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TOTAL ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENTS IN DIFFERENT TURKISH EGGPLANT (*SOLANUM MELONGENA L.*) CULTIVARS

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In this study, total water soluble antioxidant activity and phenolic content of 26 eggplant (**Solanum melongena L.*****) cultivars were investigated. Total water soluble antioxidant activity of the cultivars varied from 2664 to 8247 μmolTrolox/kg, which is a 3.1-fold difference. Cultivars also showed significant variation for total phenolic contents ranging from 615 to 1376 mg/kg, a 2.2-fold difference. The two traits were significantly correlated and results of this study suggested that breeders can use the information to develop eggplant cultivars with high antioxidant activity.***

Keywords: ***ABTS assay, Nutritional content, Phenolic compounds, Solanaceae, Water soluble antioxidant activity.***

INTRODUCTION

Many reports demonstrate that, in addition to basic nutrients, fruits and vegetables contain biologically important substances such as vitamins, minerals and antioxidant compounds that have beneficial effects on human health.[1,2] Plant-derived antioxidants and their potential to decrease the risk of diseases caused by oxidative stress are of particular interest. Oxidative stress results from the formation of an excessive amount of reactive oxygen species (ROS) and can lead to many disorders in humans including cancer, atherosclerosis, cardiovascular diseases and aging.[3,4,5] Oxygen can be converted to ROS during normal cellular metabolism and as a result of environmental effects such as UV, radiation, cigarette smoke and air pollutants. ROS, especially the hydroxyl radical ($HO^{\cdot}$), have high chemical reactivity and can immediately attack, oxidize and inhibit the normal function of biologically important molecules, such as DNA, proteins and lipids [3,6] thereby causing many degenerative processes in organisms.[5,7,8] Antioxidants protect organisms from O_2 toxicity by scavenging or quenching ROS or by breaking oxidizing chain reactions.[5,9]

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The human diet has an important role in protection against oxidative stress because many crucial antioxidants cannot be synthesized by the human body. Therefore, these antioxidants must be obtained through diet. There are several types of dietary antioxidants in plants including water-soluble antioxidants, such as vitamin C and phenolic compounds, and lipid-soluble antioxidants, such as carotenoids and vitamin E.[9] Although plants can be characterized for the content and activity of individual antioxidants, it may make more sense to consider the total antioxidant capacity of fruits and vegetables. This is because some antioxidants have synergy such that one of the antioxidants reduces a free radical and the other regenerates the antioxidant that was oxidized by the free radical. Vitamin E and vitamin C have this type of relationship.[9,10] Such synergies can only be taken into account when total antioxidant capacity is measured.

Phenolic compounds make a significant contribution to total water-soluble antioxidant activity, are the largest group of secondary metabolites produced by plants[9] and are common in fruits and vegetables. Flavonoids, lignin precursors and phenolic acids are the most important phenolic compounds.[11] The antioxidant activity of phenolic compounds depends on the number and position of their hydroxyl groups. Due to their structure, the hydroxyl groups of phenolic compounds easily donate their H^+ to ROS and reduce them.[9]

Solanum melongena L., commonly known as eggplant or aubergine, is an economically important vegetable crop with over 1.7 million ha produced worldwide.[12] Turkey ranks fourth in production of eggplant with 880,000 metric tons/year while China ranks first with 17,030,000 metric tons/year.[12] Eggplant originated in India and China and spread to tropical and temperate parts of the world. Eggplant fruits have great phenotypic variability. For example, they have a wide range of shapes (ovoid, globular, oblong, semi-long, long and serpentine), sizes (varies from a few grams to more than a kilo) and colors (green, white, violet, purple, striped, black or orange).[13] Eggplant is an important component of the human diet in many countries including Turkey. It can be used fresh, dried or preserved and can be cooked in many ways. As well as its use as a food, eggplant has been used in traditional medicine for the treatment of several human disorders such as asthma, bronchitis, diabetes and arthritis.[14] Recent studies also suggest that eggplant can modestly decrease blood cholesterol rates in humans.[14,15] Eggplant is a good source of dietary fiber, vitamins (vitamins B1 and B6), minerals (potassium, magnesium) and phytochemicals especially phenolic compounds.[16] Eggplant peel is a good source of anthocyanin, one of the most important flavonoids, which determines eggplant color.[17]

The main goal of this study was to measure total water-soluble antioxidant activity and total phenolic compounds contents of Turkish eggplant accessions. In this way it was possible to determine the genetic diversity of eggplant for antioxidant activity and phenolic content and also to determine the relationship between these two traits. Identification of eggplant accessions with high antioxidant activity will allow plant breeders to map the loci controlling this trait and to develop new cultivars that have higher antioxidant activity for improvement of human health.

