Diversity in protein secondary structure, molecular weight, mineral and amino acid composition of lentil and horse gram germplasm

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Abstract Lentil and horse gram germplasm was assessed for variety in seed and flour properties. Horsegram grains showed higher a^* and b^* and lower L^* values as compared to lentil grains indicating lentil grains were lighter in color as compared to horse gram. Both the pulses showed significant differential accumulation of minerals. Flours from horse gram lines showed higher Mn, K, Mg, Na, Zn and Ca content and lower Cu and Fe content as compared to lentil lines. Polypeptide of 42 kDa was present in IC94636 and IC139555 only and 35 kDa PP subunit was absent in all the horse gram lines except IC94636. Major polymorphism among lentil lines was observed in 10, 35–37 and 55–49 kDa PP subunits. Amount of β -sheets and β -turns was the highest whereas that of antiparallel β -sheets was the lowest. NIC17550, NIC17551 and NIC17552 showed higher content of antiparallel β -sheets and random coils among lentil lines. PL1 showed the highest portion of α -helixes and β -turns whereas PL57 showed the highest proportion of β -sheets among lentil lines. Lentil flours showed higher proportion of aspartic acid, glutamic acid, asparagine, serine, citrulline and serine and lower proportion of histidine, threonine, GABA, tyrosine and cystine as compared to horse gram.

Keywords SDS-PAGE · Minerals · Amino acids · FTIR · *Lens culinaris* · *Macrotyloma uniflorum*

Introduction

Pulses are considered to be substantially enriched with nutrients (Dogan et al. 2005). Horse gram and lentil are an important part of healthy diet consumed in India and are source of protein, important minerals and B-complex vitamins. Eaten directly as seeds or sprouts mainly in southern parts of India, horse gram, apart from being rich in proteins is an important source of iron and molybdenum. Lentils are rich in dietary fibre, helps in managing blood-sugar disorders, have few calories and negligible fat which reduce chances of many heart diseases. Horse gram shows antioxidant, constringent and diuretic action. It's use in treatment of many diseases such as diarrhea, hypertension leucorrhea, hemorrhage, kidney and gall stones, bleeding during pregnancy have been reported (Prasad and Singh 2015). Lentil is the most widely used and studied legume. On the other hand, horse gram is under-exploited legume grown extensively in India, mainly for animal feed. Korhonen and Pihlanto (2003) stated that pulses can be used as ingredients for production of functional foods as well as pharmaceutical preparations. Seed storage proteins (SSPs), which are synthesized at the stage when cell division is complete, help in identification and characterization of diversity in crop varieties, cultivars and their wild varieties and establishing phylogenetic relationship between accessions (Nisar et al. 2007). Polymorphism in SSP has been associated with geographical origin of germplasm (Ghafoor et al. 2003; Satija et al. 2002). Data on genetic diversity in germplasm is beneficial in gene bank management and establishment of core collection. However, similar information about pulses grown in Himalayan region is limited. In order to exploit full agricultural potential, it is imperative that diversity among different characteristics of lentil and horse gram grain be

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investigated. This will be crucial in development of high yielding commercially viable cultivars. Therefore, the aim of this study was to explore the diversity in protein profiling, secondary structure, mineral and amino acid composition of lentil and horse gram lines.

Materials and methods

Thirty lentil (*Lens culinaris*) (PL1, PL4, PL5, PL19, PL23, PL25, PL26, PL36, PL40, PL57, PL234, PL639, OPL62, 406, L617, 635, L649, L830, L4076, L4147, L4188, L5227, MC6, PERCOZ, VIPASHA, NIC17552, NIC17551, NIC17550, NIC14398 and NIC17549) and forty-eight horse gram (*Macrotyloma uniflorum*) (NIC14350, NIC14351, NIC14411, NIC17543, NIC17545, IC94634, IC94636, IC94637, IC106914, IC107188, IC107337, IC107344, IC107346, IC107484, IC108079, IC139555, IC278826,

IC278827, IC278831, IC280031, IC313262, IC321237, IC321242, IC469259, IC469266, IC469271, IC469272, IC469273, IC544826, IC544827, IC544828, IC544829, IC544830, IC544831, IC544833, IC544834, IC544835, IC544836, IC544837, IC544840, IC544841, IC544842, IC547542, IC547543, IC107660 IC 107568 and IC94638) varieties were collected from NBPGR, Phagli, Shimla, India and were 2012–2013 harvest.

Methods

Hunter color parameter

The color parameters (L^* , a^* and b^*) of lentil and horse gram grains was determined using a Hunter colorimeter (Model D 25, Hunter Associates Laboratory, USA) as described earlier by Kaur and Singh (2007). High L^* values

Table 1 Hunter color parameters, protein and ash content of lentil lines

Lines	Protein content (%)	Ash content (%)	L^*	a^*	b^*
PL-1	22.8 ± 0.13 ^f	2.48 ± 0.02 ^c	51.1 ± 0.27 ⁱ	6.74 ± 0.13 ^f	13.70 ± 0.32 ⁱ
PL-4	24.3 ± 0.15 ^h	2.96 ± 0.04 ^{hi}	44.5 ± 0.29 ^d	5.28 ± 0.14 ^d	7.03 ± 0.15 ^c
PL-5	26.6 ± 0.11 ^l	2.83 ± 0.05 ^g	42.7 ± 0.50 ^c	2.83 ± 0.25 ^a	6.18 ± 0.42 ^{bc}
PL-19	25.6 ± 0.14 ^j	2.55 ± 0.05 ^d	52.3 ± 0.39 ^{ij}	5.53 ± 0.35 ^{de}	12.17 ± 0.20 ^h
PL-23	27.5 ± 0.16 ⁿ	2.92 ± 0.03 ^h	48.9 ± 0.66 ^g	4.96 ± 0.25 ^{cd}	9.81 ± 0.52 ^f
PL-25	27.3 ± 0.14 ⁿ	2.60 ± 0.06 ^{de}	51.0 ± 0.49 ⁱ	5.68 ± 0.59 ^{de}	11.48 ± 0.10 ^{gh}
PL-26	26.9 ± 0.15 ^m	2.68 ± 0.03 ^{ef}	49.8 ± 0.46 ^{gh}	5.57 ± 0.23 ^{de}	10.50 ± 0.33 ^g
PL-40	26.6 ± 0.14 ^l	3.30 ± 0.07 ^k	46.0 ± 0.66 ^c	4.83 ± 0.12 ^{cd}	7.35 ± 0.23 ^{cd}
PL-234	25.9 ± 0.13 ^k	2.16 ± 0.02 ^a	47.7 ± 0.54 ^f	4.49 ± 0.51 ^c	8.26 ± 1.44 ^d
PL-639	25.6 ± 0.11 ^j	2.49 ± 0.05 ^c	46.7 ± 0.24 ^{ef}	4.74 ± 0.08 ^{cd}	7.77 ± 0.22 ^{cd}
OPL-62	23.3 ± 0.16 ^g	2.34 ± 0.03 ^b	44.0 ± 0.78 ^d	3.31 ± 0.14 ^a	6.31 ± 0.65 ^{bc}
406	20.7 ± 0.18 ^c	3.12 ± 0.04 ^j	48.4 ± 0.33 ^{fg}	5.41 ± 0.23 ^d	9.16 ± 0.15 ^e
L-617	19.4 ± 0.12 ^a	3.55 ± 0.04 ^m	49.3 ± 0.70 ^{gh}	5.05 ± 0.37 ^d	9.13 ± 0.44 ^e
L-635	21.4 ± 0.17 ^d	3.00 ± 0.01 ⁱ	46.5 ± 0.37 ^e	4.80 ± 0.32 ^{cd}	8.04 ± 0.34 ^d
L-649	19.6 ± 0.09 ^a	3.37 ± 0.05 ^{dk}	47.9 ± 0.08 ^f	4.34 ± 0.07 ^{bc}	8.22 ± 0.04 ^d
L-830	25.4 ± 0.17 ^j	2.63 ± 0.03 ^e	48.2 ± 0.29 ^{fg}	6.12 ± 0.05 ^e	9.45 ± 0.10 ^{ef}
L-4076	27.5 ± 0.15 ⁿ	2.61 ± 0.02 ^{de}	46.7 ± 0.57 ^{ef}	3.35 ± 0.28 ^a	7.33 ± 0.33 ^{cd}
L-4147	27.3 ± 0.18 ⁿ	2.34 ± 0.02 ^b	53.6 ± 1.15 ^j	6.53 ± 0.66 ^{ef}	15.40 ± 0.57 ^j
L-4188	25.6 ± 0.14 ^j	2.58 ± 0.02 ^{de}	41.4 ± 0.45 ^b	4.11 ± 0.11 ^b	5.64 ± 0.27 ^b
L-5227	19.8 ± 0.13 ^b	3.08 ± 0.03 ^j	50.0 ± 0.49 ^h	6.05 ± 0.32 ^e	11.01 ± 0.23 ^g
MC-6	25.1 ± 0.14 ⁱ	3.46 ± 0.06 ^l	48.9 ± 0.83 ^g	7.17 ± 0.47 ^f	12.52 ± 0.54 ^h
PERCOZ	22.1 ± 0.15 ^c	2.60 ± 0.05 ^{de}	49.5 ± 0.73 ^{gh}	9.05 ± 0.17 ^g	12.56 ± 0.36 ^h
VIPASHA	19.3 ± 0.14 ^a	2.40 ± 0.03 ^{bc}	49.4 ± 0.54 ^{gh}	7.14 ± 0.25 ^f	10.36 ± 0.33 ^{fg}
PL-36	27.1 ± 0.18 ⁿ	2.74 ± 0.02 ^f	46.5 ± 0.15 ^e	6.75 ± 0.13 ^f	9.79 ± 0.03 ^{ef}
PL-57	27.7 ± 0.19 ⁿ	2.67 ± 0.06 ^{ef}	38.8 ± 0.15 ^a	1.26 ± 0.07 ^a	1.77 ± 0.10 ^a
NIC14398	25.9 ± 0.12 ^k	3.05 ± 0.04 ⁱ	46.6 ± 0.21 ^{ef}	4.44 ± 0.10 ^c	7.99 ± 0.23 ^d
NIC17549	25.7 ± 0.13 ^j	3.34 ± 0.07 ^d	45.3 ± 0.11 ^{de}	4.68 ± 0.08 ^c	7.88 ± 0.33 ^{cd}
NIC17550	24.3 ± 0.15 ^h	3.12 ± 0.01 ^j	45.8 ± 0.38 ^{de}	5.23 ± 0.22 ^d	9.22 ± 0.45 ^{ef}
NIC17551	25.4 ± 0.12 ^j	2.67 ± 0.02 ^e	46.2 ± 0.19 ^e	5.35 ± 0.15 ^d	9.17 ± 0.27 ^e
NIC17552	24.7 ± 0.15 ^h	2.54 ± 0.03 ^d	44.4 ± 0.27 ^d	6.45 ± 0.09 ^{ef}	9.45 ± 0.16 ^{ef}

Data represented as mean value ± SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

Table 2 Hunter color parameters, protein content and ash content of horse gram lines

