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The DNA fingerprint in food forensics: the Basmati rice case

KEYWORDS: Microsatellites, SSRs (simple sequence repeats), food fraud, Code of Practice, fragrance gene fgr, Basmati NJPlot dendrogram, analytical technologies.

ABSTRACT

Due to its exceptional aroma and cooking characteristics Basmati rice is one of the most popular rice specialities in the EU and the Middle East and is attracting a premium price. In the EU the strict authenticity definitions by the UK Code of Practice on Basmati Rice (CoP) of 2005 contributed significantly to improve the quality of this product and thereby its success on the market. Fifteen varieties were defined as authentic and a DNA fingerprinting method was determined for authenticity testing. Twenty-six new varieties had been released since then by India and Pakistan as Basmati and had to be included in the revised CoP of 2017. This study reports the analysis of the DNA fingerprints of these cultivars from reference materials from official sources to enable the application of the CoP. Results not only allow the enforcement of the revised CoP, but provide further insights into the genetic relationships between the varieties and their descent from common ancestors. The Basmati cultivars of major economic importance can be grouped in four types due to their close relationship: Basmati 370, Kernel/Taraori, Super Basmati and Pusa Basmati 1. The genotype fgr is supposedly the major cause of the Basmati aroma and is missing in 6 of the new varieties. Because it is not the only functional polymorphism associated with fragrance of rice the content of aroma in these new varieties should be studied and further requirements should be defined including the organoleptic characteristics of Basmati.

INTRODUCTION

Due to recent food scandals like melamine in dairy products and horse meat in beef products the topic food fraud is receiving increasing attention. Innovations in laboratory analysis provide tools to detect adulterations and particularly DNA analysis is gaining increasing importance. Two principal methods in DNA analysis for food control can be distinguished (1, 2): (a) DNA barcoding to identify species of plants, animals and microorganisms like horse meat in beef products and (b) DNA fi ngerprinting to differentiate animal breeds and plant varieties, like for the detection of cheap long grain rice mixed into high value Basmati (3). DNA fi ngerprinting is based on allele differences in hypervariable sequences. These are for example microsatellites (also called simple sequence repeats, SSRs, or short tandem repeats, STRs) and single nucleotide polymorphisms, SNPs, in fi ngerprinting of the second and third generation, respectively. It was fi rst used for the identifi cation of human individuals and has revolutionized forensics and the combat against crime. Basmati authenticity testing is a prominent example for its application in food forensics.

During the last decades Basmati became the favourite rice in the UK partly due to the large population originating from the Indian subcontinent (1). But adulterations with common long grain rice were frequently observed, which cost less than half the price of Basmati.

Therefore the British Retail Consortium BRC, Rice Association and the British Rice Millers Association issued in 2005 their Code of Practice on Basmati Rice and defi ned 15 rice varieties as authentic (4). Cultivation is geographically limited to 7 States in Northern India and the Punjab in Pakistan. The CoP defi nes in its article 6.1 DNA fi ngerprinting as the standard method to differentiate authentic Basmati from non-Basmati adulterants. Developed by Bligh (5) in 2000 the method has proven its reliability and robustness in practice and in numerous inter-laboratory ring trials and profi ciency tests. The CoP has signifi cantly improved the Basmati quality on the EU market. Whereas 13 out of 54 Basmati products were found adulterated in various consumer tests from 2006 to 2010 in Germany and France (6, 7, 8), in two recent German studies (9, 10) only one out of 36 products was not compliant with the CoP.

Since 2005 26 new Basmati varieties were notifi ed in India and Pakistan and many of these are now cultivated at large commercial scale. Consequently the CoP had to be revised in 2017 and now includes all 41 cultivars released as Basmati in the countries of origin (4). In forensics criminals can be only identifi ed based on the DNA traces left behind at the site of crime, if their DNA fi ngerprints are included in the police data base. Similarly rice varieties can be only identifi ed, if their DNA fi ngerprint is known. Therefore also the DNA fi ngerprinting method originally defi ned by the UK Food Standards Agency (FSA) had to be adapted and the Rice Association provided reference materials of all new varieties from offi cial sources to our laboratory to determine the fi ngerprints. This report reveals the results of this study, which allows Basmati authenticity testing under the new CoP and provides further information about the breeding history of Basmati varieties and their descent from traditional ancestors.

