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In vitro evaluation of berries of various *Vitis* genotypes for disease resistance to *Botrytis cinerea*

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Summary

Berries of 41 *Vitis* genotypes were evaluated for resistance to *B. cinerea*. Evaluation revealed that four genotypes were highly resistant (HR), eight resistant (R), eighteen susceptible (S) and eleven highly susceptible (HS). We further evaluated HR genotype 'Dong fang zhi xing' and HS genotype 'Gold finger' by comparing the fungal growth, reactive oxygen species (ROS) responses, jasmonic acid (JA) levels, anti-oxidants, e.g., Peroxidase (POD), Superoxide dismutase (SOD) and Malondialhydrate (MDA) content changes after infection with *B. cinerea*. Our results confirmed that the elevated resistance of 'Dong fang zhi xing' was due to weak fungal development, low ROS production, timely elevation of anti-oxidative functions, and high JA levels. Moreover, HS 'Gold finger' infection was severe and sustained ROS production which may be due to its relatively unchanged anti-oxidative activities and low JA level. Our results could help grape breeders to select suitable germplasm for future research work.

Key words: *Vitis vinifera* L.; resistant levels; jasmonic acid; ROS; Anti-oxidant; microscopy.

Introduction

Grape (*Vitis vinifera* L.) is a widely cultivated crop that has immense economic value as it is a source of many products, including wine, jam, juice and jelly, grape seed extracts, raisins, vinegar and grape seed oil (Tu *et al.* 2016). However, the yield and berry quality of grape is limited by a range of biotic and abiotic stresses (Li 2015). *Botrytis cinerea* is a polyphagous fungus that infects more than 1400 species of cultivated plants (Elad *et al.* 2016). This fungus causes one of the most serious diseases on the grapevine called botrytis bunch rot. This necrotrophic fungus actively attempts to destroy living host tissue to use them as nutrients (Mengiste 2012) and uses naturally senesced plant tissue in the environment as well (Kohler *et al.* 2015). The pathogen reduces both the yield and quality of the wine (Ribéreau-Gayon et *al.* 1998) severely. Host disease development depends on various genetic and phenotypic traits, such as bunch compactness and morphological, anatomical, and chemical features of the berry skin, which are highly dependent on the grapevine cultivar (Latorre *et al.* 2015). The post-veraison period is essential for the development of key grapevine berry quality parameters, important in terms of wine production (Davies and Robinson 1996). To control this disease, fungicides have long been used, leading not only to the generation of fungicide resistant strains (Hahn 2014) but also harmful to both human health and the environment (Damalas and Eleftherohorinos 2011).

Reactive oxygen species (ROS) play important role in plant physiology, such as in plant development, cellular signaling, and biotic and abiotic stress tolerance. Thus ROS production needs to be firmly regulated to balance its physiological functions (Liu and He 2016). Multiple cellular signaling actions mostly rely on redox reactions. Thus ROS may be straightly involved in the cellular redox metabolism. ROS regulate the redox regulative network in each cell, gene expression, translation, and metabolism (Dietz 2016). The plant–fungus relationship correlates with ROS production. Oxidative rupture is an initial and universal plant response to pathogen attack. In *B. cinerea*, plant cell death is favorable to the pathogen and causes susceptibility of the host (Govrin and Levine 2000).

Antioxidants prevent and protect the cell from the damage caused by free radicals which help in sustaining the rate of oxidation reactions in a cell (Sies 1997). Antioxidants play a very crucial role in mitigating or preventing the process of oxidation of other molecules (Ahamadi *et al.* 2015). To avoid the oxidative damage caused by these toxic ROS, the level of the endogenous antioxidant defense system is raised in higher plants (Sharma *et al.* 2010).

Plant hormones such as jasmonic acid (JA), salicylic acid (SA), and ethylene are involved in biotic stress neutralization (Durrant and Dong 2004). JA participates in plant response to injury and biotic stresses, such as during insect and pathogen attacks (Wasternack and Hause 2013). JA plays important roles in the stimulation of induced systemic resistance in plants to pathogen or pest attack and wounding (Rosahl and Feussner 2004).The accumulation of JA occurs

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rapidly in plant tissues and cells after wounding or exposure to fungal elicitors (Li *et al.* 2005, Kang *et al.* 2006). JA and its methyl ester (MeJA), as naturally occurring plant growth regulators, have been proven to be a plant signal molecule that is involved in plant defense mechanisms against biotic and abiotic stresses (Creelman and Mullet 1997).

The main objective of this work was to evaluate different grapevine genotypes to *B. cinerea* by using grape berries under controlled conditions. A total of 41 grape genotypes were evaluated for their phenotypic, physiological and histochemical disease signs and symptoms post *B. cinerea* infection. Furthermore, we investigated the critical levels of ROS, antioxidant enzymes and JA contents both in highly resistant (HR) and highly susceptible (HS) *Vitis* genotypes. Our present study could serve as a basis for future grape breeding programs.

