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Characterization of Unique and Differentially Expressed Proteins in Anthracnose-Tolerant Florida Hybrid Bunch Grapes

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Abstract Anthracnose is a major disease in Florida hybrid bunch grapes, caused by a fungus viz. *Elsinoe ampelina*. Florida hybrid bunch grapes are grown in southeastern USA for their superior wine characteristics. However, the effect of anthracnose on grape productivity and wine quality is a major concern to grape growers. Our research is aimed at determining biochemical basis of anthracnose tolerance in Florida hybrid bunch grape. Leaf samples were collected from the plants infected with *E. ampelina* at different periods and analyzed for differential protein expression using high throughput two-dimensional gel electrophoresis. Among the 32 differentially expressed leaf proteins, two were uniquely expressed in tolerant genotypes in response to *E. ampelina* infection. These proteins were identified as mitochondrial adenosine triphosphate synthase and glutamine synthetase, which are known to play a major role in carbohydrate metabolism and defense. Several proteins including ribulose 1-5 bisphosphate-carboxylase involved in photosynthesis were found to be suppressed in susceptible genotypes compared to tolerant genotypes following *E. ampelina* infection. The results indicate that the anthracnose-tolerant genotypes have the ability to up-regulate and induce new proteins upon infection to defend the invasion of the pathogen as well as maintain the normal regulatory processes.

Keywords Grape · Anthracnose · Differential protein expression · Mitochondrial ATP synthase · Glutamine synthetase · Ribulose 1-5 bisphosphate-carboxylase

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Abbreviations

HPLC	High performance liquid chromatography
LC/MS/MS	liquid chromatography/mass spectrometry/mass spectrometry
NCBI	National Center for Biotechnology Information
2-D	two-dimensional electrophoresis

Introduction

Grape is an important fruit crop worldwide and has variable applications in food and wine industry. Most of the major fungal diseases of grape, including black rot, downy mildew, powdery mildew, anthracnose and several kinds of blights, and fruit rots, persist in the southeastern USA where hot and humid climatic conditions favor rapid spread of the infection leading to crop destruction. Commercial production of grapes (*Vitis* spp. and their hybrids) in Florida requires cultivars that are resistant to diseases such as Pierce's disease (PD, *Xylella fastidiosa*), anthracnose (*Elsinoe ampelina*), black rot (*Guignardia bidwellii*), and downy mildew (*Plasmopora viticola*). Despite their economic importance, there has been little research on Florida hybrid bunch grape diseases in the southeast.

Until 1980s, most research efforts were focused on development of disease-resistant bunch grape varieties [1] through hybridization of local grape species with the table wine group, *Vitis vinifera*. The common trait of resulting hybrids is resistance to PD. Collectively, the PD-resistant bunch varieties are considered a distinct race (termed "Florida hybrid bunch grape") because of their combination of quality and PD resistance. However, anthracnose is a serious problem in Florida hybrid bunch grapes, especially during the years of heavy rainfall [2]. Anthracnose affects both plant foliage and fruit. Young, green succulent shoots are most susceptible and the growing points of these shoots are often killed. Disease symptoms on berry consist of whitish-gray lesions with a dark margin. Florida hybrid bunch grape cultivars show wide genetic variation for anthracnose tolerance [2]. Cultivars which are tolerant to anthracnose yield inferior wines, while susceptible cultivars produce good wines. Because of their superior characteristics, the wine industry prefers the cultivars such as Blanc du Bois and Suwannee, which are highly susceptible to anthracnose. Spraying with fungicides, although provides some disease control, is expensive and not environmental friendly. Therefore, it is necessary to understand biochemical basis of anthracnose tolerance in order to develop anthracnose-tolerant grape cultivars with superior enological traits. It is known that pathogen infection leads to induction of defense response and changes in carbohydrate metabolism of plants [3]. Upon sensing the invasion, plants can evoke one or many defense mechanisms in an attempt to restrict pathogen growth [4, 5]. When plants are infected by pathogens, several qualitative and quantitative changes will occur in plant protein composition in both infected and in adjacent non-infected tissues which show changes in metabolism that involve the stimulation of synthesis and degradation of specific proteins. These changes are directly associated with those in enzyme complexes, and are involved in protein changes. Enzymatic reactions are also influenced by changes in concentrations of activators, inhibitors, and effectors as well as substrates and coenzymes, in response to infection. Such changes in enzymatic reactions should be considered in protein changes [6]. The pathogen infection reduces the photosynthetic ability and increases contribution of energy-dissipating processes for protecting PSII integrity [7, 8]. Hence, it is important to study polypeptides that are induced during fungal infection in order to understand the plant tolerance mechanism and role of these proteins in plant protection. In this research, we report for the

first time the enzymes uniquely expressed in tolerant Florida hybrid grape in response to *E. ampelina* infection.

