Variation in composition, protein and pasting characteristics of different pigmented and non pigmented rice (*Oryza sativa* L.) grown in Indian Himalayan region

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Abstract This present study was aimed to evaluate proximate composition, antioxidant activity, amino and phenolic acids, protein profile and pasting characteristics of pigmented rice (PR) and non pigmented rice (NPR) landraces grown in Himalayan region. Higher antioxidant activity, total phenolic content, fat and protein content were observed for PR as compared to NPR. Histidine, iso-leucine, lysine, methionine, phenyl-alanine, valine and threonine were significantly higher for PR. Paste viscosities were higher for NPR (except IC568266) as compared to PR. IC568266 was observed to be waxy landrace. Higher free (gallic acid, catechin, caffeic acid, vanillic acid, sinapic acid and luteolin) and bound phenolics (ferulic acid, *p*-coumaric acid and quercetin) were observed in PR as compared to NPR. Majority of PR landraces showed higher accumulation of prolamines with low molecular weight polypeptides of 18, 20 and 29 kDa as compared to NPR. PR showed the higher essential amino acids, phenolic acids (*p*-coumaric acid, ferulic acid and quercetin) and antioxidant activity with improved nutritional value and showed potential for developing nutraceutical and functional foods.

Keywords Rice · Protein · Amino acids · Phenolic acids · Pasting

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Introduction

Rice (*Oryza sativa* L.) is the most popular food known for its high nutritional and health beneficial properties. In order to breed rice with higher phenolic compounds and other nutritional components, it is necessary to explore the genetic diversities of their composition. Higher amount of red rice are produced in south Asia including India, Sri Lanka, Philippines, China, and Japan. In India, rice is distributed in East, South, and the hilly tracts of the West and Northeast and in plains Western Uttar Pradesh, Punjab, Gujarat etc. Nowadays we have sufficient amount of white rice landraces but identified nutritionally beneficial rice landraces for PR has become highly interested for good source of bioactive compounds. Higher levels of protein (well-balanced amino acid profile) improve human nutrition in rural families in Asia, which is considered as an important objective of rice breeding. In the recent years, PR landraces have increased attention from the researchers and consumer preferences due to its high nutritional properties and used as a functional food (Li et al. 2016). Many traditional landraces were released in various regions of India and have been used in breeding programs. An appropriate conservation of landraces and broadening the gene pool of rice landraces has become important for breeding rice varieties with high yields and superior qualities in India. By combining nutritional care with the quality improvement of rice landraces, sustainable solutions for problems caused by malnutrition could be achieved (Verma and Shrivastva 2017). Traditional rice breeding has focused on improving agronomic traits and grain quality standards for the food industry and good yield for farmers. Phytochemicals was concentrated in rice bran so it was also studied for its phenolic acids content (Ti et al. 2014). The present work was designed to investigate and

compare the effect of pigments and genetic diversity on total phenolic content (TPC), antioxidant activity (AOA) as well as nutritional components (composition) and pasting properties of PR and non pigmented rice (NPR) landraces grown in Indian Himalayan region. This study helps to improve the scientific understanding of complete composition and processing quality of Indian PR and NPR landraces as functional foods.

Materials and methods

Materials

The experiment comprised of six landraces of Himalayan upland paddy grown in the month of May to October of 2014–2015 were procured from the Regional Station of the National Bureau of Plant Genetic Resources (NBPGR), Phagli, Shimla, India. Out of these IC611163, IC274376 and IC329617 were PR grains while IC611150, IC568266 and IC611195 were NPR grains. These landraces were grown in different agro climatic conditions of hills and known for their specific quality and agronomic traits. The area where the crop was grown had an average rainfall of 1450 mm and temperature ranging from 0 to 27 °C. The topography was sub-mountainous while soils were shallow to moderately shallow, having loam to clay loam texture with the varying proportions of gravel. The paddy was dehusked on a McGill sample sheller (Rapsco, Brookshire, TX, 77423, USA) and milled in a McGill mill number 2 (Rapsco, Brookshire, TX, 77423, USA) to remove 6% bran, as described earlier by Singh et al. (2014).

Methods

Color parameters

Color parameters (L^* , a^* and b^* values) are measured using a color meter (Hunter Associates Laboratory Inc., USA) following the method described earlier (Singh et al. 2014).

Whiteness was determined as follows:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

Proximate composition

Moisture (MC), protein (PC), ash (AC), fat (FC) and carbohydrate (CHO) content of different milled rice landraces were determined in triplicate using AOAC (1990). The food energy (FE) was estimated using the equation described in Verma and Shrivastva (2017).

$$\text{FE (kCal/g)} = (\text{PC} \times 4) + (\text{FC} \times 9) + (\text{CHO} \times 4) \text{ in } (\%).$$

Antioxidant activity and total phenolic content

TPC and AOA of extracts prepared from milled rice were determined by method described in Xu and Chang (2007) and Pal et al. (2016b).

