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WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Fourteenth Session
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ADDENDUM TO DOCUMENT BMT/14/8

IDENTIFICATION OF RICE VARIETIES USING GENIC MARKERS FOR THREE DUS CHARACTERISTICS

Document prepared by experts from Islamic Republic of Iran

The Annex to this document contains a copy of a presentation “Identification of Rice Varieties using Genic Markers for Three DUS Characteristics” made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Seyed Hossein Jamali, Leila Sadeghi, Ehsan Pourabed, Mohammad Reza Jazayeri, Samad Mobasser
Plant Variety Identification & Registration Department of Seed & Plant Certification & Registration Institute (SPCRI), Iran (Islamic Republic of)

[Annex follows]

Identification of Rice Varieties using Genic Markers for Three DUS Characteristics

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S.H. JAMALI

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, and especially is of the main dish of Iranian consumers.

New varieties of rice are bred through public research breeding programs mostly carried out by

- Rice Research Institute of Iran (RRII), and
- Genetic and Agricultural Biotechnology Institute of Tabarestan (GABIT) of SANR University,

with some of them bearing genetic material of varieties of International Rice Research Institute (IRRI), known as IR varieties.

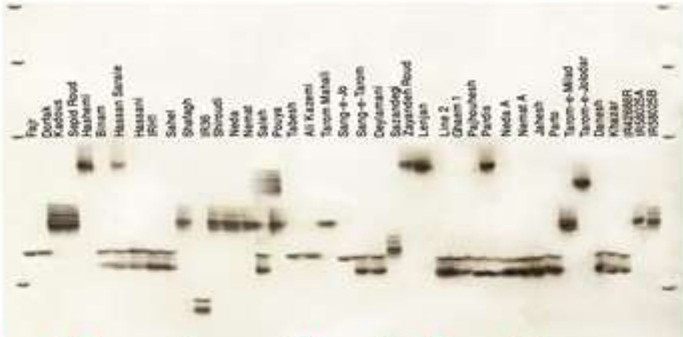
Molecular Markers in DUS Testing of Rice Varieties

So far, a number of neutral molecular markers have been used in identification and distinctness testing of rice varieties. These markers include

RAPDs (Shukla *et al.* 2011; Patra and Chawla, 2010),
ISSRs (Shukla *et al.* 2011), and
SSRs or microsatellites (Singh *et al.*, 2004; Sarao *et al.* 2009)

However EST-SSRs and MADS-box genes of rice have been used in identification and DUS testing of rice varieties (Bonow *et al.*, 2009)

Identification and Distinctness of Iranian Rice Varieties with SSR Markers



Although these markers can differentiate the varieties,

they do not reflect the expressed characters

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model 1

"Characteristic-specific molecular markers"

Could the breeder-friendly markers used in MAS breeding programs

be deployed as examiner-friendly markers in Variety Identification and DUS testing?

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Plant Material

A total of 43 rice varieties including

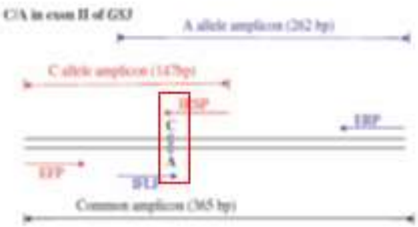
- 17 varieties bred by RRII,
- 10 varieties bred by GABIT,
- 12 Iranian local varieties, and
- 4 lines from IRRI

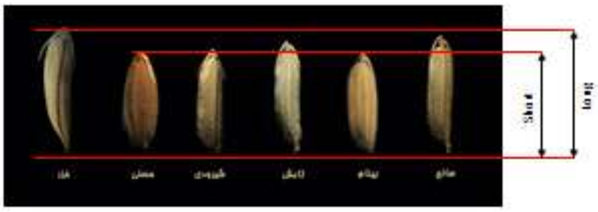
were used in this study

Genotyping & Phenotyping

Characteristic	Ch. No in DUS test guideline	Gene	Marker	Marker Type
Grain length	54 and 58, Grouping Ch.	*GS3*	SF28	CAPS, *Pst*I
			EFP ERP IRSP IFLP	ASP
Amylose content	63	*Wx*	RM190	SSR
			W2-R	CAPS, *Acc*I
Fragrance	65, Grouping Ch.	*frg*	FMbadh2-E7	STS

Grain length





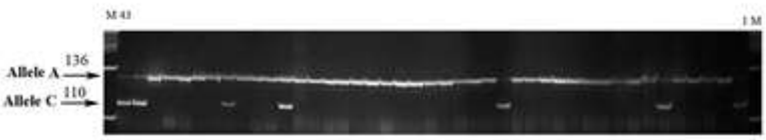


Table - Mean comparison for grain and decorticated grain length in two allelic group A and C

Allele	No. of Varieties	Grain length (mm)	decorticated grain length (mm)
C	7	8.1 *a*	6 *a*
A	36	10.1 *b*	7.2 *b*
difference		2	1.2
LSD		0.8	0.56

		Grain length Min	Grain length Max	Grain length Diff.	Decorticated Min	Decorticated Max	Decorticated Diff.
C	7	6.3	9.2	2.9	4.8	6.6	1.8
A	36	7.4	11.8	4.4	6	8.8	2.8
overlap		1.8				0.6	

Means indicated by non-common letters have significant difference ($P = 0.0002$) by LSD test

