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BIODIVERSITY IN MORPHOLOGICAL AND PHYSICO-CHEMICAL CHARACTERISTICS OF WILD RASPBERRY (*Rubus idaeus* L.) GERMPLASM COLLECTED FROM TEMPERATE REGION OF AZAD JAMMU & KASHMIR (PAKISTAN)

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Abstract. Wild raspberry (*Rubus idaeus* L.) is abundantly grown and widely distributed in temperate regions of lower Himalayan range which represents rich biological diversity for raspberry. Thirty accessions were explored from three different locations of Azad Jammu and Kashmir (Pakistan), several traits were visualized at sampling locations and some characteristics were evaluated and quantified after harvest of leaf and fruit samples. The accessions differed greatly in growth habit, blooming time, intensity of flowering, fruit setting, ripening time, productivity, fruit shape and color. Concerning quantitative characteristics, significant differences were found in plant height, leaf length and width, number of braches per plant, duration of flowering, fruit setting percentage, days to maturity, fruit length, width and weight among the samples collected from different locations. Ripe fruits were evaluated for their chemical composition i.e. moisture, carbohydrates, protein, fat, fiber, ash, TSS, acidity, pH and mineral (K, Ca, Na, Fe, Cu, Zn, Mn, Pb, and Cr) contents. The location also had significant effect on chemical composition of ripe fruits.

Key words: genetic diversity, mineral composition, morphological traits, nutritional composition, wild germplasm

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INTRODUCTION

The genus *Rubus* contains a large number of highly variably and heterogeneous species, which occur in all parts of the world except dessert regions. The genus has been divided into 12 subgenera of which only a few species have been domesticated [Jennings et al. 1990, Romoleroux 1992]. Wild raspberry (*Rubus idaeus* L.) is abundantly grown and widely distributed in temperate regions of lower Himalayan range [Maikhuri et al. 1994]. This region (State of the Azad Jammu and Kashmir and Indian held Kashmir) represents rich biological diversity, particularly in edible fruits including raspberry. According to Zaffar et al. [2004] variability in fruit characteristics of primitive varieties is partially due to their genetic makeup and is also influenced by environmental factors prevailing in the region. Patamsyte et al. [2008] intended for the management and utilization of genetic resources of wild raspberry and reported that wild raspberry represents high level of genetic variation among accessions for morphological traits.

In the most of the developing countries, the food situation is critical due to increasing population and high prices of staple foods. Therefore, study for exploring new food sources and exploiting plant resources existing in wild form is invaluable. The primitives local inhabitants have been using local fruits including wild tree fruits and various small fruits like raspberry, strawberry, blueberry, barberry etc. since long times, which grow naturally during the summer season. These fruits have wide diversity in their nutritional composition and also contain remarkable medicinal values, which are yet to be analyzed. Raspberry fruits have been eaten fresh or processed for thousands years and used for medicinal purposes. Further, raspberry juice can be extracted, concentrated and used as an edible dye for foodstuffs, and also can be transported to distinct market as dehydrated form.

Raspberry is a rich source of food components; vitamins, minerals and bioactive compounds like phenolics, anthocyanins, organic acids etc. The wild fruit species chosen for their high vitamin C content, mineral elements and medicinal value could be of interest for fruit processing industries. For their economic potential, wild fruits, semi-domesticated and less utilized fruits provide better economic return by making a variety of edible products such as jam, jelly, juice, squash and sauce [Maikhuri et al. 1994]. Evaluation of wild raspberry for nutritional aspect and bioactive compounds has great benefit for breeders, food and pharmaceutical industries [Badjakov et al. 2008]. Therefore, the maintenance and study of the natural germplasm of raspberry as a potential gene donor is important for the conservation and utilization of raspberry genetic resources. Chemical aspects of fruits such as carbohydrates, protein, lipids, vitamin C, acidity, total soluble solids (TSS), and pH and mineral elements may also provide important information to the consumers. Only few studies concerning morphological, chemical and nutritional properties of wild raspberry has been performed yet [Han et al. 2008, Celik and Ercisli 2009, HanPing et al. 2009]. The exploitation of both types of characteristics is valuable in studies of plant genetic resources [Patamsyte et al. 2008]. Thus, an attempt was made to characterize available germplasm of wild raspberry on the basis of their physico-chemical traits of horticultural interest and for exploiting their potential as a source of nutrition.

MATERIALS AND METHODS

Geographical features and ecological characteristics of the study areas. For characterization of wild raspberry (*R. idaeus* L.) germplasm, initial survey of raspberry growing area in temperate belt of Azad Jammu and Kashmir (Pakistan) was conducted in 2009 and various sites from three locations (Topa, Banjosa and Neriyan Sharif) of two districts i.e. Rawalakot and Sudhnoti were explored for in situ and ex situ observations. The geographic area surveyed is located in the foothills of Himalayas between 73 to 75ºE longitudes and 33 to 35ºN latitudes. The topography of the area is mainly hilly and mountainous with valleys and stretches of plains. The climate is moist subtropical to cold temperate with an average rain fall varying from 800 to 1600 mm. Other ecological characteristics of the three locations are given below.

Topa. Elevation ranges from 1674 to 1981 m from sea level. Aspect and topography; top with steep slope towards northern side. Winter temperature ranges from -5 to 5°C with severe snowfall during late December to late February or sometimes in early March. Summer (April to September) temperature ranges from 6 to 22°C.

