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Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype

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Abstract

Consumption of tomato products has been associated with decreased risk of some cancer types. Epidemiological findings confirm the observed health effects due to the presence of varied antioxidants in tomato. The bio-antioxidant content and antioxidant activity of 12 tomato genotypes was therefore studied. Significant differences were found between lycopene, ascorbic acid and phenolic contents among various genotypes. Lycopene and ascorbic acid contents showed 1–4 fold and 1–2 fold variation on both fresh and dry weight basis, respectively. Antioxidant activity was found to vary significantly among genotypes. In both free radical quenching assay and FRAP assay, significant activity was found. Activity was higher in the hexane fraction containing lycopene than the methanol fraction containing phenolics. Tomato peels, in addition to lycopene, contain significantly high amounts of ascorbic acid and phenols. Cherry tomatoes, particularly variety 818 cherry, with the highest contents of antioxidants (lycopene, ascorbic acid and phenols) and highest antioxidant activity represents a valuable genotype not only for improving the status of dietary antioxidants in our diet but also for increasing nutritional value through germplasm enhancement programmes. The cherry varieties also merit considerable attention for processing because of their high total soluble solids and titrable acidity.

Keywords: Tomato; Genotype; Antioxidants

1. Introduction

Tomato fruit has undoubtedly assumed the status of a functional food considering the overwhelming epidemiological evidence for its reducing risk of certain types of cancers (Nguyen & Schwartz, 1999). It is a reservoir of diverse antioxidant molecules, such as ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids. The disease-preventing potential of a food is a consequence of a several such constituents which may show some synergistic interactions. Lycopene, the biomolecule of interest, has been shown to have strong antioxidant activity and exhibits the highest physical quenching rate constant with singlet oxygen (Di Mascio, Kaiser, & Sies, 1989). It has the highest antioxidant activity among all dietary antioxidants. In addition to these properties, lycopene has also been shown to induce cell-to-cell communications and modulate hormones, immune systems and other metabolic pathways (Rao & Agarwal, 1999). Dietary intake of lycopene is epidemiologically correlated with diminished risk of prostate cancer and it has been found to be superior to α- and β-carotene in inhibiting cell proliferation in various human epithelial cancer cell lines (Giovannucci, 1999). Tomatoes also contain moderate amounts of α- and β-carotene and lutein. β-Carotene is known for its provitamin A activity and lutein for reduced risk of lung cancer (Sies, 1991). Consumption of tomato and tomato products is thus being considered as a nutritional indicator of good dietary habits and healthy life styles.

Some recent papers report the presence of flavonoids in tomato which are also important in conferring antioxidative health benefits (Bourne & Rice-Evans, 1998; Takeoka, 2001; Vinson, Hao, Su, & Zubik, 1998). Some varieties of tomatoes contain high amounts of flavonols, primarily as quercetin (Crozier, Lean, McDonald, & Black, 1997). Flavanols and flavones are of particular interest as they are potential antioxidants and have been

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found to possess antioxidative and free radical scavenging activities in foods and their consumption is associated with a reduced risk of cancer (Kaur & Kapoor, 2001).

Tomatoes constitute the predominant source of lycopene and phenols in the Indian diet because of their year-round availability and high utility in Indian culinary preparations. In American diet too, tomato has been found to be a leading source of phenol, followed by corn, pinto beans, potato and onion (Vinson et al., 1998). Although the phenolic content in tomatoes is only moderate compared to other potential vegetables, such as onion, their high consumption in Indian diet makes them a good source of phenols. So far, tomato varieties in India have only been screened for their lycopene and ascorbic acid contents and there has been no report on the variation of phenolic content and antioxidant activity in different genotypes. Thus the present study undertakes a study of the variations in contents of different antioxidants, such as lycopene, ascorbic acid, and phenolics, in some selected tomato genotypes and their contribution to the antioxidant activity. An insight into various antioxidant components of tomato would help to define its quality.

