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Assessing Agronomic Characteristics and Physicochemical Properties of Selected Watermelon Varieties Grown in Tunisia

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ABSTRACT

Watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) is a popular vegetable. Interest in assessing agronomic and bioactive compounds with antioxidant capacity and potential health benefits in watermelon is increasing. Besides some agronomic characteristics, the variability of lycopene and total phenolic contents of six watermelon varieties (four commercial cultivars 'Aramis', 'Crimson Sweet', 'Dumara', 'Giza', and two new selections P503 and P403 developed by the National Agricultural Research Institute of Tunisia) as influenced by sampling area was determined. 'Giza' and P503 were characterized by small fruits with a thin rind and a relatively high amount and large seeds. Significant differences were found in lycopene and phenolic contents between watermelon varieties. Lycopene content in P503 and 'Giza' was more than 2-fold higher than that in 'Dumara' and P403. The highest phenolic value (90.28 mg GAE kg^{-1} FW) was shown by 'Dumara'. The lycopene and total phenolic contents varied significantly between studied sampling areas but not in all varieties. Generally, highest values for lycopene and total phenolics were obtained for heart and stem end areas. For all studied watermelon varieties, lycopene was best correlated with rind thickness and 100-seeds weight. This study demonstrates that the amount of lycopene and total phenolics were both influenced by genotype and sampling area, emphasizing the need to adopt standardized and documented sampling methods when assessing quality attributes, and to evaluate watermelon biodiversity in order to improve its nutritional value.

Keywords: *Citrullus lanatus* (Thunb.) Mansfeld, lycopene, phenolics, quality, sampling area

INTRODUCTION

Watermelon is one of the main vegetable crops grown and consumed in Tunisia, and is much appreciated as an excellent refreshing summer fruit. In fact, it ranks fourth in surface among vegetable crops (Jebari 2003). In 2006, approximately 12,400 ha were dedicated to this crop, producing up to 340,000 t of watermelon (DGPA 2006).

Watermelon contains, in addition to vitamin A, C, E, potassium, citrulline and arginine, a variety of natural antioxidants such as carotenoids and phenolics (Perkins-Veazie 2002; Perkins-Veazie *et al*. 2007). In recent years, natural compounds, particularly lycopene and phenolics, have received great interest because of their antioxidant activity against free radicals, suggesting protective roles in reducing risk of chronic diseases, such as cancer and cardiovascular disease (Rice-Evans *et al*. 1996; Giovanucci 1999; Agarwal and Rao 2000).

Watermelon accumulates lycopene as major mesocarp carotenoids (70-90%) giving the fruit its typical red colour (Tomes *et al*. 1963; Tadmor *et al*. 2005). This red pigment has the highest antioxidant activity among all dietary antioxidants (Di Mascio *et al*. 1989; George *et al*. 2004). It has been reported that watermelon serves as a bioavailable source of lycopene in the diet and that this bioavailability to humans from fresh watermelon juice is similar to that of heat-processed tomatoes (Edwards *et al.* 2003).

In addition, it has been reported that watermelon contains a moderate amount of phenolics (Perkins-Veazie 2002; Brat *et al*. 2006; Mélo *et al*. 2006). These compounds are important secondary metabolites in plants and because of their structure, phenolic compounds are very efficient scavengers of peroxyl radicals (Halliwell 1996; Aruoma 1999). In addition, many phenolic compounds can exhibit pharmacological effects (Larson 1988; Manach *et al*. 1998).

Despite their great health benefit, few studies have reported on lycopene and total phenolic contents of watermelon varieties. In Tunisia, attention is now paid to antioxidant component studies. In fact, their estimate is becoming an important evaluation parameter for the nutritional quality of food (Lenucci *et al*. 2006). Tlili *et al*. (2007) recently highlighted that watermelon can be considered an important source of lycopene. However, further studies on antioxidant components of watermelon varieties grown under Tunisian environmental conditions are needed. In tomato fruit, Hdider *et al*. (2007) showed a large variation in lycopene content between cultivars. Also, Ilahy and Hdider (2007) showed that lycopene content in tomato vary with the stage of maturity.

