Comparative transcriptome analysis reveals the resistance regulation mechanism and fungicidal activity of the fungicide phenamacril in *Fusarium oxysporum*

Zhitian Zheng1, Huaqi Liu1, Yunyong Shi1, Zao Liu1, Hui Teng1, Sheng Deng2*, Lihui Wei2, Yunpeng Wang1* & Feng Zhang3

*Fusarium oxysporum* (Fo) is an important species complex of soil-borne pathogenic fungi that cause vascular wilt diseases of agricultural crops and some opportunistic diseases of humans. The fungicide phenamacril has been extensively reported to have antifungal activity against *Fusarium graminearum* and *Fusarium fujikuroi*. In this study, we found that the amino acid substitutions (V151A and S418T) in Type I myosin FoMyo5 cause natural low resistance to phenamacril in the plant pathogenic Fo isolates. Therefore, we compared the transcriptomes of two phenamacril-resistant Fo isolates FoII5, Fo1st and one phenamacril-sensitive isolate Fo3_a after 1 μg/mL phenamacril treatment. Among the 2728 differentially expressed genes (DEGs), 14 DEGs involved in oxidation–reduction processes and MFS transporters, were significantly up-regulated in phenamacril-resistant isolates. On the other hand, 14 DEGs involved in ATP-dependent RNA helicase and ribosomal biogenesis related proteins, showed significantly down-regulated expression in both phenamacril-resistant and -sensitive isolates. These results indicated that phenamacril not only seriously affected the cytoskeletal protein binding and ATPase activity of sensitive isolate, but also suppressed ribosome biogenesis in all the isolates. Hence, this study helps us better understand resistance regulation mechanism and fungicidal activity of phenamacril and provide reference for the development of new fungicides to control Fo.

The *Fusarium oxysporum* (Fo) species complex contains many destructive fungal plant pathogens and causes vascular wilt diseases on a broad range of host plants, which involves fungal colonization of the xylem via the roots and the growing mycelium eventually causes vessel obstruction, blocks transport of water to the aerial parts of the plant1–5. Based on host specificity, the species complex includes more than 150 formae speciales4, such as Fo f. sp. *lycopersici*, Fo f. sp. *cubense*, Fo f. sp. *niveum* and so on, which infect tomato, banana, and watermelon, respectively5–11. In addition, some Fo also cause life-threatening invasive fusariosis in immunocompromised animals and humans12–18. Therefore, it is important to find an effective method to control diseases caused by Fo.

Currently, resistant cultivars, crop rotation and biocontrol using microorganisms are used to control vascular wilt disease16–18. In addition, the use of chemical fungicides significantly reduce Fusarium wilt. However, these Fo species typically show broad resistance to antifungal drugs and become more difficult and persistent to control19–21. Therefore, finding an effective antifungal compound is crucial for controlling diseases caused by Fo. Phenamacril (experimental code JS399-19; a.i. 2-cyano-3-amino-3-phenylacrylic acetate) is a Fusarium-specific fungicide and shows excellent control of Fusarium head blight (FHB) and Rice bakanae disease in the field caused by *Fusarium graminearum* and *Fusarium fujikuroi*, respectively22,23. In our previous studies, we...
found that FoMyo5 motor domain substitutions (V151A and S418T) cause natural low resistance (EC_{50} value varies from 1.5 to 15 μg/mL) to fungicide phenamacril in Fo3a. Thus, it is critical to elucidate the low resistance mechanism in Fo species for developing efficient control methods of phenamacril-resistant populations in the fields or immunocompromised individuals.

In F. graminearum, we previously reported that mutations occurred in Myosin5, encoded by FGSG_01410.1, confer to resistance to phenamacril18. As we know, Myosin5 are eukaryotic, actin-dependent ATPase motors that play important roles in actin filament bundle organization, vesicle/organelle transport, transcriptional regulation, intracellular transport, and signal transduction27,28. In Fusarium asiaticum, the mutation types of A135T, V151A, P204S, I424M, A577T, R580G/H or I581F led to low resistance to phenamacril25. The mutation types of S418R, I424R or A577G were responsible for moderate resistance and K216R/E, S217P/L or E420K/G/D conferred high resistance25. In F. fujikuroi, the point mutation S219P or S219L in Myosin-5 conferred high resistance to phenamacril23.

Transcriptome refers to the sum of all RNA transcribed by a specific tissue or cell at a certain time or in a certain state, mainly including mRNA and non-coding RNA. Transcriptome sequencing is to study all mRNA transcribed by specific tissues or cells at a certain period29. It is the basis of gene function and structure research and plays an important role in understanding the development of organisms and the occurrence of diseases and exploring regulation pathways involved in pathogens response to fungicides stress30–32. With the development of gene sequencing technology and the reduction of sequencing cost, RNA-seq has become the main method of transcriptome research with its advantages of high throughput, high sensitivity and wide application range. RNA-seq method allows the characterization of the transcriptome even in species for which no reference genome is available. In both instances, RNA-seq reads can be assembled de novo into a transcriptome33,34. For example, transcriptome analysis of F. f. sp. nivene after treatment with 80 μg/mL of fungicide thymol demonstrated that thymol produced reactive oxygen species (ROS) accumulation and destroyed the integrity of the cell wall and cell membrane so as to explain the mechanism of antifungal32.

The objectives of this paper were to study how the mutation types of V151A and S418T in FoMyo5 regulate low resistance to phenamacril, elucidate the resistance regulation mechanism of phenamacril in Fo and determine the key genes or pathways where phenamacril could inhibit the growth of Fo. For this purpose, we aligned FoMyo5 motor domains of eight Fo isolates and compared the transcriptomes of one phenamacril-sensitive isolate Fo3_a and two phenamacril-resistant isolates Fo1st and FoII5 after treated with 1 μg/mL phenamacril. The comparative transcriptome analysis of those Fo isolates could provide insight into the resistance regulation mechanism and fungicidal activity of phenamacril and provide reference for the development of new fungicides to control diseases caused by Fo.

**Results and discussion**

**The activity of phenamacril against eight Fo isolates.** Recently, the fungicidal activities and resistance mechanism of phenamacril have been extensively reported23–26,35–37. In our previous studies, we found that FoMyo5 motor domain substitutions (V151A and S418T) cause natural resistance to fungicide phenamacril in Fo3a. However, we just tested the activity of phenamacril against six reference Fo strains before, including plant-pathogenic and -nonpathogenic or human-pathogenic strains38. Here, we first isolated 7 isolates of Fo from six kinds of horticultural crops suffering from Fusarium vascular wilt and one kind of cornea of patients with fungal keratitis. The majority of the isolates exhibited pink colonies and reduced aerial hyphal, except FoII5 and Fo3_a showed white colonies (see Supplementary Fig. S1). The mycelial growth rate was recorded in Table 1.

