International rice research notes

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TITLE OF ARTICLE: POLYMERASE CHAIN REACTION AMPLIFICATION OF DNA FROM BACTERIAL POPULATIONS OF RICE USING SPECIFIC OLIGONUCLEOTIDE PRIMERS

Aseptic mass collection of anthers for increasing efficiency of anther culture in rice breeding

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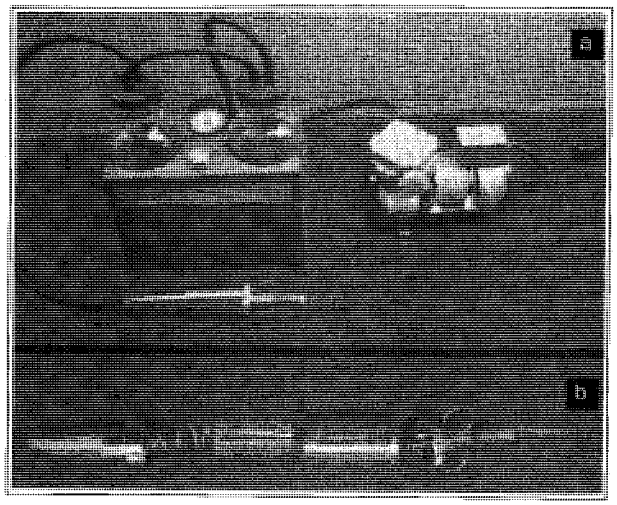
Anther culture is widely used in rice varietal improvement programs in Korea. The first anther-derived rice cultivar, Hwaseongbyeo, was developed in 1985. Five more have been developed since: Hwacheongbyeo, Hwajinbyeo, Hwaryeongbyeo, Joryeongbyeo, and Hwaseonchalbyeo. These cultivars are being grown on more than 10% of the rice area in Korea.

Recent advances have resulted in increased callus induction and plant regeneration, thus increasing the efficiency of anther culture breeding. Methods normally used for extracting anthers from rice florets and placing them on media, however, are laborious and time-consuming. They are not appropriate for breeding programs requiring many regenerated plants.

We devised a simple apparatus for aseptic mass collection of anthers. Assembled, it consists of a vacuum source (Fig. 1a) and an aseptic anther-collecting part, made up of a stainless steel cylinder body, 10-ml tube, and extracting glass pipet-tip (Fig. 1b). After being autoclaved, it is used directly in the assembled apparatus.

Rice florets with anthers at the proper stage (uninucleate-binucleate) are clipped and then sucked up by the vacuum with power pressure of 14-20 mm Hg. The collected anthers are immediately transferred from the tube to callus induction medium with a sterilized spatula or forceps in a laminar flow cabinet or clean benches.

We conducted a simple test that showed the anther-plating efficiency of vacuum-collecting to be three times more efficient than that of the conventional method. It took 592 min to plate 5,980 anthers on callus medium with vacuum collection and 601 min to plate 1,743 anthers using the conventional method (see table).



a) The assembled apparatus for aseptic mass collection of anthers consists of a vacuum source and an aseptic anther-collecting part; b) close-up of the anther-collecting part, made of a stainless steel cylinder body, 10-ml tube, and extracting tip.

Comparison of anther-plating efficiency and anther culture response between vacuum collection and the conventional method used in rice anther culture.[a]

Anther-collecting method	Anther plating			Anther culture response			
	Anthers plated (no.)	Time elapsed (min)	Time/100 anthers (min)	Callusing		Plant regeneration[b]	
				no.	%	no.	%
Vacuum	5980	592	9.9	1046	17.5	2224	40.5
Conventional	1743	601	34.5	303	17.4	364	20.5

[a]Mean of 6 replications with 5 japonica genotypes.

[b]Plant regeneration (%) = $\frac{\text{No. of plants regenerated}}{\text{No. of anthers plated}} \times 100$.