Banjosa. Elevation ranges from 1219 to 1828 m from sea level. Aspect and topography; depressions with gentle slope from all sides. Winter temperature ranges from -2 to 9°C with moderate snowfall during January. Summer temperature ranges from 12 to 28°C.

Neriyan Sharif. Elevation ranges from 1219 to 1981 m from sea level. Aspect and topography; southern and eastern slopes exposed to sunlight. Winter temperature ranges from -1 to 10°C, snowfall occurs during January but due to expose from all sides, it melts immediately. Summer temperature ranges from 12 to 30°C.

Plant material and its collection. A total of 30 wild-growing raspberry accessions (10 from each location) of distinct characteristics were selected and labeled for qualitative traits, for example Topa (TP1 To TP10), Banjosa (BJ1 to BJ10) and Neriyan Sharif (NS1 to NS10). Extensive surveys comprised of three trips of the selected accessions at the times of flowering, fruit setting and fruit maturity. From each location, fruits from the ten plants used for the qualitative observations, were collected for physico-chemical evaluations.

***In situ* observations**. *In situ* investigations for qualitative traits were visualized as growth habit of plants (drooping, pyramidal and broad spreading), time of flowering (early, early to mid season, mid season and late), intensity of flowering (low, medium and high), fruit setting (low, medium and high), time of ripening (early, mid season and late), productivity (low, medium and high), fruit shape (slightly oblong and round), exterior color of immature fruits (green to light yellow, light green and green) and exterior color of mature fruits (purple, dark red and blackish). Fruit color was recorded following the horticultural color chart issued by the Royal Horticultural Society of London [RHS 2001].

Quantitative evaluations. Plant height was measured by using a measuring tape and leaf size (length and width) was measured by Portable Laser Leaf Area Meter (Model: CI-202, Bio Sciences, USA), while number of branches per plant was counted and average number of branches was calculated. For duration of flowering, observations were recorded on the date of opening of first flower and date of dropping last petal of each accession, as described by Singh et al. [2004]. Fruit setting percentage was estima-

ted by counting number of flowers per selected branches and number of fruit set on those branches. Days to fruit maturity were recorded by counting days from first day of fruit set to last day of fruit maturity. Fruit lengths and widths were measured by Vernier calliper (Model: Digital Guo Gen Shanghai, China) and fruit weight was taken in grams using an electric balance (Model: BL 220-H, Japan).

Chemical Analysis. Fully ripe fruits were harvested from each location and were stored in icebox to avoid physico-chemical changes during transportation from field area to Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratory at Peshawar for chemical analysis. Edible portion (pulp) or juice of the fruits was analyzed for following parameters with the methods as described below.

Nutritional composition. Moisture content of the fruits (pulp) was determined by following the gravimetric method [AOAC 2000]. Total soluble carbohydrates were estimated by using a spectrophotometer (model: T60U, PG Instruments, UK) [Pearson et al. 1976]. Crude protein was determined by using Kjeldahl digestion and distillation method [AOAC 2000]. Crude fat percentage estimation was performed by solvent extraction method [AOAC 2000]. Crude fiber content of the samples was estimated by the method as described by AOAC [2000]. Total inorganic matter (ash percentage) was determined by incinerating the samples at 600°C for 3 hours [AOAC 2000]. Total soluble solids were determined by using a hand refractometer (Model: HR-032, AFAB, USA) at 20°C [AOAC 2000]. Titratable acidity was determined by following the method as described by AOAC [2000]. The pH of the juice sample was recorded by using a digital pH meter (model: Digital, Portugal).

Mineral composition. The samples of fruit pulp were digested separately by the wet digestion method as described previously [Ahmed et al. 2013]. Electrolytes i.e. K, Ca and Na (mg kg^{-1}) from the digested samples were estimated by flame photometery. The minerals of the fruit pulp Fe, Cu, Zn, Mn, Pb and Cr (mg kg^{-1}) were quantified by using Atomic Absorption Spectrophotometer Spectra AA 220 (Agilents Technologies, USA) as previously described [AACC 2000].

Data analysis. Data collected on quantitative traits and chemical constituents were statistically analyzed by employing analysis of variance (ANOVA) technique and least significant difference (LSD) test $p \leq 0.05$, by using MSTAT-C software (Crop and Soil Sciences Department, Michigan State University, USA).

RESULTS

Qualitative traits. *In situ* qualitative evaluations were made visually by establishing raspberry descriptor for the traits of growth habit of plants, intensity and time of flowering, fruit setting, ripening time, productivity, fruit shape, exterior color of immature fruit and exterior color of mature fruits (tabs 1, 2 and 3). Flowering and fruiting stages of raspberry germplasm growing at different locations is shown in Figs 1 and 2.

