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Exposure to the Endophytic Fungi Regulates the Anthocyanin Profiles in the Post-Veraison Grape Berries of 'Cabernet Sauvignon'

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Abstract: The potential of endophytes to initiate changes in host secondary metabolism is expected to be applied to improve the biochemical qualities of the crop. Our previous study revealed the significant impacts of fungal endophytes on the biochemical properties and the anthocyanin profiles in grape berries of the local cultivar 'Rose Honey' (RH). To validate the effects, our present work further assessed the impacts of the same fungal endophytes on grape berries of the worldwide planted grapevine cultivar 'Cabernet Sauvignon' (CS). Consistent with the results of RH, exposure to most of the used endophytic fungi shaped the biochemical traits and anthocyanidin profile of the CS grape berries. Among the detected biochemical traits, the phenylalanine ammonia-lyase (PAL) activity in berries had the strongest response to endophytic fungal exposure, and the fungal strains RH32, RH36, and MDR1 had the greatest biochemical impacts on the grape berries. Interestingly, the most anthocyanidin species were detected in the two grape berry varieties when exposed to fungal strains MDR36 and RH34. In both varieties, the total anthocyanin concentrations were quantitatively promoted by strains RH36, RH44, MDR1, and MDR36, but suppressed by strain RH7. Malvidin derivatives and delphinidin derivatives accounted for the majority of the relative abundance of the total detected anthocyanins in CS berries. The acylation degree of anthocyanins in grape berries was also significantly promoted by exposure to fungal endophytes. In CS grape berries, a seldom-distributed anthocyanidin, pelargonidin-3-*O*-glucoside, as well as the diglucoside anthocyanidin were detected when exposed to fungal strains as RH32, RH34, RH36, MDR1, MDR4, and MDR36. Overall, the endophytic fungal strains MDR36, RH36, and RH34 have the ability to promote metabolite profiles in both grape varieties. This work confirms the possibility of using certain endophytic fungal strains as a strategy for shaping grape pigmentation in vinification at the post-veraison or post-harvest stages.

Keywords: grape berries; endophytic fungi; biochemical traits; anthocyanidin profile; grape pigmentation

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1. Introduction

Endophytes are microorganisms that live within healthy plant tissues or organs without causing disease [1], and that can transform the bioactive compounds synthesized by the host plant into multifunctional products [2]. Endophytic fungi are found to grow both intracellularly and intercellularly within the internal tissues of the host plants [3]. They exhibit various degrees of host specificity in plant colonization patterns and different lifestyles ranging from facultative to obligate [3,4]. It is generally believed that endophytes can be transmitted vertically or horizontally [5].

In recent years, the effects of environmental microbes, especially endophytes, on grape quality characteristics such as anthocyanins, tannins, stilbenes, and other metabolites, have

sparked increasing interest in terms of their organoleptic characteristics and potential applications in human health [6–8]. Anthocyanins, as water-soluble pigments, are synthesized from phenylalanine through the phenylpropanoid synthesis pathway [9], and a correlation between phenylalanine ammonia-lyase (PAL) activity and the total anthocyanin content was observed [10]. Anthocyanins were shown to be present in the skins of red grapes and also in the flesh of the 'teinturier' varieties [11]. Anthocyanins are responsible not only for color in grapes and red wines, but also for contributing to the astringency and bitterness. Additionally, anthocyanins possess anticancer, anti-inflammatory, antimicrobial, and neuroprotective activities, as well as the ability to prevent cardiovascular disease [12]. Among all the factors that influence the synthesis and metabolism of plant anthocyanins, endophytes are one of the most important effectors of plants, and their roles in grape anthocyanins are still poorly understood.

A previous study showed that the total stilbene content of *Vitis amurensis* cells was increased by 2.2–5.3-fold after co-culture with different endophytic bacteria for 2 weeks, and increased by 2.6–16.3-fold when co-cultured with endophytic fungi [13]. In our previous study, it was shown that exposure to endophytic fungi could differentially shape the biochemical properties and the anthocyanin profiles of post-veraison grape berries of 'Rose Honey' (RH, *V. vinifera* L. × *V. labrusca* L., the main local varieties in Yunnan, China) [14]. However, it is unclear whether the above results were variety-specific responses in RH grapes or common effects in different grape varieties. Here, to validate the effects, our present work further assessed the impacts of the same fungal endophytes on berries of another grapevine cultivar, 'Cabernet Sauvignon' (CS, *V. vinifera* L.), which is widely cultivated around the world, by using an in vitro aseptic grape berry–fungus co-culture system. The biochemical properties and the anthocyanin profiles were analyzed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS).

2. Materials and Methods

2.1. Fungal Strains and Grape Berries

Twelve strains of fungal endophytes were used in this study, isolated from grapevine leaves of 'Rose Honey' (RH) from local vineyards (Yunnan province, China) using the tissue patch method described previously [15]. The isolated fungal strains were identified using internally transcribed spacer (ITS) DNA sequences [16]. Briefly, the total DNA of fungal isolates was first extracted with cetyltrimethylammonium bromide (CTAB) methods and ITS sequences were amplified with the primer pairs ITS4 and ITS5 [17]. The PCR mixture (50 µL) contained 1 µL ITS5 (10 µmol L^{-1}), 1 µL ITS4 (10 µmol L^{-1}), 5 µL 10×PCR buffer, 4 µL dNTPs, 1 µL DNA template, 0.3 µL Taq (5 U), and 37.7 µL ddH_2O. The reaction conditions were as follows: pre-denaturation at 94 °C for 4 min, with one cycle; denaturation at 94 °C for 1 min, renaturation at 54 °C for 1 min, and extension at 72 °C for 1 min, with 33 cycles; and extension at 72 °C for 3 min. PCR products were commercially sequenced (Shenggong, Shanghai) and the BLASTn search option of the NCBI database was used for species identification. The nucleotide sequences of fungal strains have been deposited in GenBank under the accession numbers ON740926-ON740939 (Table 1). All strains were inoculated in potato dextrose agar (PDA) medium, then incubated at 26 °C for 7 days.

The grape berries of 'Cabernet Sauvignon' (CS) in fully mature stages were harvested from 8-year-old field-grown grapevines. Grape clusters were selected and well-packaged in bags and taken to the laboratory within 3 h for further experiments.

Table 1. Physio-chemical values of grape berries after co-culture with different strains of fungal endophytes.

