Vitis **56**, 71–77 (2017) DOI: 10.5073/vitis.2017.56.71-77

In vitro specific interactions revealed the infective characteristics of fungal endophytes to grapevine

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Summary

In the present study a method for co-culture of fungal endophytic strains and grape cells was developed in order to study their interactions, and filter candidates for further safe inoculation in the vineyard. Analysis of morphological and physiological traits was performed by measuring the plant callus and fungal growth, plant cells viability, degree of cell oxidation and the scale of contact or its absence as reaction of the fungal endophyte to the presence of the plant callus. Accordingly, endophytic fungal strains (EFS) were classified on scale of invasion into categories (strong - medium - weak invasive), as well as the contact between the two partners (grow into - grow onto - contact - no contact) and the grape cell oxidation degree (normal (no oxidation) - light - moderate - serious). More included the dominance and distribution of EFS in the plant host, and correlation plots of physiological traits during plant callus and endophytic fungi co–culture were calculated.

K e y w o r d s : co-culture; grape callus; categorized fungal endophytes; grape quality management.

Introduction

Endophytes are symbiotic organisms that live within plant tissues or organs but cause no obvious symptoms of infection (Stone *et al.* 2000). It was proved to exert multiple impacts on their host plants, such as growth promotion (Lu *et al.* 2000, Khan *et al.* 2012, Doty 2015), increased adaptability to stresses (e.g. Marks and Clay 1996, Kuldau and Bacon 2008, Ownley *et al.* 2008), as well as metabolites regulations (Rasmussen and Newman 2008, Yang *et al.* 2016). Some endophytic fungi produce compounds similar to those found in the host plant, and these fungi are potential sources for active compounds that may have medical, agricultural and industrial applications (Rodriguez *et al.* 2009, Aly *et al.* 2010, Zhao *et al.* 2010, Kaul *et al.* 2012, Kusari and Spiteller 2012). On the other side, metabolic profiles of plants could be purposely induced or modified by certain kinds of endophytes (Rasmussen and Newman 2008, Wang *et al.* 2010, Yang *et al.* 2016). And parameters of appearance of grape berries were shaped using fungal endophytes (Huang *et al.* 2015). This implies the possibility to manage crop qualities by tools of endophytes, especially to those crops used to produce organoleptic sensitive products, such as wine, coffee and others. However, the under-covered mechanisms involved in plant-endophytes interactions greatly hindered the application of endophytes in crop quality management. More how to select candidate fungal endophytes from tremendous fungal strains for purpose use need effective methods.

In studying interactions of fungi and plant hosts, it is often advantageous to employ simplified systems, such as dual culture. Using dual cultures of plant calli and endophytes, Peters *et al.* (1998) found that in interactions of endophytes with their own hosts, metabolites secreted by the host calli into the growth media resulted in positive growth responses of the endophytes. However, in dual culture with the callus of a non-host, this was not the case, suggesting that the endophytes responded to specific stimuli produced by their respective hosts (Peters *et al.* 1998). Similarly, growth of an endophyte, *Cryptodiaporthe hystrix*, and of an EM fungus was greater in dual culture with callus of the host than it was with that of a non-host (Sieber *et al.* 1990, Sirrenberg *et al.* 2010). Same effect with the grass endophyte *Aktinsonella* was found, and suggested that growth of fungi correlates positively with host compatibility (Lu 1994). Growth stimulation in the host-interactions seemed to be due to chemotaxic signalling with non-volatile substances, and not to diffusion of specific nutrients, since the dual cultures grew on a complex medium (Peters *et al.* 1998). This study however introduced another simplified method of solid co-culture which allowed to analyses the interactions between plant callus and fungal endophytes, within two weeks. And by analysing the specific interactions between fungal endophytes and grape cells during the co-culture, fungal strains were categorized and selected for different purposes in viticulture.

Material and Methods

P r e p a r a t i o n o f g r a p e (*V i t i s v i n i f e r a*, 'C a b e r n e t S a u v i g n o n') c a l l u s : Grape calli induced from grapevine shoots and sub-cultured for gener-

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ations were used in the experiments. B5 solution with 3 % sucrose, 0.2 mg·L^{-1} cytokinin, 0.1 mg·L^{-1} naphthylacetic acid (NAA) and 0.75 % agar was prepared as the medium for callus sub-culture and the following co-culture. All prepared grape calli were in the logarithmic growth phase.

Preparation of endophytic fungi: All foliar endophytic fungal strains (EFS, Tab. 1) were isolated from 'Cabernet Sauvignon' (*Vitis vinifera)* and another local variety, 'Rose honey' (*V. Vinifera* L.× *V. labrusca* L.) in local vineyards (Yunnan province, China). Four EFS (ECS2, ECS11, ECS13 and ECS16) were host variety originated, and the left 17 fungal strains were isolated from a non-host variety (Tab. 1). Endophytic fungal strains were then identified using ITS DNA sequences (MA *et al.* 2014). Before the co-culture with plant cells, fungal strains were plate cultured on potato dextrose agar (PDA) medium in 9 cm diameter petri dishes for one week.

Construction of fungus-callus co-culture system: Sterilized 30 mL callus medium was added to each sterilized petri dish to generate solid culture plates. Approximately 1.5 g of prepared grape callus was weighed and inoculated into the centre of every Petri dish, and adjusted to a height of 0.3 cm. The plates were dark cultured at 25 °C for 7 d. After one week of culture, the callus entered the rapid propagation stages (the 5th to 16th d after inoculation, according to pilot experiment). Then, fungal strains were inoculated on the same plate (7th d after the callus inoculation). When starting the co-culture, mycelial discs were generated using a sterilized 0.8 cm diameter puncher from the PDA plate for every fungal strain. Three mycelial discs of one fungal strain were inoculated onto one plate at approximately the same distance (~2 cm) from the callus, as shown in Fig. 1. Calli without fungal inoculation were used as callus control and plates without calli but inoculated with mycelial discs of fungal strains were used as fungus control (Fig. 1). Every treatment and control contains 3-5 biological replicates.

Measuring the growth rate of endophytic fungi and grape calli during the co-culture: The diameter of fungal colonies was measured daily in both the fungal controls and the co-cultures. Growth rates were then calculated using the following formulae: Growth rate (GR) = (D1-D0)/T (D1: colonial diameter of the harvest day; D0: diameter of mycelial disc

Table 1

Strains of fungal endophytes used in the experiment

Fungal stain ID	Species	Distribution [a)]	Dominance (%) [b)]
ERH48	*Colletotrichum Gloesporioides*	rare	5.32
ERH37	*Epicoccum Nigrum*	medium	32.73
ERH46	*Alternaria Arborescens*	Wide	60.67
ERH12	*Niqrospora Sphaerica*	medium	10.77
ERH38	*Epicoccum Nigrum*	medium	32.73
ERH6	*Alternaria Alternaria*	wide	78.26
ERH45	*Daldinia Eschscholtzii*	rare	3.19
ERH28	*Alternaria Alternaria*	wide	78.26
ERH32	*Alternaria Alternaria*	wide	78.26
ERH34	*Trichothecium Roseum*	rare	3.13
ERH7	*Epicoccum Nigrum*	medium	32.73
ERH5	*Trichothecium* sp.	rare	5.76
ERH43	*Alternaria Arborescens*	wide	60.67
ERH44	*Alternaria Arborescens*	wide	60.67
ERH31	*Alternaria Alternaria*	wide	78.26
ERH16	*Epicoccum Nigrum*	medium	32.73
ERH24	*Alternaria Arborescens*	wide	60.67
ECS2	*Colletotrichum Gloesporioides*	rare	5.42
ECS11	*Epicoccum Nigrum*	medium	32.73
ECS13	*Fusarium Oxysporum*	medium	0.28
ECS16	*Alternaria* sp.	wide	25.21