2. Materials and methods

2.1. Tomato sampling and sample preparation

Twelve genotypes of tomato fruit (*Lycopersicon esculentum*) (Table 1) grown in the fields of the Indo-Israel Project of Indian Agricultural Research Institute, New Delhi, were taken for experimentation. Freshly harvested, uniformly ripened healthy fruits, at the red ripe stage, were taken for analysis. One-kilogramme of fruit of each variety was selected randomly to study the variation in contents of various biomolecules, such as lycopene, phenolics and ascorbic acid in tomato pulp and peel, respectively. The tomato fruits were crushed in a Waring blender and the homogeneous mass was taken for analysis.

Table 1
Variations in lycopene content in tomato as a function of genotype

Varieties	Lycopene content (mg 100 g^{-1})			
	Peel		Pulp	
	fwb	dwb	fwb	dwb
818 cherry[a]	14.1 (1)	115 (1)	6.94 (1)	81.9 (6)
DT-2	8.1 (8)	81.1 (9)	5.22 (2)	125 (1)
BR-124 cherry[a]	10.2 (5)	90.8 (6)	4.89 (3)	76.8 (5)
5656	10.7 (4)	104 (4)	4.50 (4)	90.9 (4)
7711	8.97 (7)	87.9 (7)	4.37 (5)	106 (2)
Rasmi	10.8 (3)	99 (5)	4.30 (6)	101 (3)
Pusa Gaurav	10.2 (6)	107 (3)	3.98 (7)	84.3 (5)
T56 cherry[a]	12.0 (2)	109 (2)	3.78 (8)	54.6 (10)
DTH-7	4.83 (12)	63.3 (11)	2.73 (9)	57.2 (9)
FA-180	7.58 (9)	82.4 (8)	2.48 (10)	63.6 (7)
FA-574	6.12 (11)	68.0 (10)	2.18 (11)	54.8 (10)
R-144	6.24 (10)	72.1 (9)	2.04 (12)	51.1 (12)
CD at 5%	0.81	5.63	0.47	7.43

The values are means of three samples done in duplicate. Values in parentheses represent the ranking order. fwb—fresh weight basis; dwb—dry weight basis.

[a] Cherry variety.

2.2. Lycopene estimation

Lycopene from tomato products was extracted with hexane: methanol: acetone (2:1:1), containing 2.5% BHT (butylated hydroxy toluene). Optical density of the hexane extract was measured spectrophotometrically at 502 nm against a hexane blank. Concentration of lycopene was calculated using the extinction coefficient (E%) of 3150 (Rao et al., 1998). Results are expressed as mg/100 g fresh weight (fw) and dry weight (dw) respectively.

2.3. Ascorbic acid estimation

Ascorbic acid content was determined by the method as described by Albrecht (1993), titrating a known weight of sample against 2,6-dichlorophenol-indophenol dye using 3% metaphosphoric acid as extracting medium.

2.4. Total phenols

A total phenol was determined using the Folin-Ciocalteau reagent (Singleton & Rossi, 1965). Results were expressed as catechin (mg/100 g) of fw and dw respectively.

2.5. Measurement of antioxidant activity

Antioxidant activity was measured in both water-soluble and water-insoluble fractions using two different methods, FRAP assay (Benzie & Strain, 1996) and free radical quenching activity assay (Huang & Frankel, 1997).

FRAP assay was done with FRAP reagent, i.e. 1 mM 2,4,6-tripyridyl-2-triazine (TPTZ) and 20 mM ferric chloride in 0.25 M sodium acetate, pH3.6. 100 μl of tomato extract (2 g/10 ml in methanol) were added to 1 ml of FRAP reagent and mixed thoroughly.

