 1 mg/mL was used as the standard and the total phenolic compounds of the samples were expressed in milligram Gallic acid equivalent (GAE) per 100 mL (mg GAE/100 mL).

Statistical analysis

The data were analyzed, to compute phenotypic correlation coefficients between the characters (Snedecor, 1956), cluster and principal component analysis (Sneath and Sokal, 1973) by the use of computer software 'Statistica' (www.statsoft.com), PAST (Hammer *et al.,* 2001) and 'SPSS' 20 (www.spss.com). Cluster analysis was conducted on

the basis of average distances and ecotypes in each cluster were then analyzed for basic statistics.

Results and discussion

Mean values and simple correlation coefficient

Plant height for 9 ecotypes of barberry showed variation in a range of 134 cm to 188 cm, maximum plant height was recorded in ecotypes of district Poonch (Topa 188 cm and Hurnamaira 174 cm. While minimum plant height was observed in ecotype Tolipeer (134 cm) belonging to district Poonch. Table 1 indicated that plant height showed highly significant and positive correlation with plant canopy and number of branches, significant positive correlation was observed with main stem diameter where as non-significant positive correlation was observed with number of thorns, days to fruit maturity and 100-fruit weight. Negative and non significant correlation was observed with days to 50% flowering and fruit diameter. Findings were aided by the findings of Razi *et al.* (2013).

Table 1. Simple correlation coefficients for morphological traits in barberry ecotypes.

	PH	PC	NB	NT	MSD	DTF	DFM	FD
PC	0.854**							
NB	0.875**	0.563						
NT	0.473	0.281	0.569					
MSD	0.603*	0.200	0.73*	0.466				
DTF	-0.398	0.60*	-0.18	-0.042	-0.004			
DFM	0.006	0.366	0.173	-0.246	0.380	0.188		
FD	-0.200	0.066	-0.213	-0.355	-0.401	-0.032	-0.362	
100-FW	0.171	0.181	0.070	0.171	0.283	-0.108	0.027	0.653*

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Plant canopy variation in response to diverse areas revealed significant differences among these ecotypes. Mean values for plant canopy ranged from 46 cm-9 cm. Maximum mean value was observed in location Hurnamaira (91.2 cm) from district Poonch and minimum was recorded in Tolipeer (46 cm) also belonging from district. Table 1 verified that plant canopy was highly significant positive correlated with plant height non significantly positively correlated with number of branches, number of thorns, main stem diameter, fruit diameter and 100-fruit weight. While it was significantly but negatively correlated with days to 50% flowering while negatively and non significantly correlated with days to 90% fruit maturity. Razi *et al.* (2013) also performed similar kind of studies.

Table 2. Principal components for morphological traits in barberry ecotypes.

PC	Eigen value	% variance	Cumulative variance%
1	3.464	38.48	38.48
2	2.12095	23.56	62.04
3	1.27789	14.19	76.23
4	1.01147	11.23	87.46
5	0.59204	6.57	94.03
6	0.44957	4.99	99.02
7	0.07497	0.83	99.85
8	0.00911	0.10	99.95

Joliff cut off = 0.7.

Number of branches per plant of barberry in response to different areas showed wide range of differences among each other. Mean values for this parameter ranged from 15-25 branches per plant in different Barberry ecotypes. Maximum mean value was recorded in ecotype Topa (25) from District Poonch population whereas ecotype Hullar from District Bagh had mean value (15) and showed lowest ranking in

this parameter. Table 1 showed that number of branches per plant is highly significant and positively correlated plant height significantly and positively correlated with main stem diameter, non significantly correlated with plant canopy, number of thorns, days to 90% fruit maturity, fruit diameter and 100-fruit weight. While it was negatively and non-significantly correlated with days to 50% flowering and fruit diameter. Results were revealed with the results of Razi *et al.* (2013).

