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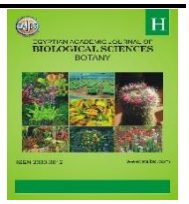

Impact of Epigenetic Regulatory Genes on Biological Functions in *Magnaporthe oryzae* the Causal Agent of Rice Blast Disease

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ABSTRACT

Magnaporthe oryzae is a heterotrophic plant pathogenic fungus that developed the capability to colonies of living plant cells, A lot of genes play key roles in biological function, especially during the invasion process and bio-trophic growth. but the mechanisms through which they establish bio-trophic growth by modifying gene expression are still unclear, we conducted an integrated analysis involving PRC2 genes which includes this description focusing on SUZ 12, EZH2 and EED1 proteins, revealing its crucial effect in the development and infection processes of the rice blast fungus, *Magnaporthe oryzae*. Deletion of SUZ 12, EZH2 and EED1 in *M. oryzae* resulted in a modest reduction in vegetative growth and a significant decrease in conidiation. Notably, the SUZ 12, EZH2 and EED1 mutants exhibited a marked reduction in virulence towards host plants. Observation of the infection process indicated that the mutants were halted in invasive growth, leading to the accumulation of substantial host reactive oxygen species (ROS). Additionally, the SUZ 12, EZH2 and EED1 mutants displayed sensitivity to cell wall-disturbing agents, These results suggest that the PRC2 complex plays an important role in bio-trophic growth, thereby facilitating invasive growth in *M. oryzae*.

INTRODUCTION

Magnaporthe oryzae, which infects rice and barley and serves as a standard plant fungal pathogen for studying plant-pathogen interactions. (Wilson-RA *et al.*, 2009) has not been extensively explored in terms of the poly-comb group (PcG) function. The infection process of *M. oryzae* involves the formation of appressorium to penetrate the upper layer of the host plant, followed by the differentiation of the penetration peg into primary hyphae, then further differentiates into bulbous secondary hyphae, establishing a bio-trophic stage, and ultimately progressing into a necrotrophic growth stage (Kankanala-P *et al.*, 2007). During the early stages of host cell infection, *M. oryzae* secretes effector proteins to suppress host defenses (Mosquera-G *et al.*, 2009, Khang-CH *et al.*, 2010). However, the establishment of bio-trophic growth in *M. oryzae* remains largely unknown.The PcG initially identified in Drosophila, these proteins were found to be essential for maintaining the silenced state of developmental regulators, particularly those associated with Hox genes (Lewis-EB., 1978). Recent advancements have elucidated the important mechanisms and biological implications

of PcG proteins in various organisms (Schwartz-YB et al., 2006, Boyer-LA. *et al.,* 2006, Lee-TI. *et al.,* 2006). The global silencing system orchestrated by PcG proteins, through the repression of various target genes, plays pivotal roles in development, stem cell biology, and cancer (Sparmann-A. *et al.,* 2006, Schwartz-YB *et al.,* 2007).

Previous studies have delineated two essential PcG protein complexes, namely Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). PRC2, in particular, methylates histone H3 on Lys27 (H3K27), contributing to the establishment of a repressive chromatin state (Margueron-R. *et al.,* 2011, Simon-JA. *et al.,* 2013). PRC1 acts as an executor for the silencing of target genes (Merini-W. *et al.,* 2015). In Drosophila, PRC2 comprises many proteins, such as the catalytic SET domain- has lysine methyltransferase EZH (enhancer of zest), WD40 essential protein EED (enhanced ectoderm development), (inhibitor of zeste-12) SuZ12, and nucleosome remodeling factor 55 (NURF55) (Kingston-RE. *et al.,* 2002). While the essential functions of PcG components and PcG-mediated chromatin process have been extensively investigated in different studies in humans on stem cell and cancer biology, and multicellular development in plants (Sparmann-A et al., 2006, Rajasekhar-VK. *et al.,* 2007), Studying the function of PcG in filamentous fungi are currently less extensive. Many studies investigated the PRC2 in various plant pathogens, including *Zymoseptoria tritici*, *Fusarium graminearum* and *Fusarium fujikuroi* (Schotanus-K. *et al.,* 2015, Studt-L. *et al.,* 2016). Studies on human fungal pathogen *Cryptococcus neoformans* (Dumesic-PA. *et al.,* 2015) and the endophytic symbiont fungus *Epichloë festucae* (Chujo-T. *et al.,* 2014) have also indicated the involvement of PRC2 in suppressing secondary metabolite metabolism genes.

