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GENETIC DIVERSITY IN FRUIT NUTRITIONAL COMPOSITION, ANTHOCYANINS, PHENOLICS AND ANTIOXIDANT CAPACITY OF PLUM (*Prunus domestica*) GENOTYPES

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Abstract. In the present work, genetic diversity in nutritional composition of sixteen plum genotypes growing at four different locations of Tehsil Rawalakot, District Poonch of Azad Jammu and Kashmir (Pakistan) were studied. Various parameters like moisture, dry matter, ash and total soluble solids contents, acidity, pH, vitamin C and sugar content, shelf-life and sensory/organoleptic evaluation, anthocyanins, phenolics and antioxidant activity were evaluated and variation in these characteristics has been discussed. The results suggested that the genotypes differed in their nutritional composition of fruits, anthocyanin and phenolic contents and antioxidant activity of fruit. The results of the present study regarding the nutritional status of existing plum germplasm will contribute and increase our knowledge about the genus *Prunus* and broaden the gene pool available for future plant breeding programs.

Key words: antioxidants, biochemical analysis, biodiversity, genotypes, proximate composition

INTRODUCTION

Plum is a temperate zone fruit crop that belongs to the genus *Prunus* of subfamily *Prunoidae*. There were described seventy seven species of plum in the genus *Prunus* [Rehder 1967]. Plum is originated from five centers; these include Western Asia for *Prunus insititia* (Damson plum), Europe for *Prunus domestica* (European plum), We-

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stern and Central Asia for *Prunus cerasifera* (Cherry plum), North America for *Prunus americana* (American plum) and China for *Prunus salicina* (Japanese plum) [Watkins1976]. In Pakistan plum is being grown commercially on an area of about 6.8 thousand hectares with a total production of 56.00 thousand tons annually [FAO 2012]. Several wild plum (*Prunus* spp.) having distinct fruits are also found widely in some areas of the country. Almost half of the plums production in the world is consumed as fresh whereas the rest of fruits are used after processing.

Plums are an excellent source of nutrients and contribute extensively to human nutrition [Cao et al. 1997], which encourage human health and prevent incidence of various diseases [Stacewicz-Sapuntzakis et al. 2001, Hooshmand and Arjimani 2009]. It contains organic acids (malic and citric acid), carbohydrates (glucose, sucrose and fructose), fibers (pectins), aromatic substances, tannins and enzymes. Every one of these substances is a vital component for taste and nutritive value of fruits [Ertekin et al. 2006]. Apart from these basic food contents, plum fruits are affluent source of phenolic compounds, characterized by relatively high anthocayanin and antioxidant activity which is higher than apples, oranges and strawberries [Kayano et al. 2002, Leong and Shui 2002, Kim et al. 2003b, Cevallos-Casals and Cisneros-Zevallos 2004]. Fruits from different plum cultivars and wild relatives vary in their physico-chemical characteristics and organoleptic properties [Robertson et al. 1992, Nergiz and Yildiz 1997, Gil et al. 2002, Lozano et al. 2009, Walkowiak-Tomczak et al. 2008, Erturk et al. 2009, *Ajenifujah-Solebo* and Aina 2011]. Such variation was due to varietal characters and also due to environmental and growth conditions [Vursavus et al. 2006]. Differences for phenolic compounds, total flavonoids, total anthocyanins and antioxidant capacity [Gil et al. 2002, Chun et al. 2003, Kim et al. 2003a, b, Cevallos-Cassals et al. 2006, Rupasinghe et al. 2006, Rop et al. 2009, Kristl et al. 2011] have also been recorded among plum cultivars.

The state of Azad Jammu and Kashmir (AJK), Pakistan lays between the two foremost centers of origin i.e. the Caucasus Mountains and China. It is mountainous area that possesses a distinctive environment with diverse climates ranging from temperate to subtropical and produces a variety of temperate fruit crops such as, apple, pear, plum, peach, apricot, almond and walnut. A large amount of genetic diversity exists in these fruit crops accumulated through mutation, hybridization and natural seed propagation [Lone and Wafal 2000, Zaffar et al. 2004, Ahmed et al. 2009]. Plums are grown profusely along with their wild relatives, which exist in their naturalized form in the area, that have not been explored yet. Indigenous fruits restrain a good percentage of diet for inhabitants who had commonly used wild edible fruits, including wild plums as food and for medication for thousands of years [Ercisli 2004, Ahmed et al. 2009]. There are few citations on the nutritional composition of local plums and no effort has been made for enhancement and exploitation of indigenous plum species for food and other uses. Therefore, this study was conducted to evaluate the indigenous plum germplasm for their sustainable use. The results of the present study regarding the nutritional status of existing plum germplasm will contribute and increase our knowledge about the genus *Prunus* and broaden the gene pool available for future plant breeding programs.

