Australasian Plant Pathol. (2016) 45:73–82
DOI 10.1007/s13313-015-0390-3

ORIGINAL PAPER

Tracing the signature of *Peronosclerospora maydis* in maize seeds

Rudy Lukman[1] · **Ahmad Afifuddin**[1] · **Thomas Lübberstedt**[2]

Received: 6 September 2015 / Accepted: 10 November 2015 / Published online: 16 November 2015

Abstract Downy mildew (*Peronosclerospora maydis*) is considered one of the most destructive diseases for maize. Beside being dispersed by air, it is suspected that contaminated seeds also play a role in the dissemination of this disease. To answer this hypothesis we collected sweet corn and field corn samples from three districts in East Java province, Indonesia. A number of representative infected and healthy looking ears were collected from those regions. Twenty seeds were randomly taken from each cob. DNA derived from twenty seeds was pooled and genotyped with multiplexing using maize microsatellite (bnlg1189) and downy mildew microsatellite (DM38) primer. Allele in the size of 153 bp can be observed after the infected samples were amplified with DM38, while the size of the maize microsatellite allele depends on the genotype of infected maize. All seeds collected from infected ears positively carry the DM allele. Meanwhile, our genotyping data revealed that 30–80 % of healthy sweet corn ears positively contained the DM allele, while only 8.3 % of healthy field corn ears contained the DM allele. Specificity of the primers was verified by the absence of cross-reaction with DNA from 6 common contaminants on maize seeds, while sensitivity tests indicated that 10 pg is the threshold for the detection of *Peronosclerospora maydis*. The results obtained from this study implicate contaminated seeds as possible sources of initial inoculums of maize downy mildew in the field and also provided a simple and accurate diagnostic method to assess the presence of downy mildew in maize seeds.

Keywords seedborne · downy mildew detection · maize disease · *Peronosclerospora maydis*

Introduction

Maize downy mildew, caused by the Oomycete pathogen *Peronoclerospora maydis* is one of the most destructive diseases of maize [*Zea mays*]. Disease can occur at any stage of maize development from seedling to harvest, though it primarily affects seeds and seedlings. A number of studies have demonstrated the debilitating effect of this pathogen on maize. The most severe damage occurs when maize is planted late or the rainy season begins early, especially if the crop has been over fertilized with nitrogen or planted after maize or sugarcane (Smith and Renfro 1999). Semangoen (1970) reported nearly 100 % loss of late-planted maize in Java, Indonesia. Considering this devastating threat, the US government has listed this pathogen as one of 100 high consequence plant pathogens having significant potential for damage to U.S. agricultural and natural ecosystems in The Microbial Rosetta Stone (MRS) database (Kamenidou et al. 2013).

P. maydis is an obligate parasite that will not grow on artificial media. In Indonesia, conidia of *P. maydis* are produced in the early morning during dew formation (Semangoen 1970) and after release can infect maize up to 42 m away, although 70–85 % of infections occur within 20 m (Mikoshiba 1983). White to yellow stripes/streaks, which become necrotic and brown are usually the first symptom observed. When systemic, the pathogen causes severe chlorosis in the upper leaves. Infected plants may be stunted and sterile and often lodge (fall over). Plants may develop

✉ Rudy Lukman
lukman_pdf@yahoo.com; rudy.lukman@bisi.co.id

[1] Biotech Department of PT. BISI International, Tbk, Kediri, East Java, Indonesia

[2] Department of Agronomy, Iowa State University, Ames, IA, USA

multiple and deformed cobs, leaflike tassels and cobs, combined tassels and cobs, and either elongated or shortened stalks (Smith and Renfro 1999).

The control of this disease with fungicides, although necessary, often does not give satisfactory results. Deployment of resistant cultivars has been the preferred means for managing the disease, and considerable effort has been directed toward understanding genetic resistance. However, the management of this disease through the use of disease-resistant cultivars has become difficult because of the highly variable nature of the pathogen. A recent survey revealed considerable genetic diversity among maize downy mildew isolates in Indonesia. Enviromental pressures derived from the use of resistant varieties, pesticides and seed treatments might accelerate the genetic evolution of this pathogen (Lukman et al. 2013).

In the past, it was assumed that *Peronosclerospora maydis* evolved on a wild, grammaceous host in the region where Java downy mildew occurs since the pathogen is not present in the Americas where maize originated. However, Raciborski (1897) and Semangoen (1970) failed to detect a grass with symptoms of downy mildew in Java. Semangoen (1970) inoculated several grasses, but only Z. *mexicana*. Which was an exotic introduction, was susceptible. He concluded that wild hosts did not play any role in the perpetuation of Java downy mildew and that the pathogen survived in maize, which was grown throughout the year. Since then, it is often believed that the disease is disseminated solely through air and no one pays attention the possibility of disease outbreak due to contaminated seeds. So far, there is no report about the seed borne nature of *P. maydis*. Recent report from Nigeria about the seed borne transmission of *P. sorghi* (Adenle and Cardwell 2000) has prompted us to investigate the possibility that *P. maydis* is also disseminated through infected maize seeds.

Testing seeds for plant pathogens can be a difficult task. Unlike infected vegetative plant tissues, infested seeds can be asymptomatic, making visual detection impossible. Additionally, pathogen populations on seeds may be low, and infested seeds may be non-uniformly distributed within a lot (Walcott 2003). Hence, a sensitive, specific, robust, and simple method to detect the seedborne pathogens is highly desired. Accordingly, this paper aims to present the development of molecular diagnostic techniques for use in the detection of downy mildew in maize seeds.

