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Proceeding Paper

Silencing of *FaPG1*, a Fruit Specific Polygalacturonase Gene, Decreased Strawberry Fruit *Fungal Decay during Postharvest* †

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Abstract: Plant health is a major target in breeding programs because crops are under constant biotic stress, and climate change is exacerbating pests and diseases that have negative impacts on agriculture. Obtaining crop varieties armed with better defences is a potential strategy to reduce losses from biotic attacks. Plant cell walls perform crucial roles in many physiological processes, and under biotic stress, play crucial defensive roles as protecting barriers, as well as a source of integrity signalling molecules. In this work, a FaPG1 mutant line with an endopolygalacturonase gene silenced was analysed to determine if the modification of this activity, which potentially alters the release of oligogalacturonides, could have a role in modified plant immunity responses. First, postharvest assays of FaPG1 fruits showed the increased fruit firmness typical of this mutant and confirmed an increased resistance to fungal decay during postharvest, enhancing fruit shelf life in comparison with control fruits. Ongoing works are aiming to characterize the pattern of OGAs production in this transgenic line.

Keywords: food security; plant innate immunity; plant cell wall; resilience; pathogen resistance; damage-associated molecular patterns (DAMPs); *fungal decay*; oligogalacturonic acid (OGA); postharvest shelf life; strawberry; *Fragaria x ananassa*

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1. Introduction

Important economic losses are due to pathogen diseases which compromise crop yields and quality. Global warming and all the associated climate changes are aggravating the negative impacts of pests in agriculture [1]. Plant immunity has evolved into a complex multi-layered system in which the first line of defence is initiated by conserved molecular patterns coming from pathogens, named pathogen-associated molecular patterns or PAMPs, or from their own corrupted cell walls due to pathogen invasion, named damaged-associated molecular patterns or DAMPs. These molecular patterns constitute pattern-triggered immunity (PTI) and launch a wide range of cellular mechanisms to defend plants from pathogen attacks. This first layer of defence, also known as plant innate immunity, suppose a general and non-specific defence response that shares common elements under abiotic and biotic stressors, providing basal resistance not only against many microbial pathogens but also against abiotic stresses [2].

Plant cell walls are carbohydrate-rich extracellular matrices with crucial roles in many physiological processes. Their complexity at the chemical and structural level and their highly dynamic metabolism prevent the complete understanding of how they perform all their functions. Accumulating evidence from cell wall mutants has unveiled several

components and mechanisms of plant innate immunity under biotic stresses, mostly in Arabidopsis [3], but still little is known about species with agronomic interest such as strawberry. Our group has an established strawberry transgenic collection of cell wall mutants. Among them, we selected the PG29 transgenic line, with the FaPG1 gene strongly downregulated (>99% by qPCR), because FaPG1 encodes an endopolygalacturonase enzyme which hydrolyses deesterified domains of polygalacturonic acid, the major pectic component in the primary cell wall of fruits, to produce oligogalacturonides (OGAs). OGAs are small pectin fragments that can activate plant innate immunity, acting as damage-associated molecular patterns (DAMPs) [4,5]. Thus, we hypothesised that a FaPG1 altered expression could potentially modify the pattern of OGAs release in these transgenic fruits with subsequent effects on their susceptibility to a/biotic stresses.

This *FaPG1* transgenic line has been well characterised previously and its main characteristics are less polygalacturonase activity, fruit cell walls enriched in pectins, and pectin fractions with a longer and more branched structure at the nanostructural level, among others. All these features could be related to their better fruit tissue preservation and the firmer fruit phenotype of transgenic in comparison with wild-type fruits at the ripe stage [6–8]. Recently, the transcriptomic analysis of FaPG1 fruits by RNA-seq expression profiles showed that 15 genes were differentially expressed (DEGs) relative to the wild type, including other cell wall-related genes [9]. As previously stated, the *FaPG1* gene encodes for an enzyