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Nutritional profiling and antioxidant assessment of Indian and exotic accessions of lentil

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Abstract

A study involving a very diverse set of 96 lentil (*Lens culinaris* Medikus. *culinaris*) genotypes including germplasm lines from India and ICARDA, Mediterranean landraces, advanced breeding lines and released varieties from India was conducted to evaluate the genotypes for protein and zinc contents, anti-nutritional traits (phenol and flavonoid) and antioxidant capacity to identify the promising lines. ANOVA indicated highly significant variations for the studied traits among the genotypes. Protein and zinc contents were recorded in the range of 18.61 to 27.77% and 27.4 to 87.3 mg/kg with an average value of 24.29% and 56.83 mg/kg, respectively. Considerable variations were recorded for total phenolic content which ranged from 4.52 to 21.67 mg GAE/ g. The total flavonoid content varied from 2.65 to 23.48 mg QE/g. Genotypes IC 262839, P 8115, LC 282896 recorded high protein and zinc contents and good antioxidant capacity with low to medium total phenol and flavonoid contents. Among the released varieties, PL04, PL06 and PL02 showed very good nutritional value and modest antioxidant capacity. Interestingly, protein content was found negatively correlated with anti-oxidant capacity (r = -0.22) and total flavonoid content (r = -0.15), whereas total flavonoid content was found positively correlated with anti-oxidant capacity (r = 0.55) and total phenol content (r = 0.24) but negatively correlated with zinc content (r = -0.12). The promising accessions identified in this study can be utilized for developing nutritionally rich lentil genotypes and varieties with balanced anti-nutritional profile to serve the resource poor and health-conscious population.

Keywords: Lentil, protein content, zinc content, phenolic content, flavonoid content, antioxidant capacity

Introduction

Lentil (*Lens culinaris* Medikus. *culinaris*) is a temperate food legume contributing towards human nutritional requirements since ancient times. Lentil occupies 6.10 million hectares of area to yield 6.33 million tons of produce globally (FAOSTAT 2018). Lentil stands as fourth most important grain legume being consumed in more than 100 countries. Lentil grains are rich source of quality protein, carbohydrates, micronutrients (iron, zinc, selenium and â-carotene), vitamins (Taleb et al. 2013). Lentil is considered nutritious legume owing to abundant macro- and micronutrients along with favorable proportions of mineral bioavailability promoting factors. Besides high nutritional value, lentil possesses various bioactive phytochemicals like polyphenols and flavonoids.

Protein is indispensable macronutrient which is irreplaceable by any other nutrients as nitrogen and sulfur are offered exclusively by amino acids. Protein performs structural (collagen), hormonal (insulin), carriers (hemoglobin), and enzymatic (amylase) functions. Protein malnutrition causes metabolic disorders (kwashiorkor and marasmus), edema, loss of muscle mass, stunting, skin lesions in humans. Phenolic compounds form protein-polyphenol complexes thereby reduce digestibility of proteins, bio-availability of amino acids and inhibit enzyme activity (Velickovic and Stanic-Vucinic 2018). Zinc is an important micronutrient and essential trace mineral in human diet. Zinc is essential for cell division, DNA

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and protein synthesis (Hotz and Brown 2004). Zinc is required for metabolic activity of several enzymes of human body. Despite of undisputable significance, zinc deficiency is prevalent worldwide affecting approximately two billion global populations (Prasad 2003). Zinc deficiency leads to attenuated immune system, reproductive abnormalities, skin disorders, retarded bones, neuro-sensory dysfunction, growth retardation, low blood pressure, diarrhoea, etc. (Hambidge and Walravens 1982). Pregnant women face stillbirths, premature labour and miscarriages and other pregnancy related complications due to zinc deficiency.

Phenolic compounds are bioactive phytochemicals possessing aromatic ring bearing at least one hydroxyl group. Around 8000 naturally occurring plant phenolics have been reported (Harborne and Harbert, 1993). Flavonoids are the phenolic compounds reported from different plant parts in free state or as glycosides. Flavonoids possess two benzene rings separated by a propane unit. The antioxidant activity of plant product is mainly due to the presence of phenols (Okpuzar et al. 2009). Marinova et al. (2005) reported anticarcinogenic and antimutagenic activities of phenolic compounds in addition to antioxidant activity.

