



Antioxidative properties of cherry tomato

P. R. PRASANNA, P. PANDA, S. BANERJEE,
S. DOLUI AND *A. BHATTACHARYA

Department of Agricultural Biochemistry, Bidhan Chandra Krishi Viswavidyalaya
Mohanpur-741252, Nadia, West Bengal

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ABSTRACT

In an effort to select the most promising line(s) out of eight advanced breeding lines of cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) in terms of antioxidative properties, the content of antioxidant constituents and activity of antioxidative enzymes were analyzed from the fruit samples at 'red ripe' stage of harvest in an experiment conducted during November, 2018. The content of lycopene, ascorbic acid and phenol were found to range between 1.89 and 3.31 mg 100⁻¹g, 21.15 and 39.77 mg 100⁻¹g and 6.94 and 12.97 mg TE g⁻¹, respectively whereas antioxidant activity under three different systems of assay (DPPHRAC, FRAP and LP) yielded values from 8.16-15.69 mg TE g⁻¹, 2.06-3.82 mg TE g⁻¹ and 7.85-55.29 μ mol MDA g⁻¹ of fresh sample respectively. The activity of antioxidative enzymes (SOD, CAT and POD) varied from 2.11 to 3.28 mg ml⁻¹ (IC₅₀), 0.038 to 0.140 μ mol H₂O₂ destroyed g⁻¹min⁻¹ and 0.0008 to 0.0044 μ mol guaiacol oxidized g⁻¹min⁻¹. The observed activity of PAL, the key enzyme in phenyl propanoid pathway, was found in the range of 33.70-43.17 μ mol-cinnamate produced g⁻¹hr⁻¹ in fresh harvested sample tissues. On the basis of PCA and average values of all the parameters contributing to antioxidative property, 'Cherry round yellow' was selected as the most promising advanced breeding line followed by 'Cherry round red (big fruit)', '2016 Cherry 4' and '2016 Cherry 3', with better scavenging of ROS and can be used as improved materials for breeding of cherry tomato in future.

Keywords: Antioxidant activity, ascorbic acid, CAT, cherry tomato, phenol, POD and SOD

[Abbreviations: CAT: Catalase; DPPH:2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; LP: Lipid Peroxidation; PAL: Phenylalanine ammonia lyase; PCA: Principal component analysis; POD: Peroxidase; SOD: Superoxide dismutase; TP: Total phenol; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid]

Tomato (*Solanum lycopersicum*) is one of the most important staple vegetables cultivated widely across the world. India produced 18.73 million tons of tomatoes sharing 10.44% of the world production in 2016 (Anon., 2018). Tomatoes are enriched with important bioactive principles and attribute that help to designate tomatoes as an important member of the group of so called 'functional foods'. Tomato is consumed both cooked and raw as salad dressings and in various processed and preserved forms as well. Its lustrous, brilliant red colour of lycopene, an antioxidant carotenoid pigment principle, attracts consumers overwhelmingly, being an inherent instinct of them endowed by nature. The protective role of tomato carotenoids and polyphenols have been established well by Campbell *et al.* (2004) as important phytochemicals to be useful in the etiology of prostate cancer. In an overall sense, tomato usually possesses arsenal of protective compounds e.g., phenolics (phenolic acids and flavonoids), carotenoids (lycopene, α - and β -carotenes), vitamins (ascorbic acid and vitamin A) and glycoalkaloids (tomatine) that fight with stressful conditions leading to various cancers, cardiovascular and neurodegenerative diseases. Besides, the bioavailability

of tomato phytochemicals are usually unaffected by routine cooking processes (Chaudhary *et al.*, 2018). It is *esp.* the raw mode of consumption that additionally increases its importance to the researchers to investigate on the scope for its improvement in terms of possessing properties apart from providing nutrition.

Normal aerobic metabolism possesses a mechanism to detoxify the harmful chemical species called reactive oxygen species (ROS) that are constantly generated as an inevitable consequence. A situation termed 'oxidative stress' is arrived when such ROS including superoxide radical anion, hydrogen peroxide and various peroxy radicals, hydroxyl radical, singlet oxygen etc. are formed at an elevated level following exposure to extreme adversities of the environment. To fight with the situation, the adversity is recognized by the cell that consequently alters the expression of certain genes leading to triggering of biochemical pathways for biosynthesis of a group of important phytochemicals like carotenoids, ascorbic acid, phenolics and enzymes such as superoxide dismutases (SOD), catalases (CAT) and a family of peroxidases (POD) etc. specialized for defense function, from a housekeeping mode to production at higher levels.

