Research Article

Assessment of Total Phenol Content, Total Flavonoid Content and Anti-Oxidant Capacity in Exotic Lentil Germplasm

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Abstract

Lentil is a rich source of proteins, macronutrients, micronutrients (iron, zinc and selenium) and bioactive phytochemicals. Twenty five lentil exotic germplasm lines were assessed for total phenol content (TPC), total flavonoid content (TFC) and anti-oxidant capacities. Lentil genotypes showed considerable variations for their TPC, TFC and anti-oxidant capacities. TPC ranged from 4.50 (IG129294) to 22.76 (IG134342) mg GAE g^{-1} with average value of 8.29 mg GAE g^{-1}. Total flavonoid content and anti-oxidant capacity using Ferric ion Reducing Antioxidant Power (FRAP) assay varied between 1.89 mg QE/g (IG129309) to 22.93 mg QE/g (IG195) and 2.78 µmole trolox eq/gm (IG129185) to 13.47 µmole trolox eq/gm (IG134327), respectively. Based on this study it can be concluded that lentil is a potent source of natural antioxidants for neutraceutical industries. The study can further pave way for lentil breeders, nutritionists, food scientists and food industries making use of lentil genotypes to suit their purposes.

Keywords: Lentil, anti-oxidant capacity, total phenol content, total flavonoid content

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Introduction

Lentil (*Lens culinaris* Medikus. *culinaris*) is a cool season nutritious legume indigenous to South Western Asia and the Mediterranean region. Estimated haploid (2n= 2x= 14) genome size of lentil is 4 Gbp [1]. Lentil is cultivated worldwide covering 4.52 million ha area, producing 4.82 million tons of harvest with an average yield of 1.06 ton/hectare [2]. In India lentil was grown on 1.8 million hectares of harvest area leading to production of 1.1 m tons [2]. The crop is mainly grown in arid to semi-arid regions of Central and Eastern India, of which major states are Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and West Bengal. Lentil being rich in dietary protein, carbohydrates, micronutrients (iron, zinc, selenium) and vitamins, provide balanced nourishment to human. In addition to high nutritive value, lentil possesses various bioactive compounds like phenols, flavonoids which play important role as natural anti-oxidants. These bioactive compounds are also called as phytochemicals and have health beneficial effects.

Reactive oxygen species (ROS) and free radicals are generated within cells as a result of normal aerobic respiration [3] and several metabolic processes or from external sources such as X-rays exposure, ozone, smoking, pollutants and industrial chemicals. Though low level of ROS are essential for regulation of normal physiological functions [4], ROS generated in excess leads to peroxidation of membrane lipids, fats, proteins and several biological molecules. ROS thus causes several major human ailments including stroke, diabetes, rheumatoid arthritis, cancer, cardiovascular disease, osteoporosis, degenerative disease and senescence processes [5]. Antioxidant compounds efficiently neutralize free radicals; act as reducing and metal-chelating agents and quenches singlet & triplet oxygen [6]. Natural antioxidants are gaining importance among researchers as synthetic antioxidants impose several side effects. Natural antioxidant substances prevent cell damage by reacting with free radicals and also prevent deterioration of food in food industries.

Several studies have shown that phenols comprising phenolic acids, flavonoids and anthocyanins act as antioxidant, antimutagenic and anticarcinogenic [7]. Phenols reduce the risk of cardiovascular diseases, diabetes, cancer and stroke and also exhibit anti-inflammatory and anti-allergic effect [8]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radical, quenching singlet and triplet oxygen, decomposing hydrogen peroxides [9]. Hence, the biomedical scientists and nutritionists are interested in natural antioxidants which can protect body against damage by reactive oxygen species. Phenols in higher concentration act as chelating agent thereby reducing bioavailability of micronutrients [10]. Flavonoids are another most common group of secondary metabolites in plant system. Pharmacological effects of flavonoids include anti-cancer [11], anti-diabetic [12], obesity preventive [13] and

inhibitor of neurodegenerative diseases [14]. The study was undertaken to assess total phenolic content, total flavonoid content and antioxidant potential of lentil genotypes.

