Zeina Thamer Abd ULHussein ALrufaye et al /J. Pharm. Sci. & Res. Vol. 10(10), 2018, 2531-2533

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Use Molecular Markers Analysis and Sensory Methods in the Revelation of Fragrance in Iraqi Rice

Zeina Thamer Abd ULHussein ALrufaye[1], Ban A.H.AL Khafaji[2], Warqa'a Muhammed Sheriff Al-sheikh[3]

1 Department of biology, College of science, University of kerbala, Iraq
2 Department of biology, College of Education for pure sciences, university of kerbala, Iraq.
3 College of Dentistry, University of Al- Qadisiya, Iraq

Abstract

Allele-Specific Amplification together with four primers External Sense Primer ESP, External Antisense Primer EAP, Internal Fragrant Antisense Primer IFAP, and Internal Non fragrant Sense Primer INSP) and sensory valuation with grains and leaves were executed to differentiate aromatic rice varieties. Our results such as The PCR results showed that band 355bp in seven varieties of rice represent the nonfragrant allele, and showed bands of 257 bp in nine varieties of rice represent the fragrant allele. While sensory test showed six varieties of nonfragrant and ten varieties fragrant. Sensory evaluation or molecular analysis alone couldn't define aromatic condition fully. The incorporation of sensory methods with molecular marker analysis was observed as fast and reliable for a check of aromatic varieties.

Keywords: *Oryza sativa L*, Allele Specific Amplification (ASA), fgr gene

INTRODUCTION

Aroma is the furthermost important characteristic of rice which leading a higher price. Consequently aromatic rice is playing a active role in global rice trading (1,2) .One of the main reasons for the aromatic emission of cooked rice because it contains various volatile compounds chemically (3). Aromatic rice varieties release aroma in vegetative stages more than at mature stages (4). The investigators used many methods to decide the existence or non-existence of aroma in rice, for instance, analyzing the aroma using Sensory test, gas chromatography and dilute KOH (5), and molecular markers associated with rice fragrance (6). Affording to many studies that designated the recessive gene (fgr) on chromosome eighth on chromosome eighth of rice which contains an 8bp deletion 5 GATTATGG3 and 3 single nucleotide polymorphisms in the exon 7 of the badh2gene encoding betaine aldehyde dehydrogenase2 BAD2 made a nonfunctional Betaine Aldehyde Dehydrogenase 2 (BADH2) enzyme with amplified levels of 2-acetyl-1-pyrroline 2AP is the chief composite responsible for the aroma (7). consequential in aroma in rice. On the other hand, there are other conservative aromatic rice varierties which display different volatile compounds other than 2AP such as propanol, 2-butanone, acetaldehyde, hexanol, pentanal etc (8). Several Several molecular markers for instance iso-enzymes, SSR, STSs, RAPDs, and RFLPs have been improved for aromatic rice identification and selection (9). Furthermore, an idealistic marker technique called Allele Specific Amplification was advanced by(6). This technique was considered beneficial for distinguish aromatic and non aromatic rice in breeding databases(1).

In this study we evaluate the efficiency of molecular markers and incorporation of sensory approaches for the recognition of aromatic in diverse varieties of Iraqi rice.

MATERIALS AND METHODS

The sixteen varieties were collected from Mashkhab Research Center in AL-Najaf Iraq Tab.1

Extraction of DNA.

DNA was extracted from Young leaves after 25 days from agriculture, according to (10) Quality of DNA was verified by Gel electrophoresis and quantification was achieved by using the bio drop technique .The sequences of primers were as follows TTGTTTGGAGC TTGCTG ATG (ESP), ATAGGAGCAGCTGAAATA TATACC (IFAP), CTGGTAAAAAGAT TATGGC TTCA (INSP), and AGTGCTTTACA AAGTCC CGC (EAP) According to this reference (6)