Materials and Methods

Sample Collection and Preparation

Florida hybrid bunch grape cultivars maintained at the Center for Viticulture and Small Fruit Research, Tallahassee, FL were used in this study. Two anthracnose-tolerant genotypes, viz. Lake Emerald and Blue Lake, and two anthracnose-susceptible genotypes, viz. Blanc du Bois and Suwannee, were used in this study. In addition, an anthracnose-tolerant muscadine grape cultivar (cv. Carlos) was also included in the study for comparison. During the month of July 2005, uninfected and infected leaves were collected around 9 AM from a vine of the same plant to avoid any developmental variation and temperature stress. High temperatures and rain are typical during this period which is ideal for disease occurrence and fungal growth. The characteristic symptoms of shot holes and necrotic spots were visualized on the infected leaves during collection. The leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C until further use.

Confirmation of *E. ampelina* Infestation

The infected portion of each leaf was separated from the uninfected area, sterilized with 5% Clorox (sodium hypochlorite) and placed on Czapek Dox agar (Difco) in a petri dish. After 21 days of incubation in dark at 28 °C, the plates were removed and observed for fungal growth. The cultures showed very little contamination from other microbes suggesting that they contained predominantly *E. ampelina* colonies. *E. ampelina* were selectively picked from the culture plate and the mycelia were suspended in sterile distilled water. The samples were cultured again on Czapek Dox agar and incubated in dark. The pure cultures *E. ampelina* were stored (100-ml batches) frozen in glycerol at −80 °C.

Inoculation study To fulfill Koch's postulates, the grape plants were inoculated with the *E. ampelina* spores. Mycelia were sporulated on potato dextrose agar and then incubated at 28 °C. Spores were suspended in sterile distilled water, and three experimental vines of Florida hybrid bunch (cv. Blanc du Bois) and muscadine (cv. Carlos) grape plants grown under greenhouse condition (28 °C with 16 h of light) were sprayed on both sides of the leaves with 0.5 ml of suspension (1.2×10^6 spores/ml), whereas three control plants were sprayed with sterile distilled water. Inoculation of grape leaves with this isolate produced anthracnose symptoms, similar to those caused by *E. ampelina* in 4 to 5 days, confirming that the leaf samples used in this study were infected with *E. ampelina*, and isolates were virulent.

PCR analysis Total RNA was isolated from *E. ampelina*. One hundred milligram mycelia of *E. ampelina* was ground to a fine powder using pestle and mortar in presence of liquid nitrogen. Total RNA was isolated using RNeasy protect mini kit (Qiagen Inc., CA, USA) as per manufacturer's instructions. One step RT-PCR kit from Promega was used according to manufacturer's instructions. RT-PCR amplification was performed using 100 ng of RNA and primers, which were constructed based on the conserved sequences for 18S RNA of *E. ampelina* by aligning the sequences deposited in NCBI database to confirm the identity

(forward 5′-TCCGTAGGTGAACCTGCGGA-3′; reverse 5′-TCCTACCTGATCCGAG GTCA-3′). The PCR conditions were as follows: first strand cDNA synthesis was carried out at 45 °C for 45 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. The obtained PCR product was separated on 1.2% agarose gel stained with ethidium bromide and visualized under UV transilluminator. PCR yielded the expected size of 500 bp fragment.

Identification and Characterization of Proteins

To prevent contamination of leaf protein extracts with fungal protein, all dead and necrotic regions were removed from infected leaves.

Sample preparation Leaf tissues (2 g) of both uninfected and infected samples were homogenized in trichloroacetic acid (20%) and centrifuged. The pellet was re-extracted with acetone followed by ethyl acetate and ethanol extraction (1:1, *v*/*v*). The final pellet was air dried to obtain a powdered sample.

