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Application of PCR-based Markers for off-type Detection in Rice CMS Lines

Ghaffar Kiani *

ABSTRACT

The cytoplasmic male sterile (CMS) lines that are used in production of hybrid seeds in three-line system often get contaminated with their maintainer lines. Using such impure CMS lines in rice hybrid seed production lead to un-uniformity and yield loss in hybrid rice. Laborious and time consuming phenotypic evaluations are used for elimination of off-type plants in CMS multiplication farms. The other option for off-type elimination in CMS lines is application of molecular markers. In this study, PCR-based markers were used for distinguishing five male sterile lines from other five maintainer lines. Results showed that *cms* marker had ability to differentiate male sterile lines Neda A and Nemat A from their maintainers. Also, *drrcms* marker differentiated male sterile lines Dasht A, Champa A and Amol-3 A from their respective maintainers. Results of the study demonstrate the use of these two markers for off-type detection in CMS seed multiplication process. This is helpful in promotion of hybrid seed production technology in Iran.

Keywords: Rice, CMS, maintainer, markers, impurity.

INTRODUCTION

Plant cytoplasmic male sterility (CMS) caused by lesion or rearrangement of mitochondrial genome is unable to produce functional pollens. But CMS can be restored by nuclear genes. Therefore, the CMS systems are widely used for hybrid seed production. (Nematzadeh and Kiani 2010)

In rice, more than 90% of the rice hybrids developed over these years belongs to wild abortive (WA) cytoplasmic source (Yao et al. 1997, Zhang et al. 2002). In all cases except one (Lefebvre et al. 1990), factors controlling the CMS are placed in the mitochondria (Schnable & Wise 1998). The phenomenon regarded as a mitochondrial mutation causing the normal developmental program of male gamete production to fail (Budar et al., 2003). CMS lines used in hybrid rice seed production system often get contaminated with maintainer lines. Molecular markers can be used for purification of such CMS lines. In this regard, breeders had used RFLP (Sane et al. 1997) and RAPD (Sane et al. 1997, Jena & Pandey 1999, Ichii et al. 2003) for purification of CMS lines. Because of cost and labor of RFLP and low repeatability of RAPD, these markers are not practical for large-scale purification of rice CMS lines. Use of PCR-based mitochondrial markers (mtDNA) designed from specific sequences in the genome of rice CMS lines has been reported earlier Yashitola et al. (2004) and Rajendrakumar et al. (2007). Purification of rice CMS lines using these markers is cost-effective, reliable and fast, ensuring uniform rice hybrid seed production.

In Iran, several CMS lines like Neda, Nemat, Dasht,

*Department of Biotechnology and Plant Breeding, Sari Agricultural Sciences and Natural Resources University, P.O. Box: 578, Sari, Iran
ghkiani@gmail.com
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Champa, Amol-3 have been developed (Nematzadeh et al., 2006). Their multiplication and maintenance is essential. So every year, sterility stability assessments, and purification of these lines is performed using phenotypic methods. Grow out test (GOT) is one of these methods. This method is based on morphological traits (Yashitola et al. 2002, 2004). The use of mitochondrial markers in the diagnosis A- and their maintainers (B lines) and their use for purification of rice CMS lines are the objectives of this study.

MATERIALS AND METHODS

Five rice CMS lines viz. Neda A, Nemat A, Dasht A, Chmpa A and Amol-3 A and their respective maintainers were used in this study. These genotypes were planted at Research farm of Sari University of Agricultural Sciences and Natural Resources in 2014.

Total genomic DNA was extracted according to Dellaporta et al. (1983). Mitochondrial markers *drrcms* (forward: ACCTTTGGGCGATGGTT, reverse: GGGTTTAGAGTCGCCAC) and *cms* (forward: ACTTTTTGTTTTTGTGTAGG reverse: TGCCATATGTCGCTTAGACTTTAC) were used for polymorphism detection between the CMS and maintainer lines. The former marker developed by Rajendrakumar et al. (2007) and the later developed by Yashitola et al. (2004).

