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Environmental and genetic variation of phenolic compounds and antioxidant capacity in raspberry

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Abstract

Raspberry (*Rubus idaeus L.*) is an economically important berry crop that contains numerous phenolic compounds with potential health benefits. It is known that the content of phenolics is affected by processing factors, but limited information is available on the influence of cultural factors or genotype. To carify this issue, phenolic compounds were analysed from a diverse range of raspberry species grown in North and Northwest of Iran. The content of phenolic compounds varied widely and significantly between species. The content of the total phenolics ranged in the raspberries collected in Heiran from 519.5 in R. caesius to 916.5 mg GAE/100gFW in R.hyrcanus. In the raspberries collected in Arasbaran, the R. caesius species had the lowest content and R. hyrcanus, 195.25 and 307.25 mg Q/100g FW. In the species collected in Heiran, the amount of antioxidant capacity was higher than Arasbaran. In addition, environment had a significant effect on the contents of phenolics, flavonoid and antioxidant capacity. Thus, breeding material should be evaluated for their potential health benefits over several regions in northern raspberry breeding.

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Introduction

Increasing epidemiological data (Rissanen *et al*., 2003) suggest that a high intake of fruits, berries and vegetables offers a number of health benefits against degenerative diseases and can promote longevity. In addition to high levels of vitamin C, berries contain phytochemicals that significantly contribute to their antioxidant and anticarcinogenic properties. The exploitation of health-promoting bioactive compounds of berries in diverse areas of food and health products is rapidly increasing. Many types of healthy juices and jams as well as dietary supplement containing various bioactive compounds from berries are available all over the world.

Raspberry (*Rubus idaeus L.*) is an economically important berry crop with a high free radical scavenging capacity and it contains numerous bioactive compounds with potential health benefits (de Ancos *et al*., 2000). Mullen *et al* (2002) have identified as many as 11 anthocyanins in raspberries. However, cyaniding-3-sophoroside and cyaniding-3-glucoside are the major compounds (de Ancos *et al*., 1999). Recently, it has been shown that anthocyanins are important antioxidants in raspberry (Mullen *et al*., 2002). However, in humans the bioavailability of dietary anthocyanins is low (Mazza *et al*., 2002). Raspberry anthocyanin composition seems to depend on the genotype so that late cultivars appear to have a higher content of anthocyanins than the early ones (de Ancos *et al*., 1999). Processing, in particular freezing, increased the total anthocyanin concentrations in some raspberry cultivars, but decreases it in others (de Ancos *et al*., 2000). In addition, storage temperatures above 0◦C have been shown to increase the content of anthocyanins (Kalt *et al*., 1999).

Ellagic acid is a phenolic compound found in many plants. However, more commonly it is found as ellagitanins. Ellagitanins occur in high concentrations in strawberries and raspberries. Ellagic acid is a bioactive compound of potential protective effects against certain cancer types (Stoner and Morse., 1997).

The health-promoting properties of berry plants are affected by cultural, genetic and processing factors. In raspberry, the effects of storage and processing on antioxidant activity and phenolic compounds are relatively well Known (de Ancos *et al*., 2000; Hakkinen *et al*., 2000; Rommel and Wrolstad ., 1993; Zafrilla *et al*., 2001), but limited information is available on the influences of cultural factors or genotype on health-promoting properties. However, increasing data suggest (de Ancose *et al*., 2000; Mikkonen *et al*., 2001) that genotype may have a profound influences on the content of bioactive compounds in berries. Consequently, extensive plant breeding programmers have been initiated to increase the levels of compounds with potential health benefits. In this paper, we show that the genotype and the environment both significantly affect the phenolic compounds in raspberry species grown in north and northwest Iran conditions.

Materials and methods

Fruit materials

Iranian raspberries that were evaluated in this study (*R. hircanus, R. Raddeanus R.caesius, R. anatolicus)* were collected from the north (Heiran) and northwest (Arasbaran) regions of Iran. The harvesting time of raspberries was the maturity stage that the fruit were fully colored and not crumbled, also when the fruit separated readily from the stems. Approximately 500g of ripe raspberry fruits per species were harvested manually in July 2012. The fruits were sorted according to uniformity of shape and color and then immediately transported to lab and freezed with liquid nitrogen and kept at -80 C, until needed for analysis. All chemicals used were analytical degree (Sigma- Aldrich Company, St. Louis, Mo, USA).

Extraction and measurement of total phenolic content

Total phenol in the methanol extracts was determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1972). Gallic acid (GAE) was used as a standard and results were expressed as mg gallic acid equivalents per 100 g fresh weight.

