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Expression of quality of basmati rice (*Oryza sativa* L.) within and beyond geographical indication: 'Penal test' the traditional method for quality determination has an edge over molecular tools

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Abstract
Quality of Basmati rice is largely deteriorated due to admixture of non-Basmati grains and genotype x environment (GxE) interactions within and beyond geographical indication (GI) area. Traditional morpho-physico-chemical methods supplemented with sensory evaluation could determine the deterioration in quality of Basmati rice resulted from GxE interactions as well. Sensory evaluation of quality of basmati mainly involved appearance, 'tenderness on touching and chewing', aroma and 'taste (mouth feel) the perception linked traits of human behavior. Findings of Human Genome Project have also, indicated that application of molecular markers were of limited value for prediction of human behavior. Grains of 4 varieties of Basmati rice produced within and beyond GI were analyzed for 13 quality parameters prescribed as per definition. Significant reduction was observed in expression of only three quality parameters elongation after cooking, elongation ratio and amylose contents when produced beyond GI. However, such expression pattern of quality parameters beyond GI was observed to be within tolerance limits of human sensory organs. It reflected that GI could not limit quality of Basmati rice. Limitations and relevance of molecular markers, traditional penal test methods for determination of quality of basmati were also discussed.

Keywords: Basmati quality, GXE interaction, taste perception, geographical indication, molecular markers

Introduction
'Basmati' a customary name ascribed to varieties of specialty rice with 13 specific quality parameters (Table-1) being cultivated over the traditional areas covered under Indo-Pak cross border GI for Basmati rice. Genotype x environment interaction (GXE) of important climatic components such as soil and water of the region adjoining to the Himalayas was supposed to be responsible for the specific quality of basmati rice (Mani *et al.* 2005, Sivaranjani *et al.* 2010, Singh *et al.* 2000 and Singh *et al.* 2012) [11, 21, 19]. It restricted cultivation of Basmati rice to the plains of India and Pakistan adjoining to Himalayas. The commodity produced only in GI was considered true Basmati and, therefore, is allowed for export under the name 'Basmati' with GI tag.

Recently look alike Basmati, non-Basmati (NB) varieties such as 'Sarbati' inferior in quality used as 'blend' (admixture) in export consignments, became popular among farmers and traders. So, it was felt necessary to standardize quality parameters in order to detect admixture to maintain purity of the commodity. The traditionally used morphological and chemical parameters have not been found to be discriminative enough, warranting development of more precise techniques. Nagraju *et al.* 2002 [15] considered molecular interventions more suitable to serve the purpose. Government of India has category wise fixed limits 5% to 15% of admixture in Basmati rice. In addition to Blending, impact of GxE in arable areas both under GI (minor GxE impact) and beyond GI (major GxE impact) is largely expected to deteriorate quality of Basmati rice causing variations at phenotypic level that cannot be detected by molecular markers. Therefore, application of expansive, fine high throughput molecular techniques alone has no particular relevance. Traditionally used morpho-physico-chemical methods supplemented with sensory (organoleptic) evaluation seem to have potential to develop quality parameters to provide confirmatory determination of quality of Basmati rice more efficiently.

In past, India has been dragged into litigation with the US Patents and Trademark Office, over granting patent of 'Basmati' to the Texas based US seed company Rice Tec, for novel rice lines whose grains had qualities similar to Indian Basmati. That case has now been settled for the major issues. But the same issue has now been raised within India itself in the name of protection of interests of Basmati growers beyond GI by the state governments as was experienced in a case filed for registration for protection of Basmati grown in the state of Madhya Pradesh under GI tag. While this state is far away from the GI area. Therefore, the present study was undertaken to determine quality Of Basmati rice produced within and beyond GI to assess relevance of GI. Relevance of molecular markers for determination of quality of Basmati rice was also reviewed citing examples from human genomics as well.

