

**(*Oryza sativa* L.)**  
**RAPD**

. . . . .

CMS (CMS)  
(B)

IR58025A A A A A A

RAPD

CMS

OPH01 OPH20

CMS

B

CMS

IR58025A A OPH20-950 bp

DH5 $\alpha$

Escherichia coli

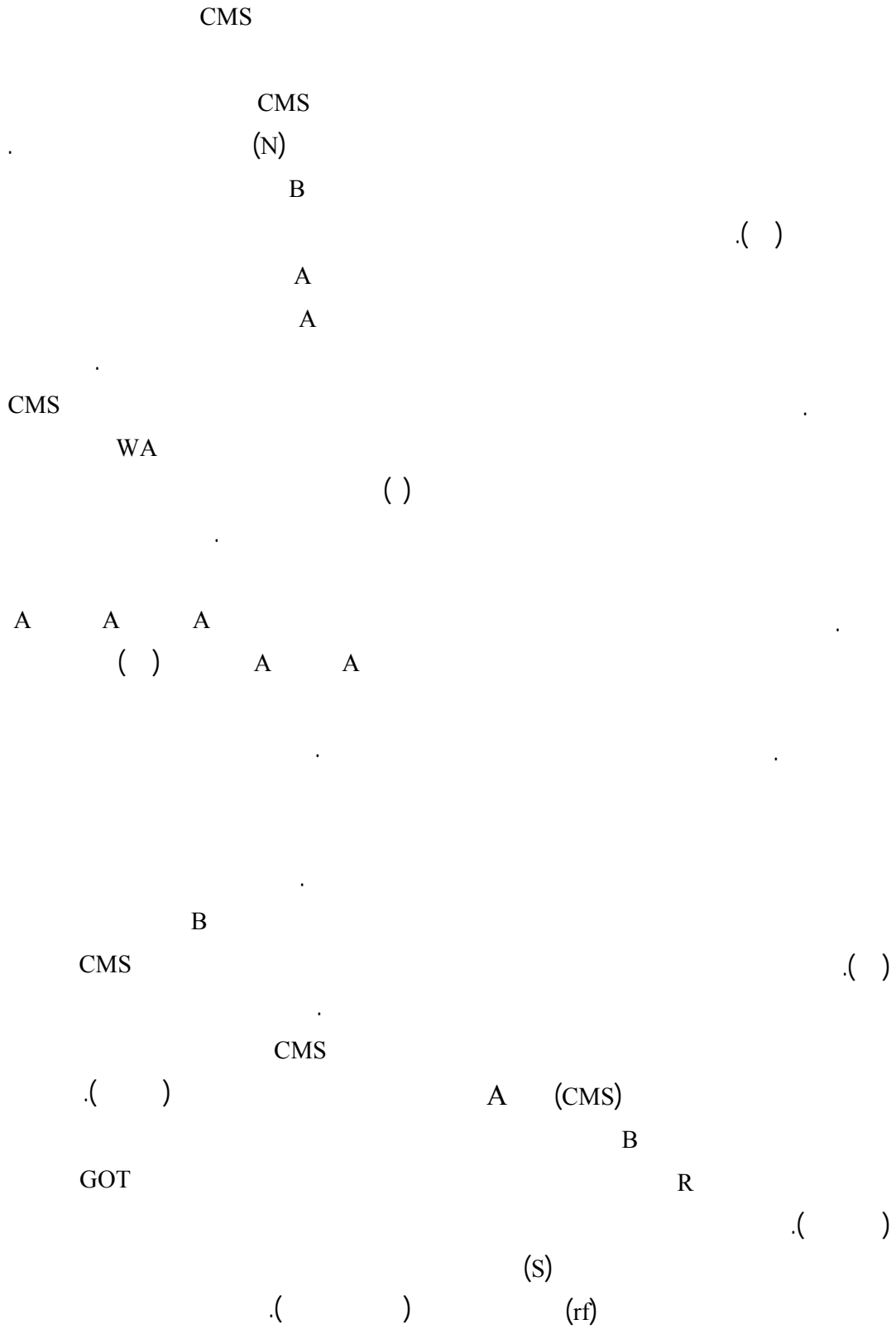
T/A

(WA ) CMS

RAPD :

