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Data Article

Confirming infection of hop plants inoculated with *Verticilium nonalfalfae*

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A B S T R A C T

Hop (*Humulus lupulus* L.) is grown mostly as flavouring and bittering ingredient for beer and is also appreciated in the herbal and cosmetic industry, as well as in pharmacology. Among several diseases that damage hop growing, the most devastating in European hop production is verticillium wilt, caused by the soil-borne fungal pathogen *Verticillium nonalfalfae*. Colonization pattern and differential expression of selected genes after artificial infection of susceptible and resistant hop cultivars with *V. nonalfalafae* in stems and roots have been analysed recently Švara et al., 2019. Here, we present the dataset related to verification of plant samples infections after artificial inoculation (fungi- and mock-inoculated). After inoculation plant samples were tested for the positive infection by PCR amplification of the *V. nonalfalfae* ITS DNA region with species specific primers developed and optimised for this purpose. For more insight please see the article “Temporal and spatial assessment of defence responses in resistant and susceptible hop cultivars during infection with Verticillium nonalfalfae”.

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Specifications Table

Subject area	Biochemistry, Genetics and Molecular Biology
More specific subject area	Plant Molecular Biology, Transcriptomics
Type of data	2 tables, 1 image (agarose gel electrophoresis image)
How data was acquired	**Primers** ITS specific primers were developed with Primer3web application based on *V. nonalfalfae* ITS sequence identified from published draft genome sequence [2]. **PCR** (PCR model- Thermal cycler 2720 Applied Biosystems) amplification on DNA of infected and mock inoculated plant material (roots). **Visualization and electrophoretic separation of amplicons** (electrophoresis tank Sub-Cell Model 192, BioRad, power supplies, UV transilluminator TFM-30V) **Electrophoretic gel image capturing** (Device for image capture of gel UVP Biospectrum 810 with cooled camera)
Data format	Figures in JPG file 300 dpi (raw data)
Experimental factors	The expression analysis experiment was performed separately on stem and root sections, which were separated from each other by cutting the stem bases using a sterile scalpel. Roots were carefully washed in fresh water. All samples were immediately frozen in liquid nitrogen and crushed to dust in the mortar for the extraction of DNA.
Experimental features	The presence and absence of *V. nonalfalfae* was confirmed by PCR using *V. nonalfalfae*-specific internal transcribed spacer (ITS) primers (Vnaa-ITS_2-F: TCATAACCCTTTGTGAACCATA and Vaa-ITS_2-R: CACGAGGCGGGCTTGTAG; amplification profile 94 °C, 5 min; 13 cycles, 93 °C, 35 s; 53 °C, 55 s; 72 °C 45 s; 13 cycles, 93 °C, 35 s, 53 °C, 55 s, 72 °C, 59 s; 9 cycles, 93 °C 35 s, 53 °C, 55 s, 72 °C 118 s; final cycle, 72 °C 10 min) followed by subsequent gel electrophoresis separation on a 1.2% gel containing 0.5 μg/mL ethidium bromide for visualisation. Only samples that were confirmed by PCR as infected and non-infected (control) were further analysed.
Data source location	University of Ljubljana, Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, Slovenia
Data accessibility	Data are within this article
Related research article	Švara A., Jakse J., Radisek S., Javornik B., Stajner N. 2019. Temporal and spatial assessment of defence responses in resistant and susceptible hop cultivars during infection with *Verticillium nonalfalfae*. Journal of Plant Physiology, 240. https://doi.org/10.1016/j.jplph.2019.153008

Value of the Data

- Specific primers developed and optimized to amplify ITS DNA region of *V. nonalfalafae* are valuable for verification of infection after artificial inoculation with *V. nonalfalafae*
- The data about success rate of the inoculation process for two hop genotypes, susceptible and resistant can aid other researchers an estimation useful for planning and designing good experiments.
- The data can serve as a benchmark for other similar studies, for example to obtain enough plant material for expression analysis; in our experiment 43% less infected plants of Wye Target and 28,6% less infected plants of Celeia were obtained due to ineffective inoculation process and it needs to be considered to have an adequate number of plants for an appropriate experiment.
- Data presented here allows experimental replication

1. Data

Table 1 describes *V. nonalfalfae* specific ITS primer information including name, sequence length, melt temperature and GC content. ITS primers were developed and optimized for verification of infection during artificial inoculation of hop with *V. nonalfalfae*.