Recent research has reported that PRC2 in *M. oryzae* can control the transcriptional effector of proteins through the repression transcription process, as demonstrated in previous studies and experiments (Zhang-W. *et al.,* 2021). Here, we collected invasive hyphae (IH) samples from infected barley leaves, revealing that PRC2 plays a pivotal role in bio-trophic growth in *M. oryzae*. The genes modulated by PRC2 have important roles in fungal cell wall construction, cell wall-degrading enzymes (CWDE), and secondary metabolism, all crucial for the infection process in plants, our research provides evidence that PRC2 is important for programming genes to facilitate bio-trophic growth in *M. oryzae.*

MATERIALS AND METHODS

Cultivation and Production of Fungal Strains:

The original strain of *M. oryzae* employed in our study was P131 (Chen-XL. *et al.,*2014). All strains were cultivated on Oatmeal Tomato Agar (OTA) media at a temperature of 28ºC. CM liquid culture inoculated with mycelia at the same temperature for 36 hours to facilitate extraction of DNA and protoplast isolation. Colony diameter and spore production observation followed the procedures outlined (Chen-XL. *et al.,* 2014). For the assessment of virulence and infection process observation, spores were collected from 7-day-old OTA cultures.

Hyphal Tip Cell Length Observation:

We stained the mycelia in CM liquid culture with 10 μg/ ml^{-1} Calcofluor White (CFW) for 10 minutes in the dark and used a fluorescence microscope (Nikon Ni90 microscope, Japan) to determine hyphal tip cell lengths.

Stress Sensitivity Test:

To evaluate fungal strains to stress response, we inoculated the CM plates supplemented with various stress agents, including 0.2 mg/ml Congo Red (CR), 0.1 mg/ml Calcofluor White (CFW), 0.005% Sodium dodecyl sulfate (SDS), 0.5 M NaCl, and 10 mM H_2O_2 with the strains,

then calculated colony diameter measurements 7- days post-inoculation (7- dpi) for further analysis.

Observation of Infection Process and Virulence Evaluation:

Evaluation of virulence was conducted on 30 days old rice seedlings (*Oryzae -sativa cv. LTH*) and ten days old barley leaves (*Hordeum vulgare cv. E9*). We sprayed the prepared spores suspensions of fungal strains (5×10^4 conidia /ml in 0.025% Tween 20) onto plant leaves, then incubated in high humidity conditions at 28°C. Disease symptoms were observed and documented five days later. An Infection process has been observed, using spores suspensions (1×10^5 conidia/ml) which were applied to barley leaves in high humidity incubation conditions at 28°C. The infected barley leaves' epidermis was examined at different times for appressorium formation, bio-trophic organisms at 24 hpi, and necrotrophic invasive growth at 30 hpi. following established protocols (Chen-XL *et al.*, 2014).

RESULTS

PRC2 Complex Impact on Vegetative Growth and Conidiation:

We investigated the impact of the PRC2 complex in vegetative growth by observing the colony diameter of Δeed1, Δezh2, and Δsuz12 mutants on (OTA) media plates. The colony diameters of these mutants were noticeably reduced compared to the wild-type. (Figs. 1 A and 1B). Hyphal tip stained with Calcofluor White (CFW) showed a significant reduction in the length of upper hyphal cells in PRC2 complex mutants according to the normal hyphal cells in the wild-type strain, (Fig.1C).