Extraction of free and bound phenolics

The free (FF) and bound (BF) phenolic compounds of the milled rice and bran were extracted using the method with minor modifications reported by Ti et al. (2014). Phenolic content in milled rice and bran were quantified using high performance liquid chromatogram of Agilent technologies (1260 Infinity Series) at wavelength of 280 nm according to Pal et al. (2016b).

Blue value and λ_{max}

Iodine absorption spectra and blue value (BV) of different milled rice landraces were determined as per the method in Pal et al. (2016a).

Pasting properties

Evaluation of the pasting properties of milled rice was done using Anton Paar Rheo Plus/32 Model MCR-301 as described earlier (Kaur et al. 2013).

Protein profiling

SDS-PAGE analysis of seed storage proteins was carried out according to the modified method by Singh et al. (2014).

Amino acids composition

Amino acids composition from milled rice was done by the method described by Dhillon et al. (2014) with minor modifications using LAB solutions software (5.54SP 5) of High performance liquid chromatography system (Shimadzu LC-30AD Model-20A, JAPAN).

Statistical analysis

The data reported in all the tables was average of triplicate observations. The data were subjected to analysis of variance and Duncan's test ($P \leq 0.05$) with Minitab statistical software (version 14.12.0, Minitab, State College, PA, USA). Pearson correlation coefficients (r) were calculated

for also to determine the relationship between different parameters. Principal component analysis (PCA) was carried out in order to determine relationships between color parameters, proximate composition, TPC, AOA and pasting characteristics amongst PR and NPR landraces. PCA loading plot (Fig. 5) provides an overview of the relationships between these parameters. The parameters whose curves lie close to one another on the loading plot are positively related, while those whose curves run in opposite directions are negatively related.

Results and Discussion

Color parameters

Color parameters were significantly ($P < 0.05$) differed amongst PR and NPR landraces. NPR were almost white and showed higher L^* (72.92–76.36) and b^* values (15.46–16.88) while lower a^* value (1.23–1.38), whereas PR exhibited darker red color and showed lower L^* (62.43–67.14) as well as b^* values (13.21–14.14) and higher a^* (6.11–7.99) (Table 1). The lower L^* and higher a^* values of PR were due to presence of higher phenolic acids in outer brany layers. The grain color has been attributed to the accumulation of phenolic acids, antioxidants and rice genotype reported earlier (Furukawa et al. 2007). The b^* value was higher for NPR as compared to PR. NPR were lighter in color and exhibited more whiteness as compared to PR (Table 1). Pigments were reported to be concentrated in periphery layers of endosperm and rare in the interior of endosperm (Lamberts and Delcour 2008). The higher a^* for

PR may be due to the presence of higher proportions of ferulic acid, *p*-coumaric acid and quercetin. Statistical analysis revealed that a^* was positively related to PC, AOA, TPC, methionine and lysine and negatively to L^* , CHO, serine, GABA and BV (Supplementary Tables 1 and 2). PR landraces with higher a^* had higher PC, AOA and TPC may be used for preparing different gluten free food products.

Proximate composition

The proximate composition of PR and NPR is presented in Table 1. MC (7.7–8.1%), (8.5–9.1%); AC (0.85–0.91%), (0.50–0.70%); PC (9.01–9.67%), (7.44–8.37%); FC (0.37–0.86%), (0.16–0.24%) and CHO (70–72%), (74–82%) for PR and NPR, respectively were significantly different for all landraces. Similar results have been observed for PR landraces by Reddy et al. (2017). It had been reported that MC (3.67–18.00%), AC (0.50–2.00%), FC (0.50–3.50%), PC (1.58–6.22%), and CHO (76.92–86.03%) in different five rice landraces in South-Eastern Nigeria by Oko and Ugwu (2011). Higher PC, FC, AC and lower CHO and MC were observed for PR as compared to NPR (Table 1). Higher FC and PC in PR increased the nutritional quality and sensory properties of cooked rice. IC274376 (PR) and IC568266 (NPR) showed the higher PC. The variation in PC might be due to seasonal and environmental differences (Singh et al. 2014). PCA revealed significant positive correlations of PC with a^* , AOA and TPC and negative with L^* and FE value (Fig. 5). The variations in FC amongst rice landraces may be due to oxidation of fat because most of the same was unsaturated type which may

Table 1 Physicochemical properties of pigmented and non pigmented milled rice from different landraces