MATERIAL AND METHODS

Plant Material

Seeds of the eggplant cultivars were obtained from the Aegean Agricultural Research Institute, Menemen, Izmir, Turkey and from Dr. Marie-Christine Daunay, INRA, Montfavet, France (only MM738). The cultivars fell into four groups based on

Table 1 List of eggplant cultivars used for antioxidant trait assays. Type of eggplant is also included.

Cultivar	TR accession	Type
Camlica	na*	Uzun
Giresun	TR55678	Uzun
Manisa Uzun	TR62668	Uzun
Kastamonu Uzun	TR37266	Uzun
TR66009	TR66009	Uzun
Zonguldak	TR68530	Uzun
Kutahya	TR66559	Uzun
Kemer-27	TR70633	Uzun
Isparta	TR66667	Uzun
Edirne Kirmizi	TR43306	Uzun
Bilecik Kemer	TR66017	Uzun
Usak	TR66572	Uzun
İzmir	TR50591	Uzun
İzmir	TR50592	Uzun
TR61892	TR61892	Uzun
MM738	na*	Beyli
Canakkale Kir	TR43010	Beyli
Gaziantep Mor	TR40300	Beyli
Burdur Yerli	TR66688	Beyli
Eskisehir Tombul	TR66012	Beyli
TR55976	TR55976	Beyli
Topan-374	TR70635	Topan
TR61986	TR61986	Topan
Trabzon	TR55995	Topan
Bursa Topan	TR66013	Topan
Rize	TR55811	Domates

*na: no accession number.

shape: Topan (broader than long), Uzun (long), Beyli (longer than broad), and Domates (tomato-shaped) (Table 1). Plants of the 25 Turkish and one foreign eggplant cultivar were grown in the field during summer 2006 with five replicate plants of each cultivar. Fruits were harvested at the normal market stage. After the fruits had been washed, they were stored at -20^oC until used for analysis. The eggplant cultivars were studied for total water soluble antioxidant activity and total phenolic content. All analyses were performed within one month after harvest.

For both assays, about three fruits of each sample were cut into pieces, well mixed and then approximately 100 g of fruit was homogenized with 200 ml cold distilled water at $+2^oC$. Eggplant homogenate was filtered through 4 layers of nylon cloth. For the antioxidant activity assay, 20 g of extract was used. For the phenolic compounds assay, 2.5 g of extract was diluted with 20 ml distilled water. These samples were centrifuged at 3000 $\times$ g for 10 min at $+2^oC$. After centrifugation, supernatants were collected and kept on ice until they were used for total water soluble antioxidant activity and total phenolic content measurements.

Total Water Soluble Antioxidant Activity

The total water soluble antioxidant activity of eggplant fruits was measured spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) using the ABTS

[2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] decolorization assay of Re et al.[18]. ABTS radical cation ($ABTS^{\cdot+}$) solution was prepared by mixing 7mM ABTS with 2.45 mM potassium persulfate and was stored in the dark for 12–16 hours. Before use, the $ABTS^{\cdot+}$ solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to adjust its absorbance to 0.700 (0.02) at 734 nm. Then 2.5, 5, and 7.5 μl aliquots of eggplant supernatant were mixed separately with 2 ml ABTS radical cation solution and decolorization of blue-green $ABTS^{\cdot+}$ solution was kinetically monitored at 734 nm for 6 min. Each assay was repeated to give three replicates for each aliquot volume. The results were calculated as the area under the curve (AUC) and expressed as μmol Trolox/kg fresh weight of eggplant fruits. To calculate AUC, the percent inhibition/concentration values for the extracts and Trolox were plotted separately against the test periods (1, 3, 6 min) and the ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

Total Phenolic Content

The total phenolic content of the eggplant extracts was spectrophotometrically measured according to the Folin-Ciocalteu procedure of Singleton and Rossi using Folin-Ciocalteu as reactive reagent and gallic acid as standard.[19] Homogenates were prepared as described in antioxidant activity determination and the clear supernatant was used for the determination of total phenolic contents. Briefly, 2 ml sample was mixed with 10 ml 2 N (10%) Folin-Ciocalteu and incubated for 3 min in dark, then 8 ml 0.7 M sodium carbonate was added. After 2 hours of incubation at room temperature in the dark, the absorbance of the reaction mixture was measured at 765 nm in spectrophotometer. There were three replicates for each sample. The results were expressed as mg gallic acid equivalents/kg fresh weight of eggplant.