Topa. Frequency of qualitative traits of the accessions from Topa is presented in Table 4. The results indicated that out of ten accessions, three (30%) had dropping growth habit, two (20%) had pyramidal and rest of five (50%) had spreading growth habit. Two (20%) accessions were early blooming, two (20%) overlapped and were early to

Table 1. Description of some morphological traits of wild raspberry germplasm in location Topa

Accession	Local name	Growth habit	Time of flowering	Intensity of flowering	Fruit setting	Ripening time	Productivity	Fruit shape	Exterior color of immature fruit	Exterior color of mature fruit
TP1	Pamnaro	broad spreading	mid season	high	medium	late	high	round	green to light yellow	purple
TP2	Pamnaro	pyramidal	early to mid season	medium	low	mid season	medium	round	green	purple
TP3	Pamnaro	pyramidal	mid season	medium	high	late	high	slightly oblong	green to light yellow	purple
TP4	Pamnaro	broad spreading	early to mid season	high	medium	early	medium	round	light green	dark red
TP5	Pamnaro	broad spreading	late	high	high	late	high	round	green to light yellow	blackish
TP6	Pamnaro	drooping	late	high	high	late	high	round	green to light yellow	purple
TP7	Pamnaro	drooping	late	medium	medium	mid season	medium	round	light green	dark red
TP8	Pamnaro	broad spreading	late	high	high	late	high	round	light green	purple
TP9	Pamnaro	drooping	early	low	low	late	high	round	green	dark red
TP10	Pamnaro	broad spreading	early	low	medium	early	medium	round	green to light yellow	purple

Table 2. Description of some morphological traits of wild raspberry germplasm in location Banjosa

Accession	Local name	Growth habit	Time of flowering	Intensity of flowering	Fruit setting	Ripening time	Productivity	Fruit shape	Exterior color of immature fruit	Exterior color of mature fruit
BJ1	Pamnarh	drooping	mid season	high	high	early	high	round	green to light yellow	purple
BJ2	Pamnarh	drooping	late	high	high	early	high	round	green	dark red
BJ3	Pamnarh	drooping	mid season	medium	low	early	medium	slightly oblong	green to light yellow	blackish
BJ4	Pamnarh	drooping	mid season	medium	low	mid season	low	slightly oblong	light green	purple
BJ5	Pamnarh	broad spreading	early to mid season	high	high	late	high	round	light green	dark red
BJ6	Pamnarh	pyramidal	mid season	high	high	late	high	slightly oblong	green to light yellow	purple
BJ7	Pamnarh	pyramidal	early	low	high	late	high	round	light green	dark red
BJ8	Pamnarh	broad spreading	early to mid season	high	medium	mid season	low	round	green	dark red
BJ9	Pamnarh	broad spreading	mid season	low	high	mid season	low	slightly oblong	green to light yellow	blackish
BJ10	Pamnarh	drooping	mid season	medium	high	mid season	high	slightly oblong	light green	dark red

Table 3. Description of some morphological traits of wild raspberry germplasm in location Neriyan Sharif

Accession	Local name	Growth habit	Time of flowering	Intensity of flowering	Fruit setting	Ripening time	Productivity	Fruit shape	Exterior color of immature fruit	Exterior color of mature fruit
NS1	Pamnarh	drooping	mid season	medium	high	mid season	medium	slightly oblong	green to light yellow	purple
NS2	Pamnarh	pyramidal	early to mid season	high	medium	mid season	low	round	green to light yellow	purple
NS3	Pamnarh	broad spreading	mid season	high	high	early	medium	round	green	dark red
NS4	Pamnarh	drooping	mid season	medium	low	early	low	round	green	purple
NS5	Pamnarh	pyramidal	mid season	high	medium	early	medium	round	light green	dark red
NS6	Pamnarh	drooping	early to mid season	high	medium	mid season	medium	slightly oblong	green to light yellow	purple
NS7	Pamnarh	pyramidal	late	medium	medium	mid season	medium	round	green	purple
NS8	Pamnarh	pyramidal	early to mid season	high	high	early	high	round	green to light yellow	purple
NS9	Pamnarh	pyramidal	mid season	high	high	mid season	medium	round	light green	dark red
NS10	Pamnarh	broad spreading	early to mid season	high	high	mid season	medium	round	green	dark red

a b c

Fig. 1. Flowering stage of wild raspberry germplasm from different locations; (a) Topa, (b) Banjonsa, (c) Neriyan Sharif

a b c

Fig. 2. Fruiting stage of wild raspberry germplasm from different locations; (a) Topa, (b) Banjonsa, (c) Neriyan Sharif

mid season. Two (20%) bloomed in mid season and remaining four (40%) bloomed late. As for intensity of flowering, five (50%) had high, only two (20%) had low and the rest of three (30%) were in the middle. Four accessions (40%) had high fruit set, four (40%) had medium and rest of two (20%) had low fruit set. Two accessions (20%) ripened in early and same number of accessions were mid season in their ripening of fruits, while rest of accessions i.e. six (60%) were late season in their ripening of fruits.

Table 4. Summary for frequency of morphological traits (qualitative) of wild raspberry germplasm in location Topa

Trait	Category	No. of plants	% age
Growth habit	drooping	3	30
	pyramidal	2	20
	broad spreading	5	50
Time of flowering	early	2	20
	early to mid season	2	20
	mid season	2	20
	late	4	40
Intensity of flowering	low	2	20
	medium	3	30
	high	5	50
Fruit setting	low	2	20
	medium	4	40
	high	4	40
Ripening time	early	2	20
	mid season	2	20
	late	6	60
Productivity	low	0	0
	medium	4	40
	high	6	60
Fruit shape	slightly oblong	1	10
	round	9	90
Exterior color of immature fruit	green to light yellow	5	50
	light green	3	30
	green	2	20
Exterior color of mature fruit	purple	6	60
	dark red	3	30
	blackish	1	10

Concerning the productivity, six accessions (60%) were found highly productive and four (40%) were in the middle while no accession were low in productivity. Nine accessions (90%) had round-shaped and remaining one (10%) had slightly oblong shaped fruits. Regarding exterior color of immature fruits, five accessions (50%) had green to light yellow, three (30%) were light green and rest of two accessions (20%) had green color. As for as exterior color of mature fruits, the fruit color of six accessions (60%) was purple while three accessions (30%) had dark red and remaining one accession (10%) had blackish color of their mature fruits.