Lines	Protein content (%)	Ash content (%)	<i>L</i> *	<i>a</i> *	<i>b</i> *
NIC-14350	21.54 ± 0.11 ^a	3.32 ± 0.11 ^{ab}	47.09 ± 0.80 ^{fg}	11.23 ± 0.48 ^g	12.59 ± 0.54 ^f
NIC-14351	23.99 ± 0.12 ^{ef}	3.41 ± 0.13 ^{be}	44.27 ± 0.92 ^{de}	10.84 ± 0.24 ^g	10.83 ± 0.54 ^e
NIC-14411	24.16 ± 0.14 ^f	3.67 ± 0.11 ^{bc}	41.17 ± 0.29 ^b	4.92 ± 0.13 ^{bc}	5.61 ± 0.12 ^b
NIC-17543	22.23 ± 0.09 ^{bc}	3.47 ± 0.11 ^{bc}	44.90 ± 0.82 ^c	11.43 ± 0.54 ^{gh}	11.22 ± 0.80 ^e
NIC-17545	23.98 ± 0.11 ^{ef}	3.61 ± 0.12 ^{bc}	43.16 ± 0.40 ^{cd}	4.23 ± 0.08 ^b	6.09 ± 0.25 ^b
IC-94634	24.86 ± 0.15 ^g	3.60 ± 0.15 ^{bc}	47.77 ± 0.92 ^g	10.57 ± 0.06 ^{fg}	12.76 ± 0.59 ^f
IC-94636	25.04 ± 0.14 ^g	3.82 ± 0.14 ^{cd}	46.97 ± 0.56 ^{fg}	5.26 ± 0.09 ^c	8.47 ± 0.28 ^d
IC-94637	23.82 ± 0.18 ^e	3.67 ± 0.11 ^{bc}	43.19 ± 0.27 ^{cd}	5.44 ± 0.13 ^c	7.25 ± 0.15 ^c
IC-106914	23.99 ± 0.08 ^{ef}	4.03 ± 0.12 ^{de}	45.11 ± 0.38 ^e	5.58 ± 0.16 ^{cd}	7.98 ± 0.06 ^{cd}
IC-107188	22.06 ± 0.12 ^b	3.52 ± 0.15 ^{bc}	42.46 ± 0.63 ^c	5.33 ± 0.19 ^c	7.02 ± 0.36 ^{bc}
IC-107337	22.59 ± 0.15 ^c	3.82 ± 0.11 ^{cd}	42.91 ± 0.11 ^{cd}	6.10 ± 0.07 ^{cd}	7.52 ± 0.14 ^c
IC-107344	23.11 ± 0.21 ^d	3.78 ± 0.11 ^{cd}	44.91 ± 0.26 ^e	5.34 ± 0.06 ^c	7.84 ± 0.12 ^{cd}
IC-107346	23.11 ± 0.18 ^d	4.64 ± 0.12 ^f	42.35 ± 0.35 ^c	4.84 ± 0.14 ^{bc}	6.25 ± 0.20 ^b
IC-107484	22.41 ± 0.11 ^c	4.30 ± 0.12 ^e	43.60 ± 0.78 ^d	6.21 ± 0.15 ^{cd}	7.82 ± 0.42 ^{cd}
IC-108079	22.59 ± 0.22 ^c	3.99 ± 0.13 ^d	48.33 ± 0.71 ^{gh}	11.61 ± 0.08 ^{gh}	13.20 ± 0.39 ^{fg}
IC-139555	21.89 ± 0.15 ^b	3.67 ± 0.11 ^c	42.88 ± 0.20 ^{cd}	4.98 ± 0.11 ^{bc}	6.72 ± 0.13 ^{bc}
IC-278826	21.89 ± 0.11 ^b	3.97 ± 0.15 ^{cd}	43.29 ± 0.43 ^{cd}	6.20 ± 0.29 ^{cd}	7.29 ± 0.33 ^{bc}
IC-278827	22.06 ± 0.13 ^b	3.90 ± 0.17 ^{cd}	46.52 ± 0.06 ^f	11.08 ± 0.09 ^g	12.53 ± 0.14 ^c
IC-278831	25.91 ± 0.16 ⁱ	4.13 ± 0.19 ^{de}	43.51 ± 0.33 ^d	4.59 ± 0.25 ^b	6.16 ± 0.43 ^f
IC-280031	24.86 ± 0.18 ^g	3.95 ± 0.12 ^{cd}	40.07 ± 0.67 ^a	5.78 ± 0.53 ^c	4.40 ± 0.60 ^b
IC-313262	23.29 ± 0.17 ^d	4.29 ± 0.13 ^e	45.21 ± 0.27 ^e	5.82 ± 0.22 ^{cd}	7.98 ± 0.21 ^a
IC-321237	22.24 ± 0.19 ^{bc}	3.38 ± 0.13 ^b	47.77 ± 0.21 ^g	12.08 ± 0.03 ^h	13.19 ± 0.24 ^{cd}
IC-321242	23.29 ± 0.19 ^d	4.18 ± 0.14 ^{de}	50.94 ± 0.50 ⁱ	10.89 ± 0.23 ^{fg}	14.83 ± 0.39 ^{fg}
IC-469259	23.80 ± 0.21 ^e	4.25 ± 0.11 ^{de}	43.88 ± 0.13 ^d	4.73 ± 0.07 ^b	7.20 ± 0.17 ^h
IC-469266	23.64 ± 0.14 ^e	3.90 ± 0.17 ^{cd}	50.89 ± 0.50 ⁱ	11.37 ± 0.27 ^{gh}	15.30 ± 0.10 ^c
IC-469271	25.21 ± 0.19 ^{gh}	3.65 ± 0.11 ^{bc}	43.46 ± 0.89 ^{cd}	4.97 ± 0.21 ^{bc}	6.75 ± 0.51 ^h
IC-469272	23.11 ± 0.17 ^d	4.23 ± 0.12 ^{de}	45.00 ± 0.20 ^e	6.75 ± 1.11 ^d	7.36 ± 0.94 ^{bc}
IC-469273	24.69 ± 0.13 ^{fg}	3.67 ± 0.11 ^{bc}	45.11 ± 0.41 ^e	6.32 ± 0.17 ^d	8.65 ± 0.28 ^c
IC-544826	23.99 ± 0.16 ^{ef}	3.68 ± 0.11 ^{bc}	42.92 ± 0.30 ^{cd}	4.33 ± 0.28 ^b	5.60 ± 0.35 ^d
IC-544827	23.64 ± 0.22 ^e	3.60 ± 0.10 ^{bc}	49.24 ± 0.78 ^h	10.83 ± 0.31 ^{fg}	12.97 ± 0.69 ^b
IC-544828	23.64 ± 0.16 ^e	3.83 ± 0.16 ^{cd}	41.96 ± 0.92 ^{bc}	3.38 ± 0.28 ^a	4.83 ± 0.29 ^{fg}
IC-544829	24.86 ± 0.21 ^g	3.66 ± 0.14 ^{bc}	49.44 ± 0.37 ^h	10.07 ± 0.49 ^f	12.56 ± 0.87 ^a
IC-544830	23.64 ± 0.19 ^e	3.67 ± 0.13 ^{bc}	45.34 ± 0.31 ^e	4.43 ± 0.13 ^b	7.12 ± 0.26 ^f
IC-544831	23.29 ± 0.21 ^d	3.21 ± 0.13 ^{ab}	44.03 ± 0.67 ^d	4.23 ± 0.11 ^b	6.35 ± 0.33 ^c
IC-544833	26.79 ± 0.14 ^j	3.14 ± 0.11 ^a	50.30 ± 0.57 ^{hi}	10.51 ± 0.35 ^{fg}	13.86 ± 0.33 ^{bc}
IC-544834	23.46 ± 0.15 ^{de}	3.83 ± 0.18 ^{cd}	46.16 ± 0.14 ^f	4.51 ± 0.04 ^b	7.37 ± 0.09 ^g
IC-544835	24.86 ± 0.23 ^g	3.63 ± 0.17 ^{bc}	48.75 ± 0.51 ^h	9.99 ± 0.22 ^f	13.03 ± 0.47 ^{fg}
IC-544836	25.73 ± 0.22 ^{hi}	4.46 ± 0.15 ^{ef}	49.23 ± 0.36 ^h	8.96 ± 0.21 ^e	12.42 ± 0.20 ^f
IC-544837	23.11 ± 0.21 ^e	3.36 ± 0.11 ^{ab}	42.76 ± 0.38 ^{cd}	5.41 ± 0.20 ^{bc}	7.63 ± 0.20 ^c
IC-544840	23.61 ± 0.19 ^{ef}	3.51 ± 0.13 ^{bc}	42.34 ± 0.13 ^c	6.00 ± 0.16 ^{cd}	7.05 ± 0.20 ^c
IC-544841	23.81 ± 0.17 ^f	3.73 ± 0.15 ^c	45.32 ± 0.47 ^{ef}	6.63 ± 0.18 ^d	8.56 ± 0.39 ^d
IC-544842	24.86 ± 0.15 ^g	4.24 ± 0.13 ^{de}	47.24 ± 0.26 ^{fg}	9.06 ± 0.13 ^e	11.46 ± 0.16 ^c
IC-547542	27.71 ± 0.15 ^k	3.45 ± 0.12 ^b	44.95 ± 0.06 ^e	5.75 ± 0.10 ^c	8.04 ± 0.13 ^{cd}
IC-547543	26.64 ± 0.23 ^l	3.86 ± 0.14 ^{cd}	43.69 ± 0.32 ^d	6.98 ± 0.17 ^{de}	7.88 ± 0.21 ^{cd}
IC-94638	20.29 ± 0.12 ^a	3.35 ± 0.08 ^{ab}	41.21 ± 0.11 ^b	5.14 ± 0.23 ^c	6.99 ± 0.09 ^c
IC-107568	24.36 ± 0.05 ^{fg}	2.71 ± 0.02 ^a	45.32 ± .23 ^{ef}	7.88 ± 0.31 ^e	8.64 ± 0.21 ^d
IC-107660	23.64 ± 0.03 ^{ef}	3.74 ± 0.03 ^c	43.22 ± 0.13 ^d	6.88 ± 0.21 ^{de}	7.88 ± 0.16 ^{cd}

Data represented as mean value ± SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

