

Supplementary material



Fig. 1. Microscopic illustration of studied attributes of *Helminthosporium oryzae*.

Supplementary info 1. Chlorophyll contents

According to this method, we took 0.5 g of fresh leaves and cut them into small pieces, and after that, added 10mL 80% acetone (20 mL water and 80 mL acetone) into each sample and kept it overnight at 0 - 4°C. Afterwards, every sample was centrifuged for 5 min at 10 000 × g. The supernatant was collected and absorbance of supernatant was taken at 480, 645 and 663 nm wavelengths with the help of spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Chlorophyll 'a' and 'b' contents were calculated by using the following formulae:

$$\text{Chl. a mg g}^{-1} \text{ fresh weight} = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645})] \times V/1000 \times W,$$

$$\text{Chl. b mg g}^{-1} \text{ fresh weight} = [22.9 (\text{OD}_{645}) - 4.68 (\text{OD}_{663})] \times V/1000 \times W,$$

V - volume of the extract (mL), W - weight of the fresh leaf tissue (g).

Supplementary info 2. Quantification of Anthocyanin

Anthocyanins was quantified using the methodology reported by Mahajan *et al.* (2011). Following the protocol, acidic methanol of 250 μL (1% HCl w/v) was added in 50 mg of fresh plant material. Plant material was homogenized, incubated for 1 hour at 4°C and moderately shake the solution. Later the suspension was centrifuged at 14000 rpm for 5 minutes at room temperature. Absorbance was determined by photometrically at 530 and 657 nm. Anthocyanin was quantified by using following equation:

$$Q_{\text{Anthocyanins}} = (A_{530} - 0.25 \times A_{657}) \times M^{-1},$$

M - weight of the plant material (g), Q - absorption value linearly correlated with amount of anthocyanins, correction factor = 0.25.

Supplementary info 3. Flavonoids estimation

1g fresh material from each plant was grounded with 20 mL of ethanol (80%) using pestle and mortar. Whatman filter paper was used to filter the solution. 0.5 mL filtrate was used and 3 mL distilled water and 0.3 mL of 0.5% NaNO₂ was added. The solutions were mixed for 5 minutes and allowed to stand at room temperature. In the solution, 0.6 mL of 10% AlCl₃ was added and place for 6 mins. The solution was diluted with distilled water to make the final volume up to 10 mL. The absorbance was recorded at 510 nm with spectrophotometer and calculated using a standard calibration curve.

Supplementary info 4. Phenolics estimation

From each replicate, 0.05g leaf sample was homogenized in 80% acetone solution. After that homogenized leaf sample and was centrifuged at 10 000 × g for 10 minutes and supernatant was collected. Then aliquot (100 µL) of the supernatant was reacted with 1 mL of Folin-Ciocalteu's phenol reagent and 2.0 mL of distilled water. Then 20% Na₂CO₃ was added (5.0 mL) to solution and the final volume was made 10mL by adding distilled H₂O. After mixing vigorously, the OD was read at 750 nm, using a UV-Visible spectrophotometer (IRMECO U2020) (GmbH, Germany).

Supplementary info 5. Total free amino acids

Total free amino acids were estimated by the method of Hamilton (Hamilton *et al.*, 1943). In this method 0.5 g homogenized plant sample was taken and 10mL potassium phosphate buffer was added in it. After centrifugation at 1000 rpm, supernatant was collected. 1mL enzyme extract was added in supernatant and followed by 1mL pyridine and 1mL of 2% ninhydrin in 1% acidic ethanol. After that, solution was boiled at 90°C for 15 min in water bath. After cooling at room temperature 50 mL volume was maintained by adding distilled water.