Materials and methods

Samples collection

Infected and healthy looking ear samples of sweet and field maize were collected from 3 districts in East Java province (Kediri, Nganjuk and Jombang; Table 1) from December 2013 till September 2014. The number of infected ears collected from one area depended on the disease incidence in that area while sufficient numbers of ears were harvested from healthy looking plants growing around the downy mildew infected maize plants.

DNA extraction from seeds

Genomic DNA of each maize ear was assembled by mixing equal amounts of DNA from 20 maize kernels. DNA of single maize kernel was isolated according to the following protocol. A single seed was placed in a tube containing 400 μL extraction buffer (200 mM Tris-HCl (pH 8.0), 200 mM NaCl, 25 mM EDTA, 0.5 % SDS). We then employed a manual grinder to crush the seeds. Four hundred μL of CTAB solution (2 %) was then added into the seed extract, followed by gently extracting using chloroform: isoamyl alcohol (24: 1). After centrifugation at 12,000 rpm at 4 °C for 10 min, the supernatant was transferred to new eppendorf tubes containing 200 μL NaCl 5 M and incubated for 10 min at room temperature. The DNA supernatant was precipitated with equal volume of cold isopropanol and centrifuged at 12,000 g for 10 min. The pellet was washed twice with 70 % ethanol, dried and dissolved in 50 μL of Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8).

Development of molecular tool to detect downy mildew in maize seeds

Previously, the potential of sorghum and maize microsatellites (Perumal et al. 2008) for studying the genetic diversity of *P. maydis* has been demonstrated (Lukman et al. 2013). In this study, we utilized one of 41 downy mildew microsatellites (DM38, labeled with fluorescence 6FAM) and multiplexed with maize microsatellite (bnlg1189, labeled with fluorescence NED) aiming to amplify simultaneously downy mildew and maize microsatellite alleles. In this case, the maize microsatellite serves as an internal control. DM 38 was chosen in this study because this primer pair generates single fragment and can also be multiplexed with bnlg1189 since these two primer pairs possess different allele size.

PCR reactions were carried out in 20 μL containing 10–20 ng of genomic DNA, 1× PCR buffer (10 mM Tris-Cl, 50 mM KCl, 1.5 $MgCl_2$), 0.2 mM dNTP mix, 0.25 μM forward and reverse primer, 2 mM $MgCl_2$, and 1 U *Taq* polymerase enzyme (New England Biolabs, UK). PCR conditions performed in GeneAmp® PCR System 9700 (Applied Biosystems, USA) was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C for 45 s and 72 °C for 60 s with final extension 20 min at 72 °C. All amplicons were separated by electrophoresis in 2 % agarose gel, stained with ethidium bromide, and visualized on Gel Doc (Kodak MI, USA) to evaluate PCR amplification. Two bands will be

Table 1 Number of maize ears utilized in this experiment and its locations

No	Sample	Infected maize ear	Healthy maize ear	Location
1	SC1	5	10	Kencong, Kediri
2	SC2	5	5	Pace, Nganjuk
3	FC1	2	5	Ngoro, Jombang
4	FC2	3	12	Sumberagung, Kediri

generated from positive samples; one representing downy mildew allele and another derived from maize allele. Negative samples only possess one band derived from maize microsatellite allele.

Primer sensitivity & specificity

The sensitivity of diagnostic PCR was assessed by determining the minimum amount of *P. maydis* genomic DNA required for detection. *P. maydis* DNA was obtained by following the modified protocol of Perumal et al. (2008) as written in Lukman et al. (2013). Amplification reactions were conducted with a ten-fold serial dilution from 10 ng into 0.0001 ng. These templates were then amplified with standard PCR protocol. In order to test the specificity of the PCR protocol, we randomly cultured maize seeds on PDA medium (20 % potato, 2 % dextrose and 2 % agar) and incubated for ±7 days to observe the contaminants. Maize seeds contaminated with different fungi were then extracted and amplified with the standard assays to know the specificity of DM38 primer.

Visualization of microsatellite allele(s) using genetic analyzer ABI 3130

Two μL of appropriate dilution of PCR product was added to 4 μL of deionized formamide containing 0.2 μL of GeneScan 500 LIZ size standard (Applied Biosystems, USA). The mixture was incubated at 95 °C for 5 min and then cooled in the freezer for 10–15 min. The samples were then run on an ABI 3130 Genetic Analyzer using the default settings of the manufacturer-supplied run module. Microsatellite allele sizing (bp) was performed using GeneMapper v4.0 software (Applied Biosystems, USA).

Results

Samples collection

Survey in 3 districts in East Java, Indonesia successfully found two samples each of infected field corn and sweet corn plants. Though infected by downy mildew, the plants still looked normal and produced ears (Fig. 1). We also collected maize ears derived from healthy maize plants growing around downy mildew infected maize plants. The number of healthy maize ears collected depended on the location of the diseased plants. Location at the center of the field enabled us to collect more samples, while locations at the edge limited us to fewer samples. Comparison between healthy and infected maize ears is displayed in Fig. 2.

