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Quantitative Analysis of Total Flavonoids and Phenolics Contents of Ten Genotypes of *Phaseolus vulgaris* Linnaeus

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ABSTRACT

A study has been conducted to evaluate the total flavonoids and phenolics contents of ten genotypes of *Phaseolus vulgaris* Linnaeus seeds as these compounds are natural antioxidants, disease preventing, anti-ageing and health promoting. All genotypes were distinct in shape, size and colour and their total flavonoids and phenolics contents also varied significantly. Genotype Triloki (K-198) contained the highest (1.67±0.00 quercetin equivalents) and Him-1 contained the lowest (0.29±0.01 mg g^{-1} quercetin equivalents) amount of total flavonoids. In case of total phenolics, genotype Him-1 had the highest (2.48±0.02 mg g^{-1} gallic acid equivalents) and PLB 14-1 had the lowest (0.66±0.02 mg g^{-1} gallic acid equivalents) amount of total phenolics contents. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) also indicated the significant differences between total flavonoids and phenolics contents of all genotypes. The study therefore, revealed that consumption of a particular genotype of *P. vulgaris* seeds can promote the health and demote the ageing by its disease preventing potential of some natural antioxidants compounds present in cotyledons.

Key words: *Phaseolus vulgaris*, genotypes, flavonoids, phenolics, antioxidants

INTRODUCTION

India is the major pulse producing country of the world occupying an area of 228.47 lakh hectares with the production of 130.7 lakh tonnes and yield 572 tonnes kg/hectare (Anonymous, 2000). Country has an annual production potential of 15.04 million tonnes pulses recorded in year 2004-05 (Anonymous, 2006). Legumes are regarded poor man's meat and grown and used as food worldwide. *Phaseolus vulgaris* has been in cultivation since ancient times and more than 400 commercial cultivars of this crop are known worldwide. Radio carbon dating suggested that kidney beans were domesticated in Central America about 7,000 years ago (Kaplan, 1965). In India, fresh pods of common bean are used as a vegetable and dried seeds are used as a pulse known as kidney beans. Common bean was introduced in India during 17th century (Simmonds, 1976) and about 99 lakhs hectares area is under the cultivation of this crop with the contribution of nearly 2037 hectares by the state of Himachal Pradesh (FAO, 1999). Kidney bean occupies an important position among various 'kharif' pulses grown in temperate hills of India at an altitude ranging from 900 to 2,500 m. This crop is cultivated and widely grown in Himachal Pradesh, Jammu and Kashmir, Uttaranchal, Uttar Pradesh, North Eastern hills, Darjeeling, South Plateau, South Indian hills (Nilgiri and Palni hills), Mahabaleswar, Ratnagiri (Maharashtra) and Chikmangalure (Kartantaka) having mild climate. In recent years, common bean has been introduced in the plains of Haryana, Punjab, Uttar Pradesh, Bihar, Madhya Pradesh, Maharshtara and Karnataka as a rabi crop. In Himachal Pradesh common bean is widely grown in Kinnaur, Lahaul and Spiti, Sirmour, Bilaspur, Solan, Chamba, Mandi and Kangra Districts. It is grown as sole crop as well as inter crop with maize in kharif crop in hills. Common beans are important source of protein, carbohydrate, vitamins and minerals for human diets. It is considered as functional food because of its richness in phytochemicals like soluble fiber and polyphenols e.g. phenols and flavonoids with their corresponding antioxidant activity (Oomah *et al*., 2005; Parr and Bolwell, 2000; Aparicio-Fernandez *et al*., 2005; Rocha-Guzman *et al*., 2007). The presence of phenols and flavonoids in the seed coats of pulses imparts resistance against certain pest attack. Besides of nutritional composition, common beans also helps in targeting different ailments like cancer, diabetes and heart diseases (Broughton *et al*., 2003).