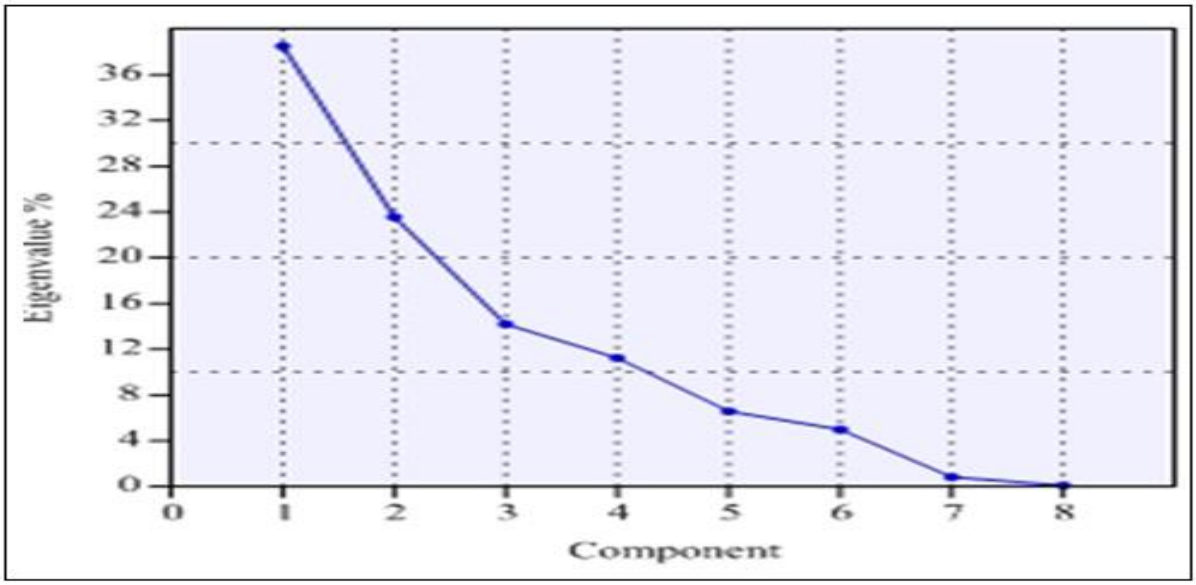

Fig. 1. Scree plot for morphological traits.

Number of thorns on main branch of each ecotype in response to different areas ranged from 21-34 thorns per main branch in different barberry ecotypes. Maximum mean value was recorded in ecotype Pullandri from district Sudhnoti with 34 thorns on main branch whereas ecotype Tolipeer from district Poonch showed minimum mean value 21 in this parameter.

