High throughput phenotype screening pipeline for functional genomics in *Magnaporthe oryzae*

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Method Article

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Abstract

Introduction

Rapid screening of mutant phenotypes is one of the integral parts in a large-scale study of gene functions. However, in filamentous fungi, strategy to address such a challenge has yet to be reported. Here we present our approach that allows rapid and systematic determination of multitude of phenotypes from insertional mutants of a filamentous fungus, _Magnaporthe oryzae_ in a high throughput manner. To increase the speed and feasibility in screening phenotypes from a large collection of mutants, we developed high throughput phenotype screening \(HPS) pipeline. Design of HPS pipeline was based on the fact that _M. oryzae_ can undergo developmental changes away from its host, which allows visual and microscopic inspection of each developmental stage. This, together with the use of 24-well plate format, underlies our screening strategy. During HPS, mutant processing and evaluation of phenotypes were carried out in 24-well plate format. The use of 24-well plate greatly diminished the time and labor needed to label and handle every mutant because a single plate number automatically specifies twenty-four mutants within a plate and the twenty-four mutants go through HPS pipeline as a single unit.

Procedure

Regeneration of mutants in 24-well plate from the library marks the entry of mutants into HPS pipeline. In our screens of mutants, alterations at seven phenotypes covering fungal development and physiology were monitored in a step-by-step manner \(Fig. 1). Phenotypes of primary interest are growth rate, pigmentation, conidiation, conidial morphology, conidial germination, appressorium formation, and pathogenicity. In each stage, phenotypes of interest were described according to the assigned numerical scale \(Table 1). 1) Measure growth of the mutants after 3 days incubation on V8 agar. Score growth by comparing relative growth rate with that of wild-type \(Fig. 1a). 2) After 6 days incubation, score mycelial color visually \(Fig. 1b). 3) Harvest conidia from 6-day old cultures of mutants by adding 1ml of Tween 20 solution \(250ppm) into each well and rubbing with cotton swab. Pipette fourty-microliters of conidial suspension drops onto 12.5×8.2cm green mirror glass, and place in a moistened plastic box to prevent the drops from drying. Incubate at room temperature for 24 hr. 4) Observe conidiation, conidial morphology, conidial germination and appressorium formation under a microscope with x100 magnification \(Fig. 1c, d, e, and f). Conidiation can be scored by comparing relative number of conidia from mutants with that of wild-type. Conidial germination rate can be measured as the percentage of germinated conidia. Ability to form appressorium can be measured as the percentage of appressorium-forming conidia among the germinated conidia. 5) After dropping conidial suspension onto the green mirror, use the rest of the conidial suspension for a pathogenicity test \(Fig. 1g). Spray-innoculate approximately 1000-microliter of spore suspension on rice plants* using an artist’s airbrush connected to compressed air. Keep the test tubes in a dew chamber to maintain the high humidity required for the fungus to penetrate the cuticle of plants. Following 20-24 hours of incubation in dew chamber, place plants at room temperature for 3 days and assess for pathogenicity. Score blast disease symptoms by

visual examination of lesions produced. *Rice plants for pathogenicity assay can be prepared as follows. Surface-sterilize plant \(Rice cv. Nagdongbyeo) seeds with clorox \(Yuhanrox regularTM) for 20 min, rinse well with distilled water, plant in test tube containing Murashige and Skoog \(MS) agar medium \(4.4gL-1and 0.9% agar), and grow for 7-to 8 days until 2 or 3 leaf-stage is reached.

Timing

It takes around two weeks to complete our protocol.

Anticipated Results

Using our HPS pipeline, it was possible for one person to screen 100 mutants for seven different phenotypes per day, suggesting the possibility of applying our strategy to mutagenesis program in other filamentous fungi.