A 25 µl mixture was prepared for the PCR assay which containing 50 ng template DNA, 2.5 µl of 10X buffer, 0.3 µl of 10 mM dNTPs, 1 µl of 50 mM MgCl2, 1 µl of each primers (2 µM), and 1 unit of *Taq* polymerase. The PCR reaction for *cms* marker was performed at 95 °C for 7 min; then for 35 cycles of 94 °C for 30 sec; 44 °C for 1 min; 72 °C for 2 min followed by 72 °C for 7 min. This marker producing 386 bp band in CMS lines (Yashitola et al. 2004). PCR products were resolved by electrophoresis in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide.

The PCR reaction for *drrcms* marker was performed at 94 °C for 5 min; then for 35 cycles of 94 °C for 30 sec; 55 °C for 30 sec; 72 °C for 1 min followed by 72 °C for 5 min. This marker produced 130 and 142 bp bands in CMS and maintainer lines, respectively (Rajendrakumar et al. 2007). PCR products were resolved by electrophoresis in 3.5% agarose gel.

RESULTS AND DISCUSSION

The result of the evaluation with *drrcms* marker on CMS and maintainers is shown in Figure 1. The result indicates that this marker distinguished CMS lines Dasht A, Champa A and Amol-3A from their respective B-lines. This marker produced expected 130 and 142 bp bands in CMS and B-lines, respectively. But this marker does not show polymorphism between CMS lines Neda A and Nemat A with their maintainers. Therefore, this marker is able to detect off-types in CMS lines Dasht A, Chmpa A and Amol-3 A in purification process. The results confirm the results of Rajendrakumar et al. (2007).

In evaluation of male sterile lines DashtA, ChampaA and Amol-3A with their cognate maintainer lines using *cms* marker, results show no polymorphism between them, but this marker had ability to distinguish between CMS lines Neda A and Nemat A from their fertile lines (Figure 1). So, this marker produced the expected band of 386 bp in CMS lines, while maintainer lines lacking the desired band. These results indicating that *cms* marker can be used for distinguishing off-type plants (maintainer) in the multiplication process of CMS seeds. The results confirm the results of Yashitola et al. (2004).

In order to validate the accuracy of *cms* marker, an attempt was made for identification and elimination of off-type plants in multiplication farm of Neda A. For this purpose, a sample consisting of ninety-six plants were chosen from multiplication farm of Neda A and used for

molecular screening for impurity detection on it. Results are shown in Figure 2. The results of this evaluation showed that there were six off-type plants in this sample and the rest were male sterile plants. Phenotypic evaluations also made in the case of seed setting on all of individual plants and the results confirm that only molecular detected off-type plants were fertile plants. Therefore, this marker can reliably detect impurities in the multiplication field of CMS lines Neda A and Nemat A.

The CMS lines were multiplied with adequate isolation distance leaving no scope for a biological contamination through alien pollens coming from the neighbouring rice fields. Under such circumstances, the only impurity that can be expected in CMS line seed lot that comes from its maintainer line which can probably be a mechanical admixture during various stages of CMS lines seed handling. Detect of contamination in hybrid rice using molecular marker reported by several researchers. Abo Youssef et al. (2011) used *drrcms* marker for distinguishing a CMS line (IR70368A) from its cognate isonuclear maintainer line (IR70368B) as well as the hybrid. Nandakumar et al. (2004) successfully employed a single restorer gene linked marker assessment for testing genetic purity of hybrid seeds. Using RAPD markers Ahmadikhah (2009) reported polymorphism of five RAPD primers between CMS lines and their maintainer lines. The developed SCAR primers (SCARmt01R-SCARmt01F) had ability to produce polymorphism between the CMS and maintainer lines. Application of markers for genetic purity testing has also been demonstrated in other crops such as maize (Wang et al., 2002), cotton (Dongre & Parkhi, 2005) and sunflower (Pallavi et al. 2011).

