Agric Res J ***62*** *(3): 373-384, June 2025*
DOI: 10.5958/2395-146X.2025.00045.6

BIOCHEMICAL ASSESSMENT OF OAT (*Avena sativa* L.) GERMPLASM FOR HIGH ANTIOXIDANT POTENTIAL

Sukhdeep Kaur[1], Rachana D Bhardwaj[1*], Rahul Kapoor[2] and Satvir K Grewal[1]

[1]*Department of Biochemistry, Punjab Agricultural University, Ludhiana-141 004, Punjab, India*

[2]*Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004, Punjab, India*

Free radicals are highly reactive, short-lived, and chemically unstable atoms or molecules with unpaired valence electrons. They accumulate as a result of an imbalance between the production and elimination of reactive oxygen species (ROS) (Mansoor *et al.*, 2022). Although the human body possesses a natural defense system to counteract these reactive species, dietary antioxidants also play a crucial role in reinforcing this defense (Gangopadhyay *et al.*, 2015). Antioxidants are molecules stable enough to donate an electron to neutralize free radicals, thereby mitigating their damaging effects. By neutralizing these toxic by-products of normal cellular metabolism, antioxidants help protect cellular structures and functions (Ahmadinejad *et al.*, 2017). Several synthetic antioxidants, such as 2,3-tert-butyl-4-methoxyphenol (BHA), 2,6-di-tert-butyl-4-methylphenol (BHT), and tert-butylhydroquinone (TBHQ), have been widely used in food products. However, concerns over their potential carcinogenicity and other adverse health effects have led to a decline in their usage across the food, cosmetics, and pharmaceutical industries (Murakami *et al.*, 2015). Consequently, there has been growing interest in identifying natural antioxidants that can safeguard the human body from oxidative damage and slow the progression of chronic diseases. Debnath *et al.* (2019) demonstrated that oat bran extracts possess exceptionally high antioxidant potential and could be effectively utilized in the development of functional foods.

The nutritional benefits of oats have garnered significant attention from researchers worldwide, and the food industry has increasingly recognized their value as a functional ingredient in a wide range of products, including infant foods. Oats are rich in immune-boosting nutrients (Chen *et al.*, 2021) and contain various bioactive compounds with health-promoting properties. These include β-glucan and antioxidants such as tocopherols, alkylresorcinols, phenolic acids and their derivatives, as well as avenanthramides - unique to oats and not found in other cereal grains (Kaur *et al.*, 2019). Tocols, a class of compounds that includes tocopherols and tocotrienols, consist of a polar chromanol ring attached to an isoprenoid-derived hydrocarbon chain. The difference between tocopherols and tocotrienols lies in the saturation state of the isoprenoid side chain (Fu *et al.*, 2017). Vitamin E, a member of the tocol family, is a potent lipid-soluble antioxidant that neutralizes peroxyl radicals, thereby preventing the propagation of lipid peroxidation in cell membranes (Rychter *et al.*, 2022). Similarly, vitamin C is a crucial water-soluble dietary antioxidant that reduces free radical-mediated cellular damage by donating two electrons, which protects other compounds from oxidation. It is absorbed in the intestine through active transport mechanisms (Putchala *et al.*, 2013). Oats also contain a diverse array of phenolic acids-including p-coumaric, ferulic, cinnamic, syringic, vanillic, 2,4-dihydroxybenzoic, and o-coumaric acids-making them an excellent dietary source of natural antioxidants with strong antiradical activity (Multari *et al.*, 2018). These phenolic compounds function as phytoalexins, which are antimicrobial substances synthesized by plants in response to pathogenic attacks, such as those caused by fungi. Avenanthramides, a unique group of hydroxycinnamoylanthranilate alkaloids found only in oats, are also known to contribute to cardiovascular health by promoting the production of nitric oxide, which aids in blood vessel dilation and blood pressure regulation (Sang and Chu, 2017).

The radical scavenging activity of cereal extracts from milled flour fractions has been reported to be highest in barley (26%), followed by oat (16%) and wheat (12%) (Ivanisova *et al.*, 2012). Singh *et al.* (2019) further highlighted that oat cultivars exhibit

*Email: rachana1981_gautam@pau.edu
Date of receipt: 19.09.2024, Date of acceptance: 25.04.2025

significant reducing power and DPPH free radical scavenging activity, indicating their strong antioxidant potential. Among the reactive oxygen species (ROS), hydroxyl radicals are the most reactive and have the shortest half-life. They are primarily generated through the Haber-Weiss reaction, involving hydrogen peroxide, superoxide anion radicals, and iron ions. Due to their high reactivity, hydroxyl radicals can cause severe damage to nearby biomolecules such as lipids, proteins, and nucleic acids (Matros *et al.*, 2015). Nitric oxide (NO), another biologically relevant free radical, is produced from L-arginine via its conversion to L-citrulline by the enzyme nitric oxide synthase (Habib and Ali, 2011). Superoxide anions, another form of ROS, are generated from multiple intracellular sources, including mitochondria during oxidative phosphorylation, as well as through the activity of enzymes such as NADPH oxidase and xanthine oxidase (Granger and Kvietys, 2015). These superoxide radicals are predominantly dismutated into hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD), yet they still pose a threat due to their ability to damage proteins, lipids, and DNA (Gurer-Orhan *et al.*, 2016). In light of the increasing interest in natural antioxidants and the emerging recognition of oats as a valuable source of such compounds, the present study involved the biochemical characterization of forty-three oat genotypes to evaluate their antioxidant potential.

The present study was carried out on 43 oat genotypes, comprising six recommended cultivars and thirty-seven advanced breeding lines, all obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (Table 1). The seeds were manually dehulled, and the resulting grains were ground into a uniform flour using a Havells Sonidoi 1200W grinder. The flour was then sieved through an 80 µm mesh to ensure consistency in particle size. The prepared flour samples were subsequently stored in plastic bags at -20 °C until further analysis.

The oat flour samples were defatted using 80% acetone. For extraction, 0.5 g of defatted flour was soaked in 80% methanol and incubated at 70 °C for one hour. The mixture was then vortexed for two hours, and the resulting extract was filtered and brought to a final volume of 5 mL with methanol. This methanolic extract was used to assess the antioxidant potential of the oat genotypes.

To estimate DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, 0.5 mL of methanolic extract was mixed with 0.5 mL of methanol and 3 mL of 100 mM DPPH solution. The mixture was kept in the dark at room temperature for 30 minutes, after which the change in colour was measured at 515 nm against a reagent blank (Blois, 1958). Total reducing power was assessed by adding 0.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 0.5 mL of 1% potassium ferricyanide to 0.5 mL of methanolic extract. The mixture was incubated at 50 °C for 20 minutes, followed by the addition of 0.5 mL of 1% trichloroacetic acid (TCA). The contents were then centrifuged at 10,000 × g for 10 minutes. Subsequently, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$ solution. The green colour that developed was measured at 700 nm. Ascorbic acid (5-40 µg) was used as the standard (Sreeramulu *et al.*, 2009).

For the Ferric Reducing Antioxidant Power (FRAP) assay, the reagent was freshly prepared by mixing 200 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1, respectively.

Antioxidant activity based upon FRAP was estimated by incubating 1.9 ml of FRAP reagent at 37°C for 10 minutes. Then 0.05 ml of the methanolic extract and 0.05 ml of methanol were added to it. After incubation at 37°C for 30 minutes, blue colour so developed was read at 593 nm (Benzie and Strain, 1996). The FRAP activity was calculated from $FeSO_4.7H_2O$ standards (5-30 µg) that were run simultaneously. Superoxide anion radical scavenging activity was estimated by adding 1 ml of 3mM pyrogallol solution into the test tubes containing 0.2 ml of methanolic extract, 0.5 ml of EDTA, and 1.3 ml of 100 mM Tris-HCl buffer (pH 8.0). Then, the absorbance of the solution was measured at 420 nm (Marklund and Marklund, 1974). Superoxide anion radical scavenging activity was determined as the percent inhibition of pyrogallol autoxidation, which was calculated from the optical density in the presence or absence of pyrogallol.