Statistical Analysis

Analysis of variance (ANOVA) and Fishers PLSD were used for statistical analysis of the data. Significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, 25 Turkish and one foreign eggplant cultivar were characterized for total antioxidant activity and phenolic content (Table 2). Of these 26 lines, 25 were cultivars of *S. melongena* and one (Rize) was a cultivar of *S. aethiopicum*. *S. aethiopicum* is a domesticated eggplant species that is predominantly cultivated in Africa. Rize is used as a local variety in Turkey and is known as "red eggplant" because it is red instead of purple.

Total Water Soluble Antioxidant Activity in Eggplant

Total water soluble antioxidant activity of the eggplant cultivars ranged from 2664 μmolTrolox/kg to 8247 μmolTrolox/kg (Table 2). The highest antioxidant activity was seen in Camlıca, which had 1.4-fold higher activity than the cultivar with the next highest activity, Giresun, and 3.1-fold higher activity than the cultivar with lowest activity, MM738, a European breeding line. Mean antioxidant activity of the 26 cultivars was 4442 ± 243 (SE) μmol Trolox/kg. When the eggplants were grouped based on type, it was seen that all types had similar total antioxidant activities with no statistically

Table 2 Antioxidant activity and total phenolics content for the eggplant cultivars. Cultivars are ordered by total antioxidant activity. Values followed by different letters are significantly different at P < 0.05 as determined by Fishers PLSD.

Cultivar	TR accession	Antioxidant activity (µmolTrolox/kg) ± SE	Rank	Phenolics content (mg/kg) ± SE	Rank
Camlica	na*	8247.2 ± 2796 a	1	1375.9 ± 13 a	2
Giresun	TR55678	5918.7 ± 145.1 b	2	1255.6 ± 3.2 c	4
Eskisehir Tombul	TR66012	5709.4 ± 108.5 bc	3	1388.9 ± 16.1 a	1
TR55976	TR55976	5596.5 ± 132.9 bcd	4	877.8 ± 5.6 hi	14
Manisa Uzun	TR62668	5442.2 ± 125.1 bcd	5	1064.8 ± 6.7 e	9
Kastamonu Uzun	TR37266	5432.2 ± 62.3 bcd	6	1022.2 ± 22.5fg	11
Canakkale Kir	TR43010	5255.3 ± 40.9 cde	7	1050 ± 16.1 ef	10
TR66009	TR66009	5216.8 ± 220.3 cde	8	911.1 ± 6.4 h	13
Zonguldak	TR68530	5105.1 ± 53.9 def	9	1013 ± 7.4 g	12
Gaziantep Mor	TR40300	4850.9 ± 229.4 efg	10	1075.9 ± 10.3 e	8
Topan-374	TR70635	4671.6 ± 142.2 fgh	11	1338.9 ± 9.6 b	3
Burdur Yerli	TR66688	4407.5 ± 92.6 ghi	12	877.8 ± 17 hi	14
TR61986	TR61986	4313.2 ± 175.5 hi	13	803.7 ± 6.7 j	17
Kutahya	TR66559	4192.9 ± 89.3 hij	14	1338.9 ± 6.4 b	3
Kemer-27	TR70633	4014.1 ± 303.3 ijk	15	635.2 ± 13 l	21
Isparta	TR66667	4005.9 ± 144.8 ijk	16	792.6 ± 14.8 j	18
Trabzon	TR55995	3935.8 ± 367.7 ijkl	17	1227.8 ± 8.5 c	5
Edirne Kirmizi	TR43306	3724 ± 122.5 jklm	18	846.3 ± 4.9 i	16
Bilecik Kemer	TR66017	3570.9 ± 260.3 klmn	19	650 ± 14.7 kl	20
Usak	TR66572	3489.2 ± 221.9 lmno	20	675.9 ± 1.9 k	19
İzmir	TR50591	3417.6 ± 46 mno	21	1127.8 ± 14 d	7
İzmir	TR50592	3337.4 ± 198.2 no	22	911.1 ± 11.6 h	13
Bursa Topan	TR66013	3296.9 ± 63.1 no	23	850 ± 14.7 i	15
TR61892	TR61892	3003.5 ± 197.2 op	24	1155.6 ± 29.4 d	6
Rize	TR55811	2690.1 ± 280.9 p	25	911.1 ± 16.1 h	13
MM738	na*	2664 ± 60.7 p	26	614.8 ± 9.8 l	22

*na: no accession number.