Specificity and sensitivity of the primer

The utilization of an ABI genetic analyzer enabled us to observe precisely the size of each allele. Blue peak allele in the size of 153 bp can be observed after the infected samples were amplified with DM38, while the size of maize microsatellite allele (black peak) depends on the genotype of infected maize. The primers proposed in the present study were tested for specificity and sensitivity. Our routine seed health evaluation indicates that under our environmental condition, maize seeds are often contaminated by *Aspergillus flavus*, *Aspergilus Niger*, *Fusarium* sp., *Rhizopus* sp., *Penicillium* sp., and *Mucor* sp. (Fig. 3). Hence, the proposed primers should be specific

Fig. 1 Performance of generative plant infected by maize downy mildew

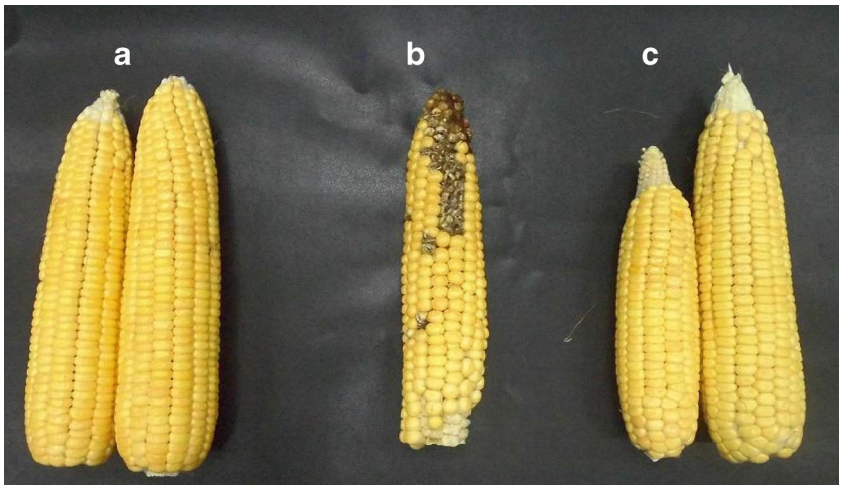


Fig. 2 Comparison between healthy maize ears (**a**, **c**) and infected maize ear (**b**). Samples were collected from Kediri, East Java, Indonesia

only for *Perenosclerospora sp.* and will not amplify those 6 contaminants. The specificity test revealed the absence of cross-reaction with DNA of other contaminants often found on maize seeds. Blue peak allele in the size of 153 bp can only be observed from DM control sample (Fig. 4). This means that DM38 SSR primer pair is specific only for the detection of downy mildew.

The analytical sensitivity of an assay is that assay's ability to detect a low concentration of a given substance in a biological sample. Analytical sensitivity is determined in one of two ways: empirically, by testing serial dilutions of specimens with a known concentration of the target substance; or statistically, by testing multiple negative specimens and using 2 or 3 standard deviations above the mean as the lower limit of detection (Saah and Hoover, 1997). In this study, sensitivity was established using a 10-fold dilution series of DNA from 10 ng into 1 pg. Figure 5 depicts that the described primers are able to generate a clear band using 10 pg of genomic DNA. A gradual decrease in band intensity is observed using 1 pg or less genomic DNA. Using pure *P. maydis* genomic DNA as template, we did not see any difference in term of primer sensitivity of genetic analyzer and agarose gel (data not shown). However, for the purpose to detect the presence of *P. maydis* in maize seeds, it was observed that genetic analyzer was more sensitive than agarose gel (Fig. 6).

Percentage of disease incidence in maize seeds

Our data showed that all seeds, both sweet corn and field corn, collected from infected plants positively carry DM (Table 2). The data also revealed that healthy looking ears also potentially carried downy mildew allele (Fig. 7). Field corn seeds derived from healthy looking ears had a significantly lower infection incidence of *P. maydis* compared with sweet corn

Fig. 3 Maize seeds contaminated with *Mucor* sp. (**a**); *Rhizopus* sp. (**b**); *Aspergilus Niger* (**c**); *Aspergilus flavus* (**d**); *Penicillium* sp. (**e**); *Fusarium* sp. (**f**)