Figures

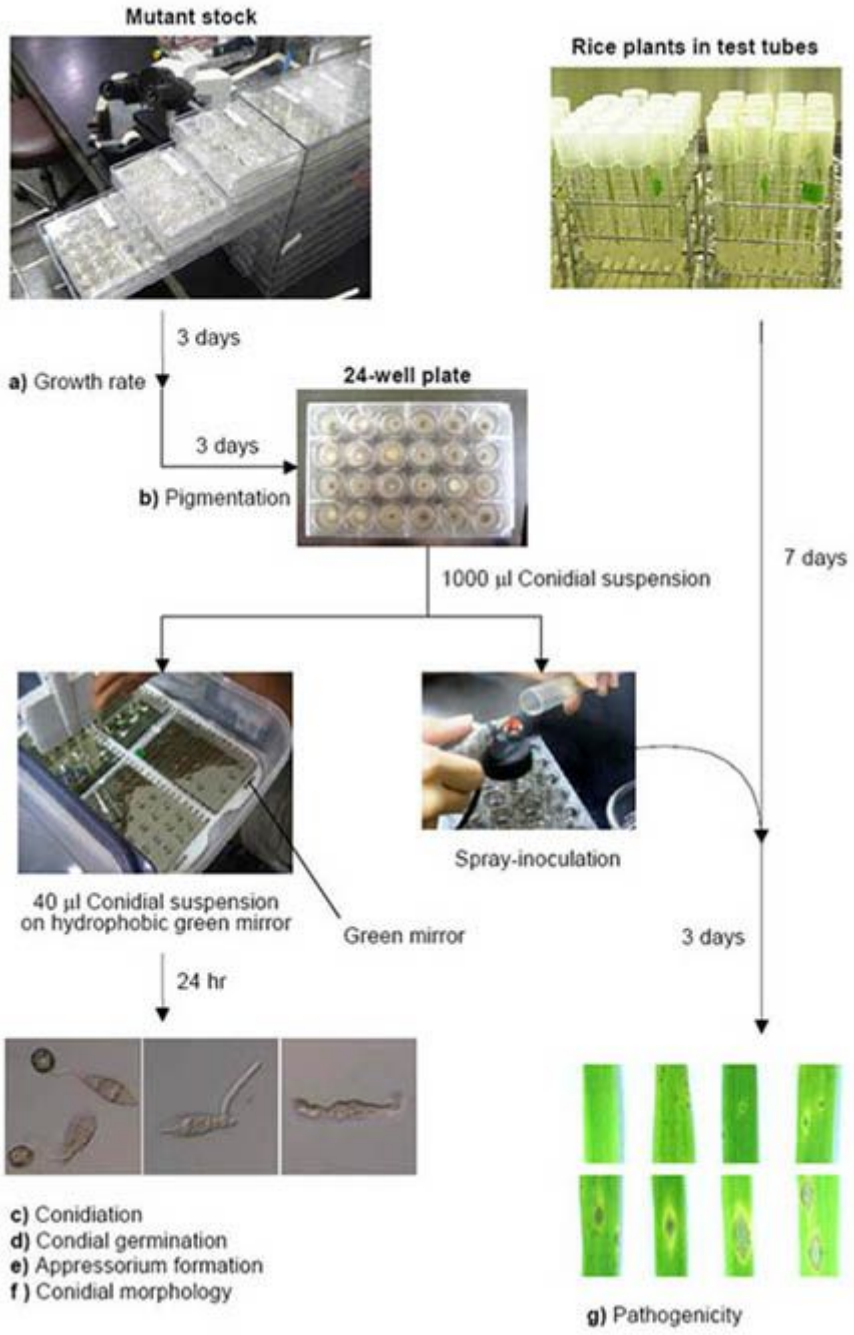

Figure 1

Schematic diagram of high throughput phenotype screening

Phenotype	Score			
	0	1	2	3
Growth rate	Slow	normal	fast	-
Pigmentation	white	normal	others	-
Conidiation	none	reduced	normal	increased
Conidial germination	none	≤ 50%	≥ 50%	-
Appressorium formation	none	≤ 50%	≥ 50%	-
Conidial morphology	normal	abnormal	-	-
Pathogenicity	none	reduced	normal	increased

Figure 2

Table 1 Numerical scale used for scoring phenotypes in high throughput screening