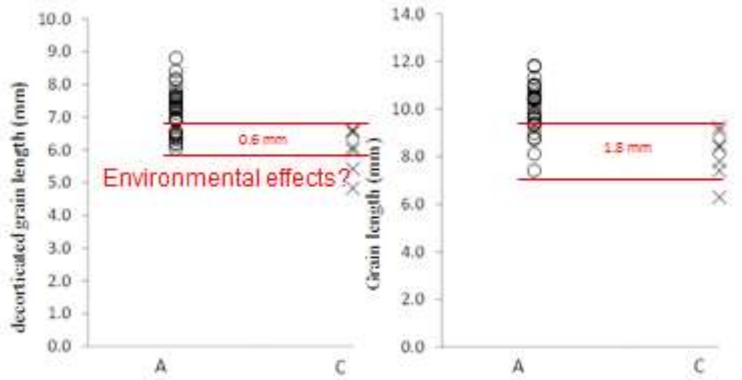


Fig . Range of decorticated grain length (left) and grain length (right) in two allelic groups A and C consisting 36 and 7 varieties respectively.

Amylose Content

SSR (RM 190) with (CT)$_n$ moif

G/T SNP

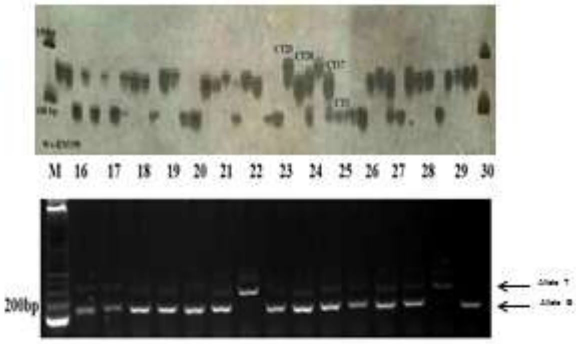


Table - Mean comparison for amylose content in four allelic group of microsatellite marker, two allelic group of SNP marker, and five allelic combination group of these loci

Allele/ Allelic combination	No. of Varieties	Amylose content (average)		*P*
$(CT)_{25}$	2	*ab*	22.5	0.0087
$(CT)_{20}$	9	*ab*	23.2	
$(CT)_{17}$	16	*b*	21.6	
$(CT)_{3}$	13	*a*	25.4	
T	3	*a*	16.8	< 0.0001
G	37	*b*	23.7	
$(CT)_{17}$ + T	3	*b*	16.8	0.0002
$(CT)_{25}$ + G	2	*a*	22.7	
$(CT)_{20}$ + G	9	*a*	23.2	
$(CT)_{17}$ + G	13	*a*	22.4	
$(CT)_{3}$ + G	13	*a*	25.4	

Means indicated by non-common letters have significant difference (P = 0.0002) by Duncan's multiple range test

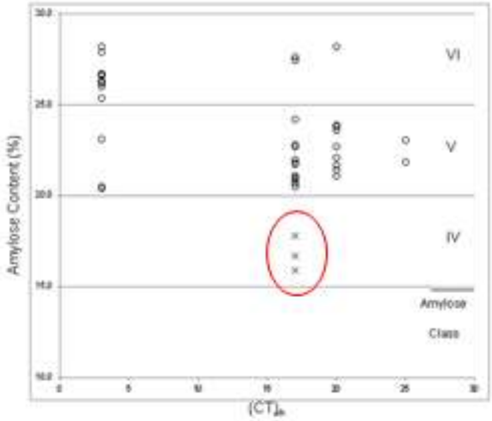
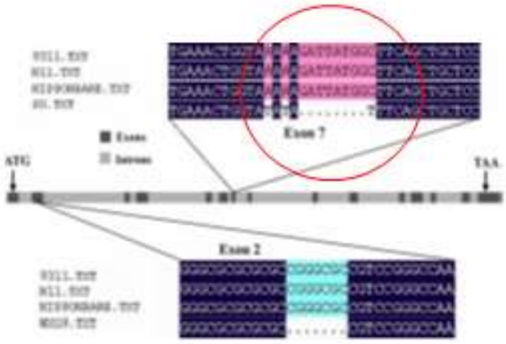


Fig - Amylose content, (CT)n allele of RM190, and Allele G/T of W2 marker (denoted by O and × respectively) in 40 rice varieties. Roman numbers indicate classes of amylose content according to the Test Guideline: IV (16-20%), V (20-25%), VI (25-30%)

Fragrance



States of Expression	No. of Varieties	Genotype
Non-fragrant or very weak	23	Allele 268
Weak	12	2 varieties bearing allele 268 10 varieties bearing allele 260
Strong	8	Allele 260

Examiner error in scoring?

Is there a match between genotype and phenotype ?

SNP and In/Del Markers produce binary alleles
DUS characteristics (in most cases) have more than two states of expression

Characteristic	Distinctness by genic markers	States of Expression
Decorticated Grain length	Allele C (Short) Allele A (Long)	A quantitative characteristic with the states of expressions 1-9 In test guideline: 3 states of short, medium and long
Amylose content	Allele G (High) Allele T (Low)	A quantitative characteristic In test guideline: 7 classes of Amylose
Fragrance	Allele 260 (fragrant) Allele 268 (non-fragrant)	A qualitative characteristic In test guideline: 3 states of non-fragrant or very weak, weak, and strong

Genic (functional) markers could be used in

grouping of varieties
Identification and distinctness of varieties,

as they are

free from environmental effects
(grain length as a quantitative trait)
cost-effective (amylose content) and
more reliable (fragrance)

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Thank You

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[End of Annex and of document]