Technical paper

Characterization of indigenous barberry germplasm in Pakistan: variability in morphological characteristics and nutritional composition

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Characterization of indigenous barberry germplasm in Pakistan: variability in morphological characteristics and nutritional composition.

Abstract – Introduction. Barberry (*Berberis aristata* DC.), a small fruit, is found growing in the wild in the temperate region of northern Pakistan. **Materials and methods**. Three different locations of Azad Jammu and Kashmir, *i.e.*, Topa, Banjosa and Neriyan Sharif, were explored; thirty accessions of barberry were identified and characterized on the basis of their growth habit, flowering, fruiting and chemical composition of fruits. **Results**. The accessions exhibited high diversity in their phenotypical/morphological traits such as growth habit, intensity and time of flowering, fruit setting, ripening time, productivity, and fruit shape and color. The location had a great impact on quantitative parameters. Accessions collected from Neriyan Sharif had significantly greater plant height, higher number of branches per plant, extended flowering duration (days), took more time to maturity (days) and had high fruit setting (%), while those from Topa had large-sized leaves and fruits with greater average fruit weight. The nutritional composition of fruits indicated that the accessions from Topa had higher carbohydrate, ash, total soluble solids and Mn contents, with significantly higher juice pH, and lower acidity; while the accessions from Neriyan Sharif had significantly higher moisture, protein, fat, fiber, K, Ca, Na, Fe, Cu, Pb and Cr contents and were highly acidic. The accessions collected from Banjosa were almost intermediate for the quantitative characteristics studied. **Conclusions**. The results suggested that not only the genotype but its growing location as well are the main factors that determine the growing habit, productivity and nutritional composition of barberry fruits, and this ultimately provides important information on how to make the best use of them.

Pakistan / *Berberis aristata* / biodiversity / fruits / physicochemical properties / proximate composition / genotype environment interaction

Caractérisation du matériel génétique indigène de l'épine-vinette au Pakistan : variabilité des caractéristiques morphologiques et de la composition nutritionnelle.

Résumé – Introduction. L'épine-vinette (*Berberis aristata* DC.), qui produit de petits fruits, se développe à l'état sauvage dans la zone tempérée du nord du Pakistan. **Matériel et méthodes**. Trois localités différentes (Topa, Banjosa et Neriyan Sharif) de l'Azad Jammu-et-Cachemire ont été explorées ; trente accessions d'épine-vinette ont été identifiées et caractérisées sur la base de leur port, floraison, fructification et composition chimique des fruits. **Résultats**. Les accessions étudiées ont présenté une forte diversité de caractéristiques phénotypiques et morphologiques (port, intensité et durée de floraison, nouaison, maturation, productivité, forme et couleur du fruit). La localisation a eu une grande incidence sur les paramètres quantitatifs. Les accessions collectées auprès de Neriyan Sharif ont significativement présenté de plus grands plants, un plus grand nombre de branches par plant, une durée de floraison plus longue, un temps de maturation plus long et ont eu un fort taux de nouaison, alors que les fruits collectés à Topa ont eu des feuilles plus grandes et des fruits de forts poids moyens. La composition nutritionnelle des fruits a révélé que les accessions de Topa produisaient des fruits à plus fortes teneurs en glucides, cendres, solides solubles totaux et Mn avec un jus à pH élevé et acidité faible, tandis que les fruits des accessions de Neriyan Sharif présentaient des taux significativement plus élevés en humidité, protéines, lipides, fibres, K, Ca, Na, Fe, Cu, Pb et teneurs en Cr, et étaient très acides. Les accessions collectées auprès de Banjosa ont été à peu près intermédiaires pour les caractéristiques quantitatives étudiées. **Conclusions**. Nos résultats suggèrent que non seulement le génotype mais aussi son lieu de culture sont les principaux facteurs qui déterminent le port, la productivité et la composition nutritionnelle des fruits de l'épine-vinette ; globalement ces informations importantes pourront permettre d'optimiser leur utilisation.

Pakistan / *Berberis aristata* / biodiversité / fruits / propriété physicochimique / composition globale / interaction génotype environnement

