DNA fingerprinting in food forensics – the Basmati and Jasmine rice cases

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Basmati varieties in the UK Code of Practice 2017

Variety	Notified by	Year	Breeding background	Ref.
Basmati varieties in the UK Code of Practice, 2005				
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	Crossing of sister lines of Pusa Basmati 1, P614-1-2 / P614-2-4-3	16
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			

Basmati rice varieties notified 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)

Source: Nader et al., in press

Agro Food Industry Hi Tech, November 21st, 2019, in press

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The DNA fingerprint in food forensics: the Basmati rice case

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, Taraori, Super Basmati and Pusa Basmati 1. An 8 base pair deletion is missing in the *bad2* gene of 6 of the new varieties. This genotype *fgr* is supposedly the major cause of the Basmati aroma, although it is not the only functional polymorphism associated with fragrance of rice. Therefore the spectrum and content of aroma in these new varieties should be studied and further requirements should be defined including the organoleptic characteristics of Basmati.

KEY WORDS: Microsatellites, SSRs (simple sequence repeats); food fraud; Code of Practice; fragrance gene *fgr*; Basmati NJPlot dendrogram

Publications, 2

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The DNA fingerprint in food forensics part II: the Jasmine rice case

ABSTRACT

Jasmine rice is the customary name for premium fragrant cultivars originating from the lowlands of the Central East of Thailand and the North-western part of Cambodia. In contrast to Basmati rice, which is well defined in the pioneering UK Code of Practice (CoP) by a joint effort of all stakeholders in the UK, India and Pakistan, the Jasmine rice authenticity definition is not that well developed. The French Rice Code only includes 3 varieties, Hom Mali (with varieties KDML105 and RD15) and Pathum Thani 1, as Thai Jasmine. Also the specification of Jasmine from Cambodia is not clearly defined. In addition the code only partially considers the Jasmine definitions in the countries of origins themselves. The success of the UK CoP is due to the restriction of Basmati rice to certain varieties, geographical regions and specific characteristics affecting the cooking and appearance. DNA fingerprinting is defined as the standard method for authenticity testing. This report reveals that the genetic fingerprinting method based on 15 microsatellites or SSRs (simple sequence repeats) markers, which was recently developed for the differentiation of all 41 Basmati varieties in the revised UK CoP, can be also applied to Jasmine rice. Based on authentic reference materials obtained from the Cambodian Research and Development Institute (CARDI) and from the trade the test not only allows the application and enforcement of the French Rice Code, but also of the Cambodian Milled Rice Standard. This might lead to the improvement of the authenticity definition of Jasmine and increase the transparency for the trade and consumers, which will be beneficial for the quality of this premium rice and in final consequence for its success on the world markets.