Strain ID	Species	GenBank Accession	PAL (U g^{-1})	SPr (mg g^{-1})	TF (mg g^{-1})	TPh (mg g^{-1})
RH7	*Epicoccum nigrum*	ON740926	19.43 ± 4.99	7.98 ± 0.48	19.79 ± 1.64	56.10 ± 1.63 **
RH12	*Nigrospora oryzae*	ON740927	21.37 ± 0.20	15.88 ± 1.34 **	16.59 ± 2.67	52.73 ± 1.61
RH32	*Alternaria alternaria*	ON740928	40.57 ± 2.91 **	24.85 ± 2.02 **	31.69 ± 0.91 **	63.99 ± 0.80 **

Table 1. *Cont.*

Strain ID	Species	GenBank Accession	PAL (U g^{-1})	SPr (mg g^{-1})	TF (mg g^{-1})	TPh (mg g^{-1})
RH34	*Trichothecium roseum*	ON740929	42.54 ± 1.32 **	15.47 ± 2.32 **	23.25 ± 2.33	63.73 ± 0.08 **
RH36	*Fusarium verticillioides*	ON740930	53.09 ± 9.91 **	12.55 ± 3.70	40.68 ± 1.98 **	87.56 ± 3.76 **
RH44	*Alternaria arborescens*	ON740931	17.45 ± 0.63	9.95 ± 0.21	23.32 ± 1.02	44.56 ± 0.44
RH47	*Fusarium proliferatum*	ON740932	22.12 ± 0.26	16.39 ± 1.66 **	20.36 ± 0.80	43.97 ± 0.68
RH48	*Colletotrichum gloesporioides*	ON740933	34.13 ± 1.78 *	9.84± 1.00	20.60 ± 1.00	40.96 ± 0.52
RH49	*Fusarium fujikuroi*	ON740934	35.43 ± 1.27 *	8.93 ± 1.10	36.86 ± 0.39 **	69.33 ± 0.44 **
MDR1	*Nigrospora oryzae*	ON740935	42.83 ± 8.76 **	26.50 ± 2.82 **	28.55 ± 2.17 **	58.96 ± 0.20 **
MDR4	*Fusarium annulatum*	ON740937	47.99 ± 10.12 **	13.62 ± 1.00 *	26.32 ± 1.11 **	75.98 ± 1.55 **
MDR36	*Colletotrichum siamense*	ON740939	76.43 ± 9.08 **	10.56 ± 1.24	20.44 ± 0.98	52.42 ± 0.37
Control			16.71 ± 2.20	8.26 ± 0.12	20.13 ± 1.95	48.76 ± 0.34

Values of physio-chemical traits were indicated as mean ± standard errors with different significance marked as * or **, compared to the control. * significant difference at 5%, and ** significant difference at 1% (Tukey's test). PAL: phenylalanine ammonia-lyase; SPr: total soluble protein; TPh, total phenol; TF: total flavonoid content.

2.2. Establishment of Berry–Fungi Co-Culture System

Murashige and Skoog (MS) medium [18] was applied for grape berry culture, containing 3% sucrose (*m/v*), 0.75% agar, and supplemented with vitamins (myo-inositol 100 mg L^{-1}, pyridoxine HCl 1 mg L^{-1}, thiamine HCl 1 mg L^{-1}, nicotinic acid 1 mg L^{-1}, D-calcium pantothenate 1 mg L^{-1}, and biotin 0.01 mg L^{-1}). Medium (pH 5.8) was dispensed into culture bottles and autoclaved for 25 min at 121 °C.

Berries with peduncles (approximately 0.5 cm) were first removed from the clusters and placed in tap water, keeping the tap water running for 20 min to clean the berries. In a laminar flow cabinet, the berries were placed in ethanol (75%) for 30 s, and washed twice with sterilized water. The berries were placed in 5% NaClO for 5 min and washed four times with sterilized water. After rinsing with 20 mM EDTA solution, the berry peduncle was cut again to approximately 0.3 cm, and then quickly inserted into nutrient medium. The treated berries were cultured at 26 °C under 12-hour photoperiods. Five to seven berries per treatment were tested and no microbial contamination was checked every day. After 7 days of preculture, grape berries were used to perform co-culture with fungi. The suspension of endophytic fungus was prepared with sterilized water (final concentration of 2.5 g L^{-1}), and added to each berry (0.5 mL). Five biological replicates were performed in each treatment and control. Grape berries without fungi were used as controls. The co-cultures and controls were cultured for another 15 days. The samples were harvested and stored at −80 °C for further assays.

2.3. Measurement of Physio-Biochemical Traits

In this study, we detected the activity of PAL, the concentrations of total soluble protein (SPr), total flavonoid (TF), and total phenol (TPh). PAL activity was assayed using phenylalanine and borate buffer as described by Pan et al. [19]. The SPr concentration was determined as described by Bradford [20]. TF was measured using the aluminum chloride colorimetric method as described by Pan et al. [19], and TPh was measured using the Folin phenol colorimetric method [21]. All analyses contained 3 biological replicates.

2.4. UPLC–MS Assay

For the anthocyanidin composition assay, grape berries were exposed to 110 °C for 10 min, and then dried at 60 °C for 2 days to achieve constant weight. After the berries were ground into powder, the powder (approximately 300 mg) was extracted with 3 mL of methanol (with 1% hydrochloric acid) for 12 h, followed by sonication for 1 h. Solutions of extraction were centrifuged at 4000× *g* for 10 min, and the supernatants were then filtered with 0.45 μM filter (Merck Millipore, Darmstadt, Germany) prior to UPLC–MS analysis.

An Agilent 1200 series liquid chromatography/mass selective detector (LC/MSD) (Agilent, Waltham, MA, USA) equipped with a UV detector and a Hypersil GOLD C18 column (2.1 × 100 mm, 1.9 µm; Thermo Fisher Scientific, Waltham, MA, USA) was used. Solvent A was 95% methanol and solvent B was Milli-Q water containing 0.1% formic acid. The flow rate was 0.3 mL min^{-1}. The injection volumes were 10 µL, and mass spectroscopy (MS) conditions were as follows: electrospray ionization (ESI) interface, positive ion model, nebulizer pressure 35 psi, drying gas flow rate 10 L min^{-1}, drying gas temperature 350 °C, HV voltage 3.5 kV, and scanning at 100–1000 *m/z*. All analyses contained 3 technical replicates.

The concentrations of anthocyanins in grape samples were quantified by calculating against the used external standard malvidin-3-*O*-glucoside chloride (Sigma–Aldrich, St. Louis, MO, USA) and expressed as mg 100 g^{-1} fresh weight.

2.5. Data Analysis

The data resulting from each treatment are presented as means ± standard errors for multiple replicates and were analyzed with statistical software SPSS 22.0. Response indexes (RI) were used to normalize the biochemical effects on grape berries in responding to the co-cultured endophytic fungal strains, and calculated by the following formula: RI = $(V_{treatment} - V_{control})/V_{control}$. In the formula, $V_{treatment}$ and $V_{control}$ represent the mean values of a certain parameter in treated and controlled grape berries. A positive RI indicates the promotion of an effect, and a negative RI indicates inhibition. The boxplots were generated by GraphPad Prism (version 7.0) according to the RI values for illustrating the integrative biochemical impacts of endophytic fungi on grape berries. A heatmap was generated in Microsoft Excel, and the principal component analysis (PCA) was performed with R software (version 3.6.1).

3. Results

3.1. Exposure of CS Grape Berries to Different Endophytic Fungal Strains Differentially Modified the Biochemistry Status

Endophytic fungal strains, such as RH32, RH34, RH36, RH49, MDR1, MDR4, and MDR36, showed different degrees of promotion effects on the detected biochemical parameters in CS berries. Strains RH7, RH12, RH44, RH47, and RH48 could initiate either promotion or inhibition impacts on the detected biochemical traits in CS berries (Table 1). Compared to non-fungal co-culture control, phenylalanine ammonia-lyase activity (PAL) was promoted in fungal strain RH36- and MDR36-exposed grape berries by more than twofold, while the concentration of total soluble protein (SPr) was promoted in fungal strain RH32- and MDR1-exposed grape berries. The total flavonoid (TF) and total phenol (TPh) contents in grape berries were both greatly promoted by strains RH32, RH36, RH49, MDR1, and MDR4 (Table 1). Generally, co-culture with the used fungal strains greatly promoted the biochemical traits PAL and SPr according to the RI-based boxplots (Figure 1a). Among the detected biochemical parameters, PAL was the most sensitive biochemical trait in responding to the co-cultured fungal endophytes (Figure 1a; Supplementary Table S1). Concerning the used endophytic fungal strains, RH32, RH36, and MDR1 conferred the most impacts on the biochemistry of CS berries, while fungal strains RH7, RH12, RH44, RH47, and RH48 had fewer biochemical influences on grape berries (Figure 1b; Supplementary Table S1).