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1. Introduction

The genus *Berberis* belonging to the family Berberidaceae is widely distributed in temperate and sub-tropical regions of the Northern Hemisphere and temperate South America [1]. It has about 650 species worldwide, of which 54 species have been reported from the Indian Himalayas. Barberry (*Berberis aristata* DC.), a spiny shrub, 1.8–3.6 m in height, is abundantly found growing in the wild in the whole range of the Himalaya Mountains up to an elevation of 3300 m. The fruit is a small, 7–10 mm- long, spherical, oblong or ovate berry. *Berberis aristata*, popularly known as 'Daruhaldi' and commonly known in different areas as 'garhwal', 'chitri' and 'sumbal', has diverse uses such as the fruits, which are edible and consumed; branches, which are used as fuel wood; leaves, as fodder for goats, and the whole plant, as a live fence or hedge [2, 3]. The berries are rich in vitamin C, with a very sharp flavor. In India, the fruits are sometimes sold in local markets [4]. The dried fruits are also used as herbal medicine. The active ingredients in the barberry stem and root bark are thought to be the isoquinoline alkaloids, especially berberine. For the first time, Saied *et al.* identified four alkaloids, (pakistanine, 1-O-methylpakistanine, pseudopalmatine chloride and pseudoberberine chloride) from *Berberis aristata* [5]. It is a mild laxative, a tonic, is useful in curing ulcers and fevers, and also useful in treating anorexia, dysentery, hepatitis and liver disorders [6]. The root is used as a medicine by local inhabitants. In its efficacy, it is almost equal to quinine and Warburg's tincture and does not produce any bad effects on the stomach, bowel, brain or organs of hearing [7]. The fruits of *Berberis* species are eaten by the villagers in the hills and also offered for sale at some places, mostly near schools, because they are very much liked by children due to their taste. *Berberis* fruits have extractable juice 26.6%, moisture 63.4%, TSS 18.90%, acidity 1.07%, total sugar 11.97%, pectin 0.37%, and vitamin C content 4.60 mg·100 mL^{-1} of juice; minerals such as potassium, calcium, sodium and iron are present in various quantities [4].

Variability in fruit characteristics of wild and primitive varieties of temperate fruits have been reported by various authors [8–11]. Barberry represents a large proportion of small fruit plants. Maikhuri *et al.* [12] stated that the genus *Berberis*, abundantly found on both sides of the Himalayas as a wild fruit and in semi-domesticated form, had high potential for exploitation. Both forms are found growing in the wild in the mountainous area of Poonch division of Azad Jammu & Kashmir (northern Pakistan). Rawalakot valley lies at an altitude of 1800–2100 m and at a latitude of 33–36° under the foothills of the Himalayas. The climate of the area is temperate and sub-humid, with annual rainfall ranging from about 500–2000 mm, most of which is irregular, with intensive storms during monsoon and winter. The mean annual temperature ranges from a few degrees below 0 °C to a maximum of 30 °C accompanied by severe cold and snowfall in winter [13].

Underexploited and underutilized natural resources, including wild fruits, with potential economic importance play a vital role in maintaining the subsistence lifestyle in traditional mountain societies. The wild fruits of many species have served as a source of food and medicine for thousands of years, particularly in the tribal and rural areas of the Himalayas [14], and barberry fruit is one of those. Characterization of wild germplasm holds promise for improving existing material, which in turn will cause local- and national-based quality products to flourish.

The objective of our study was to compare the physicochemical properties of indigenous germplasm of barberry (*B. aristata* DC.) found growing in the wild in the temperate region of northern Pakistan. This research on characterization of wild and local germplasm of barberry will increase our knowledge about its future utilization.

2. Materials and methods

2.1. Plant material and ecological characteristics of the locations

Thirty genotypes of barberry (*B. aristata* DC.) collected from three different locations

of Azad Jammu and Kashmir (Pakistan), *i.e.*, Topa (TP), Banjosa (BG) and Neriyan Sharif (NS), were used for this study.

2.1.1. Topa location

Elevation ranges from 1674 m to 1981 m from sea level. There are tops with steep slopes towards the northern side. Winter temperatures range from –5 °C to 5 °C with severe snowfall during late December to late February, or sometimes in early March. Summer (April to September) temperatures range from 6 °C to 22 °C.

2.1.2. Banjosa location

Elevation ranges from 1219 m to 1828 m from sea level. There are depressions with gentle slopes from all sides. Winter temperatures range from –2 °C to 9 °C with moderate snowfall during January. Summer temperatures range from 12 °C to 28 °C.

2.1.3. Neriyan Sharif location

Elevation ranges from 1219 m to 1981 m from sea level. Southern and eastern slopes are exposed to sunlight. Winter temperatures range from –1 °C to 10 °C, and snowfall occurs during January but, due to being exposed from all sides, it melts immediately. Summer temperatures range from 12 °C to 30 °C.

2.2. *In situ* qualitative evaluation

A comprehensive survey of the three locations mentioned above was conducted. Thirty plants found growing in the wild in these areas were selected and labeled for data collection. *In situ* investigations were recorded on the growth habit of plants, intensity and time of flowering, fruit setting, ripening time, productivity, fruit shape, and exterior color of immature and mature fruits. The color of the fruits was recorded according to the horticultural color chart issued by the Royal Horticultural Society, London, UK [15].