Landraces	IC274376 PR	IC611163 PR	IC329617 PR	IC611150 NPR	IC611195 NPR	IC568266 NPR
L^* PR	62.43a	62.86a	67.14b	74.90d	76.36e	72.91c
a^* PR	7.99d	7.51c	6.11b	1.23a	1.31a	1.28a
b^* PR	14.01b	14.14b	13.21a	15.46c	16.88d	16.77d
Whiteness	59.12a	59.56a	64.06b	70.49d	70.93d	68.11c
MC (%)	7.70a	8.10b	7.90a	8.60b	9.10c	8.50
AC (%)	0.90c	0.89c	0.85b	0.50a	0.70a	0.62a
FC (%)	0.85d	0.37c	0.86d	0.24b	0.16a	0.20a
PC (%)	9.67d	9.01c	9.34c	7.81a	7.44a	8.37b
CHO (%)	70a	71b	72c	82f	81e	74d
FE (kCal/g)	326.33b	323.37a	333.1c	361.4e	355.2d	331.28a
AOA (%)	56.36f	54.58e	46.55d	16.03b	12.93a	18.53c
TPC (%)	1.92f	1.55e	1.19d	0.83b	0.57a	0.94c

PR pigmented rice, NPR non pigmented rice, MC moisture content, AC ash content, FC fat content, PC protein content, CHO carbohydrates (starch), FE food energy value, BV blue value, AOA antioxidant activity, TPC total phenolic content

Means with similar letters in a column do not differ significantly (at $P \leq 0.05$)

have undergone oxidation easily by reactive oxygen (Verma and Shrivastva 2017). The variation in AC of traditional landraces may be due to the differences in mineral content of the soils and the water used for irrigation. FE values were appreciably differed among both PR and NPR landraces (Table 1). FE ranged from 323 to 333 kCal/g for PR while 331 to 361 kCal/g for NPR. FE values obtained in the study found similar results as reported by Verma and Shrivastva (2017). NPR showed higher FE values due to higher CHO as compared to PR. PCA revealed that FE was positively correlated to CHO, BV and paste viscosities while negatively correlated to PC (Fig. 5). FE measured the available amount of energy obtained from food via cellular respiration.

Antioxidant activity and total phenolic content

AOA and TPC of different PR and NPR landraces are reported in Table 1. AOA ranged between 46.55 and 56.36% for PR landraces while 12.93 and 18.53% for NPR landraces. PR showed higher AOA due to presence of higher phenolic acids and amino acids as compared to

NPR. PR exhibited significantly higher AOA than NPR as reported earlier (Laokuldilok et al. 2011). Higher AOA of PR was due to higher amounts of phytochemicals. Methionine gets oxidized to methionine sulfoxide by reactive oxygen and proline were reported to have potent AOA (Elias et al. 2008). Among all the amino acids, tyrosine and methionine showed higher AOA followed by cysteine, histidine and phenyl alanine. TPC ranged from 1.19 to 1.92 mg GAE/g for PR landraces, while 0.57 to 0.94 mg GAE/g for NPR (Table 1). PR landraces possessed significantly higher TPC as compared to NPR due to the presence of higher amount of phenolic acids in color pigments. Higher TPC in PR could be due to higher amount of phenolics, anthocyanin and proanthocyanidin located in the aleurone layer of rice grain (Min et al. 2012). PCA revealed that AOA and TPC were positively correlated to a*, PC, valine, methionine and lysine and negative with L*, CHO, serine, alanine, arginine and GABA (Fig. 5) (Supplementary Tables 1 and 2). The results suggested that the phenolic acids in PR would exert corresponding correlation to AOA and TPC. AOA and TPC were positively correlated to gallic acid, catechin, chlorogenic acid, sinapic acid,

Table 2 Phenolic acids composition of pigmented and non pigmented milled rice from different landraces in free form (FF) and bound form (BF)

