



Short communication

Analysis of grape polyamines from *Grapevine leafroll associated viruses* (GLRaV-2 and -3) infected vines

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ABSTRACT

This is the first report comparing the free polyamine content of 'Pinot noir' grapes from vines infected with *Grapevine leafroll associated viruses* (GLRaV)-2 or -3, with samples taken from healthy vines. Berries were collected from three different rootstock/scion combinations, and all samples were from commercial vineyards in Oregon. Three free polyamines (putrescine, cadaverine, and spermidine) were found in all 'Pinot noir' grapes analysed in this study. Berries from healthy *Vitis riparia* rootstock/'Pinot noir' clone 114 were significantly higher in putrescine (28.6 $\mu\text{mol/l}$) and total polyamines (41.9 $\mu\text{mol/l}$), compared to their GLRaV-3 infected samples (20.7 and 33.0 $\mu\text{mol/l}$, respectively). Vine virus status did not alter total or individual polyamines in berries from the other two rootstock/scion combinations (unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion [clone unknown] and self-rooted/'Pinot noir' clone Pommard). Levels of putrescine, spermidine, and total polyamines varied between berries taken from the healthy vines of the three different rootstock/scion combinations.

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1. Introduction

Plant polyamines are involved in several plant growth and development processes (Evans & Malmberg, 1989; Kusano, Yamaguchi, Berberich, & Takahashi, 2007), though more studies are required to discern relationships between plant pathogens and polyamines. Some reports on the possible roles of polyamines have been made, specifically metabolism and catabolism in relation to plant defence against certain biotic and abiotic stresses (Alkazar et al., 2006; Bouchereau, Aziz, Larher, & Martin-Tanguy, 1999; Evans & Malmberg, 1989; Groppa & Benavides, 2008; Kusano, Berberich, Tateda, & Takahashi, 2008; Kusano et al., 2007; Kuznetsov, Radyukina, & Shevyakova, 2006; Moschou, Paschalidis, & Roubelakis-Angelakis, 2008; Rhee, Kim, & Lee, 2007; Walters, 2000, 2003a, 2003b).

Walters (2000, 2003a, 2003b) summarised the involvement of plant polyamines as a response to pathogen attack. Fungal attack (i.e. *Botrytis cinerea*) has been reported to alter and increase grape polyamines (Hajos, Sass-Kiss, Szerdahelyi, & Bardocz, 2000; Kiss, Korbasz, & Sass-Kiss, 2006), likely from the fungus changing the grape's polyamines and contributing its own polyamines as well

(Kiss et al., 2006; Marshall, Russo, Van Etten, & Nickerson, 1979). Edreva (1997) reported a reduction in free polyamine, compared to control, in tobacco leaves inoculated with fungal, bacterial, or viral pathogens, and suspected the decrease was due to tissue damage rather than a direct affect of the pathogen. Gonzalez, Mosquera, San Jose, and Diaz (1997) reported an increase in free polyamines in 'Albarino' shoots that were infected with grapevine fleck disease and co-infected with GLRaV-1 or -3 or *Grapevine rupestris stem pitting associated virus* (GRSPaV), but did not report if GLRaVs affected polyamine levels in the fruit. To date, no literature has examined how GLRaVs might impact polyamine in 'Pinot noir' grapes.

According to a recent survey by the Oregon Wine Board, wine grapes contribute \$1.42 billion in economic activity for the state of Oregon. As the leading cultivar grown in Oregon is 'Pinot noir', we decided to investigate the impact of GLRaVs upon that cultivar's berry composition, as recent years have brought a heightened awareness and concern of the effects of this group of viruses of fruit quality (Lee & Martin, 2009; Martin, Eastwell, Wagner, Lampercht, & Tzanetakis, 2005). GLRaV-3 infection significantly reduced individual and total anthocyanins in 'Pinot noir' berries when measured against healthy vines of the same rootstock/scion (samples were taken from *Vitis riparia* rootstock/'Pinot noir' clone 114; Lee & Martin, 2009), and may contribute towards reduced yields, decreased colour, and other negative effects (Lee, Keller, Rennaker, & Martin, 2009; Lee & Martin, 2009). The objective of

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this study was to examine free polyamine content and composition of grapes from vines with GLRaV-2 or -3 infection, and compare them to the grapes from adjacent vines free of these viruses.