Material and Methods

Grape berries and fungal materials: Grape berries were obtained from the grape germplasm depository at the Northwest A&F University, Yangling, Shaanxi, China. *B. cinerea* spores were isolated from 'Gold finger' cultivar (*V. vinifera*) and maintained on potato dextrose agar medium at 22-23 °C. After 20 d, the conidia were removed, and conidial suspension of concentration 1×10^5 spores·mL^{-1} was prepared with the help of hemocytometer to inoculate the grape berries as earlier described by Surya *et al*. (2015).

Berry selection and inoculation: The experiment was performed using grape berries of the same size and age, E-L 39 stage (Coombe 1995), as it is the optimum stage to study *B. cinerea* disease reaction (Surya *et al.* 2015). Berries were arbitrarily selected, harvested from the grape plants and washed several times with distilled water for laboratory assessment. 72 berries from three replicates of each genotype were evaluated. The berries were sprayed evenly with the conidial suspension. Control leaves were sprayed with distilled water and kept in an incubator with a relative humidity of 90 % - 100 % at 22 °C for 9 d.

Disease severity rating: Disease severity was evaluated and rated as previously described (Li *et al.* 2008) with slight modifications. The symptoms observed on the berries were scored from 1 to 7 (Grade 1 = 0.1 % - 5.0 %, 2 = 5.1 % - 15.0 %, 3 = 15.1 % - 30.0 %, 4 = 30.1 % - 45.0 %, 5 = 45.1 % - 65 %, 6 = 65.1 % - 85.0 %, and 7 = 85.1 % - 100.0 %) on the basis of the estimated percentage of lesion over the entire berry surface. Then, the score was transformed into a severity index (SI). The resistance level was rated into four classes by SI value. Disease resistance levels of the different cultivars were categorized as highly resistant (HR) (score: 0 - 1.50), resistant (R) (score: 1.51 - 3.50), susceptible (S) (score: 3.51 - 5.50), and highly susceptible HS (score: 5.51 - 7.0). The average SI values data were used to evaluate resistance level by using following formula:

$$SI = \frac{\Sigma(Rank\ x\ number\ of\ infected\ berries\ in\ that\ rank)}{Total\ number\ of\ berries\ x\ highest\ rank} \times 100$$

Light microscopy: To characterize the colonization of *B. cinerea* on the grape berries, the berries skin were cut into segments of 2 - 3 cm^2 and fixed and decolorized in 100 % ethanol and saturated chloral hydrate. Afterward, the samples were stored in 50 % glycerol and stained with aniline blue solution at the time of observation with an Olympus BX-51 microscope (Cheng *et al*. 2012)

H_2O_2 measurement: H_2O_2 content was calculated at different time points (0, 1, 2, 3, 4, 5, 6, 7,8 and 9 d post inoculation (dpi) as previously described (Moloi and Westhuizen 2006).

O_2^- measurement: The O_2^- production rate was calculated at different time points (0, 1, 2, 3, 4, 5, 6, 7,8 and 9 dpi)in accordance with the method described by Elstner and Heupel (1976).

Enzyme extraction and activity assay: SOD activity was measured at different time points (0, 1, 2, 3, 4, 5, 6, 7,8 and 9 dpi) as previously described (Mittler *et al.* 2011). Peroxidase (POD) activity was measured at different time points (0, 1, 2, 3, 4, 5, 6, 7,8 and 9 dpi) using about 0.5 g leaves (Giannopolitis and Ries 1977). The SOD activity was measured as previously described (Maehly and Chance 1954). The MDA content was measured at different time points (0, 1, 2, 3, 4, 5, 6, 7,8 and 9 dpi) according to Zhang (2004).

JA quantification: Grape berries of the inoculated and control plants were collected at different time points (0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 dpi) and were immediately frozen in liquid nitrogen. The samples were carefully ground in liquid nitrogen and then stirred in 80 % methanol at 4 °C overnight. The extract was methylated as previously described (Royo *et al.* 1999). JA was quantified with a competitive ELISA assay (Albrecht *et al.* 1993, Royo *et al.* 1999).

Statistical analysis: Experiments were performed using three replicates in a completely randomized design. Means and standard errors were computed from independent replicates by using SPSS 13.0. LSD 0.05 was employed to compute significant differences, and correlation data of the resistance evaluation in 2017 were analyzed. All images were combined with the help of Adobe Photoshop. All graphs were prepared using Origin Pro 9.0 32-bit software.

Results and Discussion

Vitis genotypes exhibited different levels of resistance to *B. cinerea*: All the tested genotypes displayed difference in *B. cinerea* resistance after nine days (Tab. 1). LSD analysis showed that the similarity was found in repeats, and the average disease severity was significantly different ($P \leq 0.05$) among the various genotypes (Tab. 2).