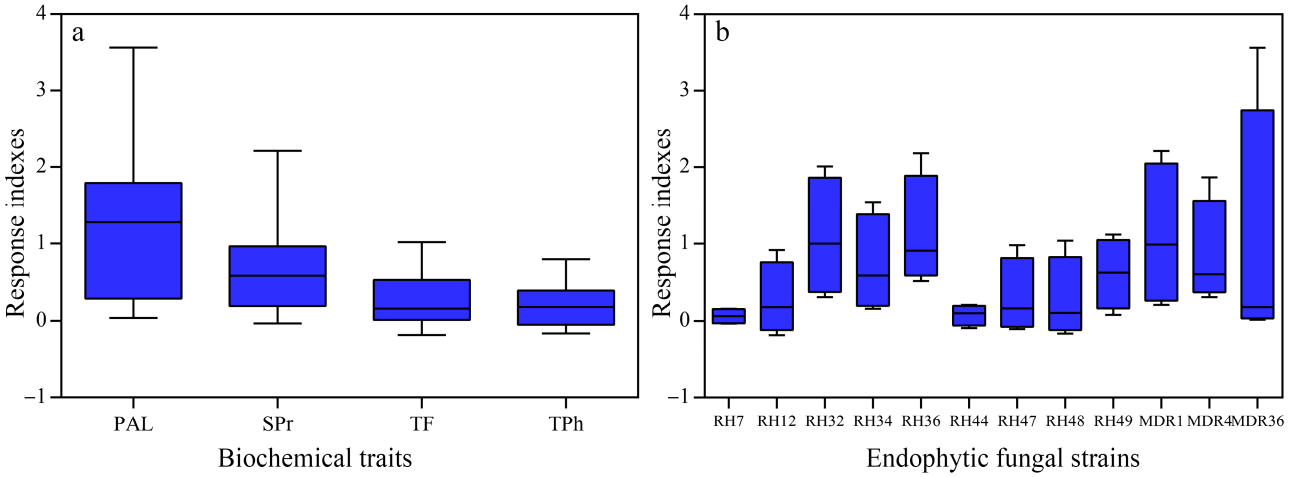

Figure 1. RI-based boxplots exhibit the integrative biochemical impacts of the co-cultured fungal strains on CS grape berries. (**a**) Comparing the sensitivities of different biochemical parameters in grape berries in responding to the used fungal endophytes. (**b**) Comparing the integrative biochemical impacts of the used fungal endophytes on CS grape berries. Median (middle black line), confidence region (boxes) and maximum non-outlying envelope (whiskers). PAL: phenylalanine ammonia-lyase; SPr: total soluble protein; TPh, total phenol; TF: total flavonoid content.

3.2. Anthocyanins in Post-Veraison CS Grape Berries Exposed to Endophytic Fungi Were Quantitatively and Compositionally Modified

In total, 16 anthocyanins were detected from extracts of CS berries, including monoglucoside, diglucoside, acetyl monoglucoside, caffeoyl monoglucoside, *p*-coumaroyl monoglucoside, and *p*-coumaroyl diglucoside (Table 2, 3). Monoglucoside was detected in all treatments, and *p*-coumaroyl diglucoside anthocyanin was detected only in RH32-treated CS grape berries. Diglucoside anthocyanins were detected in grape berries treated with RH34, RH47, RH48, and MDR36, and caffeoyl monoglucoside was detected in grape berries treated with RH32, RH48, and MDR36. In addition, *p*-coumaroyl monoglucoside anthocyanins were not detected only in RH49-treated grape berries, while acetyl monoglucoside anthocyanins were not detected in the RH44, RH48, and MDR1 treatments. Furthermore, co-culture with fungal endophytes mostly promoted the concentration of acetyl monoglucoside anthocyanins (4.05–45.56%) in CS grape berries (Table 3). In comparison with the control, all the fungal strains used, except RH7 and RH12, considerably promoted the total anthocyanin concentrations in CS berries (Table 2). Fungal strains RH48 and MDR1 promoted the total anthocyanin concentrations by 107.49% and 104.07%, respectively (Table 2).

In addition to the quantitative effects, the CS berries co-cultivated with fungal endophytes experienced differential effects on the compositional patterns of the anthocyanins. Different species of anthocyanins were detected and are shown in Table 2 and Table S2. Derivatives of all these anthocyanin categories were detected in grape berries from most treatments, while no pelargonidin species were detected in berries treated with fungal strains RH7, RH12, RH44, RH48, and RH49 and from the non-fungal co-culture control, and no cyanidin species were detected in berries treated with strains RH7 and RH48 (Table 2; Figure 2a). Compared to the non-fungal co-culture control, pelargonidin-3-*O*-glucoside, delphinidin-3-O-acetylglucoside, petunidin-3-O-acetylglucoside, peonidin-3-O-acetylglucoside, petunidin-3,5-O-diglucoside, pelargonidin-3,5-O-diglucoside, petunidin-3-O-caffeoylglucoside, peonidin-3-O-coumarylglucoside, and cyanidin-3-O-coumarylglucoside-5-O-glucoside exclusively accumulated in CS berries that were co-cultivated with fungi (Table 2). Among all the detected anthocyanin species, cyanidin-3-O-coumarylglucoside-5-O-glucoside was detected only in RH32-treated grape berries, petunidin-3,5-O-diglucoside was detected only in RH48- and MDR36- treated grape berries, and pelargonidin-3,5-O-diglucoside was detected only in RH34- and RH47-treated grape berries (Table 2). Co-culture with strains RH36, RH44, MDR1, and MDR36 triggered greater promotion of malvidin derivative concentrations, but co-culture with

strains RH7 and MDR4 had an inhibitory effect compared to other treatments. Additionally, co-culture with strains RH47, RH48, and MDR1 considerably promoted the concentrations of delphinidin derivatives in CS berries, but co-culture with RH7 had a strong inhibitory effect. For petunidin derivatives, all the fungal strains used, especially RH32, RH49, and MDR36, triggered significant effects on anthocyanin concentration (Table 2; Figure 2a). The acylated anthocyanins were promoted due to co-culture with most fungal strains, while strains RH44, RH48, and MDR1 suppressed these anthocyanin species in CS berries (Figure 2b).