It is known that the amount of each antioxidant in vegetables is strongly influenced by varietal differences and a large number of external factors such as agrotechnical process, climatic conditions and ripeness during harvest and post harvest manipulation (Waterman and Mole 1994; Abushita *et al*. 2000; Dumas *et al*. 2003). Recently, Perkins-Veazie *et al*. (2006) and Perkins-Veazie and Davis (2007) emphasized the importance of cultivars and sampling areas when assessing the lycopene and soluble solids content in watermelons. In fact, in large fruited watermelon where only a portion of the fruit is feasibly tested for quality, an accurate and reproducible sampling method must be used.

Therefore, and based on these facts the aim of this study was to investigate some agronomic and physicochemical properties of selected watermelon varieties grown in Tunisia as influenced by sampling area.

MATERIALS AND METHODS

Field experiment

Field experiments were conducted in 2007 at the Research and Experimental Station of Teboulba, Monastir, Tunisia. A total of six

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watermelon varieties including four commercial cultivars considered important in Tunisia and two new selections P503 and P403, selected by the National Agricultural Research Institute of Tunisia, were used in this experiment. The commercial cultivars were 'Crimson Sweet' (Clause), 'Dumara' (Nunhems), 'Aramis' (Nunhems) and 'Giza' (Egyptian variety selected and improved by the National Agricultural Research Institute of Tunisia). Sowing was carried out on the 5th March 2007 in plug-seedling trays. Watermelons were transplanted on the 24th April 2007 into a sandy soil on black plastic mulch, with an intra-row spacing of 125 cm and an inter-row spacing of 150 cm. Four blocks were used with 10 plants per variety. After transplanting, drip irrigation was applied with 4 L h^{-1} drippers placed at 0.4 m intervals along the irrigation line. Drip irrigation ran for 1-3 h, at 1-2 day intervals, depending on potential evapotranspiration for research station, climate data and crop coefficient. The production methods were in accordance with the procedures utilized by the research and experimental station of Teboulba, Monastir, Tunisia and recommended by INRAT. They included fertilization with synthetic chemical fertilizers (145 kg N ha^{-1}, 140 kg P_2O_5 ha^{-1}, 210 kg K_2O ha^{-1}). Chemical fertilizer solution was added to water irrigation by pump injection twice a week. The production methods also included a hand weeding control and plant pathogen control with synthetic chemical pesticides. Imidaclopride (Promochimie, Tunis, Tunisia) (200 g L^{-1}) was used to reduce aphids. Acetamipride (SEPCM, tunis, Tunisia) (200 g L^{-1}) was applied to reduce thrips. Abamectine (Bioprotection, tunis, Tunisia) (18 g L^{-1}) was used to reduce mites. All these pesticides were applied once a cycle.

All varieties were simultaneously grown in an open field and subjected to identical cultural practices in order to minimise the effects of environmental conditions and maximize those related to genotype.

Ripe watermelons were harvested in July. Field ripeness was judged by various methods including tendril browning, yellowing of the ground spot, and loss of surface gloss and by a thumping sound which changes from a metallic ringing when immature to a soft hollow sound at maturity. Watermelons were selected randomly from the different blocks. Four ripe fruits were harvested per block per variety. All the fruits were transported carefully to the laboratory for analysis to avoid internal bruising.

Fruit sampling

Fruit were cut longitudinally from stem end to blossom end through the ground spot, and tissue samples were taken from four different areas: blossom end, heart, stem end and peripheral (between locular and rind area). Soluble solids content (°Brix) and pH was carried out immediately on the juice obtained from mixed tissue fruit. For further analysis, about 250 g of flesh without seeds per sampling area per fruit was collected, wrapped with aluminium, placed into plastic bags and placed quickly at -80°C.