Table 3 Mineral composition (mg/kg) of different lentil lines

Sample	Cu	Mn	Fe	K	Mg	Na	Zn	Ca
PL-1	1.58 ± 0.05 ^b	0.30 ± 0.04 ^b	1.31 ± 0.17 ^a	119 ± 42 ^b	24.7 ± 3.6 ^a	69.5 ± 18 ^{ab}	4.15 ± 0.37 ^a	49 ± 11 ^a
PL-4	0.56 ± 0.07 ^{ab}	0.32 ± 0.04 ^{bc}	1.06 ± 0.05 ^{ab}	131 ± 34 ^b	24.8 ± 5.5 ^a	201 ± 34 ^b	8.17 ± 0.65 ^{ab}	58 ± 12 ^{ab}
PL-5	2.36 ± 0.33 ^b	0.48 ± 0.06 ^c	1.40 ± 0.01 ^{ab}	153 ± 2.1 ^b	24.1 ± 2.4 ^a	70.4 ± 6.9 ^a	3.53 ± 0.28 ^a	257 ± 34 ^b
PL-19	1.03 ± 0.13 ^{ab}	0.37 ± 0.02 ^{bc}	1.09 ± 0.02 ^{ab}	115 ± 4.3 ^b	25.8 ± 6.6 ^{ab}	54.6 ± 7.0 ^a	2.83 ± 0.44 ^a	20 ± 8.9 ^a
PL-23	0.78 ± 0.05 ^{ab}	0.36 ± 0.05 ^{bc}	1.14 ± 0.03 ^{ab}	117 ± 12 ^b	29.5 ± 8.3 ^{ab}	88.3 ± 4.9 ^{ab}	10.3 ± 0.14 ^{ab}	68 ± 16 ^{ab}
PL-25	1.17 ± 0.06 ^{ab}	0.41 ± 0.05 ^{bc}	1.18 ± 0.03 ^{ab}	144 ± 28 ^b	20.6 ± 1.9 ^a	79.7 ± 5.1 ^a	3.43 ± 0.35 ^a	36 ± 9.5 ^a
PL-26	0.60 ± 0.03 ^{ab}	0.39 ± 0.04 ^{bc}	1.32 ± 0.05 ^{ab}	114 ± 6 ^{ab}	22.3 ± 2.1 ^a	68.6 ± 7.3 ^a	5.92 ± 0.99 ^a	39 ± 3.9 ^a
PL-40	0.24 ± 0.03 ^{ab}	0.59 ± 0.10 ^d	2.38 ± 0.31 ^c	117 ± 9 ^b	31.0 ± 2.6 ^{ab}	54.2 ± 2.3 ^a	4.73 ± 0.18 ^a	53 ± 14 ^{ab}
PL-234	0.51 ± 0.02 ^{ab}	0.30 ± 0.06 ^b	1.03 ± 0.19 ^a	69 ± 21 ^a	22.1 ± 8.9 ^a	43.4 ± 3.3 ^a	5.33 ± 0.92 ^a	41 ± 9.2 ^a
PL-639	0.17 ± 0.04 ^a	0.38 ± 0.04 ^{bc}	1.09 ± 0.09 ^{ab}	103 ± 16 ^{ab}	23.8 ± 6.7 ^a	58.9 ± 5.5 ^a	7.99 ± 0.56 ^{ab}	50 ± 3.3 ^{ab}
OPL-62	0.16 ± 0.04 ^a	0.29 ± 0.08 ^b	1.07 ± 0.07 ^{ab}	89 ± 4.5 ^{ab}	44.9 ± 8.4 ^c	46.1 ± 4.5 ^a	5.01 ± 0.43 ^a	282 ± 44 ^c
406	0.18 ± 0.02 ^a	0.46 ± 0.08 ^c	2.25 ± 0.62 ^c	114 ± 24 ^{ab}	25.3 ± 4.9 ^{ab}	69.5 ± 4.7 ^a	4.60 ± 0.75 ^a	58 ± 6.5 ^a
L-617	0.17 ± 0.02 ^a	0.44 ± 0.05 ^c	2.01 ± 0.13 ^c	130 ± 6.9 ^b	21.1 ± 5.5 ^a	80.5 ± 6.8 ^a	6.40 ± 0.25 ^a	51 ± 9.7 ^a
L-635	0.90 ± 0.03 ^{ab}	0.33 ± 0.05 ^b	1.33 ± 0.15 ^{ab}	145 ± 17 ^b	30.6 ± 0.5 ^{ab}	54.5 ± 0.6 ^a	2.43 ± 0.73 ^a	145 ± 42 ^b
L-649	1.46 ± 0.48 ^b	0.48 ± 0.08 ^c	1.47 ± 0.19 ^b	120 ± 31 ^b	23.3 ± 2.7 ^a	81.9 ± 14 ^{ab}	5.20 ± 0.66 ^a	57 ± 9.5 ^a
L-830	1.89 ± 0.11 ^b	0.24 ± 0.02 ^{ab}	1.31 ± 0.03 ^{ab}	134 ± 19 ^b	26.2 ± 2.6 ^{ab}	86.5 ± 19 ^a	2.80 ± 0.47 ^a	145 ± 48 ^b
L-4076	1.67 ± 0.10 ^b	0.35 ± 0.04 ^{bc}	1.28 ± 0.20 ^{ab}	102 ± 15 ^{ab}	19.0 ± 3.8 ^a	24.51.7 ^a	4.08 ± 0.99 ^a	28 ± 1.6 ^a
L-4147	0.72 ± 0.03 ^{ab}	0.42 ± 0.04 ^{bc}	1.20 ± 0.14 ^{ab}	86 ± 16 ^{ab}	23.1 ± 6.7 ^a	56.3 ± 7.4 ^a	27.1 ± 1.18 ^b	42 ± 0.8 ^a
L-4188	0.18 ± 0.00 ^a	0.38 ± 0.01 ^{bc}	1.19 ± 0.03 ^{ab}	94 ± 5.5 ^{ab}	19.0 ± 5.3 ^a	21.2 ± 1.9 ^a	4.45 ± 0.66 ^a	22 ± 2.5 ^a
L-5227	0.28 ± 0.08 ^a	0.42 ± 0.03 ^{bc}	1.30 ± 0.09 ^{ab}	113 ± 22 ^{ab}	21.5 ± 4.1 ^a	48.3 ± 2.1 ^a	3.51 ± 0.94 ^a	82 ± 3.8 ^{ab}
MC-6	0.15 ± 0.03 ^a	0.18 ± 0.04 ^a	1.11 ± 0.27 ^{ab}	116 ± 39 ^b	17.9 ± 6.8 ^a	74.3 ± 7.4 ^a	9.09 ± 0.74 ^{ab}	31 ± 3.4 ^a
PERCOZ	0.19 ± 0.01 ^a	0.21 ± 0.08 ^{ab}	1.05 ± 0.02 ^a	103 ± 38 ^{ab}	19.5 ± 1.1 ^a	65.5 ± 5.1 ^a	3.95 ± 0.63 ^a	14 ± 1.9 ^a
VIPASHA	0.16 ± 0.01 ^a	0.45 ± 0.02 ^c	1.34 ± 0.11 ^{ab}	125 ± 33 ^b	19.1 ± 1.8 ^a	60.6 ± 2.6 ^a	8.97 ± 0.39 ^{ab}	21 ± 9.9 ^a
PL-36	0.20 ± 0.02 ^a	0.43 ± 0.04 ^c	1.12 ± 0.01 ^{ab}	98 ± 17 ^{ab}	22.6 ± 1.0 ^a	47.7 ± 3.3 ^a	4.88 ± 0.18 ^a	32 ± 1.7 ^a
PL-57	0.20 ± 0.01 ^a	0.41 ± 0.02 ^b	1.36 ± 0.09 ^{ab}	95 ± 11 ^{ab}	23.7 ± 4.3 ^a	44.3 ± 6.4 ^a	4.87 ± 0.78 ^a	36 ± 2.5 ^a
NIC14398	0.14 ± 0.01 ^a	0.32 ± 0.02 ^{bc}	1.25 ± 0.01 ^{ab}	130 ± 0.7 ^b	18 ± 0.001 ^a	40.0 ± 0.9 ^a	1.64 ± 0.01 ^a	45 ± 11 ^a
NIC17549	0.16 ± 0.07 ^a	0.28 ± 0.03 ^{ab}	1.15 ± 0.11 ^{ab}	119 ± 7.8 ^b	12.8 ± 7.5 ^a	64.0 ± 6.4 ^a	1.56 ± 0.04 ^a	53 ± 19 ^{ab}
NIC17550	0.19 ± 0.01 ^a	0.20 ± 0.00 ^a	1.36 ± 0.04 ^{ab}	339 ± 51 ^c	17.1 ± 1.2 ^a	14.7 ± 2.7 ^a	5.35 ± 1.3 ^a	88 ± 22 ^{ab}
NIC17551	0.20 ± 0.01 ^a	0.25 ± 0.01 ^b	1.88 ± 0.09 ^b	133 ± 12 ^b	34.1 ± 4.9 ^b	68.5 ± 16 ^a	5.99 ± 0.7 ^a	77 ± 18 ^{ab}
NIC17552	0.27 ± 0.05 ^a	0.22 ± 0.01 ^{ab}	1.57 ± 0.01 ^b	114 ± 1.8 ^{ab}	47.5 ± 4.3 ^c	77.3 ± 3.2 ^{ab}	3.38 ± 0.3 ^a	64 ± 22 ^{ab}

Data represented as mean value ± SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

is an indicator of lightness; high a^* value suggests redness and low a^* value suggests greenness and b^* value specifies yellowness–blueness of the grains.

Preparation of flour

Grains of different lentil and horse gram lines were ground and passed through 60 No. (BIS) sieve to obtain flour. Flours obtained were packed in airtight containers and kept in refrigerator for further analysis.

Proximate composition

Flours of different lines were evaluated for ash and protein (% N * 6.25) content using standard AOAC methods (1990).

Gel electrophoresis

Electrophoretic analysis of proteins was carried out using method of Laemmli (1970) as described by Kaur et al. (2013b). Proteins (80 µg) were resolved on SDS-PAGE under reducing conditions. The electrophoresis was run at 35 mA constant current. The gels were stained with Coomassie Brilliant Blue R-250 staining solution to visualize polypeptides. Gels were analyzed using AlphaEase software.

Mineral composition

Minerals (Cu, Mn, Fe, Zn, K, Mg, and Ca) were determined using Atomic Absorption Spectrometer (Agilent Technologies) using method described by Ghuman et al.

(2017). Instrument was calibrated with standard stock solutions of all minerals.

Amino acid analysis

The amino acids (AAs) were analysed by Shimadzu Amino Acid Analyzer using method described by Dhillon et al. (2014) after digestion with 6 N HCL at 110 °C for 24 h.

Protein secondary structure analysis

Secondary structure analysis of proteins was carried out using FTIR spectrophotometer (Vertex 70, Bruker Optics Inc., Germany). For FTIR analysis moisture was completely removed from flour by keeping in P₂O₅ in a dessicator for about a month. A background spectrum of an empty cell was taken using wavelength between 800 and 2000/cm with 4/cm resolution. Flour samples were then pressed against crystal of Attenuated Total Reflectance (ATR) cell (PIKE Technology Inc., USA) and spectra obtained was recorded. For determination of conformation present in protein structures spectra between 1600 and 1700 cm (amide I) was selected. Fourier self-deconvolution was applied (FSD) and was subject to second derivative. The analysis of individual peaks was carried out using Omnic software (Thermo Nicolet Cooperation, WI). The relative proportions of different conformations (α -helix, β -sheets, antiparallel β -sheets, β -turns and random coils) of secondary structures were determined by calculating their respective areas under graph.

Statistical analysis

The data were reported as average of triplicate readings and was subjected to analysis of variance (ANOVA) using Minitab Statistical Software (State College, PA).

