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Differential Response of Guar genotypes to *Alternaria* Infection

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ABSTRACT

Background: Leaf blight caused by *Alternaria* spp. is one of the serious concerns in guar production worldwide. Despite this, the interaction of host-pathogen and responses of different guar genotypes against necrotrophic fungus *Alternaria* is still not much explored.

Methods: In the present investigation, six guar genotypes have been infected with *Alternaria* and observed differential responses by using various parameters, including biochemical assays, antioxidant activities (SOD, CAT, PO, GPx, APx PAL, β-1,3-glucanase enzyme, total phenolic content) and physiological parameters (chlorophyll content, carotenoid and photosynthetic yield-II).

Result: After the pathogenicity of *Alternaria* there was a substantial shift in the biochemical activity in all the inoculated resistant genotypes compared to their respective controls. Moreover, a significant reduction was also observed up to 65% in terms of physiological performance, such as total chlorophyll content, carotenoids and photosynthetic yield-II content, after 14 days of pathogenicity. The genotypes RGC-1066 and RGC-936 showed relatively more resistance and RGC-936 showed relatively more susceptibility towards *Alternaria* infection. These findings also imply that host-pathogen interactions factors have a role in the differential expression of defence-related enzymes and antioxidant activities in response to *Alternaria* inoculation.

Key words: *Alternaria alternata,* Alternaria leaf blight, Defence-related enzymes, Differential response, Guar.

INTRODUCTION

Cluster bean, commonly known as guar [*Cyamopsis tetragonoloba* (L.) Taub], is an economically important drought-resistant legume crop. Guar gum or galactomannan polysaccharide present in the endosperm of guar, have great demand globally have great demand globally and is used in a wide range of industries (Thakur and Randhawa, 2018). India is the world's largest producer and exporter of guar gum. India contributed 72-82% of the world's total guar production and exported 0.38 million MT of guar gum worth $ 457 million during the fiscal year 2019-20 (Anonymous, 2020). However, this demand was severely affected by various biotic and abiotic stresses. Out of them the biotic stress caused by several plant invaders including bacteria, fungi and viruses resulted in 60% reduction in the yield (Saharan and Saharan, 2004; Kumar *et al*., 2018). Leaf blight caused by *Alternaria* spp. has become a severe concern in the guar industry (Thakur and Prasad, 2020). The necrotrophic fungus is responsible for widespread guar crop loss, particularly in Rajasthan and Haryana states of India. Moreover, in severely infected areas the leaves and pods show typical concentric rings and necrotic lesions, which give blighted symptoms, substantial defoliation and loss of seed (Mamgain *et al*., 2013). The preventive measures have had limited efficacy in controlling leaf blight. In modern agriculture, the use of resistant cultivars is thus the most cost-effective, long-term and ecologically benign way of disease management (Bisht *et al*., 2021).

At various stages of infection and disease severity depends on existing defence mechanisms to slow down and halt infection. The underlying defence mechanism, which includes ongoing physiological and biochemical activity, will assist in a better understanding of any crop's defence strategy (Chaerani, 2006). Understanding the role of inducible defence responses in plants, such as upward or downward regulation of numerous physiological and biochemical processes, is critical. This will aid in developing resistant genotypes with great economic importance to meet rising demand (Feys *et al*., 2000). Fungal hyphae penetrate in the plants through the stomatal opening and damaged cell surfaces (cuts and wounds) and thereby triggers various microbial elicitors, enhanced levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These ROS/RNS are highly reactive and causes internal cell damage. To control ROS/RNS, plant intern releases respective scavenging molecules to prevent internal cell damage. These in turn help in the activation of defence mechanism in the host plant by producing defence-related enzymes *viz*., superoxide dismutase (SOD), catalase (CAT), peroxidase (PO), glutathione peroxidase (GPx), ascorbate

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peroxidase (APx), phenylalanine ammonia-lyase (PAL), β-1,3-glucanase and phenolic compounds. Plant resilience was aided by enhanced antioxidant activity and ROS scavenging machinery (Ray, 2015; Nafisa *et al*., 2020).

Disease resistance is a complex mechanism and comparative host-pathogen interaction studies of resistant and susceptible hosts will provide a deep insight into the resistance mechanism and help in breeding for biotic resistance. So, the present investigation was undertaken to study differential response of guar genotypes against *Alternaria* infection during the early stages of infection.

MATERIALS AND METHODS

The present study was conducted during the year 2021-22 at Division of Plant Genetic Resources and Improvement, CSIR-NBRI, Lucknow.

Fungal and plant material

The plant pathogen causing leaf blight or leaf spot of guar, *Alternaria alternata* f. sp. *Cyamopsidis* was obtained from the Indian Type Culture Collection (ITCC-6982), Division of Plant Pathology, ICAR-IARI, New Delhi. The culture was sub-cultured on Potato Dextrose Agar (PDA) plates at 28±2°C for 14 days. The spore or conidia were examined using a fluorescence microscope (LEICA DM2500, Germany). A total of six guar varieties were selected for challenge inoculation, showing varying resistance and susceptibility to *Alternaria* (Table 1).

Fungal culture inoculum

For inoculation, *A. alternata* spores were grown on PDA plates were scraped off and suspended in 5 ml sterile distilled water. The spore suspension was then filtered using a multi-layered muslin cloth. The spore count was adjusted using a haemocytometer to 3×10^5 spore/ml. The spore suspension was sprayed on 4 weeks old guar plants using an atomizer until runoff and for control distilled water is used. After 14 days plants of infection plant were evaluated for per cent disease index (PDI).

Detached leaf assay

Detached leaves of 4 weeks old plants were placed in petri plates containing agar-agar media. Fungal disc of 1 cm diameter scooped out from 10 days *A. alternata* culture was kept on the centre of each leaf, whereas in control agar disc was placed. The disease symptom was evaluated based on tissue maceration by the pathogen (Ray *et al*., 2015).

Per cent disease index (PDI)

The PDI was calculated according to Vakalounakis (1983) after 14 days post-inoculation (Wheeler, 1969).

$$PDI = \frac{\text{Total sum of rating}}{(\text{Number of leaves examined} \times \text{Maximum rating})} \times 100$$

The disease severity rating was calculated using progressive disease scale given by Sangeetha and Siddaramaiah (2007). To satisfy Koch's postulates, the pathogen was re-isolated from artificially inoculated leaves and the resulting cultures were compared to the original culture.

Biochemical and physiological response

Mock-inoculated and inoculated leaves of one month guar genotypes were harvested at 0, 24, 48, 72 and 96 hours after pathogen inoculation (hapi). Plant material (100 g) from each treatment was homogenized in extraction buffer (10 mM sodium phosphate buffer, pH 6.0, containing 1% (w/v) PVP and 1 mM EDTA) at 4°C. All the enzymatic assays were carried out in 6 replications (Mishra *et al*., 2018). The PAL activity was determined using the calibration curve of mole trans-cinnamic acid $gram^{-1}$ protein $hour^{-1}$ (Kim and Huang, 2014). Gallic acid equivalent $gram^{-1}$ plant tissue was used to calculate Total Phenolic Content (TP) (Ainsworth, 2007). SOD activity was examined by evaluating its ability to prevent photochemical reduction of NBT (Nitro Blue Tetrazolium) utilising the riboflavin methionine system (Fridovich, 1974). Furthermore, the activity of PO activity was determined by adding enzymatic extract in 0.05 M pyrogallol and 1% H_2O_2 (Hammer-schmidt *et al*., 1982). The rate of oxidation of H_2O_2 was used to evaluate CAT activity (Aebi, 1983). The APx was measured and expressed as nanomoles of ascorbate oxidised per $minute^{-1}$ $milligram^{-1}$ protein following Nakano and Asada (1981). Hemeda and Klein's methodology was used to measure GPx, which was expressed as units at 470 nm mg $protein^{-1}$ $minute^{-1}$ (Hemeda and Klein, 1990). The activity of β-1,3-glucanase enzyme was measured in grams of glucose $minute^{-1}$ mg^{-1} of soluble protein (Miller, 1959). Physiological parameters like total chlorophyll and

Table 1: List of genotypes used in the present study.

Genotypes	Specific traits
RGC-936	Early, branched type, dwarf wide adoption, drought hardy, high yielding, grains medium sized with light pink colour, most popular variety of Rajasthan.
RGC-1066	This variety is resistant to bacterial blight and root rot, unbranched early high yielding suitable for intercropping and mixed cropping, seeds are high in endosperm content and photo thermo insensitive and cultivated in *Kharif* and *Zaid* season.
PNB	Non branching variety with pod quality of Pusa Mausmi. Developed by crossing Pusa Domausami and Pusa Sadabahar.
HG-563	Branched, pubescent with smooth leaves, early maturing.
HG-365	Branched, pubescent and serrated leaves, early maturing.

