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Identification of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice cultivars

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Abstract

Physical, cooking and protein characteristics of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice cultivars were studied for establishing criteria of identification. Paddy grains of *Basmati-386* had the least thousand seed weight while milled rice had maximum kernel length and L/B ratio. *Basmati-386* took minimum cooking time and exhibited the highest elongation ratio. The $\Delta L/\Delta W$ ratio better distinguished the three cultivars as *Basmati-386* had a significantly higher value. SDS–PAGE of globulin and glutelin could be used as an identification tool for differentiating the three cultivars by protein patterns.

Keywords: Rice cultivars; Identification; Basmati-386; PR-106; IR-8; Physical; Cooking; Protein characteristics

1. Introduction

Rice is a staple food for nearly one half of the world's population. Although rice has a relatively low protein content, brown rice ranks higher than wheat in available carbohydrates, digestible energy and net protein utilization. Also, rice protein is superior in lysine content to wheat, corn and sorghum (Hegsted, 1969).

Rice cultivars differ significantly in their morphological, chemical and cooking properties. A harmonious combination of intensity of aroma, texture, elongation, palatability, digestibility and longer shelf life makes the *Basmati* superior among rice cultivars. These factors strongly depend on the kernel morphology, as well as the starch and proteins. Different techniques have been employed to assess these characters, e.g., electron microscopy for kernel morphology (Beerh & Srinivas, 1991), image analysis for size and shape (Lan, Fang, Kocher, & Hanna, 2002), gas liquid chromatography for aroma (Buttery, Ling, Juliano, & Turnbaugh, 1983), micro-satellite techniques for DNA fingerprinting (Henry, 2001), RP-HPLC for proteins (Huebner, Bietz, Webb, & Juliano, 1990) and cooking properties for quality (Hirannaiah, Bhashyam, & Ali, 2001). Rice proteins have been analyzed by many investigators using gel electrophoresis (Hussain, Scanlon, Juliano, & Bushuk, 1989; Steenson & Sathe, 1995). Iwasaki, Shibuya, Suzuki, and Chikubu (1982) supported the idea of classifying rice varieties into groups by differences in electrophoretic patterns of proteins.

The current study was undertaken to differentiate three rice varieties, IR-8, PR-106 and Basmati-386, chosen as representatives of their own classes – coarse, fine and superfine, respectively, based on their physical, cooking and protein characteristics.

2. Materials and methods

2.1. Plant material

Three rice cultivars *IR-8*, *PR-106* and *Basmati-386*, representing coarse, fine and superfine varieties, respectively, were obtained from Punjab Agricultural University, Ludhiana, India.

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2.2. Methods

2.2.1. Preparation of defatted flour

Paddy samples were dehusked (HT McGill Inc., USA), polished (Baldor Electric Co., USA) and ground in a Brabender Quadramatic Junior Mill (Duisburg, Germany). The flour was extracted with petroleum ether (flour/solvent ratio 1:10 w/v) under constant stirring (30 min) and then filtered (Whatman No. 1). The extraction was repeated twice on residues with the flour/solvent ratio 1:5 (w/v). Residues were desolventized at 60 °C and defatted flour was stored in airtight glass jars at ambient temperature.

2.2.2. Proximate composition

Moisture, lipid, ash and protein were determined by the method AOAC (1990). Carbohydrate content was determined by difference.

2.2.3. Physical characteristics

Length and breadth were measured by using a vernier caliper. Weights of 100 seeds and kernels multiplied by 10 were expressed as thousand seed weight and thousand kernel weight, respectively. Bulk density was determined as weight of grains per unit volume for paddy as well as polished rice. Surface area (S) was expressed per unit weight of raw rice kernels (Bhattacharya & Sowbhagya, 1971).

2.2.4. Cooking behaviour

The polished grains were cooked in a boiling water bath. Minimum time taken to gelatinize the core was reported as cooking time. Length and breadth of cooked rice kernels were measured by vernier caliper. Elongation ratio was determined as ratio of length of cooked rice to the length of raw polished rice while elongation index was calculated as the ratio of L/B of cooked rice to L/B of raw polished rice. Ratio of difference in length of raw and cooked kernels to the difference in width of raw and cooked kernels was expressed as $\Delta L/\Delta W$. Water absorption (g/g) was determined on the basis of gain in weight of grains after cooking. Swelling number was calculated as the ratio of weight of cooked rice to the weight of raw rice. Ratio of the water absorption to the surface area per unit weight of uncooked rice was expressed as W/S. The gruel left after cooking was evaporated at 100 °C for 10 h and the solids left were expressed as per cent solid loss on the basis of raw rice.