RESULTS AND DISCUSSION

The UK Code of Practice and Basmati authenticity defi nition in the EU

Table 1 lists the 41 Basmati varieties included in the CoP of 2017 with further information regarding their descent, where it was available in the public domain.
Six traditional and 35 evolved cultivars are currently notifi ed as Basmati in India and Pakistan. Traditional varieties were selected and cultivated by farmers over generations and rice research institutes selected pure lines from these seeds. New varieties were evolved by crossing the traditional lines with high yielding non-Basmati indica cultivars. In 2005 it was a common understanding among Indian and Pakistani plant breeders that authentic Basmati has to be either traditional or evolved from breeding with at least one traditional parent. The 15 varieties in the fi rst CoP followed this rule.

Shortly after its release in 2005 the CoP became the gold standard for Basmati authenticity beyond the UK in most EU member states. Nine of the 15 varieties are furthermore eligible for a zero import duty (4), if brought into the EU as brown cargo rice. In 2005 Pusa 1121 was introduced, which soon became the major Basmati consumed in the Middle East due to its exceptional high quality (25). But it was not evolved from a traditional Basmati parent and was not accepted as Basmati in the EU, until the French Rice Code of 2015 (26) and the revised CoP of 2017 defi ned it as Basmati. The commercial success of Pusa 1121 made the agreement among Indian and Pakistani breeders obsolete and most of the newly notifi ed Basmati varieties do not have a traditional parent anymore, as indicated in the pedigree of major Basmati varieties in Figure 1.

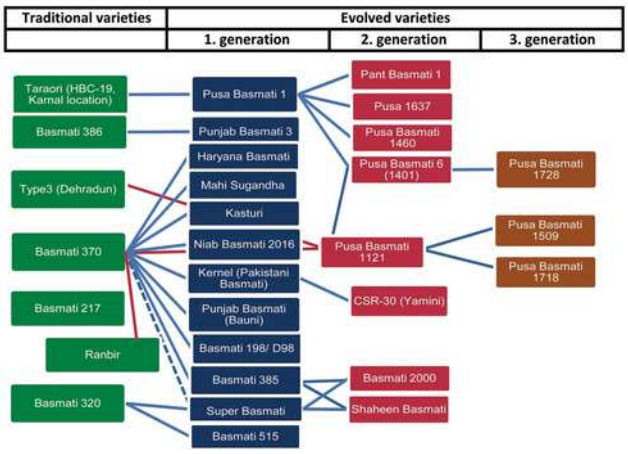

Figure 1. Pedigree of Basmati rice varieties based on information about their breeding history, which was available in the public domain (Table 1). For other cultivars no information was available. Pusa Basmati 1609 and Chenab are not included, because their ancestors in the Basmati family are not clearly defi ned (19, 22). The descent of Super Basmati and Basmati 515 from either Basmati 320 or 370 is reported contradictorily in literature (see below, 14, 15). Pusa Basmati 1121 was evolved by crossing lines P614-1-2 and P614-2-4-3, derived from Basmati 370 and Type 3 (25). Pusa 1718 is a new Basmati variety from India (25), which is not yet included in the CoP.

DNA fi ngerprinting and UPGMA cluster analysis of genetic similarities from SSR data The DNA fi ngerprinting method for the identifi cation of the 15 varieties in the CoP of 2005 was published by the FSA in 2004 and is based on the 10 microsatellite markers RM1, RM44, RM55, RM171, RM201, RM202, RM223, RM229, RM241, and RM348 (also M16) (27). Several further qualitative and quantitative methods based on SSR and InDel (insertion/ deletion) markers were developed by Steele (28) and published on the FSA website as standard protocols (29). Another method by Steele was based on the 8 markers RM44, RM201, RM110, RM212, RM252, RM263, RM282 and RM339 and was applied in a profi ciency test in 2007 (30).

In order to differentiate all varieties in the CoP of 2017 the original spectrum of 10 SSR markers was extended to 15 and furthermore by the genotype fgr, an 8 base pair deletion in the gene bad2 coding for the betain aldehyde dehydrogenase 2 (31). This mutation was detected in all Basmati, Jasmine (originated from Thailand, Cambodia and Vietnam) and Sadri (Iran) rice varieties, which have been tested so far and supposedly causes their aroma (2, 31, unpublished results). Screening protocols to test Basmati authenticity are based on the quantifi cation of this gene in relation to the wildtype (32).