Frequency of callus induction from anthers was similar for both methods. Plant regeneration rate and absolute number of plantlets were far greater using the vacuum-collecting method (see table). Possible damage and desiccation of immature anthers from suction did not harm anther culturability.

With the vacuum-collecting method, large amounts of anthers can be gathered quickly and easily, with reduced contamination during collection and plating. This method can enhance efficiency, particularly when sampling large breeding populations. It can also be useful in pollen culture where many isolated pollens are needed for culturing and in in vitro selection at haploid level. ■

Polymerase chain reaction amplification of DNA from bacterial pathogens of rice using specific oligonucleotide primers

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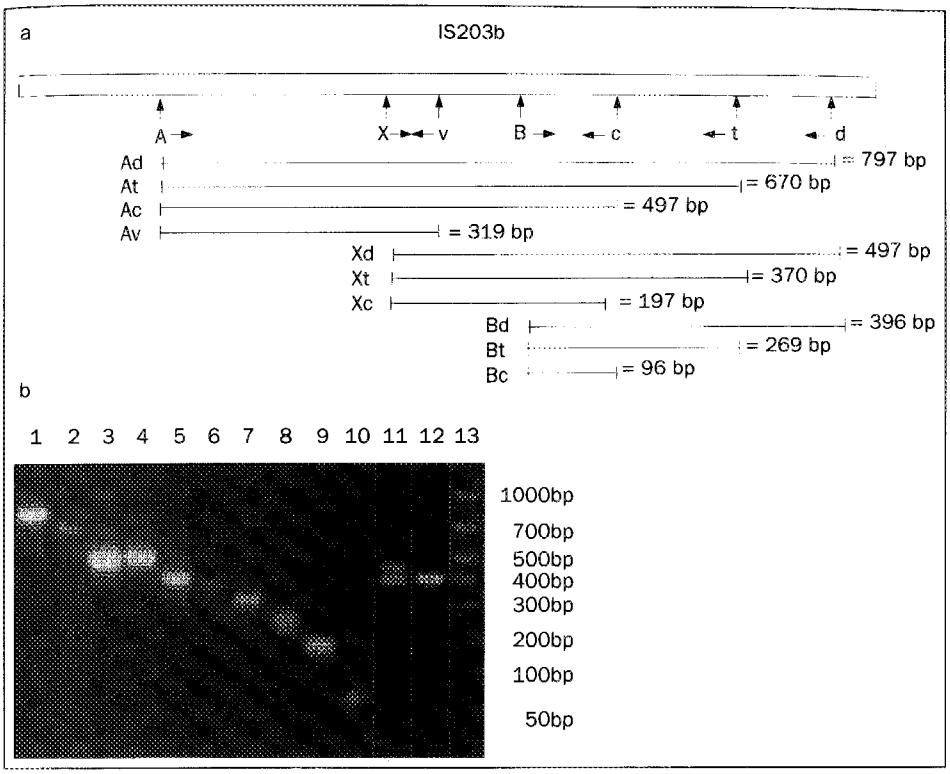
The polymerase chain reaction (PCR) is a powerful molecular technique that allows a segment of DNA to be amplified more than a millionfold even when the template DNA is present in a very tiny amount. The technique involves cycles of denaturation of duplex template DNA, annealing of two oligonucleotide primers to specific regions in the DNA, and extension of the region flanked by the two primers. This process results in an exponential increase in copy number of the flanked region, permitting specific DNA sequences to be detected with extraordinary sensitivity. Thus PCR can provide a useful diagnostic technique when a high degree of sensitivity is required.

We investigated the possibility of amplifying DNA from bacterial patho-



(a) Location of the primers in relation to the sequence of the repetitive element *IS1112* from Xoo. (b) Gel electrophoresis banding pattern of the different DNA fragments amplified by each primerpair. Lanes 1 to 10 depict amplification by primerpairs Ad, At, Ac, Xd, Bd, Xt, Av, Bt, Xc, and Bc, respectively, of plasmid DNA containing the repetitive element *IS1112*. Lanes 11 and 12 depict amplification of Xoo and Xocola DNA, respectively, by primerpair Bd resulting in a double band for Xoo and a single band for Xocola. Lane 13 contains a molecular weight marker.