Then we compared the susceptibility of these 7 Fo isolates and one reference Fo II5 to phenamacril based on mycelial growth in fungicide-amended and fungicide-free media at 28 °C. Sensitivity test showed that phenamacril exhibits stronger inhibitory activity against the human pathogenic one (Fo3_a) and Fo f. sp. vasinfectum (LAI0). The EC_{50} values of the two isolates are 0.516 μg/mL and 0.804 μg/mL, respectively (Table 1).

| Strains       | EC_{50}(μg/ml) | Growth rate on three media (mm/day) |
|--------------|---------------|------------------------------------|
| Fo3-2        | 7.069 ± 0.686c | 8.38 ± 0.24a                        |
| LA0          | 0.804 ± 0.03e  | 7.61 ± 0.18b                        |
| FoII5        | 5.512 ± 0.161d | 7.46 ± 0.45bc                       |
| Fo1st        | 8.422 ± 0.847bc| 7.38 ± 0.18bc                       |
| CAO0         | 11.826 ± 0.581a| 7.18 ± 0.11 cd                      |
| Fo3_a        | 0.516 ± 0.026e | 6.86 ± 0.43de                       |
| FoX-KW       | 9.488 ± 0.97b  | 6.61 ± 0.25e                        |
| FoHGKW       | 8.511 ± 0.615bc| 6.54 ± 0.18e                        |

Table 1. Sensitivity of the Fusarium oxysporum isolates to phenamacril and growth rate. *EC_{50} is the fungicide concentration resulting in 50% mycelial growth inhibition. Values are means ± standard deviation of three experiments, values in a column followed by the same letters are not significant difference at P = 0.05, Fisher’s LSD test. **Growth rate was measured after incubation for 7 days. Mean and standard deviation were calculated from three replicates. The same letter indicated there was no significant difference. Different letters were used to mark statistically significant differences (P = 0.05).*
Fo isolates collected from various hosts showed different resistance levels, the EC50 values varied from 5.512 to 11.826 μg/mL were recorded for FoII5, Fo3-2, Fo1st, FoHGKW, FoX-KW and CAO0 (Table 1). These six isolates of Fo demonstrated low resistance levels to fungicide phenamacril.

Alignment analysis of the FoMyo5 motor domains in Fo. To elucidate the difference of susceptibility to phenamacril among isolates Fo3_a, LA0, FoII5, Fo3-2, Fo1st, FoHGKW, FoX-KW and CAO0. We aligned these FoMyo5 motor domains using Bioedit 7.2 software and we also found two amino acid mutations at codon 151 and codon 418 in these resistant isolates (Fig. 1). These results showed that the two substitutions V151A and S418T conferred the low resistance levels to phenamacril in these field Fo isolates, which is consistent with our earlier research24.

RNA-seq and de novo reference transcriptome assembly in Fo. In order to elucidate deeper insight into the resistance regulation mechanism and fungicidal activity of phenamacril in Fo, we sequenced the transcriptomes of two phenamacril-resistant isolates FoII5, Fo1st and one phenamacril-sensitive isolate Fo3_a under 1 μg/mL phenamacril treatment and 0.1 μL/mL methanol treatment conditions. The number after the letters represent three biological replicates.

| Sample name | Raw reads | Clean reads | Clean bases (G) | Q20 (%) | Total mapped (%) | Uniquely mapped (%) | Multiple mapped (%) |
|-------------|-----------|-------------|-----------------|---------|------------------|---------------------|-------------------|
| Fo1st_1     | 27,940,218| 26,675,362  | 4.0             | 98.34   | 93.67            | 93.44               | 0.24              |
| Fo1st_CK1   | 31,708,052| 30,688,002  | 4.6             | 98.13   | 93.03            | 92.66               | 0.37              |
| Fo1st_2     | 28,277,756| 27,195,768  | 4.08            | 98.07   | 92.96            | 92.7               | 0.26              |
| Fo1st_CK2   | 32,973,382| 31,945,512  | 4.79            | 98.18   | 92.96            | 92.7               | 0.26              |
| Fo1st_3     | 30,706,132| 29,179,662  | 4.38            | 98.12   | 93.41            | 93.15               | 0.26              |
| Fo1st_CK3   | 31,471,120| 30,360,824  | 4.55            | 98.15   | 92.86            | 92.65               | 0.21              |
| Fo3_a1      | 29,649,918| 28,351,218  | 4.25            | 98.28   | 71.29            | 71.15               | 0.14              |
| Fo3_aCK1    | 29,679,972| 28,645,658  | 4.3             | 98.17   | 71.53            | 71.36               | 0.16              |
| Fo3_a2      | 32,405,328| 31,129,220  | 4.67            | 98.19   | 69.77            | 69.64               | 0.13              |
| Fo3_aCK2    | 31,685,548| 30,712,572  | 4.61            | 98.19   | 70.89            | 70.75               | 0.14              |
| Fo3_a3      | 33,792,602| 32,224,420  | 4.83            | 98.22   | 70.43            | 70.31               | 0.12              |
| Fo3_aCK3    | 31,611,978| 30,659,206  | 4.6             | 98.16   | 70.99            | 70.81               | 0.18              |
| Fo15_1      | 31,255,856| 29,083,840  | 4.36            | 98.25   | 89.84            | 85.81               | 4.03              |
| Fo15_CK1    | 33,743,472| 32,509,710  | 4.88            | 98.19   | 89.88            | 85.22               | 4.66              |
| Fo15_2      | 31,503,714| 29,148,128  | 4.37            | 98.22   | 87.88            | 82.58               | 5.3               |
| Fo15_CK2    | 31,323,396| 30,203,936  | 4.53            | 98.15   | 91.02            | 87.1               | 3.92              |
| Fo15_3      | 28,693,422| 26,966,280  | 4.04            | 98.22   | 91.95            | 88.4                | 3.55              |
| Fo15_CK3    | 31,046,020| 29,893,490  | 4.48            | 98.29   | 88.62            | 83.57               | 5.05              |