Table 1: List of Iraq *Oryza Sativa* L. cultivars analyzed in this research

No.	Name of Varieties	Source	Pedigree
1	Aba 1	Mashkhab Research Center in AL-Najaf	Aba Research Center
2	Eanber albaraka	Mashkhab Research Center in AL-Najaf	Indian
3	Forat 1	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
4	Ghadeer	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
5	Barnamaj4	Mashkhab Research Center in AL-Najaf	Research International Rice Institute
6	Yasmine	Mashkhab Research Center in AL-Najaf	Vietnam
7	Buhooth1	Mashkhab Research Center in AL-Najaf	Turkey
8	Eanber 33	Mashkhab Research Center in AL-Najaf	Local
9	Dijlah	Mashkhab Research Center in AL-Najaf	Chinese
10	Meshkhab 2	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
11	Meshkhab1	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
12	First genotype (T85)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
13	Second genotype(HT-1)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
14	Third genotype (LT-2)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
15	Fourth genotype (BT-7)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
16	Fifth genotype (aromatic 64)	Mashkhab Research Center in AL-Najaf	Research International Rice Institute

PCR amplification

The volume of the PCR reaction mixture is 20µL that included 2.0 μL of 10X reaction buffer (with 20mM Mg+), 0.2 μL of 10mM dNTPs mix, 0.25 μL of YEA taq DNA Polymerase (Yeastem Biotech Co. Ltd., Taiwan), DNA template 5.0 μL , 0.4 μL of each primer EAP, and ESP, 0.5 μL of primer INSP and 0.5 μL of IFAP, Amplification was carried out using a thermal cycler. The amplification procedure is as follows 5min at 94℃ followed by 35 cycles of 30 s denaturation at 94 , of 30 s annealing at 53℃ and 1min extension at 72℃, concluding with the final extension of 7min at 72℃. Amplification products were electrophoresed on 2% agarose gels and stained with ethidium bromide (EB). was done to analyse PCR products. PCR fragment size was estimated through 100 bp ladder(Bioneer – USA). The bands representing aromatic, non aromatic, for fgr gene were analyzed by Allele Specific Amplification technique

RESULTS AND DISCUSSION

Aroma Estimation by Sensual Methods In this research grain and leaf of selected16 varieties were used for aroma evaluation The results showed that ten varieties is aromatic and six varieties non aromatic character in (Table 2).

The four primers in single tube Allele Specific Amplification (ASA)

aroma analysis for sixteen varieties by Allele Specific Amplification resulted THIS Aba 1, Barnamaj4 ,Buhooth1 ,Dijlah, Meshkhab 2,First genotype (T85), Fifth genotype (aromatic 64) varieties 355 bp bands showed a PCR product amplified through two Primers (External Antisense (EAP), Internal Nonfragrant Sence (INSP))as of the nonfragrant allele. Nonaromatic rice cultivars have what seems to be a completely practical copy from the gene encoding BADH2 for the synthesis of γ-Aminobutyric acid (GABA) , while Eanber albaraka, Forat 1 ,Ghadeer ,Yasmine , Eanber 33, Meshkhab1 ,Second genotype(HT-1) ,Third genotype (LT-2) ,Fourth genotype (BT-7) varieties showed bands of 257 bp indicating fragrant a PCR product amplified through two Primers (External Sence Primer (ESP), Internal Fragrant Antisense (IFAP)) from the fragrant allele (fgr),Consistent with the resuts (11). Fragrant varieties are possessing a copy from the gene encoding BADH2 which owns the SNPs and deletion, leading to a frameshift that generates the premature stop codon that apparently disables the BADH2 enzyme that results in the synthesis of 2-acetyl-1-pyrroline (2AP).This polymorphism affords an opportunity for the production of a complete marker for aroma in rice previously, (6) revealed that it is potential to distinguish non aroma from aroma rice varieties by using this method.

