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Organic vs. Integrated-Production Agriculture Farming: Which Grapevine Stress-Responsive Genes Are Affected by the Application of Resistance Inducers and Elicitors?

Tomás Monteiro [1,*], Mariana Patanita [1], Maria do Rosário Félix [2], André Albuquerque [1], Joana A. Ribeiro [1], Filipa Santos [2], Margarida Basaloco [3], Augusto Maria da Rosa [4] and Maria Doroteia Campos [1,*]

[1] MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; mpatanita@uevora.pt (M.P.); andrealb@uevora.pt (A.A.); joanaar@uevora.pt (J.A.R.)

[2] MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; mrff@uevora.pt (M.d.R.F.); fs@uevora.pt (F.S.)

[3] InovPlantProtect Collaborative Laboratory, Estrada de Gil Vaz, Ap. 72, 7350-999 Elvas, Portugal; margaridabasaloco1@gmail.com

[4] Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; m53319@alunos.uevora.pt

* Correspondence: tomas.monteiro@uevora.pt (T.M.); mdcc@uevora.pt (M.D.C.)

Abstract: With the rising prominence of organic farming systems in European Union countries, motivated by agricultural policies, there is pressure for effective disease management strategies. To address this challenge, the use of plant resistance inducers (PRIs) and elicitors has emerged as a promising approach. In this study, we compared the impact of integrated production with organic agriculture farming practices, specifically applying PRIs and elicitors in the latter, on the expression levels of stress-responsive genes in two grapevine cultivars, 'Alicante Bouschet' and 'Trincadeira'. Our findings revealed that the organic farming system led to upregulation of eight of the 12 studied genes in at least one cultivar, indicating a significant influence of production mode. The upregulated genes were associated with plant stress-responsive genes (*PR1*, *PR2*, *PR4*, and *TLP9*), sugar metabolism (*HT5*), phenylpropanoids (*STS1*), enzymes related to jasmonic acid synthesis and response to biotic stresses, respectively (*LOX*, *PER42*). Also, the 'Alicante Bouschet' cultivar consistently displayed significantly higher levels of transcript accumulation on most of the stress-related genes compared to the 'Trincadeira' cultivar in both production modes. Our study provides valuable insights into the effectiveness of PRIs and elicitors in increasing plant expression levels of stress-responsive genes, leading to greater resilience to pathogen attacks and emphasizing their position in organic agriculture.

Keywords: Alicante Bouschet; Trincadeira; farming systems; elicitors; plant resistance inducers

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1. Introduction

In recent years, the European Union (EU) has intensified its commitment to encourage sustainable farming practices, mostly through the Common Agriculture Policy (CAP). Included in the CAP 2023-27 objectives is an action plan aligned with the Green Deal's Farm to Fork strategy, which aims to have at least 25% of all EU's agricultural land under an organic farming system until 2030 [1,2]. This commitment is in line with a larger global trend towards more socially and environmentally conscious farming methods [3].

Grapevine (*Vitis vinifera*), one of the most important perennial crops worldwide, with a global surface area of around 7.28 million hectares in 2022 [4], is inevitably included in the EU's sustainability objectives, with the goal of coordinating agricultural methods with environmental conservation and socioeconomic development. By 2019, 450,000 ha

of vineyards were already dedicated to organic farming practices [5]. In Portugal, the vineyard industry holds a significant position, covering a total of 175,791 ha. Moreover, Portugal is a prominent wine exporter, having produced a total of 6.8 million hL of wine in 2022, making it the fifth biggest wine producer in Europe [6–8].

Despite its economic and cultural importance, grapevine faces numerous threats that induce plant stress, such as water and nutrient deficiencies, temperature fluctuations, soil salinity, pests, and diseases. Addressing these challenges requires innovative approaches to farming, especially in regard to the organic production systems, which face several restrictions on the use of phytopharmaceutical products that are not applied to integrated production (IP) systems. This latter system makes use of integrated pest management strategies that are compatible with agricultural productivity, environmental preservation, utilization of natural resources, and production processes, in addition to farming practices that guarantee sustainable agriculture while maintaining higher yields. It also allows for the use of biological and chemical control methods [9]. Organic farming is a system that aims to eliminate the use of synthetic inputs including synthetic fertilizers and agrochemicals, genetically engineered seeds, and breeds, regardless of potentially lowering yields. These are replaced with site-specific management approaches that preserve and improve long-term soil fertility while also preventing pests and diseases [10].

One of the biggest challenges of organic farming is finding alternatives to synthetic chemicals, which is why substances like plant resistance inducers (PRIs) and elicitors gain relevance. When applied, they aim to improve the defensive plant mechanisms against pathogens, minimizing their potential damage and subsequential production loss. While PRIs improve the plant's readiness to protect itself against potential threats, elicitors directly induce immune responses that activate plant defensive systems; they are perceived by the plant as a signal to express resistance, locally or systemically [11]. However, induced resistance still fails to reach the level of effectiveness provided by agrochemicals [12].