Materials and Methods

Plant material

Twenty five exotic lentil genotypes were assessed for total phenolic content, total flavonoid content and anti-oxidant potential. Material was planted at Experimental Farm, Division of Genetics, IARI, New Delhi, JNKVV RRS, Sagar and RAK, Sehore during 2015-16. Experiment was conducted in randomized block design with row to row spacing of 25 cm and plant to plant spacing of 5 cm. Material was sown in the mid of November 2015 and harvested in mid-April 2016. Recommended package of practices were followed. 10 grams of working sample were cleaned and stored for analysis of various parameters.

Sample preparation

Harvested grains were dried and finely grounded using electric grinder. Fatty substances of powdered grains were percolated with n-hexane for about 8 hours. After de-fattening two grams powder was extracted using 30 ml of 80% ethanol for 3 hours**.** Extracts were sonicated for 30-40 seconds and then further centrifuged at 3000 rpm for 15 minutes. Supernatant was collected and stored at 5°C for analysis.

Determination of phenolic content

Phenolic content were assessed according to Folin- Ciocalteu method as per Singleton *et al*. [15] using gallic acid as standard. 0.5 ml aliquot was diluted with 2.5 ml of distilled water in test tubes. 1:1 diluted Folin–Ciocalteau reagent was added further. 2 ml of Na_2CO_3 (20% w/v) was mixed after 3 minutes to each test tubes. Blank was prepared similarly with the same solvents but omitting the extract. Absorbance of supernatant solution and blank was read at 750 nm using Shimadzu UV-Vis spectrophotometer. The concentration of phenol was multiplied by dilution factor. Obtained results were expressed as equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

Determination of total flavonoid content

The aluminium chloride colorimetric method [16] was used. One ml of extract was taken in test tubes containing 1.4 ml of double distilled water. Blank was prepared using doubled distilled water instead of sample extract. 0.3 ml 5% $NaNO_2$ was then added, followed by 0.3 ml 10% $AlCl_3$. 2 ml 1M NaOH was added. Absorbance of solution was measured at 510 nm as against the prepared blank. Total flavonoids were expressed as mg quercetin equivalents per gram of dry mass (mg QE/ g).

Ferric ion Reducing Antioxidant Power assay (FRAP)

Antioxidant potential was assessed using ferric ion reducing antioxidant power method according to Benzie and Strain [17]. 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tri (2-pyridyl)-s-triazine in 40 mM HCL and 20 mM $FeCl_3$ was added in a ratio of 10:1:1 to prepare FRAP reagent. 0.1 ml of sample extract was taken in test tubes and 3 ml of freshly prepared FRAP reagent was added. Absorbance value was read as against blank at 593 nm after 10 min incubation at room temperature.

Statistical analysis

All assays or tests were conducted in triplicate; data were analyzed statistically and expressed as mean ± standard deviation. One way analysis of variance was used to compare the means. F-test was performed to study any significant differences between the means. $P<0.05$ was considered significant. Correlation analysis of total phenolic content, antioxidant activity and total flavonoid content were also performed using Pearson's correlation coefficient by Microsoft Excel data analysis (v.2007) tool pack software.

Results and Discussion

Statistical values on total phenolic content, total flavonoid content and antioxidant potential (FRAP method) of 25 lentil genotypes is presented in **Table 1**. Wide variation was recorded for the studied traits. The histogram for the frequency distribution of TPC, TFC and anti-oxidant potential in lentil germplasm were represented in **Figure 1**.