Chemicals

Gallic acid was obtained from Sigma-Aldrich, Chemical Co., Milan. Other reagents were of analytical grade.

Determination of agronomic characteristics

Watermelon yield was expressed by fruit weight per plant (kg). Rind thickness (from peel to start of pink colour) was measured to 0.1 mm using callipers at the ground spot and directly above the ground spot. After separating them manually, the individual number and 100-seeds weight of fruit were determined.

Determination of physicochemical properties

Soluble solids content in watermelon (°Brix) was measured by cutting a wedge of flesh from all sampling areas and squeezing the juice into a digital refractometer (Atago PR-100, NSG Precision Cells, Inc., Farmingdale, NY, USA) calibrated with a 10% sucrose solution. Only melons with mean soluble solids content ≥ 8% were sampled for lycopene to ensure that all fruits were fully ripe. pH was assessed on the juice obtained from mixed tissue fruit from all sampling areas using an electronic pH meter (WTW, Microprocessor pH Meter, PH 539, Weilheim, Germany).

Frozen samples of different sampling areas from every fruit were ground in a mortar and pestle and again with a laboratory blender. Lycopene extraction and determination were conducted as described by Fish *et al*. (2002). The method uses a mixture of hexane/ethanol/acetone (2: 1: 1, v/v/v) containing 0.05% butylated hydroxytoluene (BHT). The absorbance of the hexane extract was measured at 503 nm with a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Zeroing was done with hexane. During analysis, some precautions like working in reduced luminosity room and wrapping glass material with aluminium were adopted to minimise lycopene loss by photo-oxidation (Fish *et al*. 2002). A molar extinction coefficient $\varepsilon = 17.2 \times 10^4$ L mol^{-1} cm^{-1} was used for lycopene content determination (Beerh and Sidappa 1959) and results were expressed in mg kg^{-1} FW.

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method as modified by Singleton *et al*. (1999) and Eberhardt *et al*. (2000). Each sample (4 g) was extracted with 10 mL of methanol for 24 h, after which 125 µL of this extract was diluted 1: 5 (v/v) with distilled water. Then 125 µL of the diluted extract was mixed with 500 µL of distilled water in a test tube, 125 µL of Folin–Ciocalteu reagent was added and the mixture was allowed to stand for 3 min. Thereafter, 1.25 mL of 70 g L^{-1} sodium carbonate solution was added and the final volume was made up to 3 mL with distilled water. Each sample was allowed to stand for 90 min at room temperature before measurement at 760 nm against a blank in a spectrophotometer (Cecil BioQuest CE 2501). The linear reading of the standard curve was from 0 to 300 µg gallic acid mL^{-1}. Results were expressed in mg gallic acid equivalent (GAE) kg^{-1} FW.

Statistical analysis

The basic plots of the experiment were spread out in a randomised experimental design in four complete blocks. The analysis of variance was performed according to the General Linear Models (GLM) procedure developed by the Statistical Analysis Systems Institute (SAS Inst., V.6.1, Cary, NC, US). Means and standard errors were calculated. Correlations were done using Pearson's correlation coefficient at $P<0.05$. LSD test was applied to establish significant differences between means with a 95% confidence level.

RESULTS AND DISCUSSION

Agronomic characteristics

The most important agronomic characteristics of the different studied watermelon varieties are presented in **Table 1**. The yield of marketable fruit of the studied watermelon varieties varied from 5.2 to 7.8 kg $plant^{-1}$ but no statistical differences were found between varieties. Regarding average fruit weight, the varieties produced fruit with an average weight ranging between 3 and 6.3 kg. 'Giza' and P503 produced small-sized fruit. Significant differences in rind thickness were found between studied watermelon varieties ($P<0.01$), varying from 8.5 to 14.5 mm for 'Giza' and 'Crimson Sweet' varieties, respectively. Similarly, differences in average seed number per fruit were significant between studied watermelon varieties ($P<0.01$), varying from 0 to 867 seeds per fruit for seedless 'Aramis' and P503 varieties, respectively. Concerning the 100-seeds weight, differences were also significant between studied watermelon varieties ($P<0.01$) with values ranging between 0 and 10.4 g for seedless 'Aramis' and P503, respectively. 'Giza' and P503 were characterized by small fruits with a thin rind. These varieties also had a relatively high amount of large seeds.