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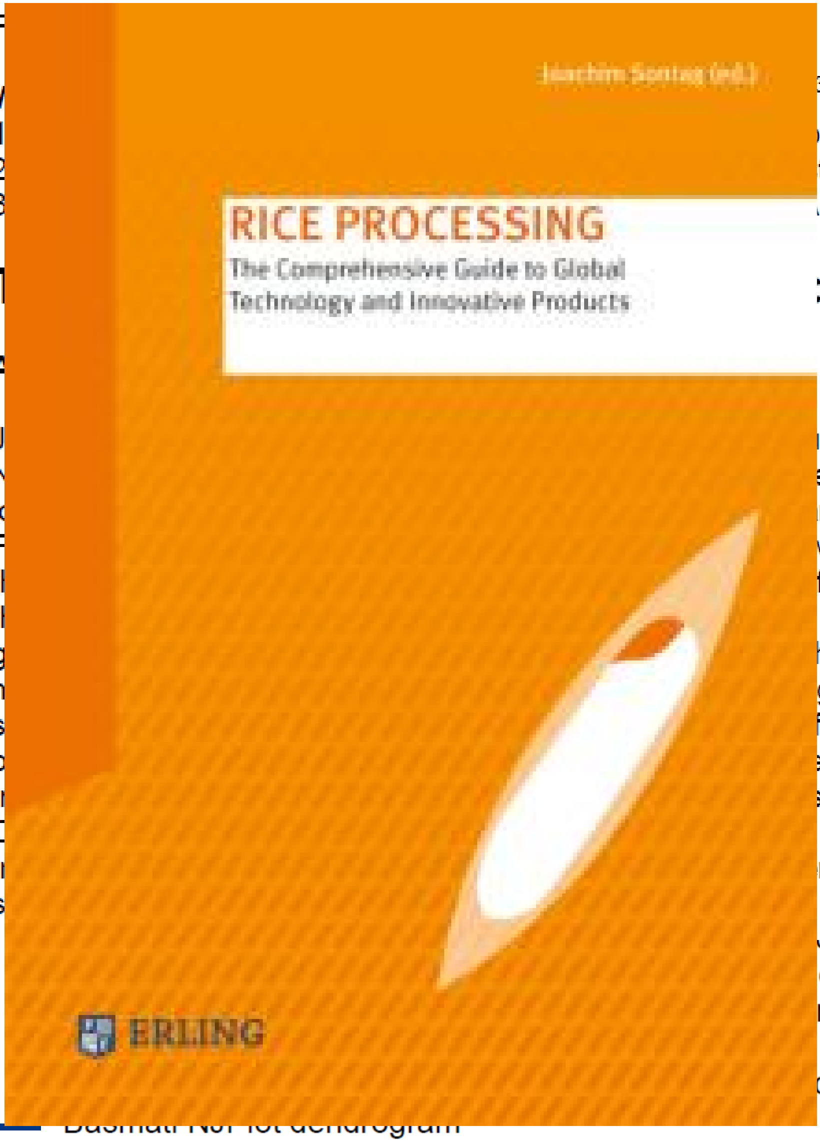
KEY WORDS: Microsatellites, SSRs (simple sequence repeats); food fraud; Code of Practice; fragrance gene *fgr*; Basmati NJPlot dendrogram

Publications



6 Impacts of food safety and authenticity issues on the rice trade

Werner Franz Nader, Anna-Karina Grote and Elyana Cuevas Montilla

6.1 Introduction and chapter overview

Prior to 2006 rice was considered a trouble-free commodity with regard to food safety issues. The only issues of major concern were dead insects and foreign objects. However, the situation changed dramatically when contaminations with genetically modified (GM) rice were detected in US exports[1]. The rising number of notifications concerning rice in the Rapid Alert System for Food and Feed (RASFF) of the European Union (EU) reflect the challenges that the rice industry and the rice trade have been through since 2006[2]. RASFF was put in place for the EU food and feed control authorities as an information exchange platform about measures taken regarding risks detected in food or feed. Intended to help the authorities act more rapidly and in a coordinated manner, it soon became a valuable tool for the food industries in the EU, which are responsible for safety issues concerning their products according to article 19 (food) and 20 (feed) of the basic EU food law[3]. RASFF, indeed, set a precedent and the International Food Safety Authorities Network (INFOSAN) was established in 2004 by the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) of the United Nations, with national focal points in over 160 member countries that provide a similar early warning system on a global scale.

Figure 6-1 summarises the number of notifications for rice and rice products in the RASFF from 2005 to 2013 for the risk categories GMOs (genetically modified organisms) and aflatoxins[2]. While only 17 notifications were about rice (0.5 % of all 3154 notifications) in 2005, this number increased to 149 or 5.3 % of all notifications in 2006. With 2.1 % of all alerts being about rice and rice products in 2012, and 1.56 % in 2013, there is clearly still room for improvement.

As shown in Figure 6-1 most rapid alerts for rice and rice products (primarily rice noodles from China) relate to transgenic lines of GM rice which are not approved within the EU. Aflatoxins in rice account for fewer notifications and primarily concern Basmati rice from Pakistan.