Results and discussion

Hunter color parameter

Hunter color values of grains from different lentil and horse gram lines are shown in Tables 1, 2. L^* , a^* , and b^* value indicates lightness to darkness, greenness (–) to redness (+) color and blueness (–) to yellowness (+), respectively. L^* , a^* , and b^* varied significantly among lentil and horse gram lines. L^* , a^* , and b^* values ranged from 38.8 to 53.6, 1.26 to 9.05 and 1.77 to 15.4, respectively for lentil lines and 40.07 to 50.94, 4.23 to 12.08 and 4.4 to 15.3, respectively for horse gram lines. Horse gram grains showed higher a^* and b^* and lower L^* values as compared to lentil grains. Higher L^* value indicates lentil

Fig. 1 a SDS-PAGE of total seed proteins from flours of different lentil lines. b SDS-PAGE of total seed proteins from flours of different lentil lines

grains were lighter in color as compared to horse gram. According to Kaur and Singh (2007) higher b^* was an indicator of higher ash content. Similar correlation was observed between b^* value and ash content in our study. PL57 showed the lowest L^* , a^* , and b^* values whereas L4147 showed the highest L^* and b^* values among different lentil lines. Among horse gram lines, IC280031 showed the lowest L^* and b^* values whereas NIC17545 showed the lowest a^* value. IC321242, IC321237 and IC469266 showed the highest L^* , a^* , and b^* value, respectively. The color of pulses depends on the presence and amounts of various phenols and pigments in the seed coats. In some studies color has been directly correlated to antioxidant content of the flours which was further correlated to their phenolic content (Parmar et al. 2014; Tiwari and Singh 2012).

Proximate composition

Protein and ash content of lentil and horse gram flours are shown in Tables 1, 2. Protein content of flours from lentil

Fig. 2 **a** SDS-PAGE of total seed proteins from flours of different horse gram lines. **b** SDS-PAGE of total seed proteins from flours of different horse gram lines. **c** SDS-PAGE of total seed proteins from flours of different horse gram lines

and horse gram lines varied from 19.3 (VIPASHA) to 27.7% (PL57) and 20.29 (IC94638) to 26.79% (IC547543), respectively. On the other hand, ash content of lentil and horse gram flours varied from 2.16 (PL234) to 3.55% (L617) and 2.71 (IC1075688) to 4.64% (IC107346), respectively. Sreerama et al. (2012) reported

22.5% protein content and 2.7% ash content in horse gram flour. Lentil flours showed higher protein and lower ash content as compared to horse gram flours. Pulse flours are incorporated as food ingredients in different food products due to their high protein content, functional characteristics and sensory attribute which they impart to the end-product.

Mineral composition

Minerals play an important role in many metabolic processes and are essential for normal functioning of various body organs. Mineral composition of flours from lentil and horse gram lines are shown in Tables 3, 4. Identification of mineral rich variety can prove beneficial in understanding the underlying genetic and physiological mechanisms which regulate transport of minerals to developing seeds (Wang et al. 2003). Cu, Mn, Fe, K, Mg, Na, Zn and Ca content of flours from lentils varied from 0.136 to 2.36 mg/kg, 0.178 to 0.594 mg/kg, 1.029 to 2.38 mg/kg, 69.7 to 339 mg/kg, 12.8 to 47.5 mg/kg, 14.7 to 201 mg/kg, 1.56 to 10.3 mg/kg and 14 to 533 mg/kg, respectively and that of flours from horse gram lines ranged from 0.105 to 1.862 mg/kg, 0.226 to 0.959 mg/kg, 0.286 to 1.514 mg/kg, 98 to 195 mg/kg, 17 to 100 mg/kg, 18 to 177 mg/kg, 1.4 to 37.9 mg/kg and 35 to 300 mg/kg. These minerals were present in the following order of concentration in lentil flours $K > Ca > Na > Mg > Zn > Fe > Mn > Cu$. Similar trend was observed for flours from horse gram lines except Cu which was higher than Mn content. These results were in agreement with earlier observations (Katoch 2013). The author showed that K, Ca, Mg and Na were present in highest amount in ricebean flours. On an average, flours from horse gram lines showed higher Mn, K, Mg, Na, Zn and Ca content and lower Cu and Fe content as compared to lentil lines. PL40 showed the highest Mn, Fe and Ca content whereas MC6, PL234 and Percoz showed the lowest content of these minerals. NIC17550 showed the highest K content and the lowest of Na content. On the other hand, NIC17549 showed the lowest Mg and Zn content. Cu, Na, Mg and Zn content was the highest for PL5, PL4, NIC17552 and PL 23, respectively among the lentil lines. While among horse gram, lines Cu and Mn content was the lowest for IC94638 whereas IC321237 showed the lowest of Fe and Na content. Cu, Mn, Fe, K, Mg, Na, Zn and Ca content was the highest for IC107344, IC94634, IC544836, IC14353, IC107337, IC313262, IC14351 and IC14350, respectively among horse gram lines. Many minerals are essential for activity of many enzyme systems and minerals like Mg which is essential for chlorophyll are part of structure of pigments.