After standing at ambient temperature (20 °C) for 4 min, absorbance at 593 nm was measured against a water blank. Calibration was against a standard curve (50–1000 μM ferrous ion) produced with freshly prepared ammonium ferrous sulphate. Values were obtained from three replications and expressed as mM FRAP. The free radical quenching activity was measured in a phosphatidyl–choline liposome solution similar to that described by Huang and Frankel (1997)

and Huang, Staue-Gracia, Frankel, and German (1999). Phosphatidylcholine (Sigma) was dissolved in water with constant stirring for 45 min to give a concentration of 8 mg/ml. The mixture was then sonicated for 5 min with a sonicating probe at half power to yield a liposome solution. An aliquot (100 μl) of previously prepared tomato fraction (100 g in hexane for the water-insoluble and in methanol for the water-soluble fraction) was placed in a 20 ml glass screw-top vial and 10 ml of the liposome solution were added. The vials were again sonicated for 5 min to disperse the tomato fraction into the solution. Oxidation was initiated by adding 10 μl of a 2 mg/ml cupric acetate solution in methanol. The samples were held in a water bath at 37 °C, and reaction rate was followed by monitoring the formation of conjugated dienes at 234 nm. Prior to spectral measurements, 100 μl of sample were diluted with 5 ml of methanol. All samples were replicated a minimum of four times. The results for all antioxidant assays were expressed as percent inhibition of conjugated diene formation as compared to control that did not contain any antioxidants. Percent inhibition was determined 35 h after initiation of oxidation.

2.6. Total soluble solids (TSS) and titrable acidity (TA)

TSS was measured using an Abbe refractometer (Carl Zeiss, Jena Germany) calibrated against sucrose. TA was measured according to AOAC Method 942.15 (AOAC, 1995) and expressed as% citric acid.

2.7. Statistical analysis

Data was subjected to analysis of variance using ANOVA, and means were compared with critical difference. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

The benefits of tomato and tomato products have mainly been attributed to their carotenoid contents. These products are an excellent source of lycopene which, although devoid of pro-vitamin activity, has been found to have antioxidant properties (Abushita, Daood, & Biacs, 2000; Stahl & Sies, 1996). Its antioxidant function is associated with lowering the risk of cancers of pancreas, breast and prostate, both in vitro and in vivo. Lycopene, the red pigment of tomato, on average constitutes about 80–90% of the total carotenoid content (Shi & Le Maguer, 2000). The present data on lycopene content showed significant variation in pulp and peel fractions of examined genotypes. A variation of 1–4 fold (fwb) and 1–2 fold (dwb) was seen in pulp. The extent of variation was 4.8–14.1 mg/100 g in peels and 2.0–6.9 mg/100 g in pulp on fwb. However, quite distinct variations were also observed when the lycopene values were expressed on dwb, resulting in changes in ranking (Table 1). When expressed on fwb, highest lycopene content was found in variety 818 cherry (6.94 mg/100 g) and lowest in variety R-144 (2.04 mg/100 g). Moderate lycopene contents (4.3–5.2 mg/100 g) were found in cultivars DT-2, BR-124, 5656 and 7711. In peels too, variety 818 showed the highest lycopene content and DTH-7 had the lowest. On an average, tomato peels had 2.5 times the lycopene content found in pulp. This is similar to the finding of Al-Wandawi, Abul Rahman, and Al Shaikhly (1985) who reported a 3 times higher lycopene content in the peel fraction. A large variation in the lycopene content of the cultivars is mainly attributed to factors such as plant nutrition, environment and genotype, which together can markedly affect the biosynthesis of carotenoids. Similar variations, ranging from 5 to 11 mg/100 g of lycopene in tomatoes have recently been reported by Abushita et al. (2000) in Hungarian varieties. A variation of 2.6–6.3 mg/100 g has also been reported by Rao and Yadav (1988) and Thakur and Kaushal (1995) in Indian varieties.