Table 2. Mapping results of RNA-Seq data from phenamacril-sensitive and -resistant Fo isolates under 1 μg/mL phenamacril treatment and 0.1 μL/mL methanol treatment conditions. ‘Letter ‘CK’ represents the control groups treated with 0.1 μL/mL methanol and others represent the treatment groups treated with 1 μg/mL phenamacril.
From the 18 high quality transcriptomes, 17,945 unigenes were obtained in total, including 970 new transcripts. These new transcripts contain transcriptional information but are not matched to the corresponding genes in the reference genome. The abundance of all the unigenes (17,945) was normalized and calculated by FPKMs (FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) method using uniquely mapped reads. Genes with FPKMs in the interval 0–1 were considered to be present at very low levels; genes with FPKMs over 60 were considered to be expressed at a very high level. The distributions of the median expression levels of all the unigenes were up regulated in phenamacril-resistant isolates than phenamacril-sensitive isolate (see Supplementary Fig. S2). In addition, functional annotation of all the unigenes were conducted and a total of 17,945 unigenes were annotated to Gene ontology (GO), Kyotoencyclopedia of genes and genomes (KEGG) and protein protein interaction network (PPI) databases, respectively. The GO annotation indicated 9586 unigenes were categorized into 596 functional terms in 3 categories. The KEGG pathway database was used to analyze intracellular metabolic processes, and 1979 unigenes were assigned to 91 KEGG pathways. ‘biosynthesis of secondary metabolites’ and ‘ribosome’ were the dominant pathways, and the proportions were 18.05%, and 10.99%, respectively. Moreover, The PPI analysis indicates that 7334 pairs of proteins encoded by the genes interacts. This high-quality transcriptome represents a valuable resource for further research on Fo isolates.

Analysis of differential expression genes (DEGs) during phenamacril treatment. One of the primary goals of the transcriptome study was to identify variations between different samples. The results indicated that these variations ranged from 226 to 2728 DEGs, based on FPKM values (Fig. 2A). When treated with 1 μg/ml phenamacril, there are 549 genes significantly up-regulated expression and 439 genes significantly down-regulated expression in phenamacril-resistant isolate Fo1st whereas there are 92 genes significantly up-regulated expression and 134 genes significantly down-regulated expression in another phenamacril-resistant isolate FoII5. However, there are as many as 1321 genes significantly up-regulated expression and 1407 genes significantly down-regulated expression in phenamacril-sensitive isolate Fo3_a (Fig. 2A). The relationships among different DEG groups from Fig. 2B were displayed as Venn diagrams, and the results indicated that 40 DEGs were identified in both phenamacril-resistant and -sensitive isolates during 1 μg/ml phenamacril treatment, of which 11 genes were significantly up-regulated and 29 genes were significantly down-regulated. In addition, a total of 55 DEGs were identified in the two phenamacril-resistant isolates but not in the phenamacril-sensitive isolate, of which 29 genes were significantly up-regulated and 26 genes were significantly down-regulated. Because these two resistant isolates are different Fo formae speciales with different genetic backgrounds, when we treated the two isolates with phenamacril, the numbers of DEGs were significantly different. However, we found that 95 DEGs of the two isolates were identical in response to phenamacril. By further studying the function of the 95 genes, we might find the mechanism of regulating resistance of Fo to phenamacril.
Clustering Analysis of DEGs during phenamacril treatment. From the transcriptomes of Fo, large numbers of DEGs under 1 μg/mL phenamacril treatment and 0.1 μL/mL methanol treatment conditions were identified in phenamacril-sensitive and-resistant isolates. To observe the gene expression patterns, we performed hierarchical clustering of all the DEGs (3482 genes) based on the log2 (FPKM + 1) for the 6 treatments. From the upper and lower part of heatmap we can see, there many of the genes that are differentially expressed in the sensitive isolate Fo3_a are different from the resistant isolates Fo1st and FoII5 after phenamacril treatment. (see Supplementary Fig. S3).

GO classification of DEGs during phenamacril treatment. To classify the functions of the predicted Fo DEGs, We performed GO enrichment analysis, which is an internationally standardised gene functional classification system in biological process, cellular component and molecular function. The DEGs were assigned to 596 functional terms by enrichment analysis of GO assignments and we chose 24 functional terms based on the significant degree of enrichment analysis from high to low for study. When treated with phenamacril, In total 6 and 2 GO terms could be assigned to resistant isolates Fo1st and FoII5, respectively. In particular, ribosome biogenesis (GO:0042254, 11DEGs), ribonucleoprotein complex biogenesis (GO:0022613, 11DEGs), rRNA processing (GO:0006364, 6DEGs), rRNA metabolic process (GO:0016072, 6DEGs), preribosome (GO:0030684, 7DEGs) and acyl-CoA dehydrogenase activity (GO:0003995, 6DEGs) were significantly enriched in Fo1st compared with Fo1stCK (CK represents the control groups), and heme binding (GO:0020037, 9DEGs) and tetrapyrrole binding (GO:0046906, 9DEGs) were the significantly enriched terms in FoII5 compared with FoII5CK (Fig. 3). However, there are 24 functional groups containing 2033 DEGs were enriched in sensitive isolate Fo3_a compared with Fo3_aCK, which involved in peptide biosynthetic and metabolic process, ribosome and non-membrane-bounded organelle, cytoskeletal protein binding and ATPase activity and so on (Fig. 3). Interestingly, as many as 72 DEGs, which involved in structural molecule activity (GO:0005198, 75 DEGs), were significantly down-regulated. And the cytoskeletal protein binding (GO:0008092, 17DEGs) and ATPase activity (GO:0016887, 36DEGs) were seriously affected in sensitive isolate after treated with phenamacril. These results are in accordance with the previous studies in F. graminearum, which revealed that phenamacril binds to FgMyoI or inhibits ATPase activity of FgMyoI motor domain and thereby reduces the stability of actin cytoskeleton37.

KEGG pathway analysis of DEGs during phenamacril treatment. To investigate the major biological pathways of the DEGs, we aligned all DEGs to KEGG pathways. In the Fo3_a vs. Fo3_aCK group, 91 KEGG metabolic pathways were identified and only the ribosome (70 DEGs) was the most significantly enriched pathways (padj < 0.05). Moreover, a total of 70 DEGs were all significantly down-regulated. In the Fo1st vs. Fo1stCK group, 76 KEGG metabolic pathways were identified and Biosynthesis of secondary metabolites (77 DEGs), Peroxisome (26 DEGs), Ribosome biogenesis in eukaryotes (22 DEGs), Fatty acid metabolism and degradation (25 DEGs), Glyoxylate and dicarboxylate metabolism (14 DEGs), Valine, leucine and isoleucine degradation (12 DEGs), Glycine, serine and threonine metabolism (15 DEGs), 2-Oxocarboxylic acid metabolism (11 DEGs) were the highly enriched pathways (padj < 0.05). In the FoII5 vs. FoII5CK group, 50 KEGG metabolic pathways were identified and only Nitrogen metabolism (4 DEGs) were the highly enriched pathways (padj < 0.05) (Fig. 4). However, a total of 44 identical KEGG pathways were annotated in both the two kinds of resistant isolates after treated with phenamacril, including Glycine, serine and threonine metabolism, Valine, leucine and isoleucine degradation, Fatty acid metabolism or degradation, Carbon metabolism, Nitrogen metabolism, etc (see Supplementary Table S3). These results demonstrate that the resistant isolates enhanced phenamacril tolerance through
amino acid, carbon, nitrogen and fatty metabolism and degradation. In particular, phenamacril inhibited the ribosome biogenesis in eukaryotes in all the isolates (Fig. 4).

