



Phytochemicals and antioxidants in watermelon (*Citrullus lanatus*) genotypes under hot arid region

B R CHOUDHARY¹, S M HALDHAR², S K MAHESHWARI³, R BHARGAVA⁴ and S K SHARMA⁵

ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan 334 006

Received: 15 May 2014; Revised accepted: 16 December 2014

ABSTRACT

Ten genotypes of red-fleshed watermelon [*Citrullus lanatus* (Thunb.)] were estimated for various health promoting bioactive compounds. The evaluated genotypes showed wide variability in total phenols, total flavonoids, tannin, total carotenoids and lycopene contents. The antioxidant activity was estimated by using *in vitro* assay of cupric reducing antioxidant capacity (CUPRAC). The significant difference ($P=0.05$) was observed among evaluated watermelon genotypes for different phytochemicals and antioxidants. The total phenols varied from 16.77 to 21.41 mg/g, total flavonoids 55.60 to 100.93 mg/100g and tannin content 35.07 to 60.83 mg/100g on dry weight basis. Total carotenoids and lycopene ranged from 4.90 to 8.06 mg/100g and 3.74 to 6.80 mg/100g, respectively on fresh weight basis. The average antioxidant activity was found to be varied from 40.13 to 84.05 $\mu\text{mol TE}/100\text{g}$ fresh weight. The results indicate that red-fleshed genotypes of watermelon are good source of antioxidants and showed significant variability for different phytochemicals and antioxidants that could be exploited to develop new cultivars/hybrids of superior quality for nutritional security.

Key words: Antioxidants, Antioxidant activity, Watermelon

Watermelon [*Citrullus lanatus* (Thunb.)] is a popular dessert crop throughout the tropics and the Mediterranean regions of the world. Now, it is not only a summer season fruit but also becoming an everyday fruit like apples, bananas and oranges because of its antioxidant properties. Fruits of watermelon contain diverse carotenoids which are responsible for different flesh colour. Watermelons are found having fruits of various sizes, shapes, rind pattern and flesh colour (Choudhary *et al.* 2012). The fruits of watermelon exhibit a number of flesh colour and therefore, have different carotenoid patterns associated with cultivars and cultivated environments (Zhao *et al.* 2013). Fruits are rich in lycopene and with a total antioxidant capacity similar to tomato (Perkins-Veazie *et al.* 2001). The fruits are also rich source of β -carotene, vitamins (B, C and E), minerals (K, Mg, Ca and Fe), amino acid (citrulline) and phenolics. Carotenoids contribute the colourful pigments of many vegetables which have antioxidant properties. Among carotenoids, β -carotene is precursor of vitamin A which is needed for eye sight. Lycopene imparts red colour in watermelon and in recent years has received much scientific attention due to its strong antioxidant properties (Edwards *et al.* 2003). Lycopene has been classified as useful in the human diet for prevention of cardiovascular diseases as well as certain types of cancer and may protect the skin from ultraviolet light damage. These beneficial effects, in many cases have been correlated with the presence of phytochemicals with antioxidant

properties (Perera and Yen 2007). Different carotenoids patterns were observed in red-fleshed and yellow-fleshed watermelon. The red-fleshed watermelon varieties contain high lycopene and varying amount of β -carotene (Tadmor *et al.* 2005). The main source of lycopene in human diet is tomato and its products in several countries. However, the red-fleshed watermelon contains more lycopene per unit fresh fruit weight than tomato and is equally bioavailable to human body (Edwards *et al.* 2003).

In recent years, consumers are aware about their health due to heavy burden of noncommunicable diseases like hypertension, diabetes, cancer, cardiovascular diseases, etc. and there has been a upsurge demand for high quality fruits. Therefore, the investigation on antioxidant composition of watermelon becomes an important field of study facilitating watermelon breeding for developing quality and nutritious fruits. Thus, it is important to characterize different watermelon genotypes for such substances to identify their nutritional value depending on different cultivars and genotypes, sampling area and fruit ripening stages (Tili *et al.* 2011). However, information on phytochemicals and antioxidants is very limited in watermelon cultivars grown in India. Thus, the objective of this study was to determine the phytochemicals and antioxidants among the selected genotypes of watermelon under hot arid conditions of India for identification of promising genotypes rich in antioxidants.