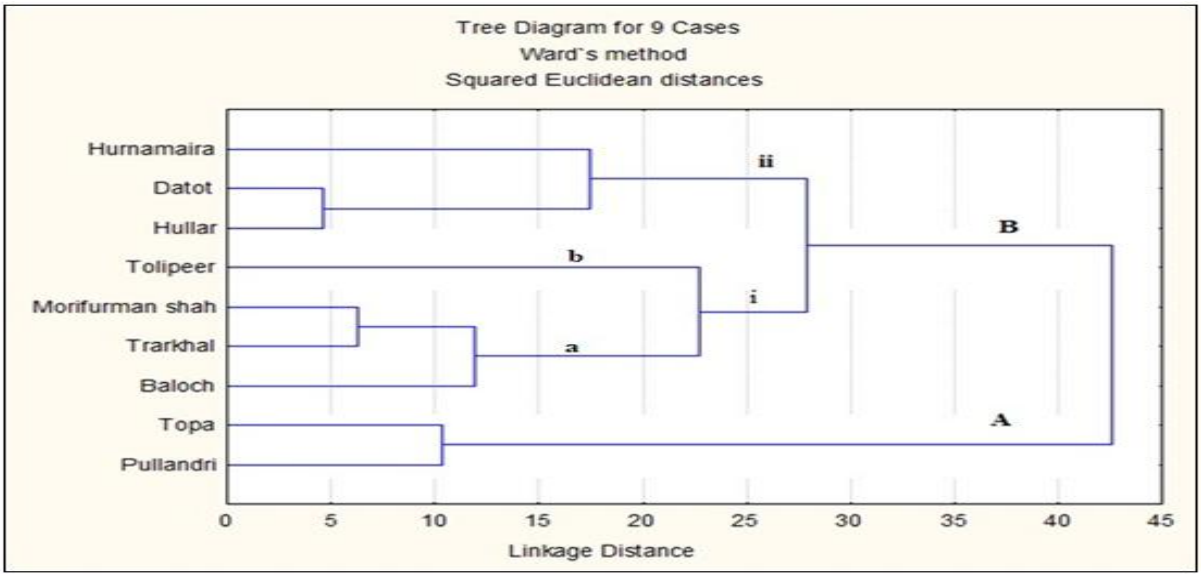

Fig. 2. Tree diagram based on cases 9 barberry ecotypes.

Table 1 showed that number of thorns on main branch has positive non-significant correlation with plant height, plant canopy, number of branches, main stem diameter and 100-fruit weight while it was

negatively and non-significantly correlated with days to 50% flowering, days to 90% fruit maturity and fruit diameter. Razi *et al.* (2013) explained similar kind of work.

Main stem diameter of barberry ecotypes in response to different areas do not showed enough variation among each other. Mean values for main stem diameter ranged from 4.8 cm-6.8 cm in different barberry ecotypes. Maximum mean value was recorded in Pullandri 6.8 cm whereas Hurnamaira had lowest plant diameter of 4.8 cm. Table 1 showed that main stem diameter was positively and significantly correlated with plant height and number of branches, positively and non-significantly correlated with plant canopy, number of thorns, days to 90% fruit maturity and 100-fruit weight. While it was negatively and non-significantly correlated with days to 50% flowering and fruit diameter.

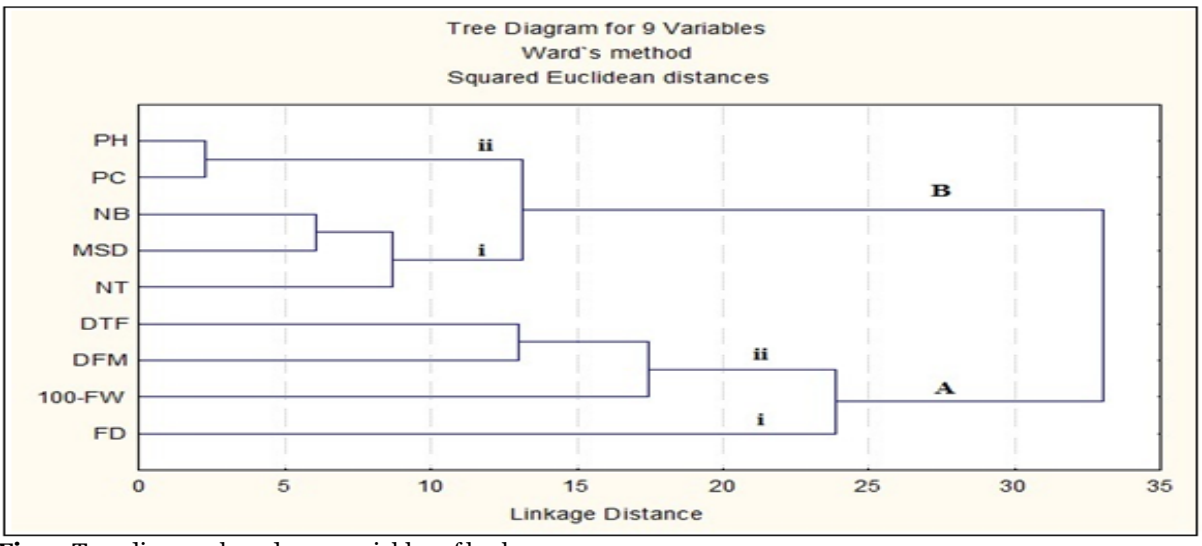

Fig. 3. Tree diagram based on 9 variables of barberry.