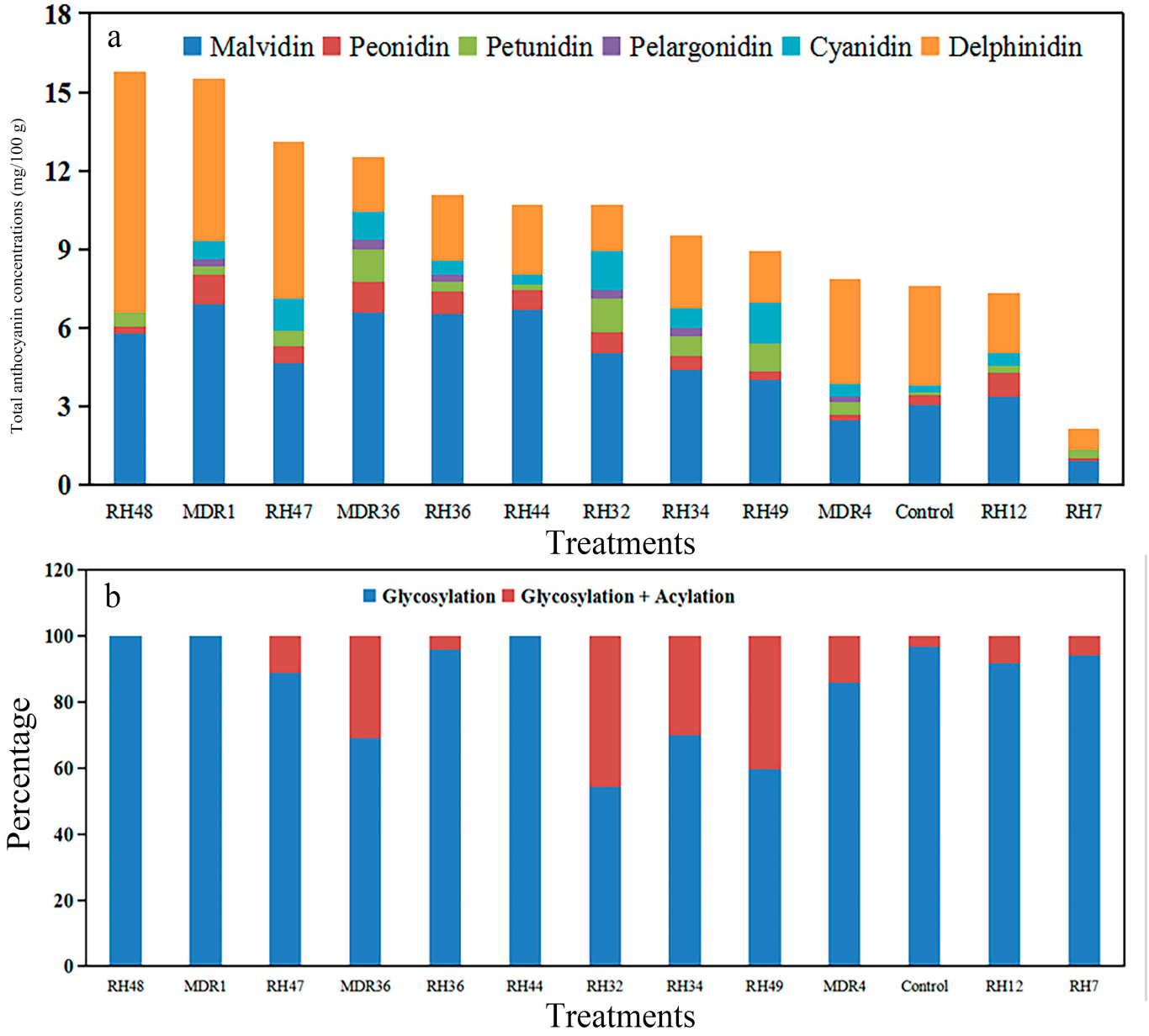

Figure 2. Assays of anthocyanin profiles in CS berries after being treated with fungal endophytes. (**a**) Total anthocyanin concentrations in CS berries and proportions of six basic anthocyanins; (**b**) ratio of glycosylation and acylation anthocyanins in CS berries.

Table 2. Anthocyanin of composition and concentration detected by UPLC–MS in CS berries after inoculated with different strains of fungal endophytes (mg 100 g^{-1}).

Treatment	RH7	RH12	RH32	RH34	RH36	RH44	RH47	RH48	RH49	MDR1	MDR4	MDR36	Control
Pg-3-glu	/	/	0.36 ± 0.01 **	0.25 ± 0.06 **	0.28 ± 0.28 **	/	/	/	/	0.30 ± 0.01 **	0.21 ± 0.01 **	0.34 ± 0.02 **	/
Cy-3-glu	/	0.45 ± 0.01	1.46 ± 0.19 **	0.75 ± 0.24	0.55 ± 0.12	0.37 ± 0.15	1.19 ± 0.18 **	/	1.55 ± 0.46 **	0.71 ± 0.12	0.52 ± 0.07	1.09 ± 0.21 **	0.26 ± 0.04
Pn-3-glu	/	0.49 ± 0.29	0.49 ± 0.16	0.54 ± 0.25	0.84 ± 0.06	0.64 ± 0.19	0.66 ± 0.25	/	0.33 ± 0.11	0.90 ± 0.02	0.18 ± 0.01	0.69 ± 0.14	0.36 ± 0.19
Dp-3-glu	0.85 ± 0.06 **	2.29 ± 0.24 **	1.20 ± 0.09 **	2.42 ± 0.37 **	2.51 ± 0.33 *	2.65 ± 0.35 *	5.71 ± 0.36 **	9.21 ± 0.64 **	1.47 ± 0.17 **	6.19 ± 0.19 **	3.80 ± 0.52	1.70 ± 0.13 **	3.78 ± 0.67
Pt-3-glu	0.19 ± 0.01	0.30 ± 0.06 *	0.10 ± 0.01	0.12 ± 0.02	0.22 ± 0.10	0.25 ± 0.03	0.18 ± 0.02	0.37 ± 0.10 **	0.11 ± 0.07	0.31 ± 0.04 *	0.17 ± 0.08	0.12 ± 0.02	0.13 ± 0.01
Mv-3-glu	0.93 ± 0.08	2.69 ± 0.06	1.88 ± 0.10	2.25 ± 0.08	5.91 ± 0.17 **	5.96 ± 0.53 **	3.56 ± 0.13	5.51 ± 2.40 **	1.85 ± 0.12	6.04 ± 0.11 **	1.65 ± 0.20	3.98 ± 0.45	2.50 ± 0.17
Dp-3-ace	/	/	0.54 ± 0.18 **	0.36 ± 0.12 **	/	/	0.31 ± 0.01 *	/	0.51 ± 0.12 **	/	0.17 ± 0.01	0.36 ± 0.18 **	/
Pt-3-ace	0.13 ± 0.01	/	1.16 ± 0.20 **	0.60 ± 0.18 *	0.17 ± 0.00	/	0.37 ± 0.06	/	0.92 ± 0.24 **	/	0.29 ± 0.01	0.92 ± 0.29 **	/
Pn-3-ace	/	0.23 ± 0.01 **	0.20 ± 0.03 **	/	/	/	/	/	/	/	/	0.23 ± 0.01 **	/
Mv-3-ace	/	0.37 ± 0.08	2.97 ± 0.30 **	1.89 ± 0.26 **	0.28 ± 0.01	/	0.81 ± 0.02	/	2.18 ± 0.23 **	/	0.66 ± 0.22	2.35 ± 0.31 **	0.26 ± 0.00
Pt-3,5-dig	/	/	/	/	/	/	/	0.13 ± 0.10 **	/	/	/	0.22 ± 0.03 **	/
Pg-3,5-dig	/	/	/	0.06 ± 0.00 **	/	/	0.05 ± 0.01 **	/	/	/	/	/	/
Pt-3-caff	/	/	0.13 ± 0.00 **	/	/	/	/	0.19 ± 0.11 **	/	/	/	0.22 ± 0.08 **	/
Pn-3-coum	0.08 ± 0.01 **	0.18 ± 0.05 **	/	0.02 ± 0.00	/	0.07 ± 0.00 **	/	0.10 ± 0.01 **	/	0.22 ± 0.01 **	0.06 ± 0.01 **	/	/
Mv-3-coum	/	0.32 ± 0.03	0.17 ± 0.03	0.26 ± 0.01	0.34 ± 0.10	0.76 ± 0.16 **	0.29 ± 0.05	0.28 ± 0.10	/	0.86 ± 0.07 **	0.15 ± 0.01	0.28 ± 0.05	0.32 ± 0.05
Cy-3-coum-5-glu	/	/	0.03 ± 0.01 **	/	/	/	/	/	/	/	/	/	/
Total	2.18 ± 0.17	7.32 ± 0.83	10.69 ± 1.31	9.52 ± 1.59	11.1 ± 0.90	10.7 ± 1.41	13.13 ± 1.09	15.79 ± 3.46	8.92 ± 1.52	15.53 ± 0.57	7.86 ± 1.15	12.5 ± 1.92	7.61 ± 1.13
RI (%)	−71.35%	−3.81%	40.47%	25.10%	45.86%	40.60%	72.54%	107.49%	17.21%	104.07%	3.29%	64.26%	