Notes: [a)] "Distribution" means the probability of the fungal endophytes isolated from tested samples, rare degree of distribution indicates probability of isolation of these fungal strains from the tested grapevine samples was less than 30 %. A probability between 30 % and 60 % is interpreted as medium degree of distribution, and more than 60 % as wide degree of distribution (MA *et al.* 2014). [b)] Dominance is the maximum degree of dominance of a specific endophytic fungus in the community of a plant (MA *et al.* 2014). The strain ID marked with "ERH" means the endophytic fungus was isolated from grape variety 'Rose honey' (*V. vinifera* L. × *V. labrusca* L.) and "ECS" indicates the fungal strain was isolated from grape variety 'Cabernet Sauvignon' (*V. vinifera*).

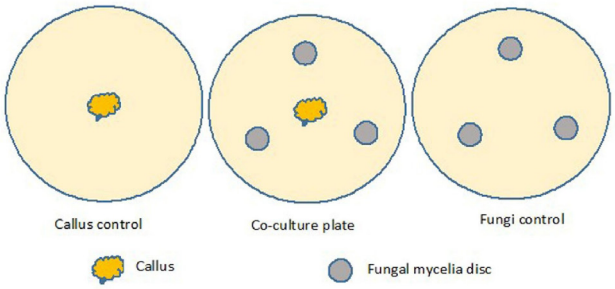

Fig. 1: The solid co-culture method for candidate fungal endophytes selection through fungi-calli interactions. Mycelia discs of fungal endophytes were harvested from potato dextrose agar (PDA) plate, and inoculated 7 d after the inoculation of calli. During the co-culture, both calli and fungi were cultured in callus medium. Two separate controls were set for each partner of the co-culture, as callus control and fungus control, respectively.

(0.8 cm) and T: days of the co-culture duration). Growth rate was presented as daily increased centimetres of fungal colonial diameter. Callus was weighed at the beginning and the end of the co-culture, and the daily increased mass (gram) was used to describe the growth rates of the calli.

Determination of morphological traits for both the fungal colonies and grape callus: After 8 d of co-culture, the scale of contact between the fungal mycelia and callus, as well as the degree of callus oxidation, was recorded. The contact or absence of fungal mycelia to callus was determined under a stereoscope. If one of the inoculated fungi contacted with (or grown onto/into) the plant cell, the assay was interpreted as a "contact", and if at least one replicate acted as so. Conversely, only when all replicates didn't contact with plant cells, the assay was interpreted as "no contact". Intimate physical contacts were apparently divided as "overgrowth" or "ingrowth". The "overgrowth" means the fungal mycelia grow on the surface of the callus and "ingrowth" means the fungal mycelium penetrates the callus, growing into the cellular spaces. Oxidation degrees of grape calli were judged according to the colour of the callus, normal (no oxidation and light yellowed); light oxidation (dark yellowed); medium oxidation (light brown) and serious oxidation (dark brown). When different degrees of oxidation occurred in replicate, the most serious degree of oxidation was used to interpret the result of treatment. Photos were taken for every treatment to record the morphological responses of both the endophytic fungi and calli.

Measurement of grape cell viability: Approximately 10 mg of the grape callus was added to a 10 mL tube, and 4.5 mL distilled water, 2-3 drops of 0.2 % prepared pectinase were then added, and well mixed. The mixture was incubated in a 40 °C water bath for 5 min to release single cells via hydrolysis. After cooled to room temperature, plant cells were then dyed by adding 0.5 mL 0.4 % trypan blue solution. Dyed cells were observed under microscope, and the percentage of undyed cells was calculated and used to represent the viability of the plant cell (Louis and Siegel 2011).