These developments have caused trade to be significantly affected, and on many occasions even disrupted. When genetically modified LibertyLink rice was found in US rice, not only was the trade with the EU disturbed, but so were exports to many other countries such as Japan, the Philippines, South Korea, Mexico and Russia. The total damage to the US rice industry has been estimated between US $ 741 million to 1.285 billion[4].

It is not only GM rice, but also pesticide residues that severely affect the trade. The fungicides isoprothiolane and tricyclazole are used in Indian Basmati rice cultivation against blast. Residues of isoprothiolane disrupted exports of Indian Pusa

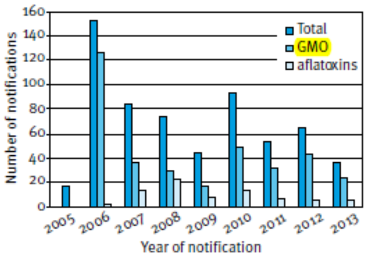


Fig. 6-1: Number of notifications in the EU Rapid Alert System for Food and Feed, RASFF, related to rice and rice products and in the categories GMOs and aflatoxins[2].

Publications

Advances in DNA Fingerprinting for Food Authenticity Testing

2

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Chapter Outline

2.1 Introduction

For the last two decades, analysis of DNA in food has become a routine procedure to detect genetically modified organisms, allergens, pathogens, and adulterations in food. Many cases of food fraud have been uncovered by DNA testing, for example, undeclared horse meat in beef products (FSA, 2013), *Pinus armandii* in Chinese pine nuts (Nader et al., 2013), persipan declared as marzipan (Brüning et al., 2011), and overfished species like yellowfin (*Thunnus albacares*) or bigeye tuna (*Thunnus obesus*) in canned tuna declared as skipjack (*Katsuwonus pelamis*) (Chuang et al., 2012). The horse meat scandal stunned the food industry and trade in the European Union (EU) in 2013. Adulteration of pine nuts by the species *P. armandii* (Chinese

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DNA-analysis:
enhancing the control of food authenticity through emerging technologies

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PEER REVIEWED

KEYWORDS: Food authenticity; Basmati rice; pine nuts; *Pinus armandii*; DNA-fingerprinting; short tandem repeats (STR); microsatellite analysis; DNA-fragment length analysis; horsemeat scandal.

ABSTRACT: DNA-analysis is one of the tools used for verifying the safety and authenticity of food. This is demonstrated with two examples – DNA-fragment length analysis to detect pine nuts causing taste disturbances and microsatellite or STR (short tandem repeat) analysis to check Basmati rice authenticity. Pine nuts from *Pinus armandii* caused dysgeusia (taste distortion) among consumers and triggered 39 biotoxin notifications in the EU Rapid Alert System for Food and Feed. Two analytical methods – DNA-fragment length analysis and chemical fingerprinting - were developed to detect *Pinus armandii* nuts and notifications have decreased rapidly after the implementation of routine controls. Microsatellite analysis became the standard method for authenticity testing in the Code of Practice for Basmati rice, defined by the retailers, traders and rice millers in the United Kingdom. As a consequence the quality of Basmati rice improved measurably. In this article the development of these DNA based methods and the impact on food safety and authenticity are described. Further applications of these technologies are discussed, e.g. for the detection of undeclared horsemeat in ready-made foods during the current food scandal hitting the European Union.