Bioactive phytochemicals were historically described as anti-nutritional (Champ 2002) as phenolic compounds play important role towards metal chelation. Flavonoids also act as chelating agent and encapsulate several minerals including zinc rendering reduced bio-availability of micronutrients (Kulbat 2016). In past few decades bioactive phytochemicals have been demonstrated to possess antioxidant activity imparting potential health benefits (Yeh and Yen 2003; Gupta et al. 2011). Human body undergoes production of free radicals and reactive oxygen species (ROS) through normal metabolic activities and exposure to radiation/ pollutants. ROS when accumulated in excess, initiates peroxidation of biological molecules resulting in the etiology of several human ailments including cancer, cardiovascular diseases, stroke, diabetes, arthritis and accelerated senescence (Menezes-Benavente et al. 2004). Antioxidant compounds act as free radical scavengers countering ROS produced within the cells. Readily available synthetic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxyltoluene (BHT) and 2-tert-butyl-4-methylphenol (TBMP) cause liver damage and suspected of being carcinogenic (Ratnam et al. 2006). Consumer awareness on safe source of antioxidant has attracted considerable importance and attention among researchers for natural alternatives to synthetic antioxidants.

Lentil has been gaining interest for health benefits as the crop has highest total phenolic content compared to other pulses (Xu and Chang 2007). Significant correlation between phenolic content and total antioxidant activity has also been established (Oomah et al. 2011) in lentil. The objectives of this study were to assess nutritional quality and antioxidant capacity of lentil accessions. This study would offer scientific reference to plant breeders, biomedical and food scientists, nutritionists and nutraceutical industries for large scale usage of lentil.

Materials and methods

Plant material

Total protein content, zinc content, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (AC) were extensively evaluated in 96 lentil accessions comprising of eight released varieties, 31 advanced breeding lines and 13 germplasm lines of Indian origin, 25 Mediterranean landraces and 19 germplasm lines from ICARDA (Table 1). The genotypes were grown at experimental farm, Division of Genetics, Indian Agricultural Research Institute, New Delhi, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Sagar and RAK, Sehore (Madhya Pradesh) following standard package of practices. Seeds were sorted out manually to remove damaged and immature ones and stored in clean containers. Working sample of 15 grams for each genotype was stored at 4°C for further extraction and analysis.

Preparation of extracts

Harvested lentil seeds were dried in dust free environment and grounded using electric grinder. Five grams of powdered sample of each genotype was percolated using n-hexane (Sigma Aldrich, St. Louis, USA) for 8 h to remove fatty substances. Two grams of defatted powder was extracted in 30 ml of aqueous ethanol (ethanol: water, 80:20 v/v) for 3 h at 25°C**.** Ethanolic extracts were sonicated for 30-40 seconds for better extraction. Sample was centrifuged at 3000 rpm for 15 minutes. Supernatant was collected and stored at 5°C for estimation of TPC, TFC and antioxidant capacity.

Protein estimation and zinc analysis

The total protein content in the seed samples were determined by Kjeldahl method. Protein values were