Data analysis: Values of physiological traits were shown as "mean" ± "standard error" for multiple replicates and were analysed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) for Windows. Data were analysed by one-way ANOVA followed by Tukey's multiple comparison test at $P < 0.05$, for the significance determination. Pearson's correlation analysis was conducted to determine the correlation between variables within or between physiological parameters. Response indexes (RI) were used to describe the effects and were calculated by following formula: Response indexes (RI) = $(V_{treatment}-V_{control})\cdot V_{control}^{-1} \times 100$. In the formula, $V_{treatment}$ is the mean value of a variable, and $V_{control}$ is the value of the control of the corresponding variable. A positive RI indicates promotion of an effect, and a negative RI indicates inhibition. Correlation plots were plotted using Sigma Plot 10.0 (Systat Software Inc., San Jose, CA).

Results

The solid fungus-callus co-culture allowed successful monitoring the interactions between endophytic fungi and plant cells *in vitro*, at both the morphological and physiological levels. After 8 days of co-culture, fungus and callus appeared morphologically different from one fungal strain to another. According to the scales of physical contact between fungal mycelium and callus, fungal strains can be divided as no contact, contact, "overgrowth" and "ingrowth" (Tab. 2 and Fig. 2). In those tested fungal strains, only ERH12 showed no physical contact, and four fungal strains ERH7 (*Epicoccum* sp.), ECS11 (*Epicoccum* sp.), ERH45 (*Daldinia* sp.) and ECS13 (*Fusarium* sp.), showed "contact" to the co-cultured grape callus. All other tested fungal

Table 2

Morphological responses of endophytic fungi and callus during co-culture

strains	Fungal genus	Scale of contact	degree of callus oxidation
ERH46	*Alternaria*	ingrowth	serious
ERH6	*Alternaria*	ingrowth	serious
ERH28	*Alternaria*	ingrowth	serious
ERH32	*Alternaria*	ingrowth	serious
ERH43	*Alternaria*	ingrowth	serious
ECS16	*Alternaria*	ingrowth	serious
ERH44	*Alternaria*	ingrowth	serious
ERH31	*Alternaria*	ingrowth	serious
ERH24	*Alternaria*	ingrowth	serious
ERH37	*Epicoccum*	ingrowth	normal (green)
ERH38	*Epicoccum*	ingrowth	normal
ERH7	*Epicoccum*	contact	normal
ECS11	*Epicoccum*	contact	light
ERH16	*Epicoccum*	ingrowth	medium (green)
ERH48	*Colletotrichum*	overgrowth	medium
ECS2	*Colletotrichum*	overgrowth	moderate
ERH34	*Trichothecium*	ingrow	light (green)
ERH5	*Trichothecium*	ingrowth	medium
ERH12	*Niqrospora*	no contact	normal
ERH45	*Daldinia*	contact	light
ECS13	*Fusarium*	contact	normal
Control	----------	----------	normal

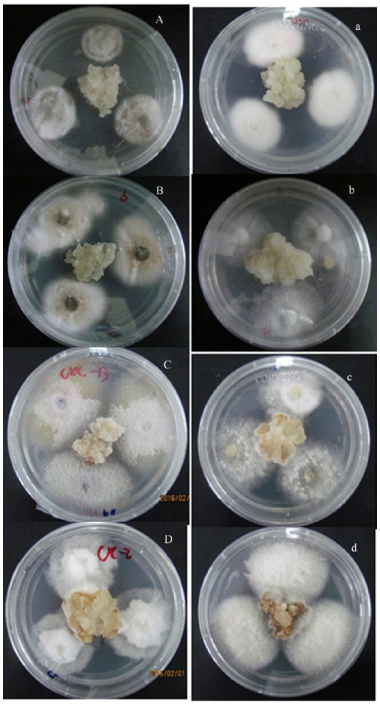

Fig. 2: Morphological interactions between co-cultured fungal endophytes and grape callus after 8 d of co-culture. For scales of contact, **A**: not contact; **B**: contact; **C**: ingrowth; and **D**: overgrowth. And as callus oxidation degrees, a: normal (no oxidation); b: light oxidation; c: moderate oxidation; and d: serious oxidation.

