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SCIENTIFIC CORRESPONDENCE

A rapid and specific detection method for blast infection caused by *Magnaporthe grisea* in *Setaria italica*

Setaria is an age-old crop of high nutritional value. It is also known for its drought tolerance and seedling vigour. However, this millet crop suffers heavy losses due to leaf and panicle phases of blast disease caused by the haploid, filamentous, heterothallic, ascomyceteous fungus, *Pyricularia grisea* Sacc. (Teleomarph: *Magnaporthe grisea* (Hebert) Barr). In our laboratory, blast diseases of rice, finger millet and grasses have been intensively researched and molecular methods have been developed for analysis of *M. grisea* populations that prevail in the country and have also been used in breeding indica rice for blast-resistance[1–3]. Because of mixed infections that occur in the field, making monoconidial isolations of *M. grisea* is more difficult than the isolation of other pathogens. Also, the use of conventional methods to detect the pathogen in a *Setaria* leaf sample may require incubation for 24 h, microscopic observation, isolation of the fungus onto an agar medium and subsequent inoculation onto seedlings of *Setaria*. This present communication, however, deals with a PCR-based rapid and specific detection procedure to facilitate early diagnosis of blast infection in *Setaria*.

Infected and healthy leaves of *Setaria* were collected from plants raised at a blast nursery at Pattambi, Kerala after artificial inoculation with *Setaria* strains of *M. grisea*. DNAs of both healthy and infected leaves were isolated following the method of Tai and Tanksley[4]. The DNA samples were subjected to PCR amplification with primers, *pfh*2a (19-mer) 5′-CGT CAC ACG TTC TTC AAC C-3′ and *pfh*2b (17-mer) 5′-CGT TTC ACG CTT CTC CG-3′, which have the ability to cause specific amplification of a 687-bp fragment of the *pot2* transposon element dispersed as muliple copies in the genome of *M. grisea* that causes blast of perennial ryegrass used in golf courses in the US[5]. The following modified PCR conditions were used in the present study: initial denaturation at 94°C for 2 min, denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. The PCR reaction mixture was prepared with 1.25 µl of 20 pmol/µl of each primer, 2.5 µl of 10 mM dNTP, 2.5 µl of 10X buffer, 1 µl of DNA *Taq* polymerase (2.5 U), 2 µl of the 50 ng/µl template DNA, 2 µl of 25 mM $MgCl_2$ in a total volume of 25 µl.

The results show that the new primers in a single amplification also amplified the *pot2* elements present in the blast fungus in infected *Setaria italica* leaf samples (Figure 1). The infection was in the form of only one blast lesion. However, they failed to amplify DNA samples obtained from leaves of uninfected or healthy *S. italica* and rice (*Oryza sativa*, L.) leaves showing multiple leaf blast lesions caused by inoculation with rice strains of the blast fungus, *Magnaporthe grisea*. *Pot2* element, an inverted transposon of 1861 bp [EMBL accession no. Z33638] is known to be present in both rice and non-rice infecting isolates of the blast fungus, *M. grisea* in equal copy numbers[1,6]. However, the primer pair chosen

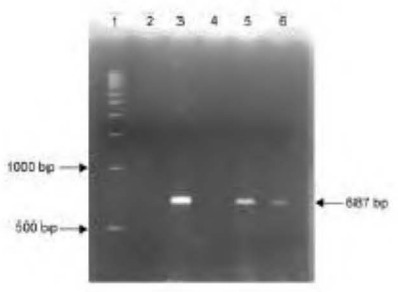

Figure 1. Specific amplification of a 687 bp DNA fragment that indicates the presence of blast infection only in infected *Setaria* leaves that contained only a single leaf blast lesion, and not in uninoculated (healthy) *Setaria* (checks) or in blast-infected rice leaves. Lane 1, 1 kb DNA marker; Lane 2, Uninfected *Setaria italica*; lanes 3, 5, 6, *M. grisea* infected *S. italica* and lane 4, *M. grisea*-infected *Oryza sativa*.

for this protocol was reported to amplify a 687-bp region (from base pair 1055 to 1741) of the total *Pot2* element for the specific detection of only the non-rice strains of *M. grisea*[5]. The results of the PCR assay were quite reproducible for amplification of the 687-bp fragment in *Setaria*-infecting *M. grisea*, suggesting that this amplified region of the genome is common to *M. grisea* isolates that infect perennial ryegrass and foxtail millet. This specific detection procedure is also rapid and can be concluded within 3–4 h.