2.3. Quantitative evaluation of morphological traits

Phenotypic/morphological characteristics of the accessions such as plant height, number of branches per plant, leaf size (length and width), fruit setting %, flowering duration, days to fruit maturity, fruit size (length and width) and weight were studied during the course of time. Plant height was measured from the ground level to the top of the plant with the help of a measuring tape. Number of branches per plant was counted randomly from the selected plants from each location and their averages were calculated. Leaf length and width were measured with the help of a leaf area meter. Duration of flowering was recorded from the date of opening of the first flower to the date of dropping of the last petal of each accession, as described by Singh *et al.* [16]. Fruit setting was recorded by counting the total number of flowers and visually observing the number of flowers developing into fruits. Days to fruit maturity were counted from the first day of fruit set to the date of fruit maturity for the accessions of the three locations. For estimation of fruit size (length and width) and fruit weight, ripe fruits were randomly collected from the plants of each location. Average fruit length and width were measured by using a Vernier caliper and average fruit weight was recorded using an electric balance.

2.4. Chemical analysis

Fully ripe fruits were harvested from each location and were stored in an icebox to avoid physicochemical changes during transportation from the field area to the laboratory for chemical analysis. The edible portion (pulp) or juice of the fruits was analyzed for various parameters as described below.

2.4.1. Moisture content

Moisture contents of the fruit edible portion were determined by the gravimetric method [17]. One gram of sample was taken in a pre-weighed dried Petri dish (W_1) and placed in a hot air oven at 105 °C for 24 h. The sample was removed from the oven and cooled in a desiccator to room temperature and re-weighed (W_2). The moisture percentage in the sample was determined by using the following formula: Moisture% = $[(W_1 - W_2) / W_1] \times 100$.

2.4.2. Carbohydrate

Total soluble carbohydrates were determined by using a spectrophotometer [18]. One gram of well-mixed sample of pulp was taken in a 100-mL conical flask. Ten mL of distilled water was added to the flask with gentle mixing followed by addition of 15 mL perchloric acid (52%). The mixture was stirred for 30 min and filtered through a No. 1 Whatman filter. One mL of the filtrate was drawn into a test tube followed by addition of anthrone reagent (4 mL). Absorbance was measured at a wavelength of 620 nm by using a spectrophotometer. Total soluble carbohydrates were determined by drawing a standard curve of glucose.

2.4.3. Crude protein

Crude protein estimation was performed by using the Kjeldahl digestion and distillation method [17]. Up to 1 g of the sample along with 5.5 g digestion mixture (5 g K_2SO_4 and 0.5 g $CuSO_4$) was taken in a round-bottomed long-neck flask. Sulfuric acid (25 mL) was taken into the digestion flask and the sample was digested until the digested material became transparent. The flask was cooled and transferred to a 250-mL volumetric flask, and the volume was made up to the mark with distilled water. Distillation of the diluted sample was carried out in a Kjeldahl distillation unit by using 25 mL NaOH (40%). Ammonia liberated from the sample during steam distillation was collected in 4% boric acid solution and titration was carried out against 0.1 N HCl solution. A blank was also run without a sample in the same manner and the crude protein was estimated by using the following formula: Crude protein% = {[(Titer reading with sample – Titer reading with blank) × 14 × 6.25] / Weight of sample} × 100.

2.4.4. Crude fat

The crude fat percentage was determined by the solvent extraction method deploying soxhlet apparatus [17]. Briefly, one g predried sample was taken in a Whatman extraction thimble and covered from the top with a cotton plug. Petroleum ether (50 mL) was taken in a receiving flask and the thimble was kept in the extraction tube. Both the extraction tube and the receiving flasks were connected with the apparatus and the extraction was carried out for 30 min followed by rinsing for a period of 1.5 h. After the completion of rinsing, the thimble was removed and the solvent was recovered in the extraction tube. Extracted fat in the receiving flask was placed in a pre-weighed Petri dish and oven-dried at 110 °C for 1 h. The crude fat percentage was determined after cooling of the Petri dish by using the following formula: Crude fat% = (Weight of extracted fat / Weight of sample) × 100.

2.4.5. Crude fiber

Crude fiber contents of the samples were determined by the Official Methods of Analysis [17]. One gram of pre-dried, defatted sample was taken in a glass beaker and 200 mL of 1.25% boiling sulfuric acid was added to it. Digestion was carried out at boiling temperature for a period of 30 min and the acid was drained by filtration through a filtration assembly. The residues on the filter paper were washed with boiling distilled water to remove the traces of acid. After washing, two hundred mL of 1.25% NaOH were added to the residues. The digestion was carried out for the next 30 min and the alkali was drained by filtration. The residues were again washed with boiling distilled water. The residues were dried overnight in a hot air oven at 110 °C. The sample was cooled in a desiccator and weighed. Incineration of the weighed sample was carried out at 550 °C in a muffle furnace until gray ash for a period of about 2 h. The crucible was cooled in a desiccator and re-weighed. Crude fiber contents were determined by using the following formula: Crude fiber% = [(Weight of digested sample – Weight of ash) / Weight of sample] × 100.