Image 1 presents gel electrophoresis assay of *V. nonalfalfae* ITS DNA amplicons. The assay was performed in order to confirm infection success in roots of two hop cultivars, susceptible Celeia and resistant Wye Target (Table 1).

Table 2 describes amplification test of infection success of Celeia and Wye Target roots after artificial inoculation with *V. nonalfalfae*. All mock-inoculated plants (marked with "–" in sample ID, Table 2) indicated the absence of the fungal DNA, while 65 of the 102 infected plants showed to be true-

Table 1
V. nonalfalfae-specific ITS primers.

ID	Sequence	Length	Tm (°C)	GC%
Vaa-ITS_2-F	TCATAACCCTTTGTGAACCATA	22	51,6	36,36
Vaa-ITS_2-R	CACGAGGCGGGCTTGTAG	18	58,7	66,67

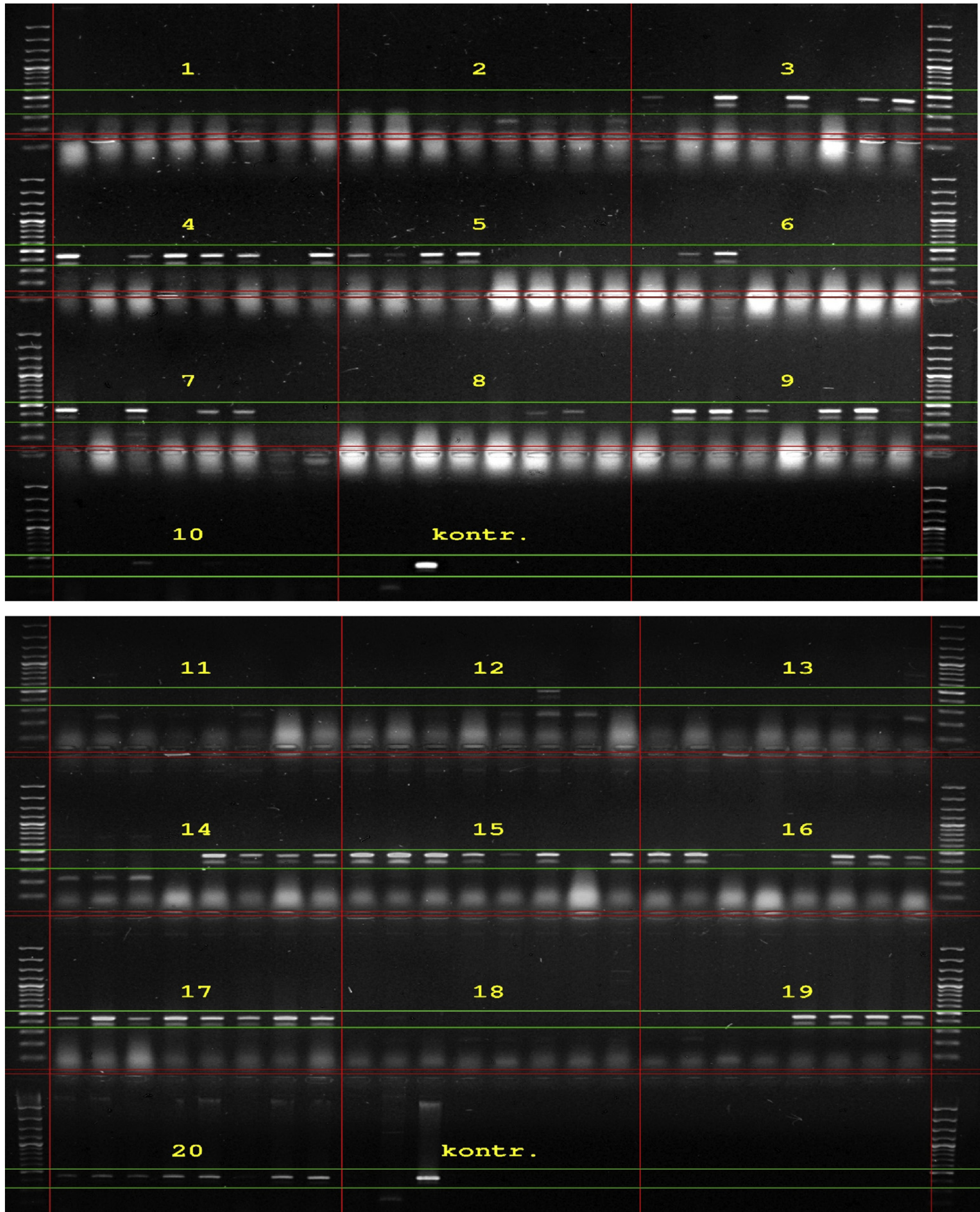

Image 1. Gel electrophoresis of *V. nonalfalfae* ITS DNA amplicons (specific numbered compartments represent samples grouped by one PCR strip represented by 8 individuals).