Extraction and measurement of total flavonoid

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle. One gram of the resultant powder was added to 10 ml of methanol containing HCl (1%, v/v) and held at room temperature for 24 h (Cordenunsi *et al.*, 2003). The slurry was centrifuged at 4000× g for 15 min at 4ºC, and the supernatant was used. The total flavonoid contents were determined by a colorimetric assay (Yanping *et al.*, 2004). One milliliter aliquot of appropriately diluted sample was added to a 15 ml tube containing 4ml of deionized water. Then 0.3 ml of 5% $NaNO_2$ was added to this mixture, which was allowed to stand for 5 min at room temperature, and 0.6 ml of 10% $AlCl_3.6H_2O$ was added. The mixture was allowed to stand for 6 min at room temperature, and 2 ml of 1 mol l^{-1} NaOH was added, and the total was made up to 10 ml with deionized water. The absorbance of the solution was measured immediately at 510 nm. Quercetin was used as a standard compound for the quantification of total flavonoid.

Determination of the antioxidant capacity by DPPH radical scavenging method

The antioxidant capacity of the raspberry fruits were evaluated by free radical 2, 2-dipheynl-1-picrylhydrazyl (DPPH) methods. For the determination of free radical scavenging capacity, raspberry samples were extracted with methanol. Then, they were centrifuged (Sigma 3K30, Germany) at 15,000× g for 10 min. The supernatants were concentrated under reduced pressure at 40º C. The dried extracts were dissolved in methanol. Free radical scavenging activity was measured according to the principle of Nakajima *et al.* (2004) with some modifications reported by Chiou *et al.* (2007). Fifty microliters of the diluted extracts (concentrations 2-20 mg ml^{-1}) were added to 1 ml of 6× 10^{-5} mol l^{-1} DPPH (free radical, 95%, sigma-Aldrich Chemie GmbH, Steinheim, Germany) in methanol. The mixture was shacked and left at room temperature for 30 min; the absorbance was measured spectrophotometrically at 515 nm. Methanol was used as an experimental control. The percent of reduction of DPPH was calculated according to the following equation

$$\%\text{ inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sampele}}{\text{Abs control}} \times 100$$

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 16.0 (SPSS Inc.,USA). Differences among the means were compared between species using one-way analysis of variance. Multiple-comparison was done using either Tukeys or Dunnett׳s T3 test. The effect of the environment was tested using t-test. Differences at $P< 0.05$ were considered to be significant. Pearson׳s correlation coefficient was used to estimate the relationship between the contents of phenolics.

Results and discussion

The aim of the study was to clarify the role of the genotype and the environment on the contents of the total phenolics, flavonoid and antioxidant capacity in raspberry species adapted to northern Iranian conditions. The influence of the species and the environment on the phenolic compounds was significant ($P<0.05$). The results are shown in Table 1. The content of the total phenolics ranged in the raspberries collected in Heiran from 519.5 in R. caesius to 916.5 mg GAE/100gFW in R.hyrcanus. In the raspberries collected in Arasbaran, the lowest amount was found in the R. caesius and the highest in R. hyrcanus, 474.25 and 848 mg GAE/100gFW, respectively (Fig.1). The differences in total phenolic content, total flavonoid and antioxidant capacity were statically significant ($P< 0.05$). The results for total flavonoid contents in the raspberry fruits are presented in Tble 1. The total flavonoid in the raspberries collected in Heiran from 201.75 in R. caesius to 321.5 mg Q/100gFW in R. hyrcanus. In the raspberries collected in Arasbaran, the R. caesius species had the lowest content and R. hyrcanus, 195.25 and 307.25 mg Q/100g FW (Fig. 2). In general, of all the species analysed, the R. caesius contained the lowest levels of antioxidant capacity and R. hyrcanus the highest in two places (Fig.3). In the species collected in Heiran, the amounts of phenol, flavonoid and antioxidant capacity was higher.

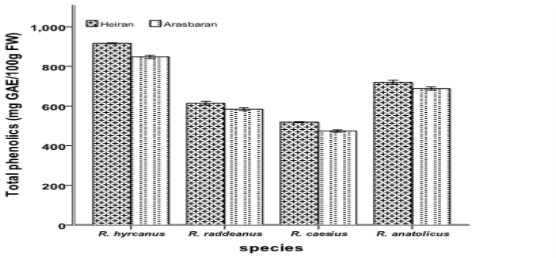

Fig. 1. Total phenolic content (TPC) of raspberry species in two regions of Iran. Results are expressed as mg GAE / 100 g FW.

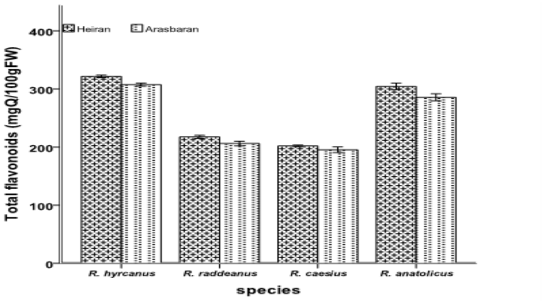

Fig. 2. Total flavonoid content (TFC) of raspberry species in two regions of Iran. Results are expressed as mg Quercetinper 100 g FW.