Overall, 41 *Vitis* genotypes were evaluated against *B. cinerea* to investigate the resistance level. All genotypes were classified according to their disease severity index (SI) at 9 dpi. Among the 41 genotypes, 11 were HS (Tab. 2). Mycelium and sporulation were observed on these geno-

Table 1

Lesions percentages on the berries surface of 41 grape cultivars infected with *B. cinerea* in 2017

Species	Name of cultivars	Lesions percentages, %	P ≤ 0.05*
V. vinifera L.	Cinsault	99.33 ± 1.15	AB
	He shi	97.73 ± 2.16	AB
	Riesling	97.66 ± 2.52	AB
	Phoenix	97.33 ± 2.52	AB
	Thompson seedless	95.00 ± 3.00	ABC
	Semillon	94.83 ± 0.76	ABC
	Ba kusi	94.66 ± 2.52	ABC
	Sauvignon Blanc	94.33 ± 3.51	ABCDE
	Merlot	91.00 ± 3.00	ABCDEF
	Gao te	90.66 ± 2.52	ABCDEF
	Se le	90.66 ± 4.04	ABCDEF
	Red hanepoot	88.13 ± 2.20	BCDEFG
	Zhana	85.33 ± 1.53	CDEFGH
	Blue French	82.83 ± 1.76	EFGHI
	Fresno	77.23 ± 1.16	GHIJ
	Weinan- B	75.06 ± 3.64	HIJ
	Hong wu zhi lu	73.16 ± 1.59	IJ
	Ma naizi	72.10 ± 2.17	IJ
	Early Muscat	69.16 ± 0.76	JK
	Tokay	68.56 ± 1.60	JKL
	Flame seedless	59.56 ± 2.65	KLM
	Sangiovese	57.12 ± 2.19	LM
	Lady finger	35.37 ± 4.45	NO
	Jing xiangyu	55.22 ± 2.54	M
	Moldova	55.13 ± 1.03	M
	Yatomirosa	5.34 ± 0.76	Q
	Bai yu	51.83 ± 3.01	M
	Ruby Seedless	37.76 ± 0.87	N
V. vinifera L. × *V. labrusca* L.	Gold finger	100.00 ± 0.00	A
	Tian shan	55.58 ± 3.39	M
	Zuijinxiang	54.99 ± 4.26	M
	Kyoho	16.33 ± 1.53	PQ
	Jumeigui	8.43 ± 0.71	Q
	Dong fang zhi xing	8.06 ± 1.02	Q
	Summer black	91.66 ± 2.08	ABCDEF
	Tian yuan qi	90.76 ± 1.19	ABCDEF
	Hu tai 8	83.12 ± 1.88	DEFGHI
	Black rose	82.50 ± 2.18	FGHI
V. vinefera L × *V. amurensis* Rupr.	Beihong	24.74 ± 1.96	OP
	Xuelanhong	95.66 ± 2.08	ABC
	Beibinghong	5.50 ± 1.50	Q

*Significance at $P \leq 0.05$. Different letters associated with each level of disease severity indicates significant differences at $P \leq 0.05$.

types. A total of 18 genotypes were found susceptible to mycelium production at 9 dpi with no/less sporulation (SI of 3.51 - 5.50). A total of 8 genotypes (Tab. 2) were found resistant with a lesser amount of mycelium production; and no sporulation was observed in genotypes with SI values of 1.51 - 3.50. Four genotypes (Tab. 2) were recorded HR with no mycelium or sporulation and with SI values of 0 - 1.50 when compared with HS genotypes.

Two prototype cultivars each from the HR and HS categories were selected for microscopic evaluation to assess fungal growth at 9 dpi (Fig. 1). The berries of HS genotypes, 'Gold finger' (Fig. 1B, F) and 'Summer black' (Fig. 1D, H) were completely moldy and roofed by mycelium along with sporulation (Tab. 1). HR genotypes, 'Dong fang zhi xing' (Fig. 1A, E) and 'Jumei gui' (Fig. 1C, G) had 2 % and 8 % lesions, respectively (Tab. 1). Moreover, only conidia with the absence of penetrating pegs were observed on the berries of HR 'Dong fang zhi xing' (Fig. 1E) and 'Jumei gui' (Fig. 1G). However, the resulting conidia/hyphae did not extend, indicating the restricted *B. cinerea* proliferation.

Resistance to gray mold was dissimilar among the berries of the 41 different *Vitis* genotypes, 'Dong fang zhi xing' and 'Jumei gui' were found to be highly resistant to *Botrytis*, which belong to *Vitis* (*V. vinifera* L. x *V. labrusca* L.) cross. Meanwhile, low or no resistance was observed in most *V. vinifera* cultivars and their hybrids (Tab. 1). These findings are in line with those of Gabler *et al.* (2003) who reported that grape genotypes vary regarding their infection resistance, the degree of fungal colonization, and disease severity. Moreover, the presence of less or no resistant phenotypes in most common table grape *V. vinifera* cultivars have been described, whereas a high level of resistance has only been found in *V. labrusca*, *V. rotundifolia*, and other grape hybrids. Wan *et al.* (2015) also reported that the HR cultivars can effectively block *B.cinerea* compared with *V. vinifera* cultivars and their hybrids. In our study, we also noted different resistance levels of various grape cultivars to *B. cinerea* (Tabs 1 and 2).