/ represents anthocyanidins that were not detected in this treatment. Values are indicated as mean ± standard errors with different significance marked as * or **, compared to control. * significant difference of 5%, and ** significant difference of 1% (Tukey's test). The abbreviations of anthocyanins are as follows: Pg−3-glu, pelargonidin-3-*O*-glucoside; Cy-3-glu, cyanidin-3-*O*-glucoside; Pn-3-glu, peonidin-3-*O*-glucoside; Dp-3-glu, delphinidin-3-*O*-glucoside; Pt-3-glu, petunidin-3-*O*-glucoside; Mv-3-glu, malvidin-3-*O*-glucoside; Dp-3-ace, delphinidin-3-O-acetylglucoside; Pt-3-ace, petunidin-3-O-acetylglucoside; Pn-3-ace, peonidin-3-O-acetylglucoside; Mv-3-ace, malvidin-3-O-acetylglucoside; Pt-3,5-dig, petunidin-3,5-O-diglucoside; Pg-3,5-dig, pelargonidin-3,5-O-diglucoside; Pt-3-caff, petunidin-3-O-caffeoylglucoside; Pn-3-coum, peonidin-3-O-coumarylglucoside; Mv-3-coum, malvidin-3-O-coumarylglucoside; Cy-3-coum-5-glu, cyanidin-3-O-coumarylglucoside-5-O-glucoside.

Table 3. Composition and proportion of anthocyanins in CS berries.

Treatment	Monoglucoside %	Acetylmonogl-ucoside %	Diglucoside %	Caffeoylmonog-lucoside %	(*p*-Coumaroyl) monoglucoside %	(*p*-Coumaroyl) diglucoside %
RH7	90.37	5.96	/	/	3.67	/
RH12	84.97	8.20	/	/	6.83	/
RH32	51.36	45.56	/	1.22	1.59	0.28
RH34	66.49	29.94	0.63	/	2.94	/
RH36	92.88	4.05	/	/	3.06	/
RH44	92.24	/	/	/	7.76	/
RH47	86.06	11.35	0.38	/	2.21	/
RH48	95.57	/	0.82	1.20	2.41	/
RH49	59.53	40.47	/	/	/	/
MDR1	93.05	/	/	/	6.95	/
MDR4	83.08	14.25	/	/	2.67	/
MDR36	63.36	30.88	1.76	1.76	2.24	/
Control	92.38	3.42	/	/	4.20	/

The total numbers of anthocyanins detected in CS grape berries exposed to endophytic fungal strains ranged from 5 to 13, and 7 anthocyanins were detected in non-fungus-treated grape berries (Figure 3; Table 4). Grape berries treated with fungal strains RH32, RH34, and MDR36 produced the most anthocyanin species (12 or 13), whereas in berries treated with RH7, only 5 species of anthocyanins were detected (Table 4). Compared to the control, 1–6 novel anthocyanidin species were introduced in CS berries due to exposure to different fungal strains (Table 4). Co-culture with strains RH32, RH34, and MDR36 introduced the most novel anthocyanidin species (five or six), whereas exposure to RH44 introduced only one novel anthocyanidin species in grape berries. In contrast, co-culture with some fungal strains considerably suppressed the anthocyanidin species compared to the control. Among all the fungal strains used, RH7 suppressed four anthocyanidin species (Table 4). Additionally, the fungal strains RH48 and MDR1 had greater impacts on anthocyanins, and the anthocyanin concentration reached a maximum (15.79 mg 100 g^{-1} and 15.53 mg 100 g^{-1}, respectively) after exposure to these strains (Table 4).

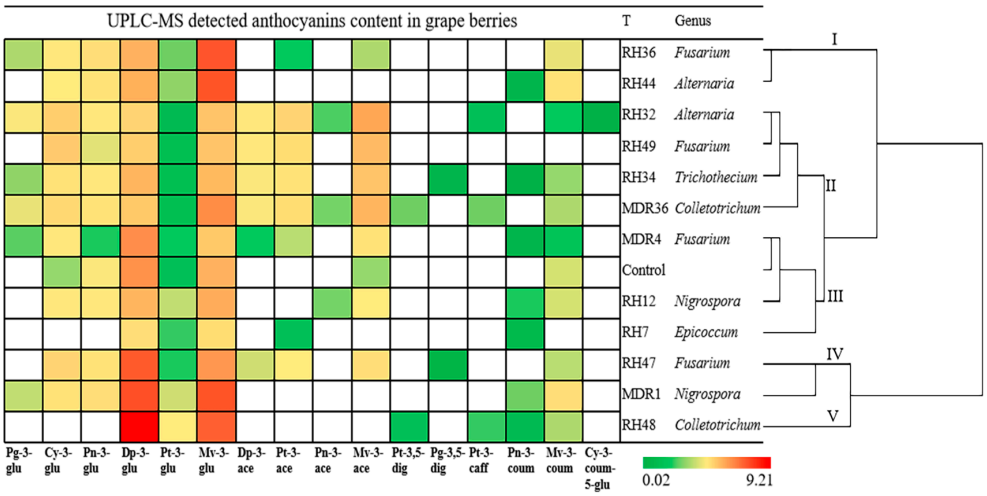

Figure 3. Heatmap and clustering of the anthocyanin content detected by UPLC–MS in CS berries after co-culture with fungal endophytes. T, treatment; Genus, genus of fungal endophytes. The abbreviations of anthocyanins are as follows: Pg-3-glu, pelargonidin-3-*O*-glucoside; Cy-3-glu, cyanidin-3-*O*-glucoside; Pn-3-glu, peonidin-3-*O*-glucoside; Dp-3-glu, delphinidin-3-*O*-glucoside; Pt-3-glu, petunidin-3-*O*-glucoside; Mv-3-glu, malvidin-3-*O*-glucoside; Dp-3-ace, delphinidin-3-O-acetylglucoside; Pt-3-ace, petunidin-3-O-acetylglucoside; Pn-3-ace, peonidin-3-O-acetylglucoside; Mv-3-ace, malvidin-3-O-acetylglucoside; Pt-3,5-dig, petunidin-3,5-O-diglucoside; Pg-3,5-dig, pelargonidin-3,5-O-diglucoside; Pt-3-caff, petunidin-3-O-caffeoylglucoside; Pn-3-coum, peonidin-3-O-coumarylglucoside; Mv-3-coum, malvidin-3-O-coumarylglucoside; Cy-3-coum-5-glu, cyanidin-3-O-coumarylglucoside-5-O-glucoside.