MATERIALS AND METHODS

Survey of the plum growing areas and selection of genotypes. A survey was conducted in the plum growing areas of District Poonch, Azad Jammu and Kashmir (northern Pakistan) during the year 2011. To get the first hand information, consultation with people was made about the present status, production and marketing of different genotypes grown in the region at various places. Locations were selected keeping in mind their suitability with respect to the availability of diverse genotypes. Bearing plants of sixteen plum genotypes with divergent characters existing at different sites were selected with visible good health, similar stem girth and size. The selected genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow), and the selected plants were tagged permanently. A short description for all the genotypes/accessions was also recorded in the form of passport data with the help of *Prunus* descriptor developed by International Board of Plant Genetic Resources [Cobianchi and Watkins 1984] (tab. 1).

Table 1. A short description about fruit skin color, pulp color, seed color and seed shape of plum genotypes

Genotype	Local name	Fruit skin colour	Pulp colour	Seed colour	Seed shape
DR1*	Alu bukhara	dark red	yellow	white	oval
DR2	Alu bukhara	dark red	yellow	light brown	oval
DR3	Alu bukhara	dark red	yellow	dark brown	elliptical
DR4	Alu bukhara	dark red	dark yellow	brown	round
SY1	Aluchi	yellow	dark yellow	off white	elliptical
SY2	Aluchi	yellow	yellow	off white	round
SY3	Aluchi	yellow	yellow	brown	round
SY4	Aluchi	yellow	light yellow	brown	elliptical
RB1	Alucha	red brown	red	brown	elliptical
RB2	Alucha	red brown	red	dark brown	oval
RB3	Alucha	red brown	red	dark brown	round
RB4	Alucha	red brown	yellow	off white	oval
LY1	Alu bukhara	yellow	yellow	dark brown	oval
LY2	Alu bukhara	yellow	light yellow	brown	elliptical
LY3	Alu bukhara	yellow	light yellow	off white	round
LY4	Alu bukhara	yellow	yellow	dark brown	oval

Means in each column with different letters are statistically significant at $p < 0.05$ (LSD test)
* – genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

Ecological characteristics of the location. The study area lies between an altitude of 1800–2100 m above sea level and latitude of 33–360 in the north-east of Pakistan under the foothills of great Himalayas in Rawalakot district of Poonch Division. 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. Some part of this regions are extremely rugged, precipitous and highly unstable.

Fruit collection and sample preparation. At marketable maturity stage (determined by visual expressions of their peel color and size), the 20 fruits of uniform size were handpicked from the selected genotypes during mid May to 1st week of June, sorted out, filled in malleable cardboard box and on the same day transported to the Post-Harvest Laboratory at the Department of Horticulture, Faculty of Agriculture, Rawalakot. Selected fruits of uniform size were washed with distilled water to eradicate any mud, dust or residuals material and then air dried. For biochemical analysis, 20 g of fruit sample was taken separately for each genotype and blended in a liquidizer with water. The mixture was filtered through a filter paper (Whatman No. 1) and total volume of sample was made 250 ml with distilled water.

Moisture content (%). One gram of fruit edible portion was taken for each genotype and moisture content of the fruits was determined by gravimetric method [AOAC 2000].

Dry matter (%). The fruit samples were dried in an oven at 70°C until a stable mass was achieved. Dry matter content was determined by using the following formula:

$$\text{Dry matter}\,(\%) = \frac{\text{Weight of sample after drying}}{\text{Initial weight}} \cdot 100$$

Ash content (%). Ash content was established by flaming a weighed sample at 550°C in a muffle furnace to a constant weight by following the method used by Ough and Amerine [1988].

Total soluble solids (%). Total soluble solid (TSS) in the juice sample was measured as described by Dong et al. [2001]. One slice of homogeneous size from ten fruits was juiced collectively to make a mixed sample for each treatment in each replication. TSS was recorded by using a hand refractometer (Abbe® model 10450).

Titratable acidity (%). Juice acidity was measured by following the method of AOAC [2000].

Juice pH. The pH of the juice samples was directly measured by using a digital pH meter [Ruck 1963].

Vitamin C content ($mg \cdot kg^{-1}$). Vitamin C content in the fruit samples was estimated by using 2, 6, dichlorophenol indophenol dye as described by Ruck [1963].

Sugar content ($g \cdot kg^{-1}$). Total sugars and reducing sugars were estimated by following the procedure illustrated by Horwitz [1960]. For total sugar content, 25 ml of prepared juice was taken in 100 ml flask; 5 ml of concentrated hydrochloric acid and 20 ml distilled water were also added for changing the non-reducing sugars into reducing sugars. For conversion it was reserved at normal room temperature for 24 hours for the purpose of complete hydrolysis. Then neutralization of mixture was done with

1 N NaOH solution by using phenolphthalein as an indicator and final volume of 100 ml was made with distilled water. This solution was taken into the burette and titrated against 10 ml Fehling's solutions for the estimation of total sugars.

Calculations for the total sugars were made according to the following formula.

$$\text{Total sugars} = 25 \cdot \frac{X}{Z},$$

where:

X – volume (ml) of standard sugar used against 10 ml of Fehling's solution,

Z – volume (ml) of sample aliquot titrated against 10 ml of Fehling's solution.