Activities of malondialhydrate (MDA), superoxide and dismutase peroxidase in HR 'Dong fang zhi xing' and HS 'Gold finger' infected by *B. cinerea*: The MDA contents in 'Dong fang zhi xing' control and inoculated samples were approximately the same at all time point's

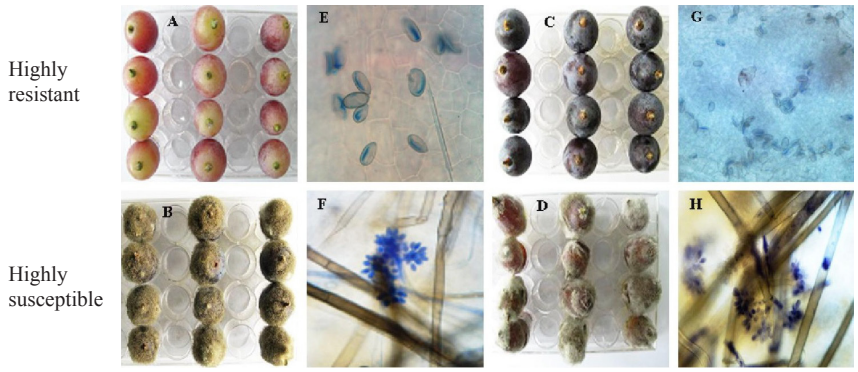

Fig. 1: Macroscopic (**A-D**) and microscopic (**E-H**) evaluation of two representative *Vitis* genotypes from each level of *B. cinerea* resistance. Highly resistant genotypes 'Dong fang zhi xing' and 'Jumei gui' are shown in (**A**, **C**) while highly susceptible 'Summer black' and 'Gold finger' are shown in (**B**, **D**).

Table 2

Laboratory evaluation of disease severity results of 41 grape cultivars against *B. cinerea*

Species	Name of cultivars	Disease severity	Scores	Resistance level	Microscopic mycelium	New sporulation
V. vinifera L.	Cinsault	88.46 ± 1.85	6.19	HS	√	√
	He shi	75.17 ± 2.10	5.30	S	√	√
	Riesling	91.85 ± 3.14	6.42	HS	√	√
	Phoenix	86.57 ± 0.88	6.05	HS	√	√
	Thompson seedless	85.33 ± 3.15	5.97	HS	√	√
	Semillon	72.20 ± 0.89	5.94	HS	√	√
	Ba kusi	65.55 ± 2.75	4.62	S	√	×
	Sauvignon Blanc	77.12 ± 1.43	5.44	S	√	√
	Bai yu	45.05 ± 2.27	3.46	R	×	×
	Ruby Seedless	23.44 ± 0.66	2.34	R	√	×
	Lady finger	55.33 ± 1.26	4.25	S	√	√
	Merlot	76.33 ± 3.22	5.38	S	√	√
	Moldova	33.88 ± 3.16	3.01	R	×	×
	Gao te	82.91 ± 1.23	5.85	HS	√	√
	Se le	64.11 ± 2.88	4.93	S	√	×
	Red hanepoot	60.52 ± 1.94	4.65	S	√	×
	Zhana	82.33 ± 3.04	5.81	HS	√	√
	Yatomirosa	7.88 ± 1.45	1.05	HR	×	×
	Blue French	73.56 ± 2.36	5.19	S	√	√
	Fresno	59.19 ± 2.65	4.55	S	√	√
	Weinan- B	63.12 ± 0.45	4.45	S	√	√
	Hong wu zhi lu	72.98 ± 0.97	5.15	S	√	×
	Ma naizi	55.23 ± 0.96	4.24	S	√	×
	Early Muscat	66.48 ± 2.35	4.69	S	√	√
	Tokay	61.54 ± 3.16	4.73	S	√	√
	Flame seedless	87.11 ± 2.5	6.09	HS	√	√
	Sangiovese	47.12 ± 1.57	3.62	S	√	×
	Jing xiangyu	29.32 ± 2.75	2.93	R	√	×
V. vinifera L. × *V. labrusca* L.	Tian shan	35.41 ± 2.23	3.14	R	×	×
	Summer black	98.73 ± 1.87	6.91	HS	√	√
	Gold finger	96.22 ± 0.75	6.73	HS	√	√
	Tian yuan qi	87.21 ± 2.74	6.10	HS	√	√
	Hu tai 8	77.56 ± 1.65	5.47	S	√	√
	Black rose	70.25 ± 3.14	4.95	S	√	√
	Kyoho	19.53 ± 2.1	1.95	R	×	×
	Jumeigui	5.72 ± 2.42	0.76	HR	×	×
	Dong fang zhi xing	4.83 ± 1.92	0.96	HR	×	×
	Zuijinxiang	25.28 ± 1.25	2.52	R	×	×
V. vinifera L × *V. amurensis* Rupr.	Beihong	18.55 ± 2.89	1.85	R	×	×
	Xuelanhong	74.87 ± 2.01	5.28	S	√	×
	Beibinghong	4.50 ± 2.14	0.9	HR	×	×

A: Disease severity: the average percentage of spreading lesions determined by observing at least 72 berries in each repeated experiment in 2017.