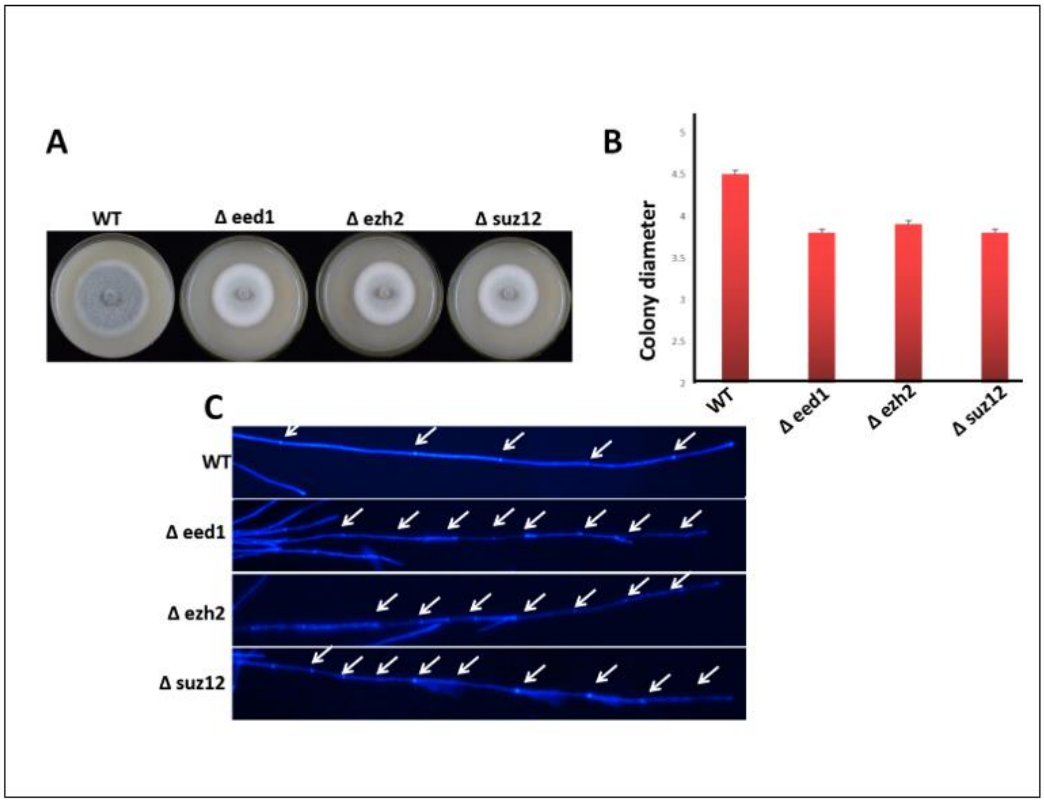

Fig 1. Illustrates the involvement of the PRC2 complex in the vegetative growth of *M. oryzae*. (A) The colony shape of the wild-type strain and PRC2 complex gene mutant strains on (OTA) plates media at 28°C for 7 days. (B) the analysis of Colony size with error bars indicating standard deviation and asterisks denoting significant differences ($P < 0.01$). (C) Hyphae tips length in the wild-type strain and PRC2 complex mutants, with arrows indicating septa between cells.

PRC2 Complex Impact on Conidiation:

Conidia formation in the Δeed1, Δezh2, and Δsuz12 mutants was approximately 45% lower than that of the wild-type strain, (Figs. 2A and B). Therefore, the disruption of the PRC2 complex led to a big malformation of the conidia forms in the mutant strains compared to the wild type, (Figs. 3A and B).