**Protein protein interaction network (PPI) analysis of DEGs during phenamacril treatment.** In order to find the interacting proteins of DEGs, we conducted the analysis of PPI using STRING protein interaction database in this paper. In the Fo3_a vs. Fo3_aCK group, we found a total of 7334 DEGs with interacting proteins, in which 6228 DEGs were down-regulated and 615 DEG were up-regulated. In addition, in the Fo1st vs. Fo1stCK and FoII5 vs. FoII5CK groups, there are 2773 and 254 DEGs with interacting proteins, respectively. Interestingly, we found that FoMyo5 (Gene ID: 42025582) was only up-regulated in the Fo3_a vs. Fo3_aCK group but not in the Fo1st vs. Fo1stCK and FoII5 vs. FoII5CK groups. In addition, the interacting protein of FoMyo5, cortactin (Gene id: 42025702) and the proteins (fimbrin, actin-like protein 3, actin cytoskeleton regulator complex protein END3 and actin-like protein 2) that interacts with cortactin were all up-regulated expression ranged from 1.38 to 2.83-fold in sensitive isolate Fo3_a after treated with 1 μg/mL phenamacril (see Supplementary Table S2). These actin cytoskeleton related proteins are required for generation, maintenance, and turnover of actin filaments, contribute to ATP-loaded and rapid filament assembly, which play important roles in vesicle/organelle transport, cell polarization, transcriptional regulation, intracellular transport, and signal transduction. Therefore, phenamacril might stimulated the transcription of FoMyo5 and actin cytoskeleton related genes to maintain stability of mycelium in the phenamacril-sensitive isolate.

**Analyzing the DEGs involved in fungicidal activity of phenamacril in Fo.** In the previous studies, crystal structure of phenamacril-bound *F. graminearum* myosin I suggesting that fungicidal activity of phenamacril results from the inhibition of the ATPase activity of the myosin I. In this paper, we found 40 DEGs were significantly expressed in both phenamacril-resistant and -sensitive isolates during phenamacril treatment (Fig. 2B). Interestingly, 14 DEGs with annotation were all down-regulated by phenamacril and these genes encoded some enzymes and proteins involved in RNA metabolism and cell membrane biosynthesis, such as ATP-dependent RNA helicase, C-8 sterol isomerase and so on (Table 3). As we know, RNA helicases are ubiquitous, highly conserved enzymes that bind or remodel RNA or RNA–protein complexes in an ATP-dependent fashion. These proteins are widely distributed in all three kingdoms of life and are associated with all biological processes involving RNA metabolism, including transcription, splicing, RNA transport, ribosome biogenesis, RNA editing, translation, and RNA decay. Many RNA helicases are essential for viability, and a growing number of these enzymes are known to play major regulatory roles in cells. The largest family of RNA helicases is the DEAD box protein family, which were shown to be involved in the ATPase and helicase activities and in their regulation. In the yeast *Saccharomyces cerevisiae*, 14 DEAD box proteins were shown to be required for ribosome biogenesis and rRNA maturation. Moreover, several DEAD box proteins have been shown to participate in several distinct pathways. In this study, 7 DEAD box proteins (ATP-dependent RNA helicase DPB2, DPB3, DPB9, DPB10, DED1, ATP-dependent RNA helicase RRP3, SPB4) were all down-regulated in both phenamacril-resistant and -sensitive isolates during phenamacril treatment (Table 3). In addition, 50S ribosomal protein L24e, ribosome biogenesis protein n, nucleolin and nucleolar GTP-binding protein, that are related to ribosome biogenesis, were also down-regulated in both phenamacril-resistant and -sensitive isolates during phenamacril treatment (Table 3). These results indicated that phenamacril not only inhibits the ATPase activ-
During cellular metabolism, the triacylglycerol in the treated with 0.1 μL/mL methanol and others represent the treatment groups treated with 1 μg/mL phenamacril. Studies have shown that the utilization of primary amines as nitrogen source by yeasts and moulds is the possession of an amine oxidase. Through these studies, we conclude that the up-regulated expression of nitric oxide synthase of 4-Aminobutyrate aminotransferase in yeast, alcohol dehydrogenase plays an important role in the production of an energy (such as ATP) to maintain mycelial growth. Researchers have reported that inhibitors reduce the catalytic activity of 4-Aminobutyrate aminotransferase. In yeast, alcohol dehydrogenase plays an important role in the conversion of alcohols to aldehydes or ketones. Phenamacril also suppressed the gene expressions of pseudouridylate synthase, PTH2 family peptidyl-tRNA hydrolase and C-8 sterol isomerase (Table 3). Some research showed that peptidyl-tRNA hydrolase played its critical role in protein biosynthesis and sterol C-8 isomerase played an essential regulation role in the ergosterol biosynthesis of Saccharomyces cerevisiae. In particular, RNA pseudouridylate synthase was studied as novel drug target to cure Malaria caused by Plasmodium Falciparum. Therefore, further research on how phenamacril inhibited the 14 functional genes, would help us to develop new fungicides to control Fo.