Amplification of different bacterial genomic DNA using 10 different primerpairs.

33 Xoo strains	Amplification with all 10 primerpairs; banding patterns all identical to each other
32 Xocola strains	Amplification with 9 primerpairs; 7 primerpairs identical to Xoo pattern
11 reference strains of *X. campestris* pathovars	
X. campestris pv. *cerealis*	Amplification with 5 primerpairs; all identical to Xoo pattern
X. campestris pv. *graminis*	
X. campestris pv. *poae*	
X. campestris pv. *armoraciae*	Nonspecific amplification
X. campestris pv. *citri*	
X. campestris pv. *campestris*	
X. campestris pv. *phaseoli*	
X. campestris pv. *holcicola*	
X. campestris pv. *vasculorum*	
X. campestris pv. *vesicatoria*	
5 *Xanthomonas* reference strains	
X. fragariae	Amplification with 6 primerpairs; all identical to Xoo pattern
X. albilineans	Nonspecific amplification
X. axonopodis	
X. maltophilia	
X. populi	
22 Philippine *Pseudomonas* rice seed isolates	Nonspecific amplification
8 *Pseudomonas* reference strains	Nonspecific amplification
P. aeruginosa	
P. avenae	
P. fluorescens	
P. fuscovaginae	
P. glumae	
P. marginalis	
P. plantarii	
P. putida	
2 Philippine *Erwinia* rice isolates	Nonspecific amplification
2 *Erwinia* reference strains	Nonspecific amplification
E. herbicola	
E. stewartii	

gens of rice by using primers constructed from the repetitive DNA element *IS1112* isolated from the genome of *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal organism of bacterial blight (BB) in rice. Three upstream and four downstream 20-basepair (bp) oligonucleotide primers were commercially synthesized based on the DNA sequence of elements *IS1112*. The upstream primers were designated with capital letters A, B, and X and the downstream primers with small letters c, d, t, and v. By combining these primers, 10 different primerpair combinations were formed: Ac, Ad, At, Av, Bc, Bd, Bt, Xc, Xd, and Xt. Based on the structure of *IS1112*, each primerpair would be expected to amplify a discrete fragment of between 96 and 797 bp (see figure).

Different bacterial DNA samples from rice pathogens and other Xanthomonads were analyzed using the primers. For DNA isolation, bacterial cultures were grown in nutrient broth to early log phase. Cells were then harvested using a centrifuge at 12,000 rpm for 10 min. The supernatant was discarded, and the cells resuspended in 2 ml of 100 mM Tris-HCl pH 8.3 and 1 mM EDTA (1X TE). Sodium dodecylsulfate (250 µl of 10%

SDS) and proteinase K (50 µl of 10 mg/ml) were added, and tubes were incubated at 37 °C for 1 h with gentle shaking. Then 0.45 ml of 5M NaCl was added. After thorough mixing, 0.4 ml of hexadecyltrimethylammonium bromide (CTAB) solution (10% CTAB in 0.7 m NaCl) was added, and the tubes were incubated at 65 °C for 20 min. An equal volume of chloroform:isoamyl alcohol (24:1) was added. The mixture was shaken for 30 min, then centrifuged for 30 min at 15,000 rpm. The aqueous (upper) phase was transferred with a bent Pasteur pipette to a fresh tube containing an equal volume of cold isopropanol and mixed gently until the DNA had precipitated. This was hooked out using a bent Pasteur pipette and washed in 70% ethanol. Purified bacteria DNA was dissolved in 1X TE and stored at -20 °C. Additional purified DNA samples were obtained from other sources.