B: Score: disease severity was scored as previously described (Li *et al.*, 2008).

C: Resistance level: highly resistant (HR: scores of 0-1.50); resistant (R: scores of 1.51-3.50); susceptible (S: scores of 3.51-5.50); highly susceptible (HS: scores of 5.51-7.0).

√: Mycelium or sporulation was observed by the naked eye on berries surfaces.

×: No mycelium or sporulation was observed by the naked eye on berries surfaces.

series (Fig. 2A). The activity of 'Gold finger' control was one fold higher than that of 'Dong fang zhi xing' control and inoculated (Fig. 2A) while higher values of MDA contents were observed in 'Gold finger' inoculated throughout the experiment, with the highest peak at 6 dpi (Fig. 2A). The MDA contents in HR 'Dong fang zhi xing' were found lower as compared to HS 'Gold finger' cultivar. Higher MDA contents were recorded in 'Gold finger', with the highest peak at 6 and 9 dpi. These findings are in line with (Korayem *et al.* 2012) who reported that increased MDA content was observed in shoots and roots of nematode-infected sugar beet genotypes in comparison to that in non-infected plants. MDA is a final product of lipid peroxidation, could be a great indicator of membrane disruption in plants exposed to pathogen colonization (Loreto and Velikova 2016). Furthermore, there is a possibility that high MDA content has been associated with overproduction of H_2O_2 in the manner of making the membranes more susceptible. Similar results were found by Radwan *et al.* (2010) who reported that higher concentration of H_2O_2 and MDA had also been found in *Vicia Faba* leaves infected by bean yellow mosaic virus. JA is recognized as signaling molecules for MDA and H_2O_2 and stimulates defense-related responses under stress (Ahmad *et al.* 2017). Therefore, it appears that JA might aid in the production of antioxidants and reduce the level of free radicals in plants under stress condition.

The SOD activities in the control samples of both 'Dong fang zhi xing' and 'Gold finger' were approximately the same, except for the elevated level at 0 dpi and 1 dpi (Fig. 2B) of 'Dong fang zhi xing'. The activity in the inoculated 'Gold