MATERIALS AND METHODS

A total of 10 diverse red fleshed genotypes of

¹email: choudharybr71@gmail.com

watermelon comprising 8 released varieties from different research institutes of India (Sugar Baby, Durgapura Lal, Charleston Grey, Asahi Yamato, Arka Manik, AHW 19, AHW 65 and Thar Manak), one advance breeding line (AHW/BR-16) and one indigenous collection (IC 582909) were selected for studies. The experiment was planned in randomized block design with three replications at Research Farm of ICAR-Central Institute for Arid Horticulture (CIAH), Bikaner, India located at 28°N latitude, 73°18'E longitude at an altitude of 234.84m above sea level and crop was sown during summer season of 2013. The soil of experimental field was loamy sand with a pH of 8.7, EC 0.20 dS/m and organic carbon 0.07%. The crop was raised on drip system maintaining row to row distance 2.5m and plant to plant 0.60m. All recommended integrated crop management practices were followed to successful crop production. Based on maturity indices the ripened fruit were harvested in the month of May and randomly selected five fruits per line from each replication for analysis of quality attributes.

The five selected fruits were carried to Plant Physiology Laboratory of ICAR-CIAH, Bikaner. The fresh fruits were cut and flesh sample was taken from the centre and locular areas of the fruit to get best homogenous sample. The flesh was dried and determined total flavonoids (Ebrahimzadeh *et al.* 2008) and tannin content (Schanderl 1970) and results were expressed as mg/100g dry weight (DW) basis. The quantification of total phenols was carried out following protocol of Malik and Singh (1980) and results were expressed as mg/g DW basis. About 200g flesh per fruit without seeds was also collected and stored at -20°C for estimation of antioxidants in laboratory. For analysis a composite sample of 50g of frozen flesh was ground for 2-3 minutes using a homogenizer. Total carotenoids and lycopene content of individual fruits was measured by spectrophotometer using wave length of 452 nm and 503 nm, respectively (Ranganna 1986). The results were expressed as mg/100g fresh weight (FW) basis. Antioxidant activity was estimated spectrophotometrically using cupric reducing antioxidant capacity (CUPRAC) and CUPRAC assay was performed according to method developed by Apak *et al.* (2007). To 100 µL of sample aliquot, 1 ml each of copper (II) chloride solution (10^{-2} M), neocuproine solution (705×10^{-3} M) and ammonium acetate buffer solution (pH 7) was mixed. The tubes were stopped and after 1 hour, absorbance at 450 nm was recorded against a reagent blank and the antioxidant activity was expressed as µmol Trolox equivalent (TE)/100g FW basis as suggested by Pentelidis *et al.* (2007).

The data obtained were statistically analyzed through one-way analysis of variance (ANOVA) using SPSS 16 software and significance was determined at $P < 0.05$. The data are presented as mean \pm SD of three replicates. Correlation between different antioxidants was also computed using correlation analysis.