Physicochemical properties

The soluble solids content, pH, lycopene and total phenolic contents values of the different studied watermelon varieties within different sampling areas are listed in **Table 2**. When averaged across sampling areas, the soluble solids varied

Table 1 Some agronomic characteristics of the different studied watermelon varieties.

Cultivars	Yield fruit weight per plant (kg)	Average fruit weight (kg)	Rind thickness (mm)	Average seed number per fruit	100-seeds weight (g)
Crimson Sweet	6.6	5.0 a	14.5 a	364 d	4.1 c
Giza	7.8	3.0 b	8.3 b	622 c	8.0 b
Dumara	6.5	5.7 a	10.3 b	789 b	4.8 c
P403	7.0	6.3 a	13.3 a	639 c	5.3 c
P503	5.2	3.5 b	9.0 b	867 a	10.4 a
Aramis	6.3	5.1 a	13.8 a	0 e	0.0 e
Significance	ns	**	**	**	**

Significance: ** Probability level of 1%; ns: not significant. Values in the same column followed by the same letters do not differ significantly (LSD test, P<0.05).

Table 2 Physicochemical properties of the different studied watermelon varieties within different sampling areas

Cultivars	Soluble solids (°Brix)	pH	Lycopene (mg kg^{-1} FW)	Total phenolic (mg GAE kg^{-1} FW)
Crimson Sweet				
blossom end area	8.9 ± 0.4 b	5.72 ± 0.1 a	49.05 ± 3.7 a	76.61 ± 3 ab
stem end area	9.3 ± 0.5 ab	5.71 ± 0.1 a	51.22 ± 7.0 a	84.60 ± 3 a
heart area	10.1 ± 0.5 a	5.77 ± 0.1 a	55.62 ± 6.8 a	74.87 ± 6 b
peripheral area	7.8 ± 0.3 c	5.77 ± 0.1 a	50.09 ± 8.7 a	62.55 ± 3 c
mean	9.0 A	5.74 C	51.50 C	74.65 B
Giza				
blossom end area	8.4 ± 0.6 ab	5.93 ± 0.1 a	86.99 ± 5.1 c	56.82 ± 7 b
stem end area	8.2 ± 0.6 ab	6.04 ± 0.1 a	99.58 ± 5.3 ab	74.35 ± 5 a
heart area	9.0 ± 0.9 a	5.90 ± 0.1 a	101.82 ± 4.6 a	69.14 ± 3 a
peripheral area	7.6 ± 0.4 b	5.97 ± 0.1 a	87.77 ± 1.8b c	71.05 ± 3 a
mean	8.3 A	5.96 A	92.04 A	67.84 C
Dumara				
blossom end area	8.4 ± 0.3 c	5.78 ± 0.1 a	40.16 ± 1.1 b	95.19 ± 3 a
stem end area	9.3 ± 0.5 b	5.89 ± 0.1 a	42.83 ± 1.7 ab	96.57 ± 3 a
heart area	10.1 ± 0.6 a	5.79 ± 0.1 a	47.68 ± 2.8 a	92.41 ± 5 a
peripheral area	7.8 ± 0.4 c	5.79 ± 0.1 a	37.55 ± 1.0 b	76.96 ± 2 b
mean	8.9 A	5.81 BC	42.10 D	90.28 A
P403				
blossom end area	8.9 ± 0.4 b	5.95 ± 0.1 a	41.67 ± 2.6 a	61.68 ± 4 c
stem end area	9.0 ± 0.6 b	5.85 ± 0.1 a	45.06 ± 3.0 a	99.70 ± 5 a
heart area	9.9 ± 0.6 a	5.97 ± 0.0 a	47.11 ± 2.0 a	74.70 ± 5 b
peripheral	7.3 ± 0.3 c	6.04 ± 0.1 a	42.40 ± 0.5 a	60.29 ± 1 c
mean	8.8 A	5.95 A	44.10 CD	74.09 B
P503				
blossom end area	7.8 ± 0.5 ab	5.78 ± 0.1 a	96.66 ± 3.9 b	54.91 ± 1 a
stem end area	8.6 ± 0.6 a	6.03 ± 0.1 a	107.27 ± 5.6 a	66.19 ± 3 a
heart area	8.8 ± 0.6 a	5.88 ± 0.1 a	106.17 ± 6.0 a	61.16 ± 3 a
peripheral area	7.4 ± 0.4 b	5.94 ± 0.2 a	82.38 ± 3.4 c	66.37 ± 6 a
mean	8.2 A	5.91AB	98.12 A	62.15 D
Aramis				
blossom end area	8.9 ± 0.6 bc	6.10 ± 0.1 a	68.11 ± 3.0 ab	58.03 ± 5 c
stem end area	9.6 ± 0.6 ab	5.95 ± 0.1 a	71.96 ± 9.2 a	71.40 ± 2 b
heart area	10.1 ± 1.1 a	6.03 ± 0.1 a	76.55 ± 4.9 a	89.28 ± 1 a
peripheral area	8.1 ± 0.7 c	6.01 ± 0.1 a	57.12 ± 1.5 b	60.64 ± 3 bc
mean	9.2 A	6.02 A	68.43 B	69.83 BC

