Genetic regulation in *Vitis vinifera* by approved basic substances against downy mildew

Llamazares-Miguel, D. [1*], Bodin, E. [2], Laurens, M. [2], Corio-Costet, M.F. [2], Nieto, J. [3]; Fernández-Navarro, J.R [3], Mena-Petite, A. [4], and AM. Diez-Navajas [1]

[1] NEIKER-BRTA, Plant Protection and Production Department, Arkaute, 01192, Spain.
[2] INRAE, UMR Santé et Agroécologie du Vignoble (1065), ISVV, Labex Cote, CS 20032, 33882 Villenave d'Ornon, France.
[3] Biopharma Research, SA (ECONATUR). P. I. Autovía Norte, C/ Montecillo s/n, E-14100, La Carlota, Córdoba (Spain)
[4] Department of Plant Biology and Ecology, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain.

1 Introduction

The oomycete *Plasmopara viticola* (Berk. & Curtis) Berl. & de Toni, causing downy mildew (DM), arrived to Europe at the end of the 19th century. Since then, a wide variety of techniques have been developed for its control. However, most techniques rely on cultural practices, which are inefficient when high pressure infections occur, and on the use of conventional fungicides, which pose a risk to the environment due to their toxicity and can generate fungicide resistance in the pathogen (Corio-Costet, 2012). Copper-based products are often used as an alternative to these chemicals, especially in organic vineyards, but their use is under strict control (European Union Regulation 473/2002), as copper accumulation in soils can lead to phytotoxicity in vines and generate other environmental problems (Garde-Cerdán et al., 2017).

Therefore, environmentally-friendly alternatives to fight DM are of great interest now more than ever, and the use of elicitors could be an efficient option. Luckily, elicitors are contemplated under the European Regulation (EC) No 1107/2009, regarding basic substances (BS). These BSs are defined as "active substances, not predominantly used as plant protection products but which may be of value for plant protection and for which the economic interest in applying for approval may be limited" (European commission, 2009). There are seven basic substances recommended for the control of *Plasmopara viticola* (Table 1).

Basic substance	Function
Chitosan hydrochloride	Elicitor
Equisetum arvense L.	Fungicide
Fructose	Elicitor
Lecithins	Fungicide
Salix cortex	Fungicide
Sucrose	Elicitor
Urtica spp	Fungicide

Table 1. List of basic substances and their function under European Regulation (EC) No 1107/2009 indicated for the control of *P. viticola*.

Regarding their mode of action, *Equisetum arvense* L., lecithins, *Salix* cortex and *Urtica* spp. are allegedly natural fungicides and should act directly against the pathogen. On the other hand, chitosan, fructose and sucrose are described as elicitors: substances capable of stimulating the natural plant defences and to subsequently overcome DM infection. Although these statements are always promising, it would be of great interest to empirically prove them, especially at the molecular level.

Hence, this works aims to shed light on the molecular mechanisms of two BS by analysing their effect at the genetic level in grapevine and against DM infection, with special focus on defence-related genes, to evaluate their putative effect as elicitors. We studied two BS-based products, previously shown to have effect against DM. One commercial product, referred to as LES, contains soy lecithin and the other, referred to as LEQ, contains soy lecithin plus a smaller amount of *Equisetum arvense* L. (EA) extract. Previous research supports that soybean is a source of defence activating damage associated molecular patterns (DAMPs) (Pearce et al., 2010; Yamaguchi et al., 2011) and, therefore, LES and LEQ could act as elicitors in grapevine. Moreover, stimulation of grapevine by DAMPs as cellodextrins and xyloglucans, has been already detected (Aziz et al., 2007; Claverie et al., 2018). To our knowledge, this is the first report of the genetic effect of soy lecithin BS based products in grapevine, with special focus on defence-related genes.

2 Materials and methods

Rooted plantlets of *Vitis vinifera* cv. 'Viura' were planted in 5 litre pots, being 3 plants per pot and considered each pot a biological replicate. Plants were grown with controlled temperature between 15 and 25ºC and 16 hours of light.