RESULTS AND DISCUSSION

Significant difference ($P=0.05$) was observed among the watermelon genotypes for total phenols, total flavonoids and tannin content (Table 1). The results revealed that total phenols showed a considerable variation among the genotypes tested and varied from 16.77 to 21.41 mg/g DW basis. The highest total phenols was noted in Asahi Yamato (21.41 mg/g DW basis) followed by AHW/BR 16 and Sugar Baby (20.67 and 20.61 mg/g DW basis, respectively). A wide variation had also been reported for total phenolics in watermelon fruits ranging from 13.05 to 18.08 mg gallic acid equivalent/100g fresh weight basis (Nagal *et al.* 2012). Likewise, significant differences in total flavonoids (55.60-100.93 mg/100g DW basis) among the different genotypes were recorded. The cultivar Asahi Yamato had the highest content of total flavonoids (100.93 mg/100g DW basis) which was significantly superior over all genotypes and about twofold higher than AHW 19 (55.60 mg/100g DW). Flavonoids are considered one of the major contributors to the antioxidant activity of vegetables and it has been also well recognized that flavonoids show antioxidant activity and have considerable effect on human health through scavenging or chelating of free radicals (Ebrahimzadeh *et al.* 2008). The results indicated that the differences in tannin content among watermelon genotypes were statistically significant and ranged from 35.07 to 60.83 mg/100g DW basis being highest in cultivar Durgapura Lal. These differences in phenolic composition might be influenced due to several factors such as genotype, sampling area and climatic conditions (Tlili *et al.* 2011 and Nagal *et al.* 2012).

The significant difference in total carotenoids, lycopene and antioxidant activity among different genotypes of watermelon were recorded (Table 1). Total carotenoid content varied from 4.90 to 8.06 mg/100g FW basis being maximum in Asahi Yamato (8.06 mg/100g FW) followed by AHW/BR 16 and Sugar Baby (6.90 and 6.65 mg/100g FW, respectively). Similar observations on carotenoids in watermelon have been previously reported by various researchers (Perkins-Veazie 2007 and Zhao *et al.* 2013). The lycopene content in red fleshed watermelon genotypes varied from 3.74 to 6.80 mg/100g FW basis showing twofold variation (Fig 1). The cultivar Asahi Yamato (6.80 mg/100 g FW) and AHW/BR 16 (6.01 mg/100 g FW) were found significantly superior over all other genotypes. However, Choo and Sin (2012) reported low content of lycopene (0.95 mg/100 g) in red-fleshed watermelon than the present study. This difference was due to red-fleshed watermelons varied in their lycopene content depending on genotype and environmental conditions (Perkins-Veazie *et al.* 2001). Lycopene provided the largest portion of the total carotenoids (84-97%) as reported by Kang *et al.* (2010). The results of total carotenoids and lycopene of this study are in close agreement with cultivars grown in different parts of world (Nagal *et al.* 2012). The varying range of lycopene content in major cultivars of watermelon has been earlier reported as 4.26 mg/100g (Tadmor *et al.* 2005), 3.3-12.0 mg/100g (Perkins-Veazie 2007) and 3.46-8.0 mg/

Table 1 Phytochemicals and antioxidants among watermelon genotypes

Genotype	Total phenols (mg/g DW)	Total flavonoids (mg/100g DW)	Tannin content (mg/100g DW)	Total carotenoids (mg/100g FW)	Lycopene (mg/100g FW)	Antioxidant activity ($\mu\text{mol TE}/100\text{g FW}$)
Sugar Baby	20.61±0.71	86.10±5.63	51.53±3.35	6.65±0.25	5.36±0.23	66.79±1.34
Durgapura Lal	20.02±0.91	80.03±1.88	60.83±4.45	6.01±0.48	4.94±0.54	60.36±2.58
Charleston Grey	17.08±1.02	59.77±4.62	57.13±4.21	5.27±0.47	4.41±0.42	52.85±1.40
Asahi Yamato	21.41±0.79	100.93±6.80	59.40±3.30	8.06±0.57	6.80±0.35	84.05±3.16
Arka Manik	17.97±0.79	58.70±3.98	58.10±1.92	5.11±0.21	4.46±0.38	51.10±0.75
AHW 19	16.86±0.99	55.60±2.89	35.07±3.07	4.90±0.60	3.74±0.34	40.13±2.01
AHW 65	16.77±1.06	57.30±4.05	42.07±1.60	4.94±0.57	4.06±0.29	43.48±1.25
Thar Manak	18.97±1.01	67.73±3.19	48.63±2.45	5.46±0.27	4.75±0.25	53.23±1.42
AHW/BR 16	20.67±0.53	87.27±5.65	51.30±4.50	6.90±0.59	6.01±0.27	72.98±0.80
IC 582909	17.55±0.66	64.53±7.50	35.77±1.39	5.37±0.52	4.65±0.51	54.35±0.45
SEm±	0.83	3.73	3.23	0.43	0.30	1.97
CD ($P=0.05$)	2.50	11.18	9.86	1.29	0.89	5.90
CV (%)	7.69	9.01	11.77	12.69	10.49	5.89

