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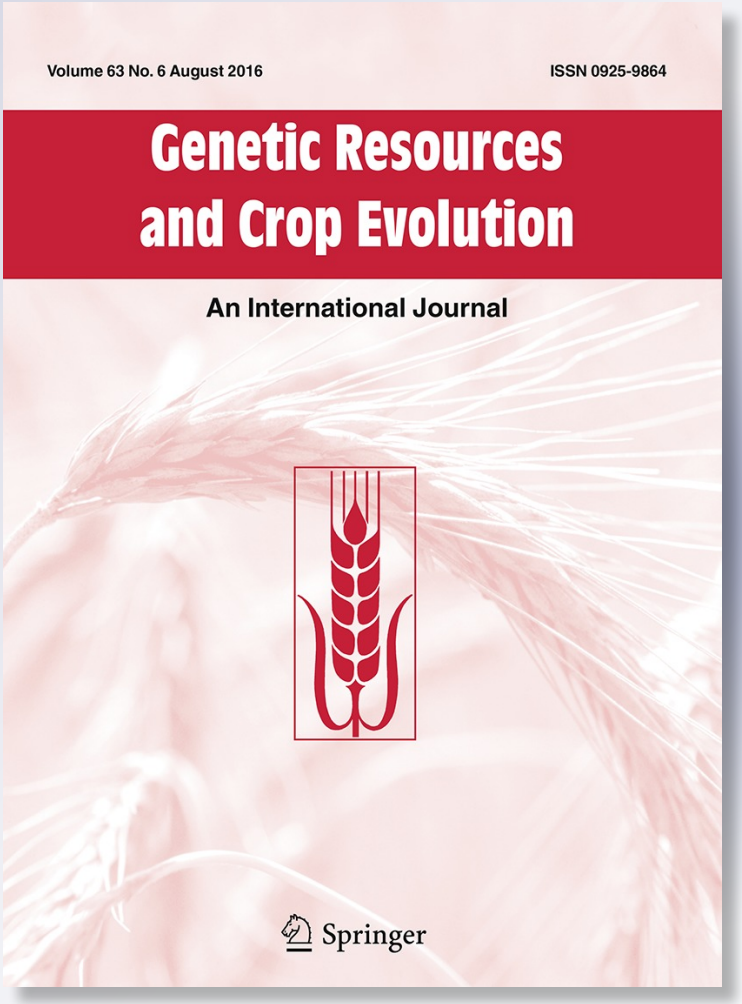

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SHORT COMMUNICATION

An analysis of variation of the aroma gene in rice (*Oryza sativa* L. subsp. *indica* Kato) landraces

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Abstract The candidature of 8-bp deletion in *badh2* gene as the predominant cause for aroma development in rice was investigated in 84 subsp. *indica* rice landraces. Presence of this functional mutation was detected in 80 % of aromatic samples and in three non-aromatic landraces which were found to be heterozygous at this locus. However, 11 landraces did not show its presence despite being aromatic during qualitative assessment. None of the wild ancestors possessed this deletion. Finally, we have discussed implications of our findings in the broader context of aroma evolution.

Keywords Aroma · *badh2* · *Indica* landraces · *Oryza* · Rice · 2-AP · 8-bp deletion

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Introduction

Aroma is one of the most acclaimed traits of rice, not only because of its culinary value, but also because of its cultural value assigning local and national identity. Scientific investigations over decades have revealed the biochemical basis of aroma, identified the gene responsible for the expression of aroma, and its polymorphism. The principal compound responsible for rice aroma production is 2-acetyl 1-pyrroline (2-AP) (Buttery et al. 1983). The candidate gene for the expression of aroma, *badh2*, located on chromosome 8, encodes betaine aldehyde deydrogenase homologue 2 (BADH2), the key enzyme regulating 2-AP production (Ahn et al. 1992). An eight base-pairs (8-bp) deletion in the exon 7 of this gene causes the loss of gene function through untimely truncation of the *badh2* transcript, thus accumulating 2-AP in aromatic landraces (Bradbury et al. 2005a).