2. Materials and methods

2.1. Plant material and virus detection

Details of the samples, replications, vineyards, vines, and harvest dates were presented in Lee and Martin (2009) and Lee et al. (2009). Briefly, grapes from visually healthy vines and those from virus-infected vines (without observed signs of fungal attack), were randomly collected from two commercially operating vineyards, with a total of three rootstock/scion combinations. Both vineyards were located in Oregon's Willamette Valley (USA), approximately 40-km apart. At commercial harvest, four clusters were taken randomly from each presumptively identified (confirmed by RT-PCR) vine, either free of or infected with GLRaV in the 2005 and 2006 growing seasons. Clusters from each vine were grouped for chemical analysis. Grapes were stored at -23°C until virus status was established for every vine by RT-PCR. Virus status was determined as reported previously (Martin et al., 2005), with alterations to the procedure being described in Lee and Martin (2009).

2.2. Reagents, chemicals, and standards

All chemicals and standards for free polyamine analysis were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and reagents for this investigation were analytical and high performance liquid chromatography (HPLC) grade.

2.3. Berry extraction and sample preparation

Frozen whole berries were puréed with a hand blender (Braun hand blender model 4169; Braun, Kronberg im Taunus, Germany) for 3 min (which macerated skin, pulp, and seeds), then centrifuged for 10 min at 4000 rpm (28 cm diameter rotor). The resulting supernatants were then filtered with disposable Millex-FH syringe filters (Millipore, Bedford, MA, USA) prior to polyamine determination. Details regarding sample preparation can be found in Lee et al. (2009).

2.4. HPLC conditions for polyamine analysis

An Agilent HP1100 system (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with a fluorescence detector (FLD), and a Hypersil ODS bonded phase analytical (150 mm \times 4.6 mm, 5 μm ; Thermo Fischer Scientific Inc., Waltham, MA, USA) and guard (20 mm \times 4.0 mm, 5 μm of the same composition) columns were used. Inline-derivatisation by *o*-phthalaldehyde/ethanethiol (OPA/ET) and 9-fluorenylmethyl chloroformate (FMOC) was performed by the autosampler immediately prior to injection, as described and evaluated in detail by Hanczko, Koros, Toth, and Molnar-Perl (2005) and Hanczko, Jambor, Perl, and Molnar-Perl (2007). Composition of mobile phases and gradient conditions were as described in Hanczko et al. (2005). Flow rate was 1.8 ml/min. 1,6-Hexanediamine was used as internal standard (IS). Peaks were collected at an excitation/emission (Ex/Em) wavelength of 334 nm/454 nm. Column compartment was held at 50°C . Polyamine values were expressed in both $\mu\text{mol/l}$ and mg/l units. One (15.63 $\mu\text{mol/l}$) of the ten concentrations of the standard mixtures (concentration ranged from 0.977 to 500 $\mu\text{mol/l}$) was injected ten times to determine single-day reproducibility. While plant polyamines are found

in free, conjugated, and bound forms, only free polyamines were examined in this study.

2.5. Statistical analysis

Statistica for Windows version 7.1 was used (StatSoft Inc., Tulsa, OK, USA) for the statistical analyses. *T*-test calculations and one-way analysis of variance (ANOVA) for values from each pair were performed. Significant differences among the three rootstock/scion values were compared using Fisher's least significant difference (LSD; $\alpha = 0.05$). Correlation between values obtained in this study, to our previous values (Lee & Martin, 2009; Lee et al., 2009), were calculated using Pearson product moment correlation coefficient (*r*) at $\alpha = 0.05$.

3. Results and discussion

Details of the vineyard location and vine virus status were reported in the first part of this project (Lee & Martin, 2009). Briefly, all vines tested positive for GRSPaV. GLRaV infected vines from unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown) vines had co-infection of GRSPaV and GLRaV-2. The other two vine combinations, *V. riparia* rootstock/'Pinot noir' clone 114 and self-rooted/'Pinot noir' clone Pommard, were GLRaV-3 positive or negative and infected with GRSPaV. The observed results are assumed to be due to GLRaV status despite co-infection with GRSPaV (Lee & Martin, 2009; Lee et al., 2009), since reports of GRSPaV have described little to no effect (Reynolds, Lanterman, & Wardle, 1997).