strains except ERH34 and ERH5, showed "ingrowth" into the callus during 8 d of co-culture. Fungal strains ERH34 and ERH5, which belong to the same genus *Colletotrichum* overgrew the surface of grape calli. Interestingly, all fungal strains from genus *Alternaria* in this experiment grew into the grape callus during the co-culture. On the other hand, in response to fungal strains, grape calli experienced different degrees of oxidization, from no oxidation (normal) to light, medium and serious oxidation. (Tab. 2 and Fig. 2). Fungal strains, such as ERH37 (*Epicoccum* sp.) and ERH34 (*Trichothecium* sp.), caused the co-cultured calli to turn green, which may be another type of oxidative responses (Tab. 2). Obvious correlations were observed between callus oxidation and physical contact between the co-culture patterns. Serious oxidation occurred only in those calli with "ingrowth" during the co-culture. However, the intimate physical contact between co-cultured callus and fungus ("ingrowth"/"overgrowth"), may cause different degrees of callus oxidation, from normal to serious (Tab. 2). Noteworthy, all used fungal strains from genus *Alternaria* in this experiment, have caused serious oxidation to the co-cultured grape callus (Tab. 2), while 5 of the total 6 fungal strains from genus *Epicoccum* almost caused no oxidation to the co-cultured grape callus (Tab. 2).

Growth rates of the fungal strains were either promoted or inhibited during the co-culture process compared to the control (Tab. 3). The growth rate of fungal strains ERH32, ERH31, ERH5, ECS2 and ERH28 were signifi-

Table 3

Significances of difference, response indexes (RI) of the detected physiological traits, and categories of the tested strains of fungal endophytes

Fungal strain	growth rate of callus value ($g \cdot day^{-1}$)	RI (%)	growth rate of fungus value ($cm \cdot day^{-1}$)	RI (%)	viability of gape cell value (%)	RI (%)	Fungal category
ERH45	0.411 ± 0.061 bcd	-33.5	0.440 ± 0.021	-17.3	68.333 ± 1.86 abcde	-23.2	Ia
ECS11	0.133 ± 0.061 ef	-78.4	0.673 ± 0.021 *	-26.8	74.667 ± 3.48 abcd	-16.1	Ia
ERH12	0.371 ± 0.025 bcde	-39.9	0.497 ± 0.012	-13.1	85.000 ± 5.13 abc	-4.5	Ib
ERH7	0.312 ± 0.053 cdef	-49.5	0.657 ± 0.137 *	-30.7	75.667 ± 4.48 abcd	-15	Ib
ECS13	0.099 ± 0.026 g	-84	0.971 ± 0.107	1.1	69.000 ± 3.46 abcde	-22.5	Ib
ERH37	0.130 ± 0.018 ef	-78.9	0.737 ± 0.019 *	-26.3	43.667 ±14.52 fg	-50.9	IIa
ERH24	0.237 ± 0.034 cdef	-61.6	0.779 ± 0.016	-14.2	44.000 ± 5.13 fg	-50.6	IIa
ERH46	0.212 ± 0.026 cdef	-65.7	0.564 ± 0.017 **	-42	61.667 ± 8.41 def	-30.7	IIb
ERH6	0.194 ± 0.044 cdef	-68.6	0.604 ± 0.084 *	-25.6	50.333 ±3.84 efg	-43.4	IIb
ERH32	0.154 ± 0.035 def	-75.1	0.747 ± 0.013 **	69.7	54.000 ± 14.29 defg	-39.3	IIb
ERH34	0.142 ± 0.028 ef	-77.1	0.765 ± 0.012 *	-21.3	55.000 ± 1.73 defg	-38.2	IIb
ERH5	0.307 ± 0.022 cdef	-50.3	0.720 ± 0.040 *	28.6	62.333 ± 2.60 def	-30	IIb
ECS16	0.136 ± 0.020 ef	-78	0.764 ± 0.057	-9	47.000 ± 1.73 efg	-47.2	IIb
ERH44	0.225 ± 0.023 cdef	-63.6	0.771 ± 0.070	-5.1	52.000 ± 7.55 efg	-41.6	IIb
ERH31	0.151 ± 0.056 def	-75.6	0.768 ± 0.042 *	37.1	58.333 ± 8.95 defg	-34.5	IIb
ERH16	0.330 ± 0.144 cdef	-46.6	0.667 ± 0.015 *	-23.2	37.000 ± 9.07 g	-58.4	IIb
ERH48	0.437± 0.035 bc	-29.2	0.693 ± 0.008	-12	74.333 ± 3.76 abcd	-16.5	IIc
ERH38	0.121 ± 0.031 ef	-80.3	0.720 ± 0.040	-10	76.000 ± 1.15 abcd	-14.6	IIc
ERH28	0.208 ± 0.035 cdef	-66.2	0.657 ± 0.074 *	23.6	67.000 ± 10.41 bcde	-24.7	IIc
ERH43	0.205 ± 0.019 cdef	-66.8	0.504 ± 0.037	-11.3	65.000 ± 4.36 cdef	-27	IIc
ECS2	0.224 ± 0.063 cdef	-63.7	0.627 ± 0.013 *	25.3	64.000 ± 3.06 cdef	-28.1	IIc
Control	0.617 ± 0.039 a				89.00 ±1.53a		

Notes: Values of physiological traits were indicated as "mean ± standard error". Lowercase letters followed each value of callus growth rate and viability, indicating the significance of difference among treatments (performed by ONE-WAY ANOVA on SPSS16.0). Only when no same letter appeared between treatments, should be interpreted as significant difference ($P < 0.05$). Significance of difference of growth rate of certain fungal strain was evaluated, respectively, after a comparison with its corresponding fungal control. *: means the significance at 0.05 level; **: means the significance at 0.01 level. Fungal strains were categorized according to different interactions with their co-cultured grape cells, and are listed as "fungal category" in the table.

cantly promoted ($P < 0.05$) during the co-culture, and the growth of fungal strain ERH32 was notably promoted by 69.7 %. Contrarily, the growth of fungal strains ERH37, ERH46, ERH6, ERH34, ERH7, ECS11 and ERH16 was significantly inhibited ($P < 0.05$) (Tab. 3). The growth of fungal strain ERH46 was inhibited mostly (42 %), among the tested fungal strains (Tab. 3). The growth of other strains, such as ERH38, ERH43 and ECS16 were less influenced by the presence of grape callus (Tab. 3). In contrast to fungus, growth of grape calli were all significantly inhibited during the co-culture, in comparison with the callus controls (Tab. 3). Co-culture with endophytic fungal strains ERH37, ERH38, ERH32, ERH34 and ECS13 greatly inhibited the growth of callus by more than 70 %. While callus growth was less inhibited by fungal strains ERH48, ERH12 and ERH45 (less than 40 %, Tab. 3). Compared to callus control, cell viabilities of the grape callus were all decreased in different degrees, depending on the co-cultured fungal strain (Tab. 3). Fungal strains ERH37, ERH16 and ERH24 imposed greatest impacts on grape cell viabilities (with the response indexes (RI ≥ 50 %). Fungal strains ERH48, ERH12, ERH7 and ECS11 conferred less effects (RI ≤ 20 %) on grape cell viability (Tab. 3). Plant cell viability significantly correlated ($P < 0.01$) with the degrees of callus oxidation (correlation analysis was performed after the digitization of oxidation degrees: serious = 4; medium = 3; light = 2 and normal = 1).