Tomato contains moderate amounts of ascorbic acid (20 mg/100 g) (Gould, 1992), thus contributing to 40% of the recommended dietary allowance for ascorbic acid. Ascorbic acid content ranged from 8.4 to 32.4 mg/100 g in tomato pulp (Table 2). Substantial amounts of ascorbic acid were also detected in peels (9.0–56.0 mg/100 g fwb and 104–462 mg/100 g dwb). Variety 818 cherry had significantly higher ascorbic acid content in both pulp and peels (32.4 mg/100 g). Gould (1992), in

Table 2
Variations of ascorbic acid content in tomato as a function of genotype

Varieties	Ascorbic acid content (mg 100 g^{-1})			
	Peel		Pulp	
	fwb	dwb	fwb	dwb
818 cherry[a]	56.0 (1)	454 (1)	32.4 (1)	383 (3)
DT-2	25.9 (6)	256 (8)	15.2 (5)	365 (4)
BR-124 cherry[a]	40.6 (5)	362 (5)	29.0 (3)	455 (1)
5656	20.1 (8)	196 (9)	13.4 (7)	270 (8)
7711	17.2 (9)	169 (10)	12.7 (8)	308 (6)
Rasmi	42.0 (4)	385 (4)	14.8 (6)	349 (5)
Pusa Gaurav	13.4 (10)	141 (11)	9.20 (11)	195 (11)
T56 cherry[a]	50.8 (2)	462 (2)	28.60 (4)	413 (2)
DTH-7	24.0 (7)	315 (6)	10.70 (9)	224 (9)
FA-180	45.5 (3)	415 (3)	9.40 (110)	171 (12)
FA-574	9.40 (11)	104 (12)	8.40 (12)	211 (10)
R-144	8.55 (12)	262 (7)	30.4 (2)	291 (7)
CD at 5%	4.59	36.48	2.54	27.5

The values are means of three samples done in duplicate. Values in parentheses represent the ranking order. fwb—fresh weight basis; dwb—dry weight basis.

[a] Cherry variety.

his recommendations for breeding varieties for processing, suggested the need for developing varieties which have ascorbic acid in excess of 20 mg/100 g. In light of this, cherry varieties, 818 cherry, T-56 and BR-124, having high ascorbic acid may be recommended as potential varieties for processing and for improvement of nutritional value in breeding programmes. Consumption of these varieties as fresh salad may also serve as a good source of dietary antioxidant.

In recent years there has been a plethora of research publications on the evaluation of phenolic content in fruits and vegetables (Donovan, Meyer, & Waterhouse, 1998; Kahkonen et al., 1999; Kaur & Kapoor, 2001; Prior & Cao, 2000). They are being viewed as "star nutrients" because of the antioxidant effects of certain phenols, such as epicatechin, often surpassing that of vitamins C and E (Stadler, 2001). The antioxidative and free radical-scavenging properties of polyphenolic compounds in several plant extracts have been recently reported, suggesting possible protective roles of polyphenolic compounds in reducing risk of cardiovascular diseases in humans (Veloglu, Mazza, Gao, & Oomah, 1998). Free phenolic content ranged from 9.20 to 22.0 mg/100 g (fw) in pulp. It was again interesting to note that three cherry varieties, namely 818 cherry, BR-124 and T-56 cherry, had higher phenolic contents than other varieties (Table 3). Peels had significantly higher phenolic contents than the pulp.

The evaluation of AOX is becoming increasingly relevant in the fields of nutrition and food technology. Rather than determining the concentration of each antioxidant molecule individually, evaluation of total antioxidant activity, using different model assay systems has become increasingly important. Total antioxidant activity is a measure of the capacity of substances extracted from the food matrix to delay the oxidation process in a controlled system (Cao, Srfie, & Prior, 1996; Fogliano, Verde, Randazzo, & Ritieni, 1999; Miller & Rice Evans, 1997; Pellegrini, Riso, & Porrini, 2000).