The HPLC elution order of the polyamines were putrescine (PUT), cadaverine (CAD), 1,6-hexanediamine (internal standard; IS), spermidine (SPD), and then spermine (SPM). A representative chromatogram of these polyamines is presented in Fig. 1. A preparation of the standard (15.63 $\mu\text{mol/l}$) was injected ten times and the % relative standard deviation (%RSD) was calculated based on fluorescence intensities. All standards %RSD were lower than 3.1% (PUT – 1.5%, CAD – 1.7%, IS – 2.0%, SPD – 2.6%, and SPM – 3.1%). Again, this derivatisation and HPLC method was comprehensively evaluated by Hanczko et al. (2005, 2007).

There was no evidence of a significant two-way interaction between vine virus status and growing season. Polyamine values were grouped as vine virus status (Table 1), for each rootstock/scion combination. All grape samples contained three identified and quantified polyamines (PUT, CAD, and SPD), but no detectable levels of SPM were found.

Vine GLRaV status did not impact the total and individual polyamines in berries from unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown), or the self-rooted/'Pinot noir' clone Pommard. When polyamine values were compared in $\mu\text{mol/l}$, PUT was the chief polyamine in both rootstock/scion combinations, followed by SPD and then CAD. In mg/l, SPD was the main polyamine in unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown), and PUT remained the main polyamine in the other rootstock/scion vines.

V. riparia rootstock/'Pinot noir' clone 114 berries had PUT as the main polyamine (in both units). Total polyamines and PUT levels were significantly lower in GLRaV-3 positive vines compared to their healthy counterparts. Though, more work is needed to determine if this is due to disruption of metabolism, catabolism, translocation, or conjugation of polyamines (Groppa & Benavides, 2008; Kuznetsov et al., 2006) as a result of GLRaV-3 infection and if these compounds are involved in plant coping mechanisms from this particular biotic stress. Berries from GLRaV-3 positive vines had a significantly lower total anthocyanin, total phenolics, and total tannin content (Lee & Martin, 2009) compared to healthy vines. Conversely, Hudec et al. (2006) found higher levels of total antho-

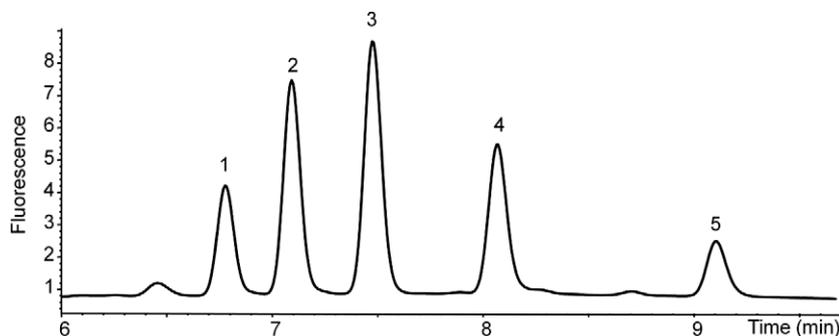


Fig. 1. Chromatogram of the polyamine standards (1 – PUT, 2 – CAD, 3 – internal standard, 4 – SPD, and 5 – SPM) detected by FLD (Ex/Em; 334 nm/454 nm). The five standards concentrations for this chromatogram were 7.8 $\mu\text{mol/l}$.

Table 1
Virus status and polyamine content at harvest from all locations are listed. All sampled vines tested positive for GRSPaV. Polyamine levels are expressed in two units. Different lower case letters indicate significantly different ($p \leq 0.05$) within the pair of virus positive and negative samples taken from one rootstock/scion combination (e.g. samples from GLRaV-3 positive versus negative vines from self-rooted/'Pinot noir' clone Pommard). Different upper case letters indicate significant difference among the healthy vines ($p \leq 0.05$). Values in parenthesis are standard errors. 'nd' indicates not detected.