Table 1 Mean values of studied lentil genotypes for total phenol content, total flavonoid content and anti-oxidant potential (FRAP)

Sr. No.	Genotype	Mean (TPC)	Mean (TFC)	Mean (FRAP)
1	IG112131	7.20	7.01	8.25
2	IG115	5.82	4.77	10.86
3	IG129185	9.46	13.31	2.78
4	IG129214	6.54	8.14	7.42
5	IG129287	6.10	13.56	3.50
6	IG129291	5.42	11.54	3.58
7	IG129293	7.92	12.39	10.41
8	IG129294	4.50	11.64	4.19
9	IG129302	6.20	12.87	5.86
10	IG129304	6.12	16.16	11.92
11	IG129309	7.58	1.89	9.60
12	IG129313	7.61	4.92	8.18
13	IG129317	8.09	15.28	6.48
14	IG129337	7.62	15.75	8.42
15	IG129560	5.38	12.96	8.43
16	IG130033	8.87	6.84	10.44
17	IG134327	9.70	9.95	13.47
18	IG134342	22.76	15.36	3.68
19	IG134656	8.62	8.86	9.66
20	IG136607	10.64	14.57	3.35
21	IG159291	5.34	16.51	7.13
22	IG195	18.29	22.93	5.97
23	IG282863	7.43	11.94	10.03
24	IG29302	6.85	3.26	9.67
25	IG334	7.29	8.53	8.26

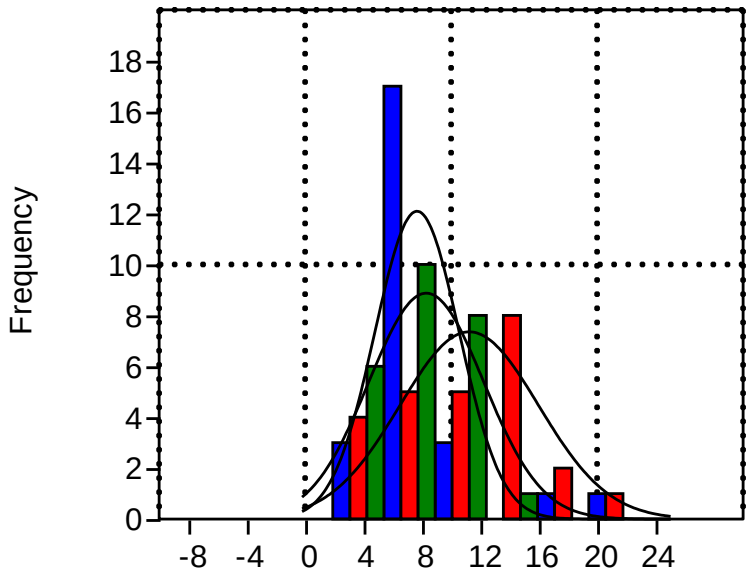

Figure 1 Histogram for the frequency distribution of Total Phenol Content (blue bars), Total Flavonoid content (green bars) and anti-oxidant potential (red bars) in lentil germplasm

Total Phenolic Content

Lentil genotypes have shown wide range of variation for total phenolic content (TPC). TPC varied from 4.50 (IG129294) to 22.76 (IG134342) mg GAE g^{-1} whereas average value for TPC was recorded to be 8.29 mg GAE g^{-1} (**Table 2**). High TPC (> 9 mg GAE/gm) was reported for IG134342, IG195, IG136607, IG134327 and IG129185 (Table 1). Genotypes having low TPC (< 6 mg GAE/gm) were IG129294, IG159291, IG129560, IG129291 and IG115 (Table 1). Wide variations for TPC have been reported [18] using ethanolic seed extracts and in different

solvent (hexane, methanol and acetone) extractions [19]. Similar results were reported by other researchers [20, 21]. Phenolics are regarded as anti-nutritional as they reduce protein digestibility and mineral bioavailability yet have the ability to serve as antioxidants [22]. Therefore low phenol containing genotypes can be utilized in development of lentil varieties with higher mineral bioavailability and improved nutritional attributes. Genotypes with high phenols can be preferred by food industries and development of disease resistant varieties.

