Omics Technologies in the Study of Fruit-Elicitor Interaction

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Fruit losses and wastage are mainly due to postharvest diseases; their control is reduced with pesticides. The excessive use of synthetic fungicides has caused harmful effects on human health and the environment, so it is therefore necessary to reduce their use. The development of new innocuous strategies has led to the use of compounds of natural or biological origin with the capacity to induce the plant defense system, which improves the fruit's response against future pathogen attacks in addition to reducing the incidence of postharvest diseases. These compounds are known as "elicitors". Although the use of molecular tools such as RT-qPCR or the measurement of the enzymatic activity of molecular markers makes it possible to determine the activation of the plant defense system in response to the application of an elicitor compound, omics technologies such as the transcriptome, proteome, or metabolome have provided new and interesting information that helps to elucidate the molecular aspects involved in the activation of the plant defense system in response to the application of elicitors.

defense system omics technologies elicitors

1. Introduction

During the last few years, there has been a growing interest in the use of "omics" technologies in biological sciences, and their use in research about the activation of defense mechanisms in fruits through the application of an elicitor has not been an exception. Omics technologies (transcriptomic, proteomic, or metabolomic) offer a global analysis that expands our knowledge and understanding of the activated metabolic processes.

Applying elicitors to induce the defense system in fruits has gained considerable popularity as an environmentally friendly alternative to generate resistance without harming the environment or human health. The use of omics technologies has contributed to expanding knowledge and identifying specific metabolic pathways involved in activating the defense system and genes/proteins that are deregulated upon elicitor application. However, many genes/proteins can still be investigated and characterized to understand their involvement in the activation of the defense system. The responses that each elicitor can induce in fruits comprise a complex network of genes that are deregulated to activate the defense system and protect the fruit against future pathogen attacks. The information generated by omics technologies allows knowing, in a global manner, the specific response in plants to each kind of elicitor and, based on that weigh-up, designing sustainable, eco-friendly strategies for disease control in fruits of agro-industrial interest.

2. Omics Technologies in the Induction of the Defense System by Biological Elicitors

Biocontrol agents (bacteria or yeasts) have several mechanisms of action for the control of postharvest diseases, and one of them is the induction of the defense system [1]. In that sense, researchers have contributed to elucidating the mechanisms of resistance induction in fruits by relying on omics sciences as a tool.

In this sense, it has been reported that *Bacillus* is a biocontrol agent that generates secondary metabolites such as cyclic lipopeptides (CLPs) that induce the vegetal defense system; for example, CLPs from *Bacillus subtilis* ABS-S14 effectively controlled green mold disease in mandarins [2]. In addition, proteomic analysis revealed the mechanisms for activating the defense system in mandarin oranges by applying CLPs. The CLP extract increased protein production in the metabolic pathways of Ca^{2+}, ABA, glycolysis, and ROS signaling, which triggered the expression of PAL, GLU, POD, and PR1 genes or proteins, resulting in the activation of the SAR pathway [3].

When evaluating the individual effects of the lipopeptides fengicin, iturin A, and surfactin from *B. subtilis* ABS-14 on mandarin fruits, the results showed that fengicin, Iturin A, and surfactin induced the expression of crucial genes involved in the ET signaling pathway as well as genes encoding CHI proteins that are important for the ISR response in plants [4].

Metabolomic studies revealed that the metabolites induced specifically by *B. subtilis* CLPs were involved in the metabolic pathways of glycine, serine, threonine, tryptophan, and tyrosine metabolism, which increased the production of secondary metabolites such as serotonin and tyramine, leading to the induction of mandarin fruit immunity [5].

The biocontrol capacity of *Bacillus cereus* AR156 on strawberry fruits was also investigated, and transcriptomic profiling showed that *Bacillus* AR156 increased the expression of numerous transcription factors, such as MYB, NAC, WRKY, ERF, bHLH, and bZIP, involved in the induction of the defense system. Transcription factors of the WRKY family are involved in metabolic pathways of plant-pathogen interaction in plants, which trigger the activation of the defense system. In addition, it was reported that a significant effect on the expression of genes related to flavonoid biosynthesis, which, as mentioned above, increased flavonoid concentration effectively controls pathogen development [6].

Similarly, to investigate the mechanisms of *Bacillus siamensis* induction, a comparative analysis of the mango fruit transcriptome during storage was established. Metabolic pathways such as plant-pathogen interaction, plant hormone signal transduction, phenylpropanoid, flavonoid, stilbenoid, diarylheptanoid, and gingerol biosynthesis were the most enriched pathways, indicating that these processes were involved in the response of mango to *B. siamensis*. Some genes (JAZ, BAK1, and PR1) were up-regulated by *B. siamensis* treatment, which triggered the stress response, induced phenol biosynthesis, and enhanced the disease resistance of mango fruit. In addition, some genes (WRKY22, HSP90, CNGCs, SOD, PAL, 4CL, CHS, and HCT) were up-regulated by *B. siamensis* in mango fruit, which stimulates the immune response and resistance to mango fruit disease [7].