Source: http://dpd.gov.in/

carotenoid concentration were measured in mg g^{-1} fresh weight (Arnon, 1949) and Net Photosynthetic Yield-II (Fv/Fm) was calculated using imaging PAM, Chlorophyll Fluorometer (Walz Effeltrich, Germany).

Statistical analysis

All the data were analysed using Graph pad prism8 software with 6 replications. Tukey's multiple comparison test ($P< 0.05$) was used to analyse the mean differences. Student t-test was used for statistical analysis of the data in the experiment on defence-related enzymes between infected and their respective controls.

RESULTS AND DISCUSSION

Disease severity and detached leaf assay

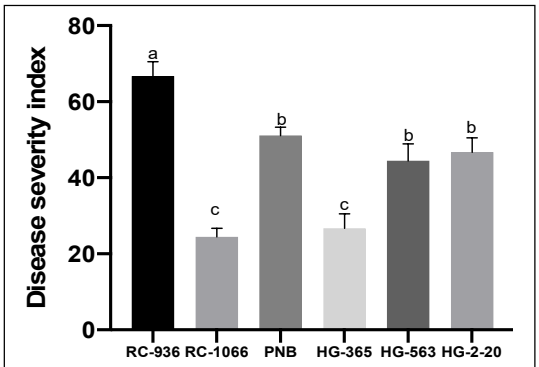
Based on host-pathogen interaction studies among guar genotypes (RGC-936, RGC-1066, PNB, HG-365, HG-563 and HG-2-20) and *Alternaria*, the PDI showed that the genotypes RGC-936 (67%), PNB (51%), showed susceptibility to *Alternaria* infection, whereas, the genotypes HG-563 (44%), HG-2-20 (47%), HG-365 (27%) were moderately susceptible (Fig 1). The genotype RGC-1066 (24%) showed moderate resistance to *Alternaria* infection. The disease severity was maximum in RGC-936 followed by PNB, HG-2-20. The genotypes RGC-936 and RGC-1066 also showed high disease severity and relatively resistant to *Alternaria in detached leaf assay,* respectively (Fig 2). A similar pattern of genotypes response against Alternaria disease showed that any one method of disease assessment might be sufficient for screening many genotypes. The colinear relationship between detached leaf assay and per cent disease index has been reported previously (Abdessemed *et al*., 2019).

Defence-related enzymes and antioxidant profiling

Upon pathogen infection, a significant alteration in the defence and antioxidant activity levels has been observed during the present investigation. When a pathogen invades the leaves through stomatal opening or cuticle it triggers microbial elicitors or pathogen receptors causing an oxidative burst by over accumulation of reactive oxygen species (ROS) like hydrogen peroxide, superoxide anion and hydroxyl group radicals. These ROS are short-lived, highly reactive and they can cause oxidative cellular damage resulting in cell dysfunction and programmed cell death. To maintain redox homeostasis plant intern release ROS scavenging molecules via endogenous defence mechanism such as PAL, SOD, PO, CAT, APX, GPX, Phenols, carotenoids *etc*. (Hasanuzzaman *et al*., 2020). This ROS scavenging molecular play a vital role in defence signalling (Torres, 2010). The PAL activity was increased ~3 fold in RGC-1066 and HG-365 at 72 hapi as compared to that of control plants (Fig 3c). The total phenolics content was steadily increased after 24 hapi and observed maximum (~4 fold) at 96 hapi in resistant genotypes RGC-1066 and HG-365 (Fig 3g). Similar pattern of observations was also reported earlier by Sahni and Prasad (2022) in the case of urdbean.

Over the period of 24 to 96 hapi, the levels of various ROS-scavenging molecules were measured. When compared to control plants, SOD and PO activity increased steadily in inoculated guar genotypes, reaching a maximum (~4 fold) at 72 hapi in genotypes RGC 1066 and HG-365 (Fig 3a and Fig 3b). The genotypes RGC 936 and HG-563 showed the lowest activity at 72 hapi. The accumulation of hydrogen peroxide in primary leaves around appressoria was reported to limit the fungal penetration and mediate defence activities (Barreto *et al*., 2007; Lehmann *et al*., 2015; Mahadik and Mali, 2018).