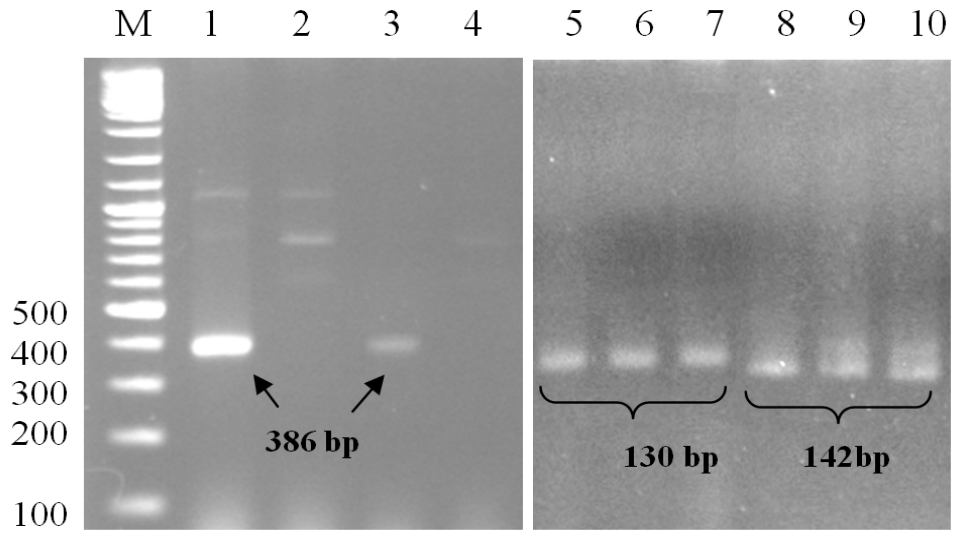

Figure 1. Molecular detection of male sterile rice lines from their maintainers using mitochondrial markers *cms* (left) and *drrcms* (right). 1= NedaA, 2= Neda, 3= NematA, 4= Nemat, 5= DashtA, 6= ChampaA, 7= Amol3A, 8= Dasht, 9= Champa, 10= Amol3. M, 100bp ladder

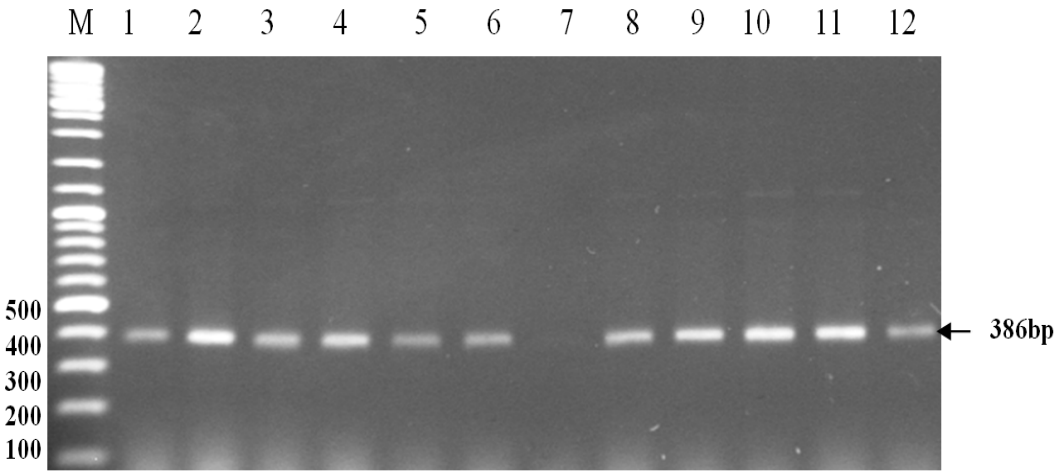

Figure 2. Molecular screening of Neda A individual plants for detection of contamination in it using mitochondrial marker *cms*. Numbers 1 to 12 are some of Neda A individual plants from multiplication farm. Plant number 7 is off-type plant. M, 100bp ladder.