Table 4 Mineral composition (mg/kg) of different horse gram lines

Lines	Cu	Mn	Fe	K	Mg	Na	Zn	Ca
NIC-14350	0.70 ± 0.07 ^b	0.86 ± 0.03 ^{cd}	0.33 ± 0.02 ^{ab}	195 ± 52 ^b	87 ± 30 ^c	136 ± 11 ^{cd}	35.2 ± 13 ^d	300 ± 31 ^c
NIC-14351	0.76 ± 0.15 ^b	0.66 ± 0.08 ^c	0.25 ± 0.03 ^a	175 ± 36 ^b	49 ± 17 ^b	103 ± 23 ^{bc}	37.9 ± 6.5 ^d	249 ± 57 ^d
NIC-14411	0.70 ± 0.07 ^b	0.74 ± 0.11 ^{cd}	0.38 ± 0.10 ^{ab}	137 ± 34 ^{ab}	34 ± 3.9 ^{ab}	64 ± 16 ^b	21.1 ± 2.2 ^c	182 ± 22 ^{cd}
NIC-17543	1.01 ± 0.06 ^{bc}	0.71 ± 0.10 ^c	0.31 ± 0.07 ^{ab}	167 ± 33 ^{ab}	34 ± 4.5 ^{ab}	95 ± 29 ^{bc}	11.6 ± 1.2 ^{bc}	149 ± 29 ^{bc}
NIC-17545	0.89 ± 0.24 ^{bc}	0.79 ± 0.04 ^{cd}	0.58 ± 0.17 ^b	142 ± 23 ^{ab}	31 ± 1.8 ^{ab}	29 ± 8.7 ^a	11.5 ± 0.4 ^{bc}	66 ± 13 ^{ab}
IC-94634	0.95 ± 0.23 ^{bc}	0.96 ± 0.07 ^d	0.54 ± 0.06 ^{ab}	169 ± 14 ^{ab}	34 ± 4.0 ^{ab}	47 ± 5.0 ^{ab}	11.2 ± 1.4 ^{bc}	48 ± 8.3 ^{ab}
IC-94636	1.03 ± 0.08 ^{bc}	0.75 ± 0.24 ^{cd}	0.61 ± 0.26 ^b	153 ± 24 ^{ab}	39 ± 5.8 ^{ab}	70 ± 18 ^b	13.9 ± 14 ^{bc}	83 ± 6.3 ^{ab}
IC-94637	1.17 ± 0.79 ^{bc}	0.50 ± 0.12 ^b	0.33 ± 0.06 ^{ab}	125 ± 12 ^a	32 ± 1.2 ^{ab}	50 ± 16 ^{ab}	9.1 ± 6.6 ^b	173 ± 36 ^c
IC-106914	1.58 ± 0.17 ^{cd}	0.67 ± 0.07 ^c	0.39 ± 0.02 ^{ab}	130 ± 14 ^a	35 ± 0.7 ^{ab}	48 ± 7.7 ^{ab}	13.5 ± 2.7 ^{bc}	111 ± 33 ^{bc}
IC-107188	0.87 ± 0.13 ^{bc}	0.59 ± 0.16 ^{bc}	0.73 ± 0.12 ^{bc}	155 ± 33 ^{ab}	30 ± 2.0 ^{ab}	127 ± 38 ^{cd}	11.5 ± 0.6 ^{bc}	44 ± 2.3 ^{ab}
IC-107337	0.78 ± 0.10 ^b	0.60 ± 0.14 ^{bc}	0.36 ± 0.06 ^{ab}	143 ± 30 ^{ab}	100 ± 46 ^c	68 ± 10 ^b	19.3 ± 5.6 ^c	263 ± 73 ^{de}
IC-107344	1.86 ± 0.10 ^d	0.62 ± 0.23 ^{bc}	0.48 ± 0.11 ^{ab}	177 ± 48 ^b	37 ± 11 ^{ab}	51 ± 23 ^{ab}	12.8 ± 1.8 ^{bc}	115 ± 11 ^{bc}
IC-107346	1.16 ± 0.35 ^{bc}	0.44 ± 0.07 ^{ab}	1.28 ± 0.14 ^d	165 ± 14 ^{ab}	34 ± 1.5 ^{ab}	87 ± 9.6 ^{bc}	2.5 ± 1.0 ^a	105 ± 8.5 ^b
IC-107484	1.09 ± 0.37 ^{bc}	0.40 ± 0.07 ^{ab}	0.79 ± 0.05 ^{bc}	167 ± 23 ^{ab}	32 ± 4.6 ^{ab}	142 ± 28 ^{cd}	5.6 ± 0.5 ^{ab}	121 ± 67 ^{bc}
IC-108079	0.71 ± 0.09 ^b	0.30 ± 0.06 ^a	0.82 ± 0.16 ^{bc}	159 ± 46 ^{ab}	26 ± 1.2 ^{ab}	143 ± 20 ^{cd}	7.1 ± 0.5 ^{ab}	158 ± 56 ^{bc}
IC-139555	0.97 ± 0.15 ^{bc}	0.43 ± 0.06 ^{ab}	0.98 ± 0.11 ^c	142 ± 56 ^{ab}	32 ± 2.7 ^{ab}	114 ± 7.8 ^{cd}	8.6 ± 1.2 ^b	161 ± 46 ^{bc}
IC-278826	0.84 ± 0.08 ^{bc}	0.31 ± 0.05 ^a	0.69 ± 0.16 ^{bc}	157 ± 36 ^{ab}	44 ± 18 ^b	105 ± 17 ^c	14.2 ± 3.2 ^{bc}	104 ± 31 ^b
IC-278827	0.96 ± 0.09 ^{bc}	0.29 ± 0.03 ^a	0.76 ± 0.11 ^{bc}	144 ± 30 ^{ab}	23 ± 1.5 ^{ab}	31 ± 5.0 ^{ab}	6.5 ± 0.7 ^{ab}	113 ± 29 ^b
IC-278831	1.06 ± 0.23 ^{bc}	0.44 ± 0.07 ^{ab}	0.82 ± 0.18 ^{bc}	136 ± 52 ^{ab}	30 ± 6.2 ^{ab}	95 ± 7.3 ^{bc}	11.5 ± 0.5 ^{bc}	199 ± 41 ^{cd}
IC-280031	1.48 ± 0.21 ^{cd}	0.43 ± 0.09 ^{ab}	0.87 ± 0.15 ^{bc}	179 ± 53 ^b	35 ± 12 ^{ab}	119 ± 14 ^{cd}	14.2 ± 2.6 ^{bc}	156 ± 56 ^{bc}
IC-313262	1.05 ± 0.15 ^{bc}	0.44 ± 0.09 ^{ab}	0.98 ± 0.16 ^c	186 ± 14 ^b	46 ± 14 ^b	177 ± 8.1 ^e	6.8 ± 1.5 ^{ab}	150 ± 15 ^{bc}
IC-321237	1.36 ± 0.16 ^{cd}	0.71 ± 0.07 ^c	0.29 ± 0.04 ^a	144 ± 34 ^{ab}	27 ± 4.5 ^{ab}	18 ± 9.5 ^a	11.8 ± 1.5 ^b	139 ± 15 ^{bc}
IC-321242	1.57 ± 0.58 ^{cd}	0.32 ± 0.02 ^a	0.96 ± 0.26 ^c	153 ± 32 ^{ab}	32 ± 2.3 ^{ab}	138 ± 33 ^{cd}	5.6 ± 1.2 ^{ab}	135 ± 27 ^{bc}
IC-469259	1.83 ± 0.39 ^d	0.50 ± 0.15 ^b	1.16 ± 0.42 ^{cd}	113 ± 10 ^a	52 ± 15 ^b	67 ± 33 ^{bc}	10.4 ± 0.1 ^b	167 ± 73 ^{bc}
IC-469266	0.74 ± 0.08 ^b	0.43 ± 0.08 ^{ab}	1.07 ± 0.23 ^{cd}	164 ± 11 ^{ab}	29 ± 2.3 ^{ab}	87 ± 8.4 ^{bc}	6.4 ± 0.7 ^{ab}	105 ± 22 ^b
IC-469271	1.20 ± 0.35 ^c	0.42 ± 0.00 ^{ab}	0.92 ± 0.02 ^c	174 ± 35 ^b	34 ± 2.8 ^{ab}	103 ± 30 ^c	3.8 ± 0.8 ^{ab}	54 ± 9.3 ^{ab}
IC-469272	0.51 ± 0.13 ^{ab}	0.42 ± 0.06 ^{ab}	1.30 ± 0.11 ^d	131 ± 13 ^{ab}	42 ± 15 ^{ab}	90 ± 41 ^{bc}	6.7 ± 0.98 ^{ab}	35 ± 7.0 ^a
IC-469273	0.57 ± 0.07 ^{ab}	0.40 ± 0.03 ^{ab}	0.78 ± 0.02 ^{bc}	144 ± 28 ^{ab}	28 ± 2.0 ^{ab}	88 ± 37 ^{bc}	3.8 ± 0.4 ^{ab}	50 ± 8.5 ^{ab}
IC-544826	0.98 ± 0.16 ^{bc}	0.54 ± 0.03 ^b	1.04 ± 0.07 ^c	167 ± 4.4 ^{ab}	34 ± 0.8 ^{ab}	47 ± 8.5 ^{ab}	15.9 ± 5.2 ^c	60 ± 15 ^{ab}
IC-544827	0.95 ± 0.01 ^{bc}	0.39 ± 0.06 ^{ab}	0.86 ± 0.21 ^{bc}	146 ± 23 ^{ab}	28 ± 0.3 ^{ab}	47 ± 6.5 ^{ab}	12.8 ± 4.0 ^{bc}	166 ± 28 ^{bc}
IC-544828	0.24 ± 0.03 ^a	0.46 ± 0.08 ^{ab}	0.78 ± 0.19 ^{bc}	144 ± 12 ^{ab}	33 ± 3.5 ^{ab}	152 ± 14 ^d	4.8 ± 0.7 ^{ab}	50 ± 17 ^{ab}
IC-544829	1.12 ± 0.24 ^{bc}	0.44 ± 0.04 ^{ab}	0.98 ± 0.02 ^c	145 ± 8.9 ^{ab}	31 ± 5.9 ^{ab}	87 ± 22 ^{bc}	5.0 ± 0.2 ^{ab}	49 ± 8.2 ^{ab}
IC-544830	0.59 ± 0.09 ^{ab}	0.39 ± 0.06 ^{ab}	0.81 ± 0.05 ^{bc}	165 ± 57 ^{ab}	40 ± 10 ^{ab}	122 ± 16 ^{cd}	6.3 ± 1.2 ^{ab}	62 ± 19 ^{ab}
IC-544831	0.56 ± 0.04 ^{ab}	0.36 ± 0.02 ^{ab}	0.79 ± 0.04 ^{bc}	140 ± 15 ^{ab}	31 ± 2.8 ^{ab}	61 ± 7.2 ^b	1.4 ± 0.1 ^a	51 ± 7.9 ^{ab}
IC-544833	0.63 ± 0.02 ^{ab}	0.34 ± 0.03 ^{ab}	0.94 ± 0.08 ^c	158 ± 14 ^{ab}	29 ± 2.8 ^{ab}	68 ± 10 ^b	2.1 ± 0.3 ^a	37 ± 17 ^a
IC-544834	0.69 ± 0.09 ^{ab}	0.42 ± 0.05 ^{ab}	0.90 ± 0.04 ^{bc}	170 ± 33 ^{ab}	32 ± 6.0 ^{ab}	51 ± 3.9 ^{ab}	8.5 ± 1.1 ^{ab}	80 ± 24 ^{ab}
IC-544835	0.91 ± 0.17 ^{bc}	0.56 ± 0.01 ^{bc}	1.20 ± 0.14 ^{cd}	173 ± 1.3 ^{ab}	30 ± 6.2 ^{ab}	60 ± 21 ^{ab}	5.0 ± 0.5 ^{ab}	43 ± 11 ^{ab}
IC-544836	1.37 ± 0.06 ^c	0.46 ± 0.05 ^{ab}	1.51 ± 0.29 ^d	130 ± 31 ^a	33 ± 5.8 ^{ab}	50 ± 12 ^{ab}	3.5 ± 0.4 ^{ab}	45 ± 8.3 ^{ab}
IC-544837	0.75 ± 0.03 ^b	0.45 ± 0.02 ^{ab}	0.83 ± 0.05 ^{bc}	164 ± 18 ^{ab}	36 ± 1.2 ^{ab}	57 ± 11 ^{ab}	3.0 ± 0.4 ^{ab}	49 ± 7.2 ^{ab}
IC-544840	0.90 ± 0.05 ^{bc}	0.44 ± 0.03 ^{ab}	0.85 ± 0.09 ^{bc}	147 ± 11 ^{ab}	28 ± 9.4 ^{ab}	36 ± 16 ^{ab}	4.5 ± 0.2 ^{ab}	54 ± 11 ^{ab}
IC-544841	0.75 ± 0.10 ^b	0.53 ± 0.03 ^{bc}	0.91 ± 0.05 ^{bc}	154 ± 9.1 ^{ab}	31 ± 7.1 ^{ab}	35 ± 4.9 ^{ab}	3.9 ± 0.4 ^{ab}	61 ± 5.3 ^{ab}
IC-544842	0.58 ± 0.05 ^{ab}	0.62 ± 0.01 ^{ab}	0.97 ± 0.05 ^c	148 ± 32 ^{ab}	17 ± 5.5 ^a	53 ± 9.0 ^{ab}	5.5 ± 0.5 ^{ab}	47 ± 6.7 ^{ab}
IC-547542	1.05 ± 0.06 ^{bc}	0.43 ± 0.02 ^{ab}	0.92 ± 0.02 ^c	140 ± 13 ^a	33 ± 1.7 ^{ab}	51 ± 5.4 ^{ab}	1.4 ± 0.2 ^a	48 ± 16 ^{ab}
IC-547543	1.11 ± 0.22 ^{bc}	0.42 ± 0.03 ^{ab}	0.82 ± 0.04 ^{bc}	171 ± 23 ^{ab}	27 ± 5.1 ^{ab}	58 ± 18 ^{ab}	4.8 ± 0.4 ^{ab}	62 ± 14 ^{ab}
IC-94638	0.11 ± 0.01 ^a	0.23 ± 0.03 ^a	0.97 ± 0.10 ^c	132 ± 6.7 ^a	18 ± 3.2 ^a	71 ± 16 ^b	3.2 ± 0.1 ^{ab}	142 ± 34 ^{bc}
IC-107568	0.16 ± 0.01 ^a	0.28 ± 0.11 ^a	0.82 ± 0.15 ^{bc}	150 ± 32 ^{ab}	19 ± 3.0 ^a	66 ± 5.1 ^b	11 ± 0.3 ^{bc}	67 ± 19 ^{ab}
IC-107660	0.18 ± 0.01 ^a	0.30 ± 0.01 ^a	0.99 ± 0.02 ^c	98 ± 25 ^a	21 ± 5.2 ^{ab}	45 ± 2.2 ^{ab}	12 ± 4.2 ^{bc}	73 ± 21 ^{ab}

Mean value ± SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

Table 5 Amino acid composition of lentil and horse gram lines

Amino acid	Lentil		Horse gram		
	PL19	L 5227	94636	94637	106914
Aspartic acid	2.87 ± 0.11 ^d	2.57 ± 0.08 ^c	1.18 ± 0.01 ^a	2.55 ± 0.03 ^c	1.77 ± 0.02 ^b
Glutamic acid	8.09 ± 0.44 ^d	7.62 ± 0.31 ^c	1.63 ± 0.02 ^a	4.85 ± 0.03 ^b	4.73 ± 0.05 ^b
Asparagine	1.23 ± 0.05 ^d	0.85 ± 0.02 ^c	0.54 ± 0.01 ^b	0.45 ± 0.01 ^a	0.89 ± 0.03 ^c
Serine	7.96 ± 0.12 ^d	8.75 ± 0.05 ^e	2.05 ± 0.10 ^a	3.88 ± 0.12 ^b	6.50 ± 0.17 ^c
Glutamine	1.43 ± 0.01 ^c	0.52 ± 0.01 ^b	6.06 ± 0.22 ^c	0.39 ± 0.04 ^a	4.29 ± 0.14 ^d
Histidine	2.75 ± 0.03 ^b	1.89 ± 0.02 ^a	5.92 ± 0.22 ^c	4.98 ± 0.34 ^d	3.03 ± 0.41 ^c
Glycine	5.48 ± 0.09 ^c	4.23 ± 0.11 ^b	5.64 ± 0.18 ^c	4.10 ± 0.25 ^b	3.76 ± 0.31 ^a
Threonine	19.0 ± 0.75 ^a	18.6 ± 0.99 ^a	20.9 ± 0.89 ^b	22.8 ± 0.79 ^c	22.4 ± 1.09 ^c
citrulline	4.34 ± 0.22 ^c	5.62 ± 0.19 ^d	3.02 ± 0.31 ^a	3.92 ± 0.23 ^b	4.98 ± 0.19 ^c
Arginine	1.20 ± 0.01 ^b	0.85 ± 0.02 ^a	2.84 ± 0.11 ^c	3.59 ± 0.09 ^d	0.77 ± 0.14 ^a
Alanine	6.19 ± 0.13 ^b	7.71 ± 0.15 ^c	8.22 ± 0.23 ^d	11.0 ± 0.31 ^e	5.30 ± 0.27 ^a
GABA	1.07 ± 0.03 ^a	2.05 ± 0.01 ^b	5.65 ± 0.13 ^d	2.78 ± 0.14 ^c	2.58 ± 0.32 ^c
Tyrosine	5.15 ± 0.03 ^b	5.97 ± 0.02 ^c	16.8 ± 0.27 ^e	4.29 ± 0.31 ^a	14.0 ± 0.21 ^d
Cystine	0.27 ± 0.01 ^b	0.12 ± 0.01 ^a	0.50 ± 0.01 ^c	0.77 ± 0.03 ^e	0.68 ± 0.02 ^d
Valine	0.47 ± 0.01 ^a	1.11 ± 0.06 ^c	0.35 ± 0.01 ^a	4.04 ± 0.32 ^d	1.18 ± 0.11 ^c
Methionine	5.96 ± 0.10 ^d	5.80 ± 0.08 ^c	3.56 ± 0.13 ^a	5.93 ± 0.14 ^d	4.57 ± 0.21 ^b
Phenylalanine	2.66 ± 0.02 ^c	2.55 ± 0.01 ^b	2.07 ± 0.08 ^a	2.10 ± 0.09 ^a	4.37 ± 0.07 ^d
Isoleucine	3.40 ± 0.04 ^c	2.79 ± 0.03 ^b	2.64 ± 0.01 ^a	6.50 ± 0.12 ^d	2.65 ± 0.02 ^a
Leucine	12.3 ± 0.78 ^c	11.8 ± 0.82 ^c	7.55 ± 0.78 ^b	2.75 ± 0.89 ^a	8.01 ± 0.69 ^b
Lysine	0.31 ± 0.01 ^c	0.15 ± 0.01 ^b	0.24 ± 0.01 ^d	0.12 ± 0.00 ^a	0.20 ± 0.01 ^c
Proline	7.83 ± 0.15 ^c	8.52 ± 0.14 ^d	2.73 ± 0.06 ^a	8.39 ± 0.16 ^d	3.53 ± 0.18 ^b