The hydroxyl radical scavenging assay is based on the Fenton-type reaction generating hydroxyl radical. It was estimated by taking the absorbance at 536 nm of the solution containing 1ml of 1.5 mM 1, 10 phenanthroline, 1ml of 1.5 mM $FeSO_4$, 0.1 ml methanolic extract, 1.9 ml of 200 mM sodium phosphate buffer (pH 7.6) and 1ml of 0.02 % hydrogen peroxide after incubation at 37°C for 1 hour (Modified method of Lee *et al.,* 2008). Sodium

Table 1. List of oat genotypes used for biochemical analysis

S.No.	Genotypes	Source	S.No.	Genotypes	Source
1	KENT	PAU, State and national release and national check	23	OL-1855	Advance breeding lines
2	OL-09	PAU, State release	24	OL-1862	Advance breeding lines
3	OL-10	PAU, State release	25	OL-1863	Advance breeding lines
4	OL-125	PAU, National release	26	OL-1864	Advance breeding lines
5	RO-19	Rahuri, Maharastra and National check	27	OL-1865	Advance breeding lines
6	UPO-212	Pantnagar, Uttrakhand and National check	28	OL-1866	Advance breeding lines
7	OL-1689	Advance breeding lines	29	OL-1867	Advance breeding lines
8	OL-1760	Advance breeding lines	30	OL-1868	Advance breeding lines
9	OL-1766	Advance breeding lines	31	OL-1869	Advance breeding lines
10	OL-1769	Advance breeding lines	32	OL-1870	Advance breeding lines
11	OL-1802	Advance breeding lines	33	OL-1871	Advance breeding lines
12	OL-1804	Advance breeding lines	34	OL-1873	Advance breeding lines
13	OL-1835	Advance breeding lines	35	OL-1874	Advance breeding lines
14	OL-1837	Advance breeding lines	36	OL-1875	Advance breeding lines
15	OL-1841	Advance breeding lines	37	OL-1876	Advance breeding lines
16	OL-1842	Advance breeding lines	38	OL-1877	Advance breeding lines
17	OL-1843	Advance breeding lines	39	OL-1878	Advance breeding lines
18	OL-1844	Advance breeding lines	40	OL-1880	Advance breeding lines
19	OL-1845	Advance breeding lines	41	OL-1881	Advance breeding lines
20	OL-1847	Advance breeding lines	42	OL-1882	Advance breeding lines
21	OL-1852	Advance breeding lines	43	OL-1883	Advance breeding lines
22	OL-1854	Advance breeding lines			

nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griss reagent. Nitric oxide radical ($NO^{\bullet}$) scavenging activity was estimated by incubating 0.050 ml of a methanolic extract with 0.2 ml of aqueous sodium nitroprusside and 0.4 ml of 200 mM sodium phosphate buffer at room temperature for $2^{1/2}$ hour. Then after adding 0.24 ml of Griss reagent (formed by mixing 2 % Phosphoric acid and 1 % Sulphanilamide), the reaction mixture was again incubated at room temperature for 15 minutes. Absorbance was read at 540 nm against a blank (Marcocci *et al.,* 1994).

Estimation of non-enzymatic antioxidants

Total phenols were estimated from the methanolic extract by using Folin-phenol's reagent (Swain and Hillis, 1959). The blue colour was read at 760 nm along with the gallic acid (10-50 µg) standard. For ascorbic acid estimation, 0.3g of flour was homogenized in 1.5 ml of 5% metaphosphoric acid and centrifuged at 10,000×g for 10 minutes. The reaction mixture comprising of 500 µl of supernatant, 200 µl 5 mM EDTA, 400 µl $FeCl_3$ (16 mM $FeCl_3$ in 0.1M sodium phosphate buffer (pH 7.5), 400 µl 1.7% TCA, 400 µl 7.6% o-phosphoric acid and 400 µl 44mM bipyridyl was incubated at 40°C for 40 minutes. The intensity of the pink colour was read at 525 nm against the reagent blank (Law *et al.*, 1983).

Flour of 0.3 g was extracted with 1.5 ml of ethanol for estimation of α-tocopherol by using a bathophenanthroline reagent. Absorbance was read at 534 nm against the reagent blank (Kayden *et al.,* 1973). Tocopherol standard (20-100 µg) was run with the samples to plot a graph against which tocopherol concentration was estimated. Extraction of flavonoids was carried out with 6 ml of distilled water. To 3 ml

extract, 1.25 ml of distilled water and 0.075 ml of 5 % $NaNO_2$ were added. After 6 minutes, 0.15 ml of 10 % $AlCl_3.6H_2O$ was added in the test tubes. After incubation for 5 minutes at room temperature, 0.5 ml of 1M NaOH was added. The absorbance was read at 510 nm after making the volume to 2.5 ml with distilled water along with the rutin (40-200 µg) standard (Zhishen *et al.,* 1999).

Statistical analysis

The data for different free radical scavenging activities and non-enzymatic antioxidants presented as Mean+SD of triplicates was analyzed by one-way ANOVA (*p≤0.05*) using Tukey's *post hoc* test by SPSS version 16.0. The varieties were then classified into low, medium, and high categories with equal class intervals using an equal distribution method by subtracting the minimum value from the maximum value and dividing the difference of the two by 3. Based upon different free radical scavenging activities and profile of non-enzymatic antioxidants, 43 oat genotypes were clustered by using XLSTAT (2017). Correlation coefficients between various free radical scavenging activities and antioxidants were also calculated using Excel.

Oat contains a number of compounds that were found to have strong antioxidant potential. In the present study, 43 oat genotypes were evaluated for antioxidant potential based upon free radical scavenging activities and non-enzymatic antioxidants. The oat genotypes were divided into low, medium and high categories on the basis of equal distribution method. DPPH free radical scavenging activity higher than 41.5 % was classified under higher DPPH free radical scavenging activity category, value between 33.4 to 41.5 % placed under medium activity and less than 33.4 % is placed under low DPPH free radical scavenging activity. Same procedure was applied for FRAP, hydroxyl radical, superoxide anion radical scavenging activity, nitric oxide and reducing power which varied as H ≥ 4.2L ≤ 2.5 (FRAP), H ≥ 33.1L ≤ 28.4 (Hydroxyl radical), H ≥ 25.5L ≤ 16.5 (Superoxide radical scavenging activity), H ≥ 82.9L ≤ 76.1 (Nitric oxide), H ≥ 60.2L ≤ 43.8 (Reducing power).

In the DPPH assay, the antioxidant effect is proportional to the disappearance of $DPPH^{\bullet}$ and the appearance of yellow-coloured DPPH compound in test samples (Viacava *et al.*, 2014). DPPH free radical scavenging activity of oat genotypes is represented in table 2. In the present study, DPPH free radical scavenging activity of oat genotypes varied from 25.3 (OL-1874) to 49.7% (OL-1843) with an average of 41.8%. Twenty-three genotypes possessed higher DPPH (41.5-49.7%) activity. Kaur *et al.* (2014) reported that oat grains have 40% DPPH free radical scavenging activity. Brindzova *et al.* (2008) observed that antioxidant components of oats reduce the DPPH radical by 53 to 82%.