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INTRODUCTION

The quality, safety and authenticity of food are of principle interest for society and strictly regulated by legislation. The basic food law of the European Union, Regulation (EC) No 178/2002, focuses in article 8 on the protection of the ability of consumers to make informed choices in relation to the foods they consume. Fraudulent or deceptive practices, including the adulteration of food, and any other practices which may mislead the consumer should be prevented. A major tool for controlling authenticity is traceability, which is primarily based on documentation. However, documents can be falsified. The most recent example is the horsemeat scandal, which is currently hitting the European Union.
Information about the origin of a food product is often encrypted in its chemical composition. Rapid developments in science and technology during the last decades allow analysis and interpretation of these codes. An example of such a code is the stable isotope composition of a food product, which provides indications on the geographical origin, agricultural practice and adulterations, e.g. with artificial flavours (1).
A similar powerful technology utilises the genetic code encrypted in the DNA. With the discovery of the polymerase chain reaction DNA-analysis became a standard technique for food control, and it is routinely applied for the detection and identification of genetically modified organisms (GMOs), allergens and pathogens (2). However, DNA-analysis is also a powerful tool to verify the authenticity of food. The DNA-barcoding system was developed to identify and classify the 10 to 100 million organisms found throughout the globe and a database of reference sequences has been established by the "Consortium for the Barcode of Life" (www.barcodeoflife.org) to identify specimens, which have not yet been identified (3). Similarly the method can be used for the identification of animal, plant or fungal species used in food production.
DNA-fingerprinting was first used as a police forensic test to identify the culprit in the sexual assault and murder of two teenagers (4). In food chemistry it can be applied to identify varieties of plant species or breeds of animal species.
In this paper we report two cases, which demonstrate the powerful contribution of two methods for DNA-analysis to improve food safety and authenticity, the detection of *Pinus armandii* nuts and the identification and quantification of Basmati rice varieties.

RESULTS AND DISCUSSION

DNA-fragment length analysis for the identification of *Pinus armandii*
Since January 2011 39 notifications appeared under the category "biotoxins" in the Rapid Alert System for Food and Feed (RASFF) of the EU, describing taste disturbances caused by pine nuts. Consumers experienced a persistent

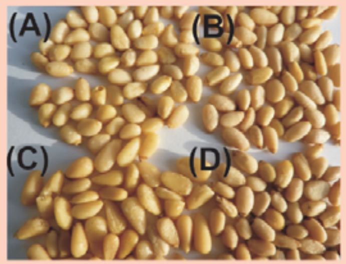
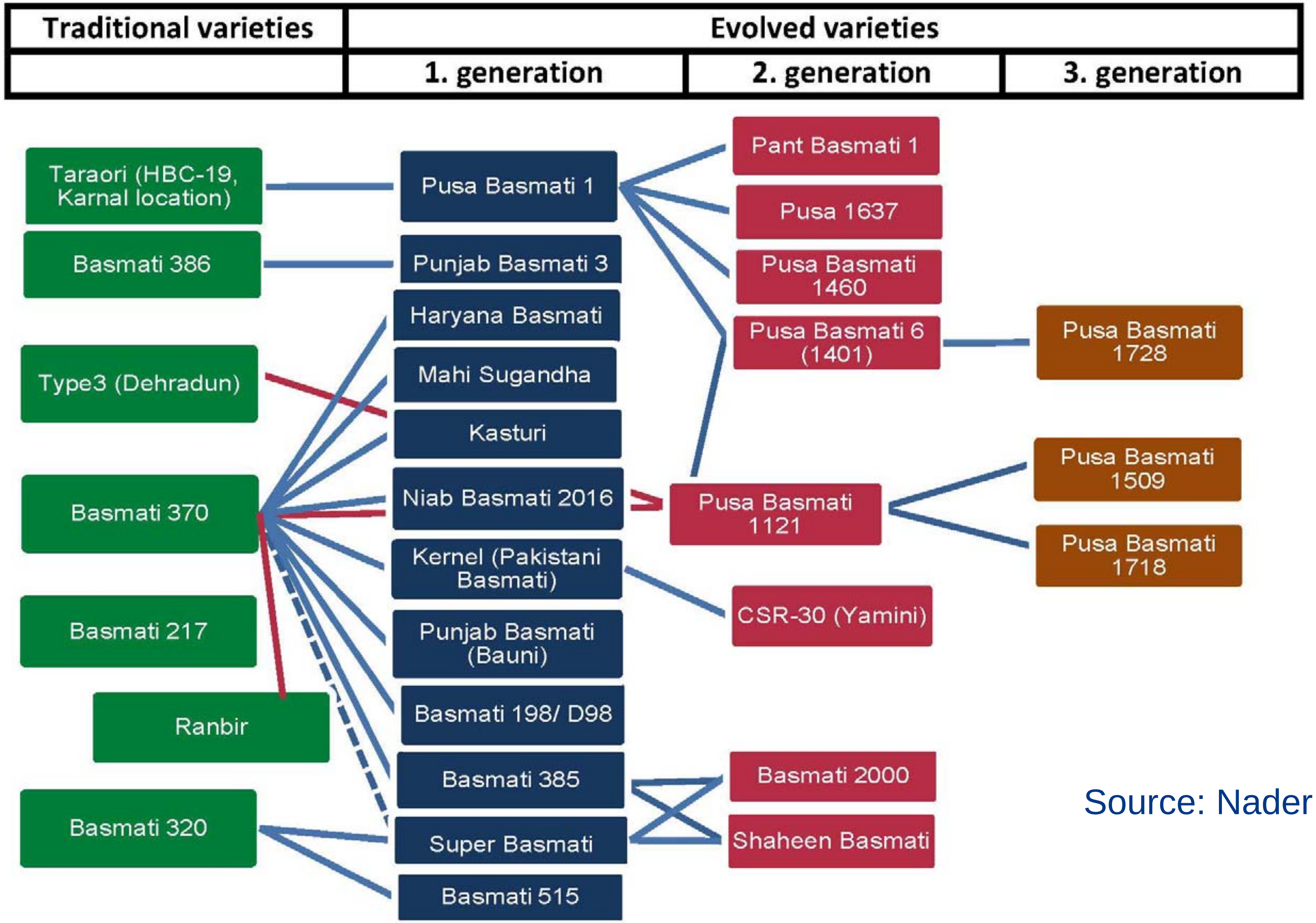