Landraces (μg/mg)	Form	IC274376 PR	IC611163 PR	IC329617 PR	IC611150 NPR	IC611195 NPR	IC568266 NPR
Gal	FF	9.73de	9.64de	9.11d	4.67b	3.89a	6.11c
Proto	FF	12.00c	10.78b	9.98a	12.34c	12.24c	13.69d
Cate	FF	23.85f	21.46d	22.81e	4.12b	3.78a	4.55bc
Chloro	FF	4.66c	2.34a	3.71b	8.56e	7.98d	9.53f
Caf	FF	6.68c	5.34b	3.65a	ND	ND	ND
Van	FF	2.45c	1.45a	1.66b	ND	ND	ND
P-cou	FF	1.41c	1.32b	1.27a	14.36e	13.86d	15.13f
Fer	FF	1.53a	1.51a	1.50a	10.56b	13.54bc	16.74d
Sin	FF	3.02c	2.95ab	2.79a	ND	ND	ND
Lut	FF	6.57c	5.02b	4.89a	ND	ND	ND
Qer	FF	3.09d	2.49b	2.39b	2.20b	1.51a	2.34c
Gal	BF	9.34a	9.32a	9.01a	17.82c	16.49b	18.04d
Proto	BF	0.95b	0.71a	0.92b	6.61d	5.51c	7.52d
Cate	BF	0.31a	0.22a	0.26a	5.45c	4.35b	6.75d
Chloro	BF	0.19a	0.17a	0.19a	1.03c	0.58b	1.16d
Caf	BF	0.31b	0.25a	0.28a	0.34b	0.33b	0.35b
Van	BF	0.05a	0.03a	0.02a	0.59c	0.45b	0.62d
P-cou	BF	17.07d	17.01d	15.89c	2.20b	1.51a	2.34b
Fer	BF	19.05d	18.46c	18.35c	1.35b	1.25a	1.44b
Sin	BF	1.24d	1.15c	1.21d	0.59a	0.51a	1.00b
Lut	BF	2.26b	1.23a	1.98b	3.90 cd	3.20c	4.54e
Qer	BF	15.50de	12.82c	15.27d	0.33a	0.34a	0.41ab

PR pigmented rice, NPR non pigmented rice, Gal gallic acid, Proto protocatechuic acid, Cate catechin, Chloro chlorogenic acid, Caf caffeic acid, Van vanillic acid, p-Cou p-coumaric acid, Fer ferulic acid, Sin sinapic acid, Lut luteolin, Qer quercetin, ND not detected

Means with similar letters in a column do not differ significantly (at $P \leq 0.05$)

luteolin and quercetin and negatively with *p*-coumaric and ferulic acid in FF. AOA and TPC were negatively correlated to protocatechuic acid and catechin while positively correlated with *p*-coumaric acid, ferulic acid, sinapic acid and quercetin in BF (Supplementary Tables 2 and 3). Furthermore, contribution of differences in amino acids composition to variation in AOA. IC274376 (PR) and IC568266 (NPR) showed the highest AOA and TPC, respectively. PCA revealed that AOA and TPC had strong correlation with PC, EAA and a^* (Fig. 5). The interactions among phenolic acids can affect antioxidant activity.

Phenolic acids composition

Phenolic acids in FF and BF extracts from PR and NPR are shown in Tables 2 and 3. Eleven phenolic compounds were detected in both PR and NPR. Amongst free phenolic acids, ferulic acid, *p*-coumaric acid, protocatechuic acid and chlorogenic acid were present in higher concentration for NPR while catechin and gallic acid were higher for PR (Table 2). Qi et al. (2010) reported that ferulic acid was the dominant phenolic acid in wheat, rice and barley. PR landraces had higher amounts of *p*-coumaric acid, ferulic acid

and quercetin in BF. In FF, caffeic acid, vanillic acid, sinapic acid and luteolin were absent in NPR. Bound phenolic acids showed higher content of gallic acid, catechin and luteolin in NPR and those of *p*-coumaric acid, ferulic acid and quercetin were predominant in PR (Table 2). Higher concentration of gallic acid, catechin, caffeic acid, vanillic acid, sinapic acid and luteolin in FF and ferulic acid, *p*-coumaric acid and quercetin in BF were observed in PR as compared to NPR. It was reported that bound phenolic acids in cereal grains were esterified to the cell wall and photochemical, mainly existed as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates and other phenols (Zhang et al. 2015). Total free and bound phenolics were higher in PR as compared to NPR (Fig. 1). The maximum amounts of phenolic compounds in rice were present in BF. IC274376 (PR) showed higher phenolic acids as compared to other landraces. Statistical analysis revealed that FF of gallic acid, catechin, chlorogenic acid, sinapic acid, luteolin and quercetin were positively correlated to a^* , AOA and TPC whereas *p*-coumaric and ferulic acid were negatively correlated with a^* , AOA and TPC. BF of *p*-coumaric acid, ferulic acid, sinapic acid

Fig. 1 HPLC chromatograms of milled rice from PR (IC274376) and NPR (IC568259), **a** free form of PR and **b** bound form of PR, **c** free form of NPR and **d** bound form of NPR. 1 = galic acid,

2 = protocatechuic acid, 3 = catechin, 4 = chlorogenic acid, 5 = caffeic acid, 6 = vanillic acid, 7 = *p*-coumaric acid, 8 = ferulic acid, 9 = sinapic acid, 10 = luteolin, 11 = quercetin

and quercetin were positively correlated with a^* , AOA and TPC while protocatechuic acid and catechin were negatively correlated to AOA and TPC (Supplementary Table 3). Phenolic acids significantly affect a^* , AOA and TPC of PR and NPR landraces. PR showed higher PC, FC, AC, phenolic acids and AOA and may be used in making colored and gluten free product such as chapati, pasta, noodles and muffins etc.

