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Role of proteins in kernel elongation after cooking in aromatic rice

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ABSTRACT

Aromatic rices constitute a small but an elite group of rice. Aroma and linear kernel elongation are two most important characteristics of high quality aromatic rices. Twelve aromatic(IR-291, IR-293, IR-294, Kalanamak, Bindli, Bindli-3132-2, Hansraj-3068, Hansraj-3083, Dehradun Basmati-3020-1, Dehradun Basmati-3041, Dehradun Basmati-3072-1 and Tapovan Basmati-5010) and four non aromatic rice cultivars (Pant Dhan-4, Pant Dhan-10, Pant Dhan-12 and Sarju-52) were used to study the role of proteins in kernel elongation after cooking. Aromatic accessions showed higher kernel elongation after cooking in comparison with non-aromatic accessions. Strong aroma was recorded in most of the scented rices except IR-291, IR-293 and IR-294, where it was medium. Total and soluble protein contents of different accessions varied in the range of 6.4 to 8.8 and 3.00 to 5.26 percent, respectively. There was no relationship between protein content and kernel elongation after cooking. Aromatic rices with high kernel elongation after cooking possess two protein bands of MW 5.20 and 33.11 kDa).

Key words: *Aromatic rice, kernel elongation, proteins*

Aromatic rices are small but distinct group of rice varieties, well known all over the world for their quality particularly high elongation (> 80%) after cooking and aroma. It possess a special place in the world rice market and is generally considered as the highest priced rice (Efferson, 1985). Lengthwise elongation after cooking without increase in girth is considered most desirable in high quality rices. High protein is reported to slow down water absorption during cooking probably by forming a thicker barrier around the starch granules (Kadan *et al.,* 1997). Chemical features like protein content have also been reported to influence the linear elongation of rice during cooking (Choudhury and Ghosh, 1978). Distribution of protein in the endosperm is as important as caryopsis protein content in determining protein retention in milled rice (Kaul *et al.,*1969). Four major types of protein *viz*., albumin, globulin, prolamin and glutelin are distributed in milled rice kernel in a ratio of 5:9:3:83 (Juliano, 1972).

Efforts are being made to screen the aromatic rice cultivars from non-aromatic cultivars based on the distribution pattern of various proteins. The relationship between these proteins and rice kernel elongation after cooking could be useful to further aromatic rice development programme. Hence the present investigation on protein profiling was undertaken using twelve aromatic and four non-aromatic rice varieties to evaluate the role of proteins in kernel elongation after cooking.

MATERIALS AND METHODS

Twelve aromatic (IR-291, IR-293, IR-294, Kalanamak, Bindli, Bindli-3132-2, Hansraj-3068, Hansraj-3083, Dehradun Basmati-3020-1, Dehradun Basmati-3041, Dehradun Basmati-3072-1 and Tapovan Basmati-5010) and four non aromatic rice cultivars (Pant Dhan-4, Pant Dhan-10, Pant Dhan-12 and Sarju-52) were used for the present study. The seeds were obtained from rice collections maintained at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal.

The rice varieties were analyzed for grain characteristics on the basis of average length of kernels and length/width ratio of milled kernels of each accession as described by (Adair *et al.,* 1973). The grain dimensions *viz.* length, width and its ratio were

Scale	Size	Length (MM)	Shape	Length/width ratio
1	Extra long	>7.50	Slender	Over 3.0
2	Long	6.61-7.50	Medium	2.1-3.0
3	Medium	5.51-6.60	Bold	1.1-2.0
4	Short	5.50 or less	Round	1.0 or less

measured and were categorized into different shape and size as per the given table.

The elongation ratio after cooking was estimated on the basis of the initial grain length and width ratio with the help of graph paper and the aroma was recorded by sensory evaluation through 1.7% KOH method.

The total rice protein and the soluble proteins of the grain were extracted according to the procedure described earlier (Padhey and Salunkhe 1979) by following the sequential steps of dehulling, polishing and grinding into fine powder by a pestle mortar. The powder was extracted with 0.05 M Tris-HCI buffer (pH 7.5) by keeping overnight. The mixture was then centrifuged at 13,000 rpm in eppondorf tube and the supernatant collected was estimated for protein content using Bradford's method (Bradford, 1976). Total protein contents were also estimated by using the grain analyzer.

Proteins in complex mixture are separated by SDS Polyacrylamide Gel Electrophoresis. The protein extract was mixed with sample buffer containing glycerol and bromophenol blue. Sample containing 100 µg of proteins was loaded in each well of 15% Polyacrylamide gel with broad range protein molecular weight markers. The gels were run in a BIO-RAD Protein II electrophoresis system at constant current of 1.5 mA per well at 15^{0}C. The run was continued till the dye front reached the bottom of the gel. The gel was stained with Coomasie brilliant blue and separated bands were scanned against the molecular weight markers using the gel scanner. After the gel electrophoresis of protein, the bands were compared with that of broad range molecular weight markers Rm value of different protein bands was calculated with the help of molecular weight marker graph (log molecular weight *vs* Rm). The plot is based on Broad Range Molecular Weight Marker : (Mol.Wt kDa :Rm) (97.40 : 0.12) (67.00 : 0.25) (43.00 : 0.43) (29.00 : 0.54) (20.00 : 0.69) (14.40 : 0.86) in Fig 1.

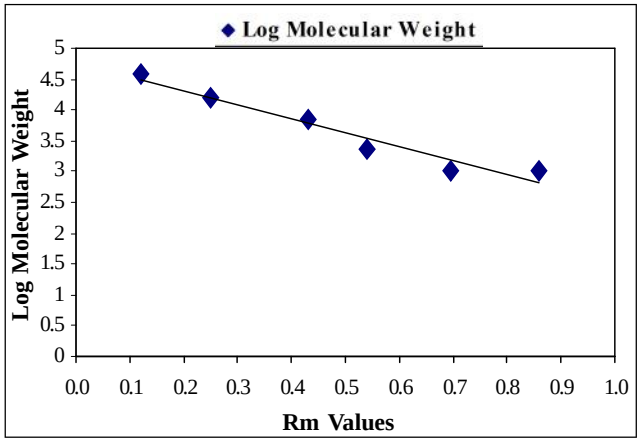

Fig. 1. Standard curve plotted with the help of broad range molecular weight marker

RESULTS AND DISCUSSION

The grain characteristics were analyzed on the basis of grain dimension *viz.*, length, width and length/width ratio. The average grain length and width varied between 4.00 mm to 7.00 mm and 1.71mm to 3.15 mm respectively. Similarly the length and width of cooked kernel varied in the range between 9.5 -19.5 mm and 2.31-3.31mm respectively. The variation of the length/ width ratio of the milled rice and cooked kernel were found to be 1.81 – 3.86 and 3.62 -7.76 respectively. The range of kernel elongation ratio after cooking varied between1.84 to 2.78. In the present study, Dehradun Basmati-3072-1 showed the maximum elongation after cooking. In general, Basmati-type accessions were longer and showed higher length width ratio of the kernel and its elongation after cooking than in non basmati types. The non aromatic cultivars like PD-4, PD-10, PD-12 and Sarju-52 showed minimum kernel elongation ratio (<2.0) among all the cultivars studied. Whereas the aromatic rice cultivars *viz.*, Dehradun Basmati-3072-1, Bindli-3132-2, IR294, Bindli, and Dehradun Basmati-3041 showed maximum kernel elongation ratio of 2.78, 2.50, 2.45, 2.37 and 2.35, respectively (Table 1).

Total grain protein content varied between 6.4

Table 1. Grain characteristics of different aromatic rice cultivars

Designation	Polished rice			Cooked kernel			Elongation Ratio	Grain shape	Grain size	Aroma	Total Protein(%)	Soluble protein(%)
	Length (mm)	Breadth (mm)	L/B Ratio	Length (mm)	Breadth (mm)	L/B Ratio						
Tapovan 5010	6.00	2.00	3.00	13.00	2.80	4.64	2.16	Med.	Med.	S	6.4	3.24
IR 293	6.00	3.15	1.90	12.00	2.80	4.28	2.00	slender	Long	M	7.9	3.71
Hansraj 3083	6.50	1.71	3.80	15.00	2.31	6.49	2.30	slender	Long	S	8.7	4.74
Dehradun Basmati-041	7.00	1.91	3.66	16.50	2.91	5.67	2.35	slender	Long	S	8.6	3.18
Bindli	4.00	2.00	2.00	9.50	2.40	3.95	2.37	bold	short	S	7.5	3.06
Pant Dhan 4	6.50	2.11	3.08	12.00	3.31	3.62	1.84	Med.	Long	NA	7.8	5.04
Pant Dhan10	6.50	2.11	3.08	12.50	3.30	3.78	1.92	Med.	Long	NA	8.3	3.19
PantDhan 12	7.00	2.12	3.30	12.75	3.31	3.85	1.85	Med.	Med.	NA	8.0	3.42
Kalanamak	6.00	2.30	2.60	13.00	2.90	3.48	2.08	Med.	Med.	S	8.6	5.26
IR 294	5.50	2.00	2.75	13.50	2.82	4.78	2.45	slender	Long	M	8.8	4.59
Sarju 52	6.50	2.92	2.22	12.50	3.29	3.79	1.92	Med.	Med.	NA	8 .0	3.18
IR 291	6.50	2.00	3.25	13.00	2.80	4.64	2.00	slender	Long	M	80	3.16
Dehradun Basmati 3020	6.50	1.71	3.80	15.00	2.69	5.57	2.30	slender	Long	S	7.9	3.34
Hansraj 3068	6.50	1.95	3.33	15.10	2.79	5.41	2.32	slender	Long	S	8.6	3.45
Bindli 3132-2	4.00	2.20	1.81	10.00	2.40	4.16	2.50	Bold	short	S	7.5	3.04
Dehradun Basmati 3072-1	7.00	1.81	3.86	19.5	2.51	7.76	2.78	slender	Long	S	7.6	3.00