With this method the DNA fi ngerprints of the 33 varieties were determined, which the Rice Association provided to us as reference materials (Table 2). The table also includes DNA fi ngerprints of 8 reference materials of the Basmati varieties in the CoP of 2005, which were obtained at that time from the FSA. The fi ngerprint of Basmati 217 is not included, as reference material of this commercially unimportant variety was not made available. According Woolfe and Steele this variety is identical with Basmati 370 in 8 SSR markers (28). For Punjab Basmati (the Indian variety, also called Bauni Basmati) 2 fi ngerprints are reported, as 2 reference materials were received in 2005. In some samples more than one allele was found in the same markers, which was due to impurities of the reference materials. The reference material of Basmati 564 was a mixture of several varieties and no conclusive fi ngerprint could be obtained.

The SSR data in Table 2 were furthermore used to calculate a dendrogram by an UPGMA (unweighted pair group method with arithmetic mean) cluster analysis (Figure 2, 33). The dendrogram separates the Basmati varieties into group I and II, which can be furthermore subdivided into 4 subgroups a to d.

Traditional Basmati varieties

Reports about aromatic rice on the Indian subcontinent can be found in literature going back to the 4th century BC. The word Basmati appeared for the fi rst time in the tragic romance Heer Ranjah of the Punjabi poet Waris Shah from 1766 (16). The fi rst pure seed line of Basmati was selected from a traditionally cultivated landrace and released as Basmati 370 in 1933 by the Kalashah Kaku Rice Research Institute, which is today in the Pakistani part of the Punjab. Further landrace selections are Type 3 or Dehradun Basmati, released 1978 by the Nagina Rice Research Station in Uttar Pradesh, Taraori (HBC-19 or Karnal location) notifi ed 1996 by the Haryana Agriculture University and Basmati 217 and 386, released in 1969 and 1997 by the Punjab Agricultural University, respectively. In 1996 the Sher-e-Kashmir University in Jammu notifi ed Ranbir Basmati from a pure line selection of Basmati 370. In the dendrogram Basmati 370, Ranbir and Type 3 form one cluster (group Ia) together with cultivar NIAB Basmati 2016. The latter rice was released by the Nuclear Institute for Agriculture and Biology in Faisalabad and was probably selected from mutants created by irradiation of Basmati 370 (34).

Ranbir differs from its ancestor Basmati 370 in 2 of 16 markers. Interestingly all samples of rice exported from Jammu as Ranbir and received by our laboratory were identifi ed as Basmati 370, which indicates that the reference material in the seed collection might not match the crop, which is actually cultivated at commercial scale.

Taraori and Basmati 386 form a separate cluster (group Ic) together with Kernel, CSR 30 (Yamini) and Punjab Basmati 3. These 5 varieties are identical in all 16 markers. Closely related to this group are Vallabh Basmati 22 and 23, both having the same 16 marker DNA fi ngerprint. Taraori and Basmati 386 were selected from landraces in the eighties, whereas Kernel (Pakistani Basmati) was evolved by breeding of Basmati 370 with the non-Basmati CM7-6 and released in 1969 by Kala Shah Kaku. Also CSR 30 and Punjab Basmati 3 cannot be distinguished from the 3 varieties above by the 16 marker fi ngerprints, although they were evolved from Pakistani Basmati and Basmati 386 by crossing with non-Basmati varieties. Further studies by DNA fi ngerprinting based on SNPs (third generation genetic fi ngerprinting, 2) or InDels (28) might solve these discrepancies.

Accordingly Basmati 370, Type 3 (Dehradun) and Ranbir can be put together under one category of traditional Basmati

(Basmati 370 type) and Taraori (HBC-19), Basmati 386, Kernel, CSR 30 and Punjab Basmati 3 under another category (Kernel/Taraori type).