Table 3 Mean values for antioxidant traits for eggplant cultivars grouped by type. The domates-type was not included as it was represented by only one cultivar. Within each column, values followed by a different letter are significantly different at P < 0.05 as determined by Fishers PLSD.

Eggplant type	Number cultivars	Mean antioxidant activity (µmol Trolox/kg) ± SE	Mean phenolics content (mg/kg) ± SE
Uzun	15	4541.2 ± 204.1 a	985.1 ± 35.1 a
Beyli	6	4747.3 ± 253.6 a	980.9 ± 57.5 a
Topan	4	4054.4 ± 179.3 a	1055.1 ± 70.1 a

significant differences between the means for each type (Table 3). But within these groups there was statistically significant variation. Uzun and Beyli types showed the most variation for total antioxidant activity with 2.7 and 2.1-fold differences between the cultivars with the highest and lowest values, respectively (Figure 1). Topan types had only 1.4-fold variation between the cultivars with the highest and lowest values for total antioxidant activity.

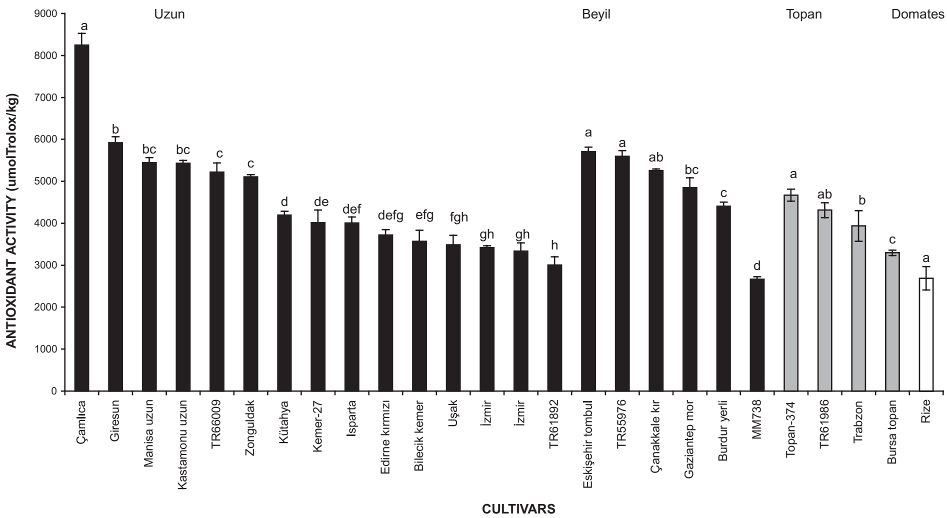

Figure 1 Antioxidant activities of the eggplant cultivars grouped by type. Within each type, columns labeled with different letters are significantly different at P < 0.05 as determined by Fishers PLSD.

Total Phenolic Content

Total phenolic content for the eggplant cultivars ranged from 615 mg/kg in MM738 to 1389 mg/kg in Eskisehir Tombul, a 2.3-fold range in content. A similar level of diversity in phenolic contents was seen by Hanson et al. [20] who examined this trait in 35 eggplant accessions from different countries. The highest phenolic content was seen in Eskisehir Tombul while MM738 had the lowest content (Table 2). Mean phenolic contents for all cultivars was 992 ± 46 (SE) mg/kg. When grouped by type, all types had similar mean phenolic content with no statistically significant differences (Table 3). All types also showed similar variation for total phenolic content with 1.7 to 2.3-fold differences in values (Figure 2).

Correlation Between Antioxidant Traits

Statistical analysis of eggplant data revealed that total water soluble antioxidant activity and phenolic content were significantly correlated (P < 0.05) for the eggplant cultivars (r = 0.52). Thus, the three cultivars that ranked highest for antioxidant activity (Camlica, Giresun and Eskisehir Tombul) also ranked within the top four for phenolic content (Table 2). Because phenolic compounds are some of the most important water-soluble antioxidants and can be present at high concentrations in plants, the correlation between these two traits was expected. Similar positive correlations between total antioxidant activity and phenolic content were also reported by Hanson et al.[20] in eggplant and have been observed in other fruits and vegetables.[21–25]

Eggplant as a Functional Food

There is worldwide interest in "functional food," foods (especially fruits and vegetables) that are not only nutritious, but also have compounds, such as antioxidants, that positively affect human health. Therefore, many studies have screened the antioxidant