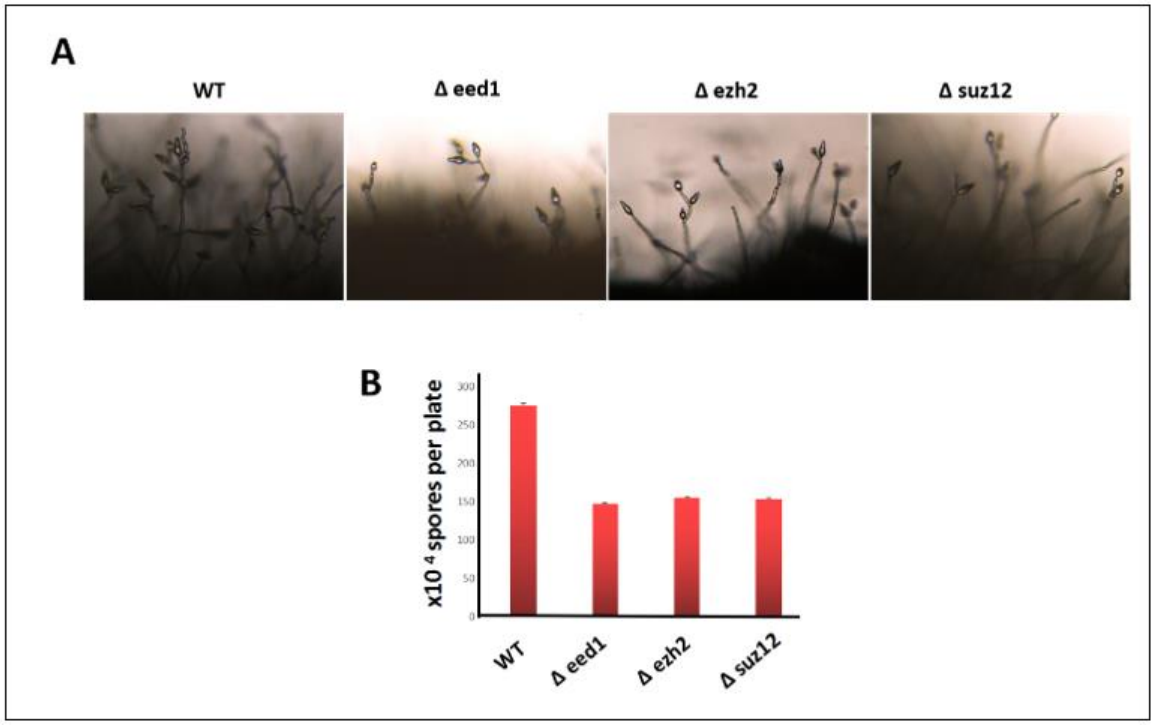

Fig 2. PRC2 complex has a vital role in spores formation (A) Observation of spores and conidiophore production using a light microscope after 5 days of growth on OTA plates. (B) Spores formation analysis with error bars representing standard deviation and asterisks denoting notable differences between strains ($P < 0.01$).

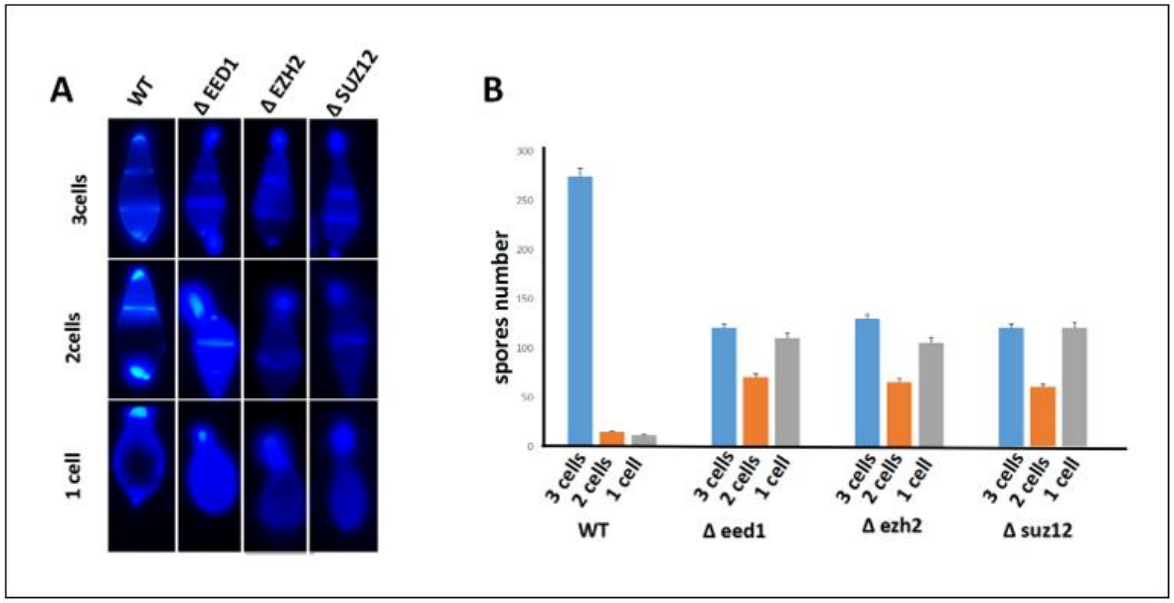

Fig 3. Disruption genes of PRC2 complex induce spores malformation (A) Observation of three-cells, two-cells and one-cell conidia in the wild-type strain and PRC2 complex strains under a light microscope after 5 days of growth on OTA plates. (B) Statistical analysis of malformed conidia, with error bars representing standard deviation and asterisks denoting significant differences among strains ($P < 0.01$).

