Supplementary Material

**Biological control potential of *Chaetomium globosum* 12XP1-2-3 and its effects on rhizosphere microorganisms of wheat under Fusarium crown rot pathogen attack**

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**supplementary inhibition rate**

**Supplementary Figure 1.** Inhibition rate of *Chaetomium globosum* 12XP1-2-3 against *Fusarium* spp. causing FCR in confrontation assay. The accession numbers of these *F.* spp. for translation elongation factor 1-alpha gene were as follows: *F.graminearum*, OM650500; *F.acuminatum*, KX663794; *F.sinensis*, OM686983; *F.equiseti*, KX663677; *F.proliferatum*, KX663598; *F.asiaticum*, KY081500; *F.culmorum*, KX702638; *F.avenaceum*, KX702713. Data were presented as mean with their standard error (SE) and analyzed using one-way factorial analysis of variance (ANOVA) and Duncan multiple range test (*p*<0.05). The same lower case letters (“a”, “b” and so on) mean that they are not significantly different at *p*<0.05.

**supplementary Fig2 final**

**Supplementary Figure 2.** Relative expression of 15 defense-related genes in wheat seedlings 7 days and 21 days after inoculation by *F. pseudograminearum* G14LY24-2. Cg+Fpg, *C. globosum* coated seeds plus inoculation of *F. pseudograminearum*; Con+Fpg, control seeds plus inoculation of *F. pseudograminearum*; Cg-Fpg, *C. globosum* coated seeds without inoculation of *F. pseudograminearum*; Con-Fpg, control seeds without inoculation of *F. pseudograminearum*. Detailed information of the genes were shown in Supplementary Table 1. Data were presented as mean with standard error (SE) and analyzed using one-way factorial analysis of variance (ANOVA) and Duncan’s multiple range test (*p* < 0.05). Lower case letters at each time period (“a”, “b” and “ab” at 7 days, and “x”, “y” and “xy” at 21 days) indicate differences among the four treatments, and the same letters mean that they are not significantly different at *p*<0.05.

**Fig S1**

**Supplementary Figure 3.** Wheat plants (cv. Aikang 58) grown in the field of Wenxian in 2019. **(A, B)** Wheat seedlings of the ‘Con’ **(A)** and ‘Cg’ **(B)** treatments on December 26, 2018; **(C, D)** Wheat plants of the ‘Con’ **(C)** and ‘Cg’ **(D)** treatments on April 11, 2019; **(E)** Wheat plants of the ‘Con’ (left) and ‘Cg’ (right, with earlier flowering) treatments on April 24, 2019. Con, control group without seed coating; Cg, treatment with wheat seeds coated with the ascospore of *C. globosum* 12XP1-2-3. Plants in the ‘Cg’ treatment showed better growth state and earlier flowering than those in the control.

**Supplementary Figure**

**Supplementary Figure 4.** Shannon index dilution curve of bacterial **(A)** and fungal **(B)** community.

**Supplementary Table 1.** Sequences of primers used for RT-qPCR analysis of gene expression.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Classification** | **Gene** | **Primer (5’to 3’)** | **Description** | **References** |
| Defence - antimicrobial | PR1 | F:CTGGAGCACGAAGCTGCAG  R:CGAGTGCTGGAGCTTGCAGT | Wheat nonexpressor of pathogenesis-related Genes 1 | Desmond et al. 2006 |
| PR2 | F:CTCGACATCGGTAACGACCAG  R:GCGGCGATGTACTTGATGTTC | Encoding beta-1,3-endoglucanase | Desmond et al. 2006 |
| PR3 | F:AGAGATAAGCAAGGCCACGTC  R:GGTTGCTCACCAGGTCCTTC | Chi1 gene | Liu et al. 2016 |
| PR4 | F:CGAGGATCGTGGACCAGTG  R:GTCGACGAACTGGTAGTTGACG | Wheatwin 1-2 gene | Desmond et al. 2006 |
| PR5 | F:ACAGCTACGCCAAGGACGAC  R:CGCGTCCTAATCTAAGGGCAG | Encoding WAS3a thaumatin-like protein | Desmond et al. 2006 |
| PR10 | F:TTAAACCAGCACGAGAAACATCAG  R:ATCCTCCCTCGATTATTCTCACG | Encoding wheat peroxidase | Desmond et al. 2006 |
| EG | F: GACAACGGGCTGACATACAC  R: TCTCGGATATGACCACCTTAACC | Endo-ß-glucanase | Powell et al. 2017 |
| Metabolism | PAL1 | F:CATACCCGCTCTACAGGTTCGT  R:CAAGGGCTCACCGTTCCAC | Phenylalanine ammonia-lyase | Duan et al.2021 |
| PAL2 | F:CGTCAAGAGCTGTGTGAAGATGG  R:GGTAGTTGGAGCTGCAAGGGTC | phenylalanine ammonia lyase | Liu et al. 2016 |
| Glu | F:TTCCGACTAGCTACACACAAAAGGT  R:AGTTCATATTCGTGTCGCTTCATC | Glucosyltransferase | Desmond et al. 2008 |
| Signalling | MAP | F:CACTGGGTCGTGACACTTCT  R:CCTCCTCTTCCTTGTATGCTG | Mitogen-activated protein kinases | Wang et al. 2011 |
| Stpk-V | F: TGGATGTATTATGAGCAGGGAG  R: GAGGATGAAGCGAAAGCAA | Serine/threonine kinase-*Haynaldia villosa* | Wang et al. 2015 |
| Defence – ROS | TaGLP5 | F:ACGAGCACAAACAGAAATAGGA  R:GAAGGAAGGAAGAGGAGGATG | A wheat germin-like protein | Wang et al. 2009 |
| RP | F: GTCATCGACAGCATCAAGAC  R: CTCGTTTGCATCTATGGAATCTC | Root peroxidase | Powell et al. 2017 |
| Transcription | WRKY | F:GCAGACGTACAACCAGAACC  R:CCCAAAGTGCTTCTCGATCTC | WRKY transcription factor | Powell et al. 2017 |
| Jasmonic acid pathway | TaAOS | F:TCCCGAGAGCGCTGTTTAAA  R:GACGATTGACGGCTGCTATGA | *Triticum aestivum* allene oxide synthase | Liu et al. 2016 |
| JAZ1 | F:GCAAGCCCTCTTTGTTCGTTC  R:AAGCTGGTAGCACTCGTCTTTATTT | Jasmonate ZIM domain-containing protein 10 | Duan et al.2021 |
| LIPASE | F:CACAAAATATCGACCCACCAC  R:ACTGGGTATTCGTCTGTCAGC | Encoding a wheat lipase | Lu et al. 2006 |
| Unknown | TaWIR1b | F:TGCCGCACAGTTTATGGATG  R:TAAGGTGGTGCGTGACAGTAGA | Wheat induced resistance 1 | Wang et al. 2017 |
| WCI3 | F:AAAGTTGGTCTTGCCACTGACTG  R:TCGACAAAGCACTTCTGGATTTC | Wheat chemically induced gene, encoding sulfur-rich/thionin-like protein | Desmond et al. 2006 |
| PDR | F: GCCAAGCAACATTCTTCTCAG  R: CTTTGCCAGTCGTTCCATACTC | Pleiotropic drug resistance 4 | Powell et al. 2017 |
| Reference gene | Plant 18S | F:CAAAGCAAGCCTACGCTCT  R:ATACGAATGCCCCCGACT | Haematococcus pluvialis 18S ribosomal RNA gene | Liu et al. 2016 |