*Email: amitava1963@yahoo.co.in

Despite being enormously important in possessing protective phytochemicals, an elaborate quantitative database on the phytochemicals and other protective constituents are still lacking for tomato, particularly under Indian context (Chandra and Ramalingam, 2011; George *et al.*, 2004; Kaur *et al.*, 2013). This information gap led the present investigator to undertake a screening programme involving eight advanced breeding lines of cherry tomato on the basis of their antioxidative properties with the following objectives aiming at selection of the best performer(s) with respect to ascertaining the important lipophilic and hydrophilic antioxidant constituent(s), and antioxidant activity under different assay systems along with measuring the activity of the key enzymes having direct and indirect bearings on antioxidative properties.

MATERIALS AND METHODS

The harvested fruit samples of the 8 selected advanced breeding lines of cherry tomato [2016 Cherry 1, 2016 Cherry 3, 2016 Cherry 4, 2016 Cherry 5, 2016 Cherry 6, Cherry round yellow, Cherry round red, Cherry round red (big fruit)] were collected in three replicates in an ice box at 'red ripe' stage available from the field maintained under AICRP on Vegetable Crops, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal at 'C Block' farm, BCKV (22°59'13"N, 88°27'17"E; 9.75m MSL), Kalyani. After collection, the representative fruit samples from each replicate were immediately washed with tap water and wiped off with a soft tissue. Then these were chopped with a sharp knife into small pieces and was analysed immediately for the content of antioxidant constituents *viz.*, lycopene, ascorbic acid (vitamin C) and total phenol, the enzymes (PAL, POD, CAT and SOD) related to the property of antioxidation as well as antioxidant activity under three different systems of assay (DPPH, FRAP and LP).

Analysis of antioxidant constituents

Lycopene from the finely chopped fresh cherry tomato fruit sample (1.0 g) was extracted with 2×10 ml portions of acetone using pestle and mortar or until the residue was colourless following centrifugation at 10,000g for 15 min. The supernatants were pooled, re-extracted with petroleum ether (40-60°C) in a separating funnel, washed with distilled water and collected through a bed of anhydrous sodium sulphate. The final extract was read at 503 nm using petroleum ether (40-60°C) as blank. The content of lycopene was calculated ($\lambda = 172 \text{ mM}^{-1}\text{cm}^{-1}$) and expressed as mg lycopene 100⁻¹g of fresh sample (Sadasivam and Manickam, 2011). Ascorbic acid was extracted with 4% oxalic acid by macerating the

finely chopped fresh fruit sample in a pestle and mortar and centrifuged at room temperature at 10,000 rpm for 30 min. The concentration of ascorbic acid in the supernatant was determined by reading absorbance at 518 nm of a reaction mixture containing 1 ml supernatant and 2 ml 1.72 mM 2,6-dichlorophenolindophenol (DCPIP) dye immediately after mixing (David and Masten, 1991). The content of ascorbic acid was expressed as μmole of ascorbic acid per gram of fresh sample.

Analysis of total phenol and antioxidant activity

Finely chopped fresh sample was macerated in a pestle and mortar with acidic (1.2 N HCl) aqueous methanol (1:1) and transferred quantitatively in a centrifuge tube. Thereafter, the contents of the tube were heated at 80-90° for 90 minutes and finally centrifuged at 10,000 rpm for 30 min. This extract was used for the estimation of total phenol and antioxidant activity (DPPH and FRAP). Total phenol content (free and bound phenolics) of the sample extract were estimated colorimetrically at 650 nm following Folin-Ciocalteu method (Gul *et al.*, 2011) and expressed as trolox equivalent (mg TE g⁻¹ fresh weight of sample), calculated from standard graph of trolox, a synthetic phenolic antioxidant.

The DPPH radical scavenging activity of extract was determined by measuring the decrease in absorbance of methanolic solution of DPPH at 517 nm (Braca *et al.*, 2001). The antioxidant activity of the sample extract in terms of scavenging DPPH radical was calculated and expressed as trolox equivalent (TE g⁻¹ FW of sample) from a standard curve prepared by plotting change in absorbance against different concentrations of trolox. The FRAP, based on the reduction of Fe (III) by the sample extract, was determined following the change in absorbance at 593 nm due to the formation of a blue coloured Fe(II)-tripyridyltriazine compound from colourless oxidized Fe(III) form in presence of a particular concentration of sample (Benzie and Strain, 1999). The results were expressed as mg TE g⁻¹ fresh weight (FW) of sample calculated from a standard curve prepared using trolox instead of sample extract. Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction following the methodology described by Liu *et al.* (2010) with some modifications. One g of fresh finely chopped sample was ground with 10 ml of 20% trichloroacetic acid (TCA) in a mortar and pestle followed by centrifugation at 10,000 rpm for 15 minutes. 1 ml of sample's aliquot, 3 ml of 20% TCA containing 0.5% TBA, and 0.2 ml of 4% butylated hydroxytoluene (BHT) were added and mixed

thoroughly and incubated at 95° for 30 minutes. After the mixture had cooled to room temperature the material was centrifuged at 10,000 rpm for 15 minutes and the absorbance of the supernatant read at 532 nm. Lipid peroxidation was calculated using the extinction coefficient of malondialdehyde ($\epsilon = 155 \text{ mM}^{-1}\text{cm}^{-1}$) and results expressed as mol MDA g^{-1} FW of the sample.