2.4.6. Ash

Total inorganic matters (ash percentage) were determined by incinerating the sample at 600 °C [17]. A one-gram sample was weighed in a pre-weighed crucible and the sample was charred to remove smoke and smell at the flame. Incineration of the charred sample was performed in a muffle furnace at 600 °C for 3 h. After completion of the incineration period, samples were

removed from the furnace and kept in desiccators, and re-weighed on cooling. Ash percentage was determined by using the following formula: Ash% = (Weight of ash / Weight of sample) × 100.

2.4.7. Total soluble solids

Total soluble solids were determined by using a hand refractometer at 20 °C as described by the Official Methods of Analysis [17]. One drop of the extracted juice was placed on the absolutely dry prism of the refractometer and the reading was recorded in °Brix.

2.4.8. Juice acidity

Titratable acidity was determined by the method as described by the Official Methods of Analysis [17]. Well-mixed extracted juice (10 mL) was diluted to 100 mL in a 250-mL flask with boiled or neutralized water. A few drops of indicator (phenolphthalein) were added to the diluted sample and titration was carried out against 0.1 N NaOH up to the pink end point persisting for at least 30 s. Acidity was calculated by using the following formula: Acidity% = (0.1 × Eq weight of acid × N of NaOH × Titer volume) / Weight of sample] × 100.

2.4.9. Juice pH

pH of the juice sample was recorded with the help of a digital pH meter.

2.4.10. Mineral detection

The minerals of the fruit pulp were determined by using an Atomic Absorption Spectrophotometer (AAS iCE 3300, Thermo Scientific, USA) as described by the Approved Methods of the American Association of Cereal Chemists [19]. Briefly, a sample of 0.5 g was digested separately by the wet digestion method. The sample was first digested with 10 mL HNO_3 at a temperature of 60–70 °C for 20 min. Further digestion was carried out with $HClO_4$ at a temperature of 190 °C until the solution became clear. The digested samples were transferred to a 250-mL volumetric flask, the volume was made up with distilled water and filtration was carried out to remove any suspended residues. Analysis was carried out for the estimation of Fe, Cu, Zn, Mn, Pb and Cr ($mg \cdot kg^{-1}$). Electrolytes, *i.e.*, K, Ca and Na ($mg \cdot kg^{-1}$) from the digested samples, were determined by flame photometry.

2.5. Statistical analysis

The quantitative data were analyzed by using one-way analysis of variance (ANOVA). Mean values were compared by employing the least significant difference (LSD) test. All analyses were performed using MSTATC software and differences at the 5% level ($p < 0.05$) were considered statistically significant.

3. Results and discussion

3.1. Qualitative traits

In situ qualitative evaluations were made visually by establishing the raspberry descriptor for the parameters, *i.e.*, growth habit of plants, intensity and time of flowering, fruit setting, ripening time, productivity, fruit shape, and exterior color of immature and mature fruits (*table I*); the frequency of qualitative traits was calculated (*table II*).

Out of thirty accessions (10 from each location), fifteen (50%) had an upright growth habit, seven (23.3%) had a pyramidal habit and eight (26.7%) had a broad-spreading growth habit. Intensity of flowering was high in thirteen accessions (43.3%). In only five accessions (16.7%) was the intensity of flowering low and the twelve other accessions (40%) were in the middle for this parameter (*figure 1*). A total of thirteen accessions (43.3%) flowered in the middle of the flowering season, which started from mid-March to the first week of April. There were only five accessions (16.7%) which were late-flowering, *i.e.*, from the second week of April to the last week of April. The remaining accessions, however, overlapped each other to some extent and were early- or early- to mid-season-flowering. Fourteen accessions (46.7%) had high fruit set. In twelve accessions (40%), the fruit set was intermediate, while

Table I.
Description of some qualitative traits of *Berberis* germplasm (Azad Jammu & Kashmir, Pakistan).

Local name	Location	Accession No.	Growth habit	Intensity of flowering	Time of flowering	Fruit setting	Ripening time	Productivity	Fruit shape	Exterior color of immature fruit	Exterior color of mature fruit
Sumbalu	Topa	1	Broad-spreading	High	Mid-season	Intermediate	Mid-season to late	High	Ovate	Green to light yellow	Purple
		2	Pyramidal	Medium	Early to mid-season	Low	Mid-season	Low	Ovate	Green	Violet
		3	Pyramidal	Medium	Mid-season	High	Late	High	Bulbous/ oblong	Green to light yellow	Purple
		4	Broad-spreading	High	Early to mid-season	Intermediate	Early to mid-season	Medium	Ovate	Light gray	Violet
		5	Upright	Medium	Late	High	Mid-season to late	High	Bulbous/ oblong	Green to light yellow	Bluish
		6	Upright	High	Mid-season	High	Early to mid-season	High	Round	Green to light yellow	Purple
		7	Upright	Medium	Early to mid-season	Intermediate	Early to mid-season	Medium	Round	Light gray	Violet
		8	Upright	High	Late	High	Mid-season to late	High	Bulbous/ oblong	Light gray	Violet
		9	Upright	Low	Mid-season	Low	Mid-season to late	High	Ovate	Green	Bluish
		10	Broad-spreading	Low	Mid-season	Intermediate	Early to mid-season	Medium	Ovate	Green to light yellow	Violet
	Neriyan Sharif	21	Upright	Medium	Early	Intermediate	Mid-season to late	Medium	Bulbous/ oblong	Green to light yellow	Bluish
		22	Pyramidal	Medium	Early	High	Mid-season to late	Low	Bulbous/ oblong	Light gray	Purple
		23	Broad-spreading	Medium	Mid-season	High	Mid-season to late	High	Ovate	Light gray	Bluish
		24	Upright	High	Early	Intermediate	Early to mid-season	Low	Round	Green to light yellow	Purple
		25	Upright	Low	Mid-season	Intermediate	Early to mid-season	Medium	Round	Green to light yellow	Violet
		26	Upright	High	Mid-season	Intermediate	Mid-season	Low	Bulbous/ oblong	Green to light yellow	Bluish