Lower case letters indicate mean separation within column and sampling area by LSD test, P < 0.05. Capital letters indicate mean separation among means within column by LSD test, P < 0.05.

between 8.2 and 9.2 °Brix and was not different among varieties (P>0.05). These values reveal that all fruits were fully ripe. In contrast, differences in soluble solids content were significant between studied sampling areas within all varieties (P<0.01). For all varieties, the highest mean value was obtained for the heart area with 9.7 °Brix and the lowest was obtained for the peripheral area with 7.7 °Brix (**Fig. 1A**). These results are consistent with those reported by Perkins-Veazie and Davis (2007), who found that soluble solids differ among sampling areas in watermelon cultivars and that the locule and heart areas have the highest values. Regarding pH, mean values were significantly different between studied watermelon varieties (P<0.05). Significantly higher values were recorded in 'Aramis', 'Giza' and P403 and lower values were recorded in 'Crimson Sweet' and 'Dumara'. Our results agree with those of Perkins-Veazie and Collins (2006) who reported a significant difference in pH between watermelon cultivars. In contrast, differences in pH were not significant between studied sampling areas in all varieties (P>0.05). This result is in disagreement with those of Perkins-Veazie and Davis (2007) who reported significant differences between measured pH in different sampling areas.

For lycopene content, the obtained data showed that values varied significantly between studied watermelon varieties (P<0.01). When averaged across sampling areas, lycopene content reached very high levels (>90 mg kg^{-1} FW) and varied from 42.10 to 98.12 mg kg^{-1} FW. The highest values were obtained for P503 and 'Giza' with 98.12 and 92.04 mg kg^{-1} FW, respectively. The lowest values were obtained for 'Dumara' and P403. P503 and 'Giza' have more than 2-fold lycopene than 'Dumara' and P403. The results are in agreement with those reported by Perkins-Veazie *et al*. (2006) who found that lycopene content varies among watermelon cultivars and can reach very high values attaining 99.8 mg kg^{-1} FW in cv. Xite, while studying the carotenoid composition of 50 watermelon cultivars. The data also proved that watermelon can constitute a predominant source of lycopene in the Tunisian diet because of its availability and high consumption, as was reported for the