Table 2
Amplification test of infection success of Celeia (CE) and Wye Target (WT) roots (R) by V. nonalfalfae-specific primers after agarose gel electrophoresis assay (where 1 stands for successful infection and 0 stands for no infection of expected and actual status, respectively).

Sample ID	Sample number	Assay	Sample ID	Sample number	Assay	Sample ID	Sample number	Assay
06CE-01R	99	0 0	12CE-01R	98	0 0	18CE-01R	79	0 0
06CE-02R	100	0 0	12CE-02R	95	0 0	18CE-02R	84	0 0
06CE-03R	101	0 0	12CE-03R	91	0 0	18CE-03R	85	0 0
06CE-04R	102	0 0	12CE-04R	92	0 0	18CE-04R	80	0 0
06CE-05R	103	0 0	12CE-05R	93	0 0	18CE-05R	88	0 0
06CE-06R	104	0 0	12CE-06R	94	0 0	18CE-06R	87	0 0
06CE-07R	105	0 0	12CE-07R	89	0 0	18CE-07R	83	0 0
06CE-08R	106	0 0	12CE-08R	90	0 0	18CE-08R	82	0 0
06CE-09R	107	0 0	12CE-09R	96	0 0	18CE-09R	86	0 0
06CE-10R	108	0 0	12CE-10R	97	0 0	18CE-10R	81	0 0
06CE+01R	124	1 0	12CE+01R	114	1 1	18CE+01R	66	1 1
06CE+02R	125	1 0	12CE+02R	115	1 1	18CE+02R	76	1 0
06CE+03R	126	1 1	12CE+03R	116	1 1	18CE+03R	75	1 ?
06CE+04R	127	1 1	12CE+04R	117	1 ?	18CE+04R	73	1 0
06CE+05R	128	1 1	12CE+05R	118	1 1	18CE+05R	68	1 1
06CE+06R	129	1 1	12CE+06R	119	1 0	18CE+06R	67	1 1
06CE+07R	130	1 1	12CE+07R	120	1 1	18CE+07R	78	1 0
06CE+08R	131	1 1	12CE+08R	121	1 1	18CE+08R	74	1 0
06CE+09R	132	1 1	12CE+09R	122	1 1	18CE+09R	65	1 0
06CE+10R	133	1 1	12CE+10R	109	1 1	18CE+10R	69	1 0
06CE+11R	134	1 1	12CE+11R	110	1 1	18CE+11R	70	1 1
06CE+12R	135	1 1	12CE+12R	111	1 1	18CE+12R	72	1 0
06CE+13R	136	1 1	12CE+13R	112	1 1	18CE+13R	71	1 1
06CE+14R	123	1 0	12CE+14R	113	1 1	18CE+14R	77	1 0
06WT-01R	139	0 0	12WT-01R	148	0 0	18WT-01R	15	0 0
06WT-02R	143	0 0	12WT-02R	8	0 0	18WT-02R	13	0 0
06WT-03R	146	0 0	12WT-03R	1	0 0	18WT-03R	14	0 0
06WT-04R	142	0 0	12WT-04R	7	0 0	18WT-04R	12	0 0
06WT-05R	137	0 0	12WT-05R	5	0 0	18WT-05R	16	0 0
06WT-06R	140	0 0	12WT-06R	3	0 0	18WT-06R	17	0 ?
06WT-07R	145	0 0	12WT-07R	6	0 0	18WT-07R	10	0 0
06WT-08R	138	0 0	12WT-08R	14	7 0 0	18WT-08R	11	0 0
06WT-09R	144	0 0	12WT-09R	2	0 0	18WT-09R	18	0 0
06WT-10R	141	0 0	12WT-10R	4	0 0	18WT-10R	9	0 0
06WT+01R	21	1 1	12WT+01R	33	1 1	18WT+01R	60	1 0
06WT+02R	25	1 1	12WT+02R	42	1 ?	18WT+02R	52	1 0
06WT+03R	149	1 1	12WT+03R	41	1 0	18WT+03R	51	1 1
06WT+04R	19	1 1	12WT+04R	35	1 1	18WT+04R	57	1 0
06WT+05R	150	1 1	12WT+05R	43	1 1	18WT+05R	53	1 1
06WT+06R	155	1 1	12WT+06R	45	1 0	18WT+06R	61	1 0
06WT+07R	22	1 0	12WT+07R	30	1 1	18WT+07R	46	1 0
06WT+08R	153	1 1	12WT+08R	44	1 0	18WT+08R	47	1 0
06WT+09R	156	1 1	12WT+09R	31	1 0	18WT+09R	56	1 0
06WT+10R	24	1 1	12WT+10R	36	1 1	18WT+10R	49	1 1
06WT+11R	20	1 0	12WT+11R	27	1 1	18WT+11R	62	1 ?
06WT+12R	159	1 1	12WT+12R	40	1 0	18WT+12R	50	1 0
06WT+13R	151	1 1	12WT+13R	29	1 1	18WT+13R	64	1 0
06WT+14R	26	1 0	12WT+14R	28	1 1	18WT+14R	55	1 0
06WT+15R	160	1 1	12WT+15R	39	1 0	18WT+15R	58	1 0
06WT+16R	157	1 1	12WT+16R	37	1 0	18WT+16R	59	1 0
06WT+17R	158	1 0	12WT+17R	48	1 0	18WT+17R	63	1 1
06WT+18R	23	1 1	12WT+18R	34	1 ?	18WT+18R	54	1 1
06WT+19R	152	1 1	12WT+19R	32	1 1			
06WT+20R	154	1 1	12WT+20R	38	1 0			

