

**ESTABLISHMENT OF MOLECULAR BREEDING LINES
FOR AROMA IN RICE**

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**FACULTY OF SCIENCE
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KUALA LUMPUR**

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**ESTABLISHMENT OF MOLECULAR BREEDING LINES
FOR AROMA IN RICE**

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Establishment of Molecular Breeding Lines for Aroma in Rice

ABSTRACT

Aroma is one of the most important traits of aromatic rice grain quality after grain appearance, amylose content, gelatinization temperature and kernel elongation. The genetic control and environmental variation of this trait are reported to be quite complex. To screen and select the F₁ individuals with aroma, four molecular markers namely, EAP, ESP, INSP, and IFAP were used in this study together with sensory evaluation. The F₁ individuals were obtained from different crosses between Malaysian local rice varieties (MR 219 and MRQ 50) with seven selected aromatic rice varieties from global popular aromatic rice cultivars and advance lines. The F₁ seeds were planted together with their parents in the experimental field at the Institute of Biological Sciences, Faculty of Science, University of Malaya and examined for their agronomic performance in the year 2011. During molecular screening and sensory evaluation, homozygous aromatic F₁ individuals were found to have better mean aroma score in leaf and grain compared to both heterozygous non-aromatic individuals and homozygous non-aromatic individuals due to crosses between two homozygous aromatic parents. However, some of the F₁ with the absence of aroma alleles, produced aroma in both leaf and grain. Moreover, the aroma emitted from those F₁ individuals was almost the same as the Pandan (*Pandanus amaryllifolius*) leaf aroma which has been linked to 2-acetyl-1-pyrroline (2AP). Thus, they were suspected follow BADH2 (betaine aldehyde dehydrogenase 2)-independent 2AP synthesis pathway. Genotypic and phenotypic analysis revealed that aroma in F₁ individuals was successfully inherited in curve shaped seeds compared to non-curve shaped seeds. The yield performance was low in most of the F₁ individuals, especially F₁ from the crosses between aromatic and aromatic parents, except for F₁ from MRQ 50/E 13. Among the F₁ from 14 different

crosses, F₁ derived from MRQ 50/E 13 produced high yields and better aroma. In the present study, the integration of molecular markers and sensory methods were observed to be reliable and fast for screening the F₁ derived from all crosses because these molecular markers distinguished homozygous aromatic, homozygous non-aromatic and heterozygous non-aromatic individuals whilst the sensory method evaluated the aroma emitted from leaf and grain during flowering to maturity stages.

Establishment of Molecular Breeding Lines for Aroma in Rice

ABSTRAK

Aroma adalah salah satu sifat paling penting dalam kualiti beras wangi selain ciri-ciri fizikal bijian, kandungan amilosa, suhu penggelatinan dan pemanjangan kernel. Kawalan genetik dan faktor alam sekitar ke atas ciri ini dilaporkan menjadi agak kompleks. Untuk penapisan dan pemilihan individu F_1 dengan aroma, empat penanda molekul iaitu, EAP, ESP, INSP dan IFAP telah digunakan dalam kajian ini bersama-sama dengan penilaian aroma. Individu-individu F_1 diperolehi dari kacukan yang berbeza antara varieti padi tempatan Malaysia (MR 219 dan MRQ 50) dengan tujuh varieti beras wangi yang dipilih daripada kultivar beras wangi global yang popular dan varieti 'advance'. Benih-benih F_1 telah ditanam bersama-sama dengan induk mereka di Institut Sains Biologi, Fakulti Sains, Universiti Malaya dan diperiksa untuk prestasi agronomi pada tahun 2011. Semasa pemeriksaan molekul dan penilaian aroma, homozigus individu F_1 aromatik didapati mempunyai aroma min skor yang lebih baik dalam daun dan biji-bijian berbanding dengan kedua-dua individu bukan aromatik heterozigot dan homozigot individu bukan aromatic disebabkan kacukan daripada kedua-dua induk homozigot aromatik. Walau bagaimanapun, sesetengah F_1 dengan ketiadaan alel aroma menghasilkan aroma dalam kedua-dua daun dan bijirin. Selain itu, aroma yang dihasilkan dari individu-individu F_1 adalah hampir sama seperti aroma daun pandan (*Pandanus amaryllifolius*) yang telah dikaitkan dengan 2-acetyl-1-pyrroline (2AP). Oleh itu, mereka telah dianggap mengikuti laluan sintesis 2AP BADH2 (betaine aldehyd dehydrogenase 2)-bebas. Analisis genotip dan fenotip mendedahkan bahawa aroma pada individu F_1 telah berjaya diwarisi dalam benih melengkung berbanding benih yang tidak melengkung. Prestasi hasil adalah rendah dalam kebanyakan individu F_1 , terutamanya F_1 dari kacukan antara induk aromatik dan aromatik, kecuali untuk F_1

dari MRQ 50 / E 13. Di kalangan 14 kacukan yang berbeza, F_1 yang berasal dari MRQ 50/ E 13 mengeluarkan lebih banyak hasil dengan aroma yang lebih baik. Dalam kajian ini, integrasi antara penanda molekul dan penilaian aroma didapati boleh dipercayai dan pantas untuk memeriksa F_1 yang diperolehi daripada semua kacukan kerana penanda molekul boleh mengenal pasti aromatik 'homozygous', 'homozygous' bukan aromatik dan heterozigot bukan aromatik individu, manakala penilaian aroma boleh menilai aroma yang dihasilkan dari daun dan biji-bijian semasa pokok padi berbunga sehingga tahap kematangan.

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RESEARCH PUBLICATION

Title: Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment

CONFERENCE PROCEEDING

Title: Aroma analysis in few rice genotypes (Oral Presentation)

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ABBREVIATION

AB-ald	4-aminobutyraldehyde
ANOVA	Analysis of variance
ASA	Allele specific amplification
BADH2	Betaine aldehyde dehydrogenase 2
bp	Base pair
cM	Centi Morgan
cm	Centimeter
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribonucleic acid
g	Gram
GABA	4-aminobutyric acid
GABald	γ -aminobutyraldehyde
GC-MS	Gas chromatography-mass spectrometry
GT	Gelatinization temperature
HYV	High yielding variety
I ₂ -KI	Iodine and potassium iodide in water (Lugol's iodine)
IRRI	International Rice Research Institute
K	Potassium
KOH	Potassium hydroxide
mA	Milli Ampere
MARDI	Malaysia Agricultural Research and Development Institute
ml	Milliliter
mm	Millimeter
N	Nitrogen
ng	Nano gram
P	Phosphorus

PCR	Polymerase chain reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
S	Sulphur
SAS	Statistical Analysis System
SES	Standard Evaluation System
SNP	Single nucleotide polymorphism
SPME	solid phase micro-extraction
SSR	Simple sequence repeat
STS	Sequence tag site
TBE	Tris/Borate/EDTA
V	Volt
2AP	2-acetyl-1-pyrroline
Δ	Delta
α	Alhpa
γ	Gamma
μl	Microliter
μM	Micromolar
$\text{\textcircled{M}}$	Male
$\text{\textcircled{F}}$	Female
%	Percentage