Table 1. List of lentil accessions along with their origin

S. No.	Genotypes	Category	Source	S. No.	Genotypes	Category	Source	S. No.	Genotypes	Category	Source
1	IC 201704	IGL	NBPGR, New Delhi	33	IG 9	ML	ICARDA	65	LL 461	ABL	PAU, Ludhiana
2	IC 208326	IGL	NBPGR, New Delhi	34	ILL 10832	ML	ICARDA	66	LL 649	ABL	PAU, Ludhiana
3	IC 262839	IGL	NBPGR, New Delhi	35	ILL 108331	ML	ICARDA	67	P 13108	ICG	ICARDA
4	IC 267663	IGL	NBPGR, New Delhi	36	ILL 147	ML	ICARDA	68	P 13129	ICG	ICARDA
5	IC 268248	IGL	NBPGR, New Delhi	37	ILL 2581	ML	ICARDA	69	P 13135	ICG	ICARDA
6	IC 560135	IGL	NBPGR, New Delhi	38	ILL 7663	ML	ICARDA	70	P 13138	ICG	ICARDA
7	IC 560169	IGL	NBPGR, New Delhi	39	L 11-243	ABL	IIPR, Kanpur	71	P 13142	ICG	ICARDA
8	IC 560181	IGL	NBPGR, New Delhi	40	L 11-273	ABL	IIPR, Kanpur	72	P 13143	ICG	ICARDA
9	IC 560206	IGL	NBPGR, New Delhi	41	L 11-279	ABL	IIPR, Kanpur	73	P 15104	ICG	ICARDA
10	IC 560212	IGL	NBPGR, New Delhi	42	L 11-282	ABL	IIPR, Kanpur	74	P 15121	ICG	ICARDA
11	IC 560333	IGL	NBPGR, New Delhi	43	L 11-289	ABL	IIPR, Kanpur	75	P 15127	ICG	ICARDA
12	IC 560372	IGL	NBPGR, New Delhi	44	L 11-291	ABL	IIPR, Kanpur	76	P 16214	ICG	ICARDA
13	IC 560812	IGL	NBPGR, New Delhi	45	L 11-294	ABL	IIPR, Kanpur	77	P 2113	ICG	ICARDA
14	IG 111996	ML	ICARDA	46	L 11-297	ABL	IIPR, Kanpur	78	P 2116	ICG	ICARDA
15	IG 112078	ML	ICARDA	47	L 4076	RV	IARI, New Delhi	79	P 2118	ICG	ICARDA
16	IG 112128	ML	ICARDA	48	L 5253	ABL	IARI, New Delhi	80	P 2125	ICG	ICARDA
17	IG 112131	ML	ICARDA	49	L 7818	ABL	IARI, New Delhi	81	P 2127	ICG	ICARDA
18	IG 115	ML	ICARDA	50	L 7903	ABL	IARI, New Delhi	82	P 3233	ICG	ICARDA
19	IG 129214	ML	ICARDA	51	L 7916	ABL	IARI, New Delhi	83	P 3234	ICG	ICARDA
20	IG 129291	ML	ICARDA	52	L 7920	ABL	IARI, New Delhi	84	P 8112	ICG	ICARDA
21	IG 129302	ML	ICARDA	53	LC 282-1444	ABL	IARI, New Delhi	85	P 8115	ICG	ICARDA
22	IG 129304	ML	ICARDA	54	LC 282-1485	ABL	IARI, New Delhi	86	PL 02	RV	GBPUAT,Pantnagar
23	IG 129317	ML	ICARDA	55	LC 282896	ABL	IARI, New Delhi	87	PL 04	RV	GBPUAT,Pantnagar
24	IG 130033	ML	ICARDA	56	LC 282907	ABL	IARI, New Delhi	88	PL 05	RV	GBPUAT,Pantnagar
25	IG 195	ML	ICARDA	57	LC 300-15	ABL	IARI, New Delhi	89	PL 06	RV	GBPUAT,Pantnagar
26	IG 49	ML	ICARDA	58	LC 300-16	ABL	IARI, New Delhi	90	PL 07	RV	GBPUAT,Pantnagar
27	IG 5320	ML	ICARDA	59	LC 300-17	ABL	IARI, New Delhi	91	PL 08	RV	GBPUAT,Pantnagar
28	IG 569608	ML	ICARDA	60	LC 300-19	ABL	IARI, New Delhi	92	PL 406	RV	GBPUAT,Pantnagar
29	IG 70230	ML	ICARDA	61	LC 74151	ABL	IARI, New Delhi	93	PL 117	ABL	GBPUAT,Pantnagar
30	IG 73798	ML	ICARDA	62	LH 90-57	ABL	CCS, HAU Hisar	94	PL 24	ABL	GBPUAT,Pantnagar
31	IG 73920	ML	ICARDA	63	LL 1122	ABL	PAU, Ludhiana	95	PL 77-12	ABL	GBPUAT,Pantnagar
32	IG 73933	ML	ICARDA	64	LL 147	ABL	PAU, Ludhiana	96	PL 97	ABL	GBPUAT,Pantnagar