Reducing sugar (g·kg^{-1}). For reducing sugar, plum juice sample (10 ml) was transferred into 250 ml volumetric flask. In the flask, 100 ml distilled water, 25 ml lead acetate solution (25%) and 10 ml potassium oxalate solution (20%) were added. Then the final volume was made by adding distilled water in 250 ml volumetric flask. This solution was filtered and filtrate was titrated against Fehling's solution. Fehling's solution (10 ml) were taken in a flask and titrated against the above prepared filtrate taken in a 50 ml burette with constant boiling on soft flame until brick red colour appeared. Then 2–3 drops of 1% methylene blue were supplemented and continued the process of titration again by adding the aliquot drop wise on boiling solution until the appearance of brick red colour again. The magnitude of aliquot used was recorded and reducing sugars were calculated as follows.

$$\text{Reducing sugars} = 6.25 \cdot \frac{X}{Y},$$

where:

X – volume (ml) of standard sugar solution titrated against 10 ml Fehling's solution,

Y – volume (ml) of sample aliquot used against 10 ml Fehling's solution.

Non-reducing sugars was deliberated by using the formula:

$$\text{non-reducing sugars} = \text{total sugars} - [\text{reducing sugars} \cdot 0.95]$$

Post-harvest life (days). Ten fruits from each accession were kept at normal room temperature to estimate the shelf-life of the fruits of each genotype. Post-harvest life was considered till 50% of the fruits retained eatable quality.

Sensory evaluation. Sensory or organoleptic evaluation for texture, aroma and flavour was carried out by a panel of ten judges, aligned with a scale of 1–10 scoring points as expressed by Krum [1955]. Ten fruits of each sample were offered to the panel for sensory consideration. The evaluation was rated as; excellent (8.01–10.00), good (5.51–8.00), fair (3.01–5.50) and poor (1.00–3.00).

Anthocayanin content (mg·100 g^{-1}). Total anthocyanin content was determined by pH differential method [Wrolstad 1993, Zheng et al. 2007]. Two dilutions of sample were prepared: one with potassium chloride buffer (pH 1.0) and the other with sodium acetate buffer (pH 4.5) and equilibrated for 15 minutes. Absorbance of each dilution was measured on a Spectrophotometer at 510 nm and 700 nm against a blank cell filled

with distilled water. Anthocyanin was calculated as milligrams of cyaniding-3-glucoside per 100 g of fresh weight.

Phenolic content (mg·g^{-1}). The total phenolics content as gallic acid equivalent (GAE) was determined by using the method of Singleton et al. [1999]. 10% Folin-Ciocalteau's reagent (v/v) was prepared, 2.5 ml of 10% Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate were added in 0.5 ml of aqueous extract of fruit. Incubation of this mixture was carried out at 45°C for 40 minutes and in the spectrophotometer absorbance was calculated at 765 nm. Gallic acid was used like a standard phenol. The mean of three readings was used and the total phenol content was expressed as milligrams of gallic acid equivalents/g extract.

Antioxidant potential (µg·ml^{-1}). The total antioxidant potential of the extracts was measured using the phosphomolybdenum reduction assay [Prieto et al. 1999]. The reducing capacity of the extracts was expressed as the ascorbic acid equivalent (AAE).

Antioxidant activity by DPPH radical scavenging. The antioxidant activity of the plum extracts was calculated by using the stable DPPH radical according to the method of Hatano et al. [1988]. Ethanol (1 ml) was added in juice of different concentration, ranging from 25–300 µg·ml^{-1}, to extract the antioxidants. Then, 0.25 mM solution of DPPH radical (0.5 ml) was added to this solution. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging}\,(\%) = \frac{A_0 - A_1}{A_0} \cdot 100,$$

where:

A_0 – absorbance of the control reaction.

A_1 – absorbance of the sample itself.

All determinations were carried out in triplicate.

Statistical analysis. The data recorded were subjected to statistical analysis using Co-Stat 6.300 Statistical Software. The means were compared by employing least significant difference (LSD) test at 5% level of probability [Steel et al. 1997].

RESULTS AND DISCUSSION

Moisture content. The plum genotypes significantly differed in moisture content of their fruits (tab. 2). The highest moisture content was recorded in SY2, followed by SY1, DR4, SY4, LY4 and DR3. All these genotypes stood at par with each other. The lowest moisture content was found in DR1 and DR2. Observed moisture content in all genotypes ranged from 70.40 to 89.17%. Values of present study are comparable with moisture percentage reported in fresh wild plum which was 93.5% [Yurdugul and Bozoglu 2009]. Similar results were also reported in sweet cherry varieties Van, Nor De Guban and 0–900 Ziraat i.e. 78.25, 75.95 and 84.27%, respectively [Vursavus et al. 2006]. Moisture content in fruits depends upon nature of fruit and environmental factors

such as water supply/precipitation. The fruits collected from a location with more precipitation may have higher water content.

Dry matter content. The highest content of dry matter was observed in DR1, followed by DR2 and SY3, all these three genotypes were statistically similar with each other but significantly differed from rest of the genotypes. The minimum dry matter content was recorded in SY2. The values of dry matter content in the genotypes studied remained between 10.83 to 29.60% (tab. 2). However, most of the observed values of dry matter were higher than reported values in plum (BY94M1945) which was 11.1% [Cevallos-Casals et al. 2006] and in cultivar 'Bluefre' with 12% dry matter [Walkowiak-Tomczak et al. 2008]. Variation in dry matter content was probably due to the variations in climatic conditions and variable growing conditions. However, plum cultivars also vary in their dry matter content [Walkowiak-Tomczak et al. 2008].