Each phenolic acid in the extracts of bran from both PR and NPR were also quantified (Table 3). PR bran showed higher proportion of protocatechuic acid, *p*-coumaric acid, ferulic acid and quercetin whereas NPR bran showed higher concentration of gallic acid, vanillic acid, caffeic acid and luteolin in FF. It had been reported that aleurone layers are rich in arabinoxylans, that might resulted into higher concentration of ferulic acid which was most abundant phenolic acid present in rice bran (Ti et al. 2014). Higher amount of phenolic acids in FF were observed in bran from both PR and NPR. Bran from PR (red and black) contained more phenolics as compared to bran from NPR as reported by Huang and Lai (2016). The major BF phenolic acids from NPR bran were *p*-coumaric acid and

ferulic acid while *p*-coumaric acid, gallic acid, vanillic acid and caffeic acid were found in PR bran. Higher phenolic acids in PR bran was also reported by Zhang et al. (2010). Total phenolic acids were higher in bran from PR as compared to NPR (Fig. 2).

BV and $\lambda_{\max}$

BV and $\lambda_{\max}$ varied from 0.1175 to 0.1987 and 583 to 590 nm, respectively for PR whereas 0.0899 to 0.2198 and 575 to 595 nm, respectively for NPR (Table 4). BV was significantly correlated to paste viscosities. The variation in BV was attributed to differences in night air temperature at the time of grain filling (Singh et al. 2014). The landraces exhibited lower BV suggesting their lower amylose content. High amylose and waxy landraces were also identified on the basis of BV as shown in Table 4. PR landraces may be considered as intermediate amylose (IA) while IC611150, IC611195 (NPR) and IC568266 (NPR) were high (HA) and low amylose (LA) landraces, respectively. Landraces showed higher BV and $\lambda_{\max}$ due to an increase in branching of chains in molecules of amylose and

Table 3 Phenolic acids composition of pigmented and non pigmented rice bran from different landraces free form (FF) and bound form (BF)

Landraces ($\mu\text{g}/\text{mg}$)	Form	IC274376 PR	IC611163 PR	IC329617 PR	IC611150 NPR	IC611195 NPR	IC568266 NPR
Gal	FF	9.89bc	8.96a	9.81b	17.82e	16.49d	19.21f
Proto	FF	20.15f	19.86de	19.12d	2.20b	1.51a	3.06c
Cate	FF	1.41 cd	1.18c	1.21c	0.59a	0.51a	0.64b
Chloro	FF	0.41c	0.31ab	0.22a	ND	ND	ND
Caf	FF	3.19b	2.94a	3.02b	5.45d	4.35c	6.90de
Van	FF	0.65ab	0.57a	0.59a	7.57d	6.56c	8.12e
<i>P</i> -cou	FF	18.99f	17.89de	17.23d	14.36b	13.86a	16.13c
Fer	FF	8.14d	8.13d	8.11d	1.99b	1.68a	2.29c
Sin	FF	0.37b	0.28a	0.31b	0.77d	0.69c	0.81de
Lut	FF	1.37bc	1.26b	1.19a	4.12e	3.25d	4.63e
Qer	FF	6.74f	6.52de	6.49d	0.76ab	0.58a	0.94c
Gal	BF	12.71f	11.23e	10.91d	0.56b	0.41a	0.64bc
Proto	BF	0.92de	0.61c	0.89d	0.29a	0.25a	0.38b
Cate	BF	0.58c	0.56c	0.46b	ND	ND	0.07a
Chloro	BF	0.28ab	0.21ab	0.21ab	0.19a	0.11a	0.28ab
Caf	BF	5.62d	4.34c	4.32c	2.78ab	2.45a	2.93ab
Van	BF	6.28d	5.98c	5.83c	1.31b	0.87a	1.39b
<i>P</i> -cou	BF	17.57ab	17.54ab	17.33a	25.46d	21.25c	30.94d
Fer	BF	1.06b	0.36a	1.02b	4.58d	3.35c	5.81e
Sin	BF	0.58bc	0.56bc	0.52b	0.45a	0.42a	0.50b
Lut	BF	3.04c	2.67ab	2.49a	ND	ND	ND
Qer	BF	1.08b	0.98a	1.04b	ND	ND	ND

PR pigmented rice, NPR non pigmented rice, Gal gallic acid, Proto protocatechuic acid, Cate catechin, Chloro chlorogenic acid, Caf caffeic acid, Van vanillic acid, *p*-Cou *p*-coumaric acid, Fer ferulic acid, Sin sinapic acid, Lut luteolin, Qer quercetin, ND not detected. Means with similar letters in a column do not differ significantly (at $P \leq 0.05$)

Fig. 2 HPLC chromatograms of bran from PR (IC274376) and NPR (IC568259), **a** free form of PR and **b** bound form of PR **c** free form of NPR and **d** bound form of NPR. 1 = galic acid, 2 = protochatechuic

acid, 3 = catechin, 4 = chlorogenic acid, 5 = caffeic acid, 6 = vanillic acid, 7 = *p*-coumaric acid, 8 = ferulic acid, 9 = sinapic acid, 10 = luteolin, 11 = quercetin

Table 4 Blue value and pasting properties of pigmented and non pigmented milled rice from different landraces

Landraces	IC274376 PR	IC611163 PR	IC329617 PR	IC611150 NPR	IC611195 NPR	IC568266 NPR
BV	0.1945c	0.1175b	0.1987d	0.2198f	0.2081e	0.0899a
$\lambda_{\max}$ (nm)	590c	583b	590c	595d	595d	575a
PT (°C)	86c	85b	87d	88e	87d	81a
PV (cP)	1270c	1130b	1960d	2760f	2490e	639a
FV (cP)	2690b	2341c	3470e	5840f	3350d	1990a
BDV (cP)	423c	201b	730e	520d	1120f	69a
SBV (cP)	1843b	2011d	2240e	3600f	1980c	1420a

PR pigmented rice, NPR non pigmented rice, BV blue value, PT pasting temperature, PV peak viscosity, SBV setback viscosity, BDV breakdown viscosity, FV final viscosity

Means with similar letters in a column do not differ significantly (at $P \leq 0.05$)

amylopectin. PCA revealed that BV showed negative correlation with PC and essential amino acids (EAA) but positive with paste viscosities (Fig. 5). IC611150 and IC611195 NPR landraces with higher BV have lower PC and EAA. BV and $\lambda_{\max}$ of starch were related to the degree of polymerization and average chain length of amylose and amylopectin (Huang et al. 2008).

Pasting properties

Pasting properties of PR and NPR landraces are shown in Table 4. PV, BDV, SBV, FV and PT ranged from 1130 to 1960 cP, 201 to 730 cP, 1823 to 2240 cP, 2690 to 3470 cP and 85 to 87 °C, respectively for PR landraces against 639 to 2760 cP, 69 to 1120 cP, 1420 to 3600 cP, 1990 to 5840 cP and 81 to 88 °C, respectively for NPR landraces. NPR showed higher paste viscosities (PV, BDV, SBV, FV) as

compared to PR as shown in Fig. 3. IC611150 (NPR) and IC611195 (NPR) showed higher PV, FV and SBV as compared to PR and waxy landrace IC568266 (NPR). Statistical data revealed that PT was positively correlated to BV, PV was positively correlated to BV, arginine, FE and negatively correlated to threonine while FV was positively correlated to arginine where as SBV was negatively correlated to citrulline (Supplementary Tables 1 and 2). IC611195 had higher BDV showed lower stability towards heating and shear stress. The differences in PV and BDV among different rice landraces could be attributed to differences in the amounts of amylose, lipids and branch chain-length distribution of amylopectin present in rice starch (Singh et al. 2006a, b). PCA analysis revealed that PT, PV, FV, SBV and BDV were closely related to BV and CHO. Landraces with higher amylose showed higher paste viscosities (Fig. 3). Variation in pasting viscosities of PR and NPR may have occurred due to variation in amylose and amylopectin fine structure, starch granule-associated proteins, PC and FC. Low amylose rice landraces had lower PV and BDV compared to those with intermediate- and high-amylose landraces. Variations in amylose content (BV) in different PR and NPR landraces were due to environmental and climatic changes (Singh et al. 2014; Kaur et al. 2016). Variation in the pasting properties of PR may have occurred due to the varied genotype, geographic and agronomic conditions, and other factors such as the

presence of starch granule-associated proteins, protein and FC (Zhang et al. 2016). Pasting behavior depends on interactions between starch and non-starch components (Waramboi et al. 2011).