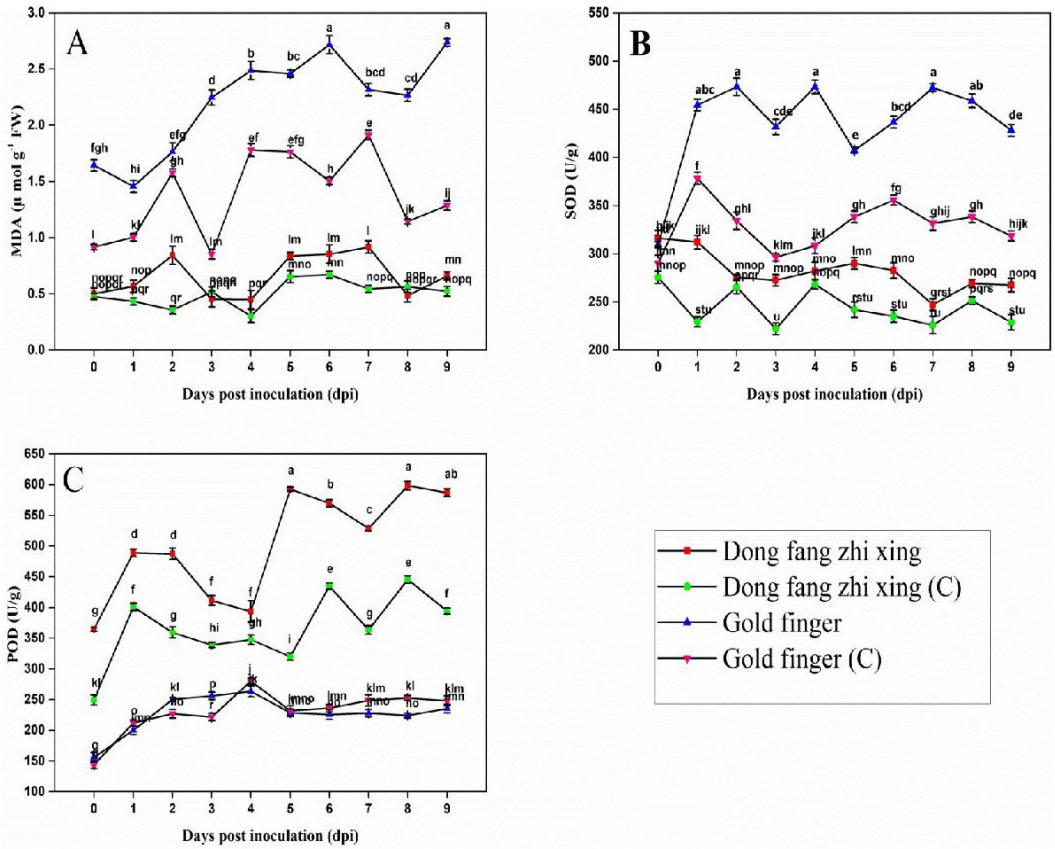

Fig. 2: MDA (**A**) and superoxide dismutase (SOD) (**B**) and Peroxidase (POD, **C**) activities of protein extracts from 'Dong fang zhi xing' and 'Gold finger' berries at 0, 1, 2, 3, 4, 5, 6, 7,8 and 9 d post-inoculation (dpi) with *Botrytis cinerea*, using sterile water as the control. Three independent experiments were used for the means and standard errors. Small letters indicate significant differences according to LSD test ($P < 0.05$).

finger' was approximately four fold higher than that of control throughout the experiment (Fig. 2B). The SOD activity of 'Gold finger' was relatively the same as that of 'Dong fang zhi xing' at 0 dpi (Fig. 2B). However, a sudden increase in 'Gold finger' was observed at 1 dpi (Fig. 2B), which was approximately maintained with no further increase detected till 9 dpi (Fig. 2B).

The POD activities in HR 'Dong fang zhi xing' and HS 'Gold finger' berries skin were tested to evaluate the robustness of the antioxidant system during *B. cinerea* infection. The control samples of 'Gold finger' cultivar displayed the same POD background activities within the all-time series (Fig. 2C). However, in 'Dong fang zhi xing' berries, POD activity increased from 0 dpi to 1 dpi and then slightly decreased at 2 to 4 dpi. An elevation POD activity was recorded at 5dpi, while the highest peak was observed at 8 dpi (Fig. 2C).

POD provides an important defense against the intrusion and extension of pathogens (Weissinger *et al.* 2013). When the plants suffer damage, more O_2^- would produce, and SOD could dismutate this O_2^-; subsequently, excessive H_2O_2 induces the overexpression of POD gene (Li *et al.* 2013). Considering enzyme activities, we discovered that the post-inoculated 'Gold finger' berries manifested slight variation in POD activities with lesion development. However, they showed increased SOD, which corresponds well with H_2O_2 production and O_2^- reduction. However, the POD activities in HR 'Dong fang zhi xing' increased during the experiment, and no significant change was observed in SOD activity. Low levels of ROS accumulation are necessary for the anti-oxidative system to sustain redox equilibrium (Foyer and Noctor 2013). Similar to the results above, we observed that the infected 'Gold finger' certainly encountered the effects of an insufficient anti-oxidative system, resulting in consistently elevated ROS levels. Meanwhile, 'Dong fang zhi xing' quickly synchronized its anti-oxidative capacity, especially the POD activities, following the inoculation. Thus, 'Dong fang zhi xing' experienced less ROS-induced stress. Given that substantial ROS was induced by 'Gold finger' but not in 'Dong fang zhi xing', the particular coordination of ROS production and scavenging mechanisms related to the anti-oxidative system during the consolidated abiotic and biotic stress (Atkinson and Urwin 2012)

O_2^- and H_2O_2 accumulation in the interactions of *B. cinerea* with HR 'Dong fang zhi xing' and HS 'Gold finger': In our study, O_2^- production was measured at various time points. Low O_2^- production levels were observed in HR 'Dong fang zhi xing' at all time points (Fig. 3A), while, in HS 'Gold finger' the O_2^- production rate was 3 fold higher than HR 'Dong fang zhi xing' from beginning till 9 dpi (Fig. 3A). Highest peak was observed at 3 dpi, which may indicate high botrytis stress on the berry skin surface (Fig. 3A). Under stress condition at 0 dpi, the H_2O_2 level in HR 'Dong fang zhi xing' and HS 'Gold finger' was the same (Fig. 3B). H_2O_2 production increased from 1 dpi and reached to a peak of 2 fold higher