Data represented as mean value ± SD. Means with similar superscripts in a row do not differ significantly ($p \leq 0.05$)

Gel electrophoresis

SDS-PAGE analysis of total seed storage proteins of lentil showed presence of 24–25 polypeptide (PP) subunits ranging from 90 to 9 kDa and that of horse gram showed 15–18 PP subunits ranging between 89 and 10 kDa. Major PP subunits observed were 85, 70, 62, 56, 46, 38–30, 24 and 21 kDa in lentil (Fig. 1a, b) and 88–50, 34–23 and 18 kDa in horse gram (Fig. 2a–c). Accumulation of low molecular weight (LMW) protein subunits ranging from 20 to > 10 kDa was observed to be lower in horse gram flours as compared to lentil flours. Horse gram exhibited two types of banding patterns, whereas lentils proteins were characterized by large similarity among different lines. Most of the PP's were highly conserved in flours from both the pulses. Major polymorphisms were found among high and medium molecular weight proteins in horse gram and among LMW proteins in lentil lines. Horse gram lines showed major variation in PP subunits of 22, 23, 30–35 and 40–45 kDa molecular weight. On the other hand, lentil lines showed major polymorphism among 10, 35–37 and 55–49 kDa PP subunits. Among horse gram lines, PP of 42 kDa was present in IC94636 and IC139555 only. On the other hand, 35 kDa PP subunit was absent in all the horse gram lines except IC94636. Another PP subunit of 22 kDa

manifested polymorphism among horse gram lines. PP's corresponding to molecular weight 37 kDa in horse gram and 30, 19, 16 and 13 kDa in lentil has been observed to be resistant to harsh processing conditions (Ghumman et al. 2016a, b). Major globular proteins observed among horse gram lines were 32 and 58 kDa and were conserved among all the accessions. IC94637 showed presence of band corresponding to 37 kDa molecular weight which was absent in other lines. Similarly, 23 kDa protein subunit was observed in IC94636 and NIC17545; while a 31 kDa band was present in IC280031 and IC321237. Among various lentil lines PL19, L4147, L5227 and PERCOZ showed maximum variation in PP subunits.

Amino acid composition

Amino acid composition of lentil and horse gram flours are shown in Table 5. Lentil and horse gram lines which showed variation in electrophoretic banding pattern were selected for amino acid analysis. Lentil flours showed higher proportion of aspartic acid, glutamic acid, asparagine, serine, citrulline and serine and lower proportion of histidine, threonine, GABA, tyrosine and cystine as compared to horse gram. Glutamic acid, serine and proline were present in higher proportion in lentil lines as

Table 6 Relative proportion of protein secondary structures in different lentil flours

Lines	Antiparallel β -sheet	β -sheets	Random coils	α -helix	β -turns	β -structures
PL-1	4.29 $\pm$ 0.65 ^b	28.5 $\pm$ 4.5 ^b	14.0 $\pm$ 3.7 ^b	18.8 $\pm$ 2.5 ^b	32.6 $\pm$ 9.2 ^b	11.7 $\pm$ 1.5 ^b
PL-4	4.19 $\pm$ 0.45 ^{ab}	26.4 $\pm$ 0.5 ^{ab}	12.3 $\pm$ 1.7 ^a	17.3 $\pm$ 0.3 ^{ab}	29.5 $\pm$ 1.8 ^{ab}	10.1 $\pm$ 0.6 ^{ab}
PL-5	3.64 $\pm$ 0.95 ^a	27.3 $\pm$ 1.6 ^{ab}	13.0 $\pm$ 0.4 ^a	16.7 $\pm$ 1.0 ^{ab}	29.8 $\pm$ 1.7 ^{ab}	9.80 $\pm$ 0.6 ^{ab}
PL-25	4.57 $\pm$ 0.51 ^b	26.7 $\pm$ 0.7 ^{ab}	13.7 $\pm$ 0.2 ^{ab}	17.0 $\pm$ 0.5 ^{ab}	27.0 $\pm$ 1.0 ^{ab}	10.9 $\pm$ 0.1 ^{ab}
PL-19	4.39 $\pm$ 0.29 ^{ab}	25.3 $\pm$ 0.6 ^{ab}	13.4 $\pm$ 0.3 ^a	17.5 $\pm$ 0.5 ^{ab}	27.9 $\pm$ 0.3 ^{ab}	11.5 $\pm$ 0.5 ^{ab}
PL-23	4.86 $\pm$ 0.47 ^b	26.8 $\pm$ 1.8 ^{ab}	13.6 $\pm$ 1.8 ^{ab}	15.8 $\pm$ 1.2 ^a	27.7 $\pm$ 0.8 ^{ab}	11.3 $\pm$ 0.9 ^{ab}
PL-26	4.04 $\pm$ 0.82 ^{ab}	25.5 $\pm$ 1.5 ^{ab}	12.8 $\pm$ 1.8 ^a	16.9 $\pm$ 0.7 ^{ab}	30.4 $\pm$ 2.4 ^b	10.3 $\pm$ 1.9 ^{ab}
PL-40	4.34 $\pm$ 0.39 ^{ab}	24.9 $\pm$ 0.7 ^{ab}	13.5 $\pm$ 0.2 ^{ab}	17.1 $\pm$ 0.3 ^{ab}	26.9 $\pm$ 1.1 ^{ab}	13.2 $\pm$ 0.4 ^b
PL-234	4.38 $\pm$ 0.19 ^{ab}	25.9 $\pm$ 0.1 ^{ab}	13.1 $\pm$ 0.1 ^a	16.9 $\pm$ 0.2 ^{ab}	29.4 $\pm$ 0.5 ^{ab}	10.3 $\pm$ 0.5 ^{ab}
PL-639	4.48 $\pm$ 0.24 ^{ab}	26.7 $\pm$ 0.5 ^{ab}	13.4 $\pm$ 0.1 ^a	16.9 $\pm$ 0.2 ^{ab}	29.2 $\pm$ 1.0 ^{ab}	9.3 $\pm$ 0.9 ^{ab}
OPL-62	4.49 $\pm$ 0.10 ^{ab}	25.2 $\pm$ 0.2 ^{ab}	13.2 $\pm$ 0.4 ^a	17.0 $\pm$ 0.2 ^{ab}	28.1 $\pm$ 0.8 ^{ab}	12.1 $\pm$ 0.1 ^b
L-406	3.99 $\pm$ 0.12 ^{ab}	25.4 $\pm$ 0.8 ^{ab}	13.3 $\pm$ 0.1 ^a	17.1 $\pm$ 0.1 ^{ab}	29.4 $\pm$ 0.3 ^{ab}	10.9 $\pm$ 1.2 ^{ab}
L-617	4.47 $\pm$ 0.09 ^{ab}	24.9 $\pm$ 0.2 ^a	14.1 $\pm$ 0.5 ^{ab}	17.5 $\pm$ 0.2 ^{ab}	27.3 $\pm$ 0.6 ^{ab}	11.8 $\pm$ 0.7 ^{ab}
L-635	4.82 $\pm$ 0.43 ^b	25.9 $\pm$ 0.6 ^{ab}	13.5 $\pm$ 0.2 ^a	17.2 $\pm$ 0.3 ^{ab}	27.7 $\pm$ 1.2 ^{ab}	10.9 $\pm$ 1.1 ^{ab}
L-649	4.43 $\pm$ 0.39 ^{ab}	25.5 $\pm$ 0.9 ^{ab}	13.1 $\pm$ 0.5 ^a	17.3 $\pm$ 0.7 ^{ab}	28.3 $\pm$ 0.5 ^{ab}	11.3 $\pm$ 1.3 ^{ab}
L-830	4.54 $\pm$ 0.29 ^{ab}	25.4 $\pm$ 0.6 ^{ab}	13.5 $\pm$ 0.1 ^a	17.1 $\pm$ 0.1 ^{ab}	27.5 $\pm$ 0.7 ^{ab}	12.0 $\pm$ 0.5 ^{ab}
L-4076	3.93 $\pm$ 0.96 ^{ab}	26.3 $\pm$ 1.6 ^{ab}	12.6 $\pm$ 0.8 ^a	17.7 $\pm$ 0.3 ^{ab}	28.3 $\pm$ 1.4 ^{ab}	11.2 $\pm$ 2.2 ^{ab}
L-4147	4.60 $\pm$ 0.02 ^{ab}	26.1 $\pm$ 0.2 ^{ab}	13.3 $\pm$ 0.1 ^a	16.7 $\pm$ 0.2 ^{ab}	28.1 $\pm$ 0.1 ^{ab}	11.2 $\pm$ 0.4 ^{ab}
L-4188	4.78 $\pm$ 0.39 ^b	26.2 $\pm$ 0.2 ^{ab}	14.4 $\pm$ 0.8 ^{ab}	17.5 $\pm$ 0.2 ^{ab}	25.5 $\pm$ 0.5 ^a	11.7 $\pm$ 0.2 ^{ab}
L-5227	4.53 $\pm$ 0.21 ^{ab}	25.2 $\pm$ 0.4 ^{ab}	13.6 $\pm$ 0.1 ^a	17.4 $\pm$ 0.1 ^{ab}	27.4 $\pm$ 0.7 ^{ab}	11.0 $\pm$ 0.7 ^{ab}
MC-6	4.32 $\pm$ 0.60 ^{ab}	25.4 $\pm$ 0.7 ^{ab}	13.5 $\pm$ 0.4 ^a	17.2 $\pm$ 0.7 ^{ab}	27.9 $\pm$ 1.8 ^{ab}	11.6 $\pm$ 0.9 ^{ab}
PERCOZ	4.41 $\pm$ 0.26 ^{ab}	27.3 $\pm$ 4.9 ^{ab}	13.2 $\pm$ 0.9 ^a	18.2 $\pm$ 1.6 ^b	27.7 $\pm$ 1.9 ^{ab}	9.8 $\pm$ 1.0 ^{ab}
VIPASHA	4.32 $\pm$ 0.28 ^{ab}	24.3 $\pm$ 2.0 ^a	12.3 $\pm$ 1.1 ^a	16.0 $\pm$ 1.6 ^{ab}	25.7 $\pm$ 2.9 ^a	17.4 $\pm$ 7.3 ^c
PL-36	5.00 $\pm$ 0.72 ^b	27.9 $\pm$ 3.4 ^b	14.5 $\pm$ 1.8 ^{ab}	15.8 $\pm$ 2.2 ^{ab}	25.4 $\pm$ 4.0 ^a	11.2 $\pm$ 0.7 ^{ab}
PL-57	4.64 $\pm$ 0.10 ^b	28.2 $\pm$ 1.2 ^b	13.6 $\pm$ 0.1 ^a	17.0 $\pm$ 0.2 ^{ab}	27.6 $\pm$ 1.0 ^{ab}	9.00 $\pm$ 0.4 ^a
NIC14398	4.66 $\pm$ 0.07 ^b	26.8 $\pm$ 1.5 ^{ab}	12.9 $\pm$ 1.0 ^a	17.0 $\pm$ 0.5 ^{ab}	28.1 $\pm$ 0.3 ^{ab}	10.7 $\pm$ 0.8 ^{ab}
NIC17549	4.64 $\pm$ 0.28 ^b	26.4 $\pm$ 0.7 ^{ab}	13.9 $\pm$ 0.4 ^{ab}	17.4 $\pm$ 0.6 ^{ab}	26.7 $\pm$ 0.9 ^{ab}	11.0 $\pm$ 0.5 ^{ab}
NIC17550	5.72 $\pm$ 0.27 ^{bc}	25.7 $\pm$ 1.0 ^{ab}	17.5 $\pm$ 0.7 ^c	13.7 $\pm$ 0.1 ^a	28.7 $\pm$ 1.1 ^{ab}	9.22 $\pm$ 0.3 ^{ab}
NIC17552	6.19 $\pm$ 0.17 ^c	26.4 $\pm$ 0.8 ^{ab}	17.3 $\pm$ 0.5 ^c	13.7 $\pm$ 0.7 ^a	27.8 $\pm$ 1.1 ^{ab}	8.78 $\pm$ 0.2 ^{ab}
NIC17551	5.49 $\pm$ 0.17 ^{ab}	24.6 $\pm$ 1.1 ^a	16.6 $\pm$ 0.7 ^c	13.5 $\pm$ 0.5 ^a	30.4 $\pm$ 1.5 ^b	9.15 $\pm$ 0.3 ^{a^b}