Table I.
Continued.

Local name	Location	Accession No.	Growth habit	Intensity of flowering	Time of flowering	Fruit setting	Ripening time	Productivity	Fruit shape	Exterior color of immature fruit	Exterior color of mature fruit
Sumbalu	Neriyan Sharif	27	Pyramidal	Medium	Late	Intermediate	Mid-season	Medium	Bulbous/ oblong	Green	Purple
		28	Pyramidal	High	Mid-season	High	Late	High	Bulbous/ oblong	Green to light yellow	Violet
		29	Pyramidal	Medium	Early to mid-season	Intermediate	Late	High	Round	Light gray	Bluish
		30	Broad-spreading	High	Late	Intermediate	Mid-season	High	Round	Green	Bluish
Sumbal	Banjosa	11	Upright	High	Early	High	Mid-season to late	High	Bulbous/ oblong	Light gray	Bluish
		12	Upright	High	Early	High	Early to mid-season	High	Ovate	Green	Violet
		13	Upright	Medium	Mid-season	Low	Early to mid-season	Medium	Bulbous/ oblong	Green to light yellow	Purple
		14	Broad-spreading	Medium	Mid-season	Low	Late	Low	Ovate	Light gray	Purple
		15	Upright	Medium	Early to mid-season	High	Late	High	Bulbous/ oblong	Green to light yellow	Violet
		16	Pyramidal	High	Mid-season	Intermediate	Mid-season to late	High	Bulbous/ oblong	Green to light yellow	Purple
		17	Upright	Low	Early	High	Mid-season	Medium	Ovate	Light gray	Bluish
		18	Broad-spreading	Low	Mid-season	High	Early to mid-season	Medium	Ovate	Green	Violet
		19	Broad-spreading	High	Early to mid-season	High	Mid-season	High	Bulbous/ oblong	Green to light yellow	Bluish
		20	Upright	High	Late	High	Mid-season	High	Ovate	Light gray	Violet

Table II.
Frequency of qualitative traits of *Berberis* germplasm (Azad Jammu & Kashmir, Pakistan).

Traits	Category	No. of plants	Percentage
Growth habit	Upright	15	50.0
	Pyramidal	7	23.3
	Broad-spreading	8	26.7
Intensity of flowering	Low	5	16.7
	Medium	12	40.0
	High	13	43.3
Time of flowering	Early	6	20.0
	Early to mid-season	6	20.0
	Mid-season	13	43.3
	Late	5	16.7
Fruit setting	Low	4	13.3
	Intermediate	12	40.0
	High	14	46.7
Ripening time	Early to mid-season	9	30.0
	Mid-season	7	23.3
	Mid-season to late	9	30.0
	Late	5	16.7
Productivity	Low	5	16.7
	Medium	9	30.0
	High	16	53.3
Fruit shape	Bulbous/ oblong	13	43.3
	Round	6	20.0
	Ovate	11	36.7
Exterior color of immature fruit	Green to light yellow	14	46.7
	Light gray	10	33.3
	Green	6	20.0
Exterior color of mature fruit	Purple	9	30.0
	Violet	11	36.7
	Bluish	10	33.3

only four accessions (13.3%) had low fruit set (*figure 2*). Observation regarding the time of ripening was recorded when fruits reached the optimum level of maturity. Nine accessions (30%) were early- to mid-season-ripening. Seven accessions (23.3%) were mid-season-ripening and nine accessions (30%) were mid-season to late in their ripening of fruits. The five other accessions (16.7%) were late-ripening. Sixteen accessions (53.3%) were found to be highly productive; nine (30%) had a moderate yield, while only five accessions (16.7%) were low in productivity. For fruit shape, eleven accessions (36.7%) had ovate fruits, thirteen (43.3%) had bulbous/oblong fruits, while six accessions had round-shaped fruits. The exterior color of immature fruits was green to light yellow for fourteen accessions (46.7%), light gray for ten accessions (33.3%) and green for the remaining six accessions (20%), while the exterior color of mature fruits was purple for nine accessions (30%), bluish for ten accessions (33.3%) and violet for the other eleven accessions (36.7%). In our study, this diversity might be