One-dimensional electrophoresis A portion (20 mg) of dried leaf powder was homogenized with 2 ml buffer (50 mM Tris–HCl, pH 8.0, 5% SDS, and 2% 2-mercaptoethanol). Total proteins were quantified using Bradford method [9]. An equal amount of protein (100 μg) was taken in individual tubes and heated at 95 °C for 3 min, then separated on 12% *w*/*v* SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) [10]. Electrophoresis was carried out at a constant current of 30 mA and the proteins were stained with Coomassie Blue R-250 (0.125%). After de-staining (15% methanol, 10% acetic acid and water), the protein bands were visualized under white light and documented using a gel doc system (Biorad).

Two-dimensional polyacrylamide gel electrophoresis Forty milligrams of powdered tissue was homogenized with 550 μl of buffer (9.3 M urea, 5 mM potassium carbonate, 5% 2-mercaptoethanol, and 2% Nonidet NP-40) and centrifuged for 15 min at 14,000 rpm. After protein quantification as above, an aliquot (100 μg) of the supernatant was fractionated by isoelectrophoresis in the first dimension followed by SDS-PAGE in the second dimension [11].

Protein identification To determine the identity and function of protein spots observed on 2-D PAGE gel, they were cut out of gel and subjected to trypsin digestion. Capillary RP HPLC separation of protein digests (desalted with a PepMap C18 cartridge) was performed using PepMap C18 column (15 cm × 75 μm i.d.) and Ultimate Capillary HPLC System (LC Packings, San Francisco, CA, USA). A linear gradient of 5% to 40% (*v*/*v*) acetonitrile for 25 min at 200 nl min^{-1} was used for separation. Tandem mass spectrometric analysis was performed online using a hybrid quadrupole time-of-flight instrument (QSTAR XL hybrid LC/MS/MS) equipped with a nanoelectrospray source (Applied Biosystems, Foster City, CA, USA) and operated with the Analyst QS v1.1 data acquisition software. Tandem mass spectra were extracted by ABI Analyst version 1.1. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 2.0.01). Mascot was set up to search NCBI non-redundant database assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.30 Da and a parent ion tolerance of 0.30 Da. Iodoacetamide derivative of cysteine is specified in Mascot as a fixed

modification. Deamidation of asparagine and glutamine and oxidation of methionine were specified in Mascot as variable modifications.

Results and Discussion

Differential Expression of Proteins among Genotypes

Qualitative and quantitative analyses of various polypeptides between uninfected and infected leaves of muscadine and Florida hybrid bunch grape were studied using polyacrylamide gel electrophoresis. The data showed that several polypeptides were either unique expressed, over expressed, suppressed, and or not expressed.

One-dimensional SDS-PAGE One-dimensional polyacrylamide gel electrophoresis (PAGE) revealed differential expression of several low molecular weight polypeptides (<40 kDa, shown with arrows) in response to *E. ampelina* infection (Fig. 1). A low molecular weight polypeptide (16 kDa) was found suppressed in the infected leaf tissue of all the genotypes studied (both Florida hybrid bunch grape and muscadine cv. Carlos cultivars). We found a new polypeptide with a molecular weight around 45 kDa induced in infected leaves of muscadine (cv. Carlos) which is unique and needs to be further characterized. Muscadine cv. Carlos is known to be an anthracnose-tolerant cultivar [12]. The results revealed major differences in the leaf polypeptide composition between tolerant and susceptible cultivars of Florida hybrid bunch grape.

Two-dimensional PAGE Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was performed to identify and characterize the proteins unique to anthracnose tolerance (Fig. 2). The 2-D gels obtained using leaf tissue of two anthracnose-tolerant (Lake Emerald and Blue Lake) and -susceptible (Blanc and Suwannee) cultivars from both uninfected (control) and