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-  
-  
-



1- Cytoplasmic male sterile  
 4- Wild abortive

2- Maintainer line  
 5- Off-type

3- Restorer line  
 6- Grow out test

(cpDNA) DNA

( ) ( )  
( )  
RAPD

DNA

A ( ) A CMS ( )  
( ) A ( ) A ( ) A ( ) RAPD  
( ) B  
( ) B ( ) B ( ) B CMS B  
( ) B

DA WA

IR58025A ( ) IR58025B ( )  
(IRRI) RAPD

( ) A B

(KI-I<sub>2</sub>) ( ) ( )  
RAPD

A B

(Diagnostics GmbH, Germany  
DNA  
High Pure PCR Product  
Roche Diagnostics) Purification  
(GmbH, Germany

**RAPD**  
DNA  
( )  
DNA

% (CMS)  
Biophotometer,) ( B)  
(Eppendorf, Germany Alpha DNA, Montreal,) RAPD  
(Canada  
T/A / /  
3'-dA /  
( ) / (10X)  
PCR / /  
DNA  
/ .  
/ )  
(10X) / )  
/ ( )  
dATP / /  
/ )  
(MJ Mini, Bio-Rad, USA)  
PCR /  
3'-dA (0.5 mg/ml)  
InsTAclone™ PCR OPH20  
Cloning (MBI Fermentas, Lithuania)  
T4 Escherichia OPH20  
pTZ57R/T DH5α coli  
Roche) MS

..... / / /

Colony PCR Escherichia coli  
(JM107) DH5α

M13

Prism-ABI 3730 XL-PerkinElmer Xgal LB IPTG

BLASTn

DNA ( )

ClustalW2

RAPD -

OPH 01	GGTCGGAGAA	OPH 18	GAATCGGCCA
OPH 02	TCGGACGTGA	OPH 19	CTGACCAGCC
OPH 03	AGACGTCCAC	OPH 20	GGGAGACATC
OPH 06	ACGCATCGCA	OPA 02	TGCCGAGCTG
OPH 07	CTGCATCGTG	OPA 03	AGTCAGCCAC
OPH 09	TGTAGCTGGG	OPA 05	AGGGGTCTTG
OPH 10	CCTACGTCAG	OPA 06	GGTCCCTGAC
OPH 11	CTTCCGCAGT	OPA 07	GAAACGGGTG
OPH 12	ACGCGCATGT	OPA 08	GTGACGTAGG
OPH 13	GACGCCACAC	OPA 09	GGGTAACGCC
OPH 14	ACCAGTTGG	OPA 11	CAATCGCCGT
OPH 16	TCTCAGTTGG	OPA 12	TCGGCGATAG
OPH 17	CACTCTCCTC		

RAPD

RAPD

(A )

(B )

B A

RAPD

1- Rapid screen

2- [Http://www.ncbi.nlm.nih.gov/genBank/index.html](http://www.ncbi.nlm.nih.gov/genBank/index.html)