The PCR reaction mix included approximately 20 ng of DNA as template in a 50 µl-reaction volume that contained 10 mM Tris-HCl pH 8.3, 50 mM KCl, 200 M each of dNTP, 0.4 M each of primer, and 1.5 units of Taq polymerase (Perkin Elmer Cetus or Boehringer Mannheim). Amplification was performed on a Perkin Elmer Cetus DNA Thermocycler with an initial denaturation step at 94 °C for 2 min. This was followed by 40 cycles of a denaturation step at 94 °C for 1.5 min, a primer annealing step at 62 °C for 2 min, and an extension step at 72 °C for 2 min. An additional extension step of 72 °C for 5 min was performed after the 40th cycle. PCR products were visualized by gel electrophoresis on 2% agarose in Tris-Borate-EDTA at 2 volts per cm and staining with ethidium bromide.

Amplification was possible for DNA from all the different bacteria (see table). The bands amplified for the two closely related rice pathogens, Xoo and *X.o.* pv. *oryzicola* (Xocola), were very similar and matched the expected band sizes. The other bacteria either showed no amplification for all 10 primerpairs or exhibited banding patterns different from the expected patterns. Banding patterns different from those expected were described as "nonspecific amplification". One primerpair, Bd, differentiated Xoo from Xocola by amplifying a double band for Xoo and a single band for Xocola (see figure).

The results show the utility of the primers for amplification and for possible PCR detection of these rice pathogens. PCR amplification of Xoo DNA could provide an extremely sensitive method for detecting and diagnosing BB pathogen. This method could be used without isolation and purification of the organism and might be particularly applicable for diagnosis of seedborne inoculum. It could be used also as a sensitive method for detecting Xoo in studies aimed at understanding the ecology and epidemiology of pathogens. ■

News about research collaboration

Computers help predict how rice blast disease will react to climate changes

How will global climate change influence rice production? IRRI scientist Paul S. Teng believes some of the greatest impact will be on fungal pathogens such as those that cause rice blast, the most devastating disease in temperate and subtropical ricefields.

IRRI and the U.S. Environmental Protection Agency are cooperating to learn how climate factors—enhanced UV-B radiation, temperature, and rainfall—could change the distribution of rice blast and the damage it could cause to rice production.

Teng and his coresearcher, Luo Yong of China, are using computer simulation models to help answer their questions. They are using historical weather data of 61 locations in seven Asian countries with a model of the rice crop and a simulation of rice blast disease.

They learned that variations in rainfall did not change where blast was found nor the severity of its epidemics. On the other hand, a 1-3° C shift in mean temperature significantly changed the disease's impact, but this varied by ecological zone. In the cool subtropical zones of Japan and northern China, raising the ambient temperature increased the risk of a rice blast epidemic. In the humid tropics and subtropics, the risk became greater with lower ambient temperatures.

The IRRI scientists have used geographic information systems technology to display these data on risk maps. The next step is to prepare maps that will help policymakers understand the issues, by graphically illustrating the potential losses that could be caused by blast under several scenarios of temperature change. This is another example of rice scientists anticipating potential problems rather than correcting damage after it occurs in farmers' ricefields. ■

Bangladesh and IRRI: more than 20 years of rice research collaboration

The completion of the Bangladesh Rice Research Institute (BRRI)-IRRI Project marks more than two decades of highly successful collaboration between IRRI and Bangladesh. The production gains achieved during these years, the result of rice research and development programs, have helped Bangladesh become nearly self-sufficient in rice.

But the close working relationship is continuing. IRRI is keeping its liaison office in Dhaka to maintain active ties with Bangladesh's dynamic rice research program. BRRI is a participant in the Rainfed Lowland Rice Research Consortium, focusing on drought problems in the ecosystem.

BRRI released 24 improved rice varieties for planting by farmers in Bangladesh, making a major contribution to doubling the country's annual produc-