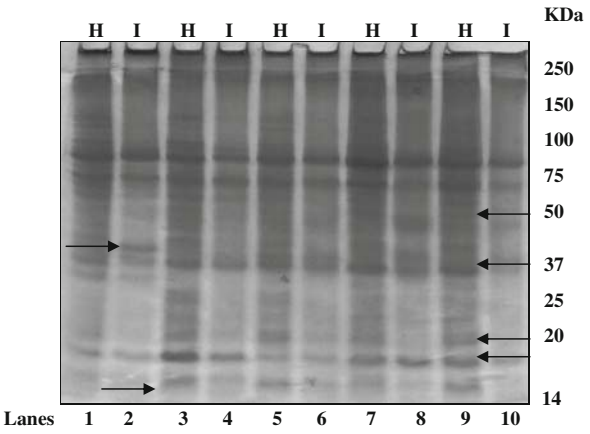

Fig. 1 Differences in the polypeptide composition of different grape genotypes upon infection with *Elsinoe ampelina*. *Lanes 1* and *2*: Carlos (Muscadine); *lanes 3* and *4*: Blue Lake (Florida hybrid); *lanes 5* and *6*: Lake Emerald (Florida hybrid); *lanes 7* and *8*: Blanc du Bois (Florida hybrid); *lanes 9* and *10*: Suwannee (Florida hybrid). *H* uninfected healthy leaf tissue, *I* infected leaf tissue. *Carlos, Blue Lake, and Lake Emerald= tolerant cultivars; Blanc du Bois and Suwannee=susceptible cultivars (*arrows* indicate affected polypeptides)

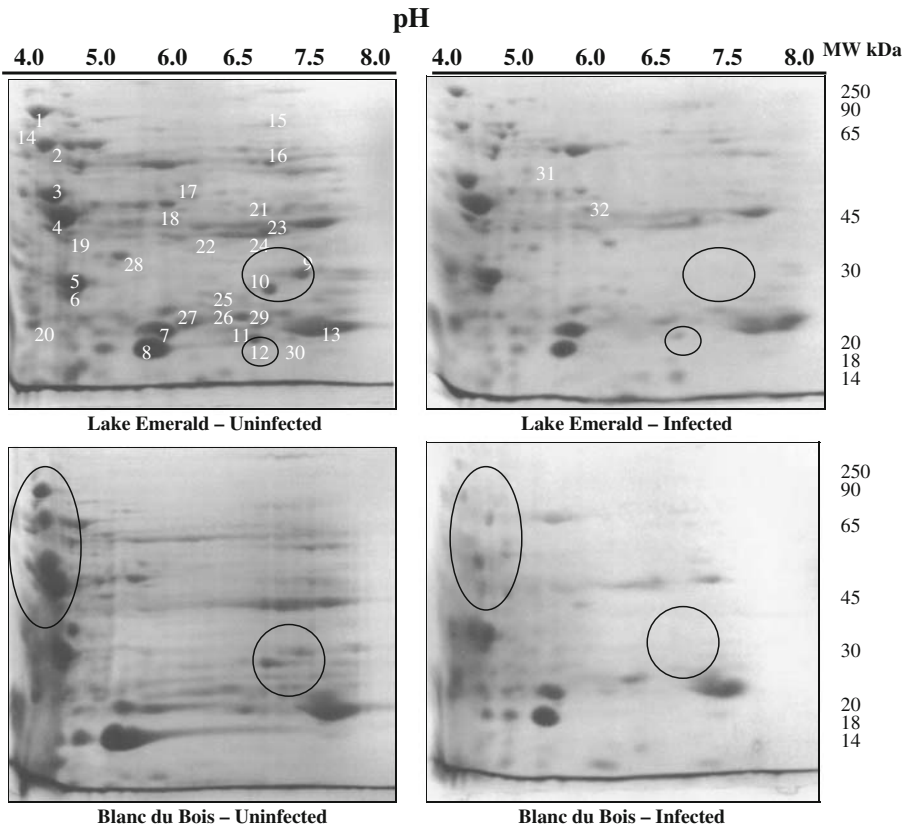

Fig. 2 2-D gels representing the protein profile of cultivars, Lake Emerald (anthracnose tolerant) and Blanc du Bois (anthracnose susceptible) induced upon infection with *Elsinoe ampelina* (*circle* indicates missing polypeptides)