By results of combined morphological and physiological interaction tested fungal strains can be categorized into 2 groups (group I and II) and 5 subgroups (subgroup Ia, Ib, IIa, IIb and IIc, Tab. 3). Fungal strains were firstly divided into two groups according to their infective ability of the callus during dual culture, *i.e.* weak infectivity (group I) and strong infectivity (Group II). Group I includes fungal strains without obvious physical contact to the plant cells, and group II were those fungal strains that easily grow into or onto the callus, during the co-culture. Group I was then further divided into two subgroups, Ia) fungal strains with weak infection ability but detrimental impacts on grape cells, such as fungal strains ECS11 and ERH45. Ib) fungal strains have less infection ability and less detrimental impact on the viability and oxidation of grape cells, such as fungal strains ERH12, ERH7 and ECS13. Similarly, group II was further divided into three subgroups, IIa) fungal strains with strong infectivity and detrimental effects to grape cells, such as fungal strains ERH24, ERH16, ERH37 and others, which decreased the viability of grape cells more than 50 % during the co-culture (Tab. 3); IIb) fungal strains with strong infection causing medium degrees of injury to plant cells, these fungal strains decreased the viability of plant cell between 30 % and 50 %, and almost half of the tested fungal strains belong to this subgroup (Tab. 3); and IIc) fungal strains with strong infective ability but less detrimental impacts on grape cells, and such as fungal strains ERH48, ERH38, ERH34 and others (Tabs 2 and 3).

Significant correlations were detected between callus and fungus growth rate (Pearson correlation = -0.602; $P < 0.01$), as well as between callus growth rate and the original host dominance of the fungal strains during the co-culture (Pearson correlation = -0.557; $P < 0.01$) (Fig. 3).

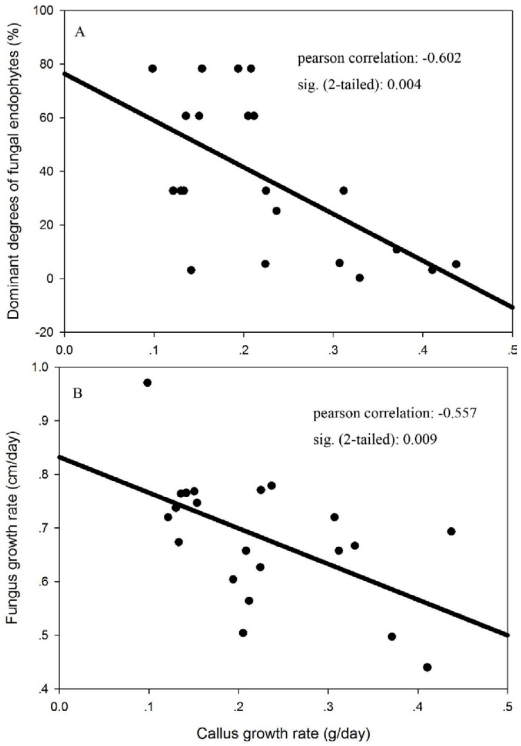

Fig. 3: Correlation plots for physiological trait pairs with critical significance during calli-fungi co-culture. **A**: Correlation between callus growth rate and dominance of the endophytic fungal strain in its host plant; **B**: correlation between callus growth rate and fungal growth rate during the co-culture. Correlation analysis was performed by using software SPSS16.0, and scatter plots were drawn with the software SigmaPlot 10.0.

Discussion

Plants sense environment or neighbours through multiple mechanisms, such as allelopathy (Inderjit and Duke 2003). Pathogen-host interactions between organisms from different species or kingdoms have been extensively studied (e.g. Cory and Myers 2004). Pathogens could be classified into categories, according to their pathogenicity and responses of host plants (Horst 1990). However, could endophytes also be classified in the same way as pathogens? Due to the difficulties in investigating the interactions between specific endophytes and their host plants *in vivo*, an *in vitro*, controllable and effective method for assessing the interactions of these cross-kingdom organisms is still useful. In this experiment, a solid co-culture system was proposed, using B5 solution as a standard medium for plant cells/calli so to balance the growth rate of fungi and plant calli. Pilot experiments demonstrated that almost all used endophytic fungi in this experiment can grow on this medium. In contrast to liquid culture methods, solid culture systems easily demonstrate the effects of one partner on another, especially at morphological level. With this system, plant cells and its co-cultured endophytic fungi showed specific interaction *in vitro*, at both morphological and physiological levels. According to the specific interactions during the co-culture, tested fungal strains have been categorized in 5 subgroups (Tab. 2), similar to the classification of pathogens (Horst 1990).