Table 3. The DEGs involved in fungicidal activity of phenamacril in Fo. "CK" represents the control groups treated with 0.1 μL/mL methanol and others represent the treatment groups treated with 1 μg/mL phenamacril.

| Gene id   | Gene name                                      | Fold change (log2 ratio)a |
|----------|-----------------------------------------------|---------------------------|
| 42037078 | FOIG_11903                                    | −2.33 −3.85 −2.94         |
| 42034116 | FOIG_08941                                    | −2.56 −4.55 −2.63         |
| 42029194 | FOIG_04019                                    | −2.94 −6.25 −2.63         |
| 42038835 | FOIG_10660                                    | −2.32 −3.85 −2.94         |
| 42029337 | FOIG_04162                                    | −2.63 −4. −1.54           |
| 42032856 | FOIG_07661                                    | −2.70 −3.23 −1.45         |
| 42030982 | FOIG_05787                                    | −2.38 −2.17 −1.22         |
| 42030757 | FOIG_05582                                    | −2.35 −3.61 −2.86         |
| 42026067 | FOIG_08892                                    | −2.30 −3.46 −1.50         |
| 42033276 | FOIG_08101                                    | −3.36 −5 −2.75            |
| 42025910 | FOIG_00735                                    | −2.42 −2.92 −2.62         |
| 42028022 | FOIG_02847                                    | −1.85 −1.84 −3.76         |
| 42034103 | FOIG_05787                                    | −2.38 −2.08 −1.88         |
| 42029667 | FOIG_04492                                    | −1.89 −1.94 −1.62         |

Analyzing the DEGs involved in resistance regulation of phenamacril in Fo. To screen for unique DEGs involved in the resistance regulation process, we compared the transcriptome of phenamacril-resistant isolates Fo1st and FoII5 with phenamacril-sensitive isolate Fo3_a during phenamacril treatment. When treated with phenamacril, 29 DEGs were significantly up-regulated and 26 DEGs were significantly down-regulated in both the phenamacril-resistant isolates Fo1st and FoII5. However, these genes were not significantly expressed in phenamacril-sensitive isolate. In particular, 14 up-regulated DEGs with annotation in both the phenamacril-resistant isolates Fo1st and FoII5. However, these genes were not significantly expressed in phenamacril-sensitive isolate. In particular, RNA pseudouridylate synthase was studied as novel drug target to cure Malaria caused by Plasmodium Falciparum. Therefore, further research on how phenamacril inhibited the 14 functional genes, would help us to develop new fungicides to control Fo.

In recent years, researchers have used transcriptome analysis to explore the response mechanism of fungicides to DMI or other fungicides. These results indicated that expression of genes involved in steroid biosynthesis, cell wall integrity, MFS transporters, ATP-binding cassette (ABC) transporters and oxidative stress response were associated with fungicide resistance in multiple fungi. In this paper, we found that MFS transporters, ABC transporter ATP-binding protein ARB1, chitinase and multiple oxidoreductases participated in the resistance regulation to phenamacril in Fo. The above mentioned data represent the first report of response to phenamacril...
in Fo and contribute to our knowledge in the mechanisms associated with fungicide resistance and fungicidal activity development in this fungal species complex.

Conclusion

In this study, we found that the amino acid substitutions (V151A and S418T) in FoMyo5 cause natural low resistance to phenamacril in the plant pathogenic Fo isolates. By a comparative transcriptome analysis of phenamacril-resistant and -sensitive isolates after 1 μg/mL of phenamacril treatment, a series of DEGs that might be associated with resistance regulation and fungicidal activity of phenamacril were identified. These genes were involved in oxidation–reduction processes, MFS transporters, ATP-dependent RNA helicase and ribosomal biogenesis related proteins. These results indicated that phenamacril not only seriously affected the cytoskeletal protein binding and ATPase activity of sensitive isolate, but also suppressed ribosome biogenesis in all the isolates. This study provides deeper insight into resistance regulation mechanism and fungicidal activity of phenamacril in Fo and reference for the development of new fungicides to control diseases caused by Fo.

Methods

Isolates, chemicals and culture conditions. The isolates used in this study are listed in Table S1 and included the Fo isolate Fo3_a from cornea of patients with fungal keratitis and seven other Fo formae speciales LA0, CAO0, Fo1st, FoII5, Fo3-2, FoX-KW and FoHGKW from the roots of chili pepper, strawberry, lotus, banana, eggplant, watermelon and cucumber, respectively. All the isolates were routinely maintained at 28 °C on Difco™ Potato Dextrose Agar plates (PDA, suspend 39 g of the powder in 1 L of purified water and autoclave at 121 °C for 15 min). For mycelial growth assays, the isolates were grown at 28 °C on PDA plates for 7 days. For sporulation assays, 15 fresh mycelial plugs taken from the periphery of a 3-day-old colony of Fo isolates Fo1st, FoII5 and Fo3_a were added to a 250-mL flask containing 150 mL of Difco™ Potato Dextrose Broth (PDB, suspend 24 g of the powder in 1 L of purified water and autoclave at 121 °C for 15 min) medium.

Technical-grade phenamacril (95%; experimental code JS399-19), which was kindly provided by the Jiangsu Pesticide Research Institute Co., Ltd, Nanjing, China, was dissolved in methanol to 10 mg/mL and stored at 4 °C. Fungicide susceptibility testing. Phenamacril was added into autoclaved PDA media for testing inhibition of mycelia growth. Mycelial plugs (5 mm in diameter) taken from the margin of a 3-day-old colony were placed on the periphery of a 3-day-old colony of Fo isolates Fo1st, FoI5 and Fo3_a were added to a 250-mL flask containing 150 mL of Difco™ Potato Dextrose Broth (PDB, suspend 24 g of the powder in 1 L of purified water and autoclave at 121 °C for 15 min) medium.

Table 4. The DEGs involved in resistance regulation of phenamacril in Fo. *NA represents no significant difference or no expression level of genes was detected. The fold change value is represented by the log2 ratio. 'CK' represents the control groups treated with 0.1 μL/mL methanol and others represent the treatment groups treated with 1 μg/mL phenamacril.