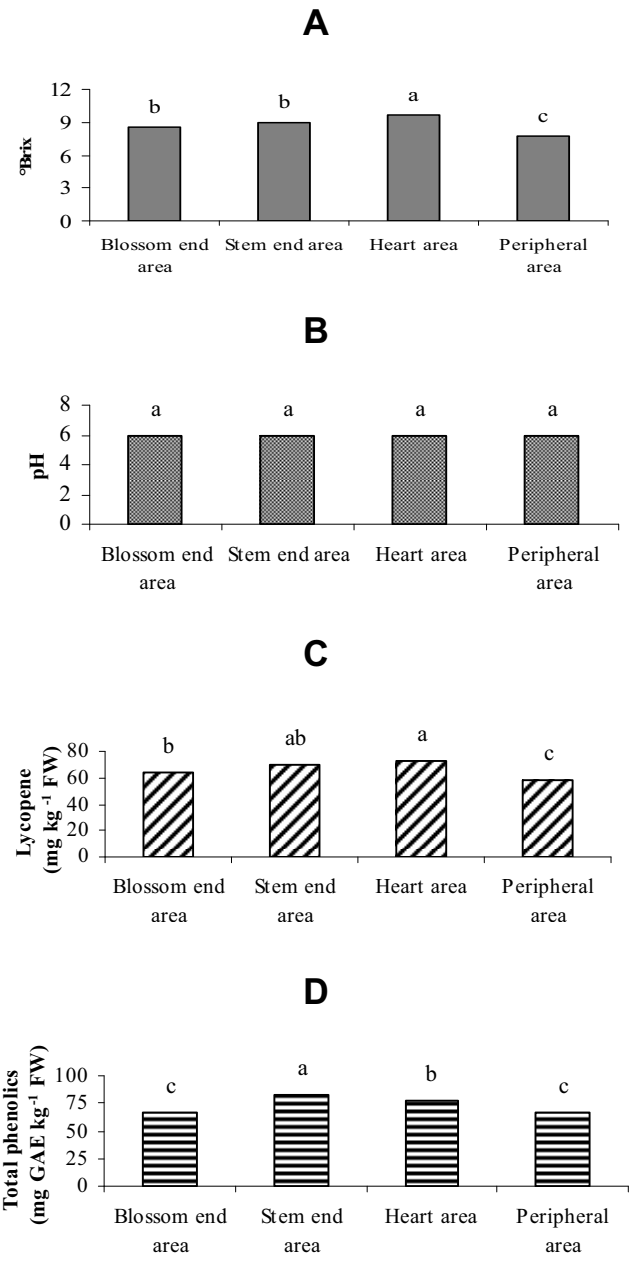

Fig. 1 Average soluble solids (°Brix) (A), pH (B), lycopene (mg kg^{-1} FW) (C) and total phenolics (mg GAE kg^{-1} FW) (D) for all watermelon cultivars within the different sampling areas. Values for each sampling area with the same letters are not significantly different (LSD test, P<0.05).

American diet (Vinson *et al*. 1998).

In addition, the results showed that lycopene content was significantly different between studied sampling areas, except for 'Crimson Sweet' and P403 (P<0.01). Nevertheless, when averaged across varieties, the highest values were obtained for heart and stem end areas with 72.49 and 69.65 mg kg^{-1} FW, respectively (**Fig. 1C**). The lowest value was obtained for the peripheral area (57.46 mg kg^{-1} FW). Our results confirm those of Perkins-Veazie and Davis (2007) who reported that lycopene content differs significantly among sampling areas in watermelon cultivars and highlights the importance of sampling area in determining lycopene content in watermelon fruit.