CONCLUSIONS

The PCR-based mitochondrial markers in this study can be used in a PCR assay to reliably distinguish impurities due to maintainer lines with low cost and labor comparing to GOT at molecular level. Application of these markers in vegetative phase in addition to time saving is helpful in promotion of hybrid seed production technology in the country.

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REFERENCES

Abo Youssef M.I., Dora S.A., Deraz S. F., Abo-Shosha A.A.M., Khalil A.A., El-Sayed M.A.A. (2011). Estimating the genetic purity in cytoplasmic male sterile (CMS) lines of Egyptian rice. ***Aust J Crop Sci*** 5(3):254-261.

Ahmadikhah A. (2009). Identification of molecular markers linked to WA-cytoplasmic male sterility in rice and development of specific SCAR markers. Mod Genet 3: 65-74. (In Persian).

Budar F., Touzet P., De Paepe R. (2003). The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited. ***Genetica*** 117: 3-16.

Dellaporta S.L., Wood J., Hicks J.B.(1983). A plant DNA mini-preparation: version II. ***Plant Mol Biol Rep*** 1: 19-21.

Dongre A., Parkhi, P. (2005). Identification of cotton hybrid through the combination of PCR based RAPD, ISSR and microsatellite markers. ***J Plant Biochem Biotechnol*** 14: 53-55.

Ichii M., Hong D.L., Ohara Y., Zhao C.M., Taketa S. (2003). Characterization of CMS and maintainer lines in indica rice (*Oryza sativa* L.) based on RAPD marker analysis. ***Euphytica*** 129:249–252.

Jena K.K., Pandey S.K. (1999). DNA markers for purification of A and B lines for hybrid rice improvement. ***Hybrid Rice Newslet*** 2:13–14.

Lefebvre A., Scalla R., Pfeiffer P. (1990). The double-

stranded RNA associated with the '447' cytoplasmic male sterility in *Vicia faba* is packaged together with its replicase in cytoplasmic membranous vesicles. ***Plant Mol Biol*** 14: 477–490.

Nandakumar N., Singh A.K., Sharma R.K., Mohapatra T., PrabhuK.V., Zaman F.U. (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice usingmicrosatellite markers. ***Euphytica*** 136:257-264.

Nematzadeh, G. A., Kiani, G. (2010). Genetic analysis of fertility restoration genes for WA-type cytoplasmic male sterility in Iranian restorer rice line DN-33-18. ***African J Biotechnol*** 9(38): 6273-6277.

Nematzadeh G.A., Ali A.J., Sattari M., Valizadeh A., Alinejad E., Nouri M.Z. (2006). Relationship between different allogamic associated trait characteristics of the five newly developed cytoplasmic male sterile (CMS) lines in rice. ***J Cent Eur Agric*** 7: 49-56.

Pallavi H.M., Rame G., Shadakshari Y.G., Bhanuprakash K., Vishwanath K. (2011). Identification of SSR markers for hibridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). ***Helia*** 34(54): 59-66.

Rajendrakumar P., Biswal A.K., Balachandran S.M., Ramesha M.S., Viraktamath B.C., Sundaram R.M. (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.

Sane A.P., Seth P., Ranade S.A., Nath P., Sane P.V. (1997). RAPD analysis of isolated mitochondrial DNA reveals heterogeneity in elite wild abortive (WA) cytoplasm in rice. ***Theor Appl Genet*** 95: 1098–1103.

Schnable P.S., Wise R.P. (1998). The molecular basis of cytoplasmic male sterility and fertility restoration. ***Trends Plant Sci*** 3(5): 175-180.