Organic farming employs copper and sulfur-based products on vineyards, primarily to combat diseases such as downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and gray mold (*Botrytis cinerea*) [12]. While effective against oomycetes and fungi [13], the overuse of copper has led to restrictions mandated by the EU to a maximum annual application rate not exceeding 4 kg/ha [14], as it poses risks including soil contamination [15,16] and negative impacts on human health and wildlife [17,18]. Consequently, urgent measures are needed to reduce reliance on copper-based products. Substitutes like PRIs and elicitors can serve as complementary products in this regard. Among the PRIs used in vineyards, cerevisane, a yeast-derived product, and chitosan, a biopolymer obtained from the exoskeletons of different marine organisms, are highlighted [19,20]. Cerevisane has shown effective results in controlling downy mildew by upregulating key defense genes in grapevines, such as one's coding for enzymes related to hormone metabolism and pathogenesis-related proteins. However, it has negative effects on certain plant growth and development processes [21]. Chitosan has demonstrated potential in controlling powdery mildew [22] and gray mold, and it can increase the activity of phenylalanine ammonia-lyase (*PAL*), a key enzyme involved in plant defense mechanisms [23]. Recent reports also suggest that chitosan may be effective in managing trunk diseases [24]. Plant extracts, such as those from *Mimosa tenuiflora* and *Quercus robur*, have demonstrated the ability to mitigate the severity of various pathogenic fungi through its antimicrobial compounds. These extracts exhibit a comparable efficacy to PRIs, bolstering multiple processes involved in the plant's innate defense mechanisms [25].

Although new developments in molecular technologies have facilitated the identification of different resistance genes, our understanding of the complex molecular mechanisms underlying grapevine-pathogen interactions is still poorly understood [26], especially in how the products applied in the organic farming system interfere to plant molecular response to biotic stress and in conferring resilience to pathogen attack.

This study aims to understand the impact of organic and IP farming systems on the expression of stress-related genes in the cultivars 'Alicante Bouschet' and 'Trincadeira'. The

selected genes are involved in sugar metabolism; pathogenesis-related, enzyme activity related to jasmonic acid synthesis; the phenylpropanoids pathway; thermotolerance; plant growth; and the response to biotic and abiotic stresses. By doing so, it facilitates the identification of genes that respond to the application of PRIs and elicitors and, thus, are responsible for plants resilience against common diseases affecting vineyards and contribute significantly to sustainable plant-disease management.

2. Materials and Methods

2.1. Study Site and Plant Materials

This study was conducted at two distinct water-fed vineyards in the Alentejo region (southern Portugal). The first vineyard, Cartuxa—Quinta de Valbom—Fundação Eugénio de Almeida, follows organic farming practices (38°58′45″ N, −7°91′92″ W). The second vineyard, Monte de Pinheiros—Adega Cartuxa—Fundação Eugénio de Almeida, operates under IP methods (38°54′93′ N, −7°87′49″ W). The cultivars selected for the present study were 'Alicante Bouschet' and 'Trincadeira', which are present in both vineyards. 'Alicante Bouschet' is known to be susceptible to downy mildew and trunk diseases and to the pest's green leafhopper and mites [27]. 'Trincadeira' is susceptible to grey mold and powdery mildew [28].

2.2. Application of Plant Resistant Inducers

Several products have been used in the organic vineyard to enhance plant resistance, including Idaicobre (IdaiNature, Valencia, Spain) (copper 6.0%), Mimetic (IdaiNature, Valencia, Spain) (manganese 1%, zinc 1%), Baslact Plus (Hubel verde, Olhão, Portugal) and Carbobasic (IdaiNature, Valencia, Spain) (sodium hydrogen carbonate 99%). These products were combined with copper and sulphur, both permitted in organic farming practices.

The 2023 technical itinerary for the PRIs application consisted of three treatments of Idaicobre, Mimetic, and Carbobasic, each. The last treatment was based on Idaicobre, Mimetic and Carbobasic, one month prior the collection of the samples. The treatments were spaced 10 days apart. Each treatment involved the application of 1.5 L/ha of both Idaicobre and Mimetic and 2 kg/ha of Carbobasic. In 2022, similar products were used with the addition of Baslact Plus.

Idaicobre, a copper-based fertilizer, aims to activate key plant enzymes [29]. Mimetic is also a fertilizer composed of botanical extracts from *Mimosa tenuiflora* and *Quercus robur* and stimulates the plants defensive system [30]. Baslact Plus, containing whey, chitosan hydrochloride, and *Equisetum arvense*, offers preventive and curative protection against fungi like powdery mildew and downy mildew [31]. Carbobasic, a sodium hydrogen carbonate, acts as a natural fungicide primarily targeting powdery mildew [32].

2.3. Sampling Collection

Samples were gathered on the morning of 10 August 2023, immediately before the grapes harvest. The samples were collected in late summer because the grapevines had experienced some stress, increasing the likelihood of overexpression of stress-related genes. Maximum and minimum temperatures in the region ranged from 29 °C to 42 °C and 10 °C to 20 °C, respectively, during July and August. There was no registered precipitation during July and August (Arquivo meteorológico Évora—meteoblue, accessed on 6 February 2024).