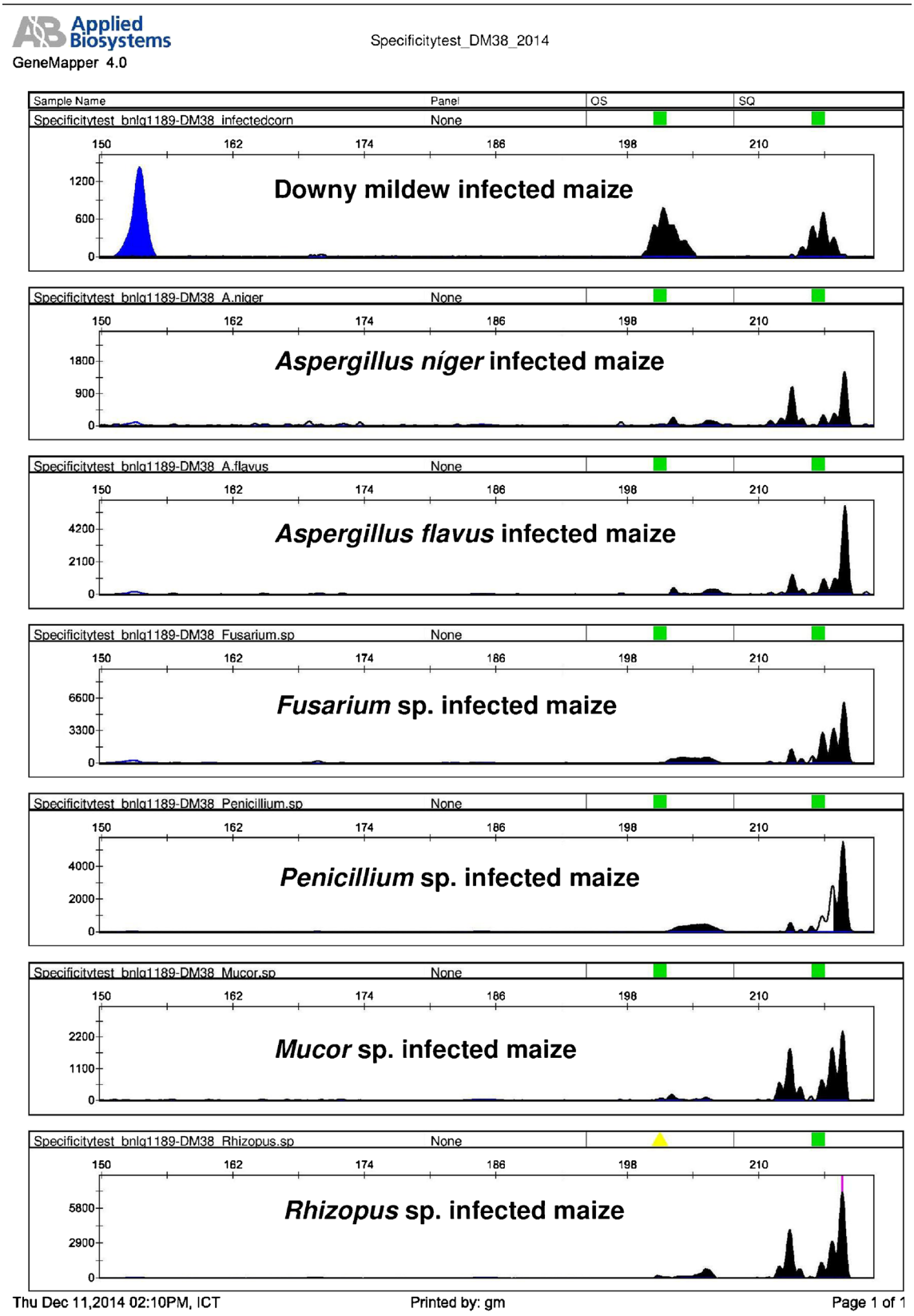


Fig. 4 Specificity test for DM38 SSR Primer pairs against fungi contaminants often found on maize seeds

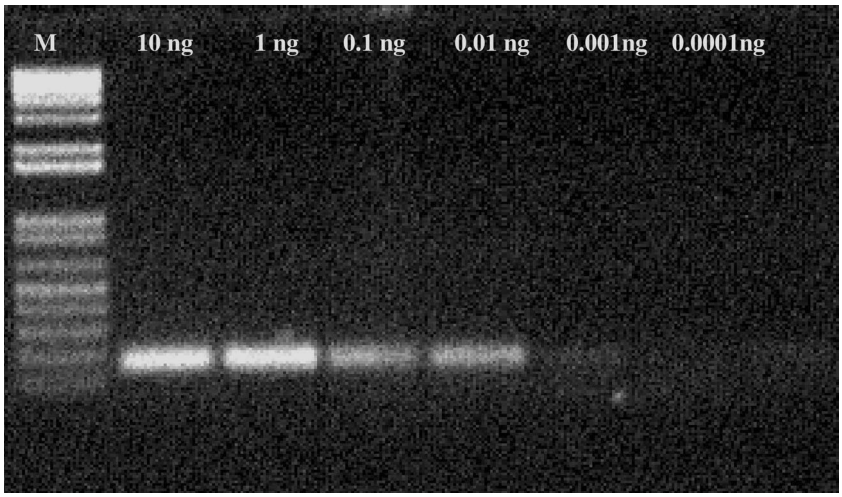


Fig. 5 Sensitivity test for DM38 SSR Primer pair to detect *Peronosclerospora sp.* Samples were loaded on 1.5 % agarose gels and visualized with EtBr. M = 1 kb Plus DNA Ladder Invitrogen

seeds. No positive sample was detected from samples derived from Ngoro, Jombang. Meanwhile, only one out of 12 (8.33 %) samples derived from Sumberagung, Kediri contained DM allele. It is interesting to note the high percentage of downy mildew incidence on healthy sweet corn seeds derived from Kencong (Kediri) and Pace (Nganjuk) reached 30–80 %, respectively.