Ash content. The maximum ash content was recorded in DR2 (5.46%) and DR1 (5.34%), followed by SY3 (4.88%) and LY2 (4.67%). These four genotypes were statically similar. On the other hand the minimum ash content was recorded in SY2 (1.24%), followed by SY1 (1.56%) and DR4 (1.57%) (tab. 2). In wild plums grown in Turkey, ash content was 3.00% [Calisir et al. 2005], while in apricot fruits, it ranged from 2.72 to 5.34% [Haciseferogullari et al. 2007]. Therefore, results of the present study are in accordance with previously reported values.

Total soluble solids (TSS). Higher TSS was recorded in the fruits of DR2, followed by those of DR1, SY3 and LY2, which were statically similar. Low TSS was recorded in the fruits of SY2, followed by those of DR4 and SY1. TSS followed almost the same trend as for ash content. TSS values in the plum genotypes studied ranged from 8.17 to 16.23% (tab. 2). Walkowiak-Tomczak et al. [2008] found that plum (*Prunus domestica*) cultivars differ in their soluble solids contents and cv. 'Wegierka Zwykła' ranked at the top in solids contents. The results of the present study are in agreement with the reported values of TSS for dark purple colored plum (11.98%) and red colored plum (14.78%) [Erturk et al. 2009], and also for some sweet cherry varieties (14.00%) [Vursavus et al. 2006].

Titratable acidity. The highest acidity content was observed in fruit juice of the genotype RB2 (2.34%), followed that of by LY1 (2.32%) and SY4 (2.32%), which were statistically at par with each other but significantly differed from other genotypes. Least acidity content was recorded in fruit juice of DR3 and LY4 (1.49%), followed by that of SY2 and RB4 (1.52%). These four genotypes were statistically similar to each other (tab. 2). In the present study recorded values were lower than previously reported values for titratable acidity in red skinned colored fruit (4.99%) and in dark purple skinned fruit 3.89% [Erturk et al. 2009], indicating that the genotypes studied were less acidic.

Juice pH. The genotypes DR3 and LY4 had higher juice pH (3.20 and 3.19, respectively), followed by SY2 (3.15) and RB1 (3.14). These four genotypes were statistically non-significant. The lowest values of juice pH were recorded in RB2 (2.76) and LY1 (2.79) which behaved statistically alike. Observed values in this study were similar with already reported values for dark purple and red colored plums having juice pH of 3.13 and 3.70, respectively [Erturk et al. 2009]. This is interesting to note that the genotypes with higher acidity had lower juice pH value and vice versa.

Table 2. Variation in moisture, dry matter, ash and total soluble solids contents, acidity and juice pH of plum genotypes

Genotype	Local name	Moisture (%)	Dry matter (%)	Ash content (%)	TSS (%)	Acidity (%)	pH of juice
DR1*	Alu bukhara	70.40 i	29.60 a	5.35 a	15.44 ab	1.89 d	3.04 cde
DR2	Alu bukhara	70.57 i	29.43 a	5.46 a	16.23 a	1.63 fg	3.07 bcd
DR3	Alu bukhara	85.54 abc	14.53 fgh	2.81 def	11.49 def	1.49 i	3.20 a
DR4	Alu bukhara	87.13 abc	12.93 fgh	1.57 ef	9.00 fg	1.86 d	3.03 de
SY1	Aluchi	87.57 ab	12.43 gh	1.56 ef	9.40 fg	1.87 d	3.02 de
SY2	Aluchi	89.17 a	10.83 h	1.24 f	8.17 g	1.52 hi	3.15 ab
SY3	Aluchi	73.37 hi	26.63 ab	4.88 ab	14.33 abc	1.67 f	3.07 bcd
SY4	Aluchi	86.59 abc	13.41 fgh	2.37 def	9.56 fg	2.32 a	2.89 f
RB1	Alucha	81.74 de	18.26 de	3.59 bcd	12.47 cde	1.58 gh	3.14 abc
RB2	Alucha	78.30 fg	21.70 cd	3.89 abcd	13.50 bcd	2.34 a	2.76 g
RB3	Alucha	85.43 bc	14.57 fgh	2.85 def	10.51 efg	1.74 e	3.04 cde
RB4	Alucha	79.26 ef	20.73 d	3.70 bcd	13.23 bcd	1.52 i	3.08 bcd
LY1	Alu bukhara	83.54 cd	16.46 ef	3.46 bcd	12.27 cde	2.32 a	2.79 g
LY2	Alu bukhara	75.50 gh	24.63 bc	4.67 abc	14.23 abc	2.02 b	2.95 ef
LY3	Alu bukhara	84.57 bcd	15.53 efg	3.11 cde	11.59 def	1.94 c	2.96 ef
LY4	Alu bukhara	86.49 abc	13.50 fgh	2.39 def	10.47 efg	1.49 i	3.19 a