Rootstock/scion	Unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown)		<i>V. riparia</i> rootstock/'Pinot noir' clone 114		Self-rooted/'Pinot noir' clone Pommard	
Harvest dates	10/17/2005 and 9/28/2006		10/03/2005 and 9/22/2006		10/03/2005 and 9/28/2006	
Virus status	GLRaV-2 positive		GLRaV-3 positive		GLRaV-3 positive	
Number of vines corresponding to the GLRaV results	7	8	5	11	6	10
Total polyamines ($\mu\text{mol/l}$)	35.5 (2.10) a	37.8 (1.87) a AB	33.0 (1.58) a	41.9 (2.99) b B	30.9 (1.73) a	31.7 (1.43) a A
1 Putrescine (PUT)	17.8 (1.95) a	19.8 (2.63) a A	20.7 (1.98) a	28.6 (2.50) b B	18.2 (0.54) a	20.9 (1.81) a A
2 Cadaverine (CAD)	3.2 (0.20) a	2.7 (0.19) a A	2.5 (0.24) a	2.4 (0.09) a A	2.5 (0.28) a	2.5 (0.35) a A
3 Spermidine (SPD)	14.6 (1.17) a	15.3 (1.44) a B	9.9 (0.49) a	10.9 (0.57) a A	10.2 (1.29) a	8.3 (1.34) a A
4 Spermine (SPM)	nd	nd	nd	nd	nd	nd
Total polyamines (mg/l)	4.01 (0.21) a	4.25 (0.17) a B	3.51 (0.13) a	4.35 (0.29) b B	3.34 (0.23) a	3.31 (0.17) a A
1 Putrescine (PUT)	1.57 (0.17) a	1.75 (0.23) a A	1.82 (0.17) a	2.52 (0.22) b B	1.60 (0.05) a	1.84 (0.16) a A
2 Cadaverine (CAD)	0.33 (0.02) a	0.27 (0.02) a A	0.25 (0.02) a	0.24 (0.01) a A	0.25 (0.03) a	0.26 (0.04) a A
3 Spermidine (SPD)	2.12 (0.17) a	2.22 (0.21) a B	1.44 (0.07) a	1.59 (0.08) a A	1.49 (0.19) a	1.21 (0.19) a A
4 Spermine (SPM)	nd	nd	nd	nd	nd	nd

cyanin and phenolics in black chokeberries applied with polyamine biosynthesis regulators, which resulted in a decrease of free polyamines in those berries.

Polyamines were compared among berries obtained from the healthy vines (Table 1). It appeared that different rootstock/scion combinations alone altered polyamine contents. *V. riparia* rootstock/'Pinot noir' clone 114 berries had higher levels of PUT compared to the other two rootstock/scion combinations. And the unknown rootstock/'Chardonnay' interstock/'Pinot noir' scion (clone unknown) was highest in SPD. The differences might have aligned with variations in management among commercial vineyards, but *V. riparia* rootstock/'Pinot noir' clone 114 and self-rooted/'Pinot noir' clone Pommard were both collected from the same working vineyard, and there was a clear difference in berry polyamines between them, in addition to their responses to GLRaV-3 infection.

There was significant positive correlation between values obtained in this study to those from previous evaluations (Lee & Martin, 2009; Lee et al., 2009): between polyamines (mg/l) and total anthocyanins by spectrophotometer and HPLC, total phenolics by spectrophotometer, total polyphenolics by HPLC, yeast assimilable nitrogen content, and total free amino acid (r ranging from 0.63 to 0.86). Additional studies need to be conducted to understand what this positive correlation signifies.

Compared to the 'Pinot noir' berries examined in this study, Sass-Kiss, Szerdahelyi, and Hajos (2000) reported similar levels of PUT (1.19–1.28 mg/l) and CAD (0.35–0.45 mg/l), but higher levels of SPD (8.79–11.83 mg/l) in their 'Harslevelu', 'Furmint', and 'Mus-

cat Ottonel' grapes. Bover-Cid, Iquierdo-Pulido, Marine-Font, and Carmen Vidal-Carou (2006) also reported higher levels of PUT (5.19 mg/l) in the 'Cabernet Sauvignon' must they examined, than our values. Del Prete, Costantini, Cecchini, Morassut, and Garcio-Moruno (2009) examined polyamine content from musts of seven grape cultivars (Merlot, Syrah, Cabernet Franc, Montepulciano, Sangiovese, Carmenere, and Cesansese d'Affile); finding PUT levels of 11.0–27.6 mg/l, which were also higher than the PUT values we measured for 'Pinot noir' berries. These findings indicate individual polyamine content varies with grape cultivar, and results from this study show grape polyamine levels to differ among 'Pinot noir' rootstock/scion combinations as well.