The presence of antioxidants caused the reduction of the ferricyanide complex to the ferrous form, thus measured the Perl·s Prussian blue at 700 nm (Ferreira *et al.*, 2007). Fourteen genotypes possessed higher reducing power ranging from 60.2 to 76.6 µg/g. Reducing power in oat genotypes varied from 27.4 (KENT) to 76.6 µg/g (OL-1855) with an average value of 56.4 µg/g (Table 2). Sreeramulu *et al.* (2009) reported that reducing power in cereal grains varied from 36 to 4540 µg/g whereas wheat and bajra were observed to have 610 µg/g and 1730 µg/g, respectively.

Ferric reducing antioxidant power of oat genotypes was estimated based on their ability to reduce the ferric 2, 4, 6-tripyridyltriazine complex (Fe^{3+}-TPTZ) to the coloured ferrous tripyridyltriazine complex (Fe^{2+}-TPTZ). In FRAP assay, yellow colour of solution changes to green or blue colour depending upon the reducing power of the antioxidants (Gulcin, 2012). The increase in absorbance is proportional to the antioxidant content (Ye *et al*., 2009). In the present investigation, FRAP activity ranged from 0.9 (OL-1874) to 5.9 µmole/g (OL-1881) in oat genotypes (Table 2). Twenty-one genotypes were found to have lower (0.9-2.5 µmole/g) FRAP activity. Twenty-one genotypes possessed medium (2.5-4.1 µmole/g) and one (OL-1881) contained higher FRAP activity. Boeck *et al.* (2018) observed that FRAP content varied from 5.8 to 16 µmole/g in oat genotypes during 2014-15 and 2015-16, whereas Sreeramulu *et al.* (2009) reported it to be 33 µmole/g in wheat grains. Krishnanunn *et al.* (2014) observed that two rice varieties namely Kuzhiadichan and Karungkavuni have 1.05 and 1.14 µmole/g FRAP activity, respectively.

Nitric oxide ($NO^{\bullet}$). a free radical, is formed from L-arginine on its conversion to L-citrulline with the help of nitric oxide synthase enzyme. It acts as both cytoprotective and tumor promoting agent. Nitric oxide on combining with superoxide anion radical generates peroxynitrite which is the main mediator of tissue and cellular injury. Nitric oxide can directly either protect or damage the cell and indirectly may cause oxidative stress (Habib and Ali, 2011). Sodium

Table 2. Variation of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) scavenging activity (%), ferric reducing antioxidant power (FRAP) (µmole/g) and total reducing power (µg/g) in oat genotypes

Sr. No.	Genotypes	DPPH activity (%)	Total reducing power (µg/g)	FRAP activity (µmole/g)	Sr. No.	Genotypes	DPPH activity (%)	Total reducing power (µg/g)	FRAP activity (µmole/g)
1	KENT	44.4±2.0^{abcdefg}	27.4±1.1^{n}	1.9±0.32^{lmnopq}	23	OL-1855	45.4±0.4^{abcde}	76.6±3.1^{a}	1.8±0.04^{lmnopqr}
2	OL-09	36.0±0.8^{klm}	53.1±3.0^{fghijk}	3.4±0.06^{bcdef}	24	OL-1862	41.9±1.5^{cdefghi}	64.9±5.6^{bcde}	1.7±0.11^{nopqr}
3	OL-10	35.0±0.4^{lm}	40.2±3.4^{lm}	2.2±0.60^{klmnop}	25	OL-1863	39.1±2.8^{fghijkl}	39.5±4.1^{lm}	1.2±0.46^{pqr}
4	OL-125	38.6±0.2^{hijkl}	55.5±2.8^{defghij}	2.2±0.28^{ijklmno}	26	OL-1864	45.5±1.3^{abcde}	42.1±3.3^{klm}	3.0±0.03^{bcdefghijk}
5	RO-19	35.5±0.6^{klm}	49.4±5.7^{ghijkl}	3.2±0.58^{bcdefghi}	27	OL-1865	45.5±0.3^{abcde}	62.2±4.0^{cdef}	3.6±0.18^{bcde}
6	UPO-212	39.1±0.9^{fghijkl}	59.9±3.1^{cdefgh}	2.2±1.80^{jklmno}	28	OL-1865	39.2±0.9^{fghijkl}	53.8±4.6^{efghij}	2.4±0.09^{hijklmno}
7	OL-1689	48.2±5.3^{ab}	60.3±3.0^{cdefg}	3.9±0.47^{b}	29	OL-1866	45.6±0.8^{abcde}	59.9±3.1^{cdefgh}	1.7±0.14^{nopqr}
8	OL-1760	40.0±1.3^{efghijkl}	64.5±2.3^{bcde}	1.0±0.17^{qr}	30	OL-1868	39.8±1.5^{efghijkl}	68.5±4.0^{abc}	3.0±0.06^{bcdefghijk}
9	OL-1766	43.0±4.2^{bcdefghij}	47.1±2.3^{ijklm}	2.1±0.06^{klmnop}	31	OL-1869	46.8±1.2^{abcd}	58.0±1.3^{cdefghi}	1.8±0.14^{mnopqr}
10	OL-1769	39.9±1.1^{efghijkl}	57.8±4.9^{cdefghi}	2.2±0.13^{ijklmno}	32	OL-1870	37.8±2.0^{ijkl}	44.4±3.3^{jklm}	2.6±0.30^{efghijklmn}
11	OL-1802	37.4±1.1^{jklm}	54.4±2.8^{efghij}	1.7±0.08^{nopqr}	33	OL-1871	44.2±1.0^{abcdefgh}	37.6±2.3^{mn}	1.6±0.02^{opqr}
12	OL-1804	44.8±2.4^{abcdef}	64.9±2.1^{bcde}	3.3±0.09^{bcdefgh}	34	OL-1873	43.1±1.2^{bcdefghij}	60.4±2.6^{cdefg}	2.6±0.17^{efghijklmn}
13	OL-1835	48.0±1.7^{ab}	64.6±1.0^{bcde}	2.2±0.38^{klmnop}	35	OL-1874	25.3±1.0^{n}	76.5±2.7^{a}	0.9±0.11^{r}
14	OL-1837	44.6±0.9^{abcdefg}	54.2±4.1^{efghij}	3.4±0.56^{bcdefg}	36	OL-1875	42.4±0.0^{bcdefghij}	53.9±3.3^{efghij}	2.2±0.03^{ijklmno}
15	OL-1841	39.8±0.7^{efghijkl}	55.9±3.3^{defghi}	3.3±0.22^{bcdefgh}	37	OL-1876	46.5±2.0^{abcd}	65.8±2.1^{abcd}	2.8±0.04^{defghijkl}
16	OL-1842	41.0±1.2^{defghijk}	60.6±4.1^{cdefg}	3.8±0.02^{bc}	38	OL-1877	44.4±0.8^{abcdefg}	67.8±2.7^{abc}	3.7±0.13^{bcd}
17	OL-1843	49.7±2.1^{a}	48.7±9.1^{hijklm}	3.3±0.10^{bcdefgh}	39	OL-1878	38.9±0.5^{ghijkl}	74.2±1.5^{ab}	2.7±0.11^{defghijkl}
18	OL-1844	31.7±0.6^{m}	58.6±1.3^{cdefgh}	3.8±0.03^{bc}	40	OL-1880	40.5±1.2^{efghijkl}	63.9±2.6^{bcdef}	2.7±0.26^{efghijklm}
19	OL-1845	41.9±0.9^{cdefghij}	40.6±3.0^{lm}	3.0±0.32^{bcdefghijk}	41	OL-1881	43.7±1.2^{bcdefgh}	59.6±2.3^{cdefgh}	5.9±0.97^{a}
20	OL-1847	45.0±1.4^{abcde}	59.3±2.0^{cdefgh}	3.1±0.03^{bcdefghij}	42	OL-1882	46.6±0.9^{abcd}	59.9±2.3^{cdefgh}	2.5±0.46^{ghijklmno}
21	OL-1852	43.4±0.8^{bcdefghi}	55.8±2.3^{defghi}	2.5±0.16^{fghijklmno}	43	OL-1883	47.6±4.2^{abc}	52.8±1.8^{fghijk}	2.5±0.13^{fghijklmno}
22	OL-1854	41.2±1.0^{defghijk}	49.9±2.4^{ghijkl}	3.2±0.06^{bcdefghi}	Mean		47.8	56.4	2.7

Values are mean ± S.D of triplicates
Within a column values followed by different alphabets differ significantly at $p<0.05$

nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griss reagent (Philips *et al.,* 2010). Oat genotypes possessed nitric oxide radical scavenging activity ranging from 69.1 (OL-1882) to 89.4 % (OL-1875) with an average value of 77.3 % (Table 3). Four genotypes (OL-1880, OL-1863, OL-1802, OL-1875) were found to have high (82.5-89.4 %) nitric oxide scavenging activity. Ghasemzadeh *et al.* (2015) observed 58-82 % nitric oxide scavenging activity in rice bran exploiting different techniques.

Superoxide anion radicals are formed by one electron reduction of oxygen, further one electron reduction of superoxide anion radical leads to the formation of hydrogen peroxide by the action of mitochondrial superoxide dismutase. Reduction of hydrogen peroxide yields the hydroxyl radical (Imlay, 2003). It can cause damage to proteins, lipids and DNA (Gurer-Orhan *et al.,* 2016). The superoxide anion radical scavenging activity of oat genotypes is presented in table 3. It varied from 7.5 (OL-1864) to 34.5 % (OL-1835) with an average value of 21.4 %. Twelve genotypes were found to have high (25.5-34.5 %) superoxide anion radical scavenging activity. Vadivel *et al.* (2011) observed that superoxide anion radical scavenging activity of legume seed extracts was between 42.2 and 61.6 %.

Hydroxyl radical is the neutral form of hydroxide ion and is a highly reactive free radical. It is formed from superoxide anion radical and hydrogen peroxide in the presence of metal ions such as copper and iron (Li *et al.,* 2008). It can strongly react with both inorganic and organic molecules including DNA, proteins, lipids and carbohydrates causing severe damage to the cells. Hydroxyl radical scavenging activity of an extract is directly related with antioxidant potential (Babu *et al.,* 2001). The capacity of oat extract to inhibit hydroxyl radical ranged from 23.6 (OL-1883) to 37.7 % (OL-1863) with the mean value of 30.2 % (Table 3). Seven genotypes were found to have high hydroxyl radical scavenging activity (33-37.7 %). Loganayaki *et al.* (2013) evaluated the two medicinal plants namely *Helicteres isora* L. and *Ceiba pentandra* L. for hydroxyl radical scavenging activity which was 80.1 and 84.1%, respectively.

Equal distribution method was applied for α-Tocopherol, total phenols, flavanoids and ascorbic acid which was H ≥ 42.7L ≤ 22 (α-Tocopherol), H ≥ 353.2L ≤ 228.2 (Total phenols) and H ≥ 227.8L ≤ 129.7 (Flavonoids) and H ≥ 125.1L ≤ 67.5 (Ascorbic acid)**.** Tocols, lipid soluble compounds are present in the germ of cereals. Tocols include tocopherol and tocotrienols. Tocopherol has 4 homologues α, β, γ and δ which differ in number and position of methyl groups on the chroman ring structure (Peterson *et al.,* 2007). It shows antioxidant activity, during the peroxidation of unsaturated lipids. If a suitable free radical is present, a non-radical product can be formed from coupling of the free radical with the tocopheroxyl radical (Yamauch, 1997). The tocopherol content in 43 oat genotypes ranged from 1.3 (OL-1871) to 63.4 µg/g (OL-1878) with an average value of 26.8 µg/g (Table 4). Eighteen genotypes possessed lower (1.3-22 µg/g) tocopherol content, sixteen were in the medium range (22 to 42.7 µg/g) and nine genotypes (OL-1835, OL-1880, OL-1878, OL-1877, OL-1766, OL-1862, OL-1883, OL-1868, OL-125) had higher content (42.7 to 63.4 µg/g). Chu *et al.* (2013) reported that oat grain contained tocopherol in the range of 9 to 16 µg/g. Sterna *et al.* (2016) reported that tocopherol content of oat grain varied from 4.5 to 12.3 µg/g.

Phenols are naturally occurring compounds with atleast one aromatic ring containing one or more hydroxyl groups. Their complexity varies from very simple to highly polymerized phenols, which are categorized as flavonoids, stilbenes, coumarins, phenolic acids and tannins (Liu, 2004). Total phenolic content varied from 102.9 (OL-1864) to 471.9 µg/g (OL-1878) with an average value of 243.5 µg/g (Table 4). Twenty genotypes had lower amount of total phenolic content (102.9 to 225.9 µg/g), 4 genotypes possessed higher amount (348.9 to 471.9 µg/g) whereas remaining nineteen genotypes lied in the medium range (225.9 to 348.9 µg/g) of total phenolic content. In earlier studies, Kahkonen *et al.* (1999) observed that total phenolic content of oat groat was 400 µg/g. Similarly, Brindzova *et al.* (2008) also reported that total phenolic content of oat groats varied from 239 to 662 µg/g.

Flavonoids consist of a large group of polyphenolic compounds, secondary metabolic compounds present in cell vacuoles of plants (Samanta *et al.,* 2011). They protect plants against various biotic and abiotic stresses and play an important role in the interaction between the plant and their environment (Pourcel *et al.,* 2007). They absorbed the harmful UV radiation which can induce cellular damage (Takahashi and Ohnishi, 2004). Total flavonoids content of oat genotypes varied from 31.6

Table 3. Variation of nitric oxide radical (%), superoxide anion (%) and hydroxyl radical scavenging activities (%) in oat genotypes

Sr. No.	Genotypes	Nitric oxide radical scavenging activity	Superoxide anion radical scavenging activity	Hydroxyl radical scavenging activity	Sr. No	Genotypes	Nitric oxide radical scav-enging activity	Superoxide anion radical scavenging activity	Hydroxyl radical scavenging activity
1	KENT	75.6±0.64^{pqrstuv}	18.9±1.5^{fghijklmnop}	33.4±0.14^{bcde}	23	OL-1855	79.8±0.49^{fghijk}	22.6±1.2^{cdefghijkl}	28.6±0.39^{ghijklmno}
2	OL-09	78.0±0.54^{ijklmnop}	23.2±1.3^{cdefghij}	36.8±0.05^{ab}	24	OL-1862	75.2±0.04^{rstuv}	22.7±0.2^{cdefghijk}	31.1±0.87^{defghijkl}
3	OL-10	74.9±0.54^{stuv}	18.6±0.8^{ghijklmnop}	27.5±0.77^{jklmnop}	25	OL-1863	85.6±0.09^{b}	14.3±2.1^{klmnop}	37.7±0.39^{a}
4	OL-125	82.3±1.67^{cde}	14.1±1.2^{mnopq}	28.9±0.77^{ghijklmno}	26	OL-1864	74.4±0.49^{tuvw}	7.5±2.5^{q}	27.6±1.30^{jklmnop}
5	RO-19	71.6±0.78^{xyzA}	20.0±0.6^{fghijklmno}	31.3±0.29^{defghijkl}	27	OL-1865	74.5±0.09^{tuvw}	26.8±3.1^{abcdefg}	27.4±0.14^{lmnop}
6	UPO-212	74.2±0.59^{uvw}	14.7±0.6^{klmnopq}	33.3±0.72^{bcdef}	28	OL-1866	74.5±0.49^{tuvw}	17.6±1.4^{ijklmnop}	32.6±1.21^{cdefg}
7	OL-1689	81.5±1.00^{defg}	21.3±2.9^{defghijklmn}	32.2±0.34^{cdefghi}	29	OL-1867	79.4±1.18^{ghijklm}	22.1±0.6^{cdefghijklm}	35.6±0.34^{abc}
8	OL-1760	78.2±1.67^{hijklmno}	22.3±1.2^{cdefghijklm}	33.7±1.98^{abcd}	30	OL-1868	80.0±0.73$_{efghij}$	25.2±0.8^{bcdefghi}	28.3±0.34^{hijklmno}
9	OL-1766	77.0±0.64^{lmnopqrs}	7.5±2.5^{q}	27.4±0.19^{klmnop}	31	OL-1869	72.1±1.13$_{wxyz}$	26.0±0.4^{bcdefgh}	28.7±0.39^{ghijklmno}
10	OL-1769	82.2±0.83$_{cdef}$	24.5±1.2^{bcdefghi}	28.4±0.39^{hijklmno}	32	OL-1870	76.2±0.34^{opqrstu}	10.6±0.4^{pq}	33.7±0.14^{abcd}
11	OL-1802	84.1±0.19$_{bc}$	27.1±0.8^{abcdef}	25.4±0.48^{op}	33	OL-1871	80.6±1.47^{efgh}	23.1±0.3^{cdefghij}	27.4±0.58^{klmnop}
12	OL-1804	70.5±0.19^{zA}	12.4±0.7^{opq}	30.7±1.26^{defghijklm}	34	OL-1873	80.4±0.14^{efghi}	28.3±3.6^{abcde}	31.7±0.82^{cdefghi}
13	OL-1835	76.7±0.83^{nopqrst}	34.5±2.4^{a}	32.6±1.16^{cdefg}	35	OL-1874	75.5±0.44^{qrstuv}	30.0±2.1^{abc}	31.5±0.14^{defghij}
14	OL-1837	73.9±0.29^{uvwx}	25.4±2.0^{bcdefghi}	30.9± 30.9±0.92^{defghijkl}	36	OL-1875	89.4±0.78^{a}	26.3±0.2^{abcdefg}	29.4±0.43^{efghijklmno}
15	OL-1841	80.4±0.78^{efghi}	25.6±1.0^{bcdefghi}	31.8±0.82^{cdefghi}	37	OL-1876	79.2±0.09^{ghijklmn}	32.0±3.3^{bcdefgh}	31.4±1.55^{defghijk}
16	OL-1842	76.2±0.29^{opqrstu}	29.4±1.6^{abcd}	32.3±0.34^{cdefgh}	38	OL-1877	75.5±0.24^{qrstuv}	13.2±1.4^{nopq}	26.2±2.00^{nop}
17	OL-1843	75.4±0.04^{qrstuv}	20.0±0.8^{fghijklmno}	29.3±0.48^{fghijklmno}	39	OL-1878	70.7±0.49^{zA}	14.6±0.3^{klmnopq}	28.2±0.82^{ijklmno}
18	OL-1844	70.8±0.10^{yzA}	21.5±0.7^{defghijklmn}	31.1±0.63^{defghijkl}	40	OL-1880	83.9±0.14^{bcd}	27.1±2.6^{abcdef}	30.1±1.00^{defghijklmn}
19	OL-1845	79.5±0.14^{ghijk}	17.8±0.6^{hijklmnop}	27.5±0.72^{jklmnop}	41	OL-1881	73.2±0.24^{vwxy}	15.0±0.8^{jklmnopq}	28.4±0.68^{hijklmno}
20	OL-1847	76.9±0.59^{mnopqrst}	26.0±1.5^{ab}	26.8±1.00^{mnop}	42	OL-1882	69.1±0.78^{A}	20.8±0.2^{efghijklmn}	28.8±1.30^{ghijklmno}
21	OL-1852	77.5±0.09^{klmnopqr}	23.4±0.5^{cdefghi}	28.7±0.29^{ghijklmno}	43	OL-1883	77.8±1.28$_{jklmnopq}$	22.6±0.1^{cdefghijkl}	23.6±0.39^{p}
22	OL-1854	81.0±1.62$_{efg}$	22.4±1.1^{cdefghijkl}	27.4±0.63^{lmnop}		Mean	77.3	21.4	30.1

Values are mean ± S.D of triplicates
Within a column values followed by different alphabets differ significantly at $p<0.05$

(OL-1869) to 326.0 µg/g (OL-1852) with an average value of 154.8 µg/g (Table 4). Sixteen genotypes were found to have lower range of total flavonoids content (31.6 to 129.7 µg/g), 21 genotypes were having medium range (129.7 - 227.8 µg/g) and 6 genotypes (OL-1876, OL-1868, OL-1852, OL-1875, OL-1855, OL-1871) possessed higher (227.8-326 µg/g) content. Salawu *et al.* (2014) reported that total flavonoids content of whole cereal grains varied from 33 to 170 µg/g, whereas in other report it was found to be 33.8-44.7 µg/g (Wahed *et al.,* 2019). Kim *et al.* (2007) found that total flavonoids content of barley grains ranged between 62.0 and 300.8 µg/g. In case of barley grains, flavonoids content was proportional to the degree of colour depth, thus blue and purple grains were discovered to possess the maximum flavonoids content among different barley varieties (Liu *et al.,* 2013). However, Ivanisova *et al.* (2012) observed that total flavonoids content in oats were 640 µg/g.

Ascorbic acid is involved in the first line of antioxidant defence and in protection of lipid membranes (Berger *et al.,* 1997). The current investigation showed that ascorbic acid content of oat genotypes varied widely from 9.9 (OL-1868) to 182.9 nmole/g (OL-1845) with an average value of 52.4 nmole/g (Table 4). Thirty two genotypes possessed lower range of ascorbic acid content (9.9 to 67.5 nmole/g), 10 genotypes were in medium range (67.5-125.1 nmole/g) whereas only one genotype possessed higher ascorbic acid content. Earlier studies (USDA 2005) showed that cereal grains contain negligible amount of ascorbic acid content.

Cluster analysis

The clustering of 43 genotypes was done according the data of antioxidant capacities and non-enzymatic antioxidants (Fig.1). The oat genotypes in this study were divided into two main clusters in the dendrogram i.e. MI and MII. Cluster MI has 2 sub clusters MIa and MIb. Genotypes which were clustered in sub cluster MIa possessed low/medium non enzymatic antioxidants whereas genotypes of MIb cluster were medium/high in free radical scavenging activities. MII has 2 sub cluster MIIa and MIIb where MIIa had two sub sub clusters MIIa1 and MIIa2. First sub sub sub cluster of MIIa1 i.e. MIIa1A had medium free radical scavenging activities and low non-enzymatic antioxidants, and MIIa1B contained medium/high free radical scavenging activities. Genotypes of sub sub clusters of MIIa2 have low/ medium non-enzymatic antioxidants. MIIb1 sub sub cluster of MIIb possessed low/medium free radical scavenging activities and non-enzymatic antioxidants, and MIIb2 further divided into 2 clusters named as MIIb2A and MIIb2B former contained genotypes of medium free radical scavenging activities and non-enzymatic antioxidants and latter had medium/high and non-enzymatic antioxidants.