Person׳s correlation coefficient between the total phenolics and antioxidant capacity was 0.87 and statistically significant ($P<0.001$). The correlation between antioxidant activity and phenolic content has been reported in fruits of raspberry (Erika *et al.*, 2011; Liagat Ali *et al.*, 2011), strawberry species (sara *et al.*, 2008) and red grape cultivars (hulya *et al.*, 2007).

Considerable data suggest that higher content of total phenolics, flavonoids in raspberry fruits contribute to their higher antioxidant capacity (Liu *et al.*, 2002; Wang and Lin., 2000). Measuring the antioxidant capacity in order to evaluate the potential health benefits of breeding material or various agronomic factors can be a tedious task. Thus, the determination of indirect parameters, such as the content of the total phenolics, flavonoids, for describing the potential health benefits, may be a more appropriate objective.

The present study shows that the genotype significantly influenced the phenol, flavonoid and antioxidant capacity in raspberry fruits. The phenolic content and composition of fruits depend on environmental factors as well as post-harvest processing conditions (Benvenuti *et al.*, 2004; Kadir *et al.*, 2009). The phenolic compounds serve in plant defence mechanism, to counteract reactive oxygen species, in order to survive and prevent molecular damage, and damaging by microorganisms, insects and herbivores (vaya *et al.*, 1997; Kadir *et al.*, 2009). The highest antioxidant capacity was observed in R. hyrcanus at 95.75%, followed by R. anatolicus (90.75%) and R. raddeanus (85.5%) (Table1).

Table 1. Total phenolic (TP), total antioxidant capacity (TAC), total flavonoid of raspberry fruits.

species	Heiran			Arasbaran		
	TAC (%)	TP (mgGAE/100gFW)	TF (mgQ/100gFW)	TAA (%)	TP (mgGAE/100gFW)	TF (mgQ/100gFW)
R. hyrcanus	95.7a	916.5a	321.5a	86.1a	848a	307.25a
R. raddeanus	85.5c	614.5c	217.2c	73.6c	584.2b	206c
R. caesius	66.2d	519.5d	201.7d	54.8d	474.2d	195.2d
R. anatolicus	90.7b	719.7b	304.2b	80.6b	688b	285.3b

Value in the same column with different lower-case letters are significantly different at $p<0.01$.

The data reported in this paper reveal a large variation in the total phenolic content among raspberry genotypes. In raspberry, the antioxidant capacity has been shown to be directly related with the total phenolic content (Wang and Lin 2000; Liu *et al.*, 2002), suggesting that breeders can be directly related with the total phenolic content as a reliable parameter for selecting genotypes for increasing the antioxidant capacity of the fruit.

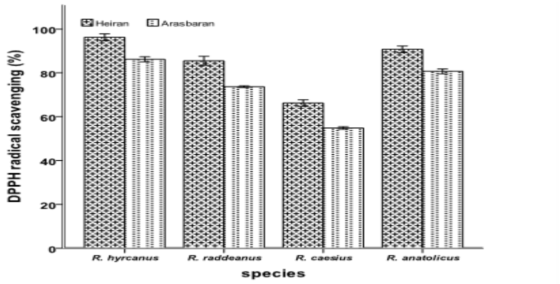

Fig. 3. DPPH free radical scavenging capacity of raspberry species in two regions of Iran. Results are expressed as mg Quercetinper 100 gFW.

Several environmental factors affect the content of phenolics in berries. It has been shown that higher growing temperatures (day and night) increase the flavonol and anthocyanin content in strawberries (Wang and Zheng., 2001). The increase of the level of carbon dioxide has also been found to lead to higher concentrations of the phenolic compounds and antioxidant capacity in strawberry (Wang *et al.*, 2003). In addition, soil conditions affect plant phenolic composition (Jeyaramraja *et al.*, 2003). An increase in soil moisture defect led to the lower activity of phenylalanine ammonia lyase and consequently to lower synthesis of phenolics in tea. Further, soil fertilization, particularly the high level of nitrogen seems to lower the levels of certain phenolics, but not of all of them (Keinanen *et al.*, 1999).

Table 2. Pearson׳s correlation coefficients for quantitative determination in raspberry species.

Variable	TAC	TP	TF
TAC	1	0.872**	0.822**
TP		1	0.929**
TF			1

ns: no significant;*P<0.05%,**P<0.01%.

As a conclusion, our results clearly demonstrate that considerable variation exists in the phenolic compounds among raspberry genotypes. These results provide a sound basis for planning breeding strategies, as well as for selecting species with high phenolic contents for producing specific material for food industry.

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