Figure 1. Pine nuts from *P. sibirica* (A), *P. armandii* (B), *P. koraiensis* (C) and *P. yunnanensis* (D)

Pedigree of Basmati rice



Source: Nader et al., in press

Workflow of routine analysis

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Rice sample

↓

grinding

↓

DNA extraction

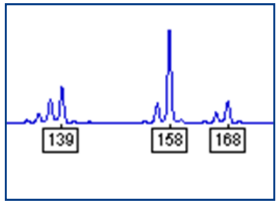
↓

PCR

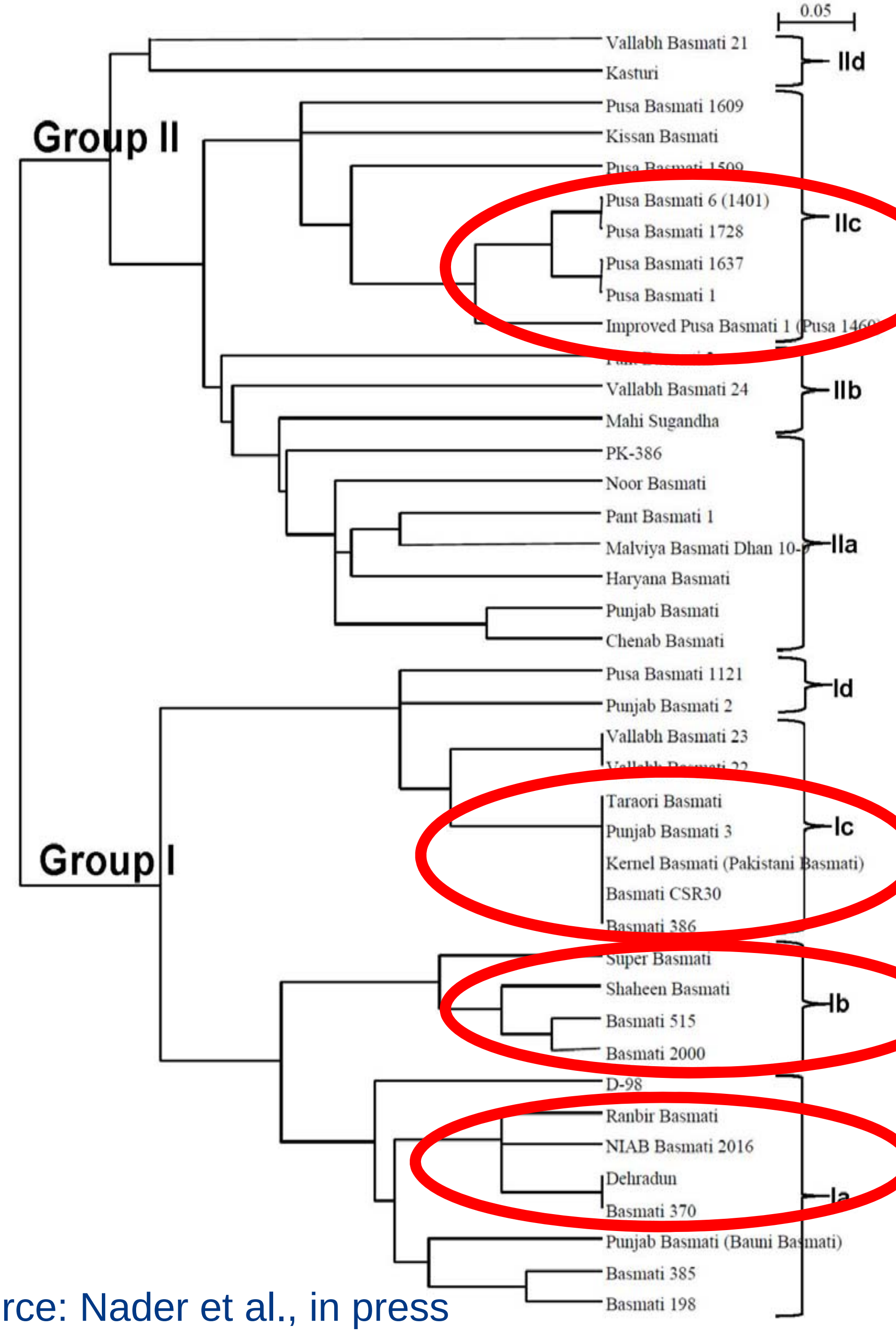
↓

CE

↓



Profile



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Pusa Basmati Family

Taraori/Kernel Basmati Family

Super Basmati Family

Basmati 370 Family

Dendrogram of the analysed Basmati rice varieties calculated with the NJPlot programme (33) and an UPGMA cluster analysis based on the SSR markers in Table 2.

Source: Nader et al., in press

Risotto Rice

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DENOMINAZIONE DELL'ALIMENTO: **RISO ARBORIO**					
Caratteristiche del granello	lunghezza (mm)	larghezza (mm)	rapporto lungh./largh.	consistenza (kg/cm^2)	perla
valori di riferimento	6,6 ÷ 7,2	3,2 ÷ 3,4	2,0 ÷ 2,2	0,65 ÷ 0,80	molto estesa
Varietà					
ALERAMO	7,1	3,4	2,1	0,79	molto estesa
ARBORIO	6,9	3,3	2,1	0,72	molto estesa
CL388	6,7	3,3	2,0	0,71	molto estesa
GENERALE	6,6	3,4	2,0	0,74	molto estesa
TELEMACO	6,8	3,3	2,0	0,68	molto estesa
VOLANO	7,1	3,4	2,1	0,80	molto estesa
VULCANO	6,7	3,2	2,1	0,72	molto estesa