Antioxidative enzymes

For determination of activity of the enzymes under study (SOD, CAT, POD and PAL) sample was prepared according to the procedure of Nayar and Gupta (2006) with some modifications. Fresh finely chopped sample from each replicate was macerated with a 10 ml solution of extraction buffer prepared with 0.1M sodium phosphate buffer, pH 7.5 (0.1 M tris-HCl buffer, pH 8.8 used only for PAL) containing 2% polyvinyl pyrrolidone and 0.25% Triton-X detergent. The content was centrifuged at 10,000 rpm for 30 min and supernatant was collected for enzyme activity assay.

The modified method of Beauchamp and Fridovich (1971) was followed for the estimation of SOD activity. The assay was based on the capacity of the extract to inhibit photochemical reduction of nitro-blue-tetrazolium (NBT) in the riboflavin-light NBT system which was spectrophotometrically determined at 560 nm wavelength. SOD activity was calculated using the following equation: % inhibition = $[(A_0 - A_c)/A_0] \times 100$, where A_c was the absorbance of sample and A_0 was the absorbance of the control. The concentration of the extract producing 50% inhibition (IC_{50} value) was calculated from a plot of concentration of the extract vs. inhibition.

CAT activity was determined by monitoring the rate of disappearance of H_2O_2 at 240 nm ($\epsilon = 40\text{mM}^{-1}\text{cm}^{-1}$) following the protocol of Aebi *et al.* (1984). Enzyme activity was expressed as μmoles of hydrogen peroxide degraded $\text{min}^{-1}\text{g}^{-1}$ FW of sample.

Peroxidase activity, determined using the guaiacol oxidation method by Lin and Kao (2001). The reaction mixture contained 4% (v/v) guaiacol, 0.1 M sodium phosphate buffer (pH 7.5) and enzyme extract. The reaction was initiated by the addition of H_2O_2 (1%). Increase in absorbance at 470 nm was recorded using UV-visible spectrophotometer. The POD activity was calculated by using an absorption coefficient ($26.6 \text{ mM}^{-1}\text{cm}^{-1}$) at 470 nm for tetraguaiacol. Enzyme activity was expressed as μmoles of guaiacol oxidized/ min/gram fresh weight (Unit g^{-1} FW) of sample.

PAL activity was assayed by using a method modified from one of Godwin *et al.*, (1996). Enzyme extract was added to the reaction mixture comprised of 0.1M Tris-HCl buffer (pH 8.8), 10 mM L-phenylalanine (prepared

in 0.1M Tris-HCl buffer pH 8.8) to initiate the reaction and was incubated at 30°C for 15 min, then terminated by the addition of 6 M HCl following recording of absorbance at 260 nm. One unit represents the amount of enzyme that produces 1 μmol of *t*-cinnamic acid ($\epsilon = 9630 \text{ M}^{-1}\text{cm}^{-1}$) per hour and expressed as Unit g^{-1} FW of sample.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) for a randomized block design and means separated with Duncan's multiple range test (DMRT). Principal component analysis (PCA), as the method of identifying the factor dimensions of the data, was used to summarize the varietal information in a reduced number of factors for selection of the best performing line(s). Statistical analyses were done using SPSS professional statistics ver. 7.5 (SPSS Inc. USA).

RESULTS AND DISCUSSION

Lycopene content in eight advanced breeding lines of cherry tomato ranged from 1.89 to 3.31 $\text{mg } 100^{-1} \text{ g}$ with an average of 2.35 (Fig.1a). The range of values obtained showed significant variation across the different lines tested forming two groups significantly differing from each other with respect to lycopene content. '2016 Cherry4' and '2016 Cherry 5' having values without significant difference between them belonged to one group and the other group consisted of rest of the lines with absence of significant difference amongst their values. Rai *et al.* (2014), Vinha *et al.* (2014), Vin Kovic *et al.* (2011), Violeta *et al.* (2013), reported values almost around 50% higher than ours and values; around 100% higher values compared to our average have been found by Dinh (2012) followed by Garcia and Barrett (2006), Lenucci *et al.* (2006) and Islam *et al.* (2019) who reported values around 300% higher than our results. However, our results were in agreement with the results obtained by the Kumar *et al.* (2018) in tomato germplasm.