NA = no aroma; M = medium aroma; S = strong aroma

Table 2. Distribution pattern of Protein in different aromatic rice cultivars as obtained by SDS-PAGE

Mol. Wt.	Aromatic cultivars											Non-Aromatic cultivars				
(kDa)	Tapovan 5010	IR 293	Hansraj 3083	Dehradun Basmati 3041	Bindli	Kalanamak	IR 294	IR 291	Dehradun Basmati 3020	Hansraj 30	Bindli 3132-2	Dehradun Basmati 3072-1	Pant Dhan 4	Pant Dhan 10	Pant Dhan 12	Saraju 52
134.28	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
124.28	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
121.51	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+
102.02	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
89.56	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
77.45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
73.69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
61.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57.30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54.59	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
52.45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
49.50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42.52	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33.11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
28.70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27.53	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14.87	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5.20	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
3 00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = present, - = absent

to 8.8 % and the soluble protein content varied from 3.0 to 5.26 % in different rice varieties (Table 1). Choudhury and Ghosh (1978) reported that protein content of fine and scented varieties ranged from 6.0 to 10.26 percent, the average being 7.83 percent and it has also been reported that linear kernel elongation was negatively correlated with protein content. Tetens *et al.*,(1997) stated a significant positive correlation between breadth and elongation ratio, and elongation ratio and protein content where as a significant negative correlation was reported between breadth and volume expansion, shape and protein content.

In the present investigation, aromatic rice cultivars showing higher kernel elongation after cooking *viz.*, Dehradun Basmati-3072-1, Bindli-3132-2, Bindli and Dehradun Basmati-3041 showed lower total protein content of 3.0, 3.04, 3.06 and 3.18 percent respectively. Non aromatic rice varieties have low soluble protein and high kernel elongation ratio. From the study it was revealed that there was no relation between the total protein content and the kernel elongation ratio after cooking both in aromatic as well as in non aromatic rice cultivars. A total of 20 protein bands, with MW in the range of 3.0 to 134.28 kDa, were observed out of which 13 bands with MW of 3.0, 14.87, 16.44, 27.53, 28.70, 42.52, 49.50, 52.45, 54.59, 57.30, 61.50, 73.69 and 77.45 bands were common to all the cultivars. Three protein bands with MW of 5.20, 33.11 and 102.02 kDa were present only in aromatic cultivars. No protein band was selective for non-aromatic cultivars. Protein band with MW of 89.56 kDa was shared by three out of four non-aromatic cultivars (PD-4, PD-10 and PD-12). These three cultivars had very poor elongation after cooking. Two unique bands with MW of 124.28 and 134.28 kDa were present only in variety IR-291 (Table 2). On the basis of grain size 16 accessions were grouped into three groups i.e. Long (Dehradun Basmati-3020, Dehradun Basmati-3072-1, Dehradun Basmati-3041, IR-293 and Hansraj-3068), Medium (Kalanamak, PD-4, PD-10, PD-12, Sarju-52 and Tapovan Basamti-5010) and short (Bindli, Bindli-3132-2). Based on shape they were categorized into 3 groups, *i.e.* Slender (all scented accessions), Medium (PD-4) and Bold (Bindli, Bindli-31322). Kernel elongation ratio after cooking varied in the range of 1.84 to 2.78. It was highest for Dehradun Basmati-30721. Strong aroma was recorded in most of the scented rices except IR-291, IR-293 and IR-294, where it was medium. No aroma was present in Sarju-52, PD-4, PD-10 and PD-12. Presence of three distinct protein bands of 5.20, 33.11 and 102.02 kDa could be useful in associating very specifically to aromatic group of rice varieties. They may play some role in either aroma or high elongation of these varieties. Thus the protein profiling by SDS-PAGE are easier and faster technique than any other conventional technique for rapid screening of aromatic rice cultivars from non-aromatic types.

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