Discussion

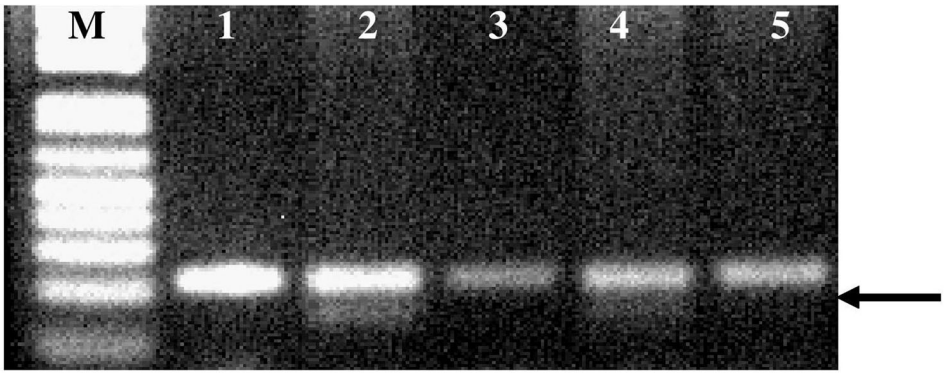
Out of three most devastating maize downy mildews (*P. sorghi*, *P. Phillipinensis* and *P. maydis*), more researches have been focused so far on *P. sorghi* and *P. philipinensis*, while *P. maydis* receives less attention. In contrast, a recent survey revealed a considerable genetic diversity of *P. maydis.* (Lukman et al. 2013). The diversity was even higher compared to the genetic similarity of six downy mildew species (*P. maydis*, *P. sacchari*, *P. sorghi*, *P. phillipinensis*, *P. sparsa* and *S. graminicola*) that shared 77 % similarity (Perumal et al. 2008). Consequently, research on the biology of *P. maydis* in maize plants and epidemiology of downy mildew is required to develop appropriate knowledge-based disease-management strategies.

Weston (1920) reported that the severity of the Philippine Downy Mildew disease in individual maize plants varies with environmental conditions and developmental stage of plants at the time of infection. When the disease infects young seedlings, there is usually a complete failure of the plant to develop and produce fertile ears. When infection attacks older plants, the plant may mature and produce stunted, malformed ears with fewer grains. In our study, we also found several downy mildew infected maize plants bearing normal maize ears. Seeds derived from those ears were then utilized as infected seeds source in our experiment, while seeds harvested from healthy plants growing around the diseased plants were utilized as healthy samples.

The results of this study support the fact that *P. maydis* is a seed borne disease in maize. Infected maize seeds are potential carriers of pathogens for long-distance dissemination. Major impact of seed borne diseases in maize is not only the yield reduction but also deterioration of marketable quality of grain. Hence, the early and correct identification of maize downy mildew is very important for the maize seed industry to set up a practical management strategy, such as disease free certified seeds. At present, there is no universal strategy for the detection of *P. maydis* in maize seeds. Therefore, the development of reliable, sensitive and specific molecular diagnostic markers could make the identification easier and could help to understand their ecological behavior in order to develop a good management strategy for disease control.

The primers proposed in the present study were tested for specificity and sensitivity. Specificity was verified by the absence of cross-reaction with DNA of other contaminants often found on maize seeds while sensitivity was established using a 10-fold dilution series of DNA and evaluated with 2 platforms i.e. genetic analyzer and agarose gel. Despite achieving the generally same result by both platforms in a sensitivity test, we observed that utilization of an ABI genetic analyzer is more convenient since the maize allele and downy mildew allele can be easily differentiated. Furthermore, use of genetic analyzer also enabled us to detect lower concentration of inoculums in seed DNA samples. One of the infected samples (Sample 5, Fig. 6) determined as negative by agarose gel platform was confirmed as positive with the genetic analyzer, although its peak height (rfu) is lower compared to other two

Fig. 6 Comparison between agarose gel and genetic analyzer to visualize downy mildew infected maize seeds. 1,3 : healthy maize samples; 2,4,5: infected maize samples. M = 1 kb Plus DNA Ladder Invitrogen. Downy mildew allele is shown by black arrow