CMS

IR58025A

950 bp

OPH01 OPH 20

CMS

OPH01

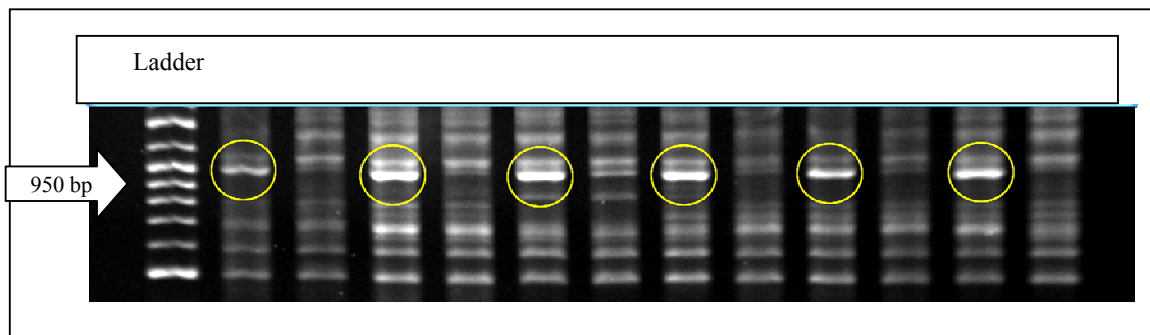
CMS

850 bp

B

CMS

OPH20



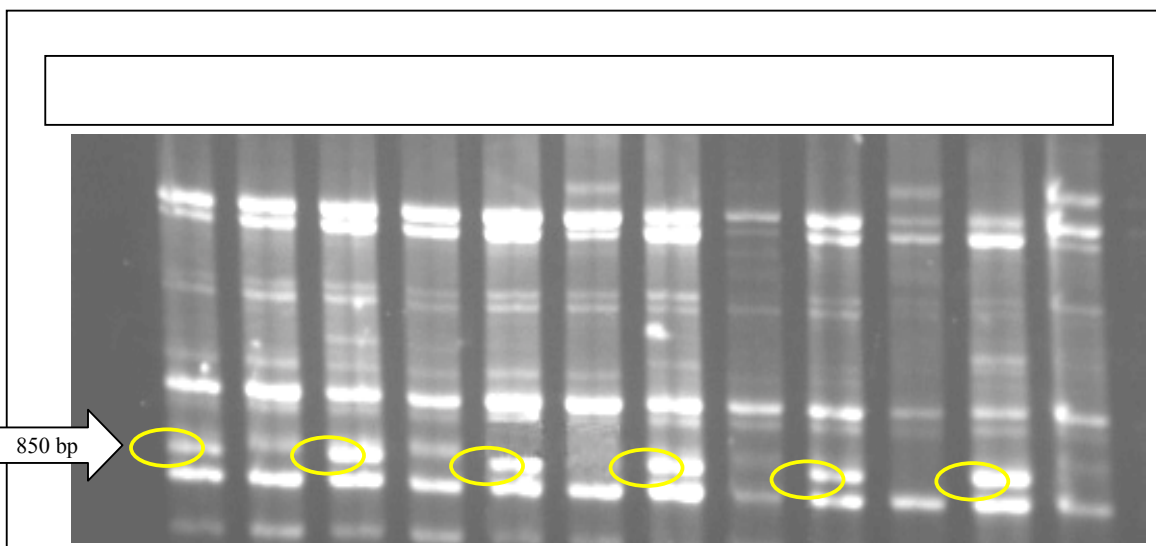
OPH 20

DNA

-

(

)



OPH 01

DNA

-

(

)

..... / / /

IR58025A A

A

OPH20-950 bp

IR58025A

DH5 $\alpha$

Escherichia coli

BLAST

(

)

BLAST

( % )

Colony PCR

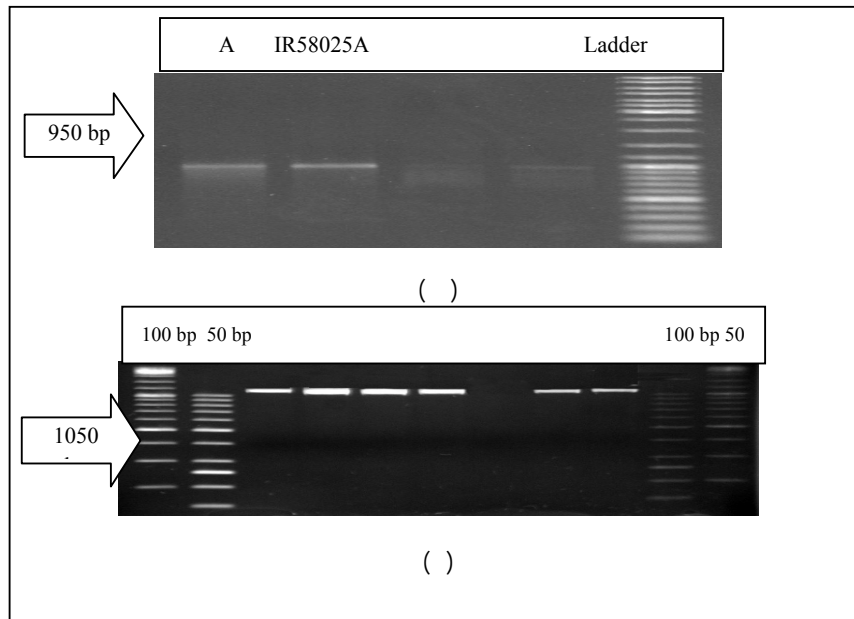
( - )

OPH20-950 bp

PCR

CLUSTALW2

M13



( )

OPH20-950

-

( )

Colony PCR

A

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Database      ---AGACATCGT-TCCAGCTGATTCAAATACCTAACTAAGCGACCTGAATGGAAGGCTAA 56
IR58025A     GGGAGACATCGT-TCCAGCTGATTCAAATACCTAACTAAGCGACCTGAATGGAAGGCTAA 59
NedaA        GGGAGACATCGTGTCCAGCTGATTCATATACCTAACTAAGCGACCTGAATGGAAGGCTAA 60
              *****