Protein profiling

SDS-PAGE analysis of PR and NPR landraces was carried out according to color. PR and NPR landraces were analyzed by SDS-PAGE which showed significant variations in molecular weight of polypeptides. A total of 24 major polypeptides (PPs) ranging from 15 to 114 kDa (± 2.0 kDa) were depicted after CBBR-250 staining of SDS-PAGE gels. Kawakatsu et al. (2008) categorized these PPs as, granule-bound starch synthase (GBSS) of 60 kDa; glutelin acidic subunits (GAS) of 32 and 33 kDa; globulins of 24 kDa; glutelin basic subunits (GBS) of 21 and 22 kDa; and prolamines of 14, 16, and 18 kDa. GAS, GBS and prolamines were highly conserved among PR and NPR landraces, however, inter-cultivar quantitative changes were observed among different PPs (Fig. 4). The change in accumulation in protein, lipid and starch synthesis in PR and NPR landraces may be due to variation in nighttime air temperature and other environmental conditions under which landraces were grown (Singh et al. 2014; Kaur et al. 2016). Prolamins are low molecular weight proteins and 15, 16 and 18 kDa PPs were highly conserved in both PR

Fig. 3 Pasting profiles of **a** pigmented and **b** non pigmented rice varieties. *IA* intermediate amylose, *HA* high amylose, *LA* low amylose

Fig. 4 SDS-PAGE analysis of pigmented (PR) and non pigmented rice (NPR) seed storage proteins showing Waxy granule-bound starch synthase (60 kDa), glutelin acidic subunits (33 kDa and 32 kDa), globulins (24 kDa), glutelin basic subunits (21 kDa and 22 kDa), and prolamins (14 kDa, 16 kDa, and 18 kDa). Molecular weights in thousands are indicated

and NPR landraces and may have also contributed higher viscosities (Kaur et al. 2018). Studies have shown that rice with Wx^a allele synthesizes ~ 10 fold higher GBSS and more apparent amylose content in endosperms, as compared to Wx^b allele. Indica rice possesses Wx^a while japonica rice showed the presence of Wx^b allele (Zhang et al. 2014). Rice plants grown at low temperature produced grains with high levels of Wx protein as well as higher levels of apparent amylose content. It has been postulated that the promoter of Wx gene was sensitive to cool temperature (18 °C) and a mutation at the 5' splicing site in the first intron of the Wx gene was coupled to the sensitivity to temperature at seed maturation (Larkin and Park 1999). PR landraces showed higher accumulation of 18, 20 and 29 kDa PPs as compared to NPR. These results confirmed that majority of Himalayan rice germplasm is conserved at PP levels. Rice proteins were also analyzed to see variation on the basis of colored outer brany layer and stored polypeptides that can be used as accession specific markers. The analysis of proteins showed the presence of 60, 32, 33, 24, 22, 21, 18, 16 and 14 kDa PPs in PR and NPR. Although these seed storage polypeptides were highly conserved but lesser quantity was found in NPR as

compared to PR was remarkable. Storage proteins can be used to distinguish colored and white rice landraces (Fig. 4). This study signifies adaptation in rice in response to diverse agro-geo-climatic conditions and provided valuable tool for the enhancement of the rice quality attributes through breeding approaches.

Amino acids composition

Arginine, alanine, serine, glutamic acid, aspartic acid, asparagine, citrulline, GABA, were significantly higher in NPR while histidine, threonine, valine, iso-leucine, methionine, phenyl alanine, lysine, proline and tyrosine were higher in PR (Table 5). PR showed higher proportions of EAA (Histidine, iso-leucine, lysine, methionine, phenyl-alanine, valine and threonine) as compared to NPR. PR comprised of almost all essential amino acids in higher proportions viz., valine, threonine, isoleucine, lysine, leucine, phenylalanine, histidine, and methionine earlier reported by Samyor et al. (2017). Higher EAA were reported in improved rice due to its higher PC (Kaur et al. 2018). It has been reported earlier that proteins and minerals contributed to yellowness and redness in cereal flours

Table 5 Amino acids (%) composition of pigmented and non pigmented milled rice from different landraces