Materials and Methods

Four varieties of quality rice involving Taroari Basmati (traditional Basmati rice) and evolved varieties Pusa basmati 1121, Pusa Basmati 1 and Pusa Sugandha 5 were planted at IARI, New Delhi and DRR, Hyderabad during the Kharif (Rainy crop season) 2011 and Kharif 2012. Grain samples taken from both the locations were analyzed following the standard DRR protocols (Anonymous, 2012) for estimation of 13 quality parameters and sensory evaluation by a penal of experts. Sensory evaluation of quality of Basmati rice was made at Quality Laboratory, DRR (ICAR), and Hyderabad by a panel of experts who could taste cooked and made observations on uncooked rice perfectly. It was based only on expertise and skill of the experts using rice aged for two months. The penal evaluation (Table 2) was used for estimation of overall acceptability ratings on account of appearance, cohesiveness, and tenderness on touching and chewing, aroma, ER, taste and flaky texture on cooking (Anonymous, 2010) [1]. Likewise, four recently developed Basmati varieties Vallabh Basmati 21, Vallabh Basmati 22, Vallabh Basmati 23, Vallabh Basmati 24 along with Taroari Basmati and Pusa Basmati1checks grown in GI were analysed (Table1).

Results and Discussion

Quality parameters of four varieties of Basmati rice involving Taroari Basmati (traditional Basmati rice) and evolved varieties Pusa 1121, Pusa Basmati 1 and PS-5 planted at IARI, New Delhi and DRR, Hyderabad during the Kharif crop seasons (Rainy season) 2011 and Kharif 2012 are depicted in Fig.1. Sensory evaluation of the same produce is presented in Fig.2.

Quality parameters and organoleptic (sensory) evaluation of the Basmati varieties Taroari Basmati, Pusa Basmati-1, Vallabh Basmati-21, Vallabh Basmati-22, Vallabh Basmati -23 and Vallabh Basmati -24 grown only in GI are given in Table-1 and Table-2, respectively.

Genotype x environment interactions may largely cause variations only at phenotypic level and, therefore, can't be detected by molecular markers available for detecting genetic differences within and among cultivars. Admixture of Sarbati type Non-Basmati rice grains into Basmati varieties and genotype x environment interactions largely deteriorate quality of Basmati rice. Admixtures cause variations at genotypic level too, that could be detected by molecular markers (Singh *et. al*. 2011; Singh *et al*. 2012, Singh *et al*. 2008; DelaCruz and Khush, 2000) [18, 19, 8]. The simple sequence repeat (SSR) markers are efficient and cost-effective and, therefore, could detect significantly higher degree of polymorphism (Zetkiewicz *et al.* 1994; Moller *et al.* 1992) [24, 13] in rice in case of admixture. An alternative method to SSR, called inter-SSR-PCR, has also been preferred to fingerprint the Basmati rice varieties (Nagaraju *et al.* 2002; Singh *et al.* 2009) [15]. These molecular tools are ideal for estimation of genetic diversity and intensive genetic mapping (Botstein *et al.* 1980; Sood and Siddiq, 1978) [4, 22] a basis of DNA fingerprinting based classification.

Quality of Basmati rice (Anonymous, 2010) [1] is a resultant effect of 13 component traits/parameters (Table-2). In addition to individual genes, some component characteristics of quality are governed by several QTLs. Basmati is itself a composite character mainly recognized by its unique aroma, taste, appearance, tenderness on touching and chewing. Basically quality parameters of Basmati rice are perceived by 4 sensory organs such as eyes, finger tips, nose and taste buds of tongue (Table-2). On average, the human tongue has about 2,000–8,000 taste buds. The taste buds generally are located on raised protrusions of the tongue surface called papillae. Taste buds contain the receptors for taste. The receptors are located around the small structures called papillae on the upper surface of the tongue. The papillae are involved in detecting salty, sour, unami, bitter and sweet the five major elements of taste perception through very small openings in the tongue known as taste pores. The food particles dissolved in mouth saliva come into contact with receptors and perceived as 'taste' (Berg *et. al.* 2002 and Chaudhari and Rope, 2010) [3, 7]. The taste or mouth feel is the natural way of determination of quality of food products. However, attempts are made to determine quality through its integrative parameters (Table-1).

Contrarily, despite having well defined genetic background most of the component traits of quality of Basmati rice are greatly influenced by environmental variations. GxE interactions largely deteriorate quality of Basmati rice. Such deteriorations in quality are caused firstly, by inadequate agronomical management. *Secondly*, biotic and abiotic stresses such as insect, diseases, lodging, drought etc. deteriorate quality of Basmati rice. *Thirdly*, cultivation of traditional basmati varieties elsewhere in traditional rice-growing areas in the world beyond its specific area of adaptation/GI (Mani *et al.* 2005) [11] will certainly lose its unique morphological and physical quality attributes (Anonymous, 2010) [1] and *lastly* exposure to sun and prolonged ageing also results into deterioration of quality. All such variations are not observed at genetic level and, therefore, cannot be detected by molecular tools (Peyman *et al.* 2009) [17]. For example, molecular markers can't serve useful purpose in determination of quality of different lots of the same variety if lot wise deterioration in quality is observed under different levels of environmental factors (no genetic variation is expected). Under such situations only traditional methods seem capable enough to work.