On the other hand, the yeast antagonist *Yarrowia lipolytica* elicited disease resistance and proved an effective biocontrol agent against *P. expansum* in apples. The proteome and transcriptome of the yeast-treated apples and the control were analyzed [8]. The authors propose metabolic pathways, such as responses to biotic stress, defense responses, protein synthesis and storage, and signal transduction, pointing out the most dynamic categories in response to biotic stimuli and defense. The analysis of the transcriptome results proved that the induced resistance was mediated by crosstalk between the SA and ET/JA pathways. *Y. lipolytica* treatment activated the ACS1 gene, and EIN2 and 4, which are involved in the ET pathway, also activated genes such as POD, thaumatin-like protein, and CH4, elicited by *Y. lipolytica* in apples [8].

The mechanisms involved in *Pichia membranaefaciens*-induced resistance in peaches were also investigated [9]. Transcriptomic analysis revealed that the MAPK signaling pathway and the regulation of transduction signals by plant hormones such as ET, JA, and AS were activated in peaches by *P. membranaefaciens*. The results showed up-regulation of defense-related genes, including PR genes (PR1, CHI4, and major allergen Pru ar 1) and glutathione S-transferase genes (MKP11.22 and Atlg10370), in addition to genes involved in plant-pathogen interaction pathways (CML48, MUK11. 19, and ROBHA) and genes involved in the synthesis of secondary metabolites (GGPS, PK55, CHS1, CYP52B2, DRF, LDOX, PAL, PNC1, and ROMT) that contributed to improving peach tree resistance potential to diseases. This induction reflected an increase in the concentration of secondary metabolites, such as flavonoids and lignin, which help to increase disease resistance [9].

3. Omics Technologies in the Induction of the Defense System by Natural Chemical Elicitors

As mentioned above, carbohydrate polymers are considered elicitors, and one of the most studied is chitosan [10], as it has proven effective in controlling various postharvest diseases [11]. Chitosan has different mechanisms of action, among which stands out is its ability to induce the defense system; in this sense, transcriptomic analysis in avocado during the development of anthracnose caused by *Colletotrichum gloeosporioides* revealed that the differential genes were located in metabolic processes regulated by chitosan, including those that prevent the propagation of *Colletotrichum* [12]. Differentially expressed genes were significantly increased in different metabolic pathways involved in the defense system, e.g., cellular processes, metabolic processes, response to abiotic stress or biotic stimulus, biological processes, transport, cellular organization and biogenesis, and signal transduction. The authors found that chitosan could induce a priming state in short times after application, which promotes effective fruit resistance against *C. gloeosporioides*, and that those fruit treatments with chitosan up-regulate some genes involved in phenylpropanoid biosynthesis such as 4CL, transcription factors such as WRKY22 and ERF, and genes involved in AFD diene biosynthesis. The results presented in this study showed that chitosan acts as a molecule capable of inducing multiple metabolic responses in avocado fruit that collectively implement a defense system capable of counteracting *C. gloeosporioides* infection [12].

Recently, transcriptomic and metabolomics analyses were used to evaluate the effect of chitosan treatment on the resistance to *B. cinerea* of two grape varieties (“Kyoho” and “Shine Muscat”) that differ in their resistance to this pathogen [13]. The authors propose a model of chitosan regulating the resistance of “Kyoho” and “Shine Muscat”

grapes to *Botritys cinerea* based on data from the transcriptome, metabolome, antioxidant enzyme activity, signal perception, plant hormones, and secondary metabolism. Interestingly, the model of resistance regulation by chitosan involved perception through PAMPs within the metabolic pathways for hormone regulation in plants and secondary metabolism deregulated genes such as PAL, ACS, ACO, EIN3, C4H, and CHS, among others. Secondary metabolites such as cinnamic acid, catechin, resveratrol, quercetin, and terpeptin A were significantly regulated by chitosan. However, chitosan inhibited the secondary metabolism of Kyoho and activated the secondary metabolism of Shine Muscat. With this information, they found that Shine Muscat had more vigorous resistance to *B. cinerea* than Kyoho but, based on the data, established a possible chitosan model regulating disease resistance [13].