Landraces (%)	IC274376 PR	IC611163 PR	IC329617 PR	IC611150 NPR	IC611195 NPR	IC568266 NPR
Asp	1.78c	1.78c	1.67b	1.59a	1.92d	1.56a
Glut	6.71a	9.37c	6.11a	9.35c	8.81b	8.68b
Aspr	0.75e	0.36c	0.10a	0.23b	0.12a	0.59d
Ser	3.08a	3.34a	3.09a	8.01c	9.07d	6.76b
Cit	2.82b	2.56b	1.90a	3.11c	1.53a	4.78d
Ala	3.59a	3.80a	4.40b	9.30d	7.50c	12.50e
Arg	4.57a	4.34a	6.89b	17.58d	13.47c	6.97b
GABA	1.92b	1.23a	1.34a	3.90d	4.20e	3.30c
Tyr	4.13c	5.12c	4.99d	4.19a	2.16a	3.82b
Cys	0.23b	0.10a	0.20b	0.90d	0.34a	0.30c
His	11.86e	9.98c	10.87d	9.12a	9.79b	9.45b
Thr	14.12b	19.78d	14.99b	9.34a	9.11a	18.23c
Val	10.29c	11.80d	13.01e	5.60a	5.80a	6.21b
Met	4.41c	4.30c	4.60d	2.13b	2.17b	1.90a
Phe	11.86f	7.00c	9.39e	4.70a	8.90d	5.80b
Ileu	12.51e	9.30c	10.40d	6.60a	9.30c	7.00b
Lys	2.78d	2.01c	2.89de	1.20a	1.40a	1.70b
Pro	2.98a	4.20c	3.60b	3.70b	4.80d	0.50e
AA	8.49b	11.15f	7.78a	10.94e	10.73d	10.24c
BA	19.20c	16.33a	20.65d	27.90f	24.66e	18.12b
Ar A	15.99f	12.12d	14.38e	8.89a	11.06c	9.62b
EAA	67.82f	64.17d	66.25e	38.69a	46.47b	50.29c
BCAA	22.80e	21.10d	23.41f	12.20a	15.10c	13.21b

PR pigmented rice, NPR non pigmented rice, Asp aspartic acid, Glut glutamic acid, Aspr asparagine, Ser serine, His histidine, Cit citrulline, Arg arginine, Ala alanine, GABA γ amino butyric acid, Tyr tyrosine, Val valine, Met methionine, Thr threonine, Phe phenyl-alanine, Ileu isoleucine, Lys lysine, Pro proline, AA acidic amino acids (Asp and Glut), BA basic amino acids (Lys, Arg and His), ArA aromatic amino acids (Phe and Tyr), BCAA branched chain amino acids (Ileu and Val)

Means with similar letters in a column do not differ significantly (at $P \leq 0.05$)

Fig. 5 Principal component analysis (PCA) loading score plot describing relationship among different properties of pigmented (PR) and non pigmented rice (NPR) from different traditional varieties. AC ash content, FC fat content, PC protein content, CHO carbohydrates (starch), FE food energy value, BV blue value, AOA antioxidant activity, TPC total phenolic content, AA acidic amino acids (Asp and Glut), BA basic amino acids (Lys, Arg and His), ArA aromatic amino acids (Phe and Tyr), BCAA branched chain amino acids (Ileu and Val), PT pasting temperature, PV peak viscosity, SBV setback viscosity, BDV breakdown viscosity, FV final viscosity

(Jamin and Flores 1998; Singh et al. 2014). Amino acids content in foods varied due to variation in variety type, genetic background, soil type, and other agro-climatic conditions (Gonzalez et al. 2012). IC274376 (PR) showed higher EAA as compared to other landraces due to higher PC. Acidic (AA) and basic amino acids (BA) were higher in NPR while PR showed higher amount of aromatic amino acids (Ar A). Branched chain amino acids (BCAA) comprised of leucine, isoleucine and valine were higher in PR as compared to NPR (Table 5). Significant correlations were found between amino acids with color values, proximate composition and paste viscosities (Supplementary Tables 1 and 2). Statistical data revealed that arginine was positively correlated to PV and FV while threonine was negatively correlated with PV (Supplementary Tables 1 and 2). PCA revealed that a^* , PC, EAA, BCAA, AOA, TPC were positively related to each other (Fig. 5). Defa and Meizu (2006) showed the presence of nutrients especially in sticky black rice (fiber, protein, essential amino acids, vitamins, minerals etc.) that stand out from the others are completely beneficial to human health. The use of PR in food products helps to prevent headaches, relieve symptoms of constipation, prevent colon cancer, adjust blood sugar levels, prevent heart disease, lower blood pressure and prevent Alzheimer's disease (Kayahara and Tukahara 2000).

Conclusion

Comparison of physicochemical composition, phenolic acids, amino acids and protein characteristics of PR and NPR originated from Himalaya were carried out. PR have higher a^* , PC, AOA, TPC, phenolic acids (mainly ferulic and *p*-coumaric acid) characteristics as compared to NPR. Higher accumulation of prolamins, aromatic amino acids, essential amino acids and branched chain amino acids were observed in PR as compared to NPR. IC274376 (PR) was the best traditional landrace. Landraces with better quality traits needs to be conserved for breeding programme. Better nutritional characteristics and health beneficial effects will be desirable to have processed food items such as puffed and flaked rice, colored noodles and snack items.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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