2.2.5. Protein fractionation

Rice protein fractions were prepared by sequentially extracting defatted flour with 0.5 M NaCl (albumin and globulin), 70% aqueous ethanol (prolamin) and 0.1 N NaOH (glutelin) at 25 °C. Following each extraction, the slurry was centrifuged (12,600 rpm, 15 min) and the supernatant was filtered (Whatman No. 1) to remove insoluble particles. Supernatants were dialyzed (pore size 2.4 nm, Hi media Laboratories Ltd., India) with double-distilled water for 24 h with six water changes. After dialysis, the albumin–globulin fractions were separated by centrifugation (13,600 rpm, 15 min). Protein fractions were stored in plastic bottles at −20 °C until further analysis. The protein content of extracts was measured by the Lowry method (Lowry, Rosebrough, Farr, & Randal, 1951).

2.2.6. Electrophoresis

SDS–PAGE of various protein fractions was carried out in a vertical slab (Mini-PROTEAN-3, Bio-Rad Laboratories, USA). The gel was run at 100 V until the dye-front reached the bottom of the resolving gel (14%). Gel was stained overnight with Coomassie gel stain solution. The extra dye was removed by repeated washings, using Coomassie destain solution. Destained gels were analyzed using the Gel Documentation System (Ultra Lum Inc., USA).

2.3. Statistical analysis

The data were analysed by ANOVA (Gomez & Gomez, 1984) to judge the significant changes among the test values due to treatment. Least significant difference (LSD) was also calculated to evaluate the significant differences among the three rice cultivars.

3. Results and discussion

3.1. Proximate composition

The moisture, ash, crude fat, protein and carbohydrate contents in the cultivars IR-8, PR-106 and

Table 1
Proximate composition of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice cultivars ($n = 3$)

Parameters	IR-8	PR-106	Basmati-386	LSD
Moisture (%)	$13.29^{a} \pm 0.69$	$13.75^{a} \pm 0.11$	$14.46^{b} \pm 0.41$	0.66
Ash (%)	$0.69^{a} \pm 0.07$	$0.78^{a} \pm 0.01$	$0.60^{a} \pm 0.07$	0.20
Crude fat (%)	$0.95^{a} \pm 0.07$	$0.75^{b} \pm 0.09$	$0.70^{c} \pm 0.14$	0.15
Protein (%)	$6.61^{a} \pm 0.17$	$8.04^{b} \pm 0.41$	$8.30^{b} \pm 0.07$	0.37
Carbohydrate (%) by difference	$78.5^{a} \pm 1.05$	$76.7^{b} \pm 0.21$	$75.9^{b} \pm 0.27$	1.28

Values reported as means ± standard deviation.

Basmati-386 are presented in Table 1. The ash, lipid and carbohydrate contents of Basmati-386 were lower than IR-8 and PR-106. The protein content was highest in Basmati-386 followed by PR-106 and IR-8. Singh, Kalia, and Malhotra (1999) observed a similar range of various constituents in nine genotypes of rice.

3.2. Physical characteristics of paddy and polished rice

Studies on paddy revealed highest grain length in PR-106, closely followed by Basmati-386, with the lowest in IR-8 (Table 2). On the same lines, PR-106 possessed the highest L/B ratio of 5.09, followed by Basmati-386 and IR-8. A marked difference was observed in thousand seed weight of the three varieties, this being significantly higher in IR-8 (26.38 g) than PR-106 (22.69 g) and Basmati-386 (21.84 g). IR-8 showed the highest bulk density, followed by Basmati-386 and PR-106.

After shelling and polishing, kernel length of *Basmati* grains significantly exceeded that of PR-106 and IR-8. Basmati-386 appeared to be 'extra long', PR-106 as 'long' and IR-8 as 'medium' varieties, according to the length scale (Jennings, Coffman, & Kauffman, 1979). Classification, based on the shape, identified Basmati-386 and PR-106 as 'slender'. IR-8 was classified as a 'medium' variety (L/B 2.1–3.0). The above results are consistent with the findings of Dutta, Lahiri, and Baset (1998). Thousand-kernel weight was highest in IR-8 and lowest in PR-106, whereas IR-8 had the highest bulk density, followed by Basmati-386 and PR-106. Basmati-386 had the highest surface area per unit weight but IR-8 had the lowest surface area. Bhattacharya and Sowbhagya (1971) observed that the surface area per unit weight was related to the shape and size of kernels.