AOX in the water-soluble fraction, as evaluated by FRAP, ranged from 0.64 to 2.3 mM FRAP. Significantly high FRAP values were observed in the variety 818 cherry (2.3 mM FRAP) and the lowest in variety FA-574 (Fig. 1). Recently, Scalfi et al. (2000) have also reported significantly high antioxidant activity in small corbarini tomatoes. Moderate values were observed in variety T-56, BR-124, 5656, Rasmi, DTH-7 and Pusa Gaurav (1.1 to 1.9 mM FRAP). Takeoka et al. (2001) have attributed AOX in the methanolic fraction to the presence of phenolic compounds, such as caffeic and chlorogenic acid. An attempt was also made to evaluate the FRAP values of hexane extracts rich in lycopene but no reproducible results were obtained. Probably carotenoids do not contribute to the ferric reducing ability of extracts. Similar observations have been made by Pulido, Bravo, and Saura Calixto (2000). For these reasons, the free radical scavenging activity was determined (Fig. 2) in both lipophilic and methanolic extracts by using a different method, that of Huang and Frankel (1997). AOX was higher in the hexane fraction containing lycopene than the methanolic fraction. Similar observations have been made by Takeoka et al. (2001). Highest AOX was found in the lipophilic, as well as the methanolic extracts of genotype 818 cherry (9.7%) and lowest in R-144 (3.10%) (Fig. 3). High antioxidant status of cherry genotype can be explained on the basis of its correspondingly high lycopene and phenolic contents.

Total solids and titrable acidity have direct implications in the tomato processing industry. Gould (1992) suggested tomato varieties with high tomato solids in

Table 3
Variations in phenolic content in tomato as a function of genotype

Varieties	Phenolic content (mg 100 g^{-1})			
	Peel		Pulp	
	fwb	dwb	fwb	dwb
818 cherry[a]	40.0 (1)	324 (2)	27.0 (1)	319 (8)
DT-2	18.4 (7)	182 (6)	15.7 (6)	377 (4)
BR-124 cherry[a]	25.0 (4)	223 (5)	22.0 (3)	345 (5)
5656	26.7 (3)	250 (4)	23.0 (2)	465 (1)
7711	15.7 (9)	144 (10)	13.0 (8)	188 (12)
Rasmi	20.4 (6)	187 (8)	17.4 (5)	421 (3)
Pusa Gaurav	24.0 (5)	253 (3)	20.0 (4)	426 (2)
T56 cherry[a]	38.0 (2)	349 (1)	22.0 (3)	319 (7)
DTH-7	12.0 (11)	158 (9)	11.4 (10)	243 (10)
FA-180	12.7 (10)	138 (11)	11.7 (9)	300 (9)
FA-574	10.4 (12)	116 (12)	9.20 (11)	231 (11)
R-144	15.7 (8)	181 (7)	13.4 (7)	344 (6)
CD at 5%	2.83	22.2	1.66	24.6

The values are means of three samples done in duplicate. Values in parentheses represent the ranking order. fwb–fresh weight basis; dwb–dry weight basis.

[a] Cherry variety.

Table 4
Variations in T.S.S. (°B) and titratable acidity (%) of Tomato as a function of genotype

Genotype	T.S.S. (°B)	Acidity (%)
818 cherry[a]	7	0.704
DT-2	5	0.512
BR-124 cherry[a]	7	0.520
5656	5	0.448
7711	5	0.370
Rasmi	5	0.256
Pusa Gaurav	5	0.512
T-56 cherry[a]	6	0.520
DTH-7	5	0.448
FA-180	5	0.384
FA 574	5	0.450
R-144	5	0.320

[a] Cherry variety.