For the defence-induction experiment, plants were treated with the two above mentioned commercial products (LES and LEQ) at the recommended dose. As controls sterile distilled water and beta-aminobutyric acid (BABA) were chosen. Briefly, three pots (nine plants) per treatment were sprayed with each of the products and controls. One day post treatment (dpt), the 3th, 4th and 5th completely extended leaves were detached and a part of them was frozen for subsequent analysis. The rest of the tissue was infected with fresh *P. viticola* sporangia at $2{\cdot}10^4$ sp/ml, and incubated in humid chambers. Samples from the infected and mock-inoculated tissue were taken 1, 3 and 7 days post infection (dpi) or 2, 4 and 8 dpt (Figure 1). At 7 dpi (8dpt), a visual evaluation of sporulation intensity was done, calculating the percentage of leaf surface presenting sporulation.

* Corresponding author: dllamazares@neiker.eus

RNA was extracted from the frozen tissue by grinding it with liquid nitrogen and following the procedure indicated in Spectrum™ Plant Total RNA Kit (Sigma-Aldrich). The presence of RNA was quantified with a NanoDrop ND-1000 (Thermo Fisher Scientific) and the integrity of the RNA was checked on a 1.2% agarose gel. The reverse transcription was performed using GoScript™ Reverse Transcription System (Promega) following the manufacturer's instructions.

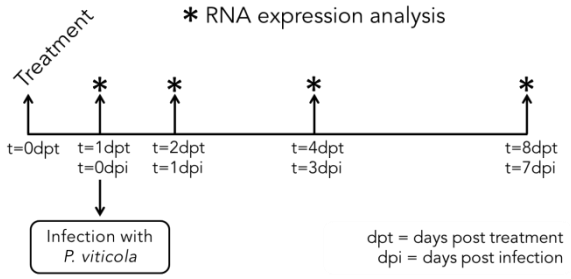

Figure 1: Experimental design of the assay.

The expression of 95 defence-related genes was analysed following the procedure described in previously (Bodin et al., 2020). Briefly, 8 groups of genes were analysed: primary metabolism, wall and cell reinforcement, PR proteins, ethylene and salicylic acid, phenylpropanoid biosynthesis, hormone signalling, redox status and oxylipins.

3 Results and discussion

Lecithins are described as a fungicide in European Regulation (EC) No 1107/2009 but the presence of DAMPs in soybean makes it plausible that lecithins could have a dual effect against DM acting both as a fungicide and by eliciting the host's defence. This dual behaviour is commonly observed in other plant extracts (Harm et al., 2011; Krzyzaniak et al., 2018). Regardless of this, it is worth noting that BABA reduced the sporulation of DM by 67%, LES by 54% and LEQ by 87% in our assay.

Here, we confirmed that LES and LEQ have an eliciting activity in grapevine by analysing the expression of defence-related genes in plants treated with these products, after which a strong modulation was observed for several gene groups. Among all, three groups were the most strongly regulated: pathogenesis-related protein (PR proteins) genes, phenylpropanoid biosynthesis genes and hormone-related genes. The percentage of significantly modulated genes changed from product to product and over time. For instance, all treatments modulated more genes at 1dpt than at any other time point. DM infection was done at 1dpt and, after that, natural induction by the pathogen overlapped with previously induced genes, decreasing the number of significantly modulated genes. Over time, LES and LEQ modulated a higher percentage of genes than BABA at 1dpt, and LEQ at 2dpt, being the percentages similar for all the treatments at 4 and 8dpt. These percentages are represented in Figure 2.

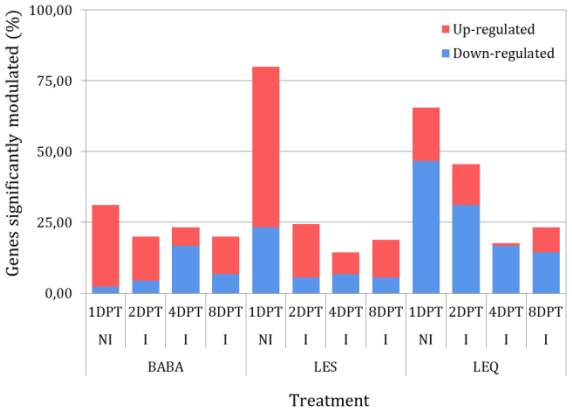

Figure 2: Percentage of significantly modulated genes for each treatment at all timepoints. NI = not infected. I = infected.