M
1
2
3
4
5

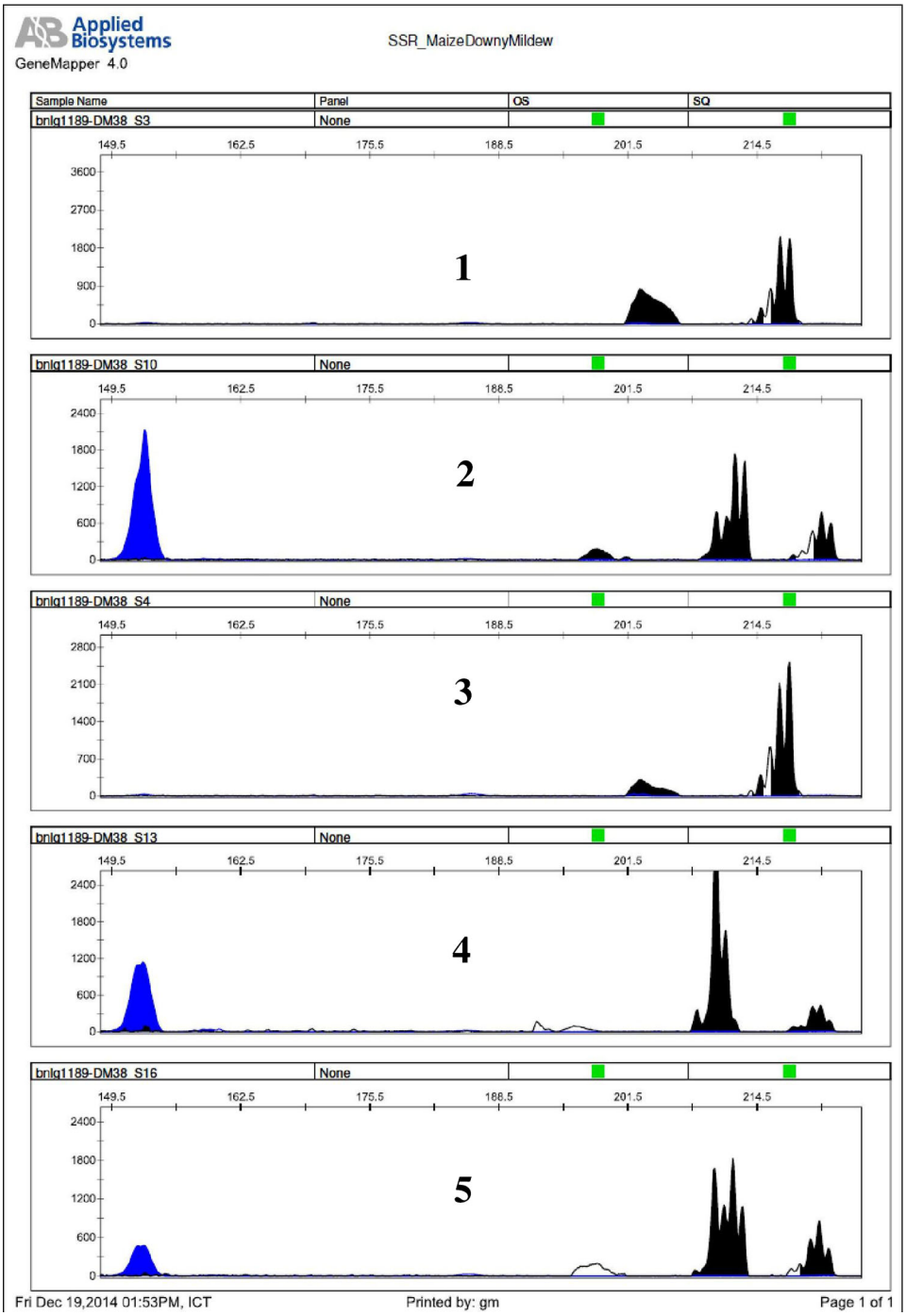

Applied Biosystems
GeneMapper 4.0
SSR_MaizeDownyMildew
Sample Name
Panel
OS
SQ
bnlg1189-DM38_S3
None
1
bnlg1189-DM38_S10
None
2
bnlg1189-DM38_S4
None
3
bnlg1189-DM38_S13
None
4
bnlg1189-DM38_S16
None
5
149.5
162.5
175.5
188.5
201.5
214.5
Fri Dec 19,2014 01:53PM, ICT
Printed by: gm
Page 1 of 1

Table 2 Percentage of disease incident on field and sweet corn seeds

No	Sample	Location	No of healthy cobs	No of infected cobs	No of positive samples	Percentage of positive samples
1	SC1	Kencong, Kediri	10	–	3	30 %
			–	5	5	100 %
2	SC2	Pace, Nganjuk	5	–	4	80 %
			–	5	5	100 %
3	FC1	Ngoro, Jombang	5	–	0	0 %
			–	2	2	100 %
4	FC2	Sumberagung, Kediri	12	–	1	8.33 %
			–	3	3	100 %

samples. The detection threshold of 10 pg of *P. maydis* genomic DNA in this study is comparable to the detection limit of 3.4 pg of *P. belbahrii* genomic DNA (Farahani-Kofoet et al. 2012), 3.0 pg of genomic *Plasmopara halstedii* DNA (Ioos et al. 2007) and 1.0 pg of genomic *Peronospora tabacina* DNA (Tsay et al. 2006).

Our observations also suggest that the surrounding healthy plants around downy mildew infected maize plants are potentially a source for disseminating symptomless, but latently infected seeds. These results support the implementation of PCR-based detection in a seed certification scheme and the necessity to control the pathogen on seeds. The PCR method can also be used for evaluation of pathogen control on seeds based on detection of the pathogen in out-growing plants. The downy mildew microsatellite (DM38) primer utilized in this study was developed from sorghum and maize downy mildew by Perumal (2008) and can be utilized to amplify *Peronosclerospora sorghi*, *P. maydis*, *P. sacchari*, *P. philippinensis*, *Peronosopora sparsa* and *Sclerospora graminicola* downy mildew isolates. Therefore, the developed assay could be used to identify unknown Peronosclerospora species that attack maize without the need of further analyses.

The percentage of oospores detected in our study was higher compared to the previous literature. Adenle and Cardwell (2000) only found three oospores out of 896 microtomed seed sections. However, the tested grains for their experiment were randomly purchased at local markets in Nigeria. Meanwhile, our samples were collected from maize plants grown in downy mildew infected fields. This may explain the higher percentage of oospores found on seeds that reached 8.33 % and 80 % in field corn seeds and sweet corn seeds, respectively. It is quite remarkable to note that sweet corn seeds are good carriers for downy mildew. One can envisage that higher water and sugar content in sweet corn than in field corn might affect the higher incidence of maize downy mildew on sweet corn seeds. However, this assumption requires further intensive researches especially related to water content since Adenle and Cardwell (2000) mentioned that there was no significant correlation of grain moisture with percentage infection of *P. sorghi*. In their study, they even observed diseased plants derived from maize seeds with 8.5 % moisture content.