PRC2 Complex Involvement in Virulence and Biotrophic Growth:

Disruption of the PRC2 complex genes was found to impact the infection capacity of *M. oryzae*. Virulence tests on susceptible rice seedlings and barley leaves revealed a dramatic reduction in lesion size and number for the PRC2 complex mutants compared to the wild-type strain, (Figs. 4A, B and C). Live-cell imaging demonstrated that at 24 hpi and 30 hpi, the Δeed1, Δezh2, and Δsuz12 mutants were defective in bio-trophic growth, Fig (4D), providing big evidence on the crucial role of the PRC2 complex in bio-trophic growth.

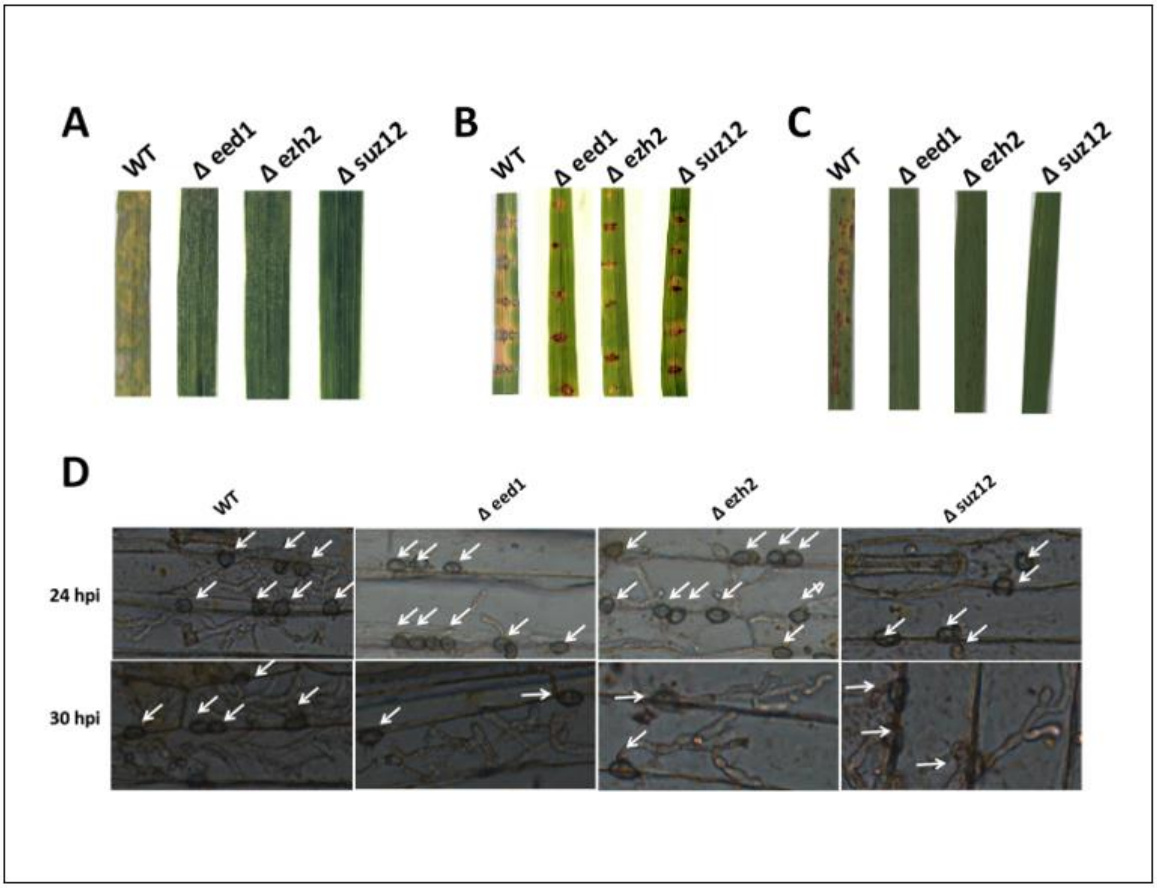

Fig 4. Disruption of the PRC2 complex genes exhibited a significant reduction in virulence. (A) Virulence test on barley leaves, sprayed with conidia suspensions and observed at 5 dpi. (B) Virulence test on wounded rice, where leaves were slightly wounded before inoculation with fungal mycelium and typical leaves were photographed at 3 dpi. (C) Virulence test on rice seedlings, with leaves sprayed with conidia suspensions and photographed at 5 dpi. (D) invasive hyphae and infection process observation at 24 and 30 hpi, arrows indicating appressoria