The first group, **PR proteins**, was activated in the two treatments and BABA. It was especially evident for LES which significantly over-expressed almost every PR protein analysed at 1dpt. LEQ and BABA, on the other hand, also induced a significant over-expression of some PR proteins. Three PR proteins were activated in all treatments, being PR4bis (chitinase), PR5 (thaumatin-like protein) and PR10 (ribonuclease-like protein). Meanwhile, PR3 (chitinase), PR4 (chitinase) and PR15 (oxalate oxidase) were exclusively activated by lecithin-based products. On the opposite, PR12 (defensin) was significantly repressed by both LES and LEQ.

The activation of these genes by LES and LEQ could be an effective way to fight DM as they code for efficient antimicrobial agents and are associated with systemic acquired resistance SAR (Ali et al., 2018; Enoki & Suzuki, 2016; La Camera et al., 2004). These proteins display variable functions and diverse target sites as fungal cell wall polymers (chitin and β-1,3-glucan), plasma membrane or even RNA, and are activated during pathogen infection leading to fungal pathogen resistance (Aziz et al., 2004; Giannakis et al., 1998). Logically, it has been shown that these proteins may be elicited by DAMPs as in *Arabidopsis thaliana* (Zhang & Mou, 2009) or *Phaseolus lunatus* (Heil et al., 2012), or even in grapevine by cellodextrins (Aziz et al., 2007). Thus, this activation prior to the infection might explain the reduced sporulation observed 7 dpi. After 4dpt, most PR proteins were down-regulated or unaffected, probably due to the reprogramming of the gene expression by the pathogen.

The other group deeply modulated by these products were the genes involved in the **phenylpropanoid biosynthesis**, which is another important pathway implicated in many plant-pathogen interactions. This pathway takes phenylalanine amino acid as a starting point. The enzymes involved in the phenylalanine biosynthesis, chorismate mutase (CHORM) and chorismate synthase (CHORS) were significantly overexpressed at some point for both BABA and LES, but not for LEQ. Hence, an increase of the phenylalanine concentration would be expected in BABA

and LES-treated plants and a subsequent increase of the phenylpropanoid pathway.

Logically, the first enzymes of the pathway, phenylalanine ammonia-lyase (PAL) and stilbene synthase (STS), were significantly overexpressed 1 dpt. These genes open the gate to flavonoid and stilbenoid as resveratrol-derived molecules (stilbenes), some of them related to biotic stresses. Previous research has demonstrated that the induction of these two genes is necessary for the synthesis of stilbenes, which have toxic activity against DM and accumulate in the infection site (Pezet et al., 2004). Moreover, the production of stilbenes has been related to different resistance levels to DM (Alonso-Villaverde et al., 2011) and stilbenic extractions from grapevine are toxic to DM zoospores (Gabaston et al., 2017). The activation of PAL and STS disappeared after DM infection and was even repressed at 4dpt by BABA. Well known elicitors as BTH, cyclodextrins and methyl jasmonate activate these two genes (Almagro et al., 2014; Burdziej et al., 2021; Dufour et al., 2016).

Inside the phenylpropanoid pathway, genes in charge of **flavonoid biosynthesis** were especially activated, although only BABA and LES induced a significant overexpression of all the genes involved in the pathway at 1dpt. The expression decreased over time and only dihydroflavonol 4-reductase (DFR) was significantly activated until 4dpt. The over-expression of this pathway could lead to a reduction of the infection by DM, as flavonoids have been long regarded as antifungal agents and have also been proven to fight against DM infection (Agati et al., 2008; Dai et al., 1995; Latouche et al., 2013; Mondolot-Cosson et al., 1997). The decreased expression of the pathway over time was also observed in grapevine for other products, considered biostimulants, where the highest expression was observed at 2 days post treatment (Bodin et al., 2020). Flavonoids are as well activated by BTH (Burdziej et al., 2021).