IGL = Indian germplasm lines, ML = Mediterranean landraces, ABL = Indian advanced breeding lines, RV = Indian released varieties, ICG- ICARDA germplasm

obtained by multiplying nitrogen percent of sample with a factor of 6.25. Seeds were washed with Milli-Q water to remove the dust and oven dried at 35°C for 5 days. Three grams of grains were grounded into fine powder using mortar and pestle manually. Grounded samples (0.5 g) were digested as per modified diacid protocol (Singh et al. 2005) using a microwave digestion system (Multiwave ECO, Anton Paar, les Ulis, France). Zinc content (in ppm) was measured using atomic absorption spectrometry (Zeeman AAS, Z-Xpress 8000, Germany).

Determination of total phenolic content (TPC)

Total phenolic content of the extract was estimated according to Folin-Ciocalteu method suggested by Singleton et al. (1999), using gallic acid as standard. 500 µL of the sample extract was mixed with 2.5 ml of deionized water, 0.5 ml of Folin-Ciocalteu reagent (Sigma Aldrich, St. Louis, USA) and 2.0 ml of 20% sodium carbonate solution. The mixture was allowed to settle for 90 minutes. The absorbance of supernatant was recorded at 750 nm (Shimadzu UV-VIS spectrophotometer, UV-2600). Absorbance value for blank was measured similarly but replacing sample extract with double distilled water. The concentration of TPC was assessed by plotting the Gallic acid calibration curve and was expressed in terms of milligram of gallic acid equivalent per gram of extract (mg GAE/g).

Determination of total flavonoid content (TFC)

Aluminum chloride colorimetric assay (Patel et al. 2010) was used to estimate total flavonoid content (TFC). 1 ml aliquot was added to test tube and diluted by adding 1.4 ml of double distilled water. 0.3 ml 5% sodium nitrite was added to the test tube, followed by 0.3 ml aluminum chloride (10%). 2 ml 1M sodium hydroxide was added after one minute and the solution was mixed thoroughly. The absorbance was measured at 510 nm (Shimadzu UV-VIS spectrophotometer, UV-2600). Absorbance value for blank was recorded similarly using double distilled water. Total flavonoids content of samples was expressed as mg quercetin equivalents per gram of dry weight (mg QE/ g).

Antioxidant capacity (AC) assay using 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH)

DPPH assay suggested by Brand-Williams et al. (1995) was used to measure free radical scavenging ability of antioxidants towards the stable 2,2'-diphenyl-1-picrylhydrazyl. 0.1 ml of extract was added to 3.9 ml of freshly prepared 80 % ethanolic solution of DPPH (Sigma Aldrich, St. Louis, USA) and mixed. The mixture was shaken and allowed to stand in the dark at 25°C for 30 min. The change in absorbance of the resulting solution (A_{samp}) was measured at 515 nm (Shimadzu UV-VIS spectrophotometer, UV-2600) against control (A_{cont}). The control contained all reagents except the sample. The radical scavenging activities of the sample was expressed in terms of IC_{50} (concentration required for a 50% decrease in absorbance of DPPH radical) relative to the control (100%) and calculated as % inhibition of DPPH. The percentage of DPPH, which was scavenged, was calculated using formulae

$$\% \text{ DPPHsc} = \{(A_{cont} - A_{samp})/ A_{cont}\} \times 100$$

The results were expressed as trolox equivalent DPPH radical scavenging activity per gram of sample (µmol TE/g).

Statistical analysis

Experimental results were documented as mean of three locations. Correlations among the studied traits were estimated using Pearson's correlation coefficient by Microsoft Excel data analysis (*v*.2007) tool pack software.