Days to 50% flowering varied from 18 days to 25 days, maximum value was observed in Baloch ecotype which was (25 days), followed by Tolipeer (23 days) and Morifurman Shah with the value of (22 days), minimum value was observed in Datot (18 days).

Table 1 showed that days to 50% flowering was non-significantly and positively correlated with days to 90% fruit maturity, while it was negatively but significantly correlated with plant canopy.

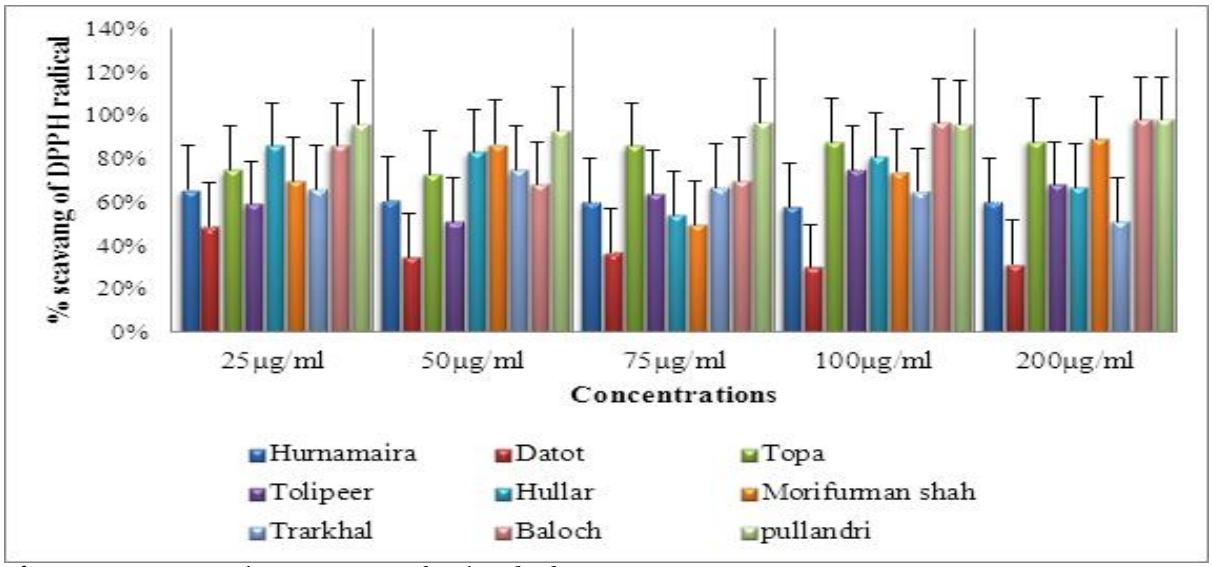

Fig. 4. DPPH Scavenging percentage of various barberry ecotypes.

Non-significantly and positively correlated with days to 90% fruit maturity non-significantly negatively correlated with plant height, number of branches, number of thorns, main stem diameter, fruit diameter and 100-fruit weight.

Data regarding the days to 90% fruit maturity of barberry in response to different areas exposed a wide range of variation. Mean values for days to 90% fruit maturity ranged from 63-108 days.

Maximum days for fruit maturity were reported in Topa (Belongs to district Poonch) 108 days and took the greater time to become mature in all population, whereas ecotype Hurnamaira from Poonch with (63 days) showed the minimum days to fruit maturity among all ecotypes in three districts.