Hormone-related genes were the last group significantly affected by these products. In the case of salicylic acid, only one gene (WRKY1) of the four analysed was clearly modulated, being significantly overexpressed for all treatments at 1dpt. This transcription factor might be related to the induction of hyper-sensitive response (HR) (Menke et al., 2005) and has demonstrated increased fungal resistance in strawberry (Encinas-Villarejo et al., 2009) and tobacco (Marchive et al., 2007). Moreover, WRKY1 has been correlated with increased DM resistance in grapevine, accompanied by an increase of jasmonic acid (JA) signalling pathway (Marchive et al., 2013).

Interestingly, this correlation between WRKY1 and JA was also observed in this study, especially for LES. In fact, at 1dpt, jasmonic synthetase (JAR1) and, at 2dpt, a closely related homolog (JAR2), were significantly over-expressed. BABA significantly activated JAR2 at 2dpt. After JAR1 and JAR2 activation, an increase of their product, JA-Ile, would be expected, which has been detected upon DM infection in a previous study (Guerreiro et al., 2016). Moreover, this study detected an increase of JA-Ile shortly after the infection and disappeared after one day. This might explain our significant over-expression of JAR1 or JAR2 shortly after the application of the product or the infection, and not later during the process. In the case of LES, the plants already had a significant overexpression of JAR1 at the moment of the infection, so the presence of JA-Ile in that moment could implicate a JA-mediated signalling against DM infection in those plants.

It is worth noting that other JA-related gene, LOX13, an enzyme involved in the production of oxylipins, precursors of JA, was significantly activated at 7dpt for all the treatments, plus at 1dpt for LES. This reinforces the idea of the SA and JA connection in early time points for LES treated plants. The general increase of LOX13 at 7dpt might be explained by the fact that JA is often related to the infection by necrotrophic pathogens (Antico et al., 2012), which may appear after the completion of the cycle by DM which lasts around 7 days.

Finally, genes controlling the homeostasis of **gibberelins** were especially affected by the application of the BS. Two genes were analysed, GA20ox for their biosynthesis and GA2ox for their catabolism. Significant overexpression of both genes was observed for all products at some time point but a clear trend was not detected. Although gibberellins are usually associated with plant growth regulation, recent studies have proposed that they may also participate in innate defence responses (De Bruyne et al., 2014). Therefore, their role in grapevine elicitation and along DM infection should be further studied and considered.

One of the main questions that may arise in this study would be the different behaviour between LES and LEQ, being both produced from soy lecithin. The production procedure might be important but the presence of EA in LEQ can also be determinant. Even if LEQ activates grapevine defences to a lesser extent, it reduced sporulation in this assay more effectively than BABA and LES. We hypothesized that the presence of EA in LEQ could have an antigerminative activity against DM, as previously demonstrated (La Torre et al., 2019), which would cause a smaller amount of zoospores to enter the tissue reducing the final sporulation. Moreover, it has been demonstrated that EA extract is rich in fatty acids, which could act as antifungal agents by entering fungal plasma membrane and destabilizing cell integrity (Langa-Lomba et al., 2021). Therefore, the EA present in LEQ could as well contribute in this manner against DM.

4 Conclusions

We hypothesized that two BS-based products containing soy lecithin could be elicitors of grapevine immunity due to the presence in soy of grapevine-eliciting DAMPs. Here we confirmed that both products modulate grapevine defence responses. However, LES displayed a wider arrange of mechanisms compared to LEQ, including flavonoid biosynthesis and JA signalling. Nonetheless, this modulation changes over time, being more obvious in the first stages and disappearing over time and after DM infection. The activation of PR proteins, phenylpropanoid

biosynthesis and SA and JA signalling before infection might explain the reduced sporulation observed for the analysed treatments but further metabolomic studies would be of great interest to confirm this.