Table 2. Mean, minimum and maximum values, standard deviation (SD) and coefficient of variation (CV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and broad sense heritability (H^2) for total protein content, zinc content, TPC, TFC and AC of 96 lentil accessions

Variable	Mean	SD	CV	Minimum	Maximum	GCV (%)	PCV (%)	H^2 (%)
Protein (%)	24.29	2.09	8.60	18.61	27.77	14.90	15.00	99.63
Zn (mg/kg)	56.83	11.92	20.97	27.4	87.3	36.18	36.32	99.30
TPC (mg GAE/g)	8.56	3.43	39.99	4.52	21.67	69.23	69.23	99.95
TFC (mg QE/g)	11.91	4.79	40.21	2.65	23.48	69.60	69.60	99.96
AC (µmol TE/g)	3.75	1.87	49.86	0.46	10.04	86.13	86.13	99.92

Results

The descriptive statistics (mean, minimum, maximum, standard deviation and coefficient of variance, genotypic coefficient of variance, phenotypic coefficient of variance and broad-sense heritability) for total protein content, zinc content, TPC, TFC and AC are presented in Table 2. Analysis of variance showed significantly high variability among the studied material for all evaluated traits (Table 3).

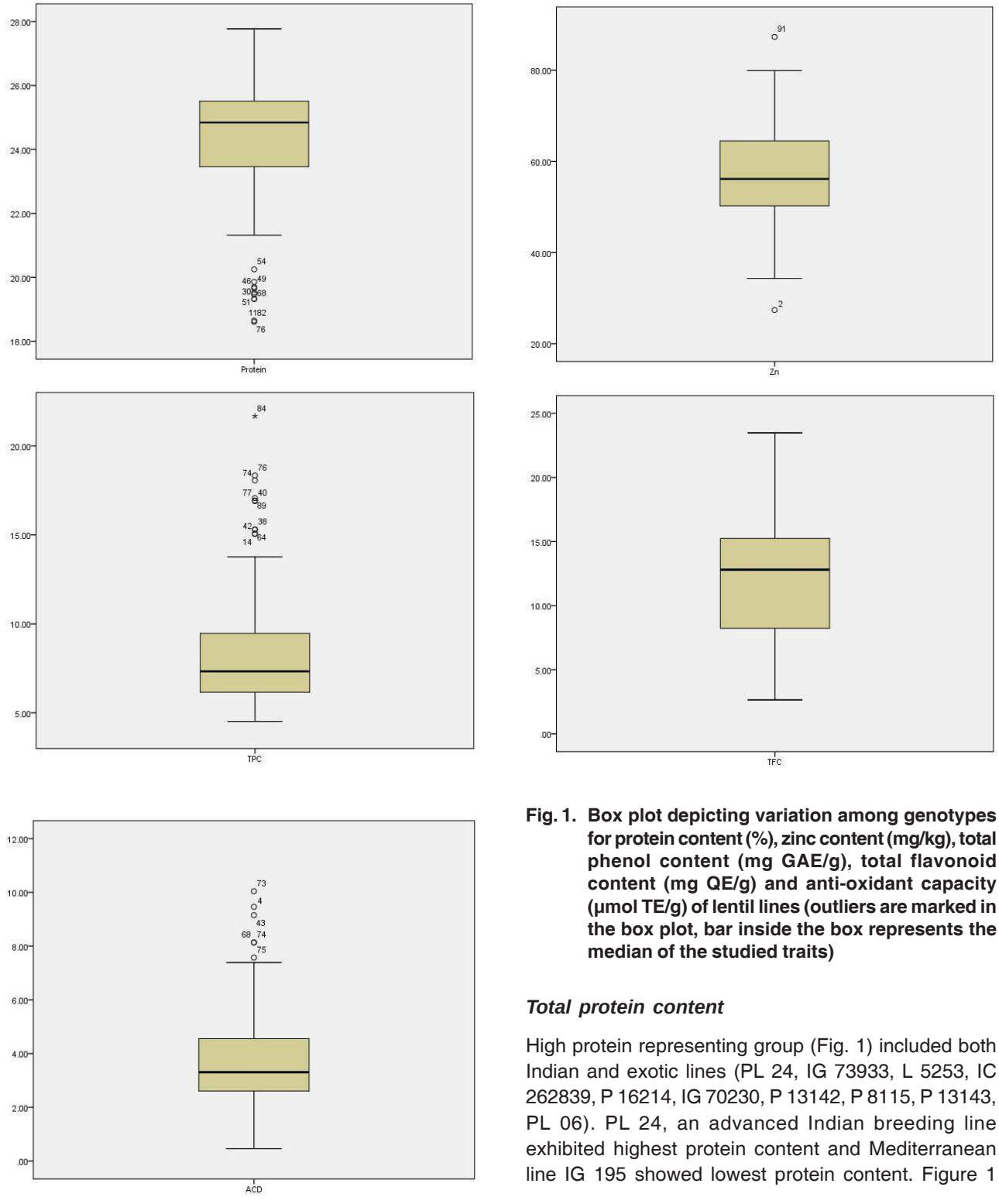

Fig. 1. Box plot depicting variation among genotypes for protein content (%), zinc content (mg/kg), total phenol content (mg GAE/g), total flavonoid content (mg QE/g) and anti-oxidant capacity (µmol TE/g) of lentil lines (outliers are marked in the box plot, bar inside the box represents the median of the studied traits)

Total protein content

High protein representing group (Fig. 1) included both Indian and exotic lines (PL 24, IG 73933, L 5253, IC 262839, P 16214, IG 70230, P 13142, P 8115, P 13143, PL 06). PL 24, an advanced Indian breeding line exhibited highest protein content and Mediterranean line IG 195 showed lowest protein content. Figure 1

Table 3. Pooled ANOVA for protein content, zinc content, total phenol content (TPC), total flavonoid content (TFC) and anti-oxidant capacity (AC) of 96 genotypes over three locations

Source of variation	DF	Mean sum of squares				
		Protein	Zinc	TPC	TFC	AC
Genotype	95	26.44**	851.84**	70.62**	137.72**	20.90**
Environment	2	8.455*	131.74*	6.32*	4.27*	1.26*