positives as they resulted in amplification of the fungal ITS region. This yields a 63.7% success rate for the inoculation process, 72% for susceptible Celeia and 59% for the resistant Wye Target cultivar, respectively. Therefore, 143% of Wye Target and 128,6% of Celeia plants should be inoculated with *V. nonalfalfae* to obtain 100% of infected plants. The calculation was done based on Table 2, determining the proportion of unsuccessfully infected plants and successfully infected plants for Wye Target and Celeia, respectively.

2. Experimental design, materials, and methods

2.1. Artificial inoculation

One year old hop plants were artificially inoculated with *V. nonalfalfae* isolate (T2) characterized as a lethal pathotype PV1 [3]. Inoculation of 60 plants of the resistant Wye Target cultivar and 42 plants of the susceptible Celeia cultivar was based on ten minute root dipping in conidial suspension adjusted to a concentration of 5×10^6 conidia/mL [4]. Mock inoculations were carried out by dipping 30 plants of each cultivar in a sterile distilled water. After inoculum treatment, the plants were transferred to 1 L pots using a sterile commercial substrate and grown in the form of a single bine in a growing chamber (12-h photoperiod of fluorescent light; 22 °C and 65% relative humidity during the light period and 20 °C and 70% during the dark period).

2.2. Primer development, PCR and gel electrophoresis

The presence and absence of fungal DNA in *V. nonalfalfae*- and mock-inoculated roots and stems of CE and WT cultivars at all three time points, respectively, were evaluated by PCR and subsequent gel electrophoresis test. The presence and absence of *V. nonalfalfae* was confirmed by PCR using *V. nonalfalfae*-specific internal transcribed spacer (ITS) primers (V naa-ITS_2-F: TCATAACCCTTTGTGAACCATA and Vaa-ITS_2-R: CACGAGGCGGGCTTGTAG; primers were developed based on *V. nonalfalfae* ITS sequence identified from published draft genome [2]; amplification profile 94 °C, 5 min; 13 cycles, 93 °C, 35 s; 53 °C, 55 s; 72 °C 45 s; 13 cycles, 93 °C, 35 s, 53 °C, 55 s, 72 °C, 59 s; 9 cycles, 93 °C 35 s, 53 °C, 55 s, 72 °C 118 s; final cycle, 72 °C 10 min) followed by subsequent gel electrophoresis separation on a 1.2% gel containing 0.5 μg/mL ethidium bromide for visualisation. Only samples that were confirmed by PCR as infected and non-infected (control) were further analysed as it is described in the article “Temporal and spatial assessment of defence responses in resistant and susceptible hop cultivars during infection with Verticillium nonalfalfae” [1].

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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