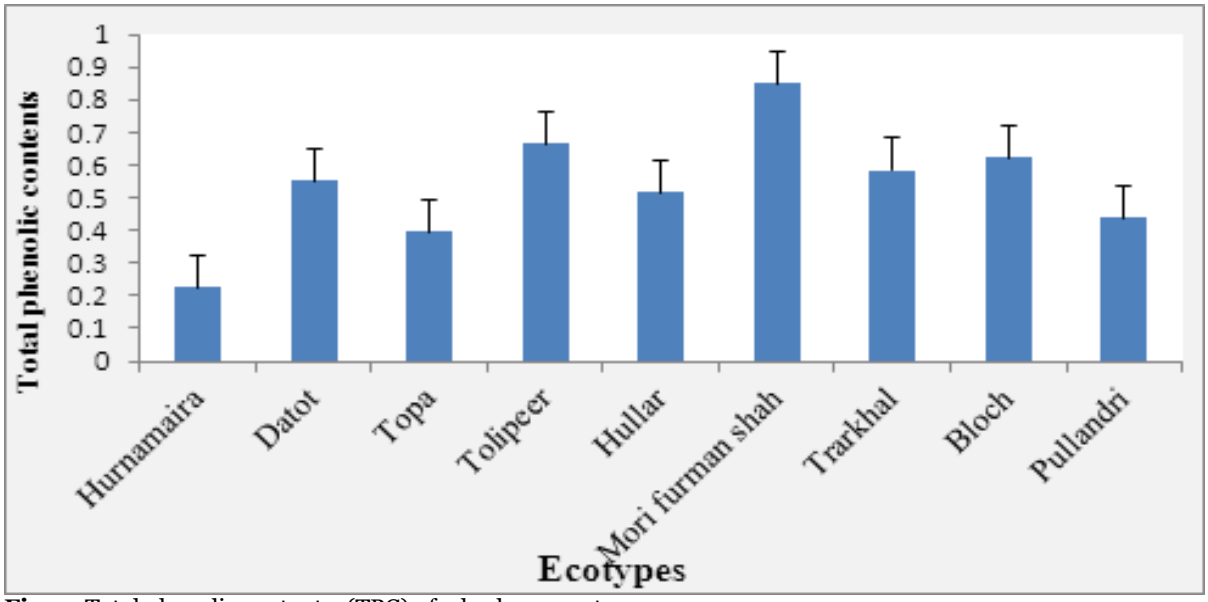

Fig. 5. Total phenolic contents: (TPC) of 9 barberry ecotypes.

Table 1 depicted that days to 90% fruit maturity was non-significantly and positively correlated with plant height, number of branches, main stem diameter, days to 50% flowering and 100-fruit weight while it was negatively and non-significantly correlated with plant canopy, number of thorns and fruit diameter.

Fruit diameter of barberry in response to different areas revealed a wide range of differences with each other. Mean values for fruit size ranged from 0.06-0.09 cm in various barberry ecotypes. Maximum mean value was reported in ecotype Datot and Morifurman Shah (0.09 cm) from district Poonch and Bagh. Whereas, ecotypes Tolipeer and Pullandri with (0.06 cm) showed minimum mean value from district Poonch and Pullandri. Table 1 indicated that fruit diameter was non-significantly and positively correlated with plant canopy significantly but negatively correlated with 100-fruit weight non-significantly and negatively correlated with plant height, number of branches, number of thorns, main stem diameter, days to 50% flowering and days to 90% fruit maturity. Similar kind of work was performed by Razi *et al.* (2013).

100-fruit weight of barberry in response to different areas showed a wide range of variation. Mean values for this parameter ranged from 19g-26g of plant in different ecotypes. Maximum mean value was reported in ecotype Pullandri (26g) from district Sudhnoti population whereas ecotypes Morifurman Shah from district Bagh had mean value 19 g and showing lowest value. Table 1 indicated that 100-fruit weight showed positive non-significant correlation with plant height, plant canopy, number of branches, number of thorns, main stem diameter and days to 90% fruit maturity. While it showed negative significant correlation with fruit diameter and

negative non-significant correlation with days to 50% flowering. Results were supported by Razi *et al.* (2013).