References

1. Agati, G., Zerovic, Z. G., Dalla Marta, A., di Stefano, V., Pinelli, P., Traversi, M. L., & Orlandini, S. (2008). Optically-assessed preformed flavonoids and susceptibility of grapevine to Plasmopara viticola under different light regimes. *Functional Plant Biology*, *35*, 77-84. https://doi.org/10.1071/FP07178
2. Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S. T., Mushtaq, M., Yadav, P., Rawat, S., & Grover, A. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological Research*, *212-213*, 29-37. https://doi.org/10.1016/j.micres.2018.04.008
3. Almagro, L., Carbonell-Bejerano, P., Belchí-Navarro, S., Bru, R., Martínez-Zapater, J. M., Lijavetzky, D., & Pedreño, M. A. (2014). Dissecting the Transcriptional Response to Elicitors in Vitis vinifera Cells. *PLoS ONE*, *9*(10), e109777. https://doi.org/10.1371/journal.pone.0109777
4. Alonso-Villaverde, V., Voinesco, F., Viret, O., Spring, J.-L., & Gindro, K. (2011). The effectiveness of stilbenes in resistant Vitaceae: Ultrastructural and biochemical events during Plasmopara viticola infection process. *Plant Physiology and Biochemistry*, *49*(3), 265-274. https://doi.org/10.1016/j.plaphy.2010.12.010
5. Antico, C. J., Colon, C., Banks, T., & Ramonell, K. M. (2012). Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. *Frontiers in Biology*, *7*(1), 48-56. https://doi.org/10.1007/s11515-011-1171-1
6. Aziz, A., Gauthier, A., Bezier, A., Poinssot, B., Joubert, J.-M., Pugin, A., Heyraud, A., & Baillieul, F. (2007). Elicitor and resistance-inducing activities of -1,4 cellodextrins in grapevine, comparison with -1,3 glucans and -1,4 oligogalacturonides. *Journal of Experimental Botany*, *58*(6), 1463-1472. https://doi.org/10.1093/jxb/erm008
7. Aziz, A., Heyraud, A., & Lambert, B. (2004). Oligogalacturonide signal transduction, induction of defense-related responses and protection of grapevine against Botrytis cinerea. *Planta*, *218*(5), 767-774. https://doi.org/10.1007/s00425-003-1153-x
8. Bodin, E., Bellée, A., Dufour, M.-C., André, O., & Corio-Costet, M.-F. (2020). Grapevine Stimulation: A Multidisciplinary Approach to Investigate the Effects of Biostimulants and a Plant Defense Stimulator. *Journal of Agricultural and Food Chemistry*, *68*(51), 15085-15096. https://doi.org/10.1021/acs.jafc.0c05849
9. Burdziej, A., Bellée, A., Bodin, E., Valls Fonayet, J., Magnin, N., Szakiel, A., Richard, T., Cluzet, S., & Corio-Costet, M.-F. (2021). Three Types of Elicitors Induce Grapevine Resistance against Downy Mildew via Common and Specific Immune Responses. *Journal of Agricultural and Food Chemistry*, *69*(6), 1781-1795. https://doi.org/10.1021/acs.jafc.0c06103