Table 4. Impact of fungal endophytes inoculation on special parameters of anthocyanins.

Treatment	Number of Total Anthocyanins Detected	Number of Novel Anthocyanins Detected	Number of Suppressed Anthocyanins	Total Content of Detected Anthocyanins (mg 100 g^{-1})	The Main Anthocyanin	Content of Main Anthocyanin (mg 100 g^{-1})
RH7	5	2	4	2.18	Malvidin-3-*O*-glucoside	0.93
RH12	9	2	0	7.32	Malvidin-3-*O*-glucoside	2.69
RH32	13	6	0	10.69	Malvidin-3-O-acetylglucoside	2.97
RH34	12	5	0	9.52	Delphinidin-3-*O*-glucoside	2.42
RH36	9	2	0	11.1	Malvidin-3-*O*-glucoside	5.91
RH44	7	1	1	10.7	Malvidin-3-*O*-glucoside	5.96
RH47	10	3	0	13.13	Delphinidin-3-*O*-glucoside	5.71
RH48	7	3	3	15.79	Delphinidin-3-*O*-glucoside	9.21
RH49	8	2	1	8.92	Malvidin-3-O-acetylglucoside	2.18
MDR1	8	2	1	15.53	Delphinidin-3-*O*-glucoside	6.19
MDR4	11	4	0	7.86	Delphinidin-3-*O*-glucoside	3.80
MDR36	13	6	0	12.5	Malvidin-3-*O*-glucoside	3.98
Control	7			7.61	Delphinidin-3-*O*-glucoside	3.78

Furthermore, when clustering based on the concentration of anthocyanidins, the strains were divided into five groups (Figure 3). Group I included fungal strains RH36 and RH44. Group II included the fungal strains RH32, RH49, RH34, and MDR36. Groups I and II had moderate impacts on anthocyanins. Group III included fungal strains MDR4, RH12, RH7, and the non-fungal co-culture control, which suggested that these fungal strains had a lower impact on the anthocyanins of CS berries. Group IV and V included three strains (RH47, RH48, and MDR1) from the genera *Fusarium*, *Nigrospora*, and *Colletotrichum*, which had great impacts on the anthocyanidins of the co-cultivated berries.

PCA was performed to visualize the effects of endophytic fungal inoculation on the biochemical traits and anthocyanidin profiles of CS grape berries and to summarize the results of the research (Figure 4). The principal components PC1 and PC2 explained approximately 73.4% of the total variance. In the biplot, PC1 was based largely on the TPh and TF contents and PAL activity, while PC2 was mainly related to the differences in total anthocyanin and SPr concentrations (Figure 4). The biplot provides a visual representation of the impacts of endophytic fungal inoculation on metabolic profiles. Based on the position of the strains on PC1 and PC2, the fungal strains RH36, RH32, RH49, and MDR4 had stronger positive impacts on TPh, TF, and PAL, and MDR1 had the strongest positive impacts on total anthocyanin and SPr concentrations. Strains RH7, RH12, RH44, RH47, and RH48 contributed negatively to the metabolites.

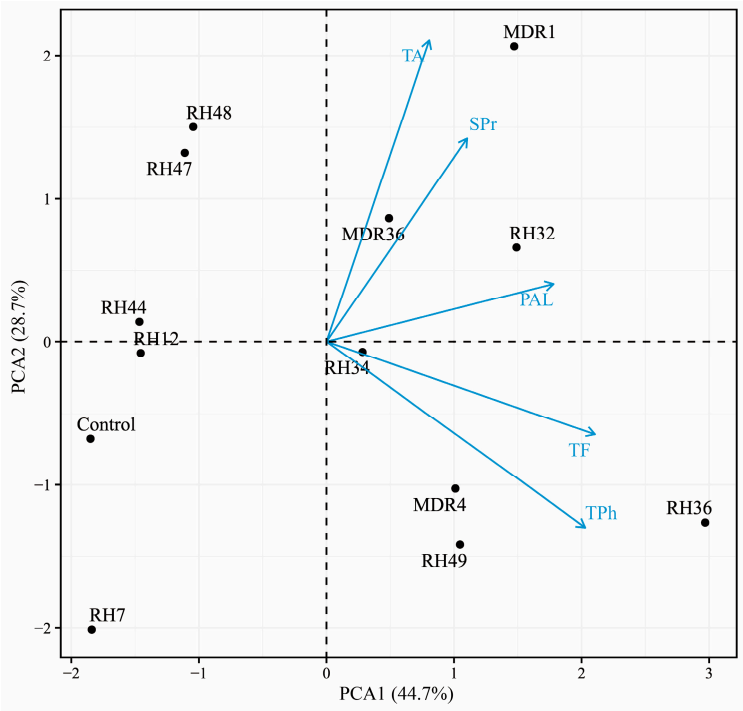

Figure 4. PCA of the impacts of endophytic fungal inoculation on the biochemical traits and anthocyanin profiles of grape berries. The blue lines represent the direction and length of variables (TA: total anthocyanin concentration; PAL: phenylalanine ammonia-lyase; SPr: total soluble protein; TPh, total phenol; TF: total flavonoid content) and indicate the contribution of the variables to the principal components.

4. Discussion

During their long-term evolution, a mutualistic and symbiotic relationship was gradually established between endophytes and grapevines, and beneficial endophytes are expected to be utilized in viticulture. The red variety of grapes is rich in anthocyanins with health benefits, which are recognized as potential pharmaceutical ingredients. Several factors could influence the composition of anthocyanins in grapes, such as variety, cultivation management, biotic and abiotic factors [10,22–26]. The anthocyanins present in grape berries are glucosides originating from delphinidin, cyanidin, petunidin, peonidin, malvidin, and pelargonidin [27,28]. Our previous studies showed that endophytic fungi in grapes may contribute to anthocyanin production [10,14], which raised the possibility of reshaping the anthocyanidin profile and thus the grape qualities by applying endophytes. As in our previous study, co-culture with 12 strains of endophytic fungi differentially shaped the biochemical properties and the anthocyanin profiles of post-veraison grape berries of 'Rose Honey' (*Vitis vinifera* L. × *V. labrusca* L., the main local variety in Yunnan, China) [14]. In this study, to further explore whether there are variety-specific differences in the response to endophytic fungal infection, we evaluated the effects of endophytic fungi on the worldwide planted wine grape variety *Vitis vinifera* L. cv. 'Cabernet Sauvignon'.