| Gene id  | Gene_name | Annotation                                                                 | Fold change (log2 ratio) |
|---------|-----------|-----------------------------------------------------------------------------|--------------------------|
| 42029749 | FOIG_04574 | Nitric oxide dioxygenase                                                    | 6.47                     |
| 42031140 | FOIG_05965 | Uroporphyrin-III C-methyltransferase/ precorrin-2 dehydrogenase/ sirohydro-| 3.42                     |
| 42035331 | FOIG_10156 | Triacylglycerol lipase                                                       | 10.78                    |
| 42031672 | FOIG_06497 | Alcohol dehydrogenase                                                       | 3.26                     |
| 42034308 | FOIG_09133 | MFS transporter, SP family, general alpha glucoside:H + symporter           | 21.08                    |
| 42026895 | FOIG_01720 | Pyridoxamine 5'-phosphate oxidase                                            | 3.63                     |
| 42031895 | FOIG_06720 | Nitrate reductase [NADPH]                                                    | 6.11                     |
| 42027369 | FOIG_02194 | Nitrite reductase [NADPH]                                                    | 1.51                     |
| 42039770 | FOIG_14595 | Primary-amine oxidase                                                        | 6.11                     |
| 42029419 | FOIG_04244 | MFS transporter, SHS family, lactate transporter                           | 10.51                    |
| 42026345 | FOIG_01170 | 2,4-diensoyl-CoA reductase [NADPH]                                          | 3.77                     |
| 42027069 | FOIG_01894 | Acetyl-CoA hydrolase                                                        | 1.66                     |
| 42030291 | FOIG_05116 | 4-Aminobutyrate aminotransferase                                            | 4.53                     |
| 42031645 | FOIG_06470 | Dihydroxy-acid dehydratase mitochondrinal                                   | 3.79                     |
| 42027968 | FOIG_02793 | Succinate dehydrogenase (ubiquinone) flavoprotein subunit                  | 2.43                     |
| 42027532 | FOIG_02357 | Elongation factor 3                                                         | −5.03                    |
| 42031693 | FOIG_06518 | ABC transporter ATP-binding protein ARBI                                    | −3.10                    |
| 42032779 | FOIG_07604 | Chitinase                                                                   | −2.06                    |
| 42029731 | FOIG_04556 | Biphynyl-2,3-diol 1,2-dioxygenase                                           | −3.79                    |
| 42030858 | FOIG_05683 | Peptidyl-prolyl cis-trans isomerase NIMA-interacting 4                     | −1.71                    |
Sequence alignment of FoMyo5 motor domains. All Fo isolates were cultured in PDB at 28 °C for 3 days and the mycelia were collected and finely ground to a powder using a mortar and pestle with liquid nitrogen. The total RNA was extracted using the E.Z.N.A. Fungal RNA Kit (Omega Bio-tek, Inc., Norcross, USA) following the manufacturer’s instructions and used for reverse transcription with the PrimeScript® RT reagent Kit (TaKaRa). The sequences of FoMyo5 motor domains were amplified from the cDNAs of all Fo isolates using the primer pairs FoMyo5F/FoMyo5R in this study (FoMyo5F: 5’-ATGGGAATAATCAAGAGCC-3’; FoMyo5R: 5’-TTTGATAAGGCCCTTTGT-3’), which were synthesized by Tsingke Biotechnology Co., Ltd. Then these amplicons were gel purified using the OMEGA BIO-TEK (Shanghai, China) gel purification kit, cloned into the PMD18-T vector and sequenced in Tsingke Biotechnology Co., Ltd. And we aligned these FoMyo5 motor domains using Bioedit 7.2 software (Isis Pharmaceuticals).

Sampling for RNA extraction. The spores harvested from 3-day-old PDB cultures of one phenamacril-sensitive Fo isolate Fo3_a and two phenamacril-resistant Fo isolates FoII5 and Fo1st were collected and suspended in sterile distilled water at 1 x 10^6 spores/mL. The freshly harvested spores of each isolate were cultured in two flasks containing 100 mL liquid YEPD medium (w/v, 1% peptone, 0.3% yeast extract, 2% glucose) on a shaking table at a speed of 175 r/min for 12 h in the dark at 28 °C and each flask was inoculated with 100 μL of the spore suspensions. Then we added 10 μL 10 mg/mL phenamacril into the flasks of treatment groups and made the final concentration of phenamacril 1 μg/mL. we added 10 μL methanol into the flasks of control groups (CK) and made the final concentration of methanol 0.1 μL/mL. A total of 3 control isolates and 3 treatment isolates continued to be cultured for 12 h at 28 °C. After 24 h, the young mycelium were collected and finely ground to a powder using a mortar and pestle with liquid nitrogen and the total RNA was extracted using the above method. The experiment was repeated three times and we got a total of 18 RNA samples.

RNA-seq libraries construction and Illumina sequencing. The RNA purity was detected with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). All samples passed the quality screening steps and were used for the subsequent steps. Total RNA was used as input material for the RNA libraries preparations. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in First Strand Synthesis Reaction Buffer5X. First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase, then use RNaseH to degrade the RNA. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and dNTP. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3’ ends of DNA fragments, Adapter with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 370–420 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. After quantity and quality monitoring, 18 cDNA libraries were sequenced using the Illumina NovaSeq 6000 platform in the Novogene Corporation at Beijing, China.

Fo de novo transcriptome assembly and analysis. Raw reads of fastq format were firstly processed through in-house perl scripts. In this step, clean reads were obtained for the 18 libraries by removing reads containing adapter, reads containing N base and low quality reads from raw data with Trimmomatic73. At the same time, QC, Q20, Q30 and GC content the clean data were calculated. All the downstream analyses were based on the clean data with high quality. Hisat2 v2.0.5 was used to align the paired-end clean reads to the reference genome of Fusarium odoratissimum NRRL 54006 reference genome (GCF_000260195.1, FO_II5_V1—Genome—Assembly—NCBI (nih.gov)) based on the gene model annotation file74. The mapped reads of each sample were assembled by StringTie (v1.3.3b) in a reference-based approach75. StringTie uses a novel network flow algorithm as well as an optional de novo assembly step to assemble and quantitate fulllength transcripts representing multiple splice variants for each gene locus. featureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene76. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels.

Identification of differential expression genes (DEGs) and clustering analysis. Differential genes expression level of the three isolates under 1 μg/mL phenamacril treatment and 0.1 μL/mL methanol treatment conditions were compared by the Wald test using the DESeq2 R package (1.20.0). The comparison groups include Fo3_a vs. Fo3_aCK, Fo1st vs. Fo1stCK and FoII5 vs. FoII5CK, that CK represents the control groups treated with 0.1 μL/mL methanol and others represent the treatment groups treated with 1 μg/mL phenamacril. DESeq2 provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P-values were adjusted using the Benjamini and Hochberg’s approach for controlling the false discovery rate. Genes with an adjusted P-value < 0.05 and
2.1 https://bioinfogp.cnb.csic.es/tools/venny/index.html. Overlapping genes within the DEG datasets and the generation of Venn diagrams were determined using Venny.

KEGG pathways. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput put experimental technologies (http://www.genome.jp/kegg/). We used cluster Profiler R package to test the statistical enrichment of differential expression genes in KEGG pathways.

GO, KEGG and PPI analysis of differentially expressed genes. Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the R (4.1.1) and clusterProfiler R package (v4.0.5), in which gene length bias was corrected. GO terms with corrected P-value less than 0.05 were considered significantly enriched by differential expressed genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput put experimental technologies (http://www.genome.jp/kegg/). We used cluster Profiler R package to test the statistical enrichment of differential expression genes in KEGG pathways.

Protein Protein Interaction network (PPI) analysis of differentially expressed genes was based on the STRING database, which known and predicted Protein–Protein Interactions. We provide differential gene protein interaction network data file, which can be directly imported into Cytoscape software for visual editing. For example, the size of nodes in the interaction network diagram is directly proportional to the degree of this node, that is, the more edges connected to this node, the greater its degree, the larger the nodes, and these nodes may be in a more core position in the network. Node1_protein and node2_protein represents interacting protein, as well as node1_gene and node2_gene represents the gene ID corresponding to the interacting protein. The score indicates the degree of interaction.