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Seedling mortality in two vulnerable tree species in the sacred groves of Western Ghats, South India

Sacred groves form a significant component of the traditional conservation movement in many parts of the tropical world[1]. The Western Ghats, one of the two mega-diversity centers in India, is dotted with sacred groves, with the highest concentrations located in the central Western Ghats[2]. Sacred groves are believed to serve as the last refugia for a number of taxa, particularly for rare, endangered and threatened species[3,4]. Of late due to encroachments and land-use changes, the sacred groves have been increasingly threatened and fragmented[4]. During the last century alone, the total area under the groves in Kodagu district in the central Western Ghats decreased by 42%. Besides, more than 46% of the sacred groves in the district are less than 0.4 ha in area. The increased fragmentation of the groves could undermine the utility of these groves in serving as a refugium for the rare, endangered and threatened (RET) species. Here we examine the effects of grove area on the seedling mortality of two economically important and vulnerable tree species.

The study was conducted in the sacred groves of Ponnampet range (12°N, 75°E), Kodagu district in the central Western Ghats of India (Figure 1). The groves are set against a matrix of coffee plantation and agricultural landscape[6,7]. The vegetation of the groves is predominantly evergreen, with a small proportion of semi-evergreen and deciduous patches. The sacred groves within 20-km radius of Ponnampet were visited and 15 groves with area ranging from 0.37 to 11.28 ha were selected for the study. The latitude and longitude of the sacred groves were recorded using a global positioning system (GPS) and digitized using GIS software (MAPINFO)[8]. Based on the GPS data, inter-grove distance was estimated for each grove.

The study was conducted on two economically important and vulnerable tree species. *Artocarpus hirsutus* Lam. (Moraceae) is a dominant canopy tree, vulnerable globally[9] and endemic to the Western Ghats[10]. The fruits are yellow, ovoid, covered with spines, containing numerous white seeds, 0.5–0.75 inches long with viability period of three weeks[11]. Because of its edible fruit collection and extensive harvesting of highly prized timber, *A. hirsutus* has been threatened in the Western Ghats. *Canarium strictum* Roxb. (Burseraceae) is reportedly vulnerable in Karnataka[9] and is known for its medicinal resin[6]. Fruits are ovoid or ellipsoid, often-trigonous drupe with 1–3 celled, 1–3 seeded stone[11]. *C. srictum* is being mainly threatened for its valuable resin extracted by partially burning the trees. The species is distributed sparsely in the evergreen forests of the Western Ghats and Eastern Himalaya in India[10]. Both species are pollinated by small insects and are animal-dispersed.

Seeds or fruits of both species were collected from randomly chosen trees from groves ($n = 13$ groves for *Artocarpus*, and $n = 11$ groves for *Canarium*). The seeds/fruits of the trees were collected during the respective fruiting phenologies (for *Canarium strictum* during January–February while for *Artocarpus hirsutus* during May–June). Immediately after collection, seeds/fruits were washed, weighed and a number of seed/fruit parameters (such as seed abortion, seed predation, etc.) were determined.

Sufficient care was taken to avoid sampling errors, including over- or under-representation for samples across the grove area. The seeds were sown separately in polythene bags filled with soil mixture, and allowed to germinate under shade in greenhouse conditions. Aborted seeds that were rudimentary and scleretorized were not considered. The germination percentage was calculated as the ratio of number of seeds germinated to the total number of sown seeds. The ratio of the number of dead seedlings (two months after germination for *Artocarpus* and three months for *Canarium*) to the total number of germinated seeds was computed for each grove and referred to as per cent seedling mortality.

We found a significant decline in per cent seedling mortality with increase in area of the grove ($P < 0.05$ in both the species; Figure 2 *a* and *b*). For *Artocarpus*, the per cent seedling mortality ranged from as high as 100% in the small groves to none in the large groves. On the other hand, for