Genotype x Environment	190	13.18**	92.46**	2.65**	5.40**	0.60**
Replication within Environment	3	0.14	9.29	0.59	0.38	0.29
Pooled Error	285	0.96	5.93	0.29	0.50	0.15

depicts high variability for protein content, which can be further utilized in development of protein rich lentil varieties. Total protein content in lentil genotypes ranged from 18.61 (IG 195) to 27.77% (PL 24) with an average value of 24.29% (Table 2). Among the released varieties PL 06 had highest protein content (26.52 %) while rest were grouped under average protein containing lines.

Zinc content

Zn is an essential micronutrient for optimal physiological function. Zn deficiency leads to stunting, crippled immune system, dysfunctioning of central nervous system and epidermal disorders (Hambidge and Walravens 1982; Prasad 1991). Zinc content in lentil genotypes varied significantly from 27.4 (P 15104) to 87.3 (P 8115) mg/kg with an average value of 56.83 mg/kg (Table 2). Genotypes P 8115, P 3234, LL 461, IC 560812, LC 282896, IC 262839 and IC 560135 exhibited higher (>75 mg/kg) zinc content (Fig. 2). Among released varieties L 4076 (70.4 mg/kg) and PL 04 (68.3 mg/kg) were reported having higher Zn content whereas rest were moderate zinc containing lines except for PL 08 (43.2 mg/kg). Figure 2 depicts high variability for zinc content in the assayed lines with the scope of their incorporation in breeding programs targeting enhancement of micronutrients in lentil.

Total phenolic content (TPC)

TPC in lentil exhibited wide array of variation ranging from 4.52 (IC 560812) to 21.67 (LC 282-1485) mg GAE/g (Fig. 3). Average value for TPC was recorded as 8.58 mg GAE/g (Table 2). Genotypes IC 560812, IC 560372 and IG 9 were the least (< 5 mg GAE/gm) while LC 282-1485, IG 195, LC 300-17 and IG 5320 were the highest (>17 mg GAE/gm) TPC containing lines. Among released varieties PL 05 was low, PL 07, PL 08, PL 06 were high whereas PL 02, PL 406, PL 04, L 4076 were medium phenol containing lines. Medium to high phenol content among released varieties call for attention of lentil breeders engaged with developing biofortified varieties.