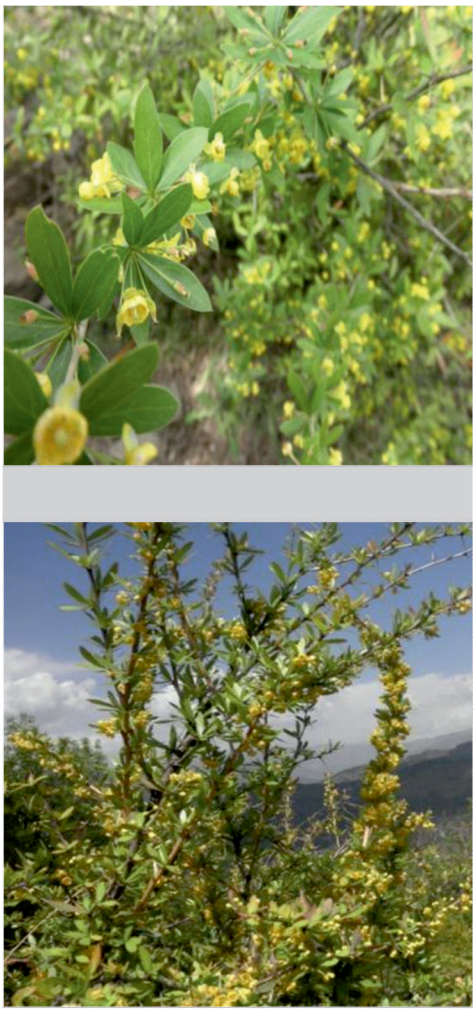

Figure 1.
Flowering stage of *Berberis* germplasm (Azad Jammu & Kashmir, Pakistan).

Figure 2.
Fruiting stage of *Berberis* germplasm (Azad Jammu & Kashmir, Pakistan).

partly due to variations in local conditions such as topography, soil conditions, climate, sunlight, rainfall and other salient features of the location. Plants bloom earlier in warm temperate regions with a southern aspect as plants receive more sunlight than those growing on northern aspects. In the localities where frost occurs during spring and low temperature prevails, plants bloom late.

3.2. Physical/morphological analysis

For quantitative parameters of a phenotypical/morphological nature (*table III*), the maximum height (2.117 m) was recorded in the plants growing in the Neriyan Sharif location as compared with those found at the other two locations, *i.e.*, Banjosa (2.033 m) and Topa (1.957 m). The maximum number of branches per plant (60.80) was counted in plants grown in Neriyan Sharif. This was followed by the plants found in the Banjosa location (57.40 branches per plant), and the minimum number of branches (54.40 branches per plant) was recorded in plants of the Topa location. The variations in height and number of branches per plant in barberry are supported by the findings of Zargari [20] and Amin [21], who stated that barberry plants are 1–3 m tall with 50–60 branches per plant. The differences in the values of these traits at different locations might be partly due to variations in local environments such as sunlight, soil conditions, climate, topography, rainfall and other salient features of the area. Palmer

Table III.
Effect of location on some quantitative characteristics of *Berberis* germplasm (Azad Jammu & Kashmir, Pakistan).

Location	Plant height (m)	Number of branches per plant	Leaf length (cm)	Leaf width (cm)	Flowering duration (days)	Fruit setting (%)	Days to fruit maturity	Fruit length (mm)	Fruit width (mm)	Fruit weight (mg)
Topa	1.957 c	54.40 c	9.69 a	3.31 a	13.40 c	69.00 c	30.00 c	9.75 a	4.34 a	122.80 a
Banjosa	2.033 b	57.40 b	7.09 b	2.98 b	17.30 b	73.80 b	33.00 b	8.34 b	3.45 b	121.20 a
Neriyan Sharif	2.117 a	60.80 a	6.67 c	2.58 c	19.50 a	78.90 a	36.40 a	7.12 c	2.65 c	115.00 b
LSD ($P \leq 0.05$)	0.070	0.58	0.43	0.12	0.66	0.78	0.38	0.87	0.66	1.63

reported that plant height and number of branches are greatly influenced by location, soil and climatic factors [22].

Leaves of the plants collected from different locations exhibited significant difference in their length and width, and those collected from the Topa location were larger in size (9.69 cm × 3.31 cm) as compared with those from the other two locations. The smallest sized leaves (6.67 cm × 2.58 cm) were found in those plants growing in the Neriyan Sharif location. Our present observations were strongly supported by the findings of Lone and Wafal, who reported remarkable diversity in leaves and flowers of rosaceous fruits [23]. Genetic as well as environmental factors are responsible for the diversity in leaf size.