Wang J., Zhong G.Y., Chin E.C.L., Register J.C., Riley R.D., Nieber W.S., Smith J.S.C.(2002). Identification of parents of F_1 hybrids through SSR profiling of maternal and hybrid tissue. ***Euphytica*** 124: 29-34.

Yao F.Y., Xu C.G., Yu S.B., Li J.X., Gao Y.J., Li X.H., Zhang Q.F. (1997). Mapping and genetic analysis of two fertility restorer loci in the wild-abortive cytoplasmic male sterility system of rice (*Oryza sativa* L.). ***Euphytica*** 98: 183-187.

Yashitola J., Thirumurugan T., Sundaram R.M., Naseerullah M.K., Ramesha M.S., Sarma N.P., Sonti R.V. (2002). Assessment of purity of rice hybrids using microsatellite and STS markers. ***Crop Sci*** 42: 1369-1373.

Yashitola J., Sundaram R.M., Biradar S.K., Thirumurugan T., Vishnupriya M.R., Rajeshwari R., Viraktamath B.C., Sarma N.P., Sonti R.V. (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.

Zhang T.X., Jan C.C., Miller J.F., Fick G.N. (2002). Inheritance of fertility restoration for two cytoplasmic male sterility sources of Helianthus pauciflorus (rigidus) Nutt. ***Crop Sci*** 42: 1873-1875.

تطبيق تقنية فحص الواسمات الوراثية (PCR) للكشف عن عن الاصناف التي تحتوي على خاصية عقم السيتوبلازم الذكري (CMS) من خطوط الأرز

***غفّار كياني* ***

ملخص

الخطوط التي تحتوي على خاصية العقم الذكوري للسيتوبلازم في نبات الارز والتي تستخدم في انتاج البذور المهجنة في نظام مكون من ثلاثة خطوط عادة ما تتلوث في كثير من الأحيان مع الخطوط المحافظة عليها. استخدام بعض الخطوط النقية التي تحتوي على خاصية عقم االستوبلازم الذكري (CMS) في انتاج بذور الارز المهجنة قادت الى عدم التماثل في الإنتاج والى خسارة المحصول في الارز المهجن. التقييم الذي يعتمد على الشكل المظهري للأصناف يعد مستهلك للوقت والأيدي العاملة ويستخدم للقضاء على النباتات التي لا تحتوي على خاصية الستوبلازم الذكري (CMS) في عدد من المزارع. الخيار الاخر للحصول على الاصناف التي تحتوي فقط على الستوبلازم الذكري (CMS) في خطوط الارز هو اللجوء الى طريقة الواسمات الجزيئية او الوراثية. في هذه الدراسة تم استخدام طريقة الواسمات الوراثية التي تعتمد على تقنية (PCR) لتمييز خمس خطوط ذكرية من خمس خطوط اخرى المحافظة عليها. النتائج اشارت الى أن واسمات (*cms*) كان لديها القدرة على تمييز الخطوط الذكرية العقيمة وهي (نيدا أ ونيمات أ) من الخطوط المحافظة عليها. كما ان واسمات (*drrcms*) استطاعت ايضا ان تميز الخطوط العقيمة الذكرية (داشات أ، شامبا أ وامول-3) من الخطوط الاخرى المحافظة عليها. نتائج هذه التجربة اشارت الى ان استخدام اثنين من الواسمات الوراثية لتمييز الاصناف التي تحتوي على عقم الستوبلازم الذكوري (CMS) في عملية إكثار البذور. وهذا أدى إلى المساعدة في تحفيز تكنولوجيا انتاج البذور المهجنة في ايران.

الكلمات الدالة: الارز، الستوبلازم العقيم الذكري (CMS)، الخطوط المحافظة، الواسمات الوراثية، عدم النقاوة.

* قسم التكنولوجيا الحيوية وتربية النبات، جامعة ساري للعلوم الزراعية والموارد الطبيعية، ايران ghkiani@gmail.com

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