This recessive allele of *badh2* carrying three single nucleotide polymorphisms (SNPs) and 8-bp deletion is known as *badh2.1* and is proclaimed to be the most abundant causal mutation occurring in aromatic rice samples. Apart from Basmati or Jasmine varieties, a large number of landraces from diverse geographic areas have been documented to have the 8-bp deletion in the *badh2* gene (Kovach et al. 2009). Recently, a series of studies have described other novel alleles responsible for aroma (Bourgis et al. 2008; Shi et al. 2008; Kovach et al. 2009; Shao et al. 2013). However, landraces with these mutations are confined to certain

geographic regions only (Shao et al. 2013). The previous studies on aroma mostly involved traditional and evolved Basmati and Basmati-type cultivars, alongside a few modern hybrid cultivars (Nagaraju et al. 2002; Sakthivel et al. 2009), thus faintly representing the indigenous subsp. *indica* landraces. In this study, we have examined the presence/absence of the primary causal mutation responsible for aroma in a suite of *indica* landraces most of which are extremely rare and a few have become extinct from farm fields. In addition, we have also included a few non-aromatic landraces and an accession each of *Oryza rufipogon* and *Oryza spontanea* from eastern India. We have finally discussed our findings in the broader context of aroma evolution.

Materials and methods

Sample collection

Seeds of indigenous rice landraces have been collected from indigenous farmers of different States of India and other countries, and conserved on-farm at Vrihi seed bank (www.cintdis.org/vrihi) (Deb 2005; Ray et al. 2013). All rice samples were collected from Vrihi's conservation farm Basudha (www.cintdis.org/basudha), located in the district of Rayagada (Odisha, India). We have also included one sample each of *O. rufipogon* and *O. spontanea* from Odisha in our analysis (Table 1).

Qualitative assessment of aroma

Aroma in rice samples was detected by smelling decorticated rice grains, following KOH method (Sood and Siddiq 1978). The samples were smelled and ranked for presence or absence of aroma by a panel consisting of four experts, chosen based on their skill to discriminate between the intensity of 2-AP aroma. The process was repeated three times for each sample. Samples are scored on a 0–3 scale, where 0 corresponded to absence of aroma, 1 for minimal aroma, 2 for moderate aroma and 3 for strong aroma (Table 1).

DNA extraction

Our germplasm set comprised of total 84 samples including 55 aromatic and 27 non-aromatic genotypes along with one sample of *O. rufipogon* and *O. spontanea* each. The genomic DNA was extracted following CTAB method (Doyle and Doyle 1987). Contaminating RNA was digested using 4 µL of DNase free RNase buffer and incubated at 37 °C for 2 h. Samples were checked on a 0.8 % agarose gel (Chromous Biotech, India) and are kept at 4 °C. The stored samples were diluted five times for PCR analysis.

Fragrance genotyping

In order to detect the functional polymorphism i.e. 8-bp deletion, we have used two sets of primers as reported by Bradbury et al. (2005b) and Shi et al. (2008). PCR amplification was carried out in a 23.5 µL reaction mixture, comprised by nuclease free water 14.5, 3 µL of 10X PCR buffer, 10 mM dNTP 2.5 µL, 0.5 U of Taq DNA polymerase (Sigma-Aldrich, USA), 1 µL BSA, and 1 µL (0.5 µM) of IFAP, ESP, INSP, EAP (hereafter referred to as Bradbury markers) and FmBADH2E7 primers (hereafter referred to as Shi marker) in PCR (Applied Biosystems, USA). The thermal cycler reactions were conducted at a preliminary 95 °C for 3 min, continued for 35 cycles of 1 min at 95 °C, 1 min at 58 °C and 2 min at 72 °C, with a 10 min extension at 72 °C.

A significant number (50) of our samples either have not amplified or showed positive control band of 577 bp for the fragrant varieties and 585 bp for the non-fragrant varieties when Bradbury markers were used, despite repeated attempts. However, the samples were amplified using the Shi marker at 55 °C.