infected tissue were analyzed using PD Quest software (PD Quest 2-D Analysis Software, BioRad). Each spot was numerically labeled and a total of 32 polypeptides were identified that showed differences in their expression levels between tolerant and susceptible cultivars, and also between uninfected and infected leaf samples (Table 1). Twenty nine polypeptides (spot no. #1–11 and 13–30) were found common to both tolerant and susceptible control samples. In the corresponding treated samples (*Elsinoe* infected), seven polypeptides out of 29 (spot no. #9, #10, and #25 to #29) were unexpressed in tolerant cultivars, while a total of 18 polypeptides (spot no. #9, #10, and #14 to #29) were unexpressed in susceptible cultivars. Nine polypeptides (spot no. #1–8 and #11) were found suppressed in susceptible cultivars upon *Elsinoe* infection, while majority of polypeptides were unaffected in tolerant cultivars (a total of 21; #1–8, #11, and #13–24). We found two uniquely expressed polypeptides (#31 and #32) in response to *Elsinoe* infection in both the tolerant cultivars (Lake Emerald and Blue Lake) studied. We also found that spot #12 continued to express in tolerant cultivar even after infection, while this protein was not expressed corresponding to infected susceptible cultivars. Using a similar approach, Kim et al. [13] were able to identify differentially expressed proteins induced upon rice blast fungus infection. They identified 14 protein spots, which were induced or increased by the treatments.

Table 1 Expression levels of unique polypeptide spots identified in uninfected and infected leaf tissues of anthracnose-tolerant and -susceptible Florida hybrid bunch grape cultivars.

Polypeptides Spots	Uninfected leaf tissue				Infected leaf tissue			
	Tolerant		Susceptible		Tolerant		Susceptible	
	LE	BL	BDB	SU	LE	BL	BDB	SU
1	++	++	++	++	++	++	+	+
2	++	++	++	++	++	++	+	+
3	++	++	++	++	++	++	+	+
4	++	++	++	++	++	++	+	+
5	++	++	++	++	++	++	+	+
6	++	++	++	++	++	++	+	+
7	++	++	++	++	++	++	+	+
8	++	++	++	++	++	++	+	+
9	++	++	++	++	−	−	−	−
10	++	++	++	++	−	−	−	−
11	++	++	++	++	++	++	+	+
12	++	++	+	+	+	+	−	−
13	++	++	++	++	++	++	++	++
14	+	+	+	+	+	+	−	−
15	+	+	+	+	+	+	−	−
16	+	+	+	+	+	+	−	−
17	+	+	+	+	+	+	−	−
18	+	+	+	+	+	+	−	−
19	+	+	+	+	+	+	−	−
20	+	+	+	+	+	+	−	−
21	+	+	+	+	+	+	−	−
22	+	+	+	+	+	+	−	−
23	+	+	+	+	+	+	−	−
24	+	+	+	+	+	+	−	−
25	++	++	+	+	−	−	−	−
26	++	++	+	+	−	−	−	−
27	++	++	+	+	−	−	−	−
28	++	++	+	+	−	−	−	−
29	++	++	+	+	−	−	−	−
30	+	+	+	+	+	+	−	−
31	−	−	−	−	+	+	−	−
32	−	−	−	−	+	+	−	−

Expression levels were analyzed manually. − No expression, + low expression, ++ high expression. *LE* Lake Emerald, *BL* Blue Lake (tolerant cultivars), *BDB* Blanc du Bios, *SU* Suwannee (susceptible cultivars)

Characterization of Uniquely Expressed Proteins in Response to *E. ampelina* Infection

Among several differentially expressed proteins, three polypeptides were found to be either uniquely expressed (#12) or newly expressed (#31 and #32) upon *E. ampelina* infection in anthracnose-tolerant cultivars. The induction of new proteins in tolerant cultivars clearly indicates their association with the anthracnose tolerance. However, no new polypeptides were induced in susceptible cultivars, albeit several polypeptides were either suppressed or unexpressed following *E. ampelina* infection. In order to further identify and characterize anthracnose-induced proteins, we eluted these spots (spots #31, #32, and #12; MW=55,000,

Table 2 Partial *Elsinoe ampelina*-induced leaf protein sequences from Florida hybrid bunch grapes (*Vitis* spp.) obtained by mass spectrometric analysis that showed high similarity (95% identity) to database entries.