Means in each column with different letters are statistically significant at $p < 0.05$ (LSD test)
* – genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

Vitamin C content. Vitamin C is one of the imperative nutritional quality factors in fruits. In the present study, results revealed significant differences among the genotypes for their vitamin C content (tab. 3). The maximum vitamin C content was recorded in fruits of DR2, which was significantly higher than all the other genotypes. The minimum vitamin C content was recorded in fruits of SY2. In present study vitamin C content ranged between 52.51 to 137.6 mg·kg^{-1}, which is lower than the vitamin C content observed in fresh European plums that was 157.9 mg·kg^{-1} [Nargiz and Yildiz 1997] and also than recorded in freeze-dried wild plums i.e. 170 mg·kg^{-1} [Yurdugul and Bozoglu 2009]. However, Gil et al. [2002] reported vitamin C content of 3–10 mg·100 g^{-1} in fresh fruits of five plum cultivars, which is lower than the values recorded in the present study.

Table 3. Variation in vitamin C and sugar content of plum genotypes

Genotype	Vitamin C (mg·kg^{-1})	Reducing sugars (g·kg^{-1})	Non-reducing sugars (g·kg^{-1})	Total sugars (g·kg^{-1})
DR1*	122.10 b	45.00 f	41.40 abc	86.40 e
DR2	137.60 a	54.10 cd	40.50 bcde	94.60 d
DR3	78.66 e	62.77 a	42.30 ab	105.07 a
DR4	62.90 g	46.52 f	41.47 abc	87.99 e
SY1	65.09 g	50.60 de	40.63 bcde	91.23 d
SY2	52.51 h	65.00 a	38.53 de	103.53 ab
SY3	112.60 c	52.33 d	42.20 ab	94.53 d
SY4	70.48 f	37.13 g	41.00 bcd	78.13 f
RB1	98.97 d	57.70 bc	43.87 a	101.57 bc
RB2	109.20 c	25.27 h	42.10 ab	67.37 h
RB3	75.39 ef	48.67 ef	43.90 a	92.57 d
RB4	100.50 d	58.24 b	41.67 abc	99.91 c
LY1	97.84 d	27.80 h	39.37 cde	67.17 h
LY2	114.40 c	35.50 g	38.13 e	73.63 g
LY3	78.31 e	37.90 g	38.73 de	76.63 f
LY4	70.80 f	63.70 a	40.50 bcde	104.20 ab

Means in each column with different letters are statistically significant at $p < 0.05$ (LSD test)
* – genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

Sugar content. Higher reducing sugar content was recorded in fruits of SY2 (65.00 g·kg^{-1}), followed by in those of LY4 (63.70 g·kg^{-1}) and DR3 (62.77 g·kg^{-1}) which were statistically at par with each other and significantly different from other genotypes. The minimum reducing content was recorded in fruits of RB2 (25.27 g·kg^{-1}) and LY1 (27.80 g·kg^{-1}), which are statistically at par (tab. 3). These results are in concurrence with the conclusion of Nergiz and Yildiz [1997]. However, the maximum non-

reducing sugar content was recorded in fruits of RB3 (43.90 g·kg^{-1}) and RB1 (43.87 g·kg^{-1}), followed by DR3 (42.30 g·kg^{-1}), SY3 (42.20 g·kg^{-1}), RB2 (42.10 g·kg^{-1}), RB4 (41.67 g·kg^{-1}) and DR1 (41.40 g·kg^{-1}), which were statically at par with each other. The minimum non–reducing content was recorded in LY2, SY2 and LY3 i.e. 38.13, 38.53 and 38.73 g·kg^{-1}, respectively. Chemical analysis of eleven plum varieties grown in Turkey showed mean value of 42.40 g·kg^{-1} for non-reducing sugars [Nergiz and Yildiz 1997]. Thus, results of the present study are in conformity with the previous study.

Regarding the total sugar content, the maximum value was recorded in DR3 (105.07 g·kg^{-1}) which was followed by SY2 (103.53 g·kg^{-1}) and LY4 (104.20 g·kg^{-1}). The minimum total sugar content was recorded in LY1 (67.17 g·kg^{-1}) and RB2 (67.37 g·kg^{-1}). Sugar is a vital constituent of fruits which directly related with sweetness and is fundamental feature of fruit quality (aroma, flavor and texture). In present study, total sugar content ranged between 67.17 to 105.02 g·kg^{-1}. Mean values of total sugar in present study are greater than reported value for European plum cultivars grown in Turkey that was 96.5 g·kg^{-1} [Nergiz and Yildiz 1997) and also for Japanese plum with 75.0 and 86.5 g·kg^{-1} [Melgarejo et al. 2012].

Post-harvest life. The maximum shelf-life was recorded in LY2 and LY4 (14 days), followed by DR2 and LY1 (13 days), these four genotypes were similar with each other. However, SY2 showed the minimum shelf-life which was 3 days, followed by SY1 and SY4 (4 days), and SY3 and RB2 (5 days). All these genotypes stood at par with each other (tab. 4). However, the differences for the shelf-life among the genotypes were due to their genetic make-up.