Sensory Methods enable the detection of fragrant and nonaromatic varieties while molecular marker promotion to recognize specific allele in single tube for detection of aroma in rice .In this investigation, we used molecular marker methods and sensory analyses for the revealing of the absence or presence of aroma in sixteen varieties, 8 varieties which were categorized as possessing the aroma alleles by the analysis of molecular marker, also discovered the presence of aroma in sensory tests.

while the Meshkhab1 varieties did not show aroma in sensory methods while transferring *fgr* gene in molecular marker analysis.First genotype (T85) and Fifth genotype (aromatic 64) varieties did not display aroma in *fgr* gene in molecular marker assay while presented presence of aroma in sensory tests may be because of a presence of another aromatic compound.

Table 2: Aromatic and non aromatic characters of Iraqi rice

No.	**Name of** Varieties	*Sensory*
1	Aba 1	non aromatic
2	Eanber albaraka	Aromatic
3	Forat 1	Aromatic
4	Ghadeer	Aromatic
5	Barnamaj4	Non aromatic
6	Yasmine	Aromatic
7	Buhooth1	Non aromatic
8	Eanber 33	Aromatic
9	Dijlah	Non aromatic
10	Meshkhab 2	Non aromatic
11	Meshkhab1	Non aromatic
12	First genotype (T85)	Aromatic
13	Second genotype(HT-1)	Aromatic
14	Third genotype (LT-2)	Aromatic
15	Fourth genotype (BT-7)	Aromatic
16	Fifth genotype (aromatic 64)	Aromatic

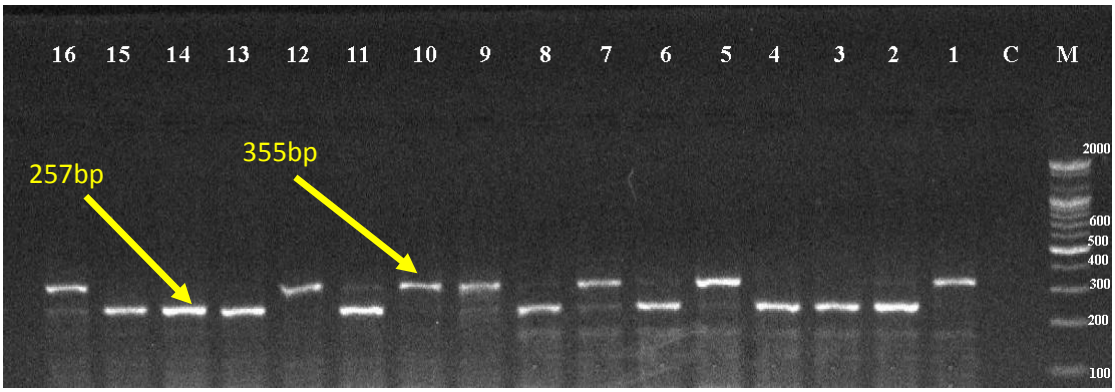

Fig. 1: Aroma analysis for sixteen cultivars rice with PCR marker (Allele Specific Amplification).

Lane 1 DNA marker(100–2,000 bp) , Lanes 2 control , Lanes3 Aba 1. Lanes4 Eanber albaraka, Lanes5 Forat 1, Lanes6 Ghadeer, Lanes7 Barnamaj4, Lanes8 Yasmine, Lanes 9 Buhooth1, Lanes10 Eanber 33, Lanes11 Dijlah, Lanes12 Meshkhab 2, Lanes13 Meshkhab1, Lanes14 First genotype (T85), Lanes15 Second genotype(HT-1) , Lanes16 Third genotype (LT-2), Lanes17 Fourth genotype (BT-7) , Lanes18 Fifth genotype (aromatic 64) respectively.

The difference in the sensory analyses may result from environmental factors or minor genes and that some rice varieties may transfer minor QTLs which own an effect on rice aroma. The difference in the sensory analyses may because of environmental factors or minor genes and that some rice cultivars may transfer minor QTLs which own an redounds on rice aroma Consistent with the results(12) .These results indicated that only sensory methods or molecular marker analysis could not clarify the complete aromatic conditions. Consistent with the results (13) stated that the molecular marker outcomes accepted well with the chemical analysis in the majority of the rice cultivars, except some covariance results(1) reported chance between results from 1.7% KOH sensory testing and molecular marker analysis for the taxonomy of non-aromatic and aromatic rice .In this study the fragrant variety ware identified can be used improving breeding programs.