In both vineyards, five random plants were selected from each cultivar (biological replicates) and leaf samples were collected, resulting in a total of 20 samples. Each sample (corresponding to a pool of three to four leaves), weighting approximately 25 g each, was immediately placed in a 50 mL tube placed in a styrofoam box with liquid nitrogen to minimize RNA degradation. Subsequently, the samples were ground to powder using sterile mortars and pestles with the assistance of liquid nitrogen. The resulting plant material was transferred to 2 mL collection tubes and stored at −80 °C, until further use.

The vines from both cultivars and production modes showed no visible signs of any disease so they were considered as asymptomatic.

2.4. RNA Extraction and Complementary DNA Synthesis (cDNA)

RNA was extracted with a NZY Total RNA Isolation kit following manufacturer's instructions (NZYTech, Lisbon, Portugal). The RNA quantification and the evaluation of its purity was performed by a Quawell Q9000 micro spectrophotometer (Quawell Technology, Beijing, China). To improve overall quality, all samples were also subjected to a purification step using the OneStep™ PCR Inhibitor Removal kit (Zymo Research, Irvine, CA, USA).

Maxima® First Strand cDNA Synthesis kit (Thermo Scientific, Waltham, MA, USA) was used to reverse transcribe the total RNA (500 ng) following manufacturer instructions.

2.5. qPCR Conditions for Gene Expression Analysis

For normalization, three reference genes were considered: *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), *PEP* (phosphoenolpyruvate carboxylase), and *UBC* (ubiquitin conjugating enzyme). Table 1 displays the amplicon sizes and primer sequences of the reference genes.

Table 1. Reference genes and primers used for qPCR normalization.

Gene	Primer Sequence (5′→3′)	AL (bp)	NCBI Accession ID	E (%)	Ref.
GAPDH	Fw: CCACAGACTTCATCGGTGACA Rv: TTCTCGTTGAGGGCTATTCCA	70	XM_002263109.3	96.3	[33]
PEP	Fw: CCTCCTCCTCCAGATTGC Rv: GGCTTGCTTGATTCCATTATC	198	AF236126.1	95.0	[34]
UBC	Fw: CATAAGGGCTATCAGGAGGAC Rv: TGGCGGTCGGAGTTAGG	161	EE253706	106.6	[34]

E—primer efficiency; AL—amplicon length.

The target genes were chosen based on previous research findings on their involvement in the grapevine response to stress [33–37]. Those genes are *PR1*, *PR2* and *PR4* (pathogenesis-related), *TLP9* (thaumatin-like protein related to *PR5*), *HT5* (hexose transporter), *cwINV* (cell wall invertase), *PAL* (phenylalanine ammonia-lyase), *STS1* (stilbene synthase), *LOX* (lipoxygenase), *PER42* (peroxidase), *HSP101* (heat shock protein), and *MAPKKK17* (mitogen-activated protein kinase kinase kinase) (Table 2). Gene-specific primers of *HSP101* and *MAPKKK17* were designed using Primer3 software version 0.4.0 from the specific sequence of *V. vinifera* deposited in the NCBI GenBank.

qPCR was performed on a LineGene9600Plus (BIOER, Hangzhou, China) using 5 µL of first-strand cDNA (previously diluted 1:10), 9 µL of NZY qPCR Green Master Mix (2x) (Nzytech, Lisbon, Portugal), and 1 µL with a 10 µM concentration of each primer, for a total volume of 18 µL. Threshold cycle (Ct) values were acquired, for each sample, with the following cycling conditions: initial denaturation for 20 s at 95 °C, followed by an amplification program of 40 cycles of 15 s at 95 °C and 20 s at 60 °C. To evaluate PCR specificity, it was added to the program a single cycle at 95 °C for 15 s, 60 °C for 1 min, and a rump-up 0.2 °C/s to 95 °C for 15 °C, to create a dissociation curve. Each sample was tested with three technical replicates and no template controls were added with every run. Efficiencies were calculated through the equation $E = (10^{(-1/\text{slope})}) - 1) \times 100$, as well as slope and linearity (coefficient of determination, R^2), using a 5-point standard curves from a 4-fold dilution series of pooled cDNA (1:10–1:2560).

The statistical tool geNorm [38] was used to assess the expression stability of the reference genes and pick the best gene combination for data normalization. To investigate target gene expression, Ct values were regressed against the log of the produced cDNA standard curve. The value of normalized arbitrary units of the target genes was then determined for each sample using the reference genes normalization parameters.

Table 2. Target genes and respective primers used for qPCR.