Gonzalez et al. (1997) demonstrated that free polyamines in 'Albarino' shoots were impacted by vine virus status (GLRaV-3, GLRaV-1+ grapevine fleck disease, and GRSPaV). They also observed an increase in PUT, SPD, SPM, and total free polyamines in the leaves of vines with GLRaV-1 and grapevine fleck disease, or GRSPaV, compared to control vines. Though not within the scope of this study, it is possible that the impact of GLRaV on polyamines might be better revealed in tissues such as flower or leaf, which generally contain higher levels of polyamines (Aziz, 2003) than healthy mature fruit. However, the levels in the fruit are most likely important in product quality. Samples taken during certain growth phases might show greater influence from GLRaV as well (Bauza, Kelly, & Blaise, 2007; Colin, Cholet, & Geny, 2002). Researchers (Kalac & Krausova, 2005; Nishimura, Shiina, Kashiwagi, & Igarashi, 2006; Silla Santos, 1996) agree that more composition examinations of polyamines in food are needed, and interest

regarding the potential roles polyamines might have in human diets is an on-going topic for clarification. Some further opportunities for research are how the polyamines found in these grapes will contribute towards a final product such as wine (Hajos et al., 2000; Kiss et al., 2006), or how the fermentation process could alter polyamines' composition and content (Del Prete et al., 2009; Herbert, Cabrita, Ratola, Laureano, & Alves, 2005).

4. Conclusion

To the best of our knowledge, this is the first preliminary report of GLRaV influences upon the polyamines of 'Pinot noir' berries. It appears berries from each rootstock/scion combination responded differently in regards to their free polyamine content. *V. riparia* rootstock/'Pinot noir' clone 114 appeared to be the most sensitive to GLRaV infection, compared to the other two rootstock/scion combinations. Our in-progress controlled GLRaV infection study, established in a research vineyard with identified rootstocks and scions, documented vine ages, and known GLRaV infection periods will provide additional data on both grape polyamine and GLRaV. That study will also increase knowledge about polyamine levels in food, in general.