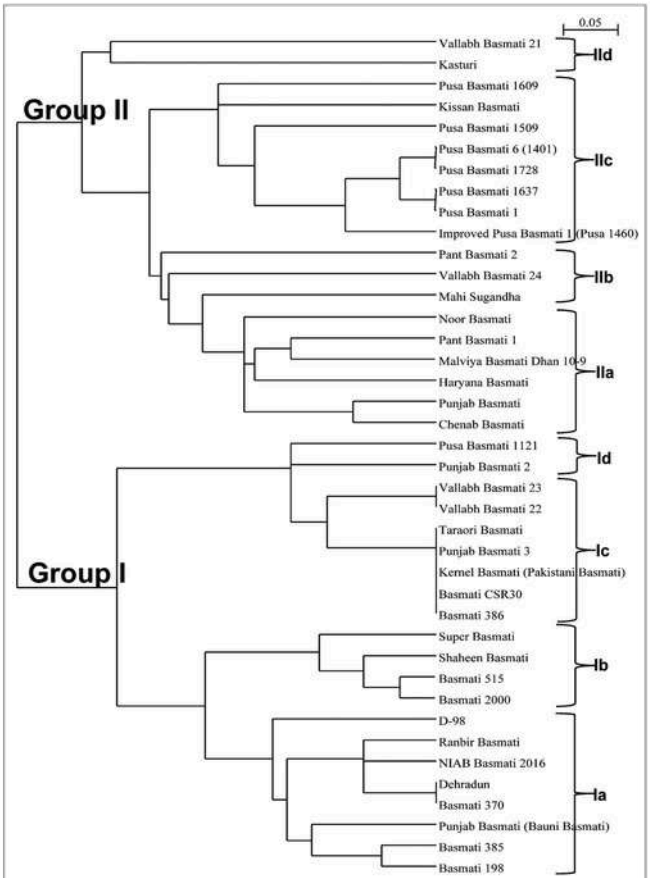

Figure 2. Dendrogram of the analysed Basmati rice varieties calculated by an UPGMA cluster analysis based on the 15 SSR markers in Table 2.

The Super Basmati family
According contradictory reports in literature Super Basmati was either evolved by crossing Basmati 370 with non-Basmati 10486 (14) or Basmati 320 with IR661 (15). This variety soon became the most successful Basmati cultivated in Pakistan due to its exceptional quality characteristics. It forms a cluster with its progeny Shaheen Basmati and 2000 and with the sister line Basmati 515 in subgroup Ib close to Ia with Basmati 370. All four varieties differ only in one marker each and additionally Super Basmati, Basmati 2000 and 515 could not be distinguished by fi rst generation DNA fi ngerprinting based on ISSRs (inter simple sequence repeats) and RAPDs (randomly amplifi ed polymorphic DNA) (2). They therefore form the relatively uniform Super Basmati type and can be put together in one category.

The varieties Basmati 198 and 385 were evolved from crossings of Basmati 370 with the non-Basmati TN1 and form their own cluster within group Ia in close proximity to their ancestor Basmati 370. D98 is genetically distinct from Basmati 198, although both are highly related and belong to the group Ia cluster in close proximity to each other and to Basmati 370. According the Trade Development Authority of Pakistan D98 is just another trade name for Basmati 198 (35). As the reference materials of both cultivars were from the trade, this statement cannot be clarifi ed with certainty. Basmati 198 was the only Pakistani Basmati variety, of which the FSA could not get references from offi cial sources before 2005 and the agency distributed a material from the trade to the laboratories instead (Mark Woolfe, personal communication). Punjab or Bauni Basmati (IET 8580) is in the same cluster and in close proximity to Basmati 198 and 385. It was created in India by pedigree breeding of Basmati 370 with either TN1 or Sona (Table 1, 15, 16). As Basmati 198 and 385 were both evolved from crossing of Basmati 370 with TN1 (11), this discrepancy in literature is now clarifi ed by the UPGMA cluster analysis.

The Pusa Basmati family Except Pusa 1121, 1509 and 1609 the other members of the Pusa Basmati family (1401, 1460, 1637 and 1728) were derived from Pusa Basmati 1 (Table 1). Pusa Basmati 1 was developed in the eighties by convergent breeding of Taraori (HBC-19) and the non-Basmati Pusa 150 at the Indian Agricultural Research Institute (16). The group forms one distinct cluster in the dendrogram (group IIc). The fi ngerprint of Pusa 1637 is the same as of Pusa Basmati 1, whereas the fi ngerprints of 1401, 1460 and 1728 differ in only one allele from their ancestor. Interestingly Pusa 1401 differs from Pusa Basmati 1 only in marker RM72 and inherited the corresponding allele from the other parent line, Pusa 1121. Pusa 1637 and 1728 are near isogenic lines of Pusa Basmati 1 and Pusa Basmati 6 (1401), respectively. They were evolved by marker assisted selection and consequently pair with their ancestors in 2 separate subgroups in the dendrogram. Pusa 1, 1401, 1460, 1637 and 1728 can be therefore put together as the Pusa Basmati 1 type.

Pusa Basmati 1509 is close to this cluster, but its ancestor Pusa 1121 (Table 1) is in a more distant cluster (group Id) together with Punjab Basmati 2. Pusa 1121 was evolved by crossing 2 lines derived from Type 3 and Basmati 370 (Table 1, 25). Pusa 1609 is also genetically distinct from group IIc, as it was created by pyramiding genes for bacterial blight and blast resistance from the non-Basmati donor parent C101A51 into the elite Basmati restorer line PRR78 through marker-assisted selection (19). Due to their special physical characteristics Pusa 1121 and 1509 form a different group apart from the Pusa Basmati 1 type.