In addition to investigating the interactions between endophytic fungi and plant cells, the categorized candidate fungal endophytes in this work are helpful in purpose applications of these fungal strains in viticulture, according to the infective and detrimental effects to grape cells. For some examples, fungal strains of group II, especially for those growing into fungi in the dual culture, are likely used for inoculation in vineyards for their effective infection abilities. Consequently, these fungal strains may have more chances to infect vines, and cause continuous interaction with host plants (SAIKKONEN *et al.* 2003). One may hypothesize using those fungal endophytes in a vineyard, to continually produce grape fruits with good quality and characters.

Endophytic fungal strains acquired from this dual culture experiment were infective and detrimental to the grape callus. Fungal strains that can grow into or onto the calli imply the strong abilities of infection (Tab. 2). Fungal strains that caused serious oxidation or greatly decreased the cell viability are detrimental to grape cells (Tab. 2). In this experiment, all fungal strains from *Alternaria* had strong ability of infection and detriment to grape cells, and these fungal strains dominantly inhabited its host grapevines (MA *et al.* 2014, see summarized in Tab. 1). The genus *Alternaria* had a great proportion in plant endophytic fungal communities (ARNOLD 2007), and strains may become pathogens under certain circumstances (SAIKKONEN *et al.* 2004). However, whether fungal strains with higher degrees of host dominance have stronger ability of invasion and damage to plant cells needs further studies. Although endophytes exert benefits to host plants (DOTY 2015, KHAN *et al.* 2012, KULDAU and BACON 2008, LU *et al.* 2000, OWNLEY *et al.* 2008), and could be purposely used in grapevine management (YANG *et al.* 2016), fungal strains with stronger infection ability causing lower damage to host, such as ERH48, ERH34, ERH5 and others, which belong to subgroup "IIc" and "IIb" should be applied for safety considerations.

In dual culture, callus initiated different growth effects on its host and non-host originated endophytes (PETERS *et al.* 1998). Host-selectivity of endophytic fungi has been well covered (COHEN 2004, PERŠOH 2013, SUN *et al.* 2012). It is still unclear whether host-specificity of one plant species covers all varieties. Grape calli used in this co-culture were induced from 'Cabernet Sauvignon' no obvious differences were observed in calli when responding to fungal endophytes originating from host/non-host cultivars. Therefore, information on invasive and detrimental effects to grape cells of these tested endophytic fungal strains will still be tested in viticulture for other grape cultivars.

Correlations provided clues to investigation of mechanisms of plant-endophytes interactions. Growth rate of plant calli significantly negatively correlated to fungus growth rate and dominance (in its original host plant; $P < 0.01$, Fig. 3) which implies that growth antagonism between endophytic fungi and plant cells, as well as fast growth rate or stronger invasive ability are necessary when an endophytic fungus can successfully infect and become the dominant species in host plants. However, details underlying the mechanism of plant-endophytes interaction need more research.

Conclusion

A simplified solid co-culture system was proposed for studying the interactions between fungal endophytes and grape cells. Endophytic fungal strains were then categorized in 5 subgroups for application in vineyard inoculation. Fungal endophytes with strong infective and less detrimental effects such as endophytic strains ERH48, ERH34, ERH5 and others will be better choices when used in inoculation of vineyards.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (NSFC 31160070 and 31560538).

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Received December 2, 2016
Accepted March 16, 2017