Consistent with the results for the RH variety [14], co-culture with most of the endophytic fungi shaped the biochemical characteristics and the anthocyanidin profile of grape berries of the CS variety. Regarding biochemical properties, PAL activity was promoted by all the strains in both RH and CS, especially strains RH36, MDR4, MDR1, and MDR36. Fungal strains RH32, RH34, RH44, RH47, RH49, MDR1, and MDR36 promoted the TF content of the two varieties of grape berries, while the fungal strain RH7 inhibited it [14]. The TPh content in both varieties of grape berries was significantly promoted by strains RH12, RH32, RH34, RH44, RH49, MDR1, MDR4, and MDR36 [14].

Regarding anthocyanin profiles, a total of sixteen anthocyanins were detected in CS berries, including six monoglucosides, four acylated monoglucosides, two diglucosides,

two (3-*O*-*p*-coumaroyl) glucosides, one 3-*O*-caffeoylglucoside, and one (3-*O*-*p*-coumaroyl)-5-O-glucoside. Regarding the composition and concentration of the detected anthocyanins, there were fewer anthocyanins in CS than in RH, which may be due to the different maturity stages of the CS and RH grape berries. Additionally, clear similarities and differences were observed between endophytic fungi in shaping berry anthocyanidins in the CS and RH cultivars. For example, both grape berry varieties exposed to fungal strains MDR36 and RH34 contained the most anthocyanidin species, and total anthocyanin concentrations were promoted by strains RH36, RH44, MDR1, and MDR36, and suppressed by strain RH7. In CS berries, the malvidin and delphinidin derivatives accounted for the majority of the relative abundance of the total detected anthocyanins, while the malvidin and cyanidin derivatives accounted for the highest proportions in the RH berries. Interestingly, owing to the exposure to fungal endophytes, the acylation degree of monoglucosides was significantly promoted in the two varieties of grape berries. Acylated anthocyanins having more than one acyl group are more stable than unacylated anthocyanins [29]. Co-culture with endophytic fungi promoted the higher acylation degree of anthocyanins in CS and RH berries, which indicated that they have the potential to improve color hue and color stability. However, acetyldiglucoside was not detected in all CS berries, but was detected in all RH berries. Furthermore, caffeoyldiglucoside was detected in RH12- and MDR1-treated RH berries, while (*p*-coumaroyl)diglucoside was detected only in RH32-treated CS grape berries.

Additionally, pelargonidin-3-*O*-glucoside and diglucosylated anthocyanins are believed to be rarely present in the cultivar *Vitis vinifera* [30,31]. Pelargonidin-3-*O*-glucoside was detected at trace levels in the berry skins of CS and Pinot Noir (*V. vinifera* L.) [32]. In our study, neither pelargonidin-3-*O*-glucoside nor anthocyanin diglucoside were detected in no-fungi-treated CS berries. However, pelargonidin-3-*O*-glucoside was detected in CS grape berries treated with strains RH32, RH34, RH36, MDR1, MDR4, and MDR36. In RH berries, pelargonidin-3-*O*-glucoside was detected both in controls and treatments, and pelargonidin-3,5-*O*-diglucoside was detected in most fungal treatments [14]. Petunidin-3,5-*O*-diglucoside was detected in RH48- and MDR36-treated CS grape berries, and pelargonidin-3,5-*O*-diglucoside was detected in RH34- and RH47-treated CS grape berries. These results indicated that co-culture with endophytic fungi could induce the production of novel anthocyanins of grape berries. Notably, diglucoside anthocyanins exhibit better color stability than their monoglucosylated counterparts [33].

5. Conclusions

A narrow sense coculture method was developed to directly evaluate the impacts of certain pure cultured endophytic fungi on grape berries in the present study. The results confirmed that exposure to endophytic fungi could reshape the physio-biochemical properties and anthocyanin profiles of grape berries in either the local variety 'Rose Honey' or the worldwide planted wine grape variety 'Cabernet Sauvignon'. The acylation degree of anthocyanins in grape berries was also significantly promoted by exposure to fungal endophytes. In 'Cabernet Sauvignon' grape berries, a seldom distributed anthocyanidin, pelargonidin-3-*O*-glucoside, as well as diglucoside anthocyanidin were detected after exposure to fungal strains such as RH32, RH34, RH36, MDR1, MDR4, and MDR36. Overall, endophytic fungal strains MDR36, RH36 and RH34 have the ability to promote metabolite profiles in both 'Rose Honey' and 'Cabernet Sauvignon'. Therefore, the study provides a practical method for screening candidate fungal endophytes to purposely shape grape metabolic profiles and suggests a strategy for grape quality management in viticulture by using fungal endophytes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020237/s1, Table S1: RI of physio-chemical traits in grape berries influenced by co-culture with fungal endophytes; Table S2: Structure of anthocyanins detected by UPLC–MS.