Varieties lacking the genotype fgr
Six varieties in the revised CoP lack the genotype fgr (see above): Chenab and Punjab Basmati, Vallabh Basmati 21 and 24, Malviya Basmati Dhan 10-9 and Pant Basmati 1.

Chenab was created at Kala Shah Kaku by crossing lines 98PP4 and 4439 (22). The line 98PP4 was found originally positive for the genotype fgr (23). Punjab Basmati was developed at the same institute, is genetically very close to Chenab and both fall under the same cluster (group IIa) with Malviya Basmati Dhan 10-9, Pant Basmati 1, Noor and Haryana Basmati. PK-386 is also included in this cluster.
It is a common long grain non-Basmati rice variety with similar cooking characteristics as Basmati, but without the aroma. Its relationship with Chenab can be explained with one of its parents, the long grain cultivar 4439.

The deletion fgr is not the only functional polymorphism associated with aroma in rice and further mutations have been reported in the bad2 gene of fragrant Indonesian Pandan or Myanmar Pearl rice (36). Further studies are therefore necessary to evaluate, whether the fgr negative Basmati varieties lack aroma.

Experimental section
DNA fi ngerprinting was performed as recently described by Nader et al. (1, 2, 3). DNA was isolated from the reference materials with the NucleoSpin® Food Kit (Macherey Nagel, Düren,

Basmati varieties in the UK Code of Practice, 2005				
Variety	**Notified by**	**Year**	**Breeding background**	**Ref.**
Basmati 370	Rice Research Station Kala Shah Kaku, today Pakistan	1933	Punjab, local selection	11
Kernel (Pakistani Basmati)	Rice Research Station Kala Shah Kaku, Pakistan	1968	Basmati 370 /CM7-6	12, 13
Basmati 217	Punjab Agricultural University, India	1969	Punjab, local selection	11
Basmati 198	Rice Research Station Kala Shah Kaku, Pakistan	1972	Basmati 370 / TN1	14
Type 3 (Dehradun)	Nagina Rice Research Station, Uttar Pradesh, India	1978	Uttar Pradesh, local selection	11
Punjab Basmati (Bauni Basmati)	Punjab Agricultural University, India	1984	Sona / Basmati 370 or TN1 / Basmati 370	15, 16
Basmati 385	Rice Research Station Kala Shah Kaku, Pakistan	1988	TN1 / Basmati 370	11
Pusa Basmati 1 (IET 10364)	Indian Agricultural Research Institute, Delhi, India	1989	Pusa 150 / Karnal local	11
Kasturi (IET 8580)	Indian Institute of Rice Research, Rajendranagar, India	1989	CK 88-17-1-5 / Basmati 370	11
Haryana Basmati (HKR 228/IET 10367)	Haryana Agricultural University, India	1991	Sona / Basmati 370	11
Mahi Suganda	Rajasthan Agricultural University, India	1995	BK 79 / Basmati 370	11
Ranbir Basmati (IET 11348)	Sher-e-Kashmir University, Jammu, India	1996	Jammu, selection from Basmati 370	11
Taraori Bas. (HBC-19, Karnal Local)	Haryana Agricultural University, India	1996	Haryana, local selection	11
Super Basmati	Rice Research Station Kala Shah Kaku, Pakistan	1996	Basmati 370/10486 or Basmati 320/IR661	14, 15
Basmati 386	Punjab Agriculture University, India	1997	Punjab, local selection	11
New varieties in the revised UK Code of Practice, 2017				
Basmati 2000	Rice Research Station Kala Shah Kaku, Pakistan	2001	Basmati 385 / Super Basmati	12
Shaheen Basmati	Soil Salinity Indistute, Pindi Bhattian, Pakistan	2001	Super Basmati / Basmati 385	12
Improved Pusa Basmati 1 (Pusa 1460)	Indian Agricultural Research Institute, Delhi, India	2007	Pusa Basmati 1 / IRBB55	16
Pusa Basmati 1121	Indian Agricultural Research Institute, Delhi, India	2008	P614-1-2 / P614-2-4-3, lines derived from Basmati 370 and Type 3	25
Vallabh Basmati 22	Sardar Vallabh Bhai Patel University of Agriculture and Technology, Uttar Pradesh, India	2009		
Basmati 515	Rice Research Station Kala Shah Kaku, Pakistan	2009	Three way cross Bas 320/10486/50021	17
Pusa Basmati 6 (Pusa 1401)	Indian Agricultural Research Institute, Pusa, India	2010	Pusa Basmati 1 / 1121	18
Punjab Basmati 2	Punjab Agricultural University, India	2012		
Basmati CSR 30 (Yamini)	ICAR Central Soil Salinity Research Institute, Haryana	2012	Buraratha 4–10 /Pakistani Basmati	11, 16
Vallabh Basmati 21 (IET 19493)	Sardar Vallabh Bhai Patel University	2013		
Malviya Basmati Dhan 10-9 (IET 21669)	Banaras Hindu University, U.P., India	2013		
Pusa Basmati 1509 (IET 21960)	Indian Agricultural Research Institute, Delhi, India	2013	Pusa Basmati 1121 / Pusa 1301	18
Basmati 564	Sher-e-Kashmir University, Jammu, India	2015		
Vallabh Basmati 23	Sardar Vallabh Bhai Patel University	2015		
Vallabh Basmati 24	Sardar Vallabh Bhai Patel University	2015		
Pusa Basmati 1609	Indian Agricultural Research Institute, Delhi, India	2015	elite Basmati restorer line PRR78 / C101A51	19
Pant Basmati 1 (IET 21665)	G. B. Pant University of Agriculture and Technology, Pantnagar, India	2016	Pusa Basmati 1 / IET 12603	20
Pant Basmati 2 (IET 21953)	G. B. Pant University of Agriculture and Technology	2016		
Punjab Basmati 3	Punjab Agriculture University, India	2016	Basmati 386/IET 17948//Basmati 386	21
Pusa Basmati 1637	Indian Agricultural Research Institute, Delhi, India	2016	MAS derived NIL of Pusa Basmati 1	18
Pusa Basmati 1728	Indian Agricultural Research Institute, Delhi, India	2016	MAS derived NIL of Pusa 6	18
NIAB Basmati 2016	Nuclear Institute for Agriculture and Biology, Faisalabad	2016	possibly mutant of Basmati 370, see text	
Noor Basmati	Nuclear Institute for Agriculture and Biology, Pakistan			
Punjab Basmati	Rice Research Station Kala Shah Kaku, Pakistan		possibly sister line of Chenab, see Figure 2	
Chenab Basmati	Rice Research Station Kala Shah Kaku, Pakistan		98PP4 / 4439	22, 23
Kissan Basmati	Rice Research Station Kala Shah Kaku, Pakistan			
PK 386 (non-Basmati and not included in the UK CoP)	Rice Research Station Kala Shah Kaku, Pakistan		4439/1053-1-2	24

Table 1. Basmati rice varieties notifi ed in India and Pakistan and included in the CoP of 2017 with further information about the breeding history, where this was available in the public domain. (MAS: marker assisted selection; NIL: near isogenic lines)

Germany) by absorption to silica membrane spin columns. The extracted DNA served as template for PCR amplifi cation with AmpliTaq Gold® DNA Polymerase (LifeTechnologies, Foster City, CA, USA) and Gold ST★R buffer (Promega Inc, Madison, WI) in ABI 9700 Thermocyclers (LifeTechnologies, Foster City, CA, USA). Primers were synthesized and labeled at the 5'-end with fl uorescent dyes FAM (6-carboxylfl uorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxy-fl uorescein) or TAMRA (carboxy-tetramethyl-rhodamine) by Eurofi ns MWG Operon (Ebersberg, Germany) or Ella Biotech (Munich, Germany). PCR products were separated on ABI 3130 Genetic Analysers with POP4® polymers on 36 cm capillary arrays in the presence of ILS 600 internal size standards (Promega Inc, Madison, WI, USA). Fragment length polymorphisms were analyzed with GeneScan®/Genotyper® or GeneMapper® software (LifeTechnologies, Foster City, CA, USA). Analysis of the fgr gene was performed by a multiplex PCR involving 2 primer pairs covering the region of the 8 base pair deletion in exon 7 of the bad2 gene (31). The dendrogram was calculated with the Populations 1.2.32 software with a hierarchical clustering algorithm and visualized with the NJPlot programme (33). This algorithm was developed for the analysis of genetic linkages based on differences in alleles of SSR markers.