The present work is a pioneer study and explored the total flavonoids and phenolics contents of ten genotypes of common bean, *P. vulgaris* who exhibit high antioxidant activity and will be helpful in developing the genotypes having high flavonoids and phenolics contents by breeding and crop improvement programmes. Phenols and flavonoids are secondary metabolites also known as antinutrient factors synthesized by the plants as a defense mechanism against different pests. Therefore, the quantitative estimation of phenols and flavonoids would also be useful to determine the susceptibility and resistance of beans to their agricultural pests both in stores and fields.

MATERIALS AND METHODS

Sample collection and authentication of genotypes: Legume samples were collected and identified with the help of plant breeders, belonging to Research Station, Bajaura, Distt. Kullu, under Chaudhry Sarvan Kumar Krishi Vishav-Vidyalaya, Palampur, Himachal Pradesh and National Bureau of Plant Genetic Resources (NBPGR) Research Station, Phagli, Shimla (Himachal Pradesh). Different cultivars of *P. vulgaris* viz. Jawala (HPR-12), Triloki (K-198), Mohini selection, Him-1, Baspa (KRC-8), Kanchan (HPR-35), PLB 14-1, EC-537968, EC-530929 and EC-530856 were evaluated for total flavonoids and phenolics contents spectrophotometrically.

Total flavonoids contents: Total flavonoid contents were determined according to the method of Ordonez *et al*. (2006). The seed were extracted in 80% ethanol and kept for overnight. To 0.5 mL of seed extract, 0.5 mL of 2% $AlCl_3$ in ethanol was added and allowed to stand for 1 h. Absorbance of golden yellow color taken at 420 nm using a UV-Vis Spectrophotometer (Ultrospec 2100 Pro, Healthcare Biosciences AB, Uppsala, Sweden) and amount of total flavonoids was determined with the help of calibration curve prepared with different concentrations of quercetin. Results were expressed as mg Quercetin Equivalents (QE)/g of seeds.

Total phenolics contents: Total phenol contents were determined according to the method of Goldstein and Swain (1963). The powdered seeds were homogenized in 0.3N HCl in methanol, centrifuged at 10,000 g for 10 min. Supernatant was collected and pellet was again extracted in 0.3N HCl in methanol and centrifuged. The supernatants were pooled and evaporated on a water bath. The residue obtained was dissolved in distilled water and final volume made to 5 mL. To this volume, 0.5 mL of Folin-phenol reagent was added and shaken vigorously. After 3 min, 1 mL 35% sodium carbonate solution was added, shaken and allowed to stand for 1 h. Absorbance was recorded at 650 nm using a UV-Vis Spectrophotometer (Ultrospec 2100 Pro, Healthcare Biosciences AB, Uppsala, Sweden). Total amounts of phenolics were determined with the help of a calibration curve prepared using different concentration of tannic acid. The results were expressed as mg Tannic Acid Equivalents (TAE)/g of seeds.

Data analysis: All experiments were carried out in triplicate. Data were subjected to analysis of variance (ANOVA) test. Duncan's multiple range test was also used to test the significance of difference between means of treatments.

RESULTS

Significant differences were found in total flavonoids and phenolics contents of ten genotypes. Genotype Triloki contained the significantly ($p \leq 0.05$) highest flavonoids contents (1.67±0.00 mg g^{-1}), followed by EC-530929 (1.33±0.00 mg g^{-1}), Jawala (0.71±0.01 mg g^{-1}), EC-530856 (0.56±0.01 mg g^{-1}), Kanchan (0.50±0.00 mg g^{-1}), Baspa (0.49±0.00 mg g^{-1}), EC-537968 (0.38±0.00 mg g^{-1}), PLB 14-1 (0.37±0.00 mg g^{-1}), Mohini-selection (0.35±0.01 mg g^{-1}) and genotype Him-1 (0.29±0.01 mg g^{-1}) had the lowest value of total flavonoids contents. Total flavonoids contents decreased in the following order based on the average values of different genotype: Triloki>EC-530929>Jawala>EC-530856>Kanchan>Baspa>EC-537968>PLB14-1>Mohini-selection> Him-1 (Table 1, Fig. 1a).

