Simple method for DNA isolation from urediospore of wheat leaf rust pathotypes

A. ABBASSI MOGHADDAM*, K.V. PRABHU1 and P. BAHADUR
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012

1 Division of Genetics, Indian Agricultural Research Institute and National Phytotron Facility, New Delhi 110 012

Key words: DNA isolation, leaf rust, urediospore

Genetic analysis of obligate parasites like rust pathogens is difficult and sometimes impossible to multiply the isolates on synthetic medium (6). DNA separation directly from urediospore, multiplied on living host is promising. Existing procedures for isolation of DNA from wheat leaf rust pathotypes yielded little amount of DNA from large amount of urediospore. Therefore, in the present study, a simple protocol was developed for DNA isolation from urediospore and RAPD analysis for different pathotypes of *Puccinia recondita tritici*.

The urediospores were multiplied on Agra-local (AL), a susceptible variety in the glasshouse. About 7-9 days old seedlings of AL were sprayed with urediospore suspension of the pathotype and incubated in the humid glass chamber for 48 h. Urediospore dust was collected after 12-15 days of inoculation. The purity of pathotype was tested on differential sets before use (4). The urediospore of different pathotypes multiplied on seedlings in the greenhouse were preserved at 5°C (1).

An amount of 50 mg urediospore was used for the isolation of genomic DNA. The DNA was extracted as per the plant DNA extraction protocol involving small quantity of tissue developed by Prabhu et al. (5). Initially 50 mg of urediospore was measured and put in a 1.5 ml micro-centrifuge tube. The above tubes were placed on a preheated heating block at 95°C specially designed for holding the tubes, with caps open. 1 ml of hot extraction buffer heated to 65-70°C containing 100 mM Tris-HCl (pH 8.0) and, 10 mM EDTA (pH 8.0), 1 M KCl was added to each tube and the cap was closed. The mixture was incubated for 10 min with intermittent vortexing of the tubes once in every 2 min. The tubes were then transferred into an icebox filled with ice flakes for 2 min. The cooled samples were centrifuged at 10,000 rpm for 10 min at 4°C. The aqueous phase was transferred to another tube and centrifuged again in the same condition for 5 min. The aqueous phase was carefully transferred to another new tube containing 5 µl of 10 mg/ml RNase and incubated at 37°C for 20 min to digest DNA. Approximately 0.6 equivalent of the volume of the supernatant (500-600 µl) of cold isopropanol (-20°C) was added to each sample, placed on microtube rack and mixed by gentle rocking for 5 min to precipitate DNA and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded.

The DNA pellet was allowed to suspend in water by adding 300 µl of sterile water. The samples were heated for 2 min at 50°C in an oven and the DNA was dispersed by intermittent flicking of the tubes. The DNA was re-precipitated by adding 15 µl of 3 M sodium acetate (pH 4.8) and 300 µl of 95% cold ethyl alcohol (-20°C) to each tube for 5 min and centrifuged at 10,000 rpm for 5 min. The supernatant was removed and the DNA pellet was washed by adding 200 µl of 70% cold ethyl alcohol for centrifugation at 10,000 rpm for 3 min. The dried DNA pellet was re-suspended in 50 µl of sterile water and stored in a refrigerator at 4°C as stock DNA. The concentration of DNA in the extract was measured using standard procedures of UV absorption at 260 and 280 nm with UV spectrophotometer. The DNA was diluted to a final concentration of 5 ng/µl and used for RAPD analysis.

---

*Email* a-abasi@Yahoo.com
RAPD analysis

The random sequence 10-mer oligonucleotide primers as given in Kolmer and Lin (2) were obtained UBC Services (2). Primers were used to study DNA extracted from 12 different leaf rust races from India. The procedure for RAPD analysis described by Williams et. al. (6) was followed. For RAPD analysis, the DNA was diluted to a final concentration of 5ng/µl. The polymerase chain reaction (PCR) was carried out in the 48-well PCR plated with thermal seal with 20 µl reaction volume containing 30 ng of genomic DNA, 200 mM of each of dATP, dGTP, dCTP and dTTP, 0.2 mM primer and 0.75 U/µl Taq DNA polymerase (Bangalore Genei Pvt. Ltd.). Amplification reactions were performed on PTC-200 Thermal Cycler (MJ Research, USA) with the following reaction profile: initial denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 1 min (denaturation), 36°C for 2 min (primer annealing), 72°C 2 min (extension), with a final extension at 72°C for 10 min.

The protocol developed for DNA isolation yielded typically 300-750 ng/µl of good quality DNA from 50mg samples of urediospore. The genomic DNA electrophoresis of random samples also indicated consistent and intact composition of the extracted DNA on 2% agarose gels. Following the above procedure DNA of 12 pathotypes of leaf rust 12-1 (5R37), 77 (45R31), 77-1 (109R63), 77-2 (109R31-1), 77-5 (121R63-1), 104 (17R23), 104B (29R23), 104-2 (21R55), 106 (2000R9), 107 (45R3), 108 (13R27) and 108-1 (57R27) was extracted. The amount of urediospores used in this study was markedly lower than used by Kolmer and Liu (2,3).

This is the first report of extracting DNA adopting a chloroform phenol free method involving only 30-50mg quantity of urediospore. The DNA could effectively be used for polymorphism analysis among the pathotypes. The preliminary attempt with ten random primers referred by Kolmer and Liu (2), generated RAPDs with one primer 5’ ‘CGGAGAGCCC3’ and distinguished all the 12 pathotypes clearly (Fig 1). More primers have to be employed for detailed satisfactory analysis which can be done for precise distinction among rust pathotypes at molecular level. Critical RAPD’s can be converted to single band SCAR’s for specific pathotypes identification in routine survey.

The senior author thanks the Head, Division of Plant Pathology, IARI for providing necessary facilities.

REFERENCES


Received for publication September 30, 2003