Furthermore, at 72 hapi, the genotype RGC-1066 had the highest catalase activity (~3 fold), followed by HG-563 (Fig 3d). From 24 to 96 hapi, GPx and Apx activity increased steadily (Singh *et al*., 2015). The GPx activity was maximum at 72 hapi (~3 fold) while maximum APX activity (~3 fold) was observed at 96 hapi in RGC-1066 and a minimum in RGC-936 (Fig 3e and Fig 3f). The β -1,3-glucanase activity was significantly higher in all the inoculated resistant genotypes as compared to their controls (Fig 3h). The enzyme activity was ~2 fold increased at 96 hapi in RGC-



Error bars indicate stand error of six replicates. Significant changes between treatments are indicated by letters on the vertical bars. (P<0.05).

Fig 1: PDI in guar genotypes under *Alternaria* infection after 14 days post-inoculation.

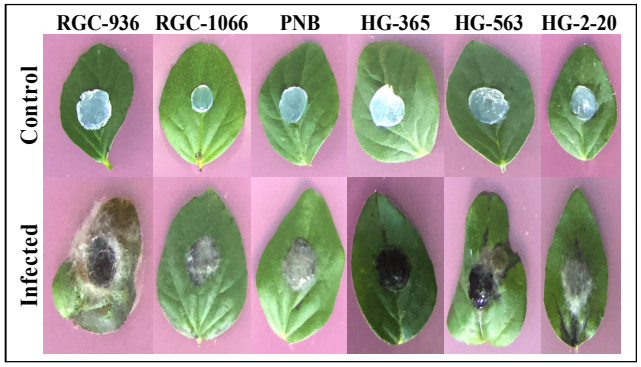
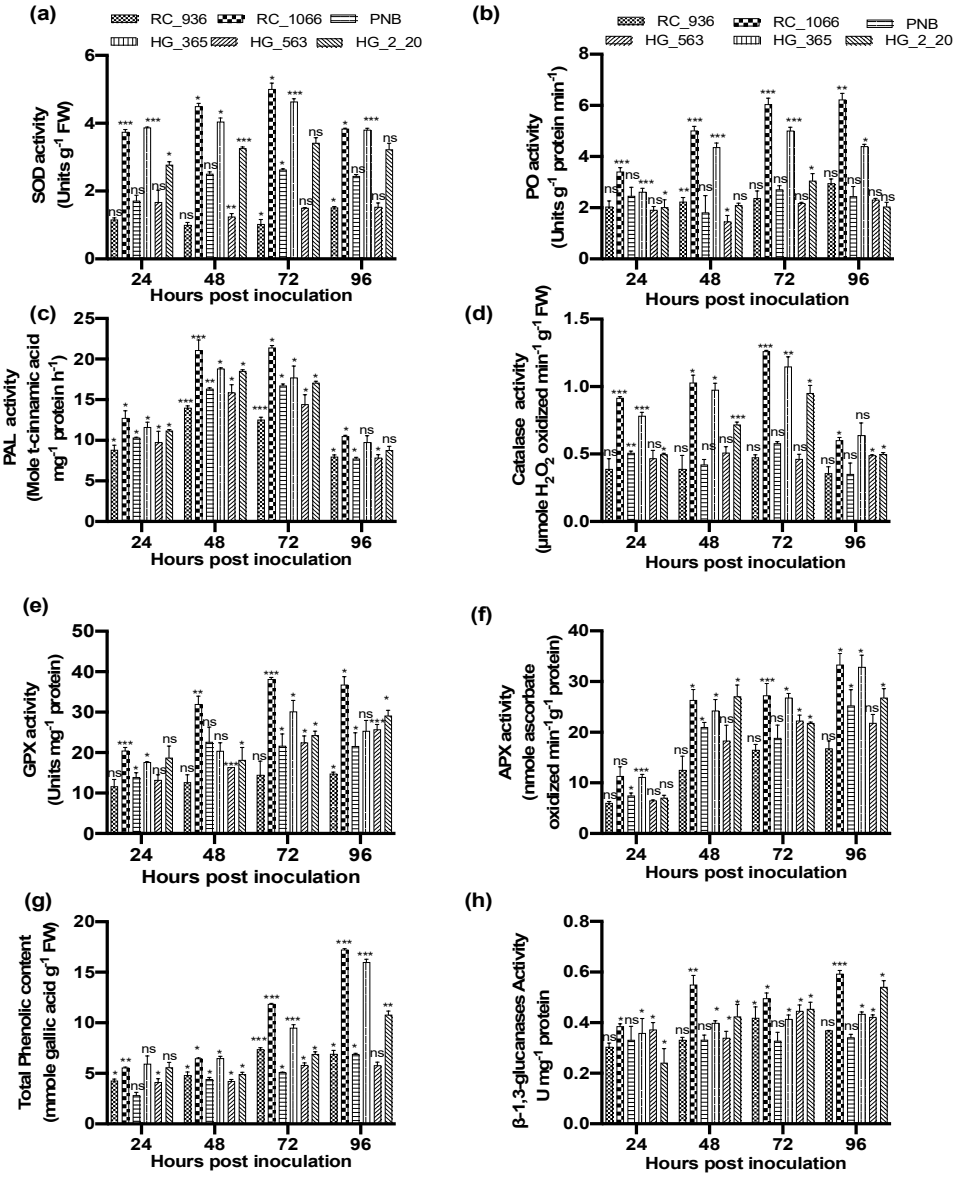