PRC2 Complex Disruption Reduce Stress Tolerance:

We investigated the impact of disrupting PRC2 Complex genes on stress response in *M. oryzae*. The findings demonstrated that the PRC2 Complex mutant displayed increased sensitivity to various stresses, such as cell wall-disrupting agents [0.1 mg/ml Calcofluor White (CFW), 0.2 mg/ml Congo Red (CR), and 0.005% Sodium dodecyl sulfate (SDS)], osmotic stress (0.5 M NaCl) and oxidative stress (10 mM H_2O_2), Fig (5A) and (5B). Notably, the mutant exhibited pronounced sensitivity specifically to cell wall-disrupting agents, whereas the wild-type strain showed only slight effects. These results indicate that PRC2 complex genes play a crucial role in responding to various types of stress.

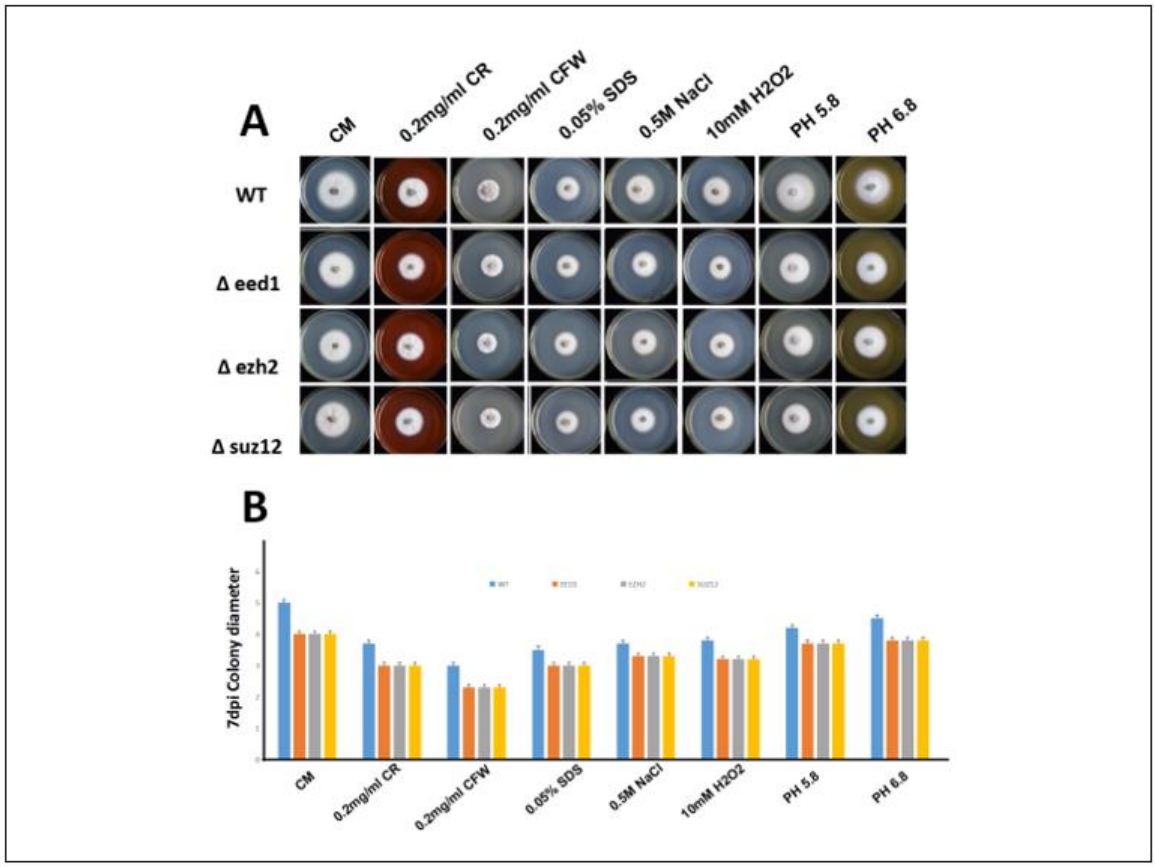

Fig. 5. Shows disrupted PRC2 complex genes cannot tolerate various stress agents. (A) The colony shape of the wild-type strain and the PRC2 complex mutant strains on (CM) media plates with different stress agents at 28°C and 7 dpi. (B) Colony diameter of PRC2 complex gene deletion mutants under different stress conditions, with colonies photographed at 7dpi on plates.