CONCLUSION

Aroma evaluation of rice varieties is complex So, incorporation of sensory methods with allele specific PCR amplification of EAP, ESP, IFAP, and INSP primers were observed as credible, fast, and cost-efficient techniques to Assess rice aroma in this search, this would play a significant role in the development of cultivars high-quality rice used in improving breeding programs

REFERENCES

1- Sarhadi, W. A. ; Hien, N. L. ; Zanjani, M.; Yosofzai, W.; Yoshihashi , T. and Hirata, Y. “Comparative analyses for aroma and agronomic traits of native rice cultivars from Central Asia,” Journal of Crop Science and Biotechnology. 2011, 11, 17–22.

2- Sakthivel, K.; Sundaram, R. M. ; Rani, N. S. ; Balachandran, S. M. and Neeraja, C. N. “Genetic and molecular basis of fragrance in rice,”J. Biotechnology Advances. 2009, 27(4), 468–473.

3- Cordeiro,G.M.; Christopher, M. J.; Henry,R. J. and Reinke,R. F. “Identification of microsatellite markers for fragrance in rice by analysis of the rice genome sequence. J. Molecular Breeding, 2002, 9(4), 245–250.

4- Vidya H.; Hemant P. and Altafhusain N. Comparative Characterization of Aroma Volatiles and Related Gene Expression Analysis at Vegetative and Mature Stages in Basmati and Non-Basmati Rice (Oryza sativa L.) Cultivars. j. Appl Biochem Biotechnol. 2015, 178(4)

5- Nguyen L. H. , Tadashi Y. , Wakil A. S. and Yutaka H. Sensory Test for Aroma and Quantitative Analysis of 2-Acetyl-1-Pyrroline in Asian Aromatic Rice Varieties. J. Plant Prod. Sci. 2006, 9(3), 294-297.

6- Bradbury, L. M. ; Henry, R. J. ; Jin, Q.; Reinke, R. F. and Waters, D. L. “A perfect marker for fragrance genotyping in rice,” Molecular Breeding. 2005, 16(4), 279–283

7- Subhas, C. R. DNA Barcoding for Wild Rice [Oryza rufipogon Griff.] of NBU Campus Based on matK gene and Assessment of Genetic Variation Using DREB and BAD2 Gene Sequences. J. Plant Gene and Trait, 2015, 6(5), 1-10.

8- Yang, D.S.; Lee, K.S.; Jeong, O.Y.; Kim, K.J.; Kays, S.J. (2008) Characterization of volatile aroma compounds in cooked black rice. J. Agricultural and Food Chemistry .56,(2)35–40.

9- Kibria, K.; Islam, M.M. and Begum, S.N. 2008. “Screening of aromatic rice lines by phenotypic and molecular markers,” Bangladesh .J. Botany. 37(2), : 141–147.

10- Dellapporta SL, Hicks JB. 1983 .A new plant DNA minipreparation: Version II. Plant Mol Biol Rep, 1:19-21.

11- Acga, C. ; Festo, M.; Ismanizan, I.; Mohamad, O.; Habibuddin, H. 2015. High resolution agarose-based system for single-tube genotyping of fgr and Waxy genes in rice: MAGE to displace PAGE? Plant omics J.P.O 8(4):348-352.

12- Yi, M.; Nwea, K.T.; Vanavichit, A.; Chai-arree, W. and Toojinda, T. 2009. “Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha,” J. Field Crops Research. 113,(2),: 178–186.

13- Bounphanousay, C.; Jaisil, P.; Sanitchon, J.; Fitzgerald, M. and Hamilton, N. S. 2008. “Chemical and molecular characterization of fragrance in black glutinous rice from Lao PDR,” Asian J. Plant Sciences,. 7(1): 1–7.