Data represented as mean value $\pm$ SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

compared to other amino acids. Proline had been reported as a key precursor of reductones and melanoidines that influence both the color and the antioxidant activity of the flours significantly (Samaras et al. 2005). PL19 showed higher relative proportion of aspartic acid, glutamic acid, asparagine, glutamine, histidine, glycine, threonine, arginine, cystine, phenylalanine, isoleucine, leucine, lysine and proline content as compared to PL5227. Lentils have been reported to have high lysine content but low sulphur-containing amino acids. So, they can be incorporated into cereal-based products which are rich source of sulphur-containing amino acids (Gomez et al. 2008). IC94637 showed the highest proportion of aspartic acid, glutamic acid, threonine, arginine, alanine, valine, methionine, isoleucine and proline among three horse gram lines. On the other hand, IC94636 showed the highest proportion of glutamine, histidine, glycine, GABA, tyrosine and lysine.

Similar amino acid composition was reported by Thirumaran and Kanchana (2000) in horse gram. GABA is a non-protein amino acid that functions as a neurotransmitter in the brain and directly affects the capability of a person to manage stress (Shiahs and Yatham 1998). It has been observed to be one of the amino acids present in highest amount in albumins isolated from lentil and horse gram (Ghumman et al. 2016).

Protein secondary structure

Secondary structure of protein present in different lentil and horse gram lines are shown in Tables 6, 7. The amide I bands arises from the C=O stretching of the peptide group and is sensitive to different conformations of the protein secondary structures. Bands at 1638 cm^{-1} , 1648 cm^{-1} and 1656 cm^{-1} were observed in the amide I region and they

Table 7 Relative proportion of protein secondary structures in different horse gram flours