Ascorbic acid content of the advanced lines of cherry tomato ranged from 21.15 to 39.77 $\text{mg } 100^{-1} \text{ g}$ with an average of 28.96 $\text{mg } 100^{-1} \text{ g}$ fresh sample (Fig. 1b). The average value obtained in our experiment is around 20% less than what found by Rai *et al.* (2014), around 11% more than that observed by Toor *et al.* (2005) and was in agreement those of Raffo *et al.* (2006) whereas George *et al.* (2004) reported an average value being 62% less in comparison to ours in tomato germplasm. Thus, cherry tomato may be regarded as moderate source of ascorbic acid meeting around 40% of the recommended dietary allowance for ascorbic acid. All the breeding lines under the present study surpassed the

recommended value of at least 20mg/100g ascorbic acid for developing varieties of tomato by Gould (1992). Therefore, the harvested produce of all the breeding lines under present experiment will serve as good sources of ascorbic acid when consumed fresh as salad.

Total phenol content ranged from lowest in '2016 cherry 3' to highest in 'Cherry round yellow' with an average value of 9.033 mg TE 100⁻¹g of fresh samples (Fig. 1c). A significant variability among all the breeding lines were obtained and three lines *viz.*, 'Cherry round yellow', '2016 Cherry6', '2016 Cherry 4' possessed considerably higher values. Therefore, these lines may be investigated further with respect to their individual phenolic compounds that confer antioxidant property as a function of their structure. Our results are in agreement with the values obtained in absolute ethanol extract (Mostapha *et al.* 2014), in tomatoes under organic cultivation (Kaur and Kapoor, 2002; Zoran *et al.*, 2014), under refrigerated storage at 6°C (Vinha *et al.*, 2013).

The antioxidant activity under three different systems of assay (DPPH radical scavenging activity, FRAP and LP) against each of the eight advanced breeding lines have been presented in fig.-2.

DPPH radical scavenging activity (Fig. 2a), expressed in terms of mg TE g⁻¹ was found to vary ranging from 8.16 in '2016 Cherry 3' to 15.69 in 'Cherry round red' with an average of 12.29 indicating a wide range of variability amongst the lines where the total phenol extract of 'Cherry round red' is around 90% more effective than '2016 Cherry 3' in scavenging DPPH free radical *via* single electron transfer (SET) mechanism *in vitro*. Our data were slightly higher than that observed by Tommonaro *et al.* (2015) who found IC₅₀ mg ml⁻¹ values of 22.22 and 27.03 mg ml⁻¹ in Red cherry tomatoes and Yellow cherry tomatoes, respectively.

The results of the FRAP assay (Fig. 2b) showed ample variation among the advanced breeding lines of cherry tomato. FRAP value expressed in mg TE g⁻¹, ranged from 2.06 ('2016 Cherry 4') to 3.82 (Cherry round red) with an average of 3.09. The lines Cherry round red, 2016 Cherry 5, Cherry round yellow and 2016 Cherry1 could be considered to be on the higher side of the efficiency with respect to antioxidative reaction through single electron transfer (SET) mechanism. However, the line 2016 Cherry 4 and 2016 Cherry 6 etc. exerted lower efficiency *in vitro*. Our results are in agreement with the results obtained by Uthairatanakij *et al.* (2017).

Our breeding lines under experiment showed significant differences between the values of malondialdehyde produced which is measured under this assay as free radical mediated oxidative degradation

product of membrane lipids leaving to membrane disintegration. The value of lipid peroxidation assay (Fig. 2c) showed a very wide range in which '2016 cherry 4' and 'cherry round red' (big fruit) were more efficient as opposed by '2016 cherry3', '2016 cherry5' and '2016 cherry1'. The average value obtained was 34.83 μmol g⁻¹ of fresh tissue. It shows that lipid peroxidation activity was nearly 3 times less than the values obtained by Rosales *et al.* (2006).

The average activity of enzymes tested relating to antioxidative property of Cherry tomato breeding lines under the present investigation has been shown in Fig.- 3.

The SOD activity (Fig. 3a), expressed in IC₅₀ values (mg ml⁻¹ showed a genotypic variation ranging between 3.28 in '2016 Cherry6' and 2.11 in 'Cherry round yellow' indicating the least and most efficient lines, respectively for dismutating superoxide radical anions by 50%. The cherry tomato lines tested are 7 to 10 times more efficient than that reported by Rabinowitch *et al.* (1982) in tomato. However, there are reports (Gomez *et al.*, 2009; Rosales *et al.*, 2009) of around 10 and 20 times more efficient superoxide inhibition as compared to ours, in cold temperature storage treatments and different samplings of green house-grown cherry tomatoes respectively, while Jung *et al.* (2011) detected approximately 4 times higher 'SOD-like activity' in water extract of eggplant pulp tissue.