Database      CCAATGAATGAACTGTTCTCCCCAGCTGTTACGAACTATCAGATGGTCAAGAATTGACAG 116
IR58025A     CCAATGAATGAACTGTTCTCCCCAGCTGTTACGAACTATCAGATGGTCAAGAATTGACAG 119
NedaA        CCAATGAATGAACTGTTCTCCCCAGCTGTTACGAACTATCAGATGGTCAAGAATTGACAG 120
              *****

Database      GAATGAAGGGAATTGATTTACAGGGATCAGATAAAGAGGGAATTTATGAAATAAGAGTTAC 176
IR58025A     GAATGAAGGGAATTGACTTCAGGGATCAGATAAAGAGGGAATTTATGAAATAAGAGTTAC 179
NedaA        GAATGAAGGGAATTGATTTACAGGGATCAGATAAAGAGGGAATTTATGAAATAAGAGCTAC 180
              *****

Database      GAGAACAACTAGTCGAACAGAAAGCATGAGTTAAGTGGTGTGCTAACTTCCTTACTCATT 236
IR58025A     TAGAACAACTAGTAGAACAGAAAGTCTGAGTTAAGTGGTGTGCTAAATTCCTTACTCATT 239
NedaA        GAGAACAACTAGTCCGACGGAAGCATGAGTTAAGTGGTGTGCTAACTTCCTTTCTCATT 240
              *****

Database      GTAGCTGCTTTGATGGGGGACATGGAAGGATTGAAATAACAAGG----- 281
IR58025A     GTACCTGCTTCAATGGGGGACATGGAAGGATTGAAATAAAAAGGAAAAAAACAACGCC 299
NedaA        GTATATGCTTTGATGGGGGACATGGTATGATTGAAATAACAAGGAAACAAAAACAGCTCA 300
              ***

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IR58025A A

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RAPD

OPH20

RAPD

PCR

DNA

CMS

B A

(B)

CMS

CMS

(N)

( )

B A

(Multiplexing)

%

(cms)

..... / / /

CMS . ( ) ( )  
( ) ( )  
( ) . % %

RAPD

. ( ) (≥ %)  
( )  
CMS (B)

CMS  
PCR

OPH20-950

1. Ballester, J. and M. Carmen. 1998. Determination of F<sub>1</sub> hybrid seed purity in pepper using PCR-based markers. *Euphytica*. 103: 223-226.
2. Clark, J.M. 1988. Novel non-templated nucleotide addition reactions catalyzed by prokaryotic and eukaryotic DNA polymerases. *Nucl. Acids Res.* 16 (20): 9677-9686.
3. Dellaporta, S.L., J. Wood and J.B. Tickes. 1983. A plant molecular DNA minipreparation version II. *Plant Mol. Biol. Rep.*, 1: 19-21.
4. Garg, A., A.K. Singh, K.V. Prabhu, T. Mohapatra, N.K. Tyagi, N. Nandakumar, R. Singh and F.U. Zaman. 2006. Utility of a fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). *Seed Sci. Tech.*, 34: 9-18.
5. Guanghua, H., H. Lei, X. Yuehua, L. Xiaoying, N. Guoqing, Y.Y. Guangwei and P. Yan. 2003. A common sequence difference between cytoplasmic male sterile lines and their maintainer lines existing in rice (*Oryza sativa* L.) chloroplast tRNA-Leu gene region. *Euphytica*. 131: 269-274.
6. Ichii, M., D.L. Hong, Y. Ohara, C.M. Zhao and S. Taketa. 2003. Characterization of CMS and maintainer lines in indica rice (*Oryza sativa* L.) based on RAPD marker analysis. *Euphytica*. 129: 249-252.
7. Jena K.K. and S.K. Pandey. 1999. DNA markers for purification of A and B lines for hybrid rice improvement. *Hybrid Rice Newsl.* 2: 13-14.
8. Jianhua, Z., M.B. McDonald and P.M. Sweeney. 1997. Testing for genetics purity in Petunia and Cyclamen seed using random amplified polymorphic DNA markers. *Hort Sci.*, 32: 246-274.
9. Komori, T. and N. Nitta. 2004. A simple method to control the seed purity of japonica hybrid rice varieties using PCR-based markers. *Plant Breed.* 123: 549-553.
10. Nancy, A.E. 2006. Cytoplasmic male sterility and fertility Restoration. *The plant Cell.* 18: 515-517.
11. Nandakumr, N., A. Singh, K. Sharma, R.K. Mohapara, T.K.V. Prabhu and F.U. Zaman. 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*. 136: 257-264.
12. Nematzadeh, G., A. JuharAli, M. Sattari, A. Valizadeh, E. Alinejad and M.Z. Nouri. 2006. Relation between different allogamic associated trait characteristics of the five newly developed cytoplasmic male sterile (CMS) lines in rice. *Central Eur. Agric. J.*, 7: 49-56.
13. Rajendran, N., R. Gandhimani, S. Singh and K. Palchamy. 2007. Development of a DNA marker for distinguishing CMS lines from fertile lines in rice (*Oryza sativa* L.). *Euphytica*. 156: 129-139.
14. Rajendrakumar, P.P., A.K. Biswal, S.M. Balachandran, M.S. Ramesha, B.C. Viraktamath and R.M. Sundaram. 2007. A mitochondrial repeat specific marker for distinguishing wild abortive type cytoplasmic male sterile rice lines from their cognate isogenic maintainer lines. *Crop Sci.*, 47: 207-211.
15. Sambrook, J. and D.W. Russell. 2001. *Molecular cloning*. New York: Cold Spring Harbor Laboratory Press. p. 1-1688.
16. Sang, X., Z. Yang, B. Zhong, Y. Li, L. Hou, Y. Pei, G. Li and G. He. 2006. Assessment of purity of rice CMS lines using cpDNA marker. *Euphytica*. 152: 177-183.