CONCLUSIONS

With the exception of Basmati 217 this report reveals the DNA fi ngerprints of all Basmati cultivars, which are notifi ed as authentic and are included in the revised CoP of 2017. As the fi ngerprints were obtained from reference materials provided by offi cial sources, they can be used to enforce the CoP. Comparison of the fi ngerprints by a UPGMA cluster analysis revealed new insights into the genetic relationships among the varieties, which is represented graphically in the form of a dendrogram. Before Pusa 1121 was notifi ed as a Basmati in 2008 a major prerequisite for Basmati authenticity was a direct descent from at least one traditional parent. Pusa 1121 and most of the varieties, which were released afterwards, are not following this rule anymore and the dendrogram reveals for some of them their increasing genetic distance from the original Basmati cultivars. In six of them the specifi c 8 basepair mutation fgr in the bad2 gene is missing, which occurs in all traditional Basmati varieties. As fgr is not the only functional polymorphism associated with aroma in rice, further chemical and organoleptic analyses become necessary to check, whether these varieties fulfi l the quality requirements of Basmati. The results furthermore reveal new insights into the history of Basmati rice. It started with the selection of pure lines from traditional landraces, was followed by crossing these with non-Basmati long grain rice to evolve high yielding second generation Basmati and is ongoing with modern breeding like marker assisted selection of near isogenic lines or gene pyramiding to obtain pest resistant cultivars of the third generation. Several cultivars (Taraori, Basmati 386, Kernel, CSR-30 and Punjab Basmati 3, furthermore Vallabh 22 and 23 and also Pusa Basmati 1 and 1637) cannot be distinguished from each other by the fi ngerprints based on the 16 markers. Third generation DNA fi ngerprinting using SNP polymorphisms is a promising tool to resolve this matter. Based on their close relationships Basmati varieties can be put together in 4 groups: Traditional varieties of the Basmati 370 and Kernel/Taraori types and evolved varieties of the Super Basmati and Pusa Basmati 1 types. Cultivars falling under these categories could be traded under these names similar to Risotto rice under Italian legislation, where 7 different varieties are approved as Arborio, 9 as Carnaroli and 6 as Baldo (37).