Furthermore, quality of Basmati rice is a resultant expression of a variety of 13 component parameters; therefore, application of potent markers for component characters may be studied *in toto* to have more reliable procedures to determine quality. It will perhaps need large number of distinct markers. However, presently there is not even a single procedure that could be used for development of molecular markers for functional mechanism of taste buds of the human tongue to describe how differently the eater may feel when he eats rice of different qualities. It also seems difficult to develop molecular markers for some of the quality traits such as appearance, cohesiveness, tenderness on touching and chewing, taste and flaky texture of cooked rice on cooling etc. On the other hand, molecular markers for so many complex traits such as aroma have been developed (Bradbury, 2005) [5].

Development of such molecular markers and their efficient exploitation for determination of quality of Basmati rice still remains to be a remote goal. Therefore, determination of quality of basmati rice based on traditional morpho-physico-chemical methods of basmati testing is still the most reliable safeguard to protect Basmati export from adulteration till better alternative approaches are made available.

In parallel, the reports from Human Genome Project reflected almost the same conclusions about different aspects of human behavior. To date, however, the utility of genetic markers based on genome analysis, to improve risk prediction has shown mixed results, even for the most promising marker, located in the 9p21 region (Talmud *et al.* 2008; Brautbar *et al.* 2009) [23]. A genetic risk score comprising 101 single nucleotide polymorphisms was not significantly associated with the prediction of activity of vital parts of human being (Paynter *et al.* 2010) [16].

To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and incidence of diseases, the use of a multilocus genetic risk score has been proposed (Morrison *et al.* 2007) [14]. One such score developed by Kathiresan *et al.* 2008 [9] including 9 genetic markers associated with increased lipid levels but showed no improvement in risk discrimination and, only a slight improvement in reclassification were observed.

In large part, however, the predictive abilities of recently discovered genetic markers could not achieve the targets in case of human behaviour. The genome has yielded one insightful surprise after another. But the primary goal, development of molecular markers of the 3 Billion US dollars Human Genome Project- to ferret out the genetic roots of common fatal disease and then generate treatments-remained largely elusive. Consequently, geneticists are almost back to square one in knowing where to look for the development of molecular markers for determination of human behavior. Paynter *et al.* 2010 [16] attempted to summarize that old fashioned traditional method of taking a record of family history of man could still be a better guide for determination of human behaviors. Likewise, 'perception' of quality of Basmati rice determined by utilizing traditional methods assumed importance. Molecular markers can adequately detect admixture/ blending, one of the major causes of quality deterioration of Basmati rice. However, application of such a sophisticated and expansive molecular technology is not required as the category wise permissible limits of admixture of grains of non-Basmati rice 5%, 8% and 15% (Table-3) can be efficiently achieved by using the old fashioned traditional morpho-physico-chemical methods (Singh *et.al.* 2011) [18].

IIRR, Hyderabad is utilizing traditional physico-chemical methods for development of variety wise database required for statutorily identification, release and notification of elite varieties of Basmati rice for commercial cultivation for domestic consumption as well as global trade. Also, Basmati Export Development Foundation, Meerut, India still mainly depends upon the same traditional physico-chemical methods for analyzing samples collected from consignments packed for export for determination of quality of Basmati rice and detection of level of admixture of non-basmati grains to verify permissible limits for Indian Basmati rice adopted by APEDA.

Furthermore, GI was also felt required to maintain quality and sanctity of 'Basmati' name as per its natural definition. The improved varieties (evolved varieties/cross breeds) such as Basmati CSR 30 and Basmati Pusa 1121 which were earlier not named 'Basmati' for many years because these could not fulfill the natural definition of Basmati. However, these semi dwarf evolved varieties of Basmati could expressed quality parameters closer to Basmati and captured lions' share in the national as well as global market. Definition of Basmati was revised twice to declare them Basmati. However, Quality expressed by such Basmati varieties beyond GI was observed to be within the limits of GxE interactions.

Presently grains of Taroari Basmati (traditional), Basmati Pusa 1121, Pusa Basmati 1 and PS-5 (evolved) grown at IARI, New Delhi and IIRR, Hyderabad during the Kharif crop seasons (Rainy season) 2011 and Kharif 2012 were analyzed for 13 quality parameters as depicted in Fig.1a and Fig1b. At IARI, only three quality parameters Kernel Length Elongation after Cooking, Elongation Ratio and Amylose Content expressed significantly higher than that did at Hyderabad. However, such differential pattern of expression of quality parameters was observed to be within the tolerance limit of sensory organs during penal test. The sensory evaluation (Penal Test) was based on appearance, cohesiveness, tenderness on touching and chewing, taste, aroma and elongation ratio of rice. Observations of the panel were used to estimate overall acceptability of Basmati varieties. During sensory evaluation no difference for overall acceptability of quality or taste (mouth feel) between the grains of Basmati grown in GI and beyond GI was experienced. Truly, no significant variation was detected in taste or mouth feel and overall acceptability during penal evaluation. Therefore, quality of Basmati varieties grown in GI and beyond GI may well be treated on par for all commercial purposes as well. Consequently, cultivation of improved varieties of basmati in areas not covered under GI doesn't seem to be against the interests of farmers of the area under GI.

Furthermore, concept of GI tag was originated to maintain quality of the product and protect interests of its developers. Geographical indication is a type of certification that the product has certain qualities. The tag also certifies that the product has been originated or improved by traditional methods in its geographical area under GI. Therefore, granting of GI tag to evolved/cross bred varieties of basmati, firstly, seems to be against the spirit of WTO agreement. Secondly, the traditional basmati types being already at the verge of extinction certainly, will disappear from cultivation due to their low yields. GI tag may well be restricted only to 'Traditional Basmati varieties', types grown in the area covered under Indo-Pak cross border GI for Basmati in order to protect and maintain the heritage of Basmati and sanctity of being to be true to its natural definition as per WTO agreement. Also, cultivation and export of evolved/cross bred varieties of basmati from all over India seems justifiable in order to protect interests of cultivators as well. Conflicts of interest do not exist

Table 1: Expression of quality parameters of basmati varieties recently released for area under GI. Prescribed quality standards were used by IIRR, Hyderabad as per latest definition of basmati rice (Anonymous, 2010) [1]

Basmati parameters	Standards prescribed for GI area	PB1	Taroari Basmati	Vallabh Basmati 21	Vallabh Basmati 22	Vallabh Basmati 23	Vallabh Basmati 24
Kernel length (mm)	6.61 (Min)	6.98	6.94	7.64	7.45	7.22	7.48
Kernel breadth (mm)	2.00(Max)	1.78	1.79	1.79	1.72	1.73	1.65
Length breadth ratio	3.5 (Min)	3.88	3.88	4.27	4.30	4.17	4.53
KLAC (mm)	12.0 (Min)	15.5	13.3	13.3	14.80	15.7	15.2
ER	1.70 (Min)	2.22	1.92	1.73	1.98	2.17	2.03
VER	3.5 (Mini)	4.85	4.63	4.7	4.74	5.3	5.1
Aroma (1-3)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Water uptake (ml)	250 (Min)	362	268	310	205.50	258	280
Amylose (%)	20-25	24.8	24.44	24.48	23.47	23.58	24.05
ASV	4.0-7.0	7.0	5.0	7.0	5.0	5.0	7.0
Hulling %	76.0 (Min)	76.0	74.0	76.0	76.0	76.8	76.5
Milling %	65.0(Min)	67.0	69.2	66.2	69.37	67.3	66. 8
HRR %	45.0 (Min)	52.1	52.2	51.0	52.01	52.1	51.5

Note: Vallabh Basmati 21 (2013) and Vallabh Basmati 23 (2014) were released for cultivation in Uttar Pradesh. Vallabh Basmati 22 (2009) was released for Haryana and Uttar Pradesh. Vallabh Basmati 24 (2014) was released for commercial cultivation in Haryana, Uttar Pradesh and Jammu and Kashmir states of India. IIRR-Indian Institute for Rice Research. Min-Minimum. Max.- Maximum. HRR- Head Rice Recovery. VER- Volume Expansion Ratio. KLAC- kernel length after cooking. ER- Elongation Ratio. ASV- Alkali Spreading Value.

Table 2: Organoleptic Panel test (DRR Protocol). Quality perceived by four sensory organs i.e. eyes, skin, tongue and nose: variety x sensory organ interaction for quality scores of Basmati rice at IIRR Hyderabad (Anonymous, 2005.)

