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Changes in Total Antioxidant Capacity of Sesame (*Sesamum* sp.) by Variety

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Total antioxidant capacity in relation to variety, species, morphological attributes and seed coat colour was investigated using 22 diverse *Sesamum* genotypes from 16 different countries. The highest total antioxidant capacity content was determined in the genotype with non-shattering capsule with a value of 24.5 µmol/g dm. This genotype was followed by 265513 with purple stem, branches and capsules. The lowest total antioxidant capacity content was observed 250946 with strong hairy stem, branches, leaves and capsule with a value of 2.6 µmol/g dm. *Sesamum radiatum* and *Sesamum prostratum* had low level of antioxidant capacity while *Sesamum* sp from Morocco had a high value of total antioxidant capacity. The relation between total antioxidant capacity content and seed colour was also investigated but no correlation was found. Instead of seed coat colour, the other properties of the accessions such as morphological attributes and regional distribution played an important role for obtaining high and low total antioxidant capacity content.

Key Words: Sesame, *Sesamum* sp., Total antioxidant capacity, Seed colour, Morphological characters, Variety.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important source of edible oil, protein and lignans. The oil has also wide medical and pharmaceutical uses. It has been used for healing burns, treating skin diseases and baldness for centuries as a folk medicine. Since sesame is primarily an oil and confectionery crop, no priority has been given to protein research[1]. However, in the last decade, sesame has been recognized as a source of unique phenylpropanoid lignans having several important implications such as antioxidative, chemopreventive, anticarcinogenic, antifungal and antibacterial properties. The research on natural antioxidants of sesame is therefore an area of interest in recent years.

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Sesame is now very attractive crop as a source of natural antioxidants, sesamin and sesamolin and their derivatives such as sesamol and sesaminol[2]. The remarkable stability and long shelf life of sesame oil are to be due to the presence of these antioxidants[3-5] although it is highly unsaturated[6-8]. These compounds are not found in other edible oils[9] and make sesame oil more resistant to oxidative rancidity and more stable than most vegetable oils[4,10].

The addition of sesame oil or these antioxidants is therefore a useful method for increasing shelf-life of lipids and lipid containing foods[11,12]. Because of this high quality oil, sesame is regarded as the queen of the oilseed crops[13].

Sesame is clearly a health-benefit crop. The studies showed that sesame has a positive influence on lowering of blood plasma cholesterol concentration in human being[14,15]. The major lignan of sesame seed, sesamin has a stimulation effect of ethanol metabolism in human and prevention of ethanol-induced fatty liver in rats[16]. Dietary sesame seeds elevate α-tocopherol in rats[17] and γ-tocopherol concentration in humans[18,19]. Sesame has also antimicrobial effects on plant and human pathogens[20] as well as chemopreventive[21,22]. Lignans and lignan glycosides, present in sesame seed, oil and cake are responsible for the important properties of sesame[5]. It is now more important issue to determine the variability of antioxidant capacity of sesame since the quantity of the health promoting component of sesame has not been investigated with regard to variety, species, seed colour and different morphologic attributes. The information about the variability of sesame antioxidant capacity is crucial for enhancing the level of these compounds in varieties by breeding programs. In the present study, our objective is to investigate the antioxidant capacity of sesame varieties and wild types with different morphologic characteristic and seed colour.

EXPERIMENTAL

The sample of sesame seeds (*Sesamum* sp.) used in this work was kindly provided by USDA, Plant Genetic Resources Conservation Unit and they all were grown in Turkey during the vegetation period of 2007. The genotypes were collections from 16 diverse countries. The material was of great variation for morphological traits including minute capsule, purple stem and capsule, hairiness, one or three capsules per leaf axil, wild types, non-shattering capsule as well as a wide spectrum of seed colours. The *S. radiatum* genotype from India, 275362 was of two different types of seed colour as dull black and light brown seed. This genotype was divided into two classes based on their seed colours and used for analyses as two subsets. The detailed information about the accessions is presented in Table-1.