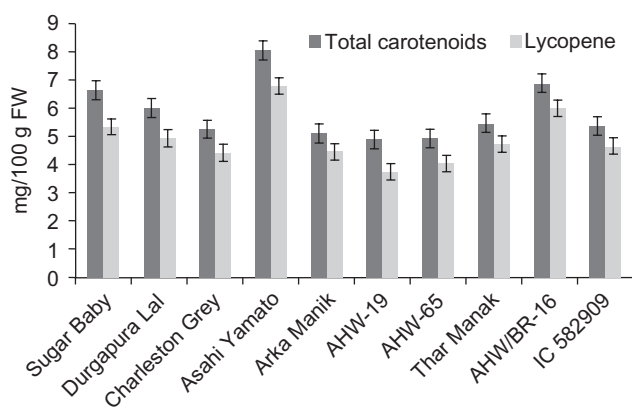


Fig 1 Total carotenoids and lycopene content of watermelon genotypes

100 g (Nagal *et al.* 2012) which was higher than tomato (Tadmor *et al.* 2005).

The chemical diversity of phenolic antioxidants compounds render it difficult to separate and quantify individual antioxidants from the plant matrix. Therefore, the total antioxidant activity is more meaningful to evaluate health benefits because it is integrated parameter of all antioxidants present in a complex sample (Apak *et al.* 2007). The average antioxidant activity of different watermelon genotypes were 40.13 to 84.05 $\mu\text{mol Trolox equivalent (TE)}/100\text{g FW}$ as determined by the CUPRAC assay. According to the results obtained, the cultivar Asahi Yamato had statistically significant antioxidant activity (84.05 $\mu\text{mol TE}/100\text{g FW}$) over all the genotypes followed by AHW/BR 16 and Sugar Baby (72.98 and 66.79 $\mu\text{mol TE}/100\text{g FW}$, respectively). The high antioxidant activity of these genotypes can be ascribed either to presence of high phenols or flavonoids or lycopene or other reducing agents which may also reduce the oxidized state of antioxidant compounds. Similar observation has been recorded by Nagal *et al.* (2012) and Choo and Sin (2012) in watermelon. The phenolic compounds are the dominant

antioxidants in vegetables, which exhibit scavenging efficiency on free radicals. Besides vitamin C, a great number of phenolic compounds have exhibited high *in vitro* antioxidant activity (Pantelidis *et al.* 2007). Since, lycopene is the main carotenoids in watermelon, accounting for 84-97% of total carotenoids (Kang *et al.* 2010) which have higher relative antioxidant potential and it is expected that the total antioxidant content of fruits also varies in the same conditions.

The correlation coefficient of total phenols ($r=0.921$), total flavonoids ($r=0.966$), total carotenoids ($r=0.979$) and lycopene ($r=0.992$) showed a high positive correlation ($P=0.01$) with antioxidant activity measured by CUPRAC assay (Table 2). But there was little correlation between antioxidant activity and tannin content. This indicates that total phenols, total flavonoids, total carotenoids and lycopene content are the major contributors towards antioxidant activity in watermelon. The highly positive correlation between antioxidant activity and phenolic content has also been reported by Nagal *et al.* (2012) in watermelon.