Table 2
Physical characteristics of paddy and polished rice of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) cultivars ($n = 5$)

Features	IR-8	PR-106	Basmati-386	LSD
Paddy				
Length (mm)	$9.1^{a} \pm 0.04$	$10.47^{b} \pm 0.079$	$9.59^{c} \pm 0.144$	0.12
Breadth (mm)	2.75 ± 0.006	$2.09 \pm . 0.022$	2.34 ± 0.012	NS*
L/B	$3.26^{a} \pm 0.138$	$5.09^{b} \pm 0.919$	$4.12^{c} \pm 0.780$	0.86
Thousand seed weight (g)	$26.38^{a} \pm 0.19$	$22.69^{b} \pm 0.11$	$21.84^{c} \pm 0.22$	0.36
Bulk density ($g\,cm^{-3}$)	$0.55^{a} \pm 0.008$	$0.45^{b} \pm 0.005$	$0.52^{c} \pm 0.015$	0.02
Polished rice				
Kernel length (mm)	$6.09^{a} \pm 0.052$	$6.64^{b} \pm 0.02$	$7.68^{c} \pm 0.045$	0.04
Kernel breadth (mm)	$2.36^{a} \pm 0.017$	$1.93^{b} \pm 0.005$	$1.64^{c} \pm 0.014$	0.01
L/B	$2.59^{a} \pm 0.27$	$3.42^{b} \pm 0.136$	$4.7^{c} \pm 0.26$	0.21
Size classification	Medium	Long	Very long	
Shape classification	Medium	Slender	Slender	
Bulk density ($g\,cm^{-3}$)	$0.77^{a} \pm 0.002$	$0.72^{b} \pm 0.006$	$0.73^{c} \pm 0.004$	0.01
Thousand kernel weight (g)	$21.41^{a} \pm 0.447$	$16.45^{b} \pm 0.253$	$17.44^{c} \pm 0.418$	0.62
Surface area per unit weight (cm^2/g)	14.86	17.87	18.39	AV**

Values reported as means ± standard deviation. * Not significant.
** Calculated on the basis of average value.

Table 3
Cooking characteristics of polished rice from coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice cultivars ($n = 5$)

Features	IR-8	PR-106	Basmati-386	LSD
Length (mm)	$9.86^{a} \pm 0.048$	$9.84^{a} \pm 0.077$	$13.76^{b} \pm 0.128$	0.11
Breadth (mm)	$3.42^{a} \pm 0.040$	$3.02^{b} \pm 0.028$	$2.50^{c} \pm 0.021$	0.04
L/B of cooked rice	$2.91^{a} \pm 0.295$	$3.28^{a} \pm 0.35$	$5.53^{c} \pm 0.65$	0.56
Elongation ratio	$1.62^{a} \pm 0.075$	$1.62^{a} \pm 0.099$	$1.69^{b} \pm 0.093$	0.18
Elongation index	$1.30^{a} \pm 0.317$	$1.07^{a} \pm 0.316$	$1.22^{a} \pm 0.448$	0.73
$\Delta L/\Delta W$*	3.557	2.98	7.07	AV**
Water absorbed (g/g)	$3.07^{a} \pm 0.17$	$3.20^{a} \pm 0.19$	$2.79^{b} \pm 0.21$	0.38
Swelling number	$4.07^{a} \pm 0.17$	$4.2^{a} \pm 0.19$	$3.79^{b} \pm 0.22$	0.39
W/S***	0.21	0.18	0.15	AV**
Solids loss (%)	$2.08^{a} \pm 0.082$	$1.42^{b} \pm 0.097$	$1.32^{b} \pm 0.149$	0.14
Time of cooking (min)	18	19	16	AV**

Values reported as means ± standard deviation.
* ΔL, difference in length between raw and cooked grains. ΔW, difference in width between raw and cooked grains.
** Calculated on the basis of average value.
*** W/S, Water absorbed (g/g) to surface area per unit weight.