In order to study seed transmission, we grew out 250 contaminated seeds. However, none of the maize seedlings showed any downy mildew symptom. We hypothesize that the micro climate inside the green house and the season at the time of the conducted experiment were not suitable for inducing diseases. Previous researches have highlighted the importance of prevailing environmental conditions (especially temperature and relative humidity) to induce the sporulation of conidia. Shetty and Safeeulla (1981) found that systemically infected sorghum leaves held in the dark at 20 °C produced a maximum of 10,800 conidia cm^{-2} at 100 % RH, but only 3600 conidia cm^{-2} at 8 5 % RH. None were produced at 80 % RH. Temperature was also shown to be an important factor for the onset and spread of sunflower downy mildew, the average air temperatures most favorable for infection by *P. halstedii* being in the range of 10–15 °C, whereas temperatures above 17 °C were detrimental for oospore germination and development of mycelia within the plant tissue (Iliescu et al., 1977). The optimum temperature for sporulation of an American isolate on maize was between 15 °C and 23 °C (Bonde et al. 1985).

The failure to observe the transmission of downy mildew from infected seeds into maize seeedlings is not a unique case as it is known that seed infection by downy mildew agents is common, but seed transmission of downy mildew diseases is rare and happens in a rather low proportion. Cohen et al. (2014) reported that vertical seed transfer of downy mildew occurred in cucumber, butternut gourd and squash are 0.25 %, 1 % and 1.5 %, respectively. Only one out 276 plants grown from infected sunflower seeds (0.36 %) produced typical systemic infection (Cohen and Sackston, 1974).

Facing this situation, we employed an indirect way to see the relationship between the disease incident in the field and the seed health status. For this purpose, we visited several maize production areas that attacked by downy mildew. We asked the farmers whether they still save the remaining seeds grown in the field. Luckily, in two heavily infected fields (disease incident more than 80 %) the farmers still kept one remaining field corn sample and one sweet corn sample. We

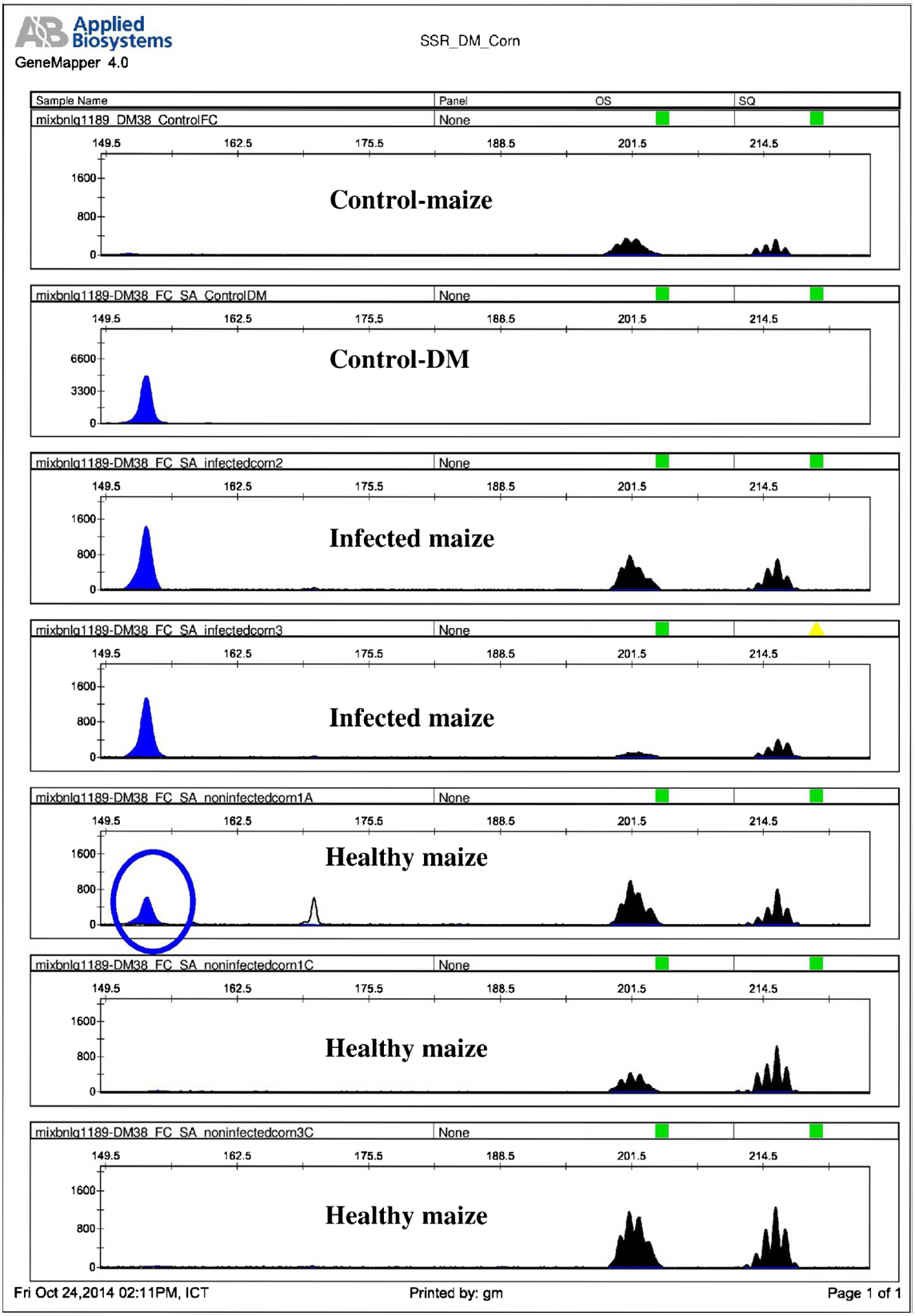


Fig. 7 Electropherograms of representative samples after amplified with multiplex bnlg1189 and DM38 primer pairs

then genotyped those samples with our primers. Surprisingly, we found that 57 and 97 out of 100 seeds of sweet and field maize, respectively positively amplified the downy mildew allele. This fact calls for immediate action to elucidate the relative importance of infected seeds as one of the possible inoculums sources of downy mildew outbreak in the field.