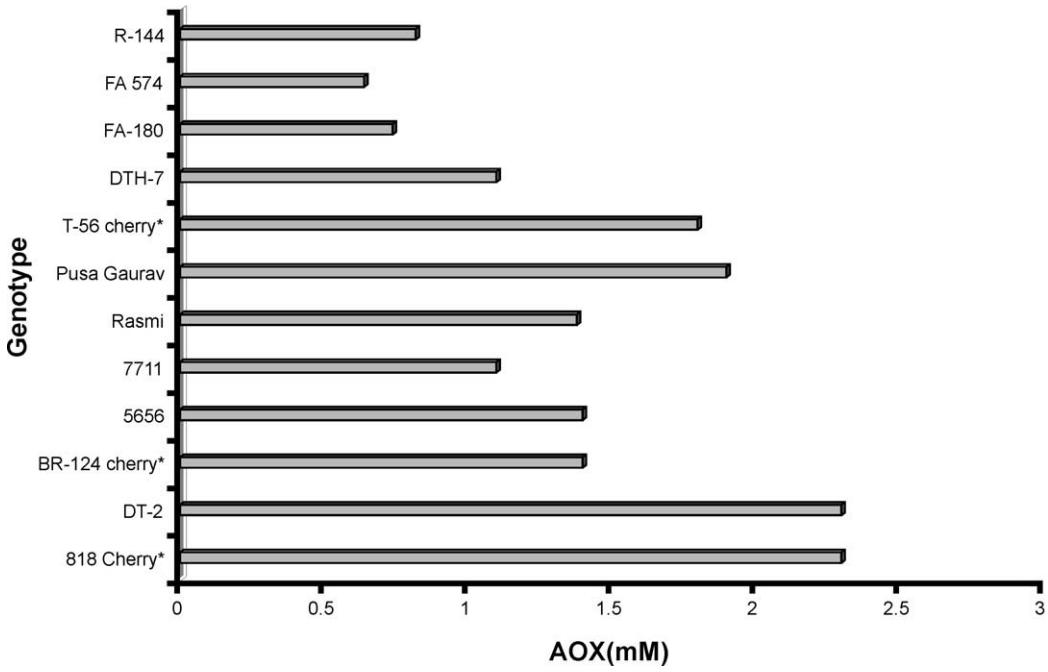

Fig. 1. Antioxidant activity in tomato genotypes based on the FRAP assay.