DISCUSSION

The PRC2 complex importance has been extensively studied in various fungi, such as *F. graminearum, F. fujikuroi, Z. tritici, C. neoformans N. crassa, and E. festucae*. These studies have revealed that the PRC2 complex is associated with gene silencing and has significant roles in developmental processes, virulence and regulating the transcription of secondary metabolite genes. newly, the functions of PRC2 in the bio-trophic growth of *M. oryzae*, have also been discovered. It was found that to involved in regulating genes essential for host invasion, particularly the effector proteins (Zhang-W *et al.*, 2021, Wu-Z *et al.*, 2021). However, the exact role of the PRC2 complex during interactions between fungi and plants remains unclear. In this study we operated biological analyses of PRC2 gene deletion mutants, to determine that PRC2 is crucial for bio-trophic growth of *M. oryzae* by reprogramming gene expression patterns. Our findings proposed that PRC2-mediated regulation is involved in suppressing genes encoding cell wall-related proteins, plant cell wall degrading enzymes (CWDEs), secondary metabolite biosynthesis proteins, and effector proteins, which are essential for the fungus to facilitate bio-trophic growth and evade host recognition.

Overall, this study provides insights into how the PRC2 complex coordinates bio-trophic growth in *M. oryzae* and highlights the potential role of regulation in fungal adaptation to host plants. Future investigations could focus on unraveling the mechanisms

of PRC2 in the regulation of AVR genes in various field strains and rice cultivars to understand the adaptation of fungal pathogens to host cells through epigenetic mechanisms.

Declarations:
Ethical Approval: Ethical Approval is not applicable.
Competing interests: The authors declare no conflict of interest.
Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.
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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.
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ARABIC SUMMARY

تأثير بعض الجينات التنظيمية على الوظائف الحيوية لفطر *Magnaporthe oryzae* المسبب الرئيسي لمرض لفحة الأرز

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Magnaporthe oryzae من الفطريات غير ذاتية التغذية الممرضة للنبات. كما له القدرة على استعمار الخلايا النباتية الحية، وتلعب الكثير من الجينات دورًا رئيسيًا في الوظائف البيولوجية خاصة أثناء عملية العدوى والنمو الغذائي الحيوي. لكن الآليات التي يتم من خلالها النمو الغذائي الحيوي عن طريق تعديل التعبير الجيني لا تزال غير واضحة، فقد أجرينا تحليلًا متكاملًا يتضمن جينات RC2P والتي تشمل البروتينات Z12SU وH2EZ وD1EE مما يوضح تأثيرها القوي في عملية النمو الخضري وكذلك الشدة المرضية للفطر. أدى حذف الجينات SUZ12 وH2EZ وD1EE في *M. oryzae* إلى انخفاض كبير في النمو الخضري وانخفاض كبير في عملية التجرثم. والجدير بالذكر أن طفرات Z12SU وH2EZ وD1EE أظهرت انخفاضًا ملحوظًا في الشراسة المرضية تجاه النباتات المضيفة. أشارت مراقبة عملية العدوى إلى توقف الطفرات في إنتاج هيفات الاختراق، مما أدى إلى تراكم أنواع الأكسجين التفاعلية (ROS). بالإضافة إلى ذلك، أظهرت طفرات Z12SU وH2EZ وD1EE حساسية تجاه المواد المثبطة لجدار الخلية، وتشير هذه النتائج إلى أن معقد PRC2 يلعب دورًا مهمًا في النمو الغذائي الحيوي، وبالتالي تسهيل النمو الغازي في فطر *M. oryzae* المسبب الرئيسي لمرض لفحة الأرز.

الكلمات الأساسية: الفطريات غير ذاتية التغذية، عملية الغزو، النمو الغذائي الحيوي، معقد PRC2.