The CAT activity (Fig. 3b) expressed in μmol H₂O₂ destroyed g min, was highest in '2016 Cherry 4' showing value of 0.140 followed by '2016 Cherry 3' (0.098) while '2016 Cherry 6' (0.038) had the lowest. Our results were in agreement with Lokhandwala and Bora (2014) who reported values at different growth stages of tomato.

The POD activity (Fig. 3c) expressed in μmol guaiacol oxidized g⁻¹ min⁻¹ ranged from '2016 Cherry 4' with a value of 0.0008 unit to '2016 Cherry1' with 0.0044 showing a wide genotypic variation. Almost all advanced breeding lines significantly differed with respect to peroxidase activity forming four groups. One of the breeding lines '2016 Cherry 6' shows similar activity with the results obtained by Kumar *et al.* (2018). Both CAT and POD react to disproportionate H₂O₂, thereby exerting their antioxidative function in keeping the level of H₂O₂, a predominant ROS, under control. However, POD differs from CAT in that the former requires a second substrate that furnishes electrons for formation of water, being one of the products in both the cases. That both CAT and POD react in a mutually exclusive manner, was evident from the significant (at 5%) negative correlation (R = - 0.436) observed in the present experiment. This relationship was also reported earlier

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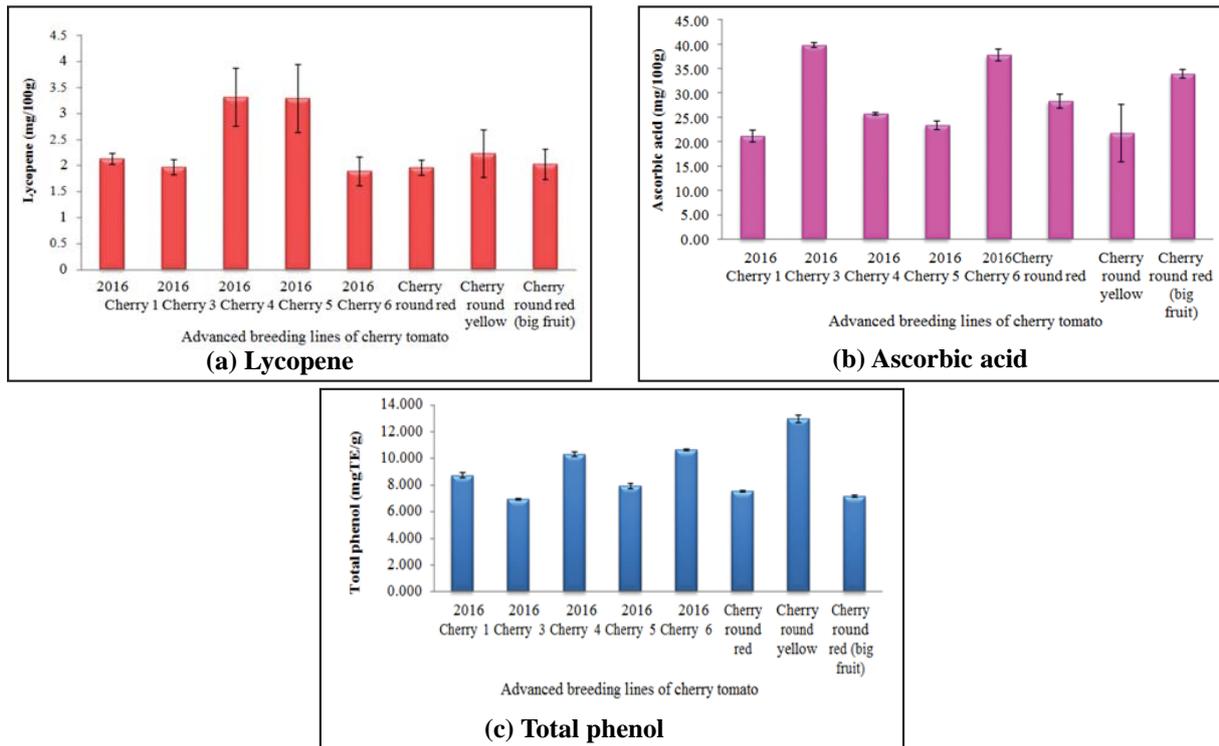


Fig. 1: Antioxidant constituents in cherry tomato fruits: (a) Lycopene (b) Ascorbic acid (c) Total phenol