Correlation between antioxidants and free radical scavenging potential

Correlation coefficients between various free radical scavenging activities and non-enzymatic antioxidants in oat varieties were calculated. Significant positive correlation was observed between nitric oxide radical scavenging activity and total flavonoids (0.343). Total reducing power was found to be positively correlated to superoxide radical scavenging activity (0.365). These results were consistent with Shim *et al.* (2003).

The average Ferric reducing antioxidant power (FRAP), total reducing power, 2, 2-Diphenyl-1-picryl hydrazyl (DPPH), superoxide anion, hydroxyl, and nitric oxide radical scavenging activities were 2.7 µmole/g, 56.4 µg/g, 47.8%, 21.4% and 30.1% and 77.3%, respectively. The oat genotypes namely OL-1769, OL-1873 and OL-1880 were observed to be in high or medium category of antioxidant potential in terms of their capacity to scavenge DPPH, hydroxyl, nitric oxide and superoxide radicals as well as in most of the non-enzymatic antioxidants i.e. tocopherol, total phenols, ascorbic acid and total flavonoids.

Positive correlation between nitric oxide radical scavenging activity with flavonoids content at 5% probability level depicted the role of flavonoids in scavenging nitric oxide radicals. The genotypes; OL-1769, OL-1873 and OL-1880, possessing higher antioxidant potential may be employed in the breeding programs to acquire superior cultivars.

Authors' contribution

Conceptualization and designing of research work (RDB); Execution of lab experiments and data collection (SK); Analysis of data and interpretation (SK, RDB, RK, SK); Preparation of manuscript (SK, RDB, SK).

Acknowledgments

The authors are thankful to the Department of Plant Breeding and Genetics for providing oat germplasm.

Table 4. Variation of α-tocopheol, total phenolic, ascorbic acid and total flavonoids contents in oat genotypes

Sr. No.	Geno-types	α-tocopher-ol content (µg/g)	Total phenolic content (µg/g)	Ascorbic acid content (nmole/g)	Total flavo-noids con-tent (µg/g)	Sr. No	Geno-types	α-tocoph-erol con-tent(µg/g)	Total phenolic content(µg/g)	Ascorbic acid content (nmole /g)	Total flavo-noids content (µg/g)
1	KENT	34.4±4.6^{hijkl}	301.3±24.2^{cdef}	78.1±3.3^{cdef}	133.4±4.1^{fghijkl}	23	OL-1855	11.6±2.3^{stuv}	463.2±29.9^{a}	47.3±4.0^{hijklm}	240.7±19.2^{bc}
2	OL-09	37.2±2.7^{fghij}	154.5±7.0^{nopqr}	61.3±8.2^{efghi}	123.8±6.8^{ijklm}	24	OL-1862	42.8±2.7^{cdefgh}	217.1±10.2^{hijklmno}	35.8±0.6^{klmno}	116.2±8.9^{lm}
3	OL-10	38.6±2.3^{defghi}	288.7±23.4^{defg}	30.6±9.1^{klmnop}	139.6±14.4^{fghijkl}	25	OL-1863	11.6±2.3^{stuv}	143.2±3.8^{qr}	33.8±6.2^{klmno}	129.3±5.5^{ghijklm}
4	OL-125	48.0±2.3^{bc}	343.8±27.4^{bcd}	40.3±4.8^{ijklmno}	138.9±9.6^{fghijk}	26	OL-1864	27.9±1.8^{klmno}	102.9±2.2^{r}	59.6±7.7^{fghij}	123.1±11.6^{ijklm}
5	RO-19	35.4±2.7^{ghijk}	253.0±20.8^{fghijkl}	23.6±6.9^{op}	158.9±7.5^{efghij}	27	OL-1865	25.6±3.2^{mnop}	191.3±16.2^{klmnop}	47.3±4.0^{hijklm}	141.0±10.3^{fghijkl}
6	UPO-212	9.7±1.3^{tuvw}	380.0±30.7^{b}	52.7±7.6^{ghijk}	160.9±2.7^{efghi}	28	OL-1866	11.6±2.3^{stuv}	293.3±11.2^{cdef}	50.5±5.0^{ghijkl}	144.4±6.8^{fghijkl}
7	OL-1689	15.3±2.3^{qrst}	150.5±9.7^{opqr}	31.7±0.3^{klmnop}	127.2±7.5^{hijklm}	29	OL-1867	36.8±1.3^{fghij}	316.8±19.7^{bcdef}	116.1±14.4^{b}	117.6±7.1^{klm}
8	OL-1760	26.1±2.7^{lmnop}	219.3±19.9^{hijklmn}	80.9±3.9^{cdef}	118.3±8.2^{klm}	30	OL-1868	44.2±1.3^{cdef}	265.8±7.0^{efghi}	9.9±2.9^{p}	246.9±28.2^{bc}
9	OL-1766	48.0±2.3^{bc}	202.0±10.3^{ijklmno}	47.3±4.0^{hijklm}	123.3±9.7^{ijklm}	31	OL-1869	21.4±1.8^{opqr}	267.3±8.7^{efghi}	27.1±8.3^{mnop}	31.6±2.7^{n}
10	OL-1769	38.6±0.4^{defghi}	261.6±14.8^{efghij}	83.3±4.7^{cde}	190.5±26.0^{de}	32	OL-1870	37.7±1.3^{efghi}	316.8±7.9^{bcdef}	22.5±6.9^{op}	136.8±7.5^{fghijkl}
11	OL-1802	18.6±1.8^{pqrs}	266.3±12.8^{efghi}	41.9±2.4^{ijklmno}	209.8±4.0^{cd}	33	OL-1871	1.3±0.4^{w}	252.0±4.1^{fghijkl}	26.8±0.4^{mnop}	247.6±20.6^{bc}
12	OL-1804	30.7±1.8^{ijklmn}	311.2±2.4^{cdef}	72.0±8.5^{defg}	165.0±12.3^{efgh}	34	OL-1873	28.9±1.8^{jklmno}	262.2±11.6^{efghij}	24.4±7.2^{nop}	137.5±5.5^{fghijkl}
13	OL-1835	47.0±0.4^{bcd}	220.9±12.9^{nijklmn}	29.8±8.8^{lmnop}	140.3±4.1^{fghijkl}	35	OL-1874	14.9±2.7^{qrst}	190.8±17.4^{lmnop}	48.3±4.5^{hijklm}	169.2±2.7^{ef}
14	OL-1837	12.1±2.7^{stuv}	161.7±12.9^{mnopq}	32.3±9.5^{klmno}	121.0±5.5^{jklm}	36	OL-1875	6.0±3.2^{uvw}	223.2±14.3^{ghijklm}	48.3±4.5^{hijklm}	257.9±22.7^{b}
15	OL-1841	8.8±2.3^{tuvw}	131.6±11.9^{pqr}	34.0±6.1^{klmno}	120.3±14.4^{jklm}	37	OL-1876	23.3±3.7^{nopq}	173.4±9.4^{mnopq}	22.8±6.7^{op}	308.8±3.4^{a}
16	OL-1842	11.1±1.8^{stuv}	187.7±19.1^{lmnopq}	46.5±3.7^{ijklmn}	122.4±8.2^{ijklm}	38	OL-1877	46.1±2.3^{bcde}	281.1±21.2^{defgh}	68.8±1.4^{cdefg}	94.2±3.4^{m}
17	OL-1843	13.9±3.7^{rstu}	151.5±13.0^{opqr}	31.7±3.6^{klmnop}	152.0±14.4^{efghijkl}	39	OL-1878	63.4±2.7^{a}	471.9±14.5^{a}	39.2±5.8^{ijklmno}	114.8±11.6^{lm}
18	OL-1844	6.0±2.3^{uvw}	149.8±3.4^{pqr}	24.7±7.5^{nop}	130.0±8.9^{ghijklm}	40	OL-1880	43.3±1.3^{cdefg}	212.2±8.3^{ijklmno}	100.3±9.9^{bc}	149.9±2.7^{fghijkl}
19	OL-1845	31.7±3.7^{ijklmn}	198.9±9.9^{jklmno}	182.9±9.4^{a}	152.7±5.5^{efghijkl}	41	OL-1881	32.1±4.1^{ijklm}	320.3±22.6^{bcde}	42.1±8.6^{ijklmno}	138.2±10.3^{fghijk}
20	OL-1847	23.3±3.7^{nopq}	163.7±14.5^{mnopq}	85.7±5.5^{cd}	156.1±2.0^{efghijk}	42	OL-1882	8.8±1.3^{tuvw}	127.5±11.8^{pqr}	37.6±1.3^{jklmno}	167.1±4.8^{efg}
21	OL-1852	4.6±2.7^{vw}	355.6±23.2^{bc}	36.0±4.8^{klmno}	326.0±24.0^{a}	43	OL-1883	53.1±2.7^{b}	257.6±8.7^{efghijk}	83.3±4.7^{cde}	118.3±11.0^{klm}
22	OL-1854	21.4±3.7^{opqr}	267.7±14.8^{efghi}	116.1±14.4^{b}	116.2±8.9^{lm}	Mean		26.8	243.5	52.4	154.8