Spot no.	Sequence	MASCOT score	No. of unique peptides	GI accession no.	Protein similar to	Organism matched	UniProtKB acc. no.
12	LTYYTPEYETK	52.8	5	62861063	Rubilose 1, 5-bisphosphate-carboxylase	*Synadenium grantii*	P85085
	GLLLHXHR	66.0					
	MSGGDHIHAGXVVGK	53.5					
	EITLGFVDLLR	61.0					
	VALEACVQAR	46.8					
31	VLNTGSPITVPVGR	91.0	4	3676296	Mitochondrial ATP synthase beta subunit	*Nicotiana sylvestris*	P85088
	VGLTGLTVAEHFR	73.9					
	FTQANSEVSALLGR	60.5					
	IPSAVGYQPTLATDLGGLQER	56.8					
32	TLSGPVSDPAK	54.2	4	1707955	Glutamine synthetase	*Vitis vinifera*	P85087
	NDGGFEVIKK	56.7					
	HKEHIAAYGEGNER	57.8					
	HETADINTFLWGVANR	65.1					

50,000, and 20,000 Da and p*I*=7.4, 6.5, and 5.5, respectively) and sequenced using LC/MS/MS. The proteomic analysis revealed that sequences of these polypeptides have 95% similarity with mitochondrial adenosine triphosphate (ATP) synthase beta subunit, glutamine synthetase (GS), and ribulose 1-5 bisphosphate-carboxylase (Table 2).

Mitochondrial ATP synthase beta subunit (spot #31) and GS (spot #33) were expressed only upon *Elsinoe* infection in the tolerant cultivars. Mitochondria control the respiratory activity of plant cells. It produces ATP using mitochondrial ATP synthase by oxidative phosphorylation. As cited in Vidhyasekaran [14], inhibition of ATP synthesis from fungal pathogen will result in suppression of defense mechanisms of the host and will facilitate extensive colonization of pathogens [15–18]. The phytotoxins produced from pathogens inhibit ATP synthesis, which results in suppression of defense mechanisms of the host, which occurs in the susceptible cells, whereas resistant cells overcomes these toxic effects. It is still unclear how resistant cells detoxify these toxins, but it has been suggested that these cells have a mechanism for excluding, exporting, or destroying the toxins [19]. Detoxification of the toxins is an important host defense mechanism. Expression of mitochondrial ATP synthase clearly shows that the anthracnose-tolerant Florida hybrid bunch grape cultivars were able to detoxify phytotoxins produced by the fungus *Elsinoe*.

Glutamine synthetase is involved in ammonia metabolism [20] which converts ammonia to amino acids. GS functions together with ferredoxin-dependent glutamate synthase, an enzyme which recycles glutamate and incorporates carbon skeletons into the cycle for transfer of amino groups to keto-acids or other amino acids utilized for protein formation. Amino groups are also transferred to nucleotides used as basic molecules for RNA and DNA synthesis [21]. GS is involved in nitrogen synthesis and nitrogen nutrition has a significant impact on plant disease development [22]. The size of lesion areas that develop when fungal pathogens infect plants is changeable depending on plant nutrition [23, 24]. Reduced availability of nitrogen often increases the susceptibility of plants to diseases [22]. Nitrogen limitation also affects pathogenesis and the form of nitrogen available to plants and pathogens can also affect the severity of the disease [25]. It has been reported that an elevated level of expression of GS occurred during the infection of tomato leaves by *Pseudomonas syringae* pv. *tomato* in resistant cultivars. Whereas in susceptible cultivars, the tabtoxin produced by *P. syringae* irreversibly inhibited the GS activity and resulted in chlorosis of the leaves [26, 27]. Expression of GS in anthracnose-tolerant Florida hybrid bunch grape cultivars upon *Elsinoe* infection indicates that they were able to maintain required nitrogen nutritional status of the plant for normal plant development.

Glutamine synthetase also helps to produce glutathione, which is a key antioxidant protein. Glutathione has been reported to have a key regulatory role on the expression of several defense-related genes such as PR1, glutathione-*S*-transferase, superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, chalcone synthase, and phenylalanine ammonia lyase participating in signal transduction pathway [28]. Our results indicate that anthracnose infection induced several changes in the expression of genes resulting in expression of certain unique proteins which play a key role in defense mechanism. This defense mechanism helps the plant to maintain normal physiological growth and defend against pathogen infection in anthracnose-tolerant Florida hybrid bunch grapes, while the susceptible genotypes suffer severe damage as they lack the ability to induce the above proteins.