Table 4
Osborne fractions of coarse (IR-8), fine (PR-106) and superfine (Basmati-386) rice proteins (n = 3)

Protein type	Protein content, g/100 g crude protein			
	IR-8	PR-106	Basmati-386	LSD
Albumin	$4.61^{a} \pm 0.72$ (5.20)	$5.32^{b} \pm 0.21$ (6.56)	$7.41^{c} \pm 0.54$ (9.58)	0.65
Globulin	$11.8^{a} \pm 2.17$ (13.32)	$9.43^{b} \pm 0.38$ (11.63)	$12.5^{c} \pm 0.55$ (16.1)	1.6
Prolamin	$1.75^{a} \pm 0.06$ (1.97)	$0.89^{b} \pm 0.22$ (1.1)	$2.51^{c} \pm 0.19$ (3.25)	0.34
Glutelin	$70.5^{a} \pm 0.58$ (79.5)	$65.4^{b} \pm 0.72$ (80.7)	$54.9^{c} \pm 3.31$ (71.1)	3.96
Residue	11	18.94	22.69	

Values in parentheses indicate percentage of soluble protein.

3.3. Cooking properties

On cooking, L/B of Basmati increased considerably (from 4.1 to 5.5) above its original L/B (Table 3). Maximum elongation was observed in *Basmati*, as both elongation ratio (ER) and elongation index (EI) were higher than those of PR-106 and IR-8. Although length and L/B ratio of paddy grains fallaciously place the PR-106 into the category of long varieties, but after cooking, PR-106 had a lower ER and EI than IR-8.

Better distinction of varieties, on the elongation basis after cooking, could be made by $\Delta L/\Delta W$ ratio as the gap between the values of two varieties was observed to be wider than both ER and EI. *Basmati*, with a $\Delta L/\Delta W$ of 7.1, was clearly distinguished by the use of this ratio from PR-106 and IR-8 with values of 2.98 and 3.36, respectively. The utility of this ratio to distinguish *Basmati* from other very similar varieties should be explored further.

Both IR-8 and PR-106 possessed similar water absorption (g/g) and swelling numbers but both these parameters were low for Basmati. Water absorption was directly related to cooking time (Bhattacharya & Sowbhagya, 1971). The ratio of water absorption to surface area per unit weight (W/S) was lowest in Basmati and highest in IR-8. Cooked Basmati grains had the best appeal with long-slender shape, fine structure and pleasant aroma. Minimum solids loss was also accredited to *Basmati*, closely followed by PR-106 and was highest in IR-8. Basmati cooked the fastest of the three varieties. Low thickness and high surface area might be the responsible factors for the rapid gelatinization of *Basmati*. Hirannaiah et al. (2001) also observed minimum solids loss with high elongation in *Basmati*.

3.4. Protein fractionation

The successive extraction of defatted rice flours of IR-8, PR-106 and Basmati-386 with different solvents yielded albumin (4.6–7.4%), globulin (9.4–12.5%), prolamin (0.9–2.5%) and glutelin (54.9–70.5%), respectively (Table 4). The major fraction of protein was glutelin in all three cultivars while prolamin was minimal. The soluble protein was highest in IR-8, followed by Basmati-386 and PR-106. The relative distributions of the soluble proteins were quite similar in the cultivars. The albumin, globulin, prolamin and glutelin contents for milled rice have been reported to be 2–5, 2–10, 1–5 and 75–90%, respectively (Simmonds, 1978).

3.5. Electrophoretic characterization

3.5.1. Albumin

The major bands in IR-8, PR-106 and Basmati-386 were observed to be 22, 23 and 24 kDa, respectively, on SDS–PAGE (Fig. 1, Table 5). However, IR-8 had one major band only, while PR-106 had five bands and Basmati-386 had four bands, suggesting that IR-8 can be easily distinguished from PR-106 and Basmati 386 on this basis. Villareal and Juliano (1981) also observed 18–

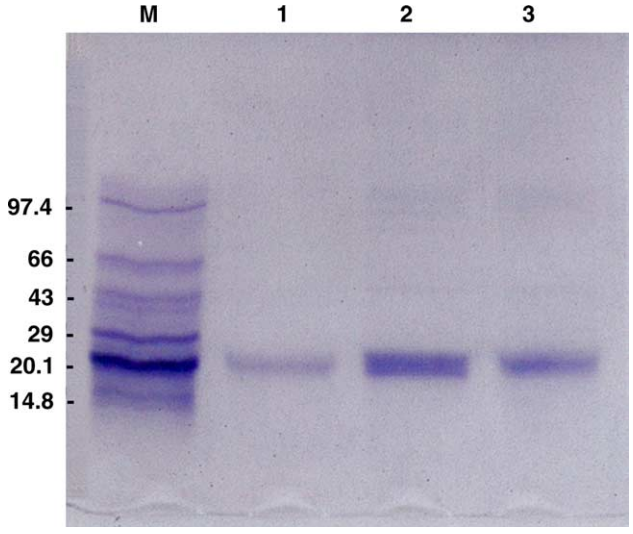

Fig. 1. SDS–PAGE of albumin fraction of IR-8, PR-106 and Basmati-386 on 14% gel. (M, Marker; Lane 1, IR-8; Lane 2, PR-106 and Lane 3, Basmati-386.)