10. Claverie, J., Balacey, S., Lemaître-Guillier, C., Brulé, D., Chiltz, A., Granet, L., Noirot, E., Daire, X., Darblade, B., Héloir, M.-C., & Poinssot, B. (2018). The Cell Wall-Derived Xyloglucan Is a New DAMP Triggering Plant Immunity in Vitis vinifera and Arabidopsis thaliana. *Frontiers in Plant Science*, *9*, 1725. https://doi.org/10.3389/fpls.2018.01725
11. Corio-Costet, M. F. (2012). Fungicide resistance in Plasmopara viticola in France and anti-resistance measures. En T. S. Thind (Ed.), *Fungicide resistance in crop protection: Risk and management* (pp. 157-171). CABI. https://doi.org/10.1079/9781845939052.0157
12. Dai, G. H., Andary, C., Mondolot-Cosson, L., & Boubals, D. (1995). Histochemical studies on the interaction between three species of grapevine, Vitis vinifera, V. rupestris and V. rotundifolia and the downy mildew fungus, Plasmopara viticola. *Physiological and Molecular Plant pathology*, *46*(3), 177-188. https://doi.org/10.1006/pmpp.1995.1014
13. De Bruyne, L., Höfte, M., & De Vleesschauwer, D. (2014). Connecting Growth and Defense: The Emerging Roles of Brassinosteroids and Gibberellins in Plant Innate Immunity. *Molecular Plant*, *7*(6), 943-959. https://doi.org/10.1093/mp/ssu050
14. Dufour, M.-C., Magnin, N., Dumas, B., Vergnes, S., & Corio-Costet, M.-F. (2016). High-throughput gene-expression quantification of grapevine defense responses in the field using microfluidic dynamic arrays. *BMC Genomics*, *17*(1), 957. https://doi.org/10.1186/s12864-016-3304-z
15. Encinas-Villarejo, S., Maldonado, A. M., Amil-Ruiz, F., de los Santos, B., Romero, F., Pliego-Alfaro, F., Muñoz-Blanco, J., & Caballero, J. L. (2009). Evidence for a positive regulatory role of strawberry (Fragaria×ananassa) Fa WRKY1 and Arabidopsis At WRKY75 proteins in resistance. *Journal of Experimental Botany*, *60*(11), 3043-3065. https://doi.org/10.1093/jxb/erp152
16. Enoki, S., & Suzuki, S. (2016). Pathogenesis-Related Proteins in Grape. En A. Morata & I. Loira (Eds.), *Grape and Wine Biotechnology*. InTech. https://doi.org/10.5772/64873
17. European Commission. (2009). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. *Official Journal of the European Union*, *309*(1), 1-50.
18. Gabaston, J., Cantos-Villar, E., Biais, B., Waffo-Teguo, P., Renouf, E., Corio-Costet, M.-F., Richard, T., & Mérillon, J.-M. (2017). Stilbenes from *Vitis vinifera* L. Waste: A Sustainable Tool for Controlling *Plasmopara Viticola*. *Journal of Agricultural and Food Chemistry*, *65*(13), 2711-2718. https://doi.org/10.1021/acs.jafc.7b00241
19. Garde-Cerdán, T., Mancini, V., Carrasco-Quiroz, M., Servili, A., Gutiérrez-Gamboa, G., Foglia, R., Pérez-Álvarez, E. P., & Romanazzi, G. (2017). Chitosan and Laminarin as Alternatives to Copper for *Plasmopara*