Banjosa. Frequency of qualitative traits of the accessions from Banjosa location is presented in tab. 5. The result showed that out of ten accession, five (50%) had dropping growth habit, two (20%) had pyramidal and rest of three (30%) had spreading growth habit. One accession (10%) bloomed early and same number of accession bloomed late, two (20%) overlapped and were early to mid season, while six accessions (60%) bloomed in mid season. As for intensity of flowering, five (50%) had high, only two (20%) had low and the rest of three (30%) were in the middle. Seven accessions (70%) had high fruit set, one (10%) had medium and rest of two (20%) had low fruit set. Three accessions (30%) ripened in early and same number of accessions were late in their ripening of fruits, while rest of accessions i.e. four (40%) were mid season in their ripening of fruits.

Table 5. Summary for frequency of morphological traits (qualitative) of wild raspberry germplasm in location Banjosa

Trait	Category	No. of plants	% age
Growth habit	drooping	5	50
	pyramidal	2	20
	broad spreading	3	30
Time of flowering	early	1	10
	early to mid season	2	20
	mid season	6	60
	late	1	10
Intensity of flowering	low	2	20
	medium	3	30
	high	5	50
Fruit setting	low	2	20
	medium	1	10
	high	7	70
Ripening time	early	3	30
	mid season	4	40
	late	3	30
Productivity	low	3	30
	medium	1	10
	high	6	60
Fruit shape	slightly oblong	5	50
	round	5	50
Exterior color of immature fruit	green to light yellow	4	40
	light green	4	40
	green	2	20
Exterior color of mature fruit	purple	3	30
	dark red	5	50
	blackish	2	20

Regarding the productivity, six accessions (60%) were found highly productive and one accession (10%) was in the middle while three accessions (30%) were low in productivity. Five accessions (50%) had round-shaped while same number of accessions

had slightly oblong shaped fruits. As for exterior color of immature fruits, four accessions (40%) had green to light yellow and the same number of accessions had light green and rest of two accessions (20%) had green color. Regarding exterior color of mature fruits, the fruit color of three accessions (30%) was purple while five accessions (50%) had dark red and remaining two accessions (20%) had blackish color of their mature fruits.

Neriyan Sharif. Frequency of qualitative traits of the accessions from Neriyan Sharif is presented in Table 6. The result demonstrated that out of ten accession, three (30%) had dropping growth habit, two (20%) had spreading and rest of five (50%) had pyramidal growth habit. No accession was early in blooming; four accessions (40%) overlapped and were early to mid season, while five accessions (50%) bloomed in mid season and one accession (10%) was late in blooming. Concerning the intensity of flowering, seven accessions (70%) had high and three accessions (30%) had medium intensity. Five accessions (50%) had high fruit set, four (40%) had medium and rest of one accession (10%) had low fruit set. Four accessions (40%) were early ripening, while rest of six (60%) accessions were mid season in their ripening of fruits.

Table 6. Summary for frequency of morphological traits (qualitative) of wild raspberry germplasm in location Neriyan Sharif

Trait	Category	No. of plants	% age
Growth habit	drooping	3	30
	pyramidal	5	50
	broad spreading	2	20
Time of flowering	early	0	0
	early to mid season	4	40
	mid season	5	50
	late	1	10
Intensity of flowering	low	0	0
	medium	3	30
	high	7	70
Fruit setting	low	1	10
	medium	4	40
	high	5	50
Ripening time	early	4	40
	mid season	6	60
	late	0	0
Productivity	low	2	20
	medium	7	70
	high	1	10
Fruit shape	slightly oblong	2	20
	round	8	80
Exterior color of immature fruit	green to light yellow	4	40
	light green	2	20
	green	4	40
Exterior color of mature fruit	purple	6	60
	dark red	4	40
	blackish	0	0

As for the productivity, one accession (10%) was found highly productive and two accessions (20%) were low in productivity while seven accessions (70%) were in middle. Eight accessions (80%) had round-shaped and two accessions (20%) had slightly oblong shaped fruits. As for exterior color of immature fruits, four accessions (40%) had green to light yellow and the same number of accessions had green and rest of two accessions (20%) had light green color. Regarding exterior color of mature fruits, the fruit color of six accessions (60%) was purple and four accessions (40%) had dark red color of their mature fruits.

Quantitative traits. Results for plant height, leaf size and number of branches plant^{-1} indicated significance differences among locations at $p \leq 0.05$ level (tab. 7). The mean values indicated that the maximum plant height (196.70 cm) and number of branches plant^{-1} (36.80) were recorded in those plants growing at Neriyan Sharif and the least in those at Topa location. Maximum leaf size was recorded in those accessions explored from Topa location and the minimum in those found at Neriyan Sharif. The results revealed that the locations had significant effect on plant height, leaf size, and number of branches plant^{-1}.