Total flavonoid content (TFC)

ICARDA and Indian germplasm lines P 3234, P 13108, IC 560206, IC 208326 and P 13135 showed least whereas exotic lines IG 195, P 3233, IG 5320, IG 111996, IG 73798, P 2125 exhibited higher (>18 mg QE/g) flavonoid content. Flavonoid content in lentil accessions varied considerably from 2.65 to 23.48 mg QE/g with mean value of 11.91 mg QE/g (Table 2). Released varieties PL 04, PL 05, PL 06, L 4076 and PL 406 had low to intermediate TFC. Three released varieties PL 07 (15.41 mg QE/g), PL 02 (16.28 mg QE/g) and PL 08 (17.26 mg QE/g) were high TFC lines. High variability for TFC can be exploited for manipulation of flavonoid content in released varieties (Fig. 4).

Antioxidant capacity

DPPH based antioxidant assay relies upon the capability of an antioxidant to donate hydrogen or electron to DPPH radical. As the odd electron pairs in the presence of free radical scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form (Paixao et al. 2007). AC exhibited by seed extracts varied from 0.46 (IG 73920) to 10.04 (L 11-282) µmol TE/g. Mean AC value was 3.75 µmol TE/g (Table 2). Some of the advanced breeding Indian lines L 11-282, LC 74151, LC 300-17, L 11-273 recorded higher (>7 µmol TE/g) AC values (Fig. 5). Released varieties were intermediate in terms of antioxidant capacity except for PL 05 and L 4076 (< 2.5 µmol TE/g).

Correlation analysis revealed total protein content to be negatively associated with anti-oxidant capacity (r = -0.22) and total flavonoid content (r = -0.15). Total flavonoid content showed a positive correlation with anti-oxidant capacity (r = 0.55) and total phenol content (r = 0.24) whereas a negative correlation was observed between TFC and zinc (r = -0.12).

Discussion

Protein content in lentil genotypes has been estimated to range from 22 to 29.3% (Alghamdi et al. 2014; Taleb et al. 2013; Ettoumi and Chibane 2015; Zaccardelli et al. 2012). Boyle et al. (2010) estimated crude protein content in green and red lentils was 23.03% and 25.88%, respectively. Zinc content in lentil genotypes is almost consistent as reported by several authors (Singh et al. 2017a, b); Alghamdi et al. 2014; Ray et al. 2014; Thavarajah et al. 2011; Khazaei et al. 2017). Recommended dietary allowance (RDA) of protein for adults is 0.8 g protein per kg body weight per day (Wu 2016). RDA of zinc is 11-8 mg per day for male and female, respectively according to national institute of health (NIH). As per this investigation, consumption of 200-250 grams of lentil lines (released variety PL 05 and L 4076) can meet RDA of protein and zinc depending upon composition and nature of anti-nutritional factors present in recommended lentil varieties (range of anti-nutritional factors other than total phenol content and total flavonoid content also affect bioavailability). Advanced breeding lines PL 24 and L 5253 can be utilized by the breeders for increasing the protein content and broadening of genetic base of Indian cultivars. Development of lentil varieties with high protein content will help in reduction of protein deficiency. Mediterranean germplasm lines P 8115 and P 3234 are rich in zinc content and can be utilized by breeders for improving the same in lentil varieties suitable to different agrological niches.

The results for TPC are in harmony with Alghamdi et al. (2014), Ettoumi and Chibane (2015) and Gupta et al. (2018). Wide range of variation for TPC (8.56 to 25.67 mg GAE/g) in lentil has been reported by Talukdar (2012). Zhang et al. (2015) assayed TPC in 20 red and green lentil cultivars to be in the range of 5 to 7 and 4.56 to 8.34 mg GAE/g, respectively. Phenols and flavonoids have dual role to play in terms of nutrition. Phenolics and flavonoid act as anti-nutritional phyto-chemicals on one hand while on other benefit humans by combating and neutralizing harmful reactive oxygen species produced within body. Hence genotypes having above as well as below-average content can be utilized in order to accomplish nutritional aspect from both ends. Higher TPC of released Indian lentil varieties PL 06, PL 07 and PL 08 indicate their potential utility for nutritionist and food technologist. Low TPC germplasm lines IC 560812 and IC 560372 can be utilized by lentil breeders to manipulate the bioavailability of micronutrients as required by the nutritionists. Released lentil varieties PL 05 and PL 02 with low TPC are likely to have better Zn bioavailability.