Table 4. Variation in shelf-life and organoleptic/sensory evaluation of plum genotypes

Genotype	Shelf-life (days)	Organoleptic score	Remarks
DR1*	12.00 bc	5.20 de	fair
DR2	13.00 ab	6.20 cd	good
DR3	11.00 c	9.33 a	excellent
DR4	12.00 bc	9.33 a	excellent
SY1	4.00 f	7.20 bc	good
SY2	3.66 f	8.83 a	excellent
SY3	5.00 ef	6.20 cd	good
SY4	4.00 f	5.20 de	fair
RB1	6.00 de	8.07 ab	excellent
RB2	5.00 ef	2.43 g	poor
RB3	6.00 de	4.17 ef	fair
RB4	7.00 d	7.20 bc	good
LY1	13.00 ab	2.83 fg	poor
LY2	14.00 a	5.17 de	fair
LY3	11.00 c	3.20 fg	fair
LY4	14.00 a	9.27 a	excellent

Means in each column with different letters are statistically significant at $p < 0.05$ (LSD test)
* – genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

Sensory evaluation. On the basis of aroma, consistency and flavor of fruits, fruit excellence was assessed by a team of judges. Fruits of the genotypes DR3 and DR4 scored the maximum value of ranking (9.33), followed by those of LY4 (9.27), SY2 (8.83) and RB1 (8.07). The minimum score were achieved by the fruits of RB2 (2.43), LY1 (2.83) and LY3 (3.20). This is interesting to note that the genotypes with higher sugar content (reducing and/or non-reducing) had the higher organoleptic score compared with those with lower sugar content. Eating quality like flavor, texture and aroma are the most important parameters that determine the consumer preferences for fruits [Harker et al. 2002]. The variation in organoleptic characteristics in the studied plum genotypes, observed by the panel of judges, was due to both their genetic make-up and prevailing environmental conditions. These results are in accordance with the findings of previous workers, who reported genotypic differences for fruit quality in apples [Oraguzie et al. 2009], peaches and nectarines [Abidi et al. 2011, Colaric et al. 2005] and apricots [Brown and Walker 1990].

Anthocyanin content. Anthocyanin is very important phytochemical for human health and have been recognized as key contributing compounds to antioxidant activity *in vivo* and *in vitro* [Cevallos et al. 2006]. The genotypes exhibited large variability in their anthocyanin content. Anthocyanin content in the studied genotypes ranged from 14.23 to 212.38 mg·100 g^{-1} of fresh weight. The higher anthocyanin content was recorded in LY3, which was significantly different from other genotypes. Lower anthocyanin content was recorded in SY2, followed by DR2 (tab. 5). The values obtained in the present study were higher than reported values for plums (33 to 173 mg·100 g^{-1}) and also for peaches (6 to 37 mg·100 g^{-1}) [Cevallos-Casals et al. 2006].

Table 5. Variation in anthocyanins, phenolics and antioxidant capacity of plum genotypes

Genotype	Anthocyanins (mg·100 g^{-1})	Phenolics (mg·g^{-1})	Antioxidant potential (µg·ml^{-1})
DR1*	125.12 f	5.95 cde	96.22 bc
DR2	16.24 m	3.44 ij	91.38 d
DR3	156.20 e	9.92 a	96.25 bc
DR4	20.31 l	3.43 ij	94.45 c
SY1	100.23 g	5.78 def	69.07 h
SY2	14.24 m	4.64 efghi	74.18 g
StY3	199.13 b	2.63 j	72.50 g
SY4	127.22 f	3.73 hij	81.52 ef
RB1	69.09 h	3.82 ghij	80.07 f
RB2	61.25 i	4.45 efghi	74.34 g
RB3	52.19 j	9.17 ab	72.07 g
RB4	162.38 d	5.55 defg	84.18 e
LY1	22.33 l	5.50 defg	80.10 f
LY2	189.34 c	4.16 fghij	99.04 ab
LY3	212.38 a	6.75 cd	96.25 bc
LY4	32.24 k	7.62 bc	100.10 a

Means in each column with different letters are statistically significant at $p < 0.05$ (LSD test)
* – genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

Phenolic content. The maximum phenolic content was recorded in DR3 (9.92 mg GAE·g^{-1}), followed by RB3 (9.17 mg GAE·g^{-1}). The minimum phenolics were estimated in SY3, DR4, DR2, SY4, RB1 and LY2, which ranged from 2.63 to 4.16 mg GAE·g^{-1} (tab. 5). Phenolics are essential components of various fruits and vegetables not only for the reason that they contribute to plant color, but also due to their contribution to the health of these fruits and vegetables. One of the main prospective health benefits of the phenolics in vegetables and fruits is their antioxidant activity, which defends low density lipoprotein (LDL) from oxidation and, hence, is considered to avoid from a variety of age-related diseases [Fang et al. 2002]. Plant genotypes may vary in their phenolic content [Gil et al., 2002, Kim et al. 2003, Scalzo et al. 2005]. Cevallos-Casals et al. [2006] reported that in plums genotypes, phenolic content ranged from 298 to 563 mg of GAE·100 g^{-1}. The total phenolic content of many plum cultivars estimated by using spectrophotometric methods was in a broad range from 174.0 to 375.0 mg of GAE·100 g^{-1} with an average of 192.1 mg of GAE·100 g^{-1} [Kim et al. 2003b]. However, in methanolic extracts of fresh plums, total phenolic content recorded was in the range of 86 to 413 mg GAE·100 g^{-1} [Rupasinghe et al. 2006]. In the present study, phenolic content ranged from 2.63 to 9.93 mg GAE·100 g^{-1} that is higher than reported values.