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17. Souframanien, J., J.G. Manjaya, T.G. Krishna and S.E. Pawar. 2003. Random amplified polymorphic DNA analyses of cytoplasmic male strils and male fertile Pigeon pea. *Euphytica*. 129: 293-299.
  18. Virmani, S.S., C.X. Mao, R.S. Toledo, M. Hossain and A. Janaiah. 2002. Hybrid rice seed production technology and its impact on seed industries and rural employment opportunities in Asia. *IRRI Technical Bull.* p. 1-13.
  19. Virmani, S.S., Z.X. Sun, T.M. Mou, A. Jauharali and C.X. Mao. 2003. Two-line hybrid rice breeding manual. Los Bonos (Philippinines), Internationahl Rice Research Institute. p. 1-88.
  20. Yashitola J., R.M. Sundaram, S.K. Biradar, T. Thirumurugan, M.R. Vishnupriya, R. Rajeshwari, B.C. Viraktamath, N.P. Sarma and R.V. Sonti. 2004. A sequence specific PCR marker for distinguishing rice lines on the basis of wild abortive cytoplasm from their cognate maintainer lines. *Crop Sci.*, 44: 920-924.
  21. Yashitola, J., T. Thirumurugan, R.M. Sundaram, M.K. Naseerullah, M.S. Ramesha N.P. Sarma and R.V. Sonti. 2002. Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Sci.*, 42: 1369-1373.

## Screening of Rice (*Oryza sativa* L.) Alloplasmic Lines Via RAPD Molecular Markers

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### Abstract

In three-line system, cytoplasmic male sterile (CMS) lines often were contaminated with cognate iso-nuclear maintainer lines during seeds multiplication processes. Therefore fingerprinting of breeding lines and identification of line-specific markers are prerequisite in genetic purity test. Six CMS lines including Neda-A, Nemat-A, Dasht-A, Amol 3-A, Champa-A, IR58025A and their iso-nuclear maintainers were used in this study. Twenty-five random amplified polymorphic DNA (RAPD) primers used for screening CMS lines and maintainer lines. The result indicated that the iso-gene lines had similar band patterns in most marker loci due to similar genetic background that present between CMS lines and co-maintainer lines. However two specific bands, 950 bp and 850 bp were produced by OPH20 and OPH01 primers that could uniquely recognize the CMS lines from their cognate maintainer lines, respectively. The genetic nature of OPH20-950 band was determined by isolation of fragment from gel, clone to *Escherichia coli* bacteria via T/A cloning system, and was sequenced. A BLAST search of OPH20-950 sequence with GenBank indicated 95% homology to a rice mitochondrial DNA. These line-specific fragments could be used as a specific feature (scare marker) for characterization and genetic purity test of WA CMS lines.

**Keywords:** Rice, RAPD, Cytoplasmic male sterile lines, Genetic purity test, Mitochondri

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