Another carbohydrate polymer used is dextran, a complex branched glucan consisting of α-1,6 glycosidic linkages and α-1,3 linkages between glucose monomers. The application of dextran to tomato fruit inhibited gray mold caused by *B cinerea* [14]. Moreover, the transcriptomic analysis revealed that the metabolic pathways of phenylpropanoid biosynthesis, flavonoid biosynthesis, linoleic acid metabolism, stilbenoid, diarylheptanoid, and gingerol biosynthesis, plant-pathogen interaction, and plant hormone signal transduction were significantly up-regulated in response to dextran elicitor treatment. In addition, the expression of Slpa1, Slpr1, Sllox1, and genes encoding TMV resistance protein were increased in dextran-treated fruit; the authors indicate that these results support the previous hypothesis that dextran may be perceived by the β-glucan-like defense system and trigger the response against *B. cinerea* infection [14].

Other authors have evaluated different elicitors in the same fruit. Illumina sequencing technology was used to investigate the transcriptome of citrus treated with SA, *P. membranaefaciens,* and oligochitosan [15]. The results showed that these elicitors caused substantial changes in mRNA relative to control fruits by activating secondary metabolite biosynthesis in citrus responses to SA, *P. membranaefaciens,* and oligochitosan. PAL, C4H, 4CL, and POD expression levels were higher, demonstrating that all three types of elicitors are involved in gene regulation of phenylpropanoid biosynthesis during the induction of fruit resistance [15].

Furthermore, the use of omics tools to evaluate the combination of two elicitors has also been reported, such as *M. guilliermondii* combined with alginate oligosaccharide in pear fruit, which was investigated by transcriptomic analysis [16]. According to the authors, this combination of elicitors increases the expression levels of related genes in the plant-pathogen interaction pathways and the WRKY signaling pathway. WRKY transcription factors are involved in signal transduction that triggers the defense response in plants. In addition, it induces multiple disease resistance genes (RPP13, RPM1, RGA3, RGA4), defense genes (TLP1b, MLO3, and MKS1), and antioxidant stress-related genes (ASO, GSTU17, RVE1, and GLP13) to improve disease resistance and antioxidant stress capacity of pear fruit and promote the synthesis and accumulation of resistant substances in pear fruit by increasing the expression levels of genes involved in the phenylpropanoid and flavonoid biosynthesis pathways (4CL, CAD1, POD1, CHI, CHI3X1, CYP75B1, and ECMP1). In addition, increased the expression of genes related to cell wall integrity (GRP, PRP, GLP13, and CYP51) and the sphingolipid metabolism pathways (AGAL1X1, GBA2X1, ASAH2, and SPHK1X1), which help maintain cell membrane integrity, which prevents the development of

pathogens. Finally, the up-regulation of several genes closely related to plant resistance (PUB23, RGLG1, LACS4, LOX1.5, and PKS5) also plays a crucial role in enhancing pear resistance [16].

4. Omics Technologies in the Induction of the Defense System by Chemical Inorganic Elicitors

Sodium silicate (Si) effectively suppresses pathogen growth and induces postharvest disease resistance in fruits and vegetables [17]. Preventive application of Si to melon fruits activates the defense response against *Trichothecium roseum*. Proteomic changes in melon fruit mitochondria after Si treatment were analyzed using a tandem mass tag (TMT)-based comparative proteomics approach. A total of 24 mitochondrial proteins were significantly altered; a comparison of protein abundance between groups showed that 19 proteins were up-regulated. Five proteins were down-regulated: metal ion binding, transmembrane hydrogen ion transporter activity, ATPase activity, and oxidoreductase activity. The identified proteins are divided into six functional groups: energy metabolism, defense and stress response, oxidation-reduction processes, glycolytic and tricarboxylic acid cycles, and amino acid metabolism (including GABA shunting). The researchers found that the proteins were differentially expressed in muskmelon fruits primed by Si treatment in response to pathogen inoculation, forming a dynamic interaction network during resistance induction. They suggest that mitochondria play an essential role during the priming of resistance against the disease by regulating energy metabolism and ROS production in Si-treated muskmelon fruits [18].

On the other hand, using gases such as carbon dioxide (CO_2) contributes to preserving the shelf life of the fruit as well as inducing defense mechanisms [19]. The cellular response of harvested strawberry fruit subjected to short-term (3 h) exposure to 30% CO_2 was investigated using transcriptomic and metabolomic analyses [20]. The CO_2 treatment reduced fruit softening and deterioration during storage at 10°C for 10 days. According to the authors, CO_2 treatment could improve fruit storage capacity by activating abiotic stress-related genes (e.g., HSPs) and down-regulating genes related to cell wall degrading enzymes (e.g., expansin, pectinesterase, and β-xylosidase). Furthermore, CO_2 treatment induced abiotic stress-related cellular responses in strawberry fruit, stimulating defense mechanisms [20].

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