Among the total phenolics contents of ten genotypes, genotype Him-1 contained the significantly ($p \leq 0.05$) highest total phenolics contents (2.48±0.02 mg g^{-1}), followed by genotype

Table 1: Decreasing order of total flavonoids and phenolics contents of different genotypes of *P. vulgaris*

Genotype	Total flavonoids (QE g^{-1})	Total phenolics (GAE g^{-1})
Triloki (K-198)	1.67±0.00^{a}	1.70±0.03^{c}
EC-530929	1.33±0.00^{b}	1.25±0.04^{e}
Jawala (HPR-12)	0.71±0.01^{c}	1.19±0.02^{e}
EC-530856	0.56±0.01^{d}	0.90±0.04^{f}
Kanchan (HPR-35)	0.50±0.00^{e}	1.20±0.01^{e}
Baspa (KRC-8)	0.49±0.00^{e}	1.48±0.03^{d}
EC-537968	0.38±0.00^{f}	2.24±0.01^{b}
PLB-1	0.37±0.00^{f}	0.66±0.02^{g}
Mohini selection	0.35±0.01^{f}	1.42±0.01^{d}
Him-1	0.29±0.01^{g}	2.48±0.02^{a}
F-value	358.73	190.00
p-value	≤0.001	≤0.001

Values are means of three replicates ±Standard error. Values with different letters (a-d) are statistically different at $p \leq 0.05$ probability level (Duncan's multiple range test)

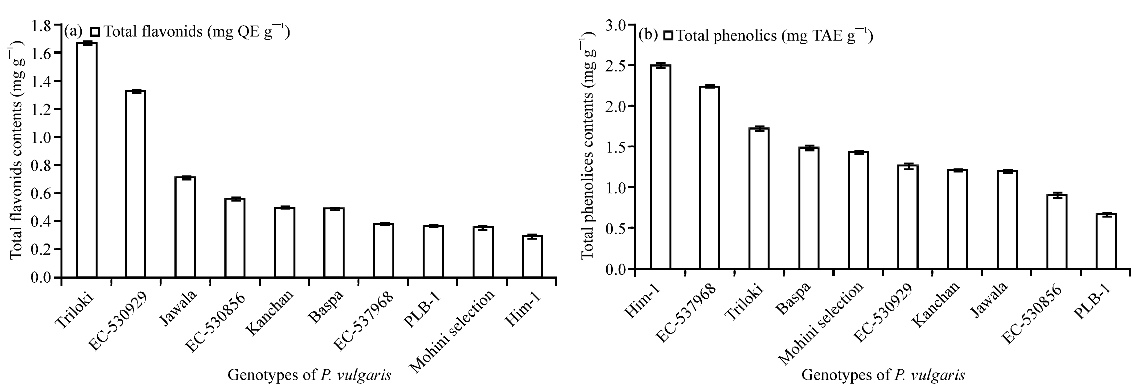

Fig. 1(a-b): Total flavonoids and phenolics contents of (mg g^{-1}) in different genotypes of *P. vulgaris*, (a) Total flavonoids (mg QE g^{-1}) and (b) Total phenolics (mg TAE g^{-1})

EC-537968 (2.24±0.01 mg g^{-1}), Triloki (1.70±0.03 mg g^{-1}), Baspa (1.48±0.03 mg g^{-1}), Mohini-selection (1.42±0.01 mg g^{-1}), EC-530929 (1.25±0.04 mg g^{-1}), Kanchan (1.20±0.01 mg g^{-1}), Jawala (1.19±0.02 mg g^{-1}), EC-530856 (0.90±0.04 mg g^{-1}) and genotype PLB 14-1 (0.66±0.02 mg g^{-1}) contained the lowest value of total phenolics contents. Total phenolics contents decreased in the following order based on the average values of different genotype: Him-1>EC-537968>Triloki>Baspa>Mohini-selection>EC-530929>Kanchan>Jawala>EC-530856>PLB 14-1 (Table 1, Fig. 1b).