Colour measuring: The L, a and b values of the whole sesame seeds' colours were analyzed by CIELAB system using a chromameter, model CR-400 (Konica Minolta, Osaka Japan) equipped with measuring head and DP-400 data processor. The L value represents the light-dark spectrum with a range of 0 (black) to 100 (white), the 'a' value represents the green-red spectrum with a range of -60 (green) to +60 (red) while the 'b' value represents the blue-yellow spectrum with a range of -60 (blue) to +60 (yellow). The chromameter was standardized with white ceramic

TABLE-1
LIST OF SESAME SAMPLES AND THEIR CHARACTERISTICS

Genotype	Local name	Species	Origin	Characteristic
211088	-	*S. indicum*	Afghanistan	Light brown seed
158921	-	*S. indicum*	China	Dull black seed
162563	-	*S. indicum*	China	Light brown seed, minute capsule
298629	-	*S. indicum*	Egypt	White seed
238989	M4363	*S. indicum*	Greece	Beige seed
156999	-	*S. prostratum*	India	Tan seed, wild type
275362	1098	*S. radiatum*	India a	Dull black seed, wild type
275362	1098	*S. radiatum*	India b	Light brown seed, wild type
222266	Konjet	*S. indicum*	Iran	Dark brown seed, purple capsule and stem
250946	K1640	*S. indicum*	Iran	Bright black seed, hairy stem, capsule and leaf
179485	9851	*S. indicum*	Iraq	Light brown seed, dense and three capsules per leaf axil
298630	Giza 24	*S. indicum*	Israel	Grey seed
612926	8	*Sesamum sp.*	Morocco	Tan seed, wild type
433891	Pb Til No. 1	*S. indicum*	Nigeria	Cream seed
292144	P 18-203	*S. indicum*	Pakistan	Dark brown seed
263469	3QO-5	*S. indicum*	Russia	White seed, multicarpel
265513	Morado	*S. indicum*	Russia	Grey seed, purple capsule and stem
490045	Kurte	*S. indicum*	S. Korea	Cream seed, dense capsule
200113	12383	*S. indicum*	Sri Lanka	Dull black seed
Muganli-57	Muganli	*S. indicum*	Turkey	Tan seed, well adapted variety
254709	Japan251	*S. indicum*	USA	White seed, long capsule
599442	Renner combine	*S. indicum*	USA	Tan seed, non-shattering capsule

calibration tile. Ten independent measurements were done for each sample. The colour differences were calculated by using the following equation: $\Delta E = (\Delta a^2 + \Delta b^2 + \Delta L^2)^{0.5}$, where ΔL, Δa and Δb are differences in colour values between the tile and seeds[23].

Determination of dry matter and thousand-grain weight: The dry matter was determined by drying the samples at 105 °C to a constant weight[24]. The thousand-grain weight of sesame seeds was determined by weighing.

Preparation of crude extracts of sesame: The seeds (≈ 0.5 g) were weighed in a tube (PPCO, Nalgene, USA) and homogenized (2000 rpm, 1 min) in 5 mL hexane with an ultraturrax (IKA Labortechnik, Germany), shaked in a shaker (Nuve, Turkey) at 100 rev./min at room temperature for 0.5 h, centrifuged (3K30, Sigma, Germany) at 5000g at 35 °C for 5 min and then discarded supernatant. This process was also repeated once for defeating. The defatted sample was dried at 50 °C. The defatted and dried sample was homogenized (2000 rpm, 1 min) in 5 mL ethanol (80 %), shaked at 100 rev./min at room temperature for 0.5 h, centrifuged at 5000 g at 35 °C

for 5 min and then collected supernatant in a beaker. This process was also repeated twice for extracting antioxidant compounds from the solid phase. The collected supernatant in the beaker was stored (12-16 h) at 4 °C until analyzed.