Table 7. Morphological characteristics of wild raspberry germplasm collected from three different locations

Location	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of branches plant^{-1}	Flowering duration (days)	Fruit setting (%)	Day to fruit maturity	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)
Topa	185.0c	12.4a	12.2a	31.5c	14.4c	78.1a	31.7c	9.1a	11.4a	3.79a
Banjosa	191.3b	11.0b	10.2b	34.0b	15.6b	72.5c	34.3b	7.2b	9.5b	3.63b
Neriyan Sharif	196.7a	9.1c	9.4c	36.8a	18.1a	76.9b	37.2a	8.3c	8.4c	3.49c
LSD ($p \leq 0.05$)	4.06	0.40	0.49	0.45	1.07	2.35	0.36	0.64	0.35	0.11

As for as fruit setting is concerned, the mean values indicated significant differences among the locations (tab. 7). The highest fruit set (78.1%) was observed in those plants growing at Topa location, followed by those at Neriyan Sharif (76.9%) and the minimum fruit set (72.5%) was recorded in the plants of Banjosa location. Days to flowering and days to maturity followed the same pattern. The plants growing at Neriyan Sharif had the maximum flowering duration (18.1 days) and took maximum time (37.2 days) to fruit maturity. Whereas, the plants found at Topa location had minimum duration for flowering (14.4 days) and took minimum days (31.7) to fruit maturity.

Data on fruit size (length and width) and weight also revealed significant differences among the locations (tab. 7). The fruits collected from Topa location had the largest size in term of length and width (9.1 and 11.4 mm, respectively), followed by those collected from Banjosa. The plants found in Neriyan Sharif had the smallest fruit size. Maximum fruit weight was recorded in accessions grown at Topa location (3.8 g) and the lowest (3.5 g) in those collected from Neriyan Sharif location.

Nutritional composition of fruits. The chemical analyses were carried out on the pulp of raspberry fruits from each location. There were significant differences ($p \leq 0.05$) for all the parameters of nutritional composition of fruits studied among the locations (tab. 8). The highest percentages 75.6, 10.8, 4.2, 4.9, 7.8 and 2.2 were recorded for the parameters of moisture, protein, fat, fiber, ash and acidity, respectively in those fruit samples collected from Neriyan Sharif. This was followed by in those fruit samples collected from Banjosa (tab. 8). The lowest values (71.0, 8.7, 2.7 2.0, 4.4 and 0.5%) were recorded in fruits collected from Topa location for these parameters, respectively. Carbohydrates, TSS and pH were found to be the highest (12.4, 11.4 and 4.4%, respectively) in fruit samples collected from Topa location and the lowest (9.5, 9.7, and 3.1%, respectively) in those collected from Neriyan Sharif.

Table 8. Nutritional composition of wild raspberry fruits collected from three different locations

Location	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	TSS (°Brix)	Acidity (%)	pH
Topa	70.99c	12.44a	8.74c	2.70c	2.01c	4.41c	11.44a	0.46c	4.43a
Banjosa	72.95b	11.99b	9.52b	3.03b	3.45b	6.16b	10.53b	1.37b	3.51b
Neriyan Sharif	75.58a	9.52c	10.78a	4.19a	4.89a	7.76a	9.74c	2.17a	3.05c
LSD ($p \leq 0.05$)	2.13	1.37	1.32	0.83	0.82	1.41	1.26	1.33	1.00

Table 9. Mineral composition (mg kg^{-1}) of wild raspberry fruits collected from three different locations

Location	K (mg)	Ca (mg)	Na (mg)	Fe (mg)	Cu (mg)	Zn (mg)	Mn (mg)	Pb (mg)	Cr (mg)
Topa	347.7c	200.0c	99.0a	123.7b	2.57c	4.00c	4.20b	1.20a	1.50b
Banjosa	351.7b	206.7b	92.7b	119.7c	2.87b	7.00b	3.63c	1.00b	1.00c
Neriyan Sharif	357.3a	213.3a	86.0c	126.0a	3.10a	9.33a	5.23a	0.84c	2.10a
LSD ($p \leq 0.05$)	3.52	4.31	4.03	3.10	0.17	1.47	0.55	0.12	0.17

Mineral composition of fruits. The results regarding mineral composition of fruits (mg kg^{-1}) collected from three different locations indicated significant differences ($p \leq 0.05$) among the locations (tab. 9). The maximum values for K (357.3 mg), Ca (213.3 mg), Fe (126.0 mg), Cu (3.1 mg), Zn (9.3 mg), Mn (5.2 mg) and Cr (2.1 mg) were recorded in those fruits taken from Neriyan Sharif. On the other hand, the maximum concentrations of Na (99.0 mg) and Pb (1.2 mg) were recorded in fruit samples collected from Topa location. The lowest mineral contents i.e. K (347.7 mg), Ca (200.0 mg), Cu (2.6 mg) and Zn (4.0 mg) were found in the fruit samples collected from Topa location, whereas, the fruit samples collected from Banjosa location had the lowest concentrations for Fe (119.7 mg), Mn (3.6 mg) and Cr (1.0 mg).

DISCUSSION

Qualitative traits. The wild raspberry plants, growing at various locations of different topographical features, showed variability in their growth habit. The wild raspberry plants had drooping, pyramidal and broad spreading growth forms. This variability in growth habit of *R. idaeus* population might be due to their genetic makeup and differences in environmental conditions prevailing in these regions. Growth habit of the germplasm studied corresponds with the findings of Ryabova [2007] who reported that wild raspberry exhibit a high level of morphological, phonological and genetic variation.