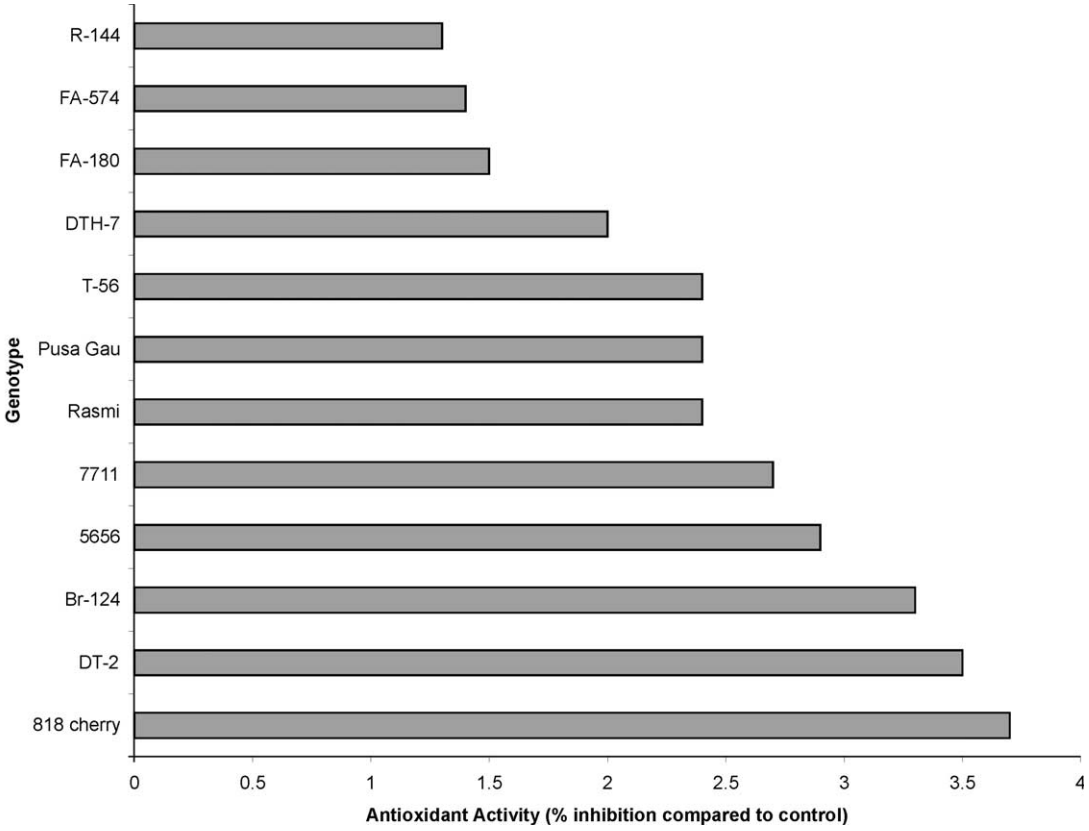

Fig. 2. Antioxidant activity in hydrophilic extracts of tomato genotypes based on free radical quenching assay.

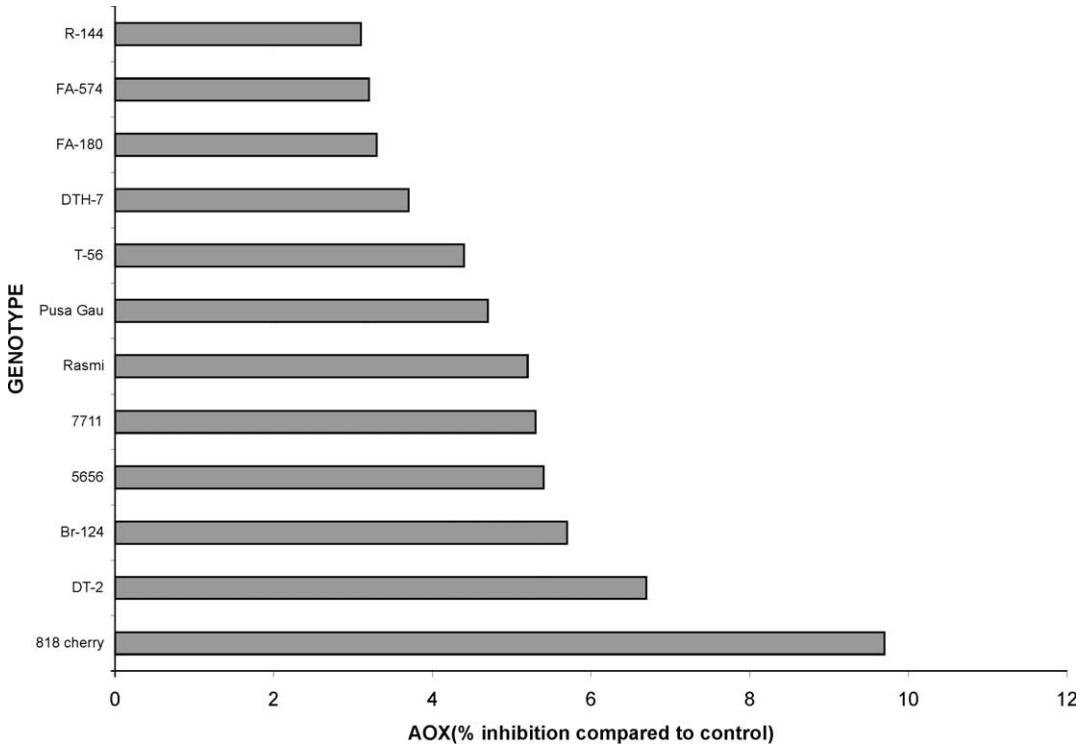

Fig. 3. Antioxidant activity in lipophilic extracts of tomato genotypes based on free radical quenching assay.

excess of 5.5%, preferably upward of 8.5% and acidity in the range 0.35–0.55% as desirable attributes for processing. In this regard, cherry varieties with high TSS (7°B) and acidity 0.70–0.51% are suitable genotypes (Table 4).

4. Conclusion

The study indicates the potential of cherry varieties 818 and DT-2, containing high levels of antioxidants, for germplasm improvement programmes.

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