Antioxidant capacity. Antioxidant capacity of hot water extracts of plum genotypes was expressed as water soluble ascorbic acid equivalents (μg·ml^{-1} of extract). The higher values were recorded in LY4 (100.01 μg·ml^{-1}), followed by LY2 (99.037 μg·ml^{-1}). The least value of antioxidant activity was 69.07 μg·ml^{-1} in SY1. The antioxidants are mainly scavengers that reduce the various free radicals and serving in the avoidance of cellular injury and other disease. Likewise, fruit antioxidants have ability to produce resistance in tissues against disease and stress conditions. However, plant genotypes may differ in their antioxidant capacity [Scalzo et al. 2005]. In an earlier investigation, antioxidant capacity expressed as vitamin C equivalent antioxidant capacity (VCEAC), in fresh plums varied from 266 to 559 mg·100 g^{-1} [Kim et al. 2003a]. Cultivar Beltsville Elite B70197 illustrated the maximum amount of VCEAC. While, fourteen red fleshed plum genotypes showed variable values from 1254 to 3244 μg trolox·g^{-1} and a positive correlation was recorded between phenolic content and antioxidants [Cevallos-Casals et al. 2006]. Walkowiak-Tomczak et al. [2008] traced the maximum antioxidant activity in cv. 'Bluefre' (159.0 μM Trolox·g^{-1} d.m.). Activity of the other two plum cultivars was greatly lesser, amounting to 100.5 and 89.7 μM Trolox·g^{-1} d.m. for cultivars 'Elena' and 'Wegierka Zwykła', respectively. Keeping in view the reported results, present study showed that plums may be used as an excellent resource of natural antioxidant. Many fruits have already been evaluated for their antioxidant activity due to their remarkable antioxidants potential [Scalzo et al. 2005].

Antioxidant activity by DPPH radical scavenging. A stable free radical 2,2--diphenyl-1-picrylhydrazyl (DPPH) was used to evaluate antioxidant activity among different genotypes of plum. Extract of five different concentrations (25, 50, 100, 150 and 300 μg·ml^{-1}) was used to measure the antioxidant activity of plum fruits. Results showed that scavenging of the extracts was dependent on the concentrations. At first concentration (25 μg·ml^{-1}) of the extract, DR4 showed the maximum radical scavenging activity (50.8%), followed by DR3 (43.7%). At second concentration (50 μg·ml^{-1}), DR4 showed 63.8% radical scavenging activity followed by SY4 that showed 58.0%. At