Principal component analysis

Principle component analysis for morphological traits in various ecotypes of barberry was shown in Table 2. Four components were found significant as Joliff cut off was 0.7, PC 1 showed maximum Eigen value of 3.464 and minimum Eigen value of 1.01 was observed in PC 4. All the components contributed 87.46% variability among all ecotypes. PC 1 explained maximum variance (38.48%) followed by PC 2 (23.56%), PC 3 (14.19%) and PC 4 (11.23%) of the total variance explained.

Scree plot for morphological traits

Scree plot of 8 principle components was drawn for morphological traits, which indicated that first four principle components have most of contribution to total variance of ecotypes for morphological traits.

It was observed that line which showing the variance contribution of PC's was going in steep manner up to about principle component number 4 and then it started to become straight, which indicates that first 4 components were main contributors of total variance and among these first six PC 1 and PC 2 contributed more than 50% of variance, which was contributed by first four PC's. It also verified the results of table which was based on principal component for morphological traits in barberry ecotypes.

Loadings for morpho-physiological traits in ecotypes of barberry

Loadings for factor 1

In loadings for factor 1 the maximum positive load was observed for plant height (0.92 and minimum negative load was observed for days to 50% flowering (-0.28). It was evident from the correlation analysis that plant height was significantly correlated with plant canopy, number of branches and main stem diameter as these traits promote vegetative growth so, it can be called as effective factor for "biomass".

Loadings for factor 2

In loadings for factor 2 the maximum positive load was observed for days to 90% fruit maturity (0.76) followed by days to 50% flowering (0.62), maximum negative load was observed for plant canopy (-0.67), fruit diameter (-0.58) and minimum negative load was observed for plant height (-0.21). Correlation analysis also verified that days to 90% fruit maturity was non-significantly correlated with vegetative growth characters, days to fruit 90% maturity is closely linked with days to 50% flowering so, it can be called as effective factor for "fruit maturity".

Loadings for factor 3

In loadings for factor 3 the maximum positive load was observed for number of branches (0.40) followed by fruit diameter (0.38) maximum negative load was observed for100-Fruit weight (-0.86), and minimum negative load was observed for plant canopy (-0.09). Correlation analysis it was also concluded that 100-fruit weight showed non-significant correlation with all other characters from this we can conclude that if number of branches per plant increases fruit weight will decrease so, it can be called as effective "factor for branches".

Loadings for factor 4

In loadings for factor 4 the maximum positive load was observed for days to 90% fruit maturity (0.59), fruit diameter (0.17) maximum negative load was observed for number of thorns (-0.614), days to 50% flowering (-0.40) and minimum negative load was observed for100-fruit weight (-0.14). In correlation analysis it was verified that days to 90% fruit maturity is non significantly correlated with all other characters, maximum negative load was observed with number of thorns, if number of thorns decreases fruit maturity will increase so, it can be called as effective factor for "fruit maturity".

Cluster analysis

Tree diagram based on 9 barberry ecotypes

The tree diagram based on 9 ecotypes of barberry displayed in figure 2. The figure indicated two main clusters at linkage distance 43. Clusters were named

as cluster A and cluster B. Main cluster B was further divided into sub-clusters i and ii based on similarities and differences. Cluster A comprised of two ecotypes Topa and Pullandri. They are present in the same cluster and have close linkage distance, They did not show much variation. Main cluster B consists of two sub clusters i and ii. Sub-cluster i is further divided in to two sub sub clusters a and b. Sub sub cluster a consists of three ecotypes Morifurman Shah, Trarkhal and Baloch.