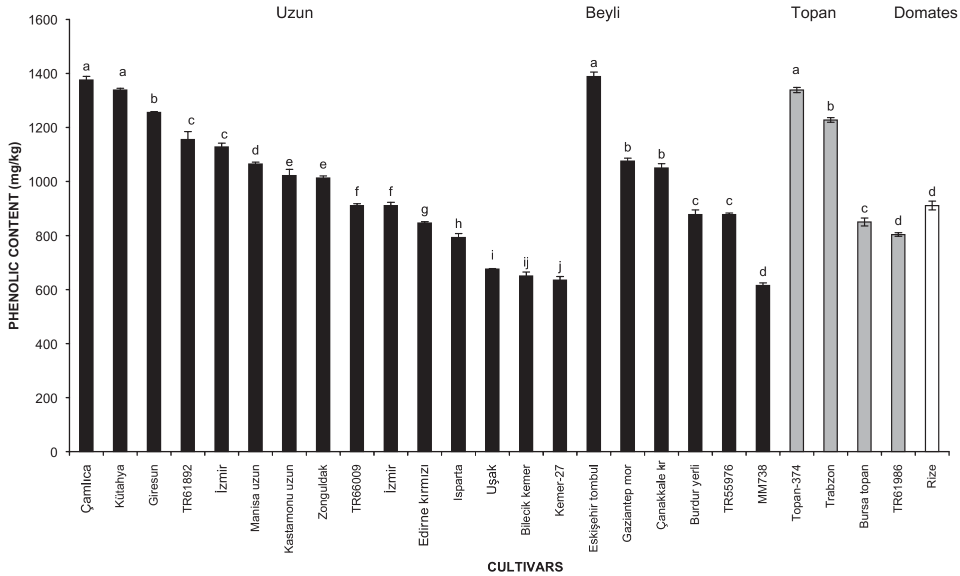

Figure 2 Total phenolic content of the eggplant cultivars grouped by type. Within each type, columns labeled with different letters are significantly different at $P < 0.05$ as determined by Fishers PLSD.

content of various crops.[21,26–31] As compared to other members of the plant family *Solanaceae* (such as pepper and tomato), there are very few studies about the antioxidant activity of eggplant. Two notable exceptions are the work of Stommel and Whitaker[17] and Hanson et al.[20] Stommel and Whitaker studied the composition of phenolic compounds in 115 eggplant accessions. They identified 14 different compounds which they classified into five groups based on their structures. Hanson et al.[20] evaluated 35 eggplant accessions for total phenolic and vitamin C contents and superoxide scavenging activity. Many different factors affect the level of antioxidant compounds in the plant. These factors include environment, genotype (cultivar) and storage conditions. Moreover, there are many valid methods of sample extraction and determination of total antioxidant activity. For these reasons, direct comparisons between results of different studies is difficult or, in some cases, impossible. However, the previous work and the current study indicate that significant diversity for antioxidant traits is present in eggplant.

The cultivar Camlica was found to have the highest antioxidant activity, followed by Giresun and Eskisehir Tombul, whereas Eskisehir Tombul has the highest phenolic, content followed by Camlica and Topan-374. Due to the positive effects of these compounds on human health,[1,2,9,32] the consumption of these three cultivars can be strongly advised. These cultivars may also be used as parents in breeding of new varieties with better functional food properties: higher antioxidant and phenolic contents. Because both of these traits are controlled by more than one gene, improvement of them will be difficult. First, the genes of interest must be mapped. For this, a suitable mapping population must be developed using parents with high variation for the desired traits. Camlica and MM738 seem to be appropriate parents for mapping of both total antioxidant activity and phenolic content, however, intraspecific crosses in *S. melongena* have very low genotypic polymorphism (variation).[33,34] Interspecific populations show higher levels of DNA polymorphism, therefore, a cross between Camlica (*S. melongena*) and Rize (*S. aethiopicum*) would be more appropriate for trait mapping. Molecular genetic mapping and identification of the most important loci controlling antioxidant activity and phenolic contents will then allow the use of marker-assisted selection for improvement of these traits in eggplant.

CONCLUSION

Our results suggest that there is significant diversity in Turkish eggplants for total water soluble antioxidant activity and total phenolic content. The work also allowed identification of eggplant cultivars with high antioxidant content that can be recommended for consumption or used as starting material for the improvement of eggplant antioxidant content by breeding.

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