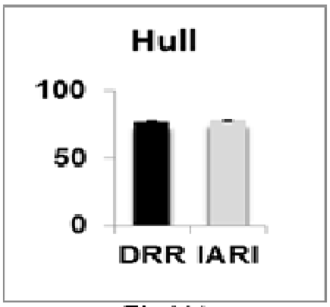
Varieties	Appearance (eyes' act)	Cohesive-ness (eyes' act)	Tenderness on		Mouth feel (Taste) (tongue's act)	Aroma (Nose's act)	Elongation ratio (eyes' act)	Overall acceptability (joint perception of four sensory organs)
			Touching (skin's act)	Chewing (tongue's act)				
Pusa Basmati-1 (yield check)	4.40	3.20	3.93	4.03	3.20	3.40	2.93	3.48 (Good)
Taroari Basmati (quality check)	5.00	4.20	3.80	4.20	3.46	3.73	3.33	3.87 (Good)
Vallabh Basmati-21	4.42 (1)	3.71 (1)	4.07(2)	4.7 0 (2)	3.14	3.50 (2)	2.57	3.47 (Good)
Vallabh Basmati-22	4.57(1)	3.92 (1)	4.42 (2)	4.57 (2)	3.64 (2)	3.92 (2)	3.57 (2)	4.00 (2) (Excellent)
Vallabh Basmati 23	4.42 (1)	3.92 (1)	4.07(2)	4.21 (2)	3.21(1)	3.71(1)	3.21 (1)	3.70 (1) (Good)
Vallabh Basmati 24	4.7(1)	4.2	4.3(2)	4.2(2)	3.4(1)	3.4(1)	3.1 (1)	3.79 (1) (Good)
Remark	4.0-4.9: Creamish; 3.0-3.9: Red Streaks	4.0-4.9: Partially Separated; 3.0-3.9: Slightly sticky	4.0-4.9: Moderately soft; 3.0-3.9: Moderately Hard	4.0-4.9: Moderately soft; 3.0-3.9: Moderately Hard	3.0-3.9: Desirable; 2.0-2.9: Tasteless	4.0 :Strong; 3.0-3.9: Optimum; 2.0-2.9 : Mild	3.0-3.9 Good: 2.0-2.9 Moderate	4.0-4.9: Excellent 3.0-3.9 : Good: 2.0- 2.9: Acceptable

Note: Value 1 in parenthesis indicated superiority over check variety Pusa Basmati-l while value 2 indicated superiority over both the check varieties Pusa Basmati-l and Taroari Basmati

Table 3: Grade (Sp, A, B) specifications for Indian Basmati rice adopted by APEDA for export (Available at http://agmarknet.nic.in/amrscheme/punerice044.htm)

Specification	Permissible limits for exports for Indian Basmati											
	Milled			Milled parboiled			Brown			Brown parboiled		
	Sp	'A'	'B'	Sp	'A'	'B'	Sp	'A'	'B'	Sp	'A'	'B'
Length of precooked rice (mm)	7.1	7.0	6.8	7.1	7.0	6.8	7.4	7.2	7.0	7.4	7.2	7.0
Min L/B ratio	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Max moisture content (%)	14	14	14	14	14	14	14	14	14	14	14	14
Max number of damaged/dis-coloured grains (%)	0.5	0.7	1.0	0.5	0.7	1.0	0.5	0.7	1.0	0.5	0.7	1.0
Max chalky grain/ black kernels (%)	3	5	7	0.1	0.5	1.0	3	5	7	0.5	1.0	2.0
Max broken grains (%)	2	3	5	2	3	5	2	3	5	2	3	5
Max foreign matter (%)	0.1	0.25	0.4	0.1	0.25	0.4	0.2	0.5	1.00	0.2	0.5	1.0
Max grains of other crops and weeds etc. (%)	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2
Max grains of other rice varieties (%)	5	8	15	5	8	15	5	8	15	5	8	15
Max paddy grains (%)	0.1	0.2	0.3	0.1	0.2	0.3	0.2	0.5	0.8	0.1	0.2	0.3
Min elongation ratio	1.7	1.7	1.7	1.5	1.5	1.5	1.7	1.7	1.7	1.5	1.5	1.5
Max green grains (%)	nil	nil	nil	nil	nil	nil	2.0	4.0	6.0	2.0	4.0	6.0