The maximum flowering duration (19.50 days) was recorded in the plants growing in Neriyan Sharif and the minimum flowering duration (13.40 days) was recorded in plants of the Topa location. The main reason for differences in flowering duration was probably due to variation in the topography, elevation and mean temperatures of the localities. The highest percentage of fruit set was recorded in plants growing in the Neriyan Sharif location (78.90%), whereas plants of the other two locations differed in their fruit setting, with significantly lower percentages (*table III*). Maximum days to fruit maturity (36.40 days) were recorded in plants of Neriyan Sharif, followed by those in Banjosa (33.00 days), and minimum days to fruit maturity (30.00 days) were recorded in the plants of the Topa location. The *Berberis* germplasm normally varies in its flowering, fruit setting and fruit yields depending upon the prevailing climatic conditions [4]. Fruits mature earlier in warm temperate regions with a southern aspect as plants receive more sunlight than those growing on northern aspects. The productivity of *Berberis* germplasm depends upon initial fruit set, its retention, and subsequent fruit growth and development, which is influenced by prevailing environmental conditions.

Fruits collected from the Topa location were of large size (9.75 mm × 4.34 mm) and differed statistically from the fruits of the Banjosa (8.34 mm × 3.45 mm) and Neriyan Sharif (7.12 mm × 2.65 mm) locations (*table III*). The maximum fruit weight (122.8 mg) was recorded in the plants found in Topa and in Banjosa (121.2 mg), which did not statistically differ; fruits of Neriyan Sharif had the minimum average weight (115.0 mg). The results indicated variations in fruit size and weight among the plants of different locations. Similar results have been reported by Akbulut *et al.* [24]. Environmental factors such as temperature, sunlight, rainfall, snowfall and hail storms vary greatly from zone to zone and locality to locality in the State of Azad Jammu and Kashmir, which ultimately affect fruit size and weight [6]. This may be due to plant selection in dense populations, which ultimately causes variations in fruit weight and size at different locations. Similar findings were reported by Garriz *et al.*, who wrote that closer spacing between plants of the same and other species affects the fruit size due to competition for light, nutrition and water [25].

Table IV.
Effect of location on chemical composition of *Berberis* fruits (Azad Jammu & Kashmir, Pakistan).

Location	Moisture	Carbohydrate	Protein	Fat	Fiber	Ash	TSS (°Brix)	Juice acidity (%)	pH of juice
			(%)						
Topa	59.67 c	25.33 a	7.003 c	3.353 b	1.663 c	5.630 a	27.63 a	0.423 c	4.43 a
Banjosa	62.61 b	22.71 b	9.443 b	2.693 c	2.693 b	2.900 c	25.36 b	0.890 b	3.75 b
Neriyan Sharif	64.70 a	20.73 c	10.580 a	4.360 a	3.533 a	3.933 b	23.60 c	1.273 a	3.13 c
LSD ($P \leq 0.05$)	0.53	1.34	1.133	0.376	0.640	1.018	1.43	0.091	0.41

3.3. Chemical analysis

The results regarding the chemical composition of barberry fruits collected from different locations revealed significant differences ($p < 0.05$) for moisture content, carbohydrate, crude protein, fat, fiber and ash contents of fruit pulp, and total soluble solids (TSS), acidity (%) and pH of fruit juice (*table IV*). The moisture content varied from 59.67% to 64.70 %, being the highest in fruits collected from Neriyan Sharif and the lowest in those from Topa. The differences in moisture content among the fruits collected from different locations might be due to the variation in the amount and distribution of rainfall and other climatic conditions prevailing at these locations. However, the amount of moisture in fruit is an important parameter, as it relates to the juice content of the fruit. The carbohydrate content varied from 20.73% to 25.33%, being maximum in fruits of Topa and minimum in those collected from Neriyan Sharif. The highest protein content (10.58%) was recorded in fruits harvested from Neriyan Sharif, then by the fruits sampled from Banjosa (9.44%), and the minimum protein content (7.00%) was found in fruits taken from Topa. However, these mean values of protein content for different locations differed significantly from each other. Fat content varied from 2.69% in fruits of the Banjosa location to 4.36% in those harvested from Neriyan Sharif. The highest fiber content (3.53%) was recorded in fruit sampled from Neriyan Sharif, which was significantly greater than from the fruits harvested from the other two locations. Maximum ash content (5.63%) was recorded in fruits sampled from Topa, which differed significantly from fruits of the other two locations. The lowest ash content (2.90%) was weighed in the fruits collected from the Banjosa locality.

The juice extracted from the fruits collected from Topa had the highest TSS and those of Neriyan Sharif had the lowest percentage. As the moisture content of the fruits increased, the TSS value decreased. The fruits collected from Neriyan Sharif were more acidic, with the lowest pH value of juice as compared with the fruits harvested from the other two locations. The differences in TSS may possibly be due to differences in genotypes, prevailing environmental conditions and also moisture/juice content of the fruits [26].