Flowering behavior, fruit setting (%), ripening and productiveness of fruit plants are generally co-related to each other. These parameters are highly influenced by geographical features of the area, environmental conditions and genetic makeup of the fruit species. This variation in their reproductive traits of wild raspberry might be due to topography, elevation and mean temperature of the locality where these accessions were growing. In warm temperate regions with southern aspect plants receive more sunlight, hence bloom and ripen earlier. Plants growing on northern aspects and localities where frost accumulates during spring; bloom late due to prevailing low temperatures [Palmer 1981] and also fruits ripen in late season. The results of the present study are also consistent with the findings of Gwozdecki et al. [1996] who stated that cultivars are the most important factor for bearing habits and productivity of the crops. Adverse weather conditions are usually the limiting factors for fruit setting and productivity of wild germplasm. Wild raspberry growing at some locations face early spring frost, hail storms, severe cold as well as heavy rainfall which are the most common feature of mountainous areas of Azad Jammu and Kashmir and may result in partial or complete failure of fruit set. The mid season and late blooming plants set normal fruit ultimately showing good cropping efficiency. Further, they escape from adverse climatic conditions of early spring. The plants growing at higher altitude where spring temperature remains low; bloom and ripen their fruits later than those growing in low lying valleys and warm temperate belts. Our observations are in agreement with Verissimo et al. [2002] who noted that duration of flowering, subsequent fruit set and cropping behavior are affected by severity of weather which varies year to year as well as depends upon aspect of locality.

Fruit shape may vary among the cultivars and wild populations. In the present study, fruit shape remained variable in the samples collected from different locations. As previously reported, variability in morphology of the fruits might be due to environmental constraints, i.e. water deficit and temperature [Latrasse and Dupuy 1973]. However, genetic makeup of the genotypes is also an important factor for the morphological characters of the fruits. Fruit color is very important indicator for quality and maturity of fruits and also a source of aesthetic pleasure for consumers. In this study, the most of the plants had green to light yellow skin color (immature fruits), some had light green and green colored fruits, while mostly developed purple or dark red color when they matured. The locations which are open and received direct sunlight for most of the day usually have light green to green color of immature fruits. The plants growing in northern aspect with low light intensities have green to light yellow fruit skin color. Fruit color is significantly influenced by temperature, location of the plant, light penetration, growth habit and plant canopy [Lotze and Bergh 2004]. Further, interaction between

environment and genotypes, growth of the tree particularly canopy, foliage and position of the fruit also influence the color of fruits [Latrasse and Dupuy 1973].

Quantitative traits. Plant height (primocane) and number of bearing branches per plant significantly differed between locations. Variability in growth behavior of the plants from different locations might be due prevailing soil and climatic conditions and topography of the location. These results are in agreement with the findings of Glisic et al. [2009] who recorded maximum height in cultivated raspberry plants growing in rich organic soil. Further, the number and arrangement of branches, plant shape, dry matter accumulation and plant size also depend upon light penetration into the plant [Palmer 1981, Glisic et al. 2009]. Wild raspberry plants studied, also exhibited diversity in foliar dimensions among the locations. This variability in leaf size is consistence with the findings of Lone and Wafal [2000] who observed remarkable diversity in leaves of Rosaceous fruits. This diversity might be due to environmental as well as genetic factors and other salient features of the area.

Wild raspberry germplasm differed in their flowering duration, fruit set and time to maturity/ripening. Blooming time and duration, fruit set and precocity also depends upon genotypes and prevailing environmental conditions at the time of flowering and fruit growth period. Rainfall and hailstorms at the time of flowering adversely affect the fruit set. Wild raspberry also showed variability in their fruit characters (size and weight), as reflected by the fruits of different locations. High cropping load results in small fruits with a low percentage of large sized fruits and reduction in crop load can favor more number of large sized fruits [Lotze and Bergh 2004]. It appears that some climatic factors such as precipitation or snow cover influence the genetic adaptation of wild raspberry populations [Patamsyte et al. 2008]. The logical reason for its diversity is also prevailing climatic conditions like temperature, sunlight, rainfall, snowfall and hailstorms. Variations in physical characters of fruits have previously been reported among different genotypes as a result of variable environmental conditions and genetic factor [Caspari et al. 1994, Behboudian et al. 1994, Garriz et al. 1998, Ahmed et al. 2013].

Nutritional composition of fruits. Water is the major component of almost all soft fruits like berries. In the present investigation, moisture content percentage varied from 71 to 75.6% in the fruits collected from three locations. Almost similar results in raspberry fruit were reported by Pritts and Handley [1989]. Differences in amount of rainfall and its distribution, and water supply during fruit growth and development and at maturity stage is probably a possible reason for variation in moisture content of fruits collected from different locations.

Carbohydrate content varied from 9.52 to 12.44% in fruit samples of three locations. This range of carbohydrates content is considerably higher than cultivated raspberry as previously recorded (10.62%) by Han et al. [2008]. Mean values for protein content differed significantly from each other. The results navigated that genotypes behaved differently against climatic conditions in term of fruit composition. Same pattern of genotypes has previously been reported by HanPing et al. [2009] who observed variation in contents of soluble sugar, starch and soluble protein of raspberry with dropping temperature from September to December. The wild species, *Rubus crataegifolius*, with strong cold resistance had the maximum contents of the three substances as compared to the cultivar 'Aozhou Hong' with weak cold resistance.

Fat is very important component of food and rarely found in fruits. In present investigation, wild fruits had reasonable quantity of fat content ranging from 2.70 to 4.19% in the fruit samples of different localities. Range of fat content in present investigation was higher than previously reported in cultivated raspberry fruits [Kafkas et al. 2008]. Celik and Ercisli [2009] also reported that wild raspberry genotypes had higher amount of fatty acids than cultivated raspberry.