Table 2 Pearson's correlation coefficients between different antioxidants in watermelon

Attributes	Total phenols	Total flavonoids	Tannin content	Total carotenoids	Lycopene
Total flavonoids	0.966**				
Tannin content	0.328	0.189			
Total carotenoids	0.922**	0.982**	0.129		
Lycopene	0.912**	0.957**	0.172	0.979**	
Antioxidant capacity	0.921**	0.966**	0.237	0.979**	0.992**

**Significant at $P=0.01$ (two-tailed)

CONCLUSION

This study suggests that watermelon is a potential

source of health promoting bioactive compounds, which may have beneficial impact on human health. The findings indicate that a wide range of phytochemicals and antioxidants exists among red-fleshed watermelon genotypes and that watermelon cultivars with very high lycopene contents are available. Such genotypes could be exploited to develop new cultivars/hybrids rich in phenolics and lycopene for health benefits and nutritional security.

REFERENCES

- Apak R, Guclu K, Demirata B, Ozyurek M, Celik S E, Bektasoglu B, Berker K I and Ozyurt D. 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* **12**: 496–547.
- Choo W S and Sin W Y. 2012. Ascorbic acid, lycopene and antioxidant activity of red-fleshed and yellow-fleshed watermelons. *Advances in Applied Science Research* **3**(5): 2 779–84.
- Choudhary B R, Pandey S and Singh P K. 2012. Morphological diversity analysis among watermelon [*Citrullus lanatus* (Thunb)] genotypes. *Progressive Horticulture* **44**(2): 321–6.
- Ebrahimzadeh M A, Pourmorad F and Bekhradnia A R. 2008. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology* **32**: 43–9.
- Edwards A J, Vinyard B T, Wiley E R, Brown E D, Collins J K, Perkins-Veazie P, Baker R A and Clevidence B A. 2003. Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. *Journal of Nutrition* **133**: 1 043–50.
- Kang B, Zhao W, Hou Y and Tian P. 2010. Expression of carotenogenic genes during development and ripening of watermelon fruit. *Scientia Horticulturae* **124**: 368–75.
- Malik C P and Singh M B. 1980. *Plant Enzymology and Histoenzymology*. Kalyani Publishers, New Delhi.
- Nagal S, Kaur C, Choudhary H, Singh J, Singh B B and Singh K N. 2012. Lycopene content, antioxidant capacity and colour attributes of selected watermelon [*Citrullus lanatus* (Thunb.)] cultivars grown in India. *International Journal of Food Science and Nutrition*. DOI: 10.3109/09637486.2012.694848.
- Pantelidis G E, Vasilakakis M, Manganaris G A and Diamantidis G. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chemistry* **102**: 777–83.
- Perera C O and Yen G M. 2007. Functional properties of carotenoids in human health. *International Journal of Food Properties* **10**: 201–30.
- Perkins-Veazie P. 2007. Carotenoids in watermelon and mango. *Acta Horticulturae* **746**: 259–64.
- Perkins-Veazie P, Collins J K, Pair S D and Roberts W. 2001. Lycopene content differs among red-fleshed watermelon cultivars. *Journal of the Science of Food and Agriculture* **81**: 983–7.
- Ranganna S. 1986. *Manual for Analysis of Fruit and Vegetable Products*, pp 83–104. Tata McGraw Hill, New Delhi.
- Schanderl S H. 1970. *Methods in Food Analysis*. Academic Press, New York.
- Tadmor Y, King S, Levi A, Davis A, Meir A, Wasserman B, Hirschberg J and Lewinsohn E. 2005. Comparative fruit colouration in watermelon and tomato. *Food Research International* **38**: 837–41.
- Tlili I, Hdider C, Lenucci M S, Riadh I, Jebari H and Dalessandro G. 2011. Bioactive compounds and antioxidant activities of different watermelon [*Citrullus lanatus* (Thunb.)] cultivars as affected by fruit sampling area. *Journal Food Composition and Analysis* **24**(3): 307–14.
- Zhao W, Lv P and Gu H. 2013. Studies on carotenoids in watermelon flesh. *Agricultural Science* **4**(7A): 13–20.