Alghamdi et al. (2014) assayed TFC in lentil was 4.12 to 8.92 mg QE/g. However, this range might be due to less number of genotypes assayed. Flavonoid content in lentil cultivars was 0.96 to 1.93 mg CAE/g (Talukdar 2012) whereas TFC in red and green lentils was 0.60 to 1.62 and 0.66 to 1.98 mg CAE/g, respectively (Zhang et al. 2015). Results obtained by other researchers might differ due to solvent used for extraction and unit of expression. Phenolic compounds and flavonoids are polar compounds hence better extracted with ethanol, methanol and water. Alam et al. (2013) advocated ethanol being organic and non-toxic solvent is predominantly used for extraction of polar compounds and antioxidant study. Flavonoids are potent antioxidant acting as free radical scavengers and metal chelators (Korkina et al. 1996). Epidemiological studies show flavonoids consumption reduces risk of cancer (Clere et al. 2011), diabetes (Zheng et al. 2011) and neurodegenerative diseases (Mandel et al. 2011). Germplasm lines IG195, P3233, IG5320, IG111996, IG73798, P2125 with higher flavonoids content can be used for studying the genetics, mapping and breeding lentil varieties possessing high antioxidant activity. Genotypes P3234, P13108, IC560206, IC208326, L7920 and P13135 can be utilized in breeding programs to ameliorate bioavailability of proteins and other micronutrients.

Antioxidants play crucial role in combating oxidative damage associated with various diseases, including cancer, cardiovascular diseases, diabetes, ageing and atherosclerosis (Lobo et al. 2010). Antioxidant capacity of seed extracts can be determined by several *in vitro* methods. Hydrogen peroxide scavenging (H_2O_2) assay, nitric oxide scavenging activity, trolox equivalent antioxidant capacity (TEAC)/ABTS radical cation decolorization assay, cupric ion reducing antioxidant capacity (CUPRAC) method, total radical-trapping antioxidant parameter (TRAP) method, ferric reducing-antioxidant power (FRAP) assay, 2,2-diphenyl-1-picrylhydrazyl

(DPPH), hydroxyl radical scavenging activity and oxygen radical absorbance capacity (ORAC) method are commonly followed methods. In this study free radical scavenging assay using DPPH reagent was used for determination of antioxidant capacity as this is most commonly used method for assaying antioxidant activity of a sample (Alam et al. 2013). DPPH free radical scavenging assay offers simple, convenient, rapid, easy and inexpensive estimation. Zhang et al. (2015) reported DPPH in the range of 23.83 to 35.03 µmol TE/g in Canadian lentils. Ettoumi and Chibane (2015) reported DPPH in lentil was 4.91 mg TE/g. DPPH in lentils varied from 10.61 to 23.26 µg/g (Alghamdi et al. 2014) and 177.43 to 212.71 µg/ml (Talukdar 2012).

Positive significant correlation between antioxidant activity (DPPH) and flavonoids ($r > 0.95$) has been reported by Shao et al. (2014, 2015), which is much higher than reported in present study. This difference may be explained as anti-oxidant activity is not an outcome of one or few phytochemicals but a synergistic and interactive effect of various bioactive compounds present within plant system at a particular stage and time. (Prochazkova et al. (2011) have also indicated high and positive correlation between TFC and antioxidant activity. It is suggested that breeding nutritionally rich crops having higher nutrient bioavailability is an effective means to sustainably tackle and alleviate micronutrient deficiencies in human beings.

Authors' contribution

Conceptualization of research (HKD, SG, AS); Designing of the experiments (HKD, SG, AS); Contribution of experimental materials (HKD, GPM, MA); Execution of field/lab experiments and data collection (SG, MA); Analysis of data and interpretation (SG, AB, AK); Preparation of manuscript (SG, GPM, HKD).

Declaration

The authors declare no conflict of interest.

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