All amplified products were segregated on a 2.0 % (w/v) agarose gel prepared in 1X TBE buffer. The gel was stained with ethidium bromide (0.5 µg/mL) and visualized using a gel documentation system (Biostep GmbH, Germany) using Argus X1 version 7 at a constant voltage of 75 V for 2 h. A 100 bp ladder (1 µg) molecular weight standard (Genei, Bangalore, India) was used to estimate PCR fragment size. The samples were then sequenced using 3100 model of ABI PRISM® Genetic Analyzer (Applied Biosystems, Shanghai, China). All generated sequence data were manually edited, assembled and aligned using the software MEGA 5.2 (Tamura et al. 2011). The presence or absence of functional mutation (i.e. the 8-bp deletion) among the amplified samples was confirmed by examinations of alignments of

Table 1 Comprehensive list of rice germplasms, primers used in the study, and corresponding Genebank accession numbers

S. no.	Landraces	Vrihi accession codes	Aroma score	Primers used	Presence of 8-bp deletion	Genebank accession numbers
1	Athi karaya	A04	0	FmBadh2E7	N	KT805282
2	Bangar sanna	B27	0	FmBadh2E7	N	KT751525
3	Bourani	B28	0	FmBadh2E7	N	KT751526
4	Brimphul	B35	2	IFAP,ESP	Y	KT860417
5	Basmati	B48	3	IFAP,ESP	Y	KT310186
6	Banshphool	B50	3	IFAP,ESP	Y	KT860418
7	Balir pinda	B51	3	IFAP,ESP	Y	KT860419
8	Batasabhog	B61	2	FmBadh2E7	N	KT877418
9	Basmati nagni	B66	0	IFAP,ESP	Y	KT877412
10	Boloi genti	B68	0	FmBadh2E7	N	KT877419
11	Burma black	B72	3	IFAP,ESP	Y	KT877413
12	Chini atap	C10	2	IFAP,ESP	Y	KT877414
13	Cheena kamini	C13	3	IFAP,ESP	Y	KU221263
14	Chatui mukhi	C15	2	IFAP,ESP	Y	KT877415
15	Cheri gadi	C16	0	IFAP,ESP	Y	KT877416
16	Chinna poni	C21	0	FmBadh2E7	N	KT877420
17	Chini-sail	C22	3	FmBadh2E7	Y	KT877417
18	Chhoto nuniya	CH01	0	FmBadh2E7	N	KT901461
19	Dahar nagra	D04	0	FmBadh2E7	N	KU198420
20	Dimru	D09	0	FmBadh2E7	N	KT953350
21	Dashahara jhuti	DD03	0	FmBadh2E7	N	KT970476
22	Dar-sal	DD06	1	IFAP,ESP	Y	KT987427
23	Dehradun-bas	DD13	2	IFAP,ESP	Y	KT987428
24	Debdulali	DD17	2	FmBadh2E7	N	KU198421
25	Durga sundari	DD26	2	IFAP,ESP	Y	KT987429
26	Garib-sal	G02	0	FmBadh2E7	N	KT953351
27	Gandha malati	G07	2	FmBadh2E7	Y	KT987430
28	Genti-sal	G10	0	FmBadh2E7	N	KT953352
29	Gopalbhog	G12	3	IFAP,ESP	Y	KT987431
30	Gandheswari	G19	3	IFAP,ESP	Y	KT987432
31	Garo joha	G20	0	FmBadh2E7	N	KT953353
32	Geng geng binni	G22	0	FmBadh2E7	N	KU198422
33	Gobindabhog	G26	1	IFAP,ESP	Y	KT987433
34	Gulvady sanna	G44	0	FmBadh2E7	N	KT953354
35	Haldi guri	H14	2	FmBadh2E7	Y	KT998658
36	Heerai joha	H18	2	IFAP,ESP	Y	KT998664
37	Jira sari	J09	1	IFAP,ESP	Y	KT998665
38	Japaka	J10	2	FmBadh2E7	Y	KT998659
39	Jubri dhan	J32	0	FmBadh2E7	N	KT953355
40	Kinari	K02	0	FmBadh2E7	N	KU198423
41	Kelas	K05	0	FmBadh2E7	N	KU206389
42	Kataribhog (BD)	K12	2	IFAP,ESP	Y	KT998666
43	Kanakchur	K16	1	IFAP,ESP	Y	KT998667