Results indicated that fiber content of wild raspberry genotypes (2.01 to 4.89%) was found close to fiber content (3.0 to 7.4%) of cultivated varieties of raspberry as observed by Pritts and Handley [1989]. Fruit samples of raspberry differed significantly for ash content which ranged from 4.4 to 7.8% in fruit samples of three locations. The results of the present study are in close conformity with those previously reported by Hristov et al. [2009] who recorded 9.3% ash content in eight raspberry cultivars. Variation for ash content in fruit samples was probably due to different localities with variable environments.

Total soluble solids and acids content contribute to fruit quality imparting sensory attributes for the berries [Plessi et al. 2007]. The mean values for TSS, acidity and pH significantly differed among the fruits of three locations, which is in agreement with the findings of various researchers for raspberry [Ochmian and Skupień 2008]. The variations observed may result not only from genetic factors but also from prevailing environmental conditions [Latrasse and Dupuy 1973, Maikhuri et al. 1994, Ahmed et al. 2013].

Mineral composition of fruits. Wild raspberry fruits contain macro- and microelements; some of these are essential for physiological equilibrium of the body [Plessi et al. 2007], while other may be toxic if present above the safer limit. Therefore, it is imperative to determine the mineral status of raspberry fruits. In the present investigation, various minerals (K, Ca, Na, Fe, Cu, Zn, Mn, Pb and Cr) were analyzed from the fruits collected from three locations which differed significantly. Similar results have been reported by previous workers [Pritts and Handley 1989, Strik 2008, Eyduran and Agaoglu 2006, Plessi et al. 2007]. The variations observed might be as a result of genetic factors and their interaction with edaphic factors and climatic conditions [Latrasse and Dupuy 1973, Ahmed et al. 2013].

CONCLUSION

The results show large variations in plant morphology, reproductive behavior (flowering, fruit setting, ripening and productivity), fruit size, color, nutritional composition (carbohydrates, protein, fat, fiber, TSS, acidity, pH etc) and mineral content in fruits of wild raspberry. Diversity observed among the genotypes originating from different locations of lower Himalayan region of the State of Azad Jammu and Kashmir, offers reliable data for selection of better genotypes. The results also imply that the nutritional components and minerals of raspberry may have substantial health benefits and health promoting effects to consumers. These genotypes are valuable source for genetic improvement of the cultivated genotypes and can be used in future breeding programs for developing superior raspberry cultivars.

REFERENCES

AACC, 2000. Approved Methods of American Association of Cereal Chemists. American Association of Cereal Chemists Inc., St. Paul Minnesota, USA.

Ahmed M., Anjum M.A., Naz R.M.M., Khan M.R. Hussain S., 2013. Characterization of indigenous barberry germplasm: variability in morphological characteristics and nutritional composition. Fruits, 68, 409–422.

AOAC, 2000. Official Methods of Analysis (17th ed.). Association of Official Analytical Chemists, Washington DC, USA.

Badjakov I., Nikolova M., Gevrenova R., Kondakova V., Todorovska E., Atanassov A., 2008. Bioactive compounds in small fruits and their influence on human health. Biotechnol. Biotechnol. Equip., 22, 581–587.

Behboudian M.H., Lawes G.S., Griffiths K.M., 1994. The influence of water deficit on water relations, photosynthesis and fruit growth in Asian pear (*Pyrus serotina* Rehd.). Sci. Hortic., 60, 89–99.

Caspari H.W., Behboudian M.H., Chalmers D.G., 1994. Water use, growth, and fruit yield of 'Housi' Asian pears under deficit irrigation. J. Amer. Soc. Hort. Sci., 119, 383–388.

Celik F., Ercisli S., 2009. Lipid and fatty acid composition of wild and cultivated red raspberry fruits (*Rubus idaeus* L.). J. Med. Plant. Res., 3, 583–585.

Eyduran S.P., Agaoglu Y.S., 2006. A preliminary examination regarding ten raspberry cultivars. Res. J. Agric. Biol. Sci., 2, 375–379.

Garriz P.I., Colavita G.M., Alvarez H.L., 1998. Fruit and spur leaf growth and quality as influenced by low irradiance levels in pear. Sci. Hortic., 77, 195–205.

Glisic I., Milosevic T., Veljkovic B., Glisic I., Milosevic N., 2009. Trellis height effect on the production characteristics of raspberry. Acta Hortic., 825, 389–393.

Gwozdecki J., Krzewińska D., Pierzga K., Bakum M., 1996. An evaluation of 'Polana' raspberry. J. Fruit Ornam. Plant Res., 2, 69–71.

Han J., HxinHua N.B., JiWen L., Patar A., Shabor M., 2008. Study on the nutritional components of raspberry fruits and their effects on antioxidant activity. Nurd. Acta Nutr. Sinica., 30, 410–413.

HanPing D., ZhiJie X., XiuWu G., 2009. Studies on physiological and biochemical characteristics of raspberry canes under natural overwintering condition. J. Jilin. Agric. Univ., 31, 573–577.

Hristov S., Stoyanova T., Minev I., 2009. Raspberry cultivars for fruit production under mountain conditions. Acta Hortic., 825, 221–224.

Jennings D.L., Daubeny H.A., Moore J.N., 1990. Blackberries and raspberries, Acta Hortic., 290, 329–391.