Determination of total antioxidant capacity by Trolox equivalent antioxidant capacity: Total antioxidant capacity (TAC) was determined from reducing 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) according to trolox equivalent antioxidant capacity (TEAC) assay described by Re *et al.*[25]. ABTS radical was generated by oxidation of 7 mM ABTS solution with 2.45 mM potassium per sulfate. The calibration curve was prepared from standard trolox in 80 % ethanol by adding 5, 10, 15 and 20 µL into 1 mL $ABTS^{\bullet+}$ radical solution and then 2.5, 5, 7.5 and 10 µM final trolox concentrations were obtained. The decreases of absorbance of mixtures were read at 734 nm 1 min intervals with a spectrophotometer (Shimadzu UV-160, Japan) during 6 min. The crude extracts (5, 10, 15 and 20 µL) were added into 1 mL $ABTS^{\bullet+}$ radical solution and the decrease of absorbance of sample was read at same condition with standard trolox. Inhibition rate was calculated from decreasing absorbance from 0 to 6 min and plotted as a function of the volume of the sample extracts and trolox solutions. The result was calculated from the rate which is proportional to the slope of sample curve against the slope of calibration curve. Appropriate solvent blanks were run in each assay and all readings were carried out three times. The results were expressed as µM TEAC/g of dry solid (dm).

Statistical analysis: All the experiments were replicated twice and analyses were done with parallels using a completely randomized design. The significance of differences among means were determined at $p < 0.05$ using one way ANOVA followed by LSD multiple range test.

RESULTS AND DISCUSSION

Total antioxidant capacity of 22 different sesame genotypes from 16 diverse countries, expressed as Trolox equivalents, is presented in Table-2. The levels of TAC in 22 sesame seeds varied considerably. Total antioxidant capacity of the sesame genotypes ranged from 2.6 to 24.5 µmol/g dry matter. 599442 from USA and 250946 from Iran contained the highest and the lowest levels of antioxidant capacity, respectively (Table-2). The genotype, 265513 from Russia with purple capsule and stem but grey seed characteristics had the second highest total antioxidant capacity with a value of 19.4. This genotype was followed by 158921 from China and 612926 from Morocco as wild type, respectively. Apart from 612926, the other wild types of sesame, *S. radiatum* and *S. prostratum* had generally low level of antioxidant capacity (Table-2).

The genotype with non-shattering capsule originating from USA, 599442 had the highest total antioxidant capacity. Instead of seed coat colour, the plant characteristics played an important role for obtaining the high level of total antioxidant capacity. The non-shattering genotypes show deleterious characteristics such as

TABLE-2
ANTIOXIDANT CAPACITY, COLOUR VALUES AND 1000 SEED WEIGHT OF DIVERSE *Sesamum* GENOTYPES

Genotype	Origin	1000 Seed weight (g)	Colour (L)	Colour (a)	Colour (b)	ΔE	Dry matter (%)	TAC (μmol/g dm)
211088	Afghanistan	3.1	35.7	6.1	7.4	61.8	95.0	5.2
158921	China	2.3	29.0	0.3	0.5	68.0	94.8	19.3
162563	China	1.8	46.4	7.0	12.2	52.1	94.3	18.3
298629	Egypt	3.8	54.9	3.9	13.8	43.9	95.1	16.4
238989	Greece	3.1	52.3	5.2	14.4	46.7	94.7	15.8
156999	India	1.9	47.7	7.5	13.1	51.1	95.2	9.8
275362	India a	1.6	28.3	1.5	1.6	68.7	94.3	7.4
275362	India b	1.3	40.4	6.6	9.8	57.4	94.3	7.8
222266	Iran	2.5	35.6	5.4	7.4	61.9	95.1	6.4
250946	Iran	2.6	31.9	3.3	4.3	65.1	95.1	2.6
179485	Iraq	1.5	44.1	6.1	11.6	54.1	94.1	16.3
298630	Israel	3.3	49.2	6.3	13.6	49.5	94.5	7.4
612926	Morocco	2.3	45.9	8.8	15.3	53.5	93.8	18.9
433891	Nigeria	2.8	55.2	4.0	12.9	43.3	94.9	13.1
292144	Pakistan	3.2	31.4	2.1	3.5	65.6	94.6	17.1
263469	Russia	1.5	53.7	4.3	13.0	44.8	94.7	17.7
265513	Russia	2.1	50.0	4.4	11.6	48.2	94.4	19.4
490045	S. Korea	1.8	49.1	5.5	12.0	49.2	94.7	9.3
200113	Sri Lanka	2.2	27.7	2.2	1.7	69.2	94.9	7.4
Muganli	Turkey	4.0	49.4	6.4	13.3	49.3	95.1	8.7
254709	USA	2.1	55.6	4.3	12.4	42.8	94.9	7.1
599442	USA	1.4	44.6	7.1	13.0	54.0	93.4	24.5
LSD (0.05)		0.3**	0.6**	0.3**	0.3**	0.6**	0.6**	5.2**