Note: Max- Maximum, Min- Minimum, Sp-Special grade,
APEDA-Agricultural products export development authority of India



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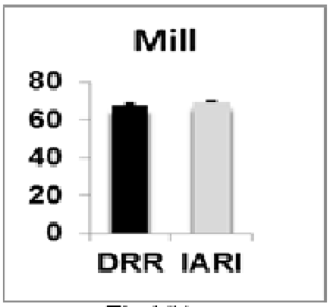

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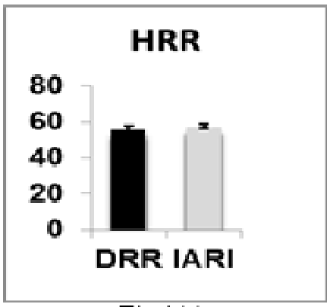

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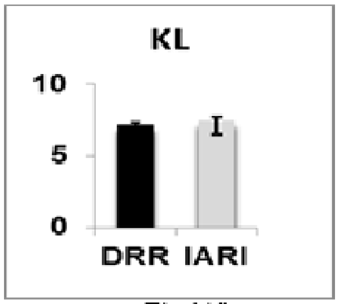

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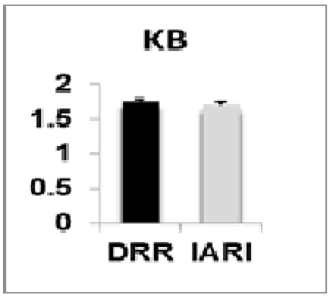

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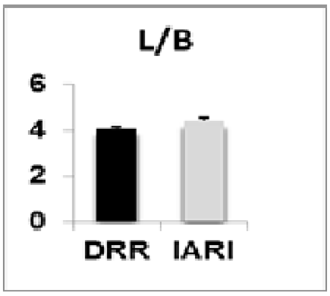

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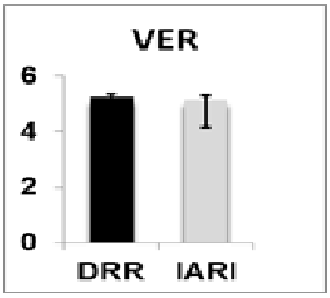

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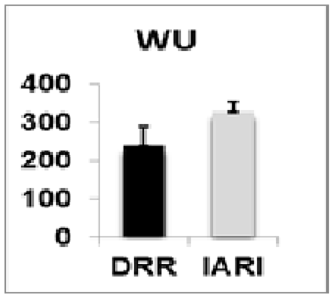

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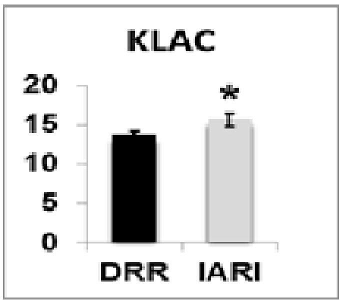

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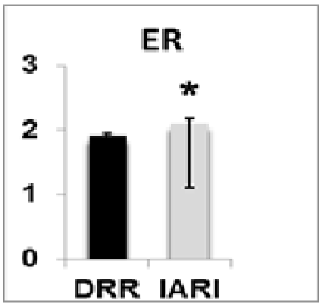

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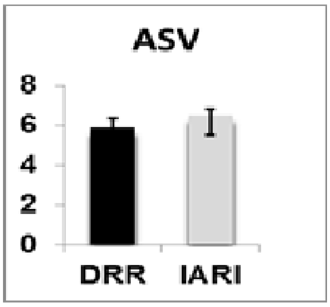

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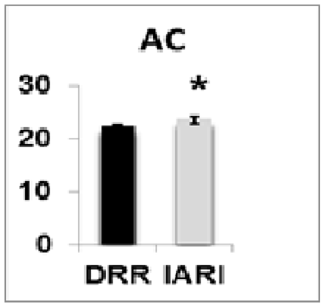

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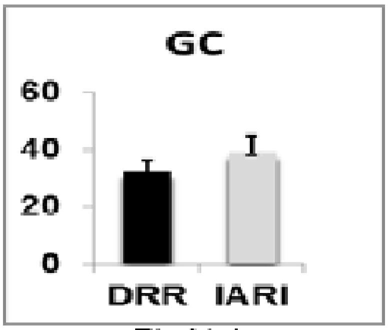

Fig 1(m)

Fig 1 (a-m): Bar diagrams represent 13 parameters of quality of Basmati produced within the GI and outside the GI. GC (Gel consistency) parameter of Basmati quality has now been removed from the latest definition of Basmati. * indicates significance of difference.