viticola Control: Effect on Grape Amino Acid. *Journal of Agricultural and Food Chemistry*, *65*(34), 7379-7386. https://doi.org/10.1021/acs.jafc.7b02352
20. Giannakis, C., Bucheli, C. S., Skene, K. G. M., Robinson, S. P., & Scott, N. S. (1998). Chitinase and β-1,3-glucanase in grapevine leaves: A possible defence against powdery mildew infection. *Australian Journal of Grape and Wine Research*, *4*(1), 14-22. https://doi.org/10.1111/j.1755-0238.1998.tb00130.x
21. Guerreiro, A., Figueiredo, J., Sousa Silva, M., & Figueiredo, A. (2016). Linking Jasmonic Acid to Grapevine Resistance against the Biotrophic Oomycete Plasmopara viticola. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00565
22. Harm, A., Kassemeyer, H.-H., Seibicke, T., & Regner, F. (2011). Evaluation of Chemical and Natural Resistance Inducers against Downy Mildew (Plasmopara viticola) in Grapevine. *American Journal of Enology and Viticulture*, *62*(2), 184-192. https://doi.org/10.5344/ajev.2011.09054
23. Heil, M., Ibarra-Laclette, E., Adame-Álvarez, R. M., Martínez, O., Ramirez-Chávez, E., Molina-Torres, J., & Herrera-Estrella, L. (2012). How Plants Sense Wounds: Damaged-Self Recognition Is Based on Plant-Derived Elicitors and Induces Octadecanoid Signaling. *PLoS ONE*, *7*(2), e30537. https://doi.org/10.1371/journal.pone.0030537
24. Krzyzaniak, Y., Trouvelot, S., Negrel, J., Cluzet, S., Valls, J., Richard, T., Bougaud, A., Jacquens, L., Klinguer, A., Chiltz, A., Adrian, M., & Héloir, M.-C. (2018). A Plant Extract Acts Both as a Resistance Inducer and an Oomycide Against Grapevine Downy Mildew. *Frontiers in Plant Science*, *9*, 1085. https://doi.org/10.3389/fpls.2018.01085
25. La Camera, S., Gouzerh, G., Dhondt, S., Hoffmann, L., Fritig, B., Legrand, M., & Heitz, T. (2004). Metabolic reprogramming in plant innate immunity: The contributions of phenylpropanoid and oxylipin pathways. *Immunological Reviews*, *198*(1), 267-284. https://doi.org/10.1111/j.0105-2896.2004.0129.x
26. La Torre, A., Righi, L., Iovino, V., & Battaglia, V. (2019). Evaluation of copper alternative products to control grape downy mildew in organic farming. *Journal of Plant Pathology*, *101*(4), 1005-1012. https://doi.org/10.1007/s42161-019-00330-6
27. Langa-Lomba, N., Buzón-Durán, L., Martín-Ramos, P., Casanova-Gascón, J., Martín-Gil, J., Sánchez-Hernández, E., & González-García, V. (2021). Assessment of Conjugate Complexes of Chitosan and Urtica dioica or Equisetum arvense Extracts for the Control of Grapevine Trunk Pathogens. *Agronomy*, *11*(5), 976. https://doi.org/10.3390/agronomy11050976
28. Latouche, G., Bellow, S., Poutaraud, A., Meyer, S., & Cerovic, Z. G. (2013). Influence of constitutive phenolic compounds on the response of grapevine (Vitis vinifera L.) leaves to infection by Plasmopara viticola. *Planta*, *237*(1), 351-361. https://doi.org/10.1007/s00425-012-1776-x
29. Marchive, C., Léon, C., Kappel, C., Coutos-Thévenot, P., Corio-Costet, M.-F., Delrot, S., & Lauvergeat, V. (2013). Over-Expression of VvWRKY1 in Grapevines Induces Expression of Jasmonic Acid Pathway-Related Genes and Confers Higher Tolerance to the Downy Mildew. *PLoS ONE*, *8*(1), e54185. https://doi.org/10.1371/journal.pone.0054185
30. Marchive, C., Mzid, R., Deluc, L., Barrieu, F., Pirrello, J., Gauthier, A., Corio-Costet, M.-F., Regad, F., Cailleteau, B., Hamdi, S., & Lauvergeat, V. (2007). Isolation and characterization of a Vitis vinifera transcription factor, VvWRKY1, and its effect on responses to fungal pathogens in transgenic tobacco plants. *Journal of Experimental Botany*, *58*(8), 1999-2010. https://doi.org/10.1093/jxb/erm062
31. Menke, F. L. H., Kang, H.-G., Chen, Z., Park, J. M., Kumar, D., & Klessig, D. F. (2005). Tobacco Transcription Factor WRKY1 Is Phosphorylated by the MAP Kinase SIPK and Mediates HR-Like Cell Death in Tobacco. *Molecular Plant-Microbe Interactions®*, *18*(10), 1027-1034. https://doi.org/10.1094/MPMI-18-1027
32. Mondolot-Cosson, L., Andary, C., Dai, G.-H., & Roussel, J.-L. (1997). Histolocalisation de substances phénoliques intervenant lors d'interactions plante-pathogène chez le tournesol et la vigne. *Acta Botanica Gallica*, *144*(3), 353-362. https://doi.org/10.1080/12538078.1997.10515380
33. Pearce, G., Yamaguchi, Y., Barona, G., & Ryan, C. A. (2010). A subtilisin-like protein from soybean contains an embedded, cryptic signal that activates defense-related genes. *Proceedings of the National Academy of Sciences*, *107*(33), 14921-14925. https://doi.org/10.1073/pnas.1007568107
34. Pezet, R., Gindro, K., Viret, O., & Richter, H. (2004). Effects of resveratrol, viniferins and pterostilbene on Plasmopara viticola zoospore mobility and disease development. *Vitis*, *43*(2), 145-148.
35. Yamaguchi, Y., Barona, G., Ryan, C. A., & Pearce, G. (2011). GmPep914, an Eight-Amino Acid Peptide Isolated from Soybean Leaves, Activates Defense-Related Genes. *Plant Physiology*, *156*(2), 932-942. https://doi.org/10.1104/pp.111.173096
36. Zhang, X., & Mou, Z. (2009). Extracellular pyridine nucleotides induce *PR* gene expression and disease resistance in Arabidopsis. *The Plant Journal*, *57*(2), 302-312. https://doi.org/10.1111/j.1365-313X.2008.03687.x