In these three ecotypes Baloch is an outlier and showed diversity, while Morifurman Shah and Trarkhal are same at the linkage distance of about 6.5. Sub sub cluster b consists of only one ecotype Tolipeer which is an outlier. Tolipeer showed maximum diversity among all the ecotypes as maximum linkage distance 22.5 from origin was observed and present as out lier in sub cluster of main cluster B. From main cluster B sub cluster ii consists of three ecotypes Hurnamaira, Datot and Hullar. Datot and Hullar which were present in same cluster at the linkage distance of about 4.8 they showed minimum diversity among all the ecotypes because very little distance from origin was observed. Hurnamaira is an outlier in this cluster and it showed maximum diversity in this cluster. Main cluster A consists of two ecotypes Topa and Pullandri which are genetically linked at the linkage distance of 10.

Tree diagram based on variables

The tree diagram based on 9 variables of barberry displayed in figure 3. The figure indicated two main clusters at linkage distance 33. Clusters were named as cluster A and cluster B. Main cluster A is further divided into two sub clusters i and ii sub-cluster i consists of only one character "Fruit diameter" which has distinct relationship with other characters and showed non-significant correlation with all other characters as indicated in table 4.10. Sub-cluster ii consists of three characters 100-Fruit weight, days to 90% fruit maturity and days to 50% flowering. 100-fruit weight showed variation while days to 90% fruit maturity and days to 50% flowering are linked at the linkage distance of 13. Main cluster B is further divided in to two sub clusters i and ii at the linkage distance of 13. Sub-cluster i consists of three traits number of thorns, main stem diameter and number of branches. Number of thorns showed distinct relation with other characters in this sub cluster while main stem diameter and number of branches are genetically linked. Sub cluster ii consists of two characters plant canopy and plant height has minimum linkage distance and showed that they have close genetic distance and significantly correlated with each other as depicted in table 1.

Biochemical Studies

DPPH radical scavenging assay

The free radical scavenging activities of the ethnolic extract of nine barberry ecotypes was evaluated by the scavenging of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assays.

The radical scavenging activity of the extract was tested against the scavenging of DPPH radicals.The extract with high antioxidant activity exhibited strong scavenging activity against the generation of OH radical. Interestingly, the crude extract was able to decolourize the DPPH at a very low concentration and the radical scavenging activity of ethanolic extract of *Berberis lycium* was even range from 30%-98% amongst different barberry populations at various concentration and indicating the highest potential as free radical scavengers (Fig. 4).

Ecotypes Baloch and Pullandri showed the highest antioxidant activity by causing 98% scavenging of DPPH radical. The ecotype Datot showed lowest antioxidant activity by causing 30% scavenging of DPPH radical. Hurnamaira, Topa, Tolipeer, Hullar and Morifurman Shah showed moderate antioxident activity.

The antioxidant activity decreased in the following order: Baloch > Pullandri > Topa > Morifurman Shah > Hullar > Trarkhal > Tolipeer > Hurnamaira > Datot. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or

hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods.

Total phenolic contents (TPC)

Determination of total phenolic contents in nine ecotypes of *Berberis lycium* fruits extracts were reported as gallic acid equivalent (GAE). Total phenolic contents differ amongst various ecotypes and found to be ranged 22.4 to 85 mg GAE/g (Figure 4). Results depicted that, TPC of fruit extracts were found to be generally higher in genotype Morifurman Shah 85 mg GAE/g as compared to the other genotypes followed by Tolipeer (66.5), Baloch (62.1), Trarkhal (58.5), Datot (55.1), Hullar (51.7), Pullandri (43.8), Topa (39.6) whereas ecotype Hurnamaira attained minimum value as 22.4 mg GAE/g.

Conclusion

Studies revealed that ecotypes from district Poonch are morphologically more diverse and they showed greater variation as compaired to other two districts this may be due to environmental factors.

Correlation studies, factor loadings and cluster analysis revealed that traits plant height, fruit diameter, 100-fruit weight and days to 90% fruit maturity are intended for most of the variation.

Ecotype Pullandri and Baloch showed maximum antioxidant activity and ecotype Morifurman Shah on the basis of total phenolic contents**.**

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