DISCUSSION

In the present study total flavonoids contents ranged from 0.29±0.01 (Him-1) to 1.67±0.00 (Triloki) mg g^{-1} Quercetin equivalents and total phenolics contents ranged from 0.66±0.02 (PLB-1) to 2.48±0.02 (Him-1) mg g^{-1} Gallic acid equivalents. Variation in total polyphenolics contents ranged from 5.87 (JaloEPP) to 14.14 (G122) mg g^{-1} Gallic acid equivalent of sample in 29 common bean from different origins, seven bean genotypes, G 122 (14.14 mg g^{-1}), BAT 93 (13.68 mg g^{-1}), T-39 (12.60 mg g^{-1}), NUA 35 (12.52 mg g^{-1}), MIB 154 (12.47 mg g^{-1}), XAN (11.73 mg g^{-1}) and Vista (11.35 mg g^{-1}) had higher total polyphenol contents (Akond *et al.*, 2011). The phenolics contents of twelve Italian cultivars ranged between 1.17 to 4.40 mg g^{-1} seed Gallic Acid Equivalents (Heimler *et al.*, 2005). The average phenolics contents were determined to be 31.2 mg 100 g^{-1}. The highest amount of phenolics was in Vista bean (48.3 mg 100 g^{-1}) and two black beans, T-39 (47.1 mg 100 g^{-1}) and Eclipse (42.5 mg 100 g^{-1}) and lowest content of phenolics was in Cranberry (19.1 mg 100 g^{-1}) (Luthria and Pastor-Corrales, 2006).

Different cultivation techniques, environmental and storage conditions, extraction and quantification procedures are responsible for the wide variation in the phenolics contents of *P. vulgaris* (Heimler *et al.*, 2005; Espinosa-Alonso *et al.*, 2006; Anton *et al.*, 2008; Granito *et al.*, 2008; Ranilla *et al.*, 2007; Boateng *et al.*, 2008).

Total phenolics and flavonoids contents vary in different pulses not only with the type of seed but also because of their colour. Genotype Him-1 (2.48±0.02) is light coloured bean and contained highest phenolics contents than genotypes PLB 14-1 (0.66±0.02) which is dark red and EC-530856 (0.90±0.04) which is black in colour. These results are in accordance with Xu and Chang (2009) who reported the light coloured beans contained higher total phenolic acid than their black counterparts. But studies of Laparra *et al.* (2008) and Akond *et al.* (2011) have reported that the black beans contained significantly high phenolics contents than white and navy beans. In three Polish cultivars of beans, cultivar Rawela (dark red) contained the highest phenolic components (90.28 mg 100 g^{-1}) followed by Tip-Top (black-brown) (35.65 mg 100 g^{-1}) and cultivar Toffi (cream) contained significantly lower phenolics contents (30.48 mg 100 g^{-1}) (Korus *et al.*, 2007). Because of antioxidant properties, polyphenols shows positive effect and should be included in the diets and it has been reported that regular intake of flavonoids rich food plays a protective role against coronary and cardiovascular diseases (Nagao *et al.*, 2007; Vita, 2005; Nardini *et al.*, 2007; Di Majo *et al.*, 2008).

The present study unveiled that these genotypes of *P. vulgaris* exhibit significant amount of flavonoids and phenolics contents and can be used as a source of natural antioxidants and denfence against various pathogens. By breeding and crop improvement programmes, it is possible to develop desired genotypes with high flavonoids and phenolics contents. That will not only improve the resistance against agricultural pests and various environmental stresses but produce the genotype containing desired flavonoids and phenolics contents.

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