Table 2 Statistical values (mean, standard error, standard deviation, variance, minimum, maximum, skewness and kurtosis values) for TPC, TFC and antioxidant potential (FRAP)

Traits	Mean	SE	SD	Variance	Minimum	Maximum	Skewness	Kurtosis
TPC (mg GAE g-1)	8.29	0.80	4.02	16.16	4.50	22.76	2.68	7.71
TFC (mg QE/100 g)	11.23	0.97	4.85	23.54	1.89	22.93	0.03	0.16
FRAP (micromole trolox eq/gm)	7.66	0.59	2.95	8.70	2.78	13.47	-0.11	-0.81

Total Flavonoid Content

Flavonoids are efficient free radical scavengers and thus possess good antioxidant property. The TFC of 25 lentil genotypes varied between 1.89 mg QE/g to 22.93 mg QE/g, with an average value of 11.23 mg QE/g (Table 2). The TFC of IG195 was highest (22.93 mg QE/g), followed by IG159291 (16.51 mg QE/g), IG129304 (16.16 mg QE/g), IG129337 (15.75 mg QE/g), IG134342 (15.36 mg QE/g), IG129317 (15.28 mg QE/g), and IG136607 (14.57 mg QE/g) (Table 1). Genotypes IG129309, IG29302, IG115, IG129313 and IG130033 exhibited least flavonoid content with values of 1.89, 3.26, 4.77, 4.92 and 6.84 mg QE/g, respectively (Table 1). The results are in agreement with previous findings [23, 24]. Plant extracts exhibiting high total phenol content show high total flavonoid content [25]. Correlation between TPC and TFC was positive and moderate (0.39) in this study. The variability for total flavonoid content can be tapped by farmers and breeders to develop cultivars with lower flavonoid content increasing the bioavailability of minerals from the food. Food industry may make use of lentil genotypes with high flavonoid content for manufacturing functional foods or nutraceutical for promoting consumer health.

Ferric ion Reducing Antioxidant Power assay (FRAP)

Anti-oxidant capacities of sample can be measured by various methods *viz.* 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, Cupric Reducing Antioxidant Capacity (CUPRAC), Ferric thiocyanate (FTC) method, Oxygen Radical Absorption Capacity (ORAC) assay and Ferric ion Reducing Antioxidant Power (FRAP) assay. In this study FRAP assay has been employed to evaluate the anti-oxidant capacities of lentil extracts. The antioxidant compounds donate H+ to ferric tripyridyltriazine (Fe3+-TPTZ) complex interrupting radical chain reaction. High FRAP value indicates higher anti-oxidant capacities of the extract. Mean values obtained for lentil genotypes have been given in table 2. Genotype IG134327 exhibited highest FRAP value (13.47 µmole trolox eq/gm) while IG129185 had the lowest value (2.78 µmole trolox eq/gm). Average FRAP value of 25 lentil genotypes recorded was 7.66 µmole trolox eq/gm (Table 2). Genotypes IG129304 (11.92 µmole trolox eq/gm), IG115 (10.86 µmole trolox eq/gm), IG130033 (10.44 µmole trolox eq/gm), IG129293 (10.41 µmole trolox eq/gm), IG282863 (10.03 µmole trolox eq/gm), IG29302 (9.67 µmole trolox eq/gm), IG134656 (9.66 µmole trolox eq/gm) and IG129309 (9.60 µmole trolox eq/gm) showed significantly higher FRAP values and thus anti-oxidant capacities (Table 1). Wide range for FRAP values has also been reported [18]. Negative correlation between FRAP and total phenol content (- 0.24) and FRAP and total flavonoid content (- 0.40) was observed in this study. In contrast, antioxidant activity as measured by the FRAP assay was found positively correlated between TPC and FRAP in lentil [18]. Positive and strong correlation of FRAP with TPC and TFC was reported in lentil [24].

TPC, TFC and anti-oxidant capacities were initially regarded as anti-nutritional factors. However in the recent years their neutraceutical properties are being identified. For enhancing the bioavailability of minerals in lentil exotic genotypes exhibiting low TPC, TFC and anti-oxidant capacities may be utilized by lentil breeders. Nutritionists and food technologists might be interested in genotypes exhibiting high TPC, TFC and anti-oxidant capacities.

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