Fig 2: Assessment of *Alternaria* blight resistance by detached leaf method in guar genotypes.

1066 (0.6 U/mg). Many studies have previously reported that β-1,3-glucanase or laminarinases found in plants have antimicrobial properties, providing resistance by hydrolyzing the β -1,3-glucan found in fungal cell walls (Tehrani *et al.,* 2020). The activity of β-1,3-glucanase suggests that it may play a role in plant protection and defence against *Alternaria* blight in guar.

After 14 DPI, physiological performance such as total chlorophyll content and carotenoids content was significantly reduced by 34-65% in the inoculated guar genotypes compared to the control among moderately susceptible to susceptible genotypes (Fig 4). To assess the maximum photochemical efficiency (PS-II yield), the Fv/Fm values were observed (Fig 5). The Fv/Fm values were decreased when compared to the corresponding control genotypes. Fv/FM values positively correlated with chlorophyll and carotenoid content (Upreti *et al*., 2021). A decrease in chlorophyll and carotenoid concentrations, implying a decrease in photosynthesis rate in inoculated plants, indicates that Alternaria has a negative effect on plant growth (Nafisa *et al*., 2020). The reduction of pigments in inoculated genotypes may be due to toxic pathogen metabolites that



(a) Superoxide dismutase (SOD) (b) Peroxidase PO (c) Phenylalanine ammonia lyase (PAL) (d) Catalase (CAT) (e) Glutathione peroxidase (Gpx) (f) Ascorbate peroxidase (APx) (g) Total phenolic content (TP) (h) b-1,3-glucanases. Error bars represent stand error of six replicates. Means having different letters indicate significant differences (P≤0.05).

Fig 3: Effect of *Alternaria* on defence-related enzymes activity in guar genotypes.