Gene	Primer Sequence (5′→3′)	AL (bp)	NCBI Accession ID	E (%)	Ref.
PR1	Fw: GCAACTATATCGGACAACGTCCTT Rv: TCACCATGCTCTAACAGTACCCA	80	XM_002273752	97.4	[35]
PR2	Fw: GCAGTCGGGAACGAAGTGAG Rv: ATGGAGGGTAGGAGTTGCCC	172	NM_001280967.2	116.9	[33]
PR4	Fw: GCCCAGAGCGCCAGCAATGT Rv: CGCCATGCCAAGGGCTTGCT	125	XM_002264684	104.7	[35]
TLP9	Fw: TGCAGCAACCTTCAACATC Rv: GTGGCGGCCTTCACAT	120	XM_002276395.4	110.1	[36]
HT5	Fw: TAGTGATGCGTCCCTCTACTC Rv: CTTCCAGCAAGAGCAATCGAC	113	NM_001281278.1	108.3	[33]
cwINV	Fw: ACGAATCATCTAGTGTGGAGCAC Rv: CTTAAACGATATCTCCACATCTGC	236	NM_001281279.1	92.2	[33]
PAL	Fw: TGCTGACTGGTGAAAAGGTG Rv: CGTTCCAAGCACTGAGACAA	114	XM_003635609.3	107.2	[33]
STS1	Fw: AGGGAAGCAGCATTGAAGGC Rv: CGGGCATTTCTACACCGGAG	97	XM_002263845.4	94.8	[33]
LOX	Fw: TGCTCTACCCCACAAGCGAA Rv: AGCAGTGTGCTCATGATTTTCCAG	95	NM_001281249.1	109.3	[33]
PER42	Fw: CTTGTGAGAGGTATGAAGATG Rv: ACCATAACGCCATTGTAAC	193	XM_002274733.3	95.1	[37]
HSP101	Fw: AATGAGACTCTTGCTGGGGC Rv: CAGCACCGATTATGGCTTGC	130	NM_001280893.1	106.8	This study
MAPKKK17	Fw: ACCTTAGGCTCTGGCTCCTC Rv: CACACCCCTTGTAGCCAACT	169	XM_002269624.3	116.5	This study

E—primer efficiency; AL—amplicon length.

2.6. Statistical Analysis

All target genes underwent a two-way ANOVA analysis using the Statistical Package for the Social Sciences (IBM SPSS 20.0), aimed at assessing significant differences in expression levels across different cultivars and production modes for each gene. Normality of the data was tested using the Shapiro–Wilk test and homogeneity of variances was tested using Levene's test. Normalized arbitrary units were used to evaluate the significant differences. Differences were considered significant when $p < 0.05$, while p-values between 0.05 and 0.10 were considered trends.

3. Results

The estimated geNorm M value for the *GAPDH*, *PEP*, and *UBC* reference genes was 0.600, 0.788, and 0.621, respectively, which is below the maximum value ($M < 1.5$) considered for gene stability. This allowed the use of the three reference genes to normalize the target gene expression.

Statistical analysis, considering a significance level of $p < 0.05$, was conducted for each target gene to allow comparisons of gene expression values obtained through normalized arbitrary units.

The genes were categorized based on their respective roles on the plant: pathogenesis-related genes (*PR1*, *PR2*, *PR4* and *TLP9*) (Figure 1), genes associated with sugar metabolism (*HT5* and *cwINV*) (Figure 2), genes involved in phenylpropanoid pathways (*PAL* and *STS1*) (Figure 3), enzyme-related genes (*LOX* and *PER42*) (Figure 4), and, finally, genes with different functions; one is associated with thermotolerance (*HSP101*) and the other with plant growth and responses to both biotic and abiotic stresses (*MAPKKK17*) (Figure 5).