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**Supplementary Table 2.** Field investigation on wheat (cv. Aikang58) height, root length, fresh weight, dry weight and chlorophyll content at Wenxian in 2019.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treat-ment | Feekes 3 | | | | Feekes 7 | | | | Chlorophyll content at Feekes 7 (SPAD) | |
| Plant height (cm) | Root length (cm) | Fresh weight (g) | Dry weight (g) | Plant height (cm) | Root length (cm) | Fresh weight (g) | Dry weight (g) | Flag leaf | Top second leaf |
| Dif | 14.1±0.6 a | 9.0±0.5 a | 0.44±0.03 a | 0.081±0.004 a | 50.0±1.5 a | 6.6±0.4 b | 5.9±0.4 a | 2.48±0.31 a | 38.9±1.2 b | 51.4±1.6 b |
| Cg | 14.4±0.7 a | 9.5±1.0 a | 0.47±0.04 a | 0.081±0.002 a | 52.1±1.7 a | 7.9±0.2 a | 6.6±0.7 a | 2.55±0.19 a | 51.5±0.9 a | 56.2±0.3 a |
| Con | 13.9±0.6 a | 9.4±0.7 a | 0.40±0.01 a | 0.082±0.005 a | 49.7±2.7 a | 6.3±0.2 b | 5.7±0.6 a | 2.18±0.13 a | 43.2±3.5 b | 56.2±1.0 a |

Dif, treatment with difenoconazole seed coating agent (Syngenta) at the dosage of 300g per 100kg seeds; Cg, treatment with wheat seeds coated with the ascospore of *C. globosum* 12XP1-2-3; Con, control group without seed coating. Data were presented as mean with their standard error (SE) and analyzed using one-way factorial analysis of variance (ANOVA) and Duncan multiple range test (*p*<0.05). Lower case letters (“a” and “b”) indicate differences of the same index among three treatments, and the same letters mean that they are not significantly different at *p*<0.05.

**Supplementary Table 3.** The characteristics of co-occurrence networks.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Networks | No. Nodes | edges | Positive edges | Negative edges | Negative edge rates (%) | Clustering coefficient | Avg. degree | Modularity | Network density |
| Cg-Bacteria | 190 | 1538 | 950 | 588 | 38.2 | 0.505 | 16.189 | 0.375 | 0.086 |
| Con-Bacteria | 193 | 1215 | 835 | 380 | 31.3 | 0.47 | 12.591 | 0.52 | 0.066 |
| Cg-Fungi | 53 | 164 | 119 | 45 | 27.4 | 0.499 | 6.189 | 0.396 | 0.119 |
| Con-Fungi | 62 | 228 | 139 | 89 | 39.0 | 0.595 | 7.355 | 0.421 | 0.121 |

Cg-Bacteria, bacterial co-occurrence network for the ‘Cg’ treatment; Con-Bacteria, bacterial co-occurrence network for the control; Cg-Fungi, fungal co-occurrence network for the ‘Cg’ treatment; Con-Fungi, fungal co-occurrence network for the control.

**Supplementary Table 4.** The taxonomic composition of bacterial phylum and fungal class in ‘Cg’ and ‘Con’ networks.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Bacterial phylum | Proportion (%) | | Fungal class | Proportion (%) | |
| Cg | Con | Cg | Con |
| Proteobacteria | 41 | 41.06 | Sordariomycetes | 27.12 | 25.76 |
| Actinobacteriota | 23 | 21.74 | Agaricomycetes | 18.64 | 21.21 |
| Bacteroidota | 16.5 | 17.87 | Dothideomycetes | 16.95 | 10.61 |
| Acidobacteriota | 3.5 | 3.38 | Tremellomycetes | 6.78 | 9.09 |
| Gemmatimonadota | 3.5 | 3.38 | Chytridiomycetes | 6.78 | 7.58 |
| Myxococcota | 3 | 3.38 | Unidentified | 6.78 | 6.06 |
| Firmicutes | 2.5 | 2.42 | Mortierellomycotina | 5.08 | 4.55 |
| Other | 7 | 6.77 | Other | 11.87 | 15.14 |

Cg, networks of the ‘Cg’ treatment; Con, networks of the control.