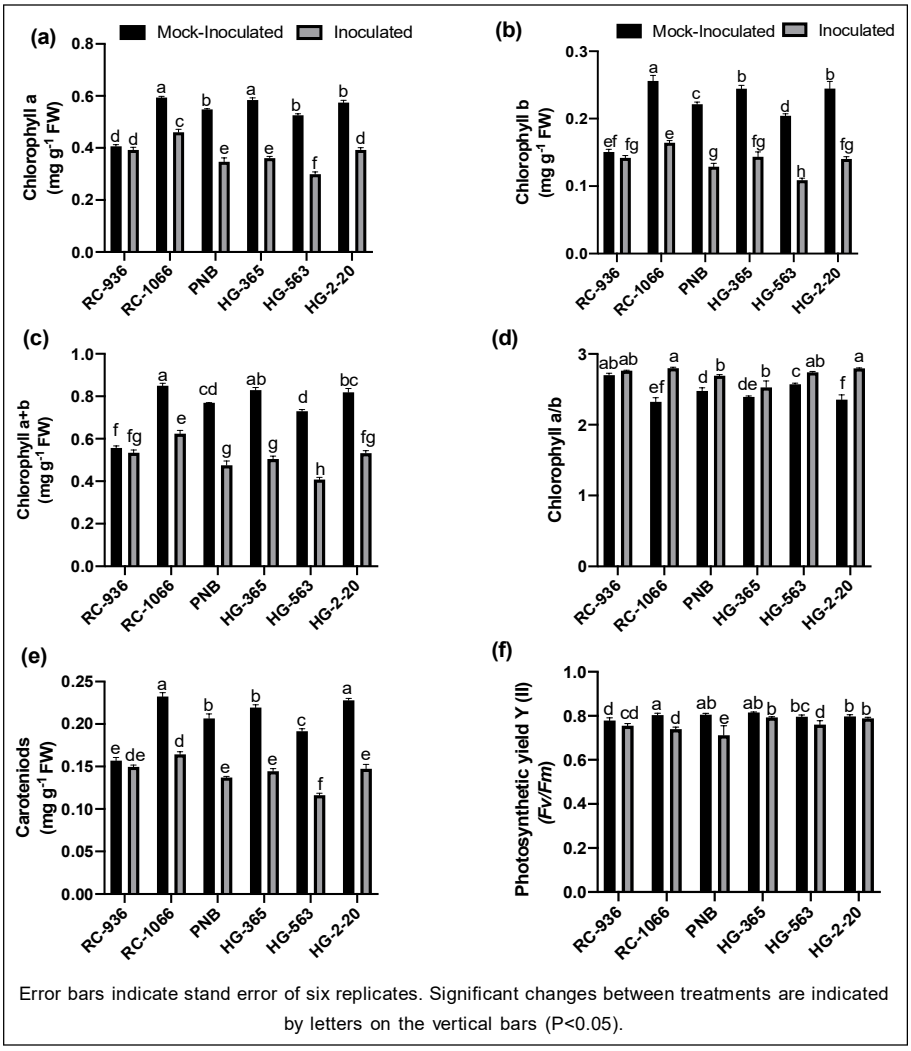


Fig 4: Effect of *Alternaria alternata* on (a) Chlorophyll *a*, (b) Chlorophyll *b*, (c) total chlorophyll *a+b*, (d) Chlorophyll *a/b, (*e) Carotenoids contents and (f) Maximum quantum yield of PSII (FV/FM) of guar 14 DPI.

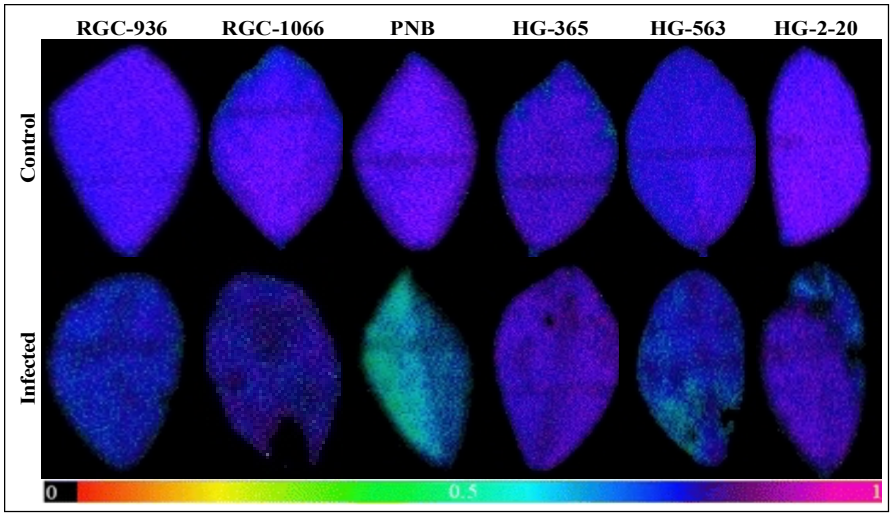


Fig 5: Imaging data of maximum photochemical efficiency of PSII in guar leaves after 14 DPI with *Alternaria* using Imaging PAM. The false colour code depicted at the bottom of the image ranged from 0 (black) to 1.0 (purple).

can cause oxidative stress in plants and overproduced ROS cause PCD and mediates defence mechanism.

CONCLUSION

Based on host-pathogen interaction, comparative physiological and biochemical analysis between mock-inoculated and inoculated, the genotype RGC-1066 was found to be relatively resistant and RGC-936 was susceptible to *Alternaria* infection. The genotype RGC-1066 was found to be potential genetic material for breeding for *Alternaria* resistance in guar and other genetic studies. The maximum enzyme activity was observed at 72 hapi, which will be best suitable for transcriptome studies for better understanding resistance pathways and pathogenesis-related interactions along with the response of host towards pathogenicity.

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Conflict of interest

All authors declared that there is no conflict of interest.

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