Total phenolic content varied significantly between studied watermelon varieties (P<0.01). When averaged across sampling areas, values obtained ranged from 62.15 to 90.28 mg GAE kg^{-1} FW. The highest total phenolic content value was shown by 'Dumara' and the lowest value by P503. Our results are consistent with those of Brat *et al.* (2006) who reported that watermelon fruit contains moderate amount of phenolic compounds reaching 116 mg GAE kg^{-1} FW. Mélo *et al*. (2006) also reported that total phenolic content in watermelon can reach 98.1 mg catechin equivalent kg^{-1} FW. Higher values ranging between 870-910 mg GAE kg^{-1} FW were obtained in red fleshed watermelon cultivars by Perkins-Veazie (2002) but without differences between them. These divergent results were probably due to variety or environmental differences. Although phenolic content in watermelons was only moderate compared to other potential vegetables, such as onion reaching 761 GAE kg^{-1} FW (Brat *et al.* 2006), its high consumption in Tunisia diet makes them a good source of phenols as was reported for American and Spanish diet. In fact, Vinson *et al*. (2001) reported that watermelon is the forth among eight fruits that provide 80% of the daily phenols in the American diet and 50% of the Spanish diet. The results also showed that the total phenolic content varied significantly between studied sampling area within all varieties, except for P503 (P<0.01). Nevertheless, for all varieties, the mean values of total phenolic content was highest in stem end area with 82.13 mg GAE kg^{-1} FW followed by the heart area with 76.92 mg GAE kg^{-1} FW. The lowest values were obtained in blossom end and peripheral areas with 67.20 mg GAE kg^{-1} FW and 66.31 mg GAE kg^{-1} FW, respectively (**Fig. 1D**). To our knowledge, these are the first results describing distribution of total phenolics in watermelon fruit.

Correlation study

Many authors studied the correlation between lycopene and phenolics and other characteristics in many fruits, particularly tomato (Giovanelli *et al*. 1999; Arias *et al*. 2000; Brandt *et al*. 2006). However, little of information is known about these types of correlations in watermelon fruit. Correlation coefficients among fruit variables measured from different evaluated watermelon varieties are listed in **Table 3**. For all studied watermelon varieties, lycopene was best correlated with rind thickness and seed weight. Rind thickness was negatively and significantly correlated with lycopene (R^2 = -0.54) indicating that varieties with thin rind thickness corresponded to higher lycopene content. Perkins-Veazie and Collins (2006) reported a weak correlation between rind thickness and lycopene (R^2 = -0.36). The 100-seeds weight was positively and significantly correlated with lycopene (R^2 = 0.56). A significant but weak negative correlation was found between phenolic and lycopene contents (R^2 = -0.35). This is unlike that found in tomato (R^2 = 0.42) by Martinez-Valverde *et al*. (2002). Soluble solids content, pH and seed number were not significantly correlated with lycopene. Soluble solids content was the only attribute positively correlated with total phenolic content (R^2 = 0.36).

CONCLUSIONS

This study confirmed the important role played by genetics in determining antioxidant components of fresh watermelon

Table 3 Correlation coefficients among variables measured of fruit from the different studied watermelon varieties.

Variables	Lycopene	Phenolics	°Brix	pH	Rind thickness	Seed number	100-seeds weight
Lycopene		-0.35**	-0.12	0.16	-0.54**	0.11	0.56**
Phenolics	-0.35**		0.36**	-0.12	0.12	0.08	-0.30
°Brix	-0.12	0.36**		0.05	0.16	-0.28	-0.36
pH	0.16	-0.12	0.05		-0.28	-0.23	-0.06

**: significant correlations at p < 0.01 level, using Pearson's correlation coefficient. Lycopene, total phenolics, °Brix and pH values were averaged across sampling areas.

and highlights the importance of standardized and documented sampling methods when determining soluble solids content, lycopene and phenols in watermelon varieties. In addition, the present study emphasizes the promising use of varieties P503 and 'Giza' for their very high lycopene content as healthy quality fruit and for future breeding programs. The variability detected among the four commercial watermelon cultivars and among the two new advanced selections emphasized an existing unexploited variability in watermelon germplasm and stresses the need to evaluate more watermelon genotypes and to support conventional breeding programs to improve watermelon nutritional value.

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