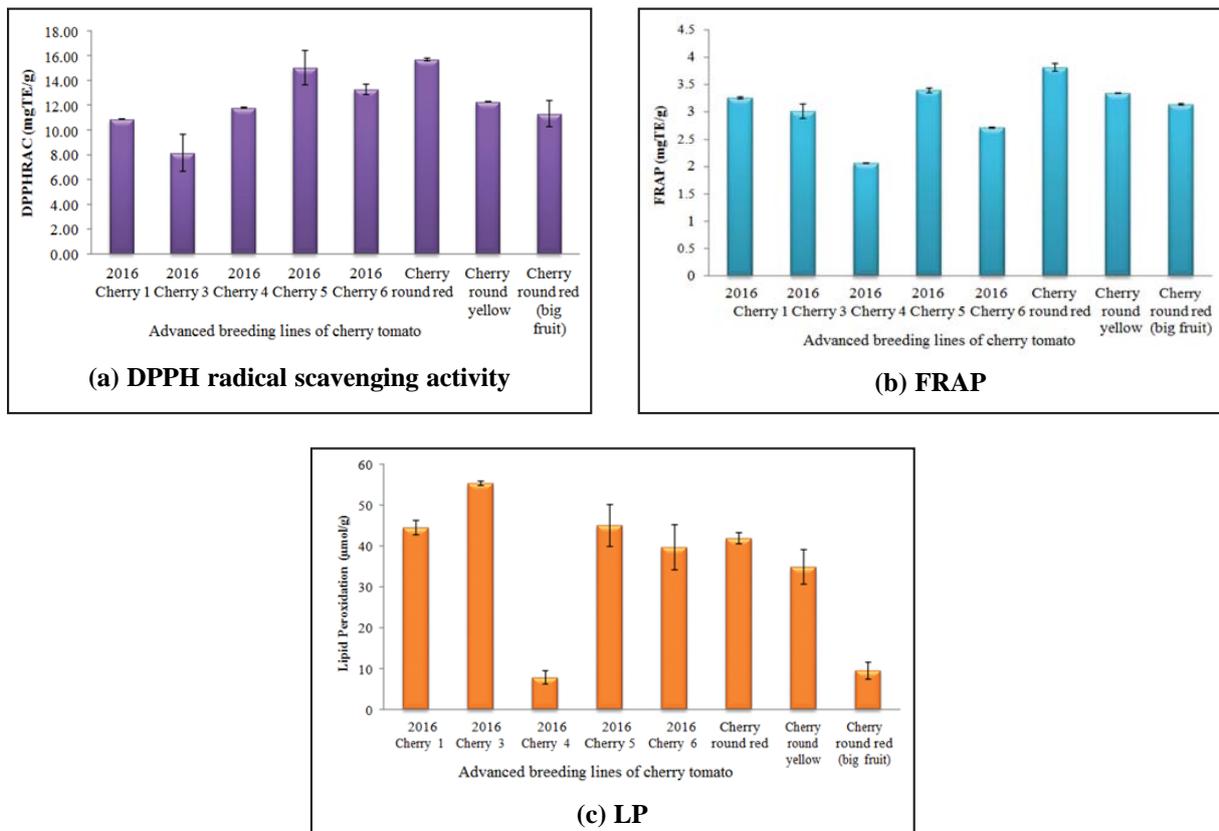


Fig. 2: Antioxidant activity of cherry tomato fruits under three different system of assay: (a) DPPH radical scavenging activity (b) FRAP (c) LP