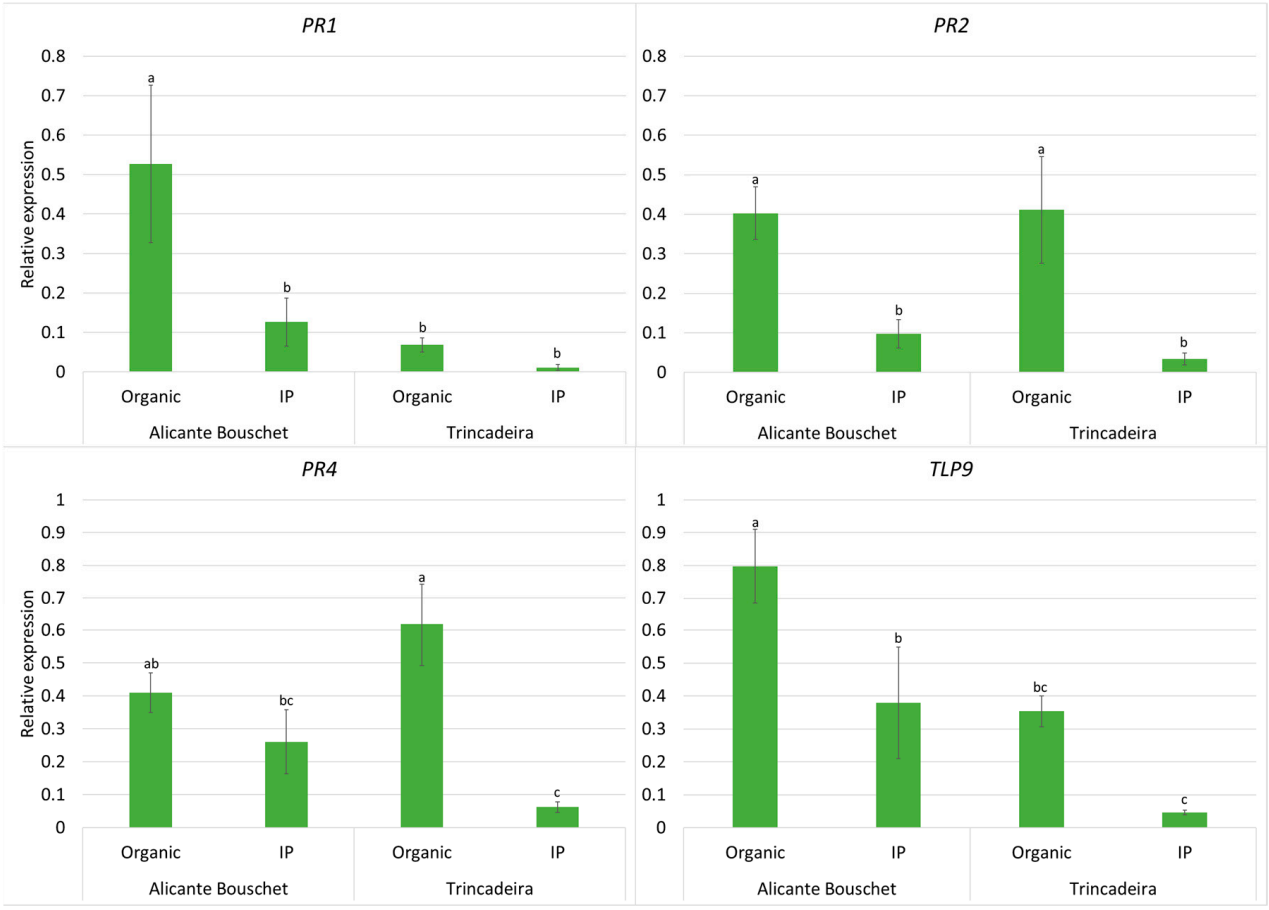

Figure 1. Transcript expression levels of plant stress-responsive genes, *PR1*, *PR2*, *PR4*, and *TLP9*, were analyzed in leaf samples from different cultivars and production modes (organic and integrated production—IP). Error bars represent the standard error of the mean. Significant differences ($p < 0.05$) are denoted by distinct lowercase letters.

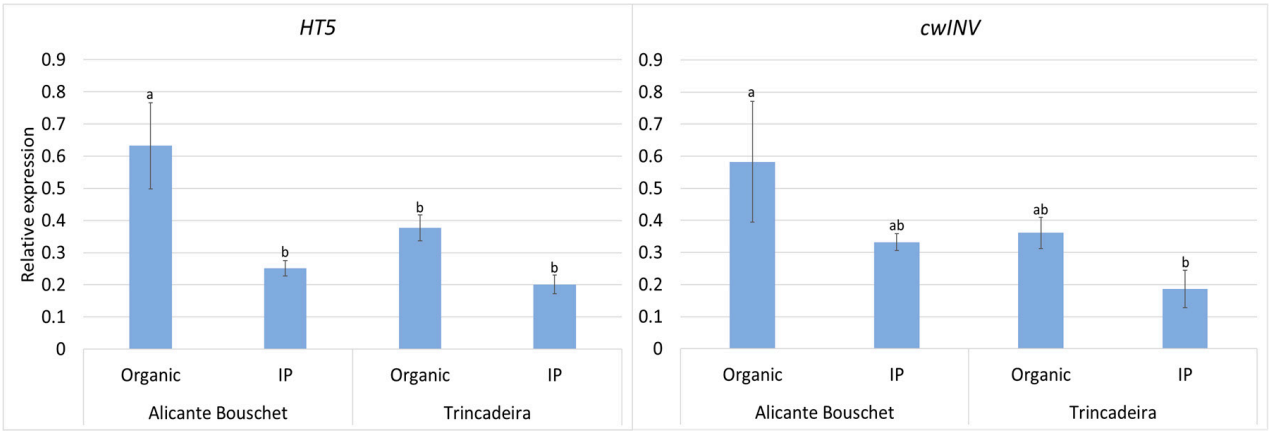

Figure 2. Transcript expression levels of sugar metabolism, *HT5* and *cwINV*, were analyzed in leaf samples from different cultivars and production modes (organic and integrated production—IP). Error bars represent the standard error of the mean. Significant differences ($p < 0.05$) are denoted by distinct lowercase letters.