Author Contributions: X.-X.P. performed most of the experiments, data acquisition, and wrote the main manuscript text; C.-X.C. and Y.-T.W. participated partially the experiments; Y.-Y.Z. conceived the research; M.-Z.Y. designed the research and reviewed the paper. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data used to support the findings are included in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol. Mol. Biol. Rev.* **2015**, *79*, 293–320. [CrossRef] [PubMed]
2. Prajapati, J.; Goswami, D.; Rawal, R.M. Endophytic Fungi: A Treasure Trove of Novel Anticancer Compounds. *Curr. Res. Pharmacol. Drug Discov.* **2021**, *2*, 100050. [CrossRef] [PubMed]
3. Patil, R.H.; Maheshwari, V.L. *Endophytes: Potential Source of Compounds of Commercial and Therapeutic Applications*; Springer: Berlin/Heidelberg, Germany, 2021; p. 60.
4. Caradus, J.R.; Johnson, L.J. Improved Adaptation of Temperate Grasses through Mutualism with Fungal Endophytes. In *Endophyte Biotechnology: Potential for Agriculture and Pharmacology, CABI Biotechnology Series*; Schouten, A., Ed.; Wageningen University and Research Centre: Wageningen, The Netherlands, 2019; p. 85.
5. Eid, A.M.; Salim, S.S.; Hassan, S.E.-D.; Ismail, M.A.; Fouda, A. Role of Endophytes in Plant Health and Abiotic Stress Management. In *Microbiome in Plant Health and Disease*; Springer: Singapore, 2019; pp. 119–144. [CrossRef]
6. Balik, J.; Kyselakova, M.; Vrchotova, N.; Triska, J.; Kumsta, M.; Veverka, J.; Hic, R.; Totusek, J.; Lefnerrova, D. Relations between Polyphenols Content and Antioxidant Activity in Vine Grapes and Leaves. *Czech J. Food Sci.* **2008**, *26*, S25–S32. [CrossRef]
7. Downey, M.O.; Harvey, J.S.; Robinson, S.P. Analysis of Tannins in Seeds and Skins of Shiraz Grapes throughout Berry Development. *Aust. J. Grape Wine Res.* **2003**, *9*, 15–27. [CrossRef]
8. Flamini, R.; Mattivi, F.; De Rosso, M.; Arapitsas, P.; Bavaresco, L. Advanced Knowledge of Three Important Classes of Grape Phenolics: Anthocyanins, Stilbenes and Flavonols. *Int. J. Mol. Sci.* **2013**, *14*, 19651–19669. [CrossRef]
9. Buchanan, B.B.; Gruissem, W.; Jones, R.L. *Biochemistry and Molecular Biology of Plants*, 2nd ed.; Wiley: Hoboken, NJ, USA, 2015. [CrossRef]
10. Yu, M.; Chen, J.C.; Qu, J.Z.; Liu, F.; Zhou, M.; Ma, Y.M.; Xiang, S.Y.; Pan, X.X.; Zhang, H.B.; Yang, M.Z. Exposure to Endophytic Fungi Quantitatively and Compositionally Alters Anthocyanins in Grape Cells. *Plant Physiol. Bioch.* **2020**, *149*, 144–152. [CrossRef]
11. Burns, J.; Mullen, W.; Landrault, N.; Teissedre, P.; Lean, M.E.J.; Crozie, A. Variations in the Profile and Content of Anthocyanins in Wines Made from Cabernet Sauvignon and Hybrid Grapes. *J. Agric. Food Chem.* **2002**, *50*, 4096–4102. [CrossRef]
12. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits. *Food Nutr. Res.* **2017**, *61*, 1361779. [CrossRef]
13. Aleynova, O.A.; Suprun, A.R.; Nityagovsky, N.N.; Dubrovina, A.S.; Kiselev, K.V. The Influence of the Grapevine Bacterial and Fungal Endophytes on Biomass Accumulation and Stilbene Production by the In Vitro Cultivated Cells of *Vitis amurensis* Rupr. *Plants* **2021**, *10*, 1276. [CrossRef]
14. Pan, X.X.; Xiang, S.Y.; Zhu, Y.Y.; Yang, M.Z. Co-cultivation with Endophytic Fungi Differentially Shaped the Anthocyanin Profiles in Post-veraison Grape Berries. *Appl. Biochem. Micro.* **2022**, *58*, 780–789. [CrossRef]
15. Chen, J.C.; Yu, M.; Liu, F.; Qu, J.Z.; Pan, X.X.; Zhang, H.B.; Yang, M.Z. Diversity Distributions and the Anthocyanin Associations of Fungal Endophytes in Different Colored Grapevine Leaves. *J. Plant Biol.* **2020**, *63*, 107–116. [CrossRef]
16. Ma, M.D.; Zhang, X.Y.; Cheng, Y.; Huang, Z.Y.; Zhang, H.B.; Yang, M.Z. Impacts on Foliar Endophytic Fungi Community Structures after Exogenous Endophytic Fungi Re-inoculation. *Microbiol. China* **2014**, *41*, 2458–2465.
17. White, T.J. Amplification and Direct Seqencing of Fungal Ribosomal RNA Genes for Phylogenetics. *Pcr. Protoc.* **1990**, *18*, 315–322.
18. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
19. Pan, X.X.; Chen, J.C.; Liu, F.; Qu, J.Z.; Zhu, Y.Y.; Yang, M.Z. Symbioses of Endophytic Fungi and Subsequent Physio-chemical Changes in Grapevine Leaves from Two Cultivars. *Russ. J. Plant Physiol.* **2021**, *68*, 735–744. [CrossRef]
20. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
21. Asami, D.K.; Hong, Y.J.; Barrett, D.M.; Mitchell, A.E. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-dried and Air-dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. *J. Agric. Food Chem.* **2003**, *51*, 1237–1241. [CrossRef]

22. Blanco-Ulate, B.; Amrine, K.C.; Collins, T.S.; Rivero, R.M.; Vicente, A.R.; Morales-Cruz, A.; Doyle, C.L.; Ye, Z.; Allen, G.; Heymann, H.; et al. Developmental and Metabolic Plasticity of White-skinned Grape Berries in Response to *Botrytis cinerea* during Noble Rot. *Plant Physiol.* **2015**, *169*, 2422–2443. [CrossRef]
23. Calderan, A.; Sivilotti, P.; Braidotti, R.; Mihelcic, A.; Lisjak, K.; Vanzo, A. Managing Moderate Water Deficit Increased Anthocyanin Concentration and Proanthocyanidin Galloylation in "Refosk" Grapes in Northeast Italy. *Agric. Water Manag.* **2021**, *246*, 106684–106693. [CrossRef]
24. Guan, L.; Dai, Z.; Wu, B.H.; Wu, J.; Merlin, I.; Hilbert, G.; Renaud, C.; Gomès, E.; Edwards, E.; Li, S.H.; et al. Anthocyanin Biosynthesis is Differentially Regulated by Light in the Skin and Flesh of White-fleshed and Teinturier Grape Berries. *Planta* **2016**, *243*, 23–41. [CrossRef]
25. Martínez-Lüscher, J.; Sánchez-Díaz, M.; Delrot, S.; Aguirreolea, J.; Pascual, I.; Gomès, E. Ultraviolet-B Radiation and Water Deficit Interact to Alter Flavonol and Anthocyanin Profiles in Grapevine Berries through Transcriptomic Regulation. *Plant Cell Physiol.* **2014**, *55*, 1925–1936. [CrossRef] [PubMed]
26. Mori, K.; Goto-Yamamoto, N.; Kitayama, M.; Hashizume, K. Loss of Anthocyanins in Red-Wine Grape under High Temperature. *J. Exp. Bot.* **2007**, *58*, 1935–1945. [CrossRef] [PubMed]
27. De Lorenzis, G.; Rustioni, L.; Parisi, S.G.; Zoli, F.; Brancadoro, L. Anthocyanin Biosynthesis during Berry Development in Corvina Grape. *Sci. Hortic.* **2016**, *212*, 74–80. [CrossRef]
28. Obreque-Slier, E.; Herrera-Bustamante, B.; Lopez-Solís, R. Ripening-associated Fattening out of Inter-varietal Differences in Some Groups of Phenolic Compounds in the Skins of Six Emblematic Grape Wine Varieties. *J. Food Compos. Anal.* **2021**, *99*, 103858. [CrossRef]
29. Sun, J.X.; Zhang, Y.; Hu, X.S.; Wu, J.H.; Liao, X.J. Structural Stability and Degradation Mechanisms of Anthocyanins. *Sci. Agri. Sin.* **2009**, *42*, 996–1008.
30. Liang, Z.; Wu, B.; Fan, P.; Yang, C.; Duan, W.; Zheng, X.; Liu, C.; Li, S. Anthocyanin Composition and Content in Grape Berry Skin in *Vitis* germplasm. *Food Chem.* **2008**, *111*, 837–844. [CrossRef]
31. Zhao, Q.; Duan, C.Q.; Wang, J. Anthocyanins Profile of Grape Berries of *Vitis amurensis*, Its Hybrids and Their Wines. *Int. J. Mol. Sci.* **2010**, *11*, 2212–2228. [CrossRef]
32. He, F.; He, J.J.; Pan, Q.H.; Duan, C.Q. Mass-spectrometry Evidence Confirming the Presence of Pelargonidin-3-*O*-glucoside in the Berry Skins of Cabernet Sauvignon and Pinot Noir (*Vitis vinifera* L.). *Aust. J. Grape Wine R.* **2010**, *16*, 464–468. [CrossRef]
33. Jánváry, L.; Hoffmann, T.; Pfeiffer, J.; Hausmann, L.; Töpfer, R.; Fischer, T.C.; Schwab, W. A Double Mutation in the Anthocyanin 5-*O*-Glucosyltransferase Gene Disrupts Enzymatic Activity in *Vitis vinifera* L. *J. Agric. Food Chem.* **2009**, *57*, 3512–3518. [CrossRef]