Table 1 continued

S. no.	Landraces	Vrihi accession codes	Aroma score	Primers used	Presence of 8-bp deletion	Genebank accession numbers
44	Kalo jira	K18	1	FmBadh2E7	N	KU206390
45	Kartik-sal	K33	0	FmBadh2E7	N	KU308248
46	Kalaturi	K37	3	IFAP,ESP	Y	KU131193
47	Krishnamukhi	K41	3	IFAP,ESP	Y	KU221261
48	Kali-sal	K54	1	FmBadh2E7	N	KU206391
49	Karnal Basmati	K57	3	FmBadh2E7	Y	KT998660
50	Kalmilata	K61	3	FmBadh2E7	Y	KT998661
51	Masineh/Kalo tudey	K63	2	IFAP,ESP	Y	KU131190
52	Kamini-sal	K67	3	FmBadh2E7	Y	KT998662
53	Krishnabhog	K72	3	IFAP,ESP	Y	KT998668
54	Kala Krishto	K82	3	IFAP,ESP	Y	KU131195
55	Kala nuniya	K86	3	IFAP,ESP	Y	KU131196
56	Laha raja	L04	3	FmBadh2E7	Y	KT998663
57	Lakkhan-sal	L15	0	FmBadh2E7	N	KU206392
58	Lilabati	L22	2	IFAP,ESP	Y	KU131191
59	Lal gobindabhog	L27	1	IFAP,ESP	Y	KU131192
60	Marich boot	M41	0	FmBadh2E7	N	KU356869
61	Mughal-sal	M43	0	FmBadh2E7	N	KU206393
62	Mohanmala	M44	0	IFAP,ESP	Y	KU308249
63	Nata	N02	0	FmBadh2E7	N	KU206394
64	Olee	O01	3	FmBadh2E7	N	KU206395
65	Parmai-sal	P08	1	FmBadh2E7	Y	KU131194
66	Pakistani Basmati	P18	3	FmBadh2E7	Y	KU131197
67	Khaskani	Q03	1	IFAP,ESP	Y	KU131198
68	Khudi khasa	Q05	2	FmBadh2E7	Y	KU131199
69	Kharishabhog	Q09	1	FmBadh2E7	N	KU206396
70	Kharah	Q11	0	FmBadh2E7	N	KU206397
71	Radhuni pagal	R07	2	IFAP,ESP	Y	KU131200
72	Rambhog	R18	2	FmBadh2E7	N	KU206398
73	Sada kaya	S01	0	FmBadh2E7	N	KU206399
74	Sundari	S07	1	FmBadh2E7	Y	KU131201
75	Subasita	S11	2	FmBadh2E7	Y	KU131202
76	Sada nuniya	S18	1	FmBadh2E7	N	KU206400
77	Tulsa	TT01	2	IFAP,ESP	Y	KU131203
78	Tulsimanjari	TT03	3	IFAP,ESP	Y	KU131204
79	Tulsibhog	TT07	1	FmBadh2E7	N	KU206401
80	Tulsibhog (N)	TT14	1	IFAP,ESP	Y	KU221262
81	Velchi	V02	3	FmBadh2E7	N	KU206402
82	Bhim-sal	V16	2	FmBadh2E7	N	KU206403
83	*Oryza rufipogon*	O.r BB	0	FmBadh2E7	N	KT970474
84	*Oryza spontanea*	O.s k	0	FmBadh2E7	N	KT970475

Y = 8-bp deletion present, N = 8-bp deletion not present, 0 = absence of aroma, 1 = minimal aroma, 2 = moderate aroma, 3 = strong aroma

sequences derived from these samples with the published genome sequence of ADT 43 (Genebank accession—HQ687206), a non-fragrant variety without the deletion.

Results

Qualitative evaluation of fragrance varieties revealed a range of fragrance intensity ranging from 1 to 3 (Table 1). We have obtained the presence of 8-bp deletion in a majority (44) of aromatic rice samples (55) of the Indica group by using either of the two markers. The Bradbury markers failed to yield all the requisite bands to completely separate fragrant and non-fragrant varieties according to the phenotypic data. A total of 34 (40.47 %) of our samples amplified with Bradbury Markers whereas 50 (59.52 %) of the same sample amplified with the Shi marker. This corroborates the observation by several authors that the Bradbury markers are not fully reliable markers to detect the presence of *badh2.1* allele (Sakthivel et al. 2009; Rai et al. 2015).

Among the amplified samples, we observed the presence of 8-bp deletion in three non-aromatic samples (Basmati Nagini, Cheri Gadi, Mohanmala), as confirmed by the fragrance phenotyping and sequence alignment. We therefore re-amplified these with Bradbury markers as these are co-dominant in nature and obtained three bands of size 580, 355 and 257 bp indicating the varieties were heterozygous non-fragrant (Table 1).