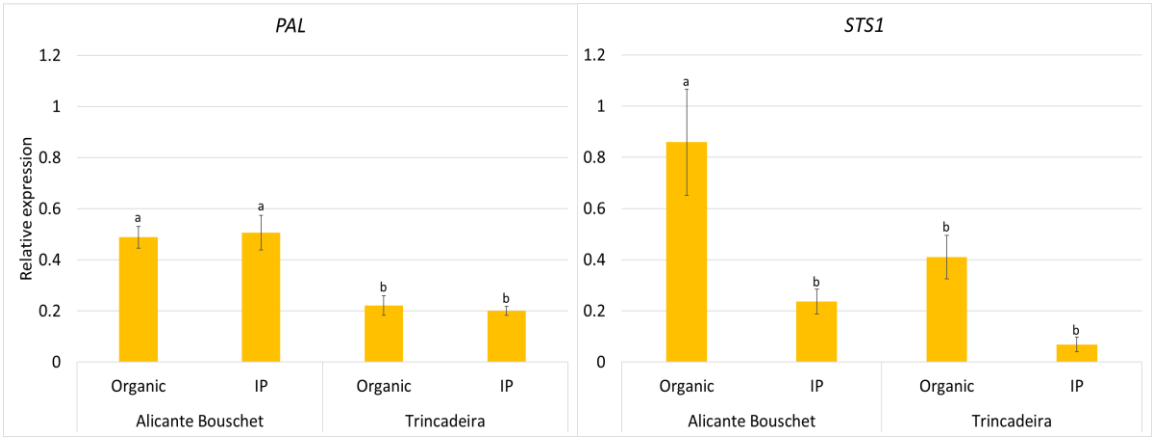

Figure 3. Transcript expression levels of phenylpropanoids, *PAL* and *STS1*, were analyzed in leaf samples from different cultivars and production modes (organic and integrated production—IP). Error bars represent the standard error of the mean. Significant differences ($p < 0.05$) are denoted by distinct lowercase letters.

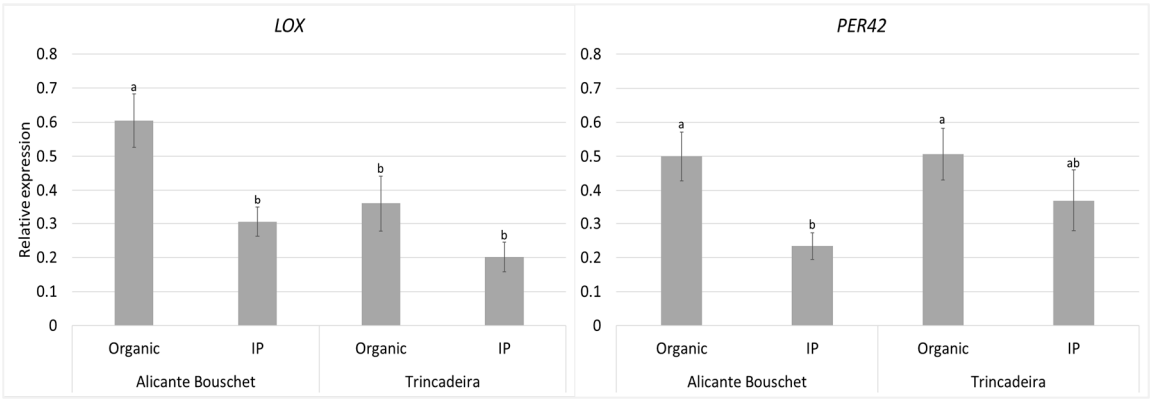

Figure 4. Transcript expression levels of enzymes involved in jasmonic acid synthesis and response to biotic stresses, *LOX* and *PER42*, were analyzed in leaf samples from different cultivars and production modes (organic and integrated production—IP). Error bars represent the standard error of the mean. Significant differences ($p < 0.05$) are denoted by distinct lowercase letters.

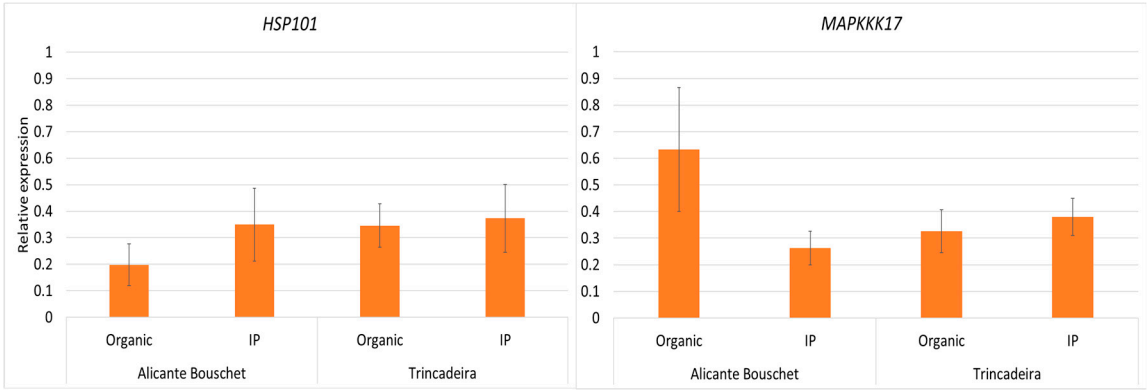

Figure 5. Transcript expression levels of thermotolerance, *HSP101*, and plant growth and response to biotic and abiotic stresses, *MAPKKK17*, were analyzed in leaf samples from different cultivars and production modes (organic and integrated production—IP). Error bars represent the standard error of the mean.

In Figure 1, significant differences were observed in the expression levels of *PR1*, *PR2*, and *TLP9* genes between grapevines grown organically and those under IP for the cultivar 'Alicante Bouschet', with the organic production mode presenting a higher transcript accumulation. 'Trincadeira' also showed significantly higher expression levels of *PR2* and *PR4* genes in organic production mode, and a trend towards a significant difference on the *TLP9* gene ($p = 0.054$). Furthermore, when comparing the differences in gene expression levels between cultivars under the organic production mode, significant differences were observed for *PR1* and *TLP9*, with expression levels consistently higher in the 'Alicante Bouschet' cultivar; in the IP mode, only *TLP9* displayed significant differences, with the 'Alicante Bouschet' cultivar showing higher expression levels.

