Supplemental File

Fluorescently tagged *Verticillium dahliae* to understand the infection process on cotton (*Gossypium hirsutum*) and its survival on other plants including weed species

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A diagram of a plant

Description automatically generated

**Figure S1.** Anatomy of a cotton seedling approximately 10 -14 days after sowing. Plant sections excised by hand for confocal microscopy include root cap, main root, lateral root, lateral root junction and tip, basal stem, and petiole of true leaves.

**Table S1**. Primers for yeast recombinantion-based cloning of to generate a plasmid for expression of mCherry in *Verticillium dahliae*.

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Sequence2** | **Target notes** |
| DG13461 | cctcaccgcggcccatggtctagaactagt**ggatcc**AACGGGC | Forward primer for TEF promoter |
| DG13471 | gcccttggagaccatGGTGAAGGTTGTGTTATGTTTTGTGGA | Reverse primer for TEF promoter |
| DG13481 | aacacaaccttcaccATGGTCTCCAAGGGCGAGGAGGA | Forward primer for mCherry |
| DG13491 | aaatcgaatgtccgcTCATTTGTACAGCTCGTCCATACCG | Reverse primer for mCherry |
| DG13501 | gagctgtacaaatgaGCGGACATTCGATTTATGCCG | Forward primer for TEF terminator |
| DG13511 | CTCGAGGTCGACAAGCTTGT | Reverse primer for TEF terminator |

1 Primers used in the cloning of TEF promoter/terminator and mCherry fragments into pPZPnat1. 2 lowercase = homology arms of primer, uppercase = sequence with homology to PCR template, bold = *Bam*HI restriction site.

**Table S2.** *Verticillium dahliae*-specific primers [1] amplifying a 200 bp ITS product were used to confirm its identity.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Primer type** | **Organism** | **Sequence (5’-3’)** | **Target** |
| ITS1-F | Forward | Fungi | CTTGGTCATTTAGAGGAAGTAA | 18S rDNA |
| ST-VE1 | Reverse | *Verticillium* spp. | AAAGTTTTAATGGTTCGCTAAGA | ITS 1 |

**Table S3**. Summary of rate of colonisation based on timing of initial observation at each infection stage throughout the confocal microscopy experiment (At each observation, n = 3 samples of plant tissue section examined).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Infection stage** | **First observed1 time** | | **Fungal structures2** | **Number of**  **observations3** |
| **Sicot** | **Siokra 1-4** |
| Germination | 24 hpi | 24 hpi | Conidia, germ tubes,  infection peg (Siokra) | 1 observation |
| Hyphal elongation | 5 dpi | 24 hpi | Conidia, hyphae | 2 observations |
| Penetration | 5 dpi | 24 hpi | Conidia, hyphae | 2 observations |
| Colonisation of the root  epidermis | 5 dpi | 5 dpi | Mycelia | 1 observation |
| Colonisation of the root vasculature | 7 dpi | 7 dpi | Mycelia, occlusion with conidia (Sicot) | 2 observations |
| Colonisation of the above  ground vasculature | - | 7 dpi | Conidia, germ tube,  hyphae | 1 observation |
| Colonisation of the petiole | - | 7 dpi | Conidia, hyphae | 1 observation |

1 Time point at which infection stage was first observed.

2 Fungal structures listed were observed on both varieties unless indicated otherwise.

3 Independent observations.

**Table S4.** mCherry transformant isolates selected for comparison against *Verticillium dahliae* VCG-1A parent, 'Vd71181', that originated from Gwydir Valley, NSW. Isolates were selected for brightness and uniformity of fluorescence. Table includes corresponding Agrobacterium strain used for transformation of mCherry protein into *V. dahliae*.

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate1** | **Origin strain** | **VCG** | **Agrobacterium strain** |
| 81T0028 | Vd71181 | 1A | AGL1 |
| 81T0029 | Vd71181 | 1A | AGL1 |
| 81T0030 | Vd71181 | 1A | AGL1 |
| 81T0069 | Vd71181 | 1A | EHA105 |
| 81T0073 | Vd71181 | 1A | EHA105 |

1 Isolate names are abbreviated to indicate Gw = Gwydir Valley and T = mCherry Transformant.

1. Lievens, B.; Brouwer, M.; Vanachter, A.C.R.C.; Cammue, B.P.A.; Thomma, B.P.H.J. Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples. *Plant Science* **2006**, *171*, 155-165, doi:https://doi.org/10.1016/j.plantsci.2006.03.009.