Values are mean ± S.D of triplicates
Within a column values followed by different alphabets differ significantly at $p<0.05$

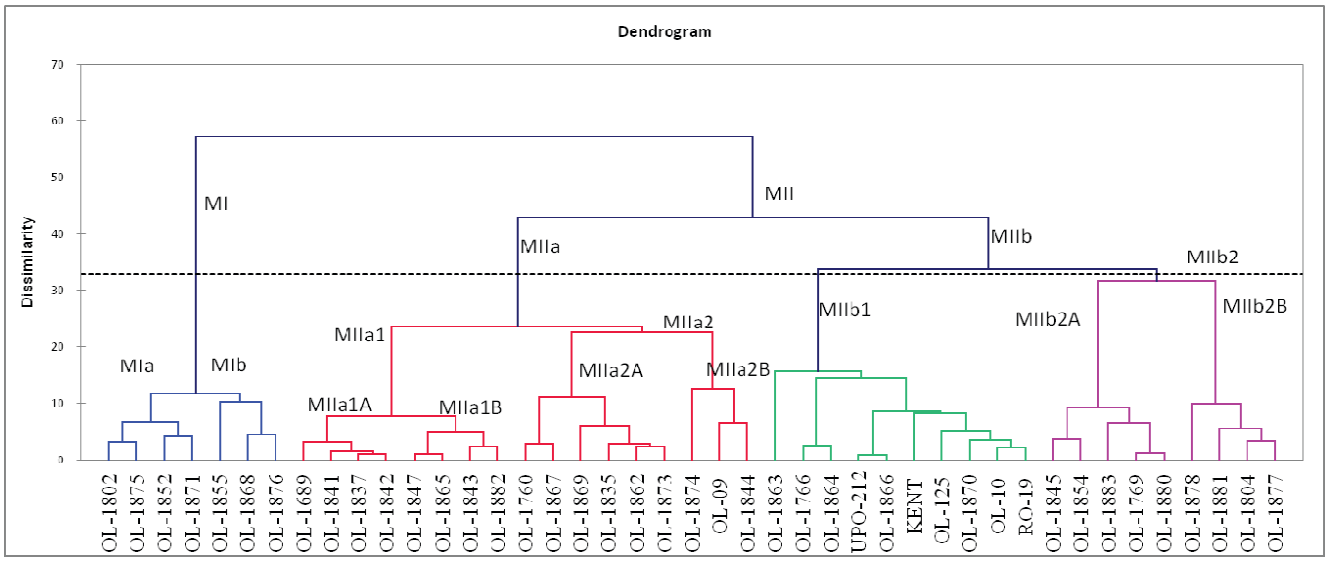

Fig. 1. Clustering of oat genotypes based upon free radical scavenging potential and non-enzymatic antioxidants

Conflicts of interest

The authors declare that they have no conflicts of interest.

LITERATURE CITED

Autran J. Abecassis J & Feillet P 1986. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. *Cereal Chemistry* **63**, 390

Ahmadinejad F, Moller S G, Hashemzadeh-Chaleshtori M, Bidkhori G and Jami M S 2017. Molecular mechanisms behind free radical scavengers function against oxidative stress. *Antioxidants* **6**: 51-65.

Babu B H, Shylesh B S and Padikkala J 2001. Antioxidant and hepatoprotective effects of *Alanthus icicfocus*. *Fitoterapia* **72**: 272-77.

Benzie I F and Strain J J 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. The FRAP assay. *Anal Biochem* **239**: 70-76.

Berger T M, Polidori M C, Dabbagh A, Evans P J, Halliwel B, Morrow J D, Roberts L J and Frei B 1997. Antioxidant activity of vitamin C in iron-overloaded human plasma. *J Biol Chem* **272**: 15656-60.

Blois M S 1958. Antioxidant determination by the use of stable free radical. *Nature* **181**: 1199-1200.

Boeck T, D'Amico S, Zechner E, Jaeger H and Schoenlechner R 2018. Nutritional properties of various oat and naked oat cultivars. *J Land Manage Food Environ* **69**: 215-26.

Brindzova L, Certik M, Rapta P, Zalibera M, Mikulajova A and Takacsova M 2008. Antioxidant activity, β-glucan and lipid contents of oat varieties. *Czech J Food Sci* **26**: 163-73.

Chen O, Mah E, Dioum E, Marwaha A, Shanmugam S, Malleshi N, Sudha V, Gayathri R, Unnikrishnan R, Anjana R M and Krishnaswamy K 2021. The role of oat nutrients in the immune system: A narrative review. *Nutrients* **24**, 1048.

Chu Y F, Wise M L, Gulvady A A, Chang T, Kendra D F, Klinken B J W V, Shi Y and Oshea M 2013. *In vitro* antioxidant capacity and anti-inflammatory activity of seven common oats. *Food Chem* **139**: 426-31.

Debnath T, Kim E K, Das G, Deb Nath N C and Lee K G 2019. Protective effect of oat (*Avena sativa*) bran extracts on acute hepatic liver damage in mice. *Food Agric Immunol* **30**: 34-46.

Ferreira I C F R, Baptista P, Vilas-Boas M and Barros L 2007. Free radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal. *Food Chem* **100**: 1511-16.

Fu J, Htar T, Silva L, Tan D M and Chuah L 2017. Chromatographic separation of vitamin E enantiomers. *Molecules* **22**: 233-50.

Gangopadhyay N, Hossain M B, Rai D K and Brunton N P 2015. Optimisation of yield and molecular weight of β-glucan from barley flour using response surface methodology. *J Cereal Sci* **62**: 38-44.

Ghasemzadeh A, Jaafar H Z E, Juraimi A S and Tayebi-Meigooni A 2015. Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of hashemi rice bran. *Molecules* **20**: 10822-38.

Granger D N and Kvietys P R 2015. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol* **6**: 524-51.

Gulcin I 2012. Antioxidant activity of food constituents: An overview. *Arch Toxicol* **86**: 345-91.

Gurer-Orhan H, Karaaslan C, Ozcan S, Firuzi O, Tavakkoli M, Saso L and Suzen S 2016. Novel indole-based melatonin analogues: Evaluation of antioxidant activity and protective effect against amyloid β-induced damage. *Bioorg Med Chem* **24**: 1658-64.

Habib S and Ali A 2011. Biochemistry of nitric oxide. *Indian J Clin Biochem* **26**: 3-17.