Regarding the selected genes involved in sugar metabolism, significant differences were identified solely in the *HT5* gene expression between production modes for the 'Alicante Bouschet' cultivar, with the organic production mode exhibiting a consistent upregulation ($p = 0.004$) (Figure 2). Inter-cultivar expression differences were only noted for the *HT5* gene in organic production mode, with the 'Alicante Bouschet' cultivar showing a higher expression level.

Once more, only the *STS1* gene exhibited significant differences between production modes for the 'Alicante Bouschet' cultivar, with the organic production mode demonstrating an upregulation (Figure 3). 'Trincadeira' only showed a tendency towards a significant difference for the *STS1* gene ($p = 0.061$) being the organic production mode upregulated. Regarding inter-cultivar expression level differences, the *PAL* gene showed significant differences for both production modes, while the *STS1* gene only showed differences between organic production modes. This upregulation is always reported in the cultivar 'Alicante Bouschet'.

LOX and *PER42* revealed significant differences between the production modes only for 'Alicante Bouschet' (Figure 4), with the organic production mode showing an upregulation of both genes. Significant differences at the gene expression level between cultivars was seen on *LOX* gene for the organic production mode, with the cultivar 'Alicante Bouschet' cultivar upregulated.

Finally, as observed in Figure 5, no significant differences were noted between production modes and cultivars regarding *HSP101* and *MAPKKK17* transcript accumulation. Only a tendency of upregulation in the organic production mode was observed for *MAPKKK17* in 'Alicante Bouschet' ($p = 0.098$).

4. Discussion

Our study aimed to assess the effects of IP and organic farming methods on stress-related gene expression in 'Trincadeira' and 'Alicante Bouschet', two grapevine cultivars well adapted to the geography and climate of the Alentejo region [39]. We found significant differences in eight out of the 12 genes studied when comparing production modes in at least one cultivar, suggesting a potential correlation between production mode and transcript accumulation. The upregulation of stress-responsive genes in organic farming systems aligns with previous studies attributing this phenomenon to the use of plant PRIs and elicitors [40–63].

The chosen target genes play various roles in plant physiology. *PR* genes have a wide range of properties and functions in plants and can be regulated by G-proteins, ubiquitin, calcium, hormones, and transcription factors [64,65]. They are associated with plant response to pathogens and directly or indirectly induce resistance against microorganisms by antifungal, antiviral, and antibacterial activity, or by causing osmotic rupture of the fungal plasma membrane [40]. *PR* genes selected for this study (*PR1*, *PR2*, *PR4*, and *TLP9*) showed significant differences in expression levels between production modes in at least one of the cultivars. Notably, even in cases where no significant differences were observed, expression values were consistently higher in organic farming compared to IP. This is in line with previous research indicating that products like chitosan, when associated with copper sulfate, upregulate *PR* gene expression in grapevine leaves [41–43],

and lead to accumulation of phytoalexins, which are antimicrobial substances produced by plants as a response to both biotic and abiotic stresses. This activation enhances the plant's protection against common vineyard diseases, such as powdery and downy mildew [41,42]. Similar outcomes have been observed in diverse plant species, including tomato and *Coffea arabica* [44,45], highlighting the importance on understanding the impact of PRIs on plants.

HT5 and *cwINV* genes, also selected for this study, are involved in sugar metabolism in plants [33,46] and are responsible for the entrance of sucrose into the plant's metabolism, as well as the sugar (hexose and sucrose) transport [47]. It was already reported that invertase genes, such as cell wall invertase (*cwINV*) and acidic vacuolar invertase (*GIN2)*, as well as sugar transport transcripts (*HT2* and *HT5*) on grapevine, were strongly induced in grapevine during acibenzolar-S-methyl treatment, which is a benzothiadiazole analogue of salicylic acid and acts as a PRI [48]. Although a different PRI was used in our study, some similarities can be observed, such as the upregulation of *HT5* expression in organically grown vineyards of the 'Alicante Bouschet' cultivar.

PAL and *STS1* genes are linked to phenylpropanoids [49,50], specialized metabolites related to plant defense against biotic and abiotic stresses. Phenylpropanoids can act indirectly by either through signaling molecules or through toxic effects caused by phytoanticipins, that are common compounds present in plant tissues, and phytoalexins, which are bioactive substances that are produced by a plant in response to the detection of pathogens, such as fungi, bacteria, and virus [51]. Although *PAL* did not exhibit significant differences in gene expression levels, we verify that *STS1* showed significant differences between production modes in 'Alicante Bouschet', with a tendency towards significance in 'Trincadeira'. A previous study carried out on a 50-year-old vineyard in Greece reported that chitosan and abscisic acid induce phenylpropanoid gene expression, including *PAL* [44]; an increase of expression and enzyme activity of both *PAL* and *STS1* was verified using a suspension of the epiphytic yeast *Aureobasidium pullulans*, which was potentially used as a PRI [45].

The selected target genes *LOX* and *PER42* are related to enzyme activity [54,55]. While *LOX* genes are oxireductase enzymes found in plants and mediate the manufacture of jasmonic acid, that serves as a stress biomarker for biotic and abiotic stressors [54]; peroxidases are involved in defensive mechanisms against pathogens and in the cell wall lignification, among other critical functions [56]. As shown in Figure 4, *LOX* and *PER42* gene expression is significantly higher in organic farming compared to IP for the 'Alicante Bouschet' cultivar, while no significant differences were observed for the 'Trincadeira' cultivar. An increased expression of *LOX*, *PAL*, and chitinase using chitosan on grapevine leaves has already been reported [57]. Also, transcript accumulation of *LOX9*, which encodes a lipoxygenase involved in the expression of jasmonic acid, *STS1*, resveratrol, and flavonol synthase genes, was positively affected by chitosan application [42]. Additionally, *LOX* genes have been shown to be upregulated in grapevines after biostimulant and laminarin application, indicating their participation in the elicitation of defense mechanisms [58].

Finally, *HSP101*, associated with plants thermotolerance [59], and *MAPKKK17*, connected to plant growth and response to biotic and abiotic stresses [60], were selected for expression analysis. Although our studies did not identify significant differences in transcript accumulation in both production modes and cultivars, mitogen-activated protein kinases (*MAPK*) were already identified as involved in defense mechanisms. The use of β-1,3-glucan laminarin, derived from the brown algae *Laminaria digitata*, has shown to be an effective elicitor of defense responses in grapevine cells, also it reduces the development of *B. cinerea* and *P. viticola* on grapevine plants, as laminarin triggered the activation of two mitogen-activated protein kinases in grapevine cells [61].

The organic farming system included the use of the botanical extracts of *M. tenuiflora* and *Q. robur*. A study on lettuce suggested a positive effect on plant defense against *Sclerotinia* [25]. This extract also demonstrated an enhancement of plant defense mechanisms against fungi like *Botrytis*, *Fusarium*, *Rhizoctonia*, and *Pythium* [62].

Despite the scarcity of literature on this matter, our results uncover a clear tendency for the 'Alicante Bouschet' cultivar to consistently display significantly higher expression

levels of stress-related genes compared to the 'Trincadeira' cultivar. This correlation could be attributed to the knowledge that the 'Alicante Bouschet' cultivar exhibits a greater susceptibility to some diseases, such as GTDs, when compared to 'Trincadeira' [63], potentially resulting in elevated levels of stress-responsive gene expression. It is essential to recognize that the application of PRIs and elicitors may downregulate genes associated with crucial processes like photosynthesis, energy metabolism, and changes in carbohydrate accumulation and distribution [48]. Therefore, further investigation into these potential impacts is needed.

Given that our study was conducted under field conditions of commercial vineyards, it reveals new insights into the impact of PRIs and elicitors on plant gene expression, showcasing potential variations compared to studies conducted under controlled conditions. Moreover, field-based research offers a comprehensive perspective, capturing the complex interactions among plants, pathogens, soil, and environmental factors. These multifaceted dynamics, often overlooked and difficult to replicate in controlled settings, contribute to a more holistic understanding of plant responses to PRIs and elicitors. The products used in the organic vineyard are described to enhance plant resistance, to activate key plant enzymes, to stimulate the plants defensive system, and were combined with copper and sulphur, both permitted in organic farming practices, contributing to the search for environmentally friendly biocontrol agents.

It is critical to recognize the limitations of our research, including variations in field conditions among the different cultivars and production modes, which could potentially affect gene expression levels. Additionally, high-throughput RNA-seq technology will be of great importance as an enabler for the identification of grapevine-specific genes that are most responsive to the application of PRIs and elicitors [66].

Plant immunity inducers in crop disease resistance have unclear modes of action and activation mechanisms. To improve crop disease resistance, it is necessary to further investigate the target, receptor recognition, key activation sites, signal transduction, and the activation mechanism of PRIs and elicitors [67]. Determining the activation mechanism of PRIs and elicitors is crucial for developing plant disease-control strategies that increase plants resilience.

With the urgent need of acquiring new knowledge on new products that fulfil the world requirements of searching of environmentally friendly biocontrol agents, our results already provide valuable insights into the effectiveness of PRIs and elicitors in organic farming, leading, for sure, to greater plant resilience to pathogen attacks and emphasizing the position of these products in organic agronomic practice. Nevertheless, we are aware that our results, although quite relevant, are preliminary.

Future research could expand upon our findings by investigating the long-term effects of organic farming techniques on plant disease susceptibility and also how the use of different concentrations and combinations of substances influences the regulation of genes, combined with evaluation of agronomic traits.

5. Conclusions

In this study, we investigated the expression of stress-responsive genes associated with various plant functions in organic and integrated-production farming systems for two grapevine cultivars, 'Alicante Bouschet' and 'Trincadeira'. Our findings highlight the significant impact of applying specific products, such as plant resistance inducers and elicitors, used in organic farming, leading to the upregulation of multiple genes when compared to integrated production farming. These results emphasize the importance of exploring the effects of these substances on plant gene expression and developing new compounds to improve the productivity of organic farming practices, thereby increasing plant resilience.

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