Liness	Antiparallel β -sheet	β -sheets	Random coils	α -helix	β -turns	β -structures
NIC-14350	4.75 $\pm$ 0.33 ^b	27.3 $\pm$ 0.86 ^{bc}	13.6 $\pm$ 0.14 ^{ab}	16.8 $\pm$ 0.30 ^{cd}	27.1 $\pm$ 0.39 ^{bc}	10.5 $\pm$ 0.37 ^{ab}
NIC-14351	4.61 $\pm$ 0.45 ^b	27.2 $\pm$ 0.46 ^{bc}	13.7 $\pm$ 0.05 ^b	16.9 $\pm$ 0.31 ^{cd}	27.0 $\pm$ 0.44 ^{bc}	10.6 $\pm$ 0.19 ^{ab}
NIC-14411	4.12 $\pm$ 0.12 ^{ab}	26.4 $\pm$ 0.82 ^b	13.6 $\pm$ 0.17 ^b	17.0 $\pm$ 0.04 ^{cd}	27.9 $\pm$ 0.50 ^c	11.0 $\pm$ 0.60 ^{ab}
NIC-17543	4.68 $\pm$ 0.27 ^b	27.9 $\pm$ 0.85 ^c	13.8 $\pm$ 0.11 ^{ab}	17.0 $\pm$ 0.29 ^{cd}	26.9 $\pm$ 0.69 ^{bc}	9.76 $\pm$ 0.05 ^a
NIC-17545	5.16 $\pm$ 0.55 ^{bc}	27.6 $\pm$ 0.21 ^{bc}	13.3 $\pm$ 0.33 ^{ab}	16.1 $\pm$ 0.40 ^c	26.5 $\pm$ 0.29 ^b	11.4 $\pm$ 0.27 ^b
IC-94634	4.20 $\pm$ 0.30 ^{ab}	26.9 $\pm$ 0.54 ^{bc}	13.7 $\pm$ 0.08 ^b	17.0 $\pm$ 0.07 ^{cd}	27.6 $\pm$ 0.57 ^{bc}	10.7 $\pm$ 0.39 ^{ab}
IC-94636	4.25 $\pm$ 0.28 ^{ab}	26.9 $\pm$ 0.76 ^{bc}	13.4 $\pm$ 0.38 ^{ab}	17.4 $\pm$ 0.59 ^d	26.7 $\pm$ 0.40 ^{bc}	11.4 $\pm$ 0.12 ^b
IC-94637	4.23 $\pm$ 0.10 ^{ab}	26.6 $\pm$ 0.59 ^b	13.8 $\pm$ 0.09 ^b	17.1 $\pm$ 0.10 ^{cd}	27.3 $\pm$ 0.30 ^{bc}	11.1 $\pm$ 0.74 ^{ab}
IC-106914	5.05 $\pm$ 0.24 ^{bc}	25.6 $\pm$ 0.60 ^{ab}	14.0 $\pm$ 0.71 ^{bc}	16.7 $\pm$ 0.60 ^{cd}	26.1 $\pm$ 0.24 ^{ab}	12.6 $\pm$ 0.51 ^{bc}
IC-107188	4.82 $\pm$ 0.18 ^b	27.2 $\pm$ 0.28 ^{bc}	14.8 $\pm$ 1.82 ^{bc}	16.6 $\pm$ 0.51 ^{cd}	26.7 $\pm$ 0.78 ^{bc}	9.98 $\pm$ 0.49 ^{ab}
IC-107337	4.60 $\pm$ 0.28 ^{ab}	26.6 $\pm$ 0.09 ^b	14.7 $\pm$ 0.30 ^{bc}	16.7 $\pm$ 0.34 ^{cd}	27.0 $\pm$ 0.45 ^{bc}	10.4 $\pm$ 0.16 ^{ab}
IC-107344	4.20 $\pm$ 0.05 ^{ab}	25.4 $\pm$ 0.20 ^{ab}	13.0 $\pm$ 0.06 ^{ab}	16.2 $\pm$ 0.10 ^{cd}	27.9 $\pm$ 0.38 ^c	13.3 $\pm$ 0.19 ^c
IC-107346	5.01 $\pm$ 0.40 ^{bc}	27.5 $\pm$ 0.58 ^{bc}	13.5 $\pm$ 0.22 ^{ab}	16.8 $\pm$ 0.25 ^{cd}	26.4 $\pm$ 0.44 ^b	10.7 $\pm$ 0.15 ^{ab}
IC-107484	4.57 $\pm$ 0.10 ^{ab}	27.2 $\pm$ 0.17 ^{bc}	13.8 $\pm$ 0.12 ^b	17.1 $\pm$ 0.13 ^d	26.7 $\pm$ 0.44 ^{bc}	10.6 $\pm$ 0.12 ^{ab}
IC-108079	4.98 $\pm$ 0.36 ^{bc}	26.2 $\pm$ 0.42 ^{ab}	13.1 $\pm$ 0.45 ^{ab}	17.1 $\pm$ 0.73 ^d	25.9 $\pm$ 0.37 ^{ab}	12.7 $\pm$ 1.13 ^{bc}
IC-139555	4.65 $\pm$ 0.01 ^{ab}	27.3 $\pm$ 0.42 ^{bc}	13.5 $\pm$ 0.35 ^{ab}	16.7 $\pm$ 0.53 ^{cd}	27.7 $\pm$ 0.60 ^c	10.2 $\pm$ 0.18 ^{ab}
IC-278826	4.61 $\pm$ 0.15 ^{ab}	26.6 $\pm$ 0.60 ^b	13.5 $\pm$ 0.14 ^{ab}	16.7 $\pm$ 0.32 ^{cd}	26.5 $\pm$ 0.61 ^b	12.1 $\pm$ 0.28 ^{bc}
IC-278827	4.79 $\pm$ 0.40 ^b	26.1 $\pm$ 0.69 ^{ab}	13.1 $\pm$ 0.23 ^{ab}	16.3 $\pm$ 0.46 ^{cd}	26.6 $\pm$ 0.21 ^b	13.2 $\pm$ 0.39 ^c
IC-278831	5.81 $\pm$ 0.54 ^c	28.8 $\pm$ 1.10 ^c	13.4 $\pm$ 0.04 ^{ab}	16.4 $\pm$ 0.24 ^{cd}	25.2 $\pm$ 0.81 ^a	10.3 $\pm$ 0.58 ^{ab}
IC-280031	4.60 $\pm$ 0.37 ^{ab}	26.8 $\pm$ 0.17 ^{bc}	13.9 $\pm$ 0.08 ^{bc}	16.7 $\pm$ 0.33 ^{cd}	26.6 $\pm$ 0.10 ^b	11.4 $\pm$ 0.89 ^b
IC-313262	4.54 $\pm$ 0.23 ^{ab}	26.2 $\pm$ 0.30 ^{ab}	14.8 $\pm$ 0.94 ^c	14.9 $\pm$ 0.86 ^{bc}	27.8 $\pm$ 0.48 ^c	11.7 $\pm$ 0.90 ^{bc}
IC-321237	5.27 $\pm$ 0.44 ^{bc}	27.2 $\pm$ 0.29 ^{bc}	13.8 $\pm$ 0.66 ^b	15.4 $\pm$ 0.11 ^{bc}	26.6 $\pm$ 0.60 ^{bc}	11.7 $\pm$ 0.47 ^{bc}
IC-321242	5.07 $\pm$ 0.56 ^{bc}	25.2 $\pm$ 0.22 ^a	15.7 $\pm$ 0.59 ^{cd}	14.6 $\pm$ 0.78 ^{ab}	26.6 $\pm$ 0.70 ^{bc}	12.9 $\pm$ 0.46 ^c
IC-469259	4.42 $\pm$ 0.37 ^{ab}	26.2 $\pm$ 0.89 ^{ab}	13.6 $\pm$ 0.02 ^{ab}	17.0 $\pm$ 0.12 ^{cd}	27.5 $\pm$ 0.51 ^{bc}	11.3 $\pm$ 1.08 ^b
IC-469266	5.25 $\pm$ 0.14 ^{bc}	25.2 $\pm$ 0.31 ^a	16.2 $\pm$ 0.51 ^d	14.7 $\pm$ 0.63 ^b	27.0 $\pm$ 0.68 ^{bc}	11.8 $\pm$ 0.47 ^{bc}
IC-469271	4.95 $\pm$ 0.45 ^{bc}	26.5 $\pm$ 1.59 ^b	12.8 $\pm$ 0.50 ^a	15.8 $\pm$ 0.47 ^{bc}	27.1 $\pm$ 0.83 ^{bc}	12.9 $\pm$ 2.28 ^b
IC-469272	5.01 $\pm$ 0.18 ^{bc}	25.3 $\pm$ 0.50 ^{ab}	15.2 $\pm$ 0.31 ^{cd}	14.3 $\pm$ 0.98 ^{ab}	27.0 $\pm$ 0.14 ^{bc}	13.2 $\pm$ 0.59 ^b
IC-469273	4.54 $\pm$ 1.20 ^{ab}	27.9 $\pm$ 0.41 ^c	14.8 $\pm$ 0.50 ^c	14.5 $\pm$ 0.42 ^{ab}	26.9 $\pm$ 0.46 ^{bc}	11.3 $\pm$ 0.73 ^b
IC-544826	5.37 $\pm$ 0.05 ^{bc}	25.7 $\pm$ 0.07 ^{ab}	15.6 $\pm$ 0.39 ^{cd}	13.4 $\pm$ 0.41 ^a	26.9 $\pm$ 0.33 ^{bc}	13.1 $\pm$ 0.33 ^c
IC-544827	4.78 $\pm$ 0.51 ^b	26.3 $\pm$ 0.46 ^{ab}	15.3 $\pm$ 0.63 ^{cd}	15.0 $\pm$ 0.81 ^{bc}	26.6 $\pm$ 0.78 ^{bc}	12.0 $\pm$ 0.16 ^{bc}
IC-544828	4.19 $\pm$ 0.15 ^{ab}	26.9 $\pm$ 0.05 ^{bc}	13.7 $\pm$ 0.05 ^b	17.0 $\pm$ 0.02 ^{cd}	28.0 $\pm$ 0.34 ^c	10.2 $\pm$ 0.15 ^{ab}
IC-544829	4.53 $\pm$ 0.17 ^{ab}	27.2 $\pm$ 0.59 ^{bc}	13.8 $\pm$ 0.22 ^b	17.0 $\pm$ 0.20 ^d	27.3 $\pm$ 0.32 ^{bc}	10.1 $\pm$ 0.84 ^{ab}
IC-544830	4.78 $\pm$ 0.04 ^b	27.4 $\pm$ 0.53 ^b	13.3 $\pm$ 0.04 ^{ab}	16.3 $\pm$ 0.20 ^{cd}	27.0 $\pm$ 0.33 ^{bc}	11.2 $\pm$ 0.11 ^{ab}
IC-544831	4.59 $\pm$ 0.27 ^{ab}	26.9 $\pm$ 1.12 ^{bc}	13.8 $\pm$ 0.36 ^b	16.8 $\pm$ 0.08 ^{cd}	26.9 $\pm$ 0.25 ^{bc}	10.9 $\pm$ 1.01 ^b
IC-544833	5.00 $\pm$ 0.01 ^{bc}	27.2 $\pm$ 0.14 ^{bc}	15.4 $\pm$ 0.42 ^{cd}	14.3 $\pm$ 0.07 ^{ab}	26.7 $\pm$ 0.94 ^{bc}	11.4 $\pm$ 0.44 ^{ab}
IC-544834	5.15 $\pm$ 0.09 ^{bc}	26.9 $\pm$ 0.51 ^{bc}	14.3 $\pm$ 0.08 ^{bc}	17.1 $\pm$ 0.61 ^d	26.6 $\pm$ 0.42 ^{bc}	9.88 $\pm$ 0.58 ^a
IC-544835	4.60 $\pm$ 0.30 ^{ab}	27.4 $\pm$ 0.87 ^{bc}	14.0 $\pm$ 0.61 ^{bc}	16.9 $\pm$ 0.58 ^{cd}	26.6 $\pm$ 0.68 ^{bc}	10.4 $\pm$ 0.55 ^{ab}
IC-544836	4.23 $\pm$ 0.13 ^{ab}	27.1 $\pm$ 0.53 ^{bc}	13.7 $\pm$ 0.09 ^b	17.4 $\pm$ 0.53 ^d	27.3 $\pm$ 0.44 ^{bc}	10.3 $\pm$ 0.36 ^{ab}
IC-544837	4.74 $\pm$ 0.60 ^b	26.9 $\pm$ 0.55 ^{bc}	15.3 $\pm$ 0.28 ^{cd}	17.0 $\pm$ 0.58 ^{cd}	25.5 $\pm$ 0.17 ^{ab}	10.5 $\pm$ 0.53 ^{ab}
IC-544840	5.06 $\pm$ 0.72 ^{bc}	26.4 $\pm$ 0.70 ^b	15.6 $\pm$ 0.71 ^{cd}	16.6 $\pm$ 0.62 ^{cd}	25.6 $\pm$ 0.66 ^{ab}	10.7 $\pm$ 1.03 ^{ab}
IC-544841	3.97 $\pm$ 0.20 ^a	26.2 $\pm$ 0.33 ^{ab}	13.5 $\pm$ 0.02 ^{ab}	16.9 $\pm$ 0.08 ^{cd}	28.6 $\pm$ 0.49 ^c	10.8 $\pm$ 0.48 ^{ab}
IC-544842	4.06 $\pm$ 0.04 ^{ab}	25.5 $\pm$ 1.07 ^{ab}	13.4 $\pm$ 0.35 ^{ab}	16.6 $\pm$ 0.39 ^{cd}	27.7 $\pm$ 0.07 ^c	12.7 $\pm$ 1.84 ^{bc}
IC-547542	4.77 $\pm$ 0.25 ^b	27.4 $\pm$ 0.86 ^{bc}	13.3 $\pm$ 0.32 ^{ab}	17.1 $\pm$ 1.32 ^d	26.9 $\pm$ 0.37 ^{bc}	10.6 $\pm$ 0.23 ^{ab}
IC-547543	4.93 $\pm$ 0.26 ^{bc}	26.8 $\pm$ 0.94 ^{bc}	13.4 $\pm$ 0.59 ^{ab}	16.5 $\pm$ 0.44 ^{cd}	26.2 $\pm$ 0.85 ^{ab}	12.2 $\pm$ 0.76 ^{bc}
IC-94638	5.18 $\pm$ 0.78 ^{bc}	26.3 $\pm$ 0.56 ^{ab}	13.4 $\pm$ 0.18 ^{ab}	17.1 $\pm$ 0.76 ^d	26.7 $\pm$ 0.16 ^{bc}	11.3 $\pm$ 0.91 ^{ab}
IC-107568	5.04 $\pm$ 0.62 ^{bc}	26.1 $\pm$ 0.65 ^{ab}	15.1 $\pm$ 0.37 ^c	16.7 $\pm$ 0.48 ^{cd}	27.0 $\pm$ 0.66 ^{bc}	10.1 $\pm$ 0.37 ^{ab}
IC-107660	4.60 $\pm$ 0.26 ^{ab}	27.3 $\pm$ 0.23 ^{bc}	15.4 $\pm$ 0.20 ^{cd}	14.3 $\pm$ 0.02 ^{ab}	26.9 $\pm$ 0.23 ^{bc}	11.5 $\pm$ 0.39 ^{ab}

Data represents as mean value $\pm$ SD. Means with similar superscripts in a column do not differ significantly ($p \leq 0.05$)

correspond to β sheets, random coil and α -helix, respectively (Kudre et al. 2013). Proportion of anti-parallel β sheets, β sheets, random coils, α -helix, β -turns and β -structures ranged from 3.64 to 6.19, 24.2 to 28.2, 12.3 to 17.5, 13.5 to 18.8, 25.3 to 32.5, and 8.78 to 17.4 respectively, for lentil lines whereas it ranged from 3.96 to 5.26, 25.2 to 28.8, 12.7 to 16.17, 13.4 to 17.4, 25.2 to 28.6 and 9.76 to 13.2 respectively for horse gram lines. Proportion of β -sheets and β -turns was the highest followed by α -helix and that of antiparallel β -sheets was the lowest. These results were in agreement with previous findings showing that proteins in raw common bean and lentil flours have a high content of β -sheets (Carbonaro et al. 2008). Mean relative proportion of β -sheets have been reported to be highest in Kidney bean and field pea proteins as well (Shevkani et al. 2015). Structural and nutritional properties have been reported to be directly correlated. Carbonaro et al. (2012) reported that high level of β -sheets may be responsible for poor digestibility of plant proteins due to limited access to proteolytic enzymes. Whole flours from broad bean, chickpea, lentil and white bean have been observed to contain higher amount of α -helix structure in comparison to protein extracts (de la Rosa-Millan et al. 2015). β -sheets and β -structures showed positive and negative correlation respectively with protein content. Lentil and horse gram lines did not show significant variation among relative proportion of different secondary structures. NIC17550, NIC17551 and NIC17552 showed higher proportion of antiparallel β -sheets and random coils and lower proportion of α -helix and β -structures as compared to other lentil lines. PL1 showed the highest proportion of α -helixes and β -turns whereas PL57 showed the highest proportion of β -sheets among lentil lines. Parmar et al. (2017) observed harder to cook grains of kidney bean and field pea grains had higher amount of β -sheets structures which resulted in higher stability of paste formed. On the other hand, PL5 showed the lowest proportion of antiparallel β -sheets and PL36 showed the lowest proportion of β -turns. Among horse gram lines, IC544841 showed the highest proportion of β -turns and the lowest proportion of anti-parallel β -sheets. IC278831 showed the highest proportion of β -sheets whereas IC321242 showed the lowest proportion. IC94636 horse gram variety showed the highest proportion of α -helix. Random coils were the highest in IC469266 and the lowest in IC469271.

Conclusion

Lentil and horse gram grains were rich in proteins, minerals and essential amino acids; however, significant differences were observed in their physico-chemical properties among lines. Lighter colored lentil grains were

indicator of lower ash content as compared to horse gram. β -sheets, which showed positive correlation with protein content, were found in the highest proportion in both the pulses. Protein subunit pattern studied electrophoretically was highly conserved among lentils whereas polymorphism among LMW horse gram proteins was observed. Lentil flours showed higher proportion of important amino acids in comparison to horse gram.

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