3.4. Mineral composition

The results regarding the mineral composition of barberry fruits collected from different locations show that the concentrations of K, Na, Fe, Cu, Zn, Pb and Cr were significantly higher in the fruits collected from the Neriyan Sharif location (*table V*). This was followed by those harvested from the Banjosa location. The lowest concentrations of these minerals were recorded in fruits taken from Topa. The maximum Ca concentration was also recorded in fruits collected from Neriyan Sharif. However, the minimum Ca concentration was found in fruits harvested from Banjosa. On the other hand, the Mn concentration was significantly higher in the fruits harvested from the Topa location, lower in fruits from the Banjosa location and intermediate in fruits from the Neriyan Sharif location (*table V*). Knowledge about mineral contents of fruits is very important

Table V.
Effect of location on mineral composition (mg·kg^{-1}) of *Berberis* fruits (Azad Jammu & Kashmir, Pakistan).

Location	Potassium (K)	Calcium (Ca)	Sodium (Na)	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Manganese (Mn)	Lead (Pb)	Chromium (Cr)
Topa	355.00 c	93.33 b	475.70 c	105.30 c	95.23 c	1.012 c	2.473 a	0.640 c	0.630 c
Banjosa	361.70 b	87.67 c	482.70 b	110.00 b	100.50 b	1.288 b	1.860 c	0.930 b	0.971 b
Neriyan Sharif	367.00 a	97.67 a	492.00 a	116.30 a	103.80 a	1.573 a	2.017 b	1.083 a	1.183 a
LSD ($P \leq 0.05$)	2.84	3.58	2.01	3.41	3.06	0.117	0.084	0.045	0.029

from a human nutrition point of view. Minerals contribute to several biological processes in the body; however, these have not been established as essential sources for a nutritional diet [27]. Variation in the mineral composition of mulberry and wild strawberry fruits has also been reported [28, 29]. In our present study, the fruits collected from different locations differed in their mineral contents. However, these values are within the range already reported in wild *Berberis* fruits [24, 30, 31]. The variation in mineral composition of fruits collected from different locations might be due to differences in topography, and soil and climatic conditions of the localities. Sood *et al.* also stated that genotypic differences, topography, and soil and climatic conditions affect the mineral composition of wild *Berberis* fruits [14].

4. Conclusion

Wild germplasm of barberry was explored from three different geo-ecological locations of Poonch Division of Azad Jammu and Kashmir (Pakistan) and plants were assessed on their qualitative and quantitative parameters. The high variability found in their traits could be helpful for genetic improvement and further evaluation for preservation of these genetic resources. The barberry fruit is a good source of various nutrients, especially carbohydrates, protein, fat, fiber and minerals. Considering their zero cost of production, easy availability, hardy nature and abundant production, they need to be popularized and exploited on a commercial scale. This will help in conserving and managing the natural environment of the area. Further, the integrity of food-chain relationships within the ecosystem can also be maintained.

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Caracterización del material genético autóctono del árbol de agracejo en Paquistán: variabilidad de las características morfológicas de la composición nutricional.

Resumen – **Introducción**. El árbol de agracejo (*Berberis aristata* DC.), que produce pequeños frutos, crece en estado salvaje en la zona templada del norte de Paquistán. **Material y métodos**. Se exploraron tres localidades diferentes (Topa, Banjosa y Neriyan Sharif) de Azad Jammu y Cachemira; se identificaron treinta accesiones de árbol de agracejo según su porte, floración, fructificación y composición química de los frutos. **Resultados**. Las accesiones estudiadas presentaron una fuerte diversidad de características fenotípicas y morfológicas (porte, intensidad y duración de la floración, cuajado, maduración, productividad, forma y color del fruto). La localización tuvo una gran incidencia sobre los parámetros cuantitativos. Las accesiones recogidas en Neriyan Sharif presentaron plantas significativamente mayores, más ramas por planta, una duración mayor de la floración, un tiempo de maduración más largo y un fuerte índice de cuajado, mientras que los frutos recogidos en Topa tenían hojas mayores y frutos con un peso medio elevado. La composición nutricional de los frutos reveló que las accesiones de Topa producían frutos con mayor contenido en glúcidos, cenizas, sólidos solubles totales y Mn con un zumo de pH elevado y poca acidez, mientras que los frutos de las accesiones de Neriyan Sharif presentaron índices significativamente mayores de humedad, proteínas lípidos, fibras, K, Ca, Na, Fe, Cu, Pb y contenido en Cr, y eran muy ácidos. Las accesiones recogidas en Banjosa presentaron índices prácticamente intermedios en las características cuantitativas estudiadas. **Conclusión**. Nuestros resultados sugieren, que, además del genotipo, el lugar de cultivo es un factor principal que determina el porte, la productividad y la composición nutricional de los frutos del árbol de agracejo; estos útiles datos podrían permitir optimizar su uso a nivel global.

Pakistán / *Berberis aristata* / biodiversidad / frutas / propiedades fisicoquímicas / composición aproximada / interacción genotipo ambiente