Table 5
Molecular weight and mass proportion of protein fractions of IR-8, PR-106 and Basmati-386 under denaturing electrophoretic conditions

	IR-8	PR-106	Basmati-386
Molecular weight (kDa)			
Albumin	22.1 (98.3)	112 (2.38)	110 (8.61)
	–	101 (0.65)	97.4 (5.52)
	–	91.9 (0.03)	59.7 (9.06)
	–	57.6 (3.81)	24 (76.81)
	–	23.1 (93.1)	–
Globulin	93.9 (20.7)	47.1 (23)	14.5 (61.1)
	41.6 (14.7)	13.2 (44.3)	0.25 (38.9)
	27.6 (18.4)	2.92 (32.6)	–
	20.6 (10.4)	–	–
	13.8 (35.9)	–	–
Prolamin	14.6 (24.4)	14.01 (63.3)	13.7 (50.7)
	11.40 (75.6)	–	–
Glutelin	33.2 (31.6)	60.3 (16.4)	9.95 (57.5)
	19.1 (22.5)	34.6 (39)	–
	13.8 (46)	28.11 (5.5)	–
		20.1 (26.5)	–
		12.9 (22.7)	–

Values in parenthesis indicate mass proportion of subunits.

20 kDa protein subunits as the major albumin of IR-36 milled rice.

3.5.2. Globulin

SDS–PAGE of globulin revealed five bands in IR-8, three in PR-106 and two in Basmati-386, with molecular weights in the range of 13–94, 2–47, and 0.2–15 kDa, respectively (Fig. 2, Table 5). Bands with molecular weight range 13–14.5 kDa were shared by all three varieties; however, a single major band in the SDS–PAGE of Basmati-386 distinguished it. PR-106 had a 47-kDa band while IR-8 had a 42-kDa band, which seemed to be quite similar visually but the 42-kDa band was preceded by a 94-kDa band in the electrophoregram of IR-8 while this pattern was missing in PR-106. Iwasaki et al. (1982) also observed that, although patterns of three rice varieties were similar for both albumins and globulins, clear differences existed, since one band found in one variety was absent in other.

3.5.3. Prolamin

Prolamin, constituting less than 5% of the total protein, was mainly composed of polypeptides with a molecular weight range 11–14.6 kDa. SDS–PAGE revealed

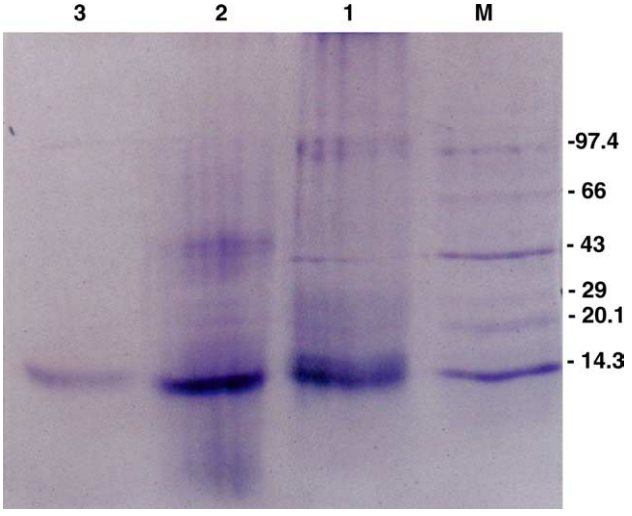

Fig. 2. SDS–PAGE of globulin fraction of IR-8, PR-106 and Basmati-386 on 14% gel. (M, Marker; Lane 1, IR-8; Lane 2, PR-106 and Lane 3, Basmati-386.)