Kafkas E., Ozgen M., Ozogul Y., Turemis N., 2008. Phytochemical and fatty acid profile of selected red raspberry cultivars: A comparative study. J. Food Qual., 31, 67–78.

Latrasse A., Dupuy P., 1973. Composition of red raspberry grown in different climatic areas in France. Jugoslav Pomol., 25–26, 107–116.

Lone A.F., Wafal B.A., 2000. Varietal diversity in the germplasm of cherries under cultivation in Kashmir. In: Environmental biodiversity and conservation, Khan M.A., Farooq S., (eds). A.P.H. Publishing, New Delhi, 319–340.

Lotze E., Bergh O., 2004. Early prediction of harvest fruit size distribution of an apple and pear cultivar. Sci. Hortic., 101, 281–290.

Maikhuri R.K., Semwal R.L., Singh A., Nautiyal M.C., 1994. Wild fruits as a contribution to sustainable rural development: a case study from the Garhwal Himalaya. Int. J. Sust. Dev. World Ecol., 1, 56–68.

Ochmian I., Skupien K., 2008. Comparison of field performance, fruit firmness, and nutritional value of yellow- and red-fruit Polish raspberry cultivars. EJPAU, 11, 4, 12, www.ejpau.media.pl

Palmer J.W., 1981. Computed effects of spacing on light interception and distribution within hedgerow trees in relation to productivity. Acta Hortic., 114, 80–88.

Patamsyte J., Zvingila D., Labokas J., Baliuckas V., Balciuniene L., Kleizaite V., Ancelis V., 2008. Study of genetic diversity in wild raspberry (*Rubus idaeus* L.) germplasm collection using morphological characters and RAPD markers. Biologia, 54, 66–74.

Pearson D., Melon H.K., Ronald S., 1976. Chemical Analysis of Food (8th ed.). Churchill Livingstone, USA, 5–63.

Plessi M., Bertelli D., Albasini A., 2007. Distribution of metals and phenolic compounds as a criterion to evaluate variety of berries and related jams. Food Chem., 100, 419–427.

Pritts M., Handley D., 1989. Bramble production guide. Northeast Regional Agricultural Engineering Services Bulletin 35. Cornell University, New York.

R.H.S., 2001. Colour chart. Royal Horticultural Society. London.

Romoleroux K., 1992. Rosaceae in the parmos of Ecuador. In: Parame: an andean ecosystem under human influence, Balslev H., Luteyn J.L., (eds). Academic Press, London, 85–94.

Ryabova D., 2007. An evaluation of *Rubus idaeus* L. Genet. Resour. Crop Evol., 54, 973–80.

Singh S.K., Bist L.D., Patel V.B., 2004. Flowering and floral biology studies in low chill pear cultivars. Ind. J. Hort., 61, 94–96.

Strik B.C., 2008. A review of nitrogen nutrition of *Rubus*. Acta Hortic., 777, 403–410.

Verissimo V., Gardin J.P., Trevisan R., de Silva J.B., 2002. Morphological and physical parameters of the flower buds of the trees of two Japanese pear cultivars grown at three different areas of Southern Brazil and their relationship with flower bud abortion intensity. Acta Hortic., 587, 381–386.

Zaffar G., Mir M.S., Sofi A.A., 2004. Genetic divergence among apricot (*Prunus armeniaca* L.) genotypes of Kargil, Ladakh. Ind. J. Hort., 61, 6–9.

RÓŻNORODNOŚĆ BIOLOGICZNA W MORFOLOGICZNYCH I FIZYKO-CHEMICZNYCH CECHACH MATERIAŁU GENETYCZNEGO MALINY WŁAŚCIWEJ (*Rubus idaeus* L.) ZBIERANEJ W REGIONIE UMIARKOWANYM AZAD JAMMU I KASZMIRU (PAKISTAN)

Streszczenie. Malina właściwa (*Rubus idaeus* L.) jest często hodowana i szeroko rozprowadzana w umiarkowanych rejonach niższego pasma Himalajów, które reprezentuje bogatą różnorodność biologiczną malin. Zbadano trzydzieści próbek z trzech różnych miejsc Azad Jammu i Kaszmiru (Pakistan), dokonano wizualizacji kilkunastu cech w miejscach pobierania próbek oraz oceniono i ilościowo określono kilka cech po zebraniu próbek liści i owoców. Próbki znacznie różniły się wzrostem, czasem kwitnienia, intensywnością zakwitania, zawiązywaniem owoców, czasem dojrzewania, plonowaniem oraz kształtem i kolorem owoców. Jeśli chodzi o cechy ilościowe, to stwierdzono istotne różnice w wysokości roślin, długości i szerokości liści, liczbie gałązek na roślinie, długości kwitnienia, procencie zawiązywanych owoców, liczbie dni do dojrzałości, długości owoców, szerokości i wadze pomiędzy próbkami zebranymi z różnych stanowisk. Dojrzałe owoce oceniono pod względem składu chemicznego, tzn. wilgotności, zawartości węglowodanów, białka, tłuszczu, błonnika, popiołu, TSS, kwasowości, pH oraz zawartości minerałów (K, Ca, Na, Fe, Cu, Zn, Mn, Pb oraz Cr). Stanowisko także miało istotny wpływ na skład chemiczny dojrzałych owoców.

Słowa kluczowe: różnorodność genetyczna, skład mineralny, cechy morfologiczne, skład odżywczy, materiał genetyczny

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