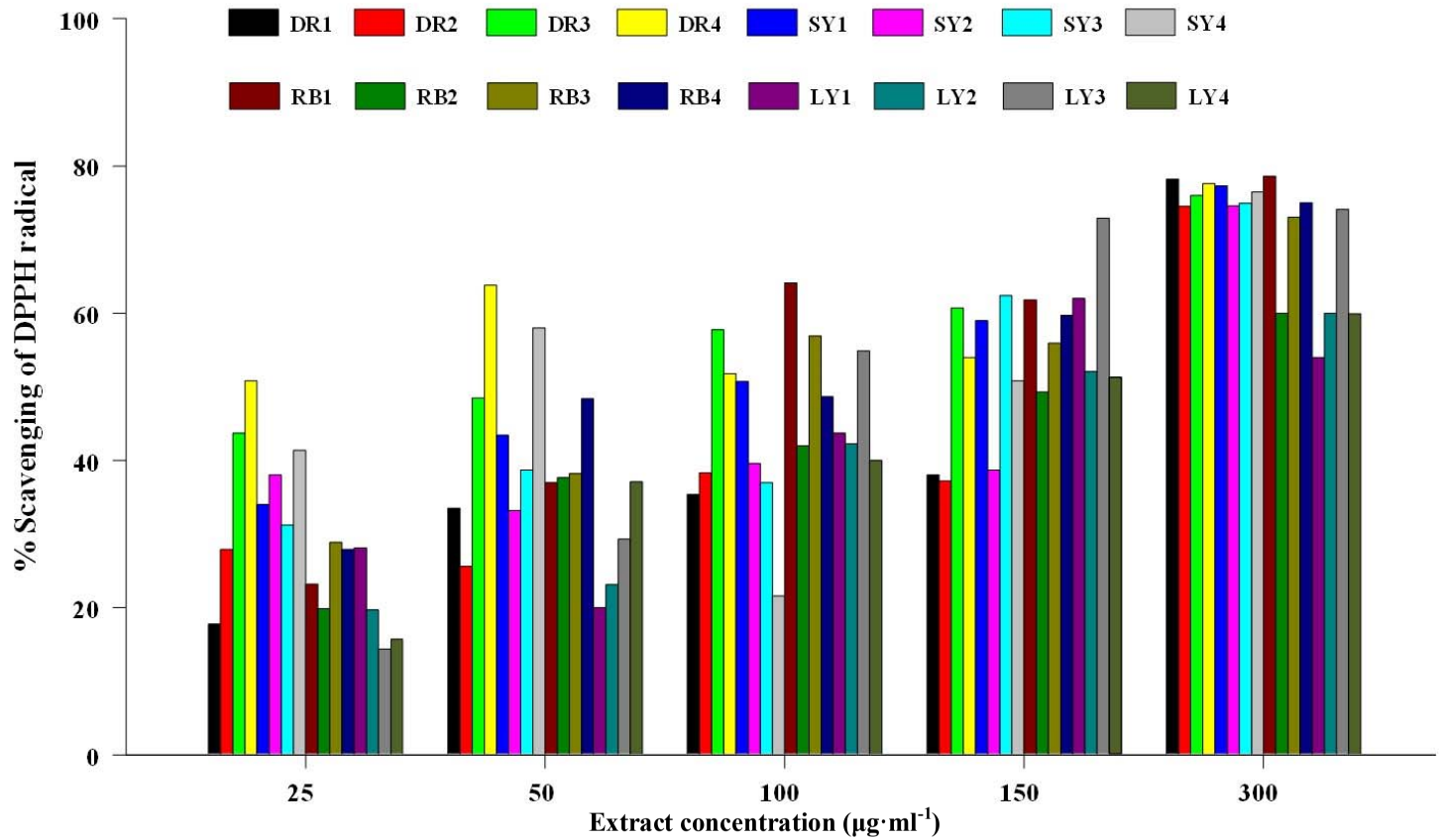

Fig. 1. Variation in antioxidant activity by DPPH radical scavenging among sixteen plum genotypes. Genotypes were denoted on the basis of skin colour and/or fruit size i.e. DR (dark red), SY (small yellow), RB (reddish brown) and LY (large yellow)

100 $\mu g \cdot ml^{-1}$ concentration, RB1, DR3 and RB3 showed maximum radical scavenging activity that was more than 56.0%, while other genotypes have the lower values. At 150 $\mu g \cdot ml^{-1}$, 72.9% scavenging activity was recorded in LY3 while other genotypes showed lower radical scavenging activities. At the last concentration (300 $\mu g \cdot ml^{-1}$), 73.0% or more radical scavenging activity was recorded in twelve genotypes, while RB2, LY1, LY2 and LY4 that have 60.0% or less radical scavenging activities (fig. 1).

To evaluate the antioxidative activity of plant extracts, DPPH radical activity has been extensively used to test the capability of compounds as free-radical scavengers or hydrogen donors [Hatano et al. 1988, Da Porto et al. 2000]. The method is also very simple. In the present study, antioxidant activity was due to presence of high vitamin C, anthocyanin and phenolic contents in fruits of the plum genotypes. Genotypic variation for antioxidant activity also exists, depending upon vitamin C, anthocynins and phenolic contents in fruits. A strong correlation has been observed between total phenolics and antioxidant activity in plums, peaches and nectarines [Gil et al. 2002].

CONCLUSION

Sixteen diverse plum genotypes were explored from different location of District Poonch of Azad Jammu and Kashmir (Pakistan). Sensory evaluation and biochemical analysis of the fruits exhibited significant differences among the genotypes for their nutritional composition. Genetic diversity found in fruit quality can be exploited for improvement of existing cultivars and promising genotypes can be popularized and planted on commercial scale.

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GENETYCZNA RÓŻNORODNOŚĆ W SKŁADZIE ODŻYWCZYM OWOCÓW, ANTOCYJANINY, FENOLE ORAZ ZDOLNOŚĆ ANTYOKSYDACYJNA GENOTYPÓW ŚLIWY (*Prunus domestica*)

Streszczenie. Badano różnorodność genetyczną w składzie odżywczym szesnastu genotypów śliw rosnących w różnych miejscach Tehsil Rawalakot, Dystrykt Poonch w Azad Jammu i Kashmirze (Pakistan). Oceniono różne parametry, takie jak wilgotność, zawartość popiołu, suchej masy i całkowitą zawartość rozpuszczalnych substancji stałych, kwasowość, pH, zawartość witaminy C, cukru, antocyjanów i fenoli, okres trwałości, cechy sensoryczne/organoleptyczne, a także omówiono zróżnicowanie tych cech. Na podstawie wyników można stwierdzić, że genotypy różniły się składem odżywczym, zawartością antocyjan i fenoli oraz zdolnością antyoksydacyjną owoców. Wyniki badania dotyczące składników odżywczych istniejącej germplazmy przyczynią się do pogłębienia wiedzy na temat gatunku *Prunus* oraz poszerzą pule genową dostępną dla przyszłych programów hodowli roślin

Słowa kluczowe: antyoksydanty, analiza biochemiczna, bioróżnorodność, genotypy, skład przybliżony

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