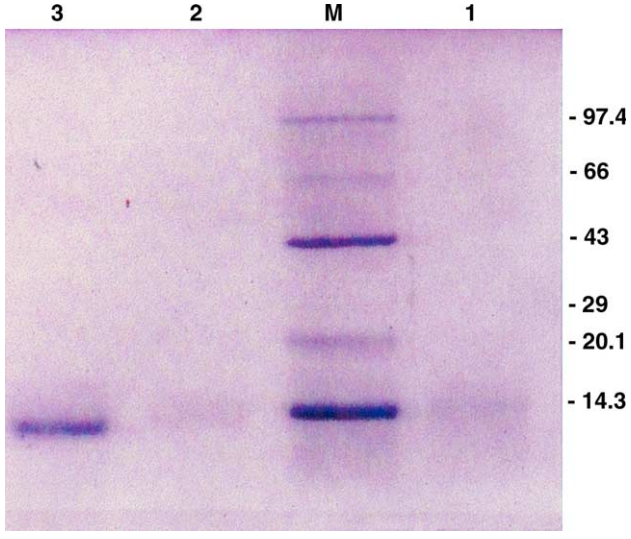

Fig. 3. SDS–PAGE of prolamin fraction of IR-8, PR-106 and Basmati-386 on 14% gel. (M, Marker; Lane 1, IR-8; Lane 2, PR-106 and Lane 3, Basmati-386.)

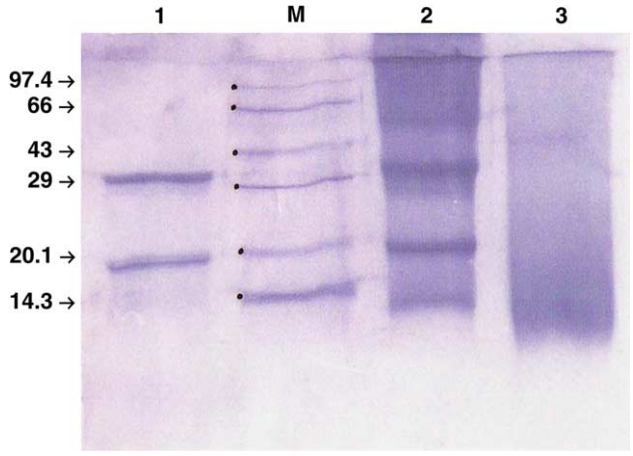

Fig. 4. SDS–PAGE of glutelin fraction of IR-8, PR-106 and Basmati-386 of 14% gel. M, Marker; Lane 1, IR-8; Lane 2, PR-106 and Lane 3, Basmati-386.

two major bands in IR-8 (14.6 and 11.4 kDa) and one band in both PR-106 and Basmati-386 of 14 and 13.7 kDa, respectively (Fig. 3, Table 5).

Steenson and Sathe (1995) reported that prolamin was the most homogeneous of the four fractions of Basmati Dehraduni with a major subunit of 13.2 kDa. A molecular weight range of 10–16 kDa has been reported by different authors (Krishnan & White, 1995; Barber, Loll, & Yang, 1998).

3.5.4. Glutelin

SDS–PAGE of glutelin provided the best varietal discrimination. IR-8 resolved into three subunits (33.2, 19 and 13.8 kDa), PR-106 into five bands (60.3, 34.6, 28.11, 20.1 and 12.9 kDa) and Basmati-386 showed just one subunit of 10 kDa (Fig. 4, Table 5). Two major bands in IR-8 best distinguished it from PR-106 and Basmati-386. A single major band of Basmati-386, of 10 kDa, clearly discriminated it from the other two varieties. Most bands were found in PR-106. Out of five, three bands (34.6, 20 and 13 kDa) of PR-106 were very similar to the bands of IR-8 (33.2, 19 and 13.8 kDa) but the high molecular weight band at 60.3 kDa was absent in IR-8 and Basmati-386. Hussain et al. (1989) obtained unique electrophoregrams for eleven varieties and confirmed the potential distinctive power of PAGE for discriminating rice cultivars.

4. Conclusion

These studies indicated that Basmati-386, PR-106 and IR-8 have different physical characteristics owing to their different shapes and sizes. *Basmati-386* took minimum cooking time and had the highest elongation ratio. The $\Delta L/\Delta W$ ratio better distinguished the three cultivars as *Basmati 386* had a significantly higher value. The glutelin was the major type of protein in rice. Electrophoresis showed different types of peptide among different fractions of the proteins. SDS–PAGE of globulin and glutelin was found capable of differentiating the three cultivars by their protein patterns. These characteristics can also form a basis to explain the functional behaviour of rice in a food system.

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