Data availability
Isolates of Fusarium oxysporum used in this study are available upon request. The raw sequence data from the 18 samples reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2022) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA006003) that are publicly accessible at https://ngdc.cnbc.ac.cn/gsa. The data and material that support the findings of this study are available from the corresponding author on request.

Received: 28 January 2022; Accepted: 20 June 2022
Published online: 30 June 2022

References
1. Thatcher, L. F., Gardiner, D. M., Kazan, K. & Manners, J. M. A highly conserved effector in Fusarium oxysporum is required for full virulence on Arabidopsis. Mol. Plant Microbe Interact. 25, 180–190 (2011).
2. Ma, L. J. et al. Fusarium pathogenomics. Annu. Rev. Microbiol. 67, 399–416 (2013).
3. Kwiatos, N., Małgorzata, R. & Stanisław, B. Diversity of laccase-coding genes in Fusarium oxysporum as a model for Fusarium pathogenomics. Mol. Plant Microbe Interact. 25, 180–190 (2011).
4. Baayen, R. P. et al. Gene genealogies and AFLP analyses in the Fusarium oxysporum complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. Phytopathology 90, 891–900 (2000).
5. Larkin, R. P. Ecology of Fusarium oxysporum f. sp. niveum in soils suppressive and conducive to fusarium wilt of watermelon. Phytopathology 83 (1993).
6. Altunk, H. H. First report of fusarium wilt of eggplant caused by Fusarium oxysporum f. sp. melongenae in Turkey. Plant Pathology 54, 577 (2005).
7. Ploetz, R. C. Fusarium wilt of banana is caused by several pathogens referred to as Fusarium oxysporum f. sp. cubense. Phytopathology 96, 648 (2006).
8. Wongna, A. & Lonthaisong, K. Changes in the 2DE protein profiles of chilli pepper (Capsicum annuum) leaves in response to Fusarium oxysporum infection. ScienceAsia 36, 259–270 (2010).
9. Fang, X., Kuo, J., Ming, P. Y., Finnegan, P. M. & Barbetti, M. J. Comparative root colonisation of strawberry cultivars Camarosa and Festival by Fusarium oxysporum f. sp. fragariae. Plant & Soil 358, 75–89 (2012).
10. Hernandez-Monjaraz et al. Isolation and expression of enolase gene in Fusarium oxysporum f. sp. lycopersici. Appl. Biochem. Biotechnol. Part A Enzyme Eng. Biotechnol. (2015).
11. Chang-Zhi, H. U. et al. Purification and characterization of cell wall degrading enzymes from Fusarium oxysporum f. sp. nelumbicola. Biol. Disaster Sci. (2016).
12. Nucci, M. & Anaisse, E. Fusarium infections in immunocompromised patients. Clin. Microbiol. Rev. 20, 695–704 (2007).
13. Jain, P. K., Gupta, V. K., Misra, A. K., Gaur, R. & Issar, S. Current status of fusarium infection in human and animal. Asian J. Anim. Vet. Adv. 6, 201–227 (2011).
14. Katja, S., Di, P. A., Gow, N., Donna, M. C. & Andes, D. R. Murine model for Fusarium oxysporum as a multihost model for the genetic dissection of fungal virulence in plants and mammals. Phytopathol. 25, 180–190 (2011).
15. Nel, B. Management of Fusarium wilt of banana by means of biological and chemical control and induced resistance. (2011).
16. Berg, G. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of bioorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18 (2009).
17. Odds, F. C., Gerven, E. V., Espinel-Ingroff, A., Bartlett, M. S. & Walsh, T. J. Evaluation of possible correlations between antifungal susceptibilities of filamentous fungi in vitro and antifungal treatment outcomes in animal infection models. Antimicrob. Agents Chemother. 42, 282–288 (1998).

log2 (Fold change)> 1 were assigned as significantly differentially expressed. Identification of unique or overlapping genes within the DEG datasets and the generation of Venn diagrams were determined using Venny.

The differential genes of all comparison groups were taken and collected as the differential gene set. We used H-cluster method to cluster the expression of differential genes after normalization with log2 (FPKM + 1). We plot the heatmap and genes or samples with similar expression patterns in the heatmap are gathered together.
20. Ana et al. Antifungal susceptibility profile of clinical Fusarium spp. isolates identified by molecular methods. *J. Antimicrobial Chemother.* 64, 805–809 (2008).
21. Bolton, M. D. & Thomma, B. P. H. J. [Methods in molecular biology] Plant fungal pathogens volume 835 | Fungicide resistance assays for fungal plant pathogens. https://doi.org/10.1007/978-1-61779-501-5, 385–392 (2012).
22. Li, H. et al. SS399-19, a new fungicide against wheat scab. *Crop Prot.* 27, 90–95 (2008).
23. Hsu, Y. P., Qu, X. P., Mao, X. W., Kuang, J. & Zhou, M. G. Resistance mechanism of Fusarium fujikuroi to phenamacril in the field. *Pest Manag. Sci.* 74, 607–616 (2018).
24. Zheng, Z. et al. FoMyo5 motor domain substitutions (Val 151 to Ala and Ser 418 to Thr) cause natural resistance to fungicide phenamcar in *Fusarium oxysporum*. *Pestic. Biochem. Physiol.* 147, 199–126 (2018).
25. Li, B. et al. Genotypes and characteristics of phenamcar-resistant mutants in *Fusarium asiaticum*. *Plant Dis.* 100, 1754–1761 (2016).
26. Zheng, Z. et al. Whole-genome sequencing reveals that mutations in myosin-5 confer resistance to the fungicide phenamcar in *Fusarium graminearum*. *Sci. Rep.* 5, 8248 (2015).
27. Mermall, V., Post, P. L. & Mooseker, M. S. Unconventional myosins in cell movement, membrane trafficking, and signal transduction. *Science* 279, 527–533 (1998).
28. Marrs, M. A., Finan, D., Sivaramakrishnan, S. & Sudich, J. A. Principles of unconventional myosin function and targeting. *Annu Rev Cell Dev Biol* 27, 133–155 (2011).
29. Wang, Z., Gerstein, M. & Snyder, M. RNA-Seq: a revolutionary tool for transcriptomics.
30. Ceragioli, M., Mols, M., Moezelaar, R., Ghelardi, E. & Abee, T. Comparative transcriptomic and phenotypic analysis of the responses of basidiospores to variable environmental treatments. *Appl. Environ. Microbiol.* 76, 3352–3360 (2010).
31. Liu, X., Jiang, J., Shao, J. & Ma, Y. Z. Gene transcription profiling of *Fusarium graminearum* treated with an azole fungicide tebuconazole. *Applied Microbiol. Biotechnol.* 85, 1105–1114 (2010).
32. Zhang, M., Ge, J. & Yu, X. Transcriptome analysis reveals the mechanism of fungicidal of thymol against *Fusarium oxysporum* f. sp. niveum. *Current Microbiology* 75, 410–419 (2015).
33. Griffith, et al. De novo assembly and analysis of RNA-seq data. *Nat. Methods* 7, 909–912 (2010).
34. Canales, J. et al. De novo assembly of maritime pine transcriptome: implications for forest breeding and biotechnology. *Plant Biotechnol.* J. 12, 286–299 (2014).
35. Zhang, C. et al. A small molecule species specifically inhibits Fusarium myosin I. *Environ. Microbiol.* 17, 2735–2746 (2015).
36. Ni, T., Yuan, M., Ji, H. H., Tang, G. & Li, X. D. Effects of mutations in the phenamcar-binding site of fusarium myosin-1 on its motor function and phenamcar sensitivity. *ACS Omega* 5, 21815–21823 (2020).
37. Zhou, Y., Zhou, X. E., Gong, Y., Zhu, Y. & Zhang, F. Structural basis of Fusarium myosin I inhibition by phenamcar. *PLoS Pathog.* 16, e1008323 (2020).
38. Skau, C. T. et al. Actin filament bundling by fimbrin is important for endocytosis, cytokinesis, and polarization in fusion yeast. *J. Biol. Chem.* 286, 26964–26977 (2011).
39. Moseley & B. Twinfilin is an actin-filament-severing protein and promotes rapid turnover of actin structures in vivo. *J. Cell Sci.* 119, 1547–1557 (2006).
40. Liang, C., Marshall, T. W., Uetrecht, A. C., Schafer, D. A. & Bear, J. E. Coronin 1B coordinates Arp2/3 complex and cofillin activities at the leading edge. *Cell* 128, 915–929 (2007).
41. David, et al. Three’s company: the fission yeast actin cytoskeleton. *Trends Cell Biol.* 21, 177–187 (2011).
42. Tanner, N. K. DExD/H box RNA helicases: from generic motors to specific dissociation functions. *Trends Biochem. Sci.* 36, 19–29 (2011).
43. Pyle, A. M. RNA helicases and remodeling proteins. *Curr. Opin. Chem. Biol.* 15, 636–642 (2011).
44. Abdelhaleem, M. Do human RNA helicases have a role in cancer?. *Biochem. Biophys. Acta.* 1704, 37–46 (2004).
45. Tanner & Kyle, N. The newly identified Q motif of DEAD box helicases is involved in adenine recognition. *Mol. Cell Biochem.* 290, 209–298 (2004).
46. Dresios, J., Derkatch, I. L., Liebman, S. W. & Synetos, D. Y.east ribosomal protein L24 affects the kinetics of protein synthesis and ergosterol biosynthesis of *Saccharomyces cerevisiae*. *Nature* 460, 1063–1068 (2009).
47. Lancellotti, L., Borsari, M., Bonifacio, A., Bortolotti, C. A. & Sola, M. Adsorbing surface strongly influences the pseudoperoxidase and nitrite reductase activity of electrode-bound yeast cytochrome c. The effect of hydrophobic immobilization. *Bioelectrochemistry* 128, 21815–21823 (2020).
48. Gurvitz, A. Agents Chemother.* 77, 1279–1288 (2014).
49. Hall, N. & Tomsett, A. Structure–function analysis of NADPH:nitrate reductase from *Aspergillus nidulans*: analysis of altered motor function and phenamcar sensitivity. *Environ. Microbiol.* 21, 527–538 (2019).
50. Jankowsky, E. RNA helicases at work: binding and rearranging. *Trends Cell Biol.* 17, 177–187 (2011).
51. Shiva, S., Zhi, H., Grubina, R., Sun, J. & Gladwin, M. T. Deoxymyoglobin is a nitrite reductase that generates nitric oxide and nitrite reductase activity of electrode-bound yeast cytochrome c. The effect of hydrophobic immobilization. *Bioelectrochemistry* 128, 21815–21823 (2020).
52. Hall, N. & Tomsett, A. Structure–function analysis of NADPH:nitrate reductase from *Aspergillus nidulans*: analysis of altered motor function and phenamcar sensitivity. *Environ. Microbiol.* 21, 527–538 (2019).
53. Lancellotti, L., Borsari, M., Bonifacio, A., Bortolotti, C. A. & Sola, M. Adsorbing surface strongly influences the pseudoperoxidase and nitrite reductase activity of electrode-bound yeast cytochrome c. The effect of hydrophobic immobilization. *Bioelectrochemistry* 128, 21815–21823 (2020).
54. Gurvitz, A. et al. The Saccharomyces cerevisiae peroxisomal 2,4-dienoyl-CoA reductase is encoded by the oleate-inducible gene *ELO5*. *Biochem. Biophys. Acta.* 1856, 1279–1288 (2014).
55. Shiva, S., Zhi, H., Grubina, R., Sun, J. & Gladwin, M. T. Deoxymyoglobin is a nitrite reductase that generates nitric oxide and nitrite reductase activity of electrode-bound yeast cytochrome c. The effect of hydrophobic immobilization. *Bioelectrochemistry* 128, 21815–21823 (2020).
56. Gurvitz, A. et al. The Saccharomyces cerevisiae peroxisomal 2,4-dienoyl-CoA reductase is encoded by the oleate-inducible gene *ELO5*. *Biochem. Biophys. Acta.* 1856, 1279–1288 (2014).
57. Shiva, S., Zhi, H., Grubina, R., Sun, J. & Gladwin, M. T. Deoxymyoglobin is a nitrite reductase that generates nitric oxide and nitrite reductase activity of electrode-bound yeast cytochrome c. The effect of hydrophobic immobilization. *Bioelectrochemistry* 128, 21815–21823 (2020).
Author contributions
Z.Z. designed the study. Z.Z., H.L., Y.S., Z.L. and H.T. performed the experiments. Z.Z., H.L., and Y.S. analyzed the data. Z.Z. and H.L. wrote the manuscript with assistance from other authors. S.D., L.W., Y.W. and F.Z. revised and edited the paper. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-15188-5.

Correspondence and requests for materials should be addressed to Z.Z., S.D. or Y.W.

Reprints and permissions information
is available at www.nature.com/reprints.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access
This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022