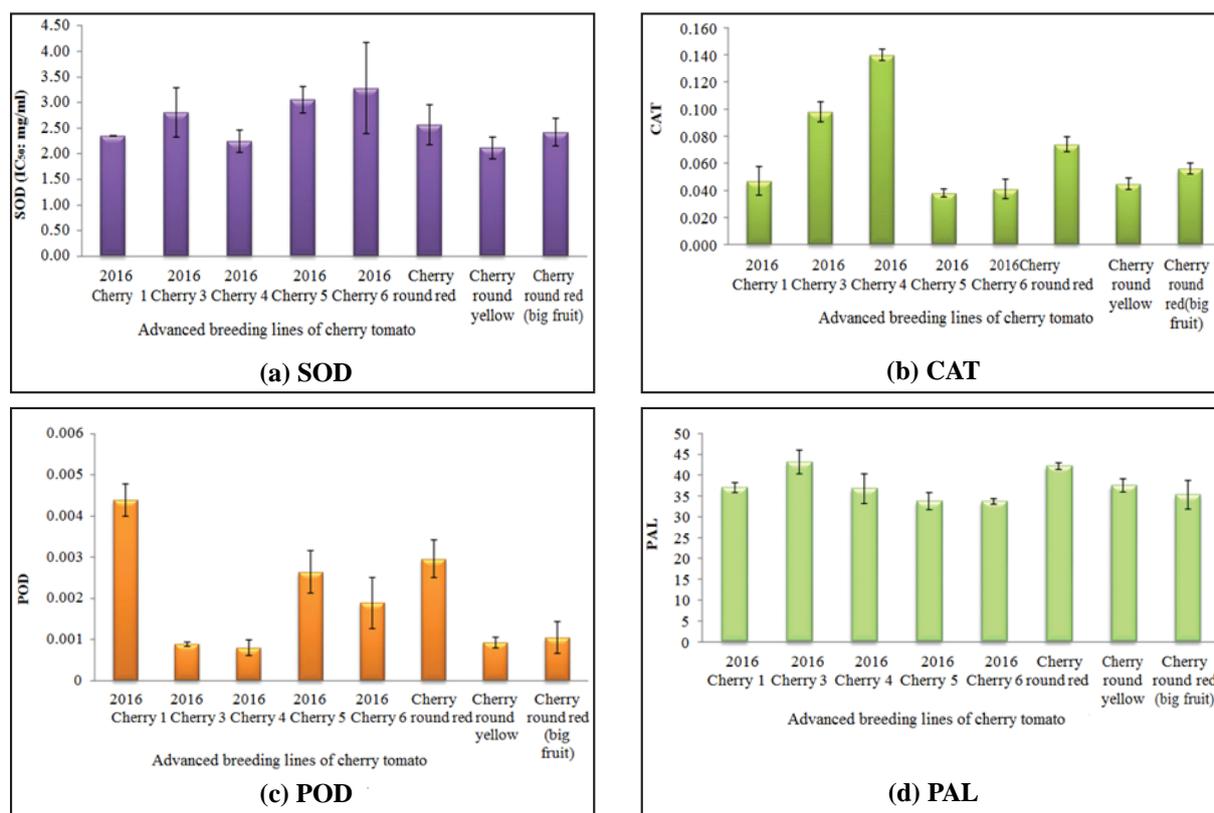


Fig. 3: Antioxidative property of cherry tomato (a) SOD (b) CAT (c) POD (d) PAL

by Braber (1980) in bean leaves. POD specific for ascorbate, therefore, might reduce the content of ascorbic acid and exert activity in an inverse relationship with it. This might have been the basis for obtaining the negative correlation ($R = -0.413$) between CAT and ascorbic acid at 5% level of significance in the present investigation, being similar to the earlier observation of Lamikanra and Watson (2001) in cantaloupe melon.

The PAL activity is one of the indications of the extent of diversion of carbon skeleton of aromatic amino acid phenylalanine from protein metabolism towards phenol metabolism through synthesis of phenyl propanoids followed by other related compounds. As phenols are the major and most predominant antioxidants in plants, the activity of the key enzyme of phenol metabolism corroborated with the content of phenolic constituents might lead to better ascertaining of the antioxidative property in any substrate. PAL activity (μmol of *t*-cinnamic acid produced g hr^{-1} ; Fig. 3d) of advanced breeding lines of cherry tomato showed significant variability with the highest activity in '2016 Cherry 6' (33.70) and the lowest in '2016 Cherry 3' (43.17) with an average value of 37.43. The values obtained by Zhou *et al.* (2012) in brinjal seem to be nearly 20 times of the values obtained in this experiment.

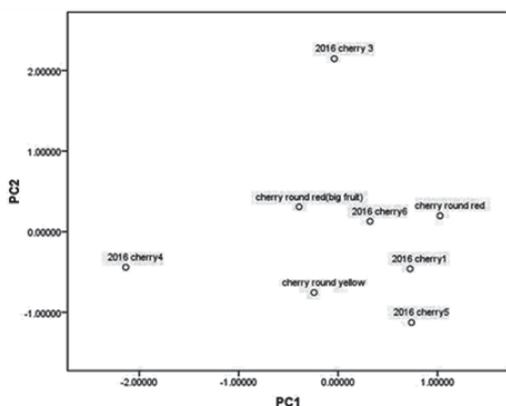
Principal component analysis

Principal component analysis (PCA) was used to obtain a simplified view of the relationship between variables and variable loadings for the components PC1, PC2, PC3 and PC4 were extracted (Table 1). These components were chosen because their eigenvalue exceeded 1.0 and explained 82.10% of total variance. The first component (PC1) explained 29.92% of total variance in which increase in the values of DPPH, FRAP, LP, SOD, POD, PAL is associated with decrease in values of lycopene, ascorbic acid, phenol and CAT. It is to be kept in mind that higher IC_{50} values of SOD imply lesser efficiency contributing to scavenging harmful ROS. Therefore, along the X-axis the lines Cherry round yellow, 2016 cherry6, Cherry round red (big fruit), 2016 Cherry3, 2016 Cherry4 can be selected as promising ones.