DENOMINAZIONE DELL'ALIMENTO: **RISO ROMA o RISO BALDO**					
Caratteristiche del granello	lunghezza (mm)	larghezza (mm)	rapporto lungh./largh.	consistenza (kg/cm^2)	perla
valori di riferimento	6,4 ÷ 7,2	2,9 ÷ 3,1	2,2 ÷ 2,4	0,60 ÷ 0,80	da poco a molto estesa
Varietà					
BACCO	6,6	2,9	2,3	0,74	poco estesa
BALDO	6,9	3,1	2,2	0,68	poco estesa
BARONE CL	6,4	3,1	2,1	0,72	molto estesa
BIANCA	6,9	3,1	2,2	0,73	poco estesa
CAMMEO	6,9	3,0	2,3	0,76	poco estesa
CASANOVA	6,6	3,0	2,2	0,78	poco estesa
ELBA	6,8	2,8	2,4	0,94	poco estesa
FEDRA	6,6	2,9	2,3	0,68	poco estesa
GALILEO	7,0	3,0	2,3	0,66	poco estesa
NEVE	7,0	3,0	2,3	0,72	poco estesa
PROTEO	6,7	2,9	2,3	0,62	molto estesa
ROMA	6,6	3,0	2,2	0,64	molto estesa

DENOMINAZIONE DELL'ALIMENTO: **RISO CARNAROLI**					
Caratteristiche del granello	lunghezza (mm)	larghezza (mm)	rapporto lungh./largh.	consistenza (kg/cm^2)	perla
valori di riferimento	6,5 ÷ 7,0	2,9 ÷ 3,1	2,2 ÷ 2,3	≥ 0,85	molto estesa
Varietà					
CARAVAGGIO	6,6	3,0	2,2	0,98	molto estesa
CARNAROLI	6,8	3,1	2,2	0,95	molto estesa
CARNAVAL	6,7	3,0	2,2	1,00	molto estesa
CARNISE	6,9	3,1	2,2	0,98	molto estesa
CARNISE PRECOCE	6,7	3,1	2,2	0,96	molto estesa
KARNAK	6,7	3,0	2,2	0,99	molto estesa
KEOPE	6,6	3,0	2,2	0,92	molto estesa
LEONIDAS CL	6,9	3,0	2,3	0,99	molto estesa
POSEIDONE	7,0	3,2	2,2	0,88	molto estesa

DENOMINAZIONE DELL'ALIMENTO: **RISO VIALONE NANO**					
Caratteristiche del granello	lunghezza (mm)	larghezza (mm)	rapporto lungh./largh.	consistenza (kg/cm^2)	perla
valori di riferimento	5,4 ÷ 5,8	3,2 ÷ 3,5	1,6 ÷ 1,8	≥ 0,85	molto estesa
Varietà					
VIALONE NANO	5,6	3,3	1,7	0,96	molto estesa

Source:

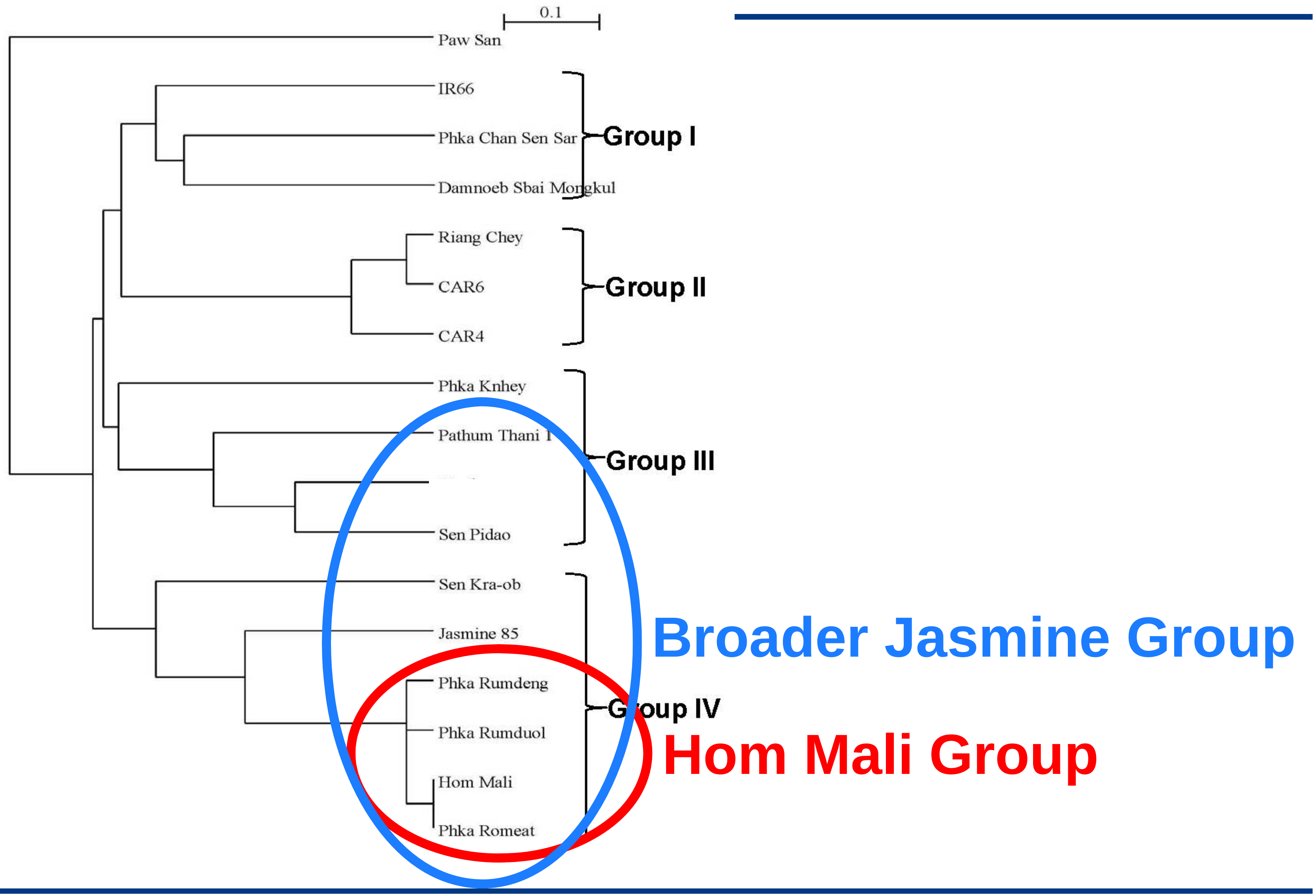
ENTE NAZIONALE RISI MILANO

REGISTRO VARIETALE
per l'annata agraria 2018/2019
aggiornato al 31 agosto 2018
(Articolo 6 del decreto legislativo 4 agosto 2017, n.131)

Cambodian Milled Rice Standard

Cambodian Rice			
(INDICA TYPE) COMPRISES OF INDIGENOUS VARIETIES			
FRAGRANT RICE		WHITE RICE	
Premium Fragrant	Fragrant Rice	Premium White	White Rice
Jasmine (Wet Season, photo sensitive period)	(Dry Season) mild natural scent	(Wet Season)	(All Seasons)
Extra-long grain, strong natural scent, soft texture, tender-fluffy when cooked with tantalizing aroma.	Long grain with similar Jasmine fragrance and characteristics, soft texture, tender when cooked, full of flavor.	Medium-length grain, kernel w/translucent endosperm, white color, distinct scent. Soft after cooking, a versatile tasty variety.	Medium-length and long grain varieties, kernel w/ white endosperm, firm texture also after cooking.
Phka Malis (Jasmine):	Sen Kra-ob	Ginger Rice:	Pearl Rice
Phka Rumduol	Sen Pidao	Phka Knhey	Neang Khon
Phka Romeat		Phka Chan Sen Sar	Ponla Pdao
Phka Rumdeng		CAR 4, CAR 6, Riang Chey	Neang Minh
Somali, Neang Malis™			IR 66
			Chul'sa
Grades: Milled and polished, 100% A, 100% B, 5%, 10%, 15%, 20%, 25%, 35%, 100% broken, A1 Super, A1 Extra Super; Premium Fragrant Organic, Organic Brown, Organic Red rice			

Dendrogram of Southeast Asian rice



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