Once established, maize downy mildew is difficult to manage, stressing the importance of seed health. These results underscore the importance of seed health testing, but seed-producing farmers lack the infrastructure and budget to test their seed lots. However, farmers can improve seed health of their samples through simple treatments like rouging the infected plants, proper storage to avoid infection, and seed dressing application to reduce disease transmission to the seedlings.

In conclusion, this paper demonstrates that *P. maydis* is a seed-borne disease. Compared with the alternative methods based on the use of grown out test of maize seedlings and histological assessment for the detection of *Peronosclerospora sp.* in maize seeds, the use of the PCR technique has definitive advantages in terms of speed, specificity and technical simplicity. More research should be devoted to further observe the interaction between inoculums from the air and infested seeds in inducing the disease outbreak of downy mildew. From practical view of disease management, it is highly desired to cultivate healthy maize seeds. Hence, beside of establishment of diagnostic tool to differentiate healthy and diseased seed lots, we also encourage to find treatments that able to eliminate the pathogen from the infected maize seeds.

Acknowledgments The authors acknowledge PT. BISI International, Tbk., Indonesia for providing financial assistance for this investigation.

References

Adenle VO, Cardwell KF (2000) Seed transmission of maize downy mildew (*peronosclerospora sorghi*) in Nigeria. Plant Pathol 49: 628–634

Bonde MR, Peterson GL, Duck NB (1985) Effects of temperature on sporulation, conidial germination, and infection of maize by *peronosclerospora sorghi* from different geographical areas. Phytopathology 75:122–126

Cohen Y, Sackston WE (1974) Seed infection and latent infection of sunflowers by *plasmopara halstedii*. Can J Bot 52:231–238

Cohen Y, Rubin AE, Galperin M, Ploch S, Runge F, Thines M (2014) Seed transmission of *pseudoperonospora cubensis*. PLoS One 9(10):e109766. doi:10.1371/journal.pone.0109766

Farahani-Kofoet RD, Römer P, Grosch R (2012) Systemic spread of downy mildew in basil plants and detection of the pathogen in seed and plant samples. Mycol Progress 11:961–966

Iliescu H, Vranceanu AV, Pirvu N (1977) Unele aspecte privind etiologia manei florii-soarelui si diseminarea ciupercii *plasmopara helianthi* novot. Annals ICCPT 42:353–361

Ioos R, Laugustin L, Rose S, Tourvieille J, Tourvieille de Labrouhe D (2007) Development of a PCR test to detect the downy mildew causal agent *plasmopara halstedii* in sunflower seeds. Plant Pathol 56:209–218

Kamenidou S, Jain R, Hari K, Robertson JM, Fletcher J (2013) The microbial Rosetta stone central agricultural database: an information resource on high-consequence plant pathogens. Plant Dis 97:1097–1102

Lukman R, Afifuddin A, Lubberstedt T (2013) Unraveling the genetic diversity of maize downy mildew in Indonesia. J Plant Pathol Microb 4:2

Mikoshiba H (1983) Studies on the control of downy mildew disease of maize in tropical countries of Asia. Tech Bull Tro Agric Res Cent 16:62

Perumal R, Nimmakalaya P, Erattaimuthu SR, No EG, Reddy UK, Prom LK, Odvody GN, Luster DG, Magill CW (2008) Simple sequence repeat markers useful for sorghum downy mildew (*peronoslerospora sorghi*) and related species. BMC Genet 9:77

Raciborski M (1897) Lijer, eine gefährliche maiskrankheit. Ber Deut Bot Ges 15:476–478

Saah AJ, Hoover DR (1997) "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. Ann Intern Med 126(1):91–94. doi:10.7326/0003-4819-126-1-199701010-00026

Semangoen H (1970) Studies on downy mildew of maize in Indonesia, with special reference to the perennation of the fungus. Indian Phytopathology 23:307–320

Shetty HS, Safeeulla KM (1981) Effect of some environmental factors on the asexual phase of *Peronosclerospora sorghi.* Proceedings of the Indian Academy of Sciences (Plant Sciences) 90: 45–51.

Smith DR, Renfro BL (1999) In: D. G. White (ed.) compendium of corn diseases, 3rd edn. APS Press, MN, p. pp78

Tsay JG, Chu C, Chuang YH, Chen RS (2006) Specific detection of *peronospora tabacina* by PCR-amplified rDNA sequences. Plant Pathol J 5:378–382

Walcott RR (2003) Detection of seedborne pathogens. HortTechnology 13(1)

Weston WH (1920) Philippine downy mildew of maize. J Agric Res 19: 97–122