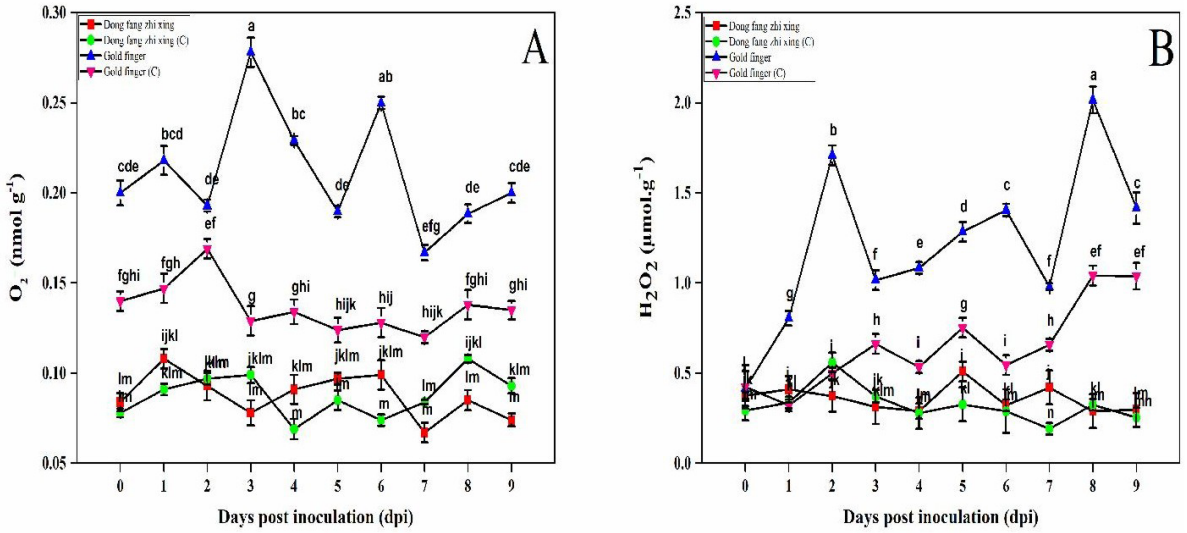

Fig. 3: Levels of O_2^- (**A**) and H_2O_2 (**B**) in berries of highly resistant 'Dong fang zhi xing' and highly susceptible "Gold finger" at 0, 1, 2, 3, 4, 5, 6, 7,8 and 9 dpi with *Botrytis cinerea* and using sterile water as the control. Three independent experiments were used for the means and standard errors. Small letters indicate significant differences according to LSD test ($P < 0.05$) between 'Dong fang zhi xing' and 'Gold finger'.

than its base line at 2dpi with a small decrease at 3 dpi and remained approximately the same till 7 dpi (Fig. 3B). A sudden increase in H_2O_2 production was observed at 8 dpi followed by a slight decrease at 9 dpi (Fig. 3B), respectively. Thus, significant differences in H_2O_2 production existed between HR and HS cultivars.

As evident in our study that HR 'Dong fang zhi xing' blocked *B. cinerea* expansion while HS 'Gold finger' did not, we investigated the possible mechanism for deeper insight. Overall, low reactive oxygen species (ROS) level was observed post-inoculation on the HR cultivar 'Dong fang zhi xing'. The ROS level after *B. cinerea* infection is low because the anti-oxidative system maintains redox equilibrium (Mittler *et al.* 2011, Foyer and Noctor 2013) and protects cells from ROS damage (Sharma *et al.* 2012). By contrast, elevated levels of ROS were accumulated on HS 'Gold finger'. Govrin and Levine (2000) reported that in host-pathogen interactions where the pathogen is a necrotroph, the pathogen-induced cell death and ROS accumulation promote pathogen growth and disease development. Thus, ROS facilitate colonization on the leaves by the necrotrophic fungus *B. cinerea* (Asai and Yoshioka 2009).

Consequently, plants accumulate ROS in the plasma membrane of host cells to trigger an oxidative burst, leading to plant cell death (Tenberge *et al.* 2002). Oxidative stress disturbs the redox equilibrium in the infected tissue, thereby promoting disease development (van Kan 2006). In the present study, ROS detection after inoculation was recorded in both HR and HS grapevine berries. Higher ROS level was detected in 'Gold finger' than in 'Dong fang zhi xing'. Thus, 'Gold finger' suffered significantly from constant ROS detection. Moreover, 'Dong fang zhi xing' did not challenge with substantial oxidative stress because of its high and timely raised anti-oxidative capacity. H_2O_2 increases either the resistance or susceptibility toward *B. cinerea*. Meanwhile, O_2^- serves as the first substrate for H_2O_2 formation (Govrin and Levine 2000, van Kan 2006, Asselbergh *et al.* 2007). Some reports have suggested that O_2^- plays a role in supporting *B. cinerea* invasion (Patykowski 2006, Zhang *et al.* 2014). H_2O_2 is induced in plant cells, accompanied by O_2-generation, which can raise programmed cell death and disease lesion development to promote *B. cinerea* infection (Asai and Yoshioka 2009). Therefore, the high and low levels of ROS production in 'Gold finger' and 'Dong fang zhi xing' are accountable for their susceptibility and resistance to *B. cinerea* infection, respectively (Simon *et al.* 2013). ROS respond to pathogen attack in plants (Torres *et al.* 2006; Foyer and Noctor 2013). Thus, we evaluated ROS accumulation and its possible outcome of antioxidant enzymes during the interactions with B. cinerea (Zhang *et al.* 2014).