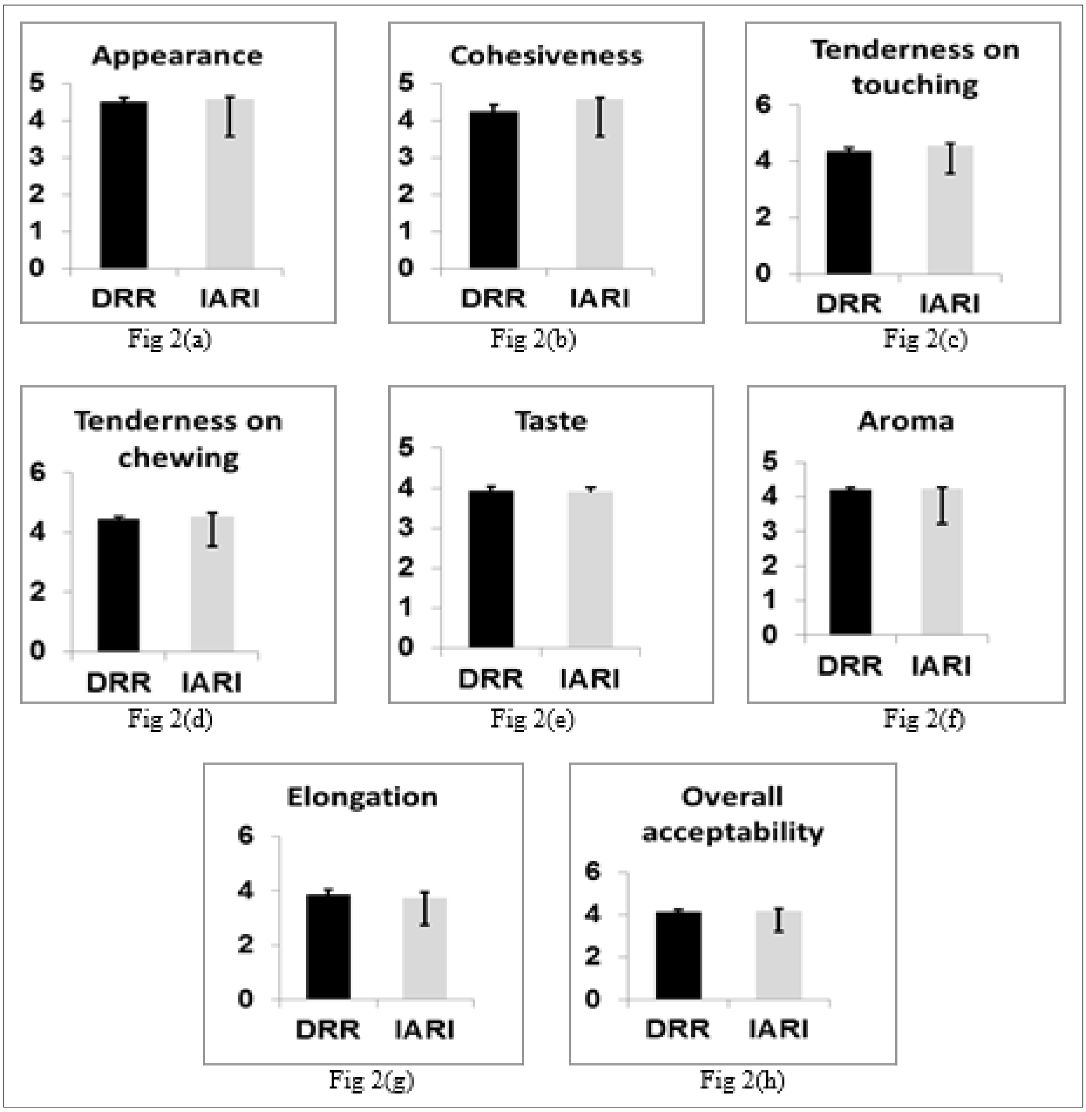

Fig 2 (a-h): Sensory evaluation of quality of Basmati rice (panel test) grown at IARI, Delhi (Within GI) and at DRR, Hyderabad (outside GI)

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