Several pathogens have been found to suppress photosynthesis of the host. Ribulose 1-5 bisphosphate-carboxylase (Rubisco, spot #12) is an important enzyme involved in carbon assimilation and is the largest functional category of proteins involved in photosynthesis [29]. Rubisco activase, a molecular chaperone, is reported to catalyze Rubisco from an

inactive closed conformation to an active open conformation [30]. Elevated levels of Rubisco activase have been documented during early stage of pathogen infection. Expression of Rubisco was seen only in the tolerant grape cultivars even upon pathogen infestation; however, the levels of expression of this protein were found low in infected leaf tissue (Fig. 2). On the contrary, this enzyme was completely absent in anthracnose-susceptible genotypes when subjected to *E. ampelina* infection. The pathogen influences the rate of net photosynthesis by affecting the mechanism of stomatal aperture and mesophyll conductance through differential expression of ribulose [31]. Suppression of ribulose 1-5 bisphosphate-carboxylase activity has been reported in sugar beet leaves (*Beta vulgaris* L.) infected with powdery mildew (*Erysiphe polygoni* D.C.) [31] and melon leaves infected with *Colletotrichum lagenarium* [32]. Further, Pinkard and Mohammed [33] noticed a negative linear relationship between light-saturated photosynthesis [*A*(max)] and leaf-level damage from MLD (*Mycosphaerella* leaf disease). Reductions in *A*[max] were greater than might be expected from the reduction in green leaf area as a result of the disease, indicating that asymptomatic tissue also was affected by MLD. The reductions in *A* [max] were not related to increase in stomatal resistance, but rather were a result of reduced activity of ribulose bisphosphate-carboxylase (Rubisco) and accompanied by changes in the capacity for ribulose bisphosphate regeneration. This shows that *Elsinoe* pathogen attempts to manipulate the carbohydrate metabolism of the plant [34]. On the other hand, the plant reorganizes carbon fluxes to ensure fight against the pathogen.

In susceptible plants, infections are known to degrade Rubisco and thereby affect normal rate of photosynthesis. Bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) has been reported to accelerate degradation of intact Rubisco in rice [29, 35]. In contrast, Tsunezuka et al. [36] reported that the expression of Rubisco continued in a rice mutant *cdr2* upon infection, which is resistant to rice blast fungus. Absence of Rubisco large subunits in anthracnose-susceptible Florida hybrid bunch grape cultivars indicates that the subunits were degraded upon infection, whereas in anthracnose-tolerant cultivars, expression of Rubisco continued. Therefore, the expression of Rubisco in tolerant cultivar indicates the ability of the plant to overcome anthracnose infection and perform normal photosynthesis.

Our results clearly indicate that the tolerant cultivars (Lake Emerald and Blue Lake) were able to express these enzymes even after infection, which helped them to maintain a desired or optimal level of gene expression for normal physiological process. But the susceptible cultivars (Blanc du Bois and Suwannee) failed to maintain expression of necessary enzymes for normal metabolism under infection and therefore were affected by *E. ampelina* to a greater extent. Further studies on the expression pattern of these enzymes should shed light on tolerance mechanism of tolerant cultivars. These are the enzymes which may be responsible for gene regulation, leading to expression of defense-related proteins conferring tolerance against anthracnose infection.

Conclusions

E. ampelina infection appeared to significantly affect enzymes associated with plant metabolism and defense pathways. The suppression levels were more pronounced in the susceptible cultivars (Blanc du Bois and Suwannee) compared to tolerant cultivars (Lake Emerald and Blue Lake) altering normal physiological processes. Several uniquely expressed polypeptides were recognized in tolerant cultivars. Expression of mitochondrial ATP synthase, glutamine synthetase, and continued expression of ribulose in tolerant

cultivars indicate that they were least affected by *E. ampelina* infection in anthracnose-tolerant Florida hybrid bunch grape cultivars. This helped tolerant cultivars to maintain normal physiological process. Further study of these uniquely expressed polypeptides should help determine their role in anthracnose tolerance.

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