TAC = Total antioxidant capacity

semi-sterility, twisted stems, cupped leaves, short capsules and low yield although it has a great potential to make sesame a modern crop by enabling mechanized harvesting[6,26,27]. The stress conditions due to its genetic structure may cause to produce anthocyanin compounds in non-shattering genotypes. Moazzami and Kamal-Eldin[28] had similar result that the grand mean of total lignan content in non-shattering genotypes was higher than those of shattering capsule ones. Fazeli *et al.*[29] also found that drought stress increased antioxidant enzymes and activities in sesame.

The second highest total antioxidant capacity content was observed in 265513 from Russia with purple capsule and stem (Table-2). To our best of knowledge, this colour structure in sesame was not reported up to date. Interestingly, the plant had purple stem, branches and capsules while the colour of its seed was grey. It is well known that purple colour is one of the most constituent to result in high level of antioxidant capacity. As expected, this genotype showed a high level of antioxidant capacity following to non-shattering genotype originating from USA.

The lowest value for total antioxidant capacity was obtained by 250946 originating from Iran. This accession had bright black seed colour. Most importantly, the plants had strong hairiness in main stem, leaves, branches and capsules. It is highly difficult to determine the relationship between hairiness and total antioxidant capacity but it seemed that hairiness was negatively affected to total antioxidant capacity in this genotype even though the seed colour of the accession was black. Based on the results of Shahidi *et al.*[30] black sesame seeds should have given more antioxidant capacity. However, the colour of seeds alone is not a determiner about the high level of total antioxidant capacity content. The morphological attributes rather than seed colour should take into consideration in sesame.

In literature, there are many studies available regarding to antioxidant capacity or lignan content of sesame. The antioxidant capacity or lignan content were associated with seed colour in most of the studies. Shahidi *et al.*[30] used two sesame varieties including white and black sesame seed and they reported that black sesame seed had higher antioxidant capacity than white sesame seed. On the other hand, Moazzami and Kamal-Eldin[28] found that the sesamin and sesamolin contents in seeds were not different black and white seeds. In addition, several varieties with different seed colour were tested for lignan content and there were no correlation found between seed colour and lignan content in sesame[31,32]. The morphological attributions and regional distribution of the varieties should take into consideration to explain the levels of total antioxidant capacity.

The L (blackness and whiteness), a (greenness), b (yellowness) and ΔE (colour difference) values are presented in Table-2. Significant differences ($p < 0.05$) in colour parameters were found in seed coat among most accessions. However, there is no linear correlation found between colour parameters and total antioxidant capacity. This result also supports that not only seed colour but also morphological attributes are an important component for affecting total antioxidant capacity in sesame.

Conclusion

Very diverse sesame accessions in worldwide and different morphological attributions such as non-shattering, purple plant, hairiness, minute capsule, wild types and wide range of seed colour were investigated for total antioxidant capacity in relation to seed coat colour and morphological characteristics. There was no absolute relationship between seed colour and total antioxidant capacity since the other factors, such as morphological structure of the crop and regional distribution masked the seed colour effect on total antioxidant capacity. It is therefore morphological structure of the crop had also an important implication on determining the level of antioxidant capacity. The results obtained in this study indicated that the genotypes with non-shattering capsule and purple plant have a great potential in plant breeding programs by enabling high level of antioxidant capacity other than having unique morphological attributions.

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