The rest of the samples (81) are either fragrant or homozygous non-fragrant. All the Basmati-type aromatic cultivars (Basmati, Dehradun-bas, Karnal basmati, Pakistani basmati, Sundari) have shown the presence of 8-bp deletion. None of the *O. rufipogon* and *O. spontanea* samples contained *badh2.1* allele. We have also found a number (11) of aromatic *indica* landraces (Batasabhog, Debdulali, Kalo jira, Kali-sal, Olee, Kharisha bhog, Rambhog, Sada nuniya, Tulsibhog, Velchi, Bhim-sal) are not possessing 8-bp deletion (Table 1).

Discussion

Despite many other polymorphisms have been uncovered over the last decade, the 8-base pair deletion at seventh exon of *badh2* gene remains most ubiquitous causal mutation conferring aroma in rice. In our study as well, the majority of the aromatic rice landraces (44), including Basmati types, possessed the 8-bp deletion as the functional polymorphism. The remaining 11 fragrant landraces did not possess 8 bp deletion, a finding that seems to indicate the presence of already reported or unknown mutation(s) in a different part of the *badh2* gene or in the promoter region (Kovach et al. 2009; Bourgis et al. 2008). Furthermore, neither of the *O. rufipogon* or *O. spontanea* samples contained the 8-bp deletion. Our sampling of the wild ancestral genotype was far from exhaustive, thus keeping the conjecture of an independent origin of aroma in *indica* rice alive (Ray et al. 2013). A set of three non-aromatic varieties which have demonstrated to harbour the deletion were heterozygous at this locus thus conforming to the existing genetic underpinning of aroma trait (Bradbury et al. 2005b).

Although we have not aimed to investigate into the genetic history of aroma, our study re-ignites the question about origin of aroma in subsp. *indica* landraces. The existence of a large number of aromatic landraces, without 8-bp deletion provokes an alternate origin hypothesis which is rooted in two primary lines of evidence. Firstly, most of the studies explored the wild pool of *O. rufipogon* almost divorcing *O. nivara* which have been luxuriantly growing across vast swaths of south and south-east Asia (Banaticla-Hilario et al. 2013). The former is reported to possess fragrance and/or other novel traits (Vanavichit 2007) or rare alleles (Sarla et al. 2003). Interestingly, *O. nivara* is genetically highly diverse in India (Juneja et al. 2006) and well-differentiated from another wild rice i.e. *O. rufipogon* (Singh et al. 2013; Wambugu et al. 2015). Closer relation between *indica* group and *O. nivara* further gained support from an in-depth study (Wambugu et al. 2015); which has demonstrated relative closeness of *O. nivara* and *O. rufipogon* from Asia to subsp. *indica* and subsp. *japonica* respectively. Surprisingly, studies unravelling evolutionary history of the key rice domestication genes has largely ignored sampling of this wild gene pool leading to a missing of an integral part of the fuller story. Secondly, certain geographic regions are over-represented in studies pertaining to rice domestication ignoring some of the most promising areas, e.g. cryptic diversity in north-eastern states and Jeypore tract has enormous potential to instigate further investigation on domestication

centers (Roy et al. 2015). A most recent study reiterating this proposition has described three domestication centres; of which north-eastern states with adjoining regions and Gangetic plain are depicted as cradle of domestication of subsp. *indica* and *aus* group (Civan et al. 2015).

Summarising, it appears that the current pool of landraces represents a significant fraction of aromatic *indica* landraces containing either 8-bp deletion or other functional polymorphisms. Interestingly, many of these landraces are rare, not accessible in public repository and some may be on the verge of extinction. These landraces carry a unique genetic legacy and signatures of the experimentation by human race towards domestication. Despite this, many are relatively less explored for novel traits and genes. Aroma is one such desirable trait subjected to complex events of domestication over time and space. In order to reconstruct the complete story, taking this quest further, deciphering the evolutionary history of the aroma summons a thorough sampling and examination of under-studied progenitors and domesticates.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. All the authors agreed with the final version of the manuscript.