Excess production of ROS such as H_2O_2 under stress conditions leads to oxidative damage such as lipid peroxidation, inactivation of proteins, and DNA mutation (Torres *et al.* 2006). Wan *et al.* (2015) reported that low ROS production and a timely increase in anti-oxidative enzymes were detected in HR cultivars, while HS cultivars massively suffered from infection and sustained ROS production due to comparatively unchanged anti-oxidative activities. These results suggest the significance of ROS response in the timely detection and defense to *B. cinerea.* POD is one of the most important enzymes involved in regulation of the intracellular level of H_2O_2 (Passardietal 2005).

JA levels in HR 'Dong fang zhi xing' and HS 'Gold finger' in interaction with *B. cinerea*: JA level was determined in both HR and HS grape cultivars at various time points. Our results indicated higher levels of JA in HR 'Dong fang zhi xing' (Fig. 4) while lower levels of JA were observed in HS 'Gold finger' throughout the experiment. These results indicated that the resistance level of different grape genotypes to *B. cinerea* might be due to the higher level of JA, this result may account for the resistance or susceptibility of the grape cultivars. Our present findings are the same with those of Jia *et al*. (2016), who stated that high JA levels block *B. cinerea* infection and strengthen grape resistance against *B. cinerea*. Moreover, JA is a major hormone concerned with plant

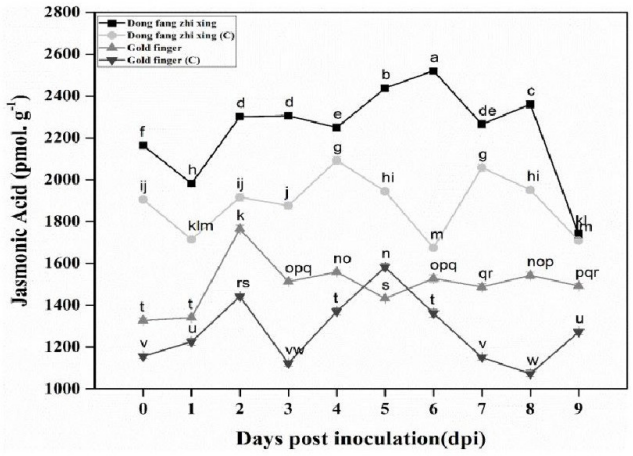

Fig. 4: Jasmonic acid (JA) levels in highly resistant 'Dong fang zhi xing' and highly susceptible 'Gold finger' berries at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 dpi with *Botrytis cinerea* and using sterile water as the control. Three independent experiments were used for the means and standard errors. Small letters indicate significant differences according to LSD test ($P < 0.05$) between 'Dong fang zhi xing' and 'Gold finger'.

defense responses (Bruinsma *et al.* 2007). More recently, several reports studying gene expression have revealed the involvement of both SA and JA/ET pathways in response to both biotrophic and necrotrophic pathogens (Toth *et al.* 2016). JA is a crucial component in the plant defense responses against insects and microbial pathogens (Bari and Jones 2009). JA accumulation occurs relatively quickly in plant tissues and cells after exposure to fungal elicitors (Li *et al.* 2005, Kang *et al.* 2006). JA is involved in plant response to injury and biotic stresses, such as insect and pathogen attacks (Shan *et al.* 2009, Wasternack and Hause 2013). JA is associated with resistance to biotrophic and necrotrophic pathogens (Glazebrook 2005, Robert-Seilaniantz *et al.* 2007). Also, JA is a naturally occurring growth regulator found in higher plants. This hormone plays several physiological roles during plant development in response to biotic and abiotic stresses (Creelman and Mullet 1995)

Conclusions

In this study, we investigated the resistance levels of different *Vitis* sp. genotypes to *B. cinerea*. Most genotypes were susceptible, but berry assay results revealed high resistance in *V. vinifera* L. x *V. labrusca* L. crosses. The results were further investigated by comparing the fungal growth, ROS responses, JA levels, and anti-oxidative activities between the HS 'Gold finger' and HR 'Dong fang zhi xing' after *B. cinerea* inoculation. Our results confirmed that low ROS production, timely elevation in anti-oxidative function, and high JA level were associated with a high level of fungal resistance in 'Dong fang zhi xing'. Meanwhile, HS 'Gold finger' suffered massive infection and sustained ROS production due to relatively unchanged anti-oxidative activities and low JA level, which showed susceptibility to *B. cinerea*. These findings can further be elucidated by studying ROS- and JA-based molecular mechanisms. This study provides an understanding of *B. cinerea* infection of grapes and could help breeders to select suitable germplasm for future research.

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