Text S1. Ultraviolet mutagenesis and screening of mutant isolates.

1. Oidia production and collection

The strain *C. cinerea A43mut B43mut pab1-1* #326 was grown at 37°C with light exposure on solid YMG medium (0.4% yeast extract, 1% malt extract, 0.4% glucose and 1.5% agar, 30 g medium per 90 mm diameter petri dish, same below) for 5 days on the 90 mm petri dish to induce oidiation. Oidia were washed out with ddH_2O and passed through three layers of autoclaved Kimwipes (Kimberly-Clark Inc., Canada). Oidia were quantified using hemocytometer (XB.K.25, Shanghai Qiujing Biochemical Reagent and Instrument, China). The concentration of oidia suspension was adjusted to 1×10^7 cells/ml.

2. Ultraviolet mutagenesis

To generate UV mutants, 5 ml of oidiation suspension was added to each 90 mm diameter petri dish and irradiated by a CL-1000S UV Crosslinker (Upland, CA, USA) with the irradiation energy of 200×100 μJ/cm^2. Such irradiation condition resulted in the survival rate of ~ 1 ‰. Irradiated oidiation suspension were diluted to 1×10^5 cells/ml with saturated cisplatin water solution. 500 μl of oidia-cisplatin solution was spread on each YMG agar plate and incubated at 37°C in dark.

3. Mutant screening

After 48 h incubation, the YMG plates with small colonies were stained with I_2 vapour (Toda et al., 1985). Each plate was directly contacted with I_2 vapour for 15 seconds. Colonies with darker red-brown colour were picked out and transferred to a new YMG medium, and cultivated and stored as the wild-type strain. Colonies formed by non-UV irradiated oidia and non-cisplatin treated oidia were used as the reference to estimate survival rate and compared I_2 staining colour.

4. Phenotype characterization

Mutants were grown on YMG solid media and checked the phenotypes. Mycelial growth

diameter under 37°C dark incubation was measured by across the two diameters at right angle on day 4 post inoculation. Oidia production was induced at 37°C with continuous light and determined on day 6 post inoculation. Fruiting body development was tested by dark incubation at 37°C for 5.5 days and transferred to 28°C with light-dark cycle. Observation stopped until the culture was dry-out.

To measure the carbohydrate contents, mutants were grown on YMG solid media with cellophane sheets and incubated at 37°C with continuous darkness. 1 ml H_2O or saturated cisplatin solution were spread on YMG media before inoculation. Mycelia were harvested on day 4 post inoculation. Glycogen content was determined with KI-I_2 staining method (Krisman, 1962).

5. Rapid DNA extraction and genotyping

Rapid DNA extraction was done using a microwave-based method (Dörnte and Kües, 2013). Mycelia of fungal cultures were scraped from the surface of plates with an inoculation loop. Sample of 3 scraping lines (~ 3 mm in length per line) was dissolved in 100 µl ddH_2O in a 1.7 ml Eppendorf tube. The closed tube was microwaved with household microwave oven in high power (800 W, NN-ST253W, Panasonic) for 30 seconds, vortexed, and microwaved for another 30 seconds or until steam droplets can be observed on the tube wall. Tubes were placed on ice immediately after microwaving and stored at –20°C until the solution was freeze. PCR amplification of targeted sequence were performed with KAPA HiFi HotStart ReadyMix PCR kit (Roche, Germany) and the following program: 95°C for 3 min, followed by 30 cycles 98°C for 20 sec, 65°C for 15 sec, and 72°C for 45 sec, and 72°C for 1 min. 2 µl of resulted supernatant from the rapid DNA extraction was added to each 25 µl PCR reactions. PCR products were detected on 1.5 % agarose gel. Sanger sequencing on PCR products were performed by Beijing Genomics Institute (BGI, Shenzhen, China).

Reference

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