References

Ahn SN, Bollich CN, Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theor Appl Genet 84:825–828

Banaticla-Hilario MCN, Sosef MSM, McNally KL, Hamilton NRS, Van den Berg RG (2013) Ecogeographic variation in the morphology of two Asian wild rice species, *Oryza nivara* and *Oryza rufipogon*. Int J Plant Sci 174(6):896–909

Bourgis F, Guyot R, Gherbi H, Tailliez E, Amabile I, Salse J, Lorieux M, Delseny M, Ghesquière A (2008) Characterization of the major fragance gene from an aromatic japonica rice and analysis of its diversity in Asian cultivated rice. Theor Appl Genet 117:353–368

Bradbury LMT, Fitzgerald TL, Henry RJ, Jin Q, Waters DL (2005a) The gene for fragrance in rice. Plant Biotechnol J 3(3):363–370

Bradbury LMT, Henry RJ, Jin QS, Reinke RF, Waters DLE (2005b) A perfect marker for fragrance genotyping in rice. Mol Breed 16:279–283

Buttery RG, Ling LC, Juliano BO, Turnbauhg JG (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agr Food Chem 31:823–826

Civan P, Craig H, Cox CJ, Brown TA (2015) Three geographically separate domestications of Asian rice. Nat Plants. doi:10.1038/NPLANTS.2015.164

Deb D (2005) Seeds of tradition, seeds of future, folk rice varieties of Eastern India. Research Foundation for Science, Technology and Ecology, New Delhi

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Juneja S, Das A, Joshi SV, Sharma S, Vikal Y, Patra BC, Bharaj TS, Sidhu JS, Singh K (2006) Oryza nivara (Sharma et Shastry) the progenitor of *O. sativa* (L.) subspecies *indica* harbours rich genetic diversity as measured by SSR markers. Curr Sci (Bangalore) 91(8):1079–1085

Kovach MJ, Calingacion MN, Fitzgerald MA, McCouch SR (2009) The origin and evolution of fragrance in rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 106:14444–14449

Nagaraju J, Kathirvel M, Kumar RR, Siddiq EA, Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR PCR and SSR markers. Proc Natl Acad Sci USA 99:5836–5841

Rai VP, Singh AK, Jaiswal HK, Singh SP, Singh RP, Waza SA (2015) Evaluation of molecular markers linked to fragrance and genetic diversity in Indian aromatic rice. Turk J Bot 39:209–217

Ray A, Deb D, Ray R, Chattopadhayay B (2013) Phenotypic characters of rice landraces reveal independent lineages of short-grain aromatic indica rice. AoB Plants 5:plt032. doi:10.1093/aobpla/plt032

Roy S, Banerjee A, Mawkhlieng B, Misra AK, Pattanayak A, Harish GD, Singh SK, Ngachan SV, Bansal KC (2015) Genetic diversity and population structure in aromatic and quality rice (*Oryza sativa* L.) landraces from North-Eastern India. Plos One. doi:10.1371/journal.pone.0129607

Sakthivel K, Rani NS, Pandey MK, Sivaranjani AKP, Neeraja CN, Balachandran SM, Madhav MS, Viraktamath BC, Prasad GSV, Sundaram RM (2009) Development of a simple functional marker for fragrance in rice and its validation in Indian Basmati and non-Basmati fragrant rice varieties. Mol Breed 24:185–190

Sarla N, Bobba S, Siddiq EA (2003) ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. Curr Sci (Bangalore) 84:683–690

Shao G, Tang S, Chen M, Wei X, He J, Luo J, Jiao G, Hu Y, Xie L, Hu P (2013) Haplotype variation at Badh2, the gene determining fragrance in rice. Genomics 101:157–162

Shi W, Yang Y, Chen S, Xu M (2008) Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. Mol Breed 22:185–192

Singh A, Singh B, Panda K, Rai VP, Singh AK, Singh SP, Chouhan SK, Rai V, Singh PK, Singh NK (2013) Wild rices of Eastern Indo-Gangetic plains of India constitute two sub-populations harbouring rich genetic diversity. Plant Omics J 6(2):121–127

Sood BC, Siddiq EA (1978) A rapid technique for scent determination in rice. Indian J Genet Plant Breed 38:268–271

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Vanavichit A (2007) In: International training workshop, the conservation and utilization of tropical/subtropical plant genetic resources, Taiwan Agricultural Research Institute, Council of Agriculture (TARIC), Taiwan, pp 131–134, Pub no 128

Wambugu PW, Furtado MBA, Waters DL, Henry RJ (2015) Relationships of wild and domesticated rices (Oryza AA genome species) based upon whole chloroplast genome sequences. Sci Rep. doi:10.1038/srep13957