Imlay J A 2003. Pathways of oxidative damage. *Annu Rev Microbiol* **57**: 395-418.

Ivanisova E, Ondrejovic M and Silhar S 2012. Anioxidant activity of milling fractions of selected cereals. *Nova Biotechnol Chimica* **11**: 45-56.

Kahkonen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, Kujala T S and Heinonen M 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* **47**: 3954-39.

Kaur J, Kaur A, Thind S S and Aggarwal P 2014. A comparison of physico-chemical properties, total phenolic content and antioxidant activity of whole grain flours. *Int J Sci Technol* **2**: 82-5.

Kaur S, Bhardwaj R D, Kapoor R and Grewal S K 2019. Biochemical characterization of oat (*Avena sativa* L.) genotypes with high nutritional potential. *LWT Food Sci Technol* **110**: 32-39.

Kayden H J, Chow C and Bjornson L K 1973. Spectrophotometric method for determination of tocopherol in red blood cells. *J Lipid Res* **14**: 533-40.

Kim M J, Hyun J N, Kim J A, Park J C, Kim M Y, Kim J G, Lee S J, Chun S C and Chung I M 2007. Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm. *J Agric Food Chem* **55**: 4802-09.

Krishnanunn K, Ramaiah S and Anbarasu A 2014. Total phenolic content and *in vitro* antioxidant assay of two medicinal rice varieties Karungkavuni and Kuzhiadichan. *Int J Pharm Bio Sci* **5**: 540-48.

Law M Y, Charles S A and Halliwell B 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts: The effect of hydrogen peroxide and of paraquat. *Biochemical J* **210**: 899-903.

Lee S T, Panter K E, Pfister J A, Gardner D R and Welch K D 2008. The effect of body condition on serum concentrations of two tetratogenic alkaloids (anagyrine and ammodendrine) from lupines (*Lupinus* species) that cause crooked calf disease. *Anim Sci* **86**: 2771-78.

Li J, Chen Z, Guan X, Liu J, Zhang M and Xu B C 2008. Optimization of germination conditions to enhance hydroxyl radical inhibition of water-soluble protein from stress millet. *J Cereal Sci* **48**: 619-24.

Liu R H 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *J Nutr* **134**: 3479-85.

Liu Z, Liu Y, Pu Z, Wang J, Zheng Y, Li Y and Wei Y 2013. Regulation, evolution, and functionality of flavonoids in cereal crops. *Biotechnol Lett* **35**: 1765-80.

Loganayaki N, Siddhuraju P and Manian S 2013. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J Food Sci Technol* **50**: 687-95.

Mansoor S, Ali Wani O, Lone J K, Manhas S, Kour N, Alam P, Ahmad A and Ahmad P 2022. Reactive oxygen species in plants: From source to sink. *Antioxidants* **11**: 225.

Marcocci L, Maguire J J, Droy-Lefaix M T and Packer L 1994. The nitric oxide scavenging property of *Ginko biloba* extract EGB 761. *Biochem Biophys Res Commun* **201**: 748-55.

Marklund S and Manklund G 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47**: 469-74.

Matros A, Peshev D, Peukert M, Mock H-P and Van den Ende W 2015. Sugars as hydroxyl radical scavengers: proof-of-concept by studying the fate of sucralose in Arabidopsis. *Plant J* **82**: 822-39.

Multari S, Pihlava J M, Ollennu-Chuasam P, Hietaniemi V, Yang B and Suomela J P 2018. Identification and quantification of Avenanthramides and free and bound phenolic acids in eight cultivars of husked Oat (*Avena sativa* L) from Finland. *J Agric Food Chem* **66**: 2900-08.

Murakami Y, Kawata A, Katayama T and Fujisawa S 2015. Anti-inflammatory activity of the artificial antioxidants 2-tert-butyl-4-methoxyphenol (BHA), 2,6-di-tert-butyl-4-methylphenol (BHT) and 2,4,6-tri-tert-butyl phenol (TBP), and their various combinations. *In Vivo* **29**: 197-206.

Peterson D M, Jensen C M, Hoffman D L and Mannerstedt-Fogelfors B 2007. Oat tocols: Saponification vs direct extraction and analysis in high-oil genotypes. *Cereal Chem* **84**: 56-60.

Philips A, Philip S, Arul V, Padmakeerthiga B, Renju V and Sethupathy S 2010. Free radical scavenging activity of leaf extracts of *Indigofera aspalathoides*- an *in vitro* analysis. *J Pharm Sci Res* **2**: 322-28.

Pourcel L, Routaboul J M and Cheynier V 2007. Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends Plant Sci* **12**: 29-36.

Putchala M C, Ramani P, Sherlin H J, Premkumar P and Natesan A 2013. Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity - A systematic review. *Arch Oral Biol* **58**: 563-74.

Rychter A M, Hryhorowicz S, Słomski R, Dobrowolska A and Krela-Kaźmierczak I 2022. Antioxidant effects of vitamin E and risk of cardiovascular disease in women with obesity - A narrative review. *Clin Nutri* **41**: 1557-65.

Salawu S O, Bester M J and Duodu K G 2014. Phenolic composition and bioactive properties of cell wall preparations and whole grains of selected cereals and legumes. *J Food Biochem* **38**: 62-72.

Samanta A, Das G and Das S K 2011. Roles of flavonoids in plants. *Int J Pharm Sci Tech* **6**: 12-35.

Sang S and Chu Y 2017. Whole grain oats, more than just a fiber: Role of unique phytochemicals. *Mol Nutr Food* Res **1**: 1-12.

Shim S I, Jun W J and Kang B H 2003. Evaluation of nutritional and antinutritional components in Korean wild legumes. *Plant Foods Human Nutr* **58**: 1-11.

Singh S, Kaur M, Sogi D S and Purewal S S 2019. A comparative study of phytochemicals, antioxidant potential and *in-vitro* DNA damage protection activity of different oat (*Avena sativa*) cultivars from India. *J Food Meas Charact* **13**: 347-56.

Sreeramulu D, Reddy C V K and Raghunath M 2009. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. *Indian J Biochem Biophys* **46**: 112-15.

Sterna V, Zute S and Brunava L 2016. Oat grain composition and its nutrition benefice. *Agri Agric Sci Procedia* **8**: 252-56.

Swain T and Hillis E 1959 The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. *J Sci Food Agric* **10**: 3-8.

Takahashi A and Ohnishi T 2004. The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the internal space station. *Biol Sci Space* **18**: 255-60.

USDA 2005. http://www.health.gov/dietaryguidelines/dga2005/document/html/appendix.htm#appB.

Vadivel V, Stuetz W, Scherbaum V and Biesalski H K 2011. Total free phenolic content and health relevant functionality of Indian wild legume grains: Effect of indigenous processing methods. *J Food Comp Anal* **24**: 935-43.

Viacava G E, Gonzalez-Aguilar G and Roura S I 2014. Determination of phytochemicals and antioxidant activity in butterhead lettuce related to leaf age and position. *J Food Biochem* **38**: 352-62.

Wahed S H A, Al-Eidani T A and Jasim A H 2019. Effect of antioxidant reagent and silicon spraying on phenol, flavonoid and anthocyanin in oat varieties. *Indian J Ecol* **46**: 175-79.

Yamauch R 1997. Vitamin E: Mechanism of its antioxidant activity. *Food Sci Technol Int* **3**: 301-09.

Ye H, Zhou C H and Sun Y 2009. Antioxidant activities *in vitro* of ethanol extract from brown seaweed *Sargassum palladium*. *Eur Food Res Technol* **230**: 101-09.

Zhishen J, Mengcheng T and Jianming W 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**: 555-59.