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References

- Alkazar, R., Marco, F., Cuevas, J. C., Patron, M., Ferrando, A., Carrasco, P., et al. (2006). Involvement of polyamines in plant response to abiotic stress. *Biotechnology Letters*, *28*, 1867–1876.
- Aziz, A. (2003). Spermidine and related-metabolic inhibitors modulate sugar and amino acid levels in *Vitis vinifera* L.: Possible relationships with initial fruitlet abscission. *Journal of Experimental Botany*, *54*, 355–363.
- Bauza, T., Kelly, M. T., & Blaise, A. (2007). Study of polyamines and their precursor amino acids in Granche noir and Syrah grapes and wine of the Rhone Valley. *Food Chemistry*, *105*, 405–413.
- Bouchereau, A., Aziz, A., Larher, F., & Martin-Tanguy, J. (1999). Polyamines and environmental challenges: Recent development. *Plant Science*, *140*, 103–125.
- Bover-Cid, S., Iquierdo-Pulido, M., Marine-Font, A., & Carmen Vidal-Carou, M. (2006). Biogenic mono-, di- and polyamine contents in Spanish wines and influence of a limited irrigation. *Food Chemistry*, *96*, 43–47.
- Colin, L., Cholet, C., & Geny, L. (2002). Relationships between endogenous polyamines, cellular structure and arrested growth of grape berries. *Australian Journal of Grape and Wine Research*, *8*, 101–108.
- Del Prete, V., Costantini, A., Cecchini, F., Morassut, M., & Garcio-Moruno, E. (2009). Occurrence of biogenic amines in wine: The roles of grapes. *Food Chemistry*, *112*, 474–481.
- Edreva, A. (1997). Tobacco polyamines as affected by stresses induced by different pathogens. *Biologica Plantarum*, *40*, 317–320.
- Evans, P. T., & Malmberg, R. L. (1989). Do polyamines have roles in plant development? *Annual Review of Plant Physiology and Plant Molecular Biology*, *40*, 235–269.
- Gonzalez, E., Mosquera, M. V., San Jose, M. C., & Diaz, T. (1997). Influence of virus on the chlorophyll, carotenoid and polyamine contents in grapevine microcuttings. *Journal of Phytopathology*, *145*, 185–187.
- Groppa, M. D., & Benavides, M. P. (2008). Polyamines and abiotic stress: Recent advances. *Amino Acids*, *34*, 35–45.
- Hajos, G., Sass-Kiss, A., Szerdahelyi, E., & Bardocz, S. (2000). Changes in biogenic amine content of Tokaj grapes, wines, and aszu-wines. *Journal of Food Science*, *65*, 1142–1144.
- Hanczko, R., Jambor, A., Perl, A., & Molnar-Perl, I. (2007). Advances in the o-phthalaldehyde derivatizations comback to the o-phthalaldehyde-ethanethiol reagent. *Journal of Chromatography A*, *1163*, 25–42.
- Hanczko, R., Koros, A., Toth, F., & Molnar-Perl, I. (2005). Behavior and characteristics of biogenic amines, ornithine and lysine derivatized with the o-phthalaldehyde-ethanethiol-fluorenylmethyl chloroformate reagent. *Journal of Chromatography A*, *1087*, 210–222.
- Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O., & Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. *Journal of Food Engineering*, *66*, 315–322.
- Hudec, J., Bakos, D., Mravec, D., Kobida, L., Burdova, M., Turianica, I., et al. (2006). Content of phenolic compounds and free polyamines in black chokeberry (*Aronia melanocarpa*) after application of polyamine biosynthesis regulators. *Journal of Agricultural and Food Chemistry*, *54*, 3625–3628.
- Kalac, P., & Krausova, P. (2005). A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. *Food Chemistry*, *90*, 219–230.
- Kiss, J., Korbász, M., & Sass-Kiss, A. (2006). Study of amine composition of botrytized grape berries. *Journal of Agricultural and Food Chemistry*, *54*, 8909–8918.
- Kusano, T., Berberich, T., Tateda, C., & Takahashi, Y. (2008). Polyamine: Essential factors for growth and survival. *Planta*, *228*, 367–381.
- Kusano, T., Yamaguchi, K., Berberich, T., & Takahashi, Y. (2007). Advances in polyamine research in 2007. *Journal of Plant Research*, *120*, 345–350.
- Kuznetsov, V. V., Radyukina, N. L., & Shevyakova, N. I. (2006). Polyamines and stress: Biological role, metabolism, and regulation. *Russian Journal of Plant Physiology*, *53*, 583–604.
- Lee, J., Keller, K. E., Rennaker, C., & Martin, R. R. (2009). Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon *Vitis vinifera* L. cv. Pinot noir: Free amino acids, sugars, and organic acids. *Food Chemistry*, *117*, 99–105.
- Lee, J., & Martin, R. R. (2009). Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon *Vitis vinifera* L. cv. Pinot noir: Phenolics. *Food Chemistry*, *112*, 889–896.
- Marshall, M., Russo, G., Van Etten, J., & Nickerson, K. (1979). Polyamines in dimorphic fungi. *Current Microbiology*, *2*, 187–190.
- Martin, R. R., Eastwell, K. C., Wagner, A., Lampercht, S., & Tzanetakis, I. E. (2005). Survey for viruses of grapevine in Oregon and Washington. *Plant Disease*, *89*, 763–766.
- Moschou, P. N., Paschalidis, K. A., & Roubelakis-Angelakis, K. A. (2008). Plant polyamine catabolism. *Plant Signaling and Behavior*, *3*, 1061–1066.
- Nishimura, K., Shiina, R., Kashiwagi, K., & Igarashi, K. (2006). Decrease in polyamines with aging and their ingestion from food and drink. *Journal of Biochemistry*, *139*, 81–90.
- Reynolds, A. G., Lanterman, W. S., & Wardle, D. A. (1997). Yield and berry composition of five *Vitis* cultivars as affected by *Rupestris stem pitting virus*. *American Journal of Enology and Viticulture*, *48*, 449–458.
- Rhee, H. J., Kim, E., & Lee, J. K. (2007). Physiological polyamines: Simple primordial stress molecules. *Journal of Cellular and Molecular Medicine*, *11*, 685–703.
- Sass-Kiss, A., Szerdahelyi, E., & Hajos, G. (2000). Study of biologically active amines in grapes and wines by HPLC. *Chromatographia*, *51*, S316–S320.
- Silla Santos, M. H. (1996). Biogenic amines: Their importance in foods. *International Journal of Food Microbiology*, *29*, 213–231.
- Walters, D. R. (2000). Polyamines in plant-microbe interactions. *Physiological and Molecular Plant Pathology*, *57*, 137–146.
- Walters, D. (2003a). Resistance to plant pathogens: Possible roles for free polyamines and polyamine catabolism. *New Phytologist*, *159*, 109–115.
- Walters, D. R. (2003b). Polyamines and plant disease. *Phytochemistry*, *64*, 97–107.