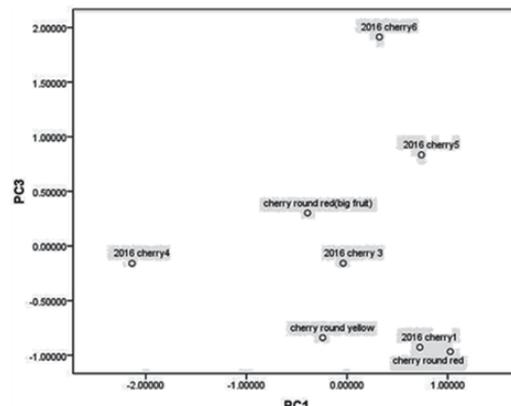
The second component (PC 2) explained additional 24.60% of total variance in which an increase in ascorbic acid, LP, SOD, CAT and PAL is associated with decrease in lycopene, phenol, DPPH, FRAP and POD. So, gradual increase in values along the Y-axis (Fig. 4a) leads to gradually increasing contributions of ascorbic acid, DPPH, LP, CAT and PAL along with gradually decreasing contributions of lycopene, phenol, FRAP,

Table 1: PCA for antioxidant content and activity relating to antioxidant property of cherry tomato

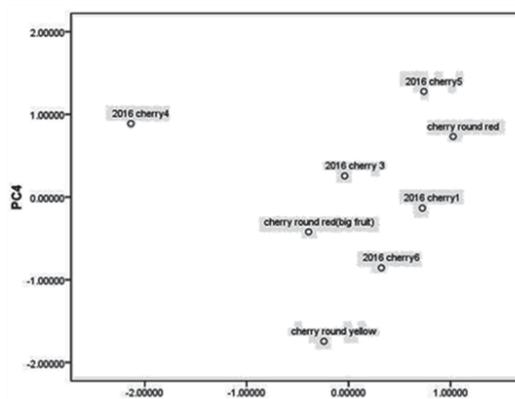
Principal component	Eigen value	%variance	% cumulative variance	
Eigen values and variance accounted for (%) by PCA based on correlation matrix				
PC1	2.992	29.920	29.920	
PC2	2.460	24.598	54.519	
PC3	1.612	16.123	70.642	
PC4	1.146	11.462	82.104	
Variables	PC1	PC2	PC3	PC4
Factor loadings due to PCs with eigen values greater than 1				
Lycopene	-.455	-.562	.095	.585
Ascorbic acid	-.025	.831	.516	-.073
Phenol	-.334	-.496	.010	-.632
DPPHRAC	.380	-.630	.156	.256
FRAP	.844	-.055	-.367	-.046
LP	.751	.284	-.037	.038
SOD	.444	.211	.812	.235
CAT	-.740	.352	-.254	.439
POD	.705	-.330	-.203	.244
PAL	.105	.671	-.642	.151



(a) PC1 vs. PC2



(b) PC1 vs. PC 3



(c) PC1 vs. PC4

Fig. 4: Scattergram of regression factor scores of all variables produced by PCs (a) PC1 vs. PC2 (b) PC1 vs. PC3 (c) PC1 vs. PC4

SOD and POD with respect to antioxidant property of cherry tomato. Therefore, along the Y-axis the promising lines that can be selected are: 2016 Cherry4, Cherry round red (big fruit), 2016 Cherry6, Cherry round red, Cherry round yellow and 2016 Cherry1.

Considering PC1 and PC2 together explaining 54.52% of total variance, the lines Cherry round yellow, 2016 Cherry6, Cherry round red (big fruit), 2016 Cherry4 are selected as being promising ones.

The third component (PC3) explaining another 16.12% of total accounted for variance where increase along Y-axis (Fig. 4b) is associated with increase mainly of ascorbic acid and SOD compromised with the prominent decrease in PAL and in FRAP, LP, CAT and POD in lesser extents. So, considering PC3 Cherry round red (big fruit), 2016 Cherry4, 2016 Cherry3, Cherry round yellow, 2016 Cherry 1, Cherry round red. Therefore, on the basis of PC1 and PC3 the lines Cherry round yellow, 2016 Cherry3, Cherry round red (big fruit), 2016 Cherry 4, turned out to be the promised ones. The fourth component (PC4) explaining another 11.46% of the total variance revealed that along Y-axis (Fig. 4c) increase in lycopene and CAT and also DPPH, SOD, POD and PAL to lesser degrees (Remembering increase in values of LP and SOD has a bad impact on antioxidant properties) is associated with considerable decrease in phenol content. Therefore, on the basis of PC1 and PC4 the promising lines under selection turned out to be Cherry round yellow, 2016 Cherry 6, 2016 Cherry 3, Cherry round red (big fruit).

Now, on the basis of all the components and considering the average values of all the antioxidant parameters investigated (see Appendix). The advanced breeding line Cherry round yellow was selected as the most promising performer followed by Cherry round red (big fruit), 2016 Cherry 4, 2016 Cherry 3 and 2016 Cherry 6 with respect to providing better antioxidant support to the consumers. These can also be utilised as improved breeding materials for developing more advanced breeding lines in future programmes.

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