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Variety	Source	RM1	RM223	M16	RM202	RM44	RM201	RM229	RM241	RM171	RM55	RM263	RM152	RM212	RM252	RM72	fgr
Traditional Basmati Varieties (UK Code of Practice 2005)																	
Basmati 370	2017 ***	W	W	Z	Y	X	X	Y	Z	Y	Z	159	144	116	233	170	+
Type 3 (Dehradun)	2005 *	W	W	Z	Y	X	X	Y	Z	Y	Z	163		116	235		+
Ranbir Basmati	2017 ***	W	W	Z	Y	X	X	Y	Z+4	Y	Z	159	144	116	221	170	+
Taraori Basmati	2017 ***	W	W	Z	Y	Y	X	Y	Y	Y	X	163	144	116	239/ 243	170	+
Basmati 386	2017 ***	W	X / W	Z	Y	Y	X	Y	Y	Y	X	163	144	116	239	170	+
Evolved Basmati Varieties (UK Code of Practice 2005)																	
Kernel	2011 ***	W	W	Z	Y	Y	X	Y	Y	Y	X	163	144	116	239	170	+
Pusa Basmati 1	2017 ***	W	W	Y	Y	Y	X	X	Y	Z	Y	163	148	116	255	155	+
Super Basmati	2011**	W	W	Z	Y	X	X	Y	Z	Z	X	163	144	116	239	170	+
Basmati 198	2005 *	Y	W	Z	X	X	X	Y	Z	Y	Z	159		134			+
Basmati 385	2005 *	Y	W	Z	X	Y	X	Y	Z	Y	Z	163		134			+
Kasturi	2017 ***	W	V	Y	Y	W	X	Y+2	Z	Z	Y	202	144	114	233	146	+
Haryana Basmati	2017 ***	Z+2	W	Y	X	W	X	X	Y	Z	Y	159	144	134	215	155	+
Mahi Sugandha	2005 *	Y	X	Z	X	W	Z	X	Z	Z	Y	159		134			+
Punjab Basmati (IET 8580)	2005 *	Y+2	Y	Z	X	X(+1)	X	Y	X-4	Y	Z+3	159		134			+
Punjab Basmati (IET 8580)	2005 *	Z+5	Y	Z	X	X(+1)	X	Y	Z	Y	Z						+
New Varieties in the UK Code of Practice 2017																	
Basmati 2000	2017 ***	W	W	Z	Y	X	X	Y	Z	Z	X	163	154	134	233	170	+
Shaheen Basmati	2017 ***	W	W	Z	Y	Y	X	Y	Z	Z	X	163	144	134	233	170	+
Improved Pusa 1 (1460)	2017 ***	W	W	Y	Y	Y	X	X	Y	Z	Y	163	154	116	253	155	+
Pusa Basmati 1121	2017 ***	W	W	Y	Y	Y	X	X	Y	Y	X	163	148	116	255	170	+
Vallabh Basmati 22	2017 ***	W	W	Z	Y	Y	X	Y	Y	Y	Z	163	144	116	255	155	+
Basmati 515	2017 ***	W	W	Z	Y	X	X	Y	Z	Z	X	163	144	134	233	170	+
Pusa Basmati 6 (1401)	2017 ***	W	W	Y	Y	Y	X	X	Y	Z	Y	163	148	116	255	170	+
Punjab Basmati 2	2017 ***	W	W	Z	Y	X	X	Y	Y	Z	X	165	144	116	237	170	+
Basmati CSR30	2017 ***	W	W	Z	Y	Y	X	Y	Y	Y	X	163	144	116	239	170	+
Malviya Basmati Dhan 10-9	2017 ***	Z	Y	Y	Y	W	X	X	Y	W	Y	159	154	134	215	155	-
Vallabh Basmati 21	2017 ***	Z+2	Y	Y	Y+4	W	X	Y+2	Z	W	Z	183	148	116	233	155	-
Pusa Basmati 1509	2017 ***	W	W	Y	Y	Y	X	Y+2	Y	Z	X	163	154	114	215/ 259	170	+
Basmati 564	2017 ***	Y / W	W (Y)	Z / Y	Y+4/ X/Y	Y/W-2	Z / X	X / Y	Z / Y	Y	Z / X	161 / 163	144/ 148/ 154	134 / 116	255/ 233 /215	170/ 161/ 155	+/-
Vallabh Basmati 23	2017 ***	W	W	Z	Y	Y	X	Y	Y	Y	Z	163	144	116	255	155	+
Vallabh Basmati 24	2017 ***	W	V-6	Y	Y / X	W	X	X	Y-2	Z	Y	163	148	134	251	-	-
Pusa Basmati 1609	2017 ***	Z	V	Y	X+2	W	X	X	Y	Z	Y	163	154	114	215	155	+
Pant Basmati 1	2017 ***	Z+2	Y	Y	X	W	W	X	Y	W	Y	176	154	134	215	155	-
Pant Basmati 2	2017 ***	Z	W	Z	X	W	Z	X+2	Y	W	Y	202	148	134	233	155	+
Punjab Basmati 3	2017 ***	W	W	Z	Y	Y	X	Y	Y	Y	X	163	144	116	239	170	+
Pusa Basmati 1637	2017 ***	W	W	Y	Y	Y	X	X	Y	Z	Y	163	148	116	255	155	+
Pusa Basmati 1728	2017 ***	W	W	Y	Y	Y	X	X	Y	Z	Y	163	148	116	255	170	+
NIAB Basmati 2016	2017 ***	W	W	Z	Y	X	X	Y	Z	Z	Z	163	144	116	233	170	+
Noor Basmati	2017 ***	Y+2	W	Y	Y	W	W	Y+2	Y	Z	Y	180	154	134	215	155	+
Chenab Basmati	2017 ***	Y+2	Y	Y	X / Y	W	Z	X	Y	Z	Y	183	148	134	215	155	-
Punjab Basmati (Pakistan)	2017 ***	Y+2	Y	Y	Y	W	Z	X	Y	Z	Y	180	154	134	215	155	-
Kissan Basmati	2017 ***	W	V	Y	Y	W	X	Y+2	Y	Z	Y	163	134	116	215	170	+
D-98	Trade	Y	W-2	Z	X	X+2	X	Y	Y-2	Y	Z	160	144	116	233	168	+
PK- 386 (non-Basmati and not included in the UK CoP)	2011**	Y+2	X	Y	Y	W	Z	Y+2	Y	Z	Y	202	155	112	215	146	-

Table 2. Allele patterns of 40 Basmati rice varieties, approved according the CoP of 2017 and based on 15 SSR marker genes (38). For the first 10 SSR markers the letter code from V to Z was adapted from the DNA fingerprinting method published by the FSA in 2004 (24). Each character defines a DNA fragment of a distinct length for each SSR marker. Reference materials were obtained in 2005* from the Food Standards Agency, in 2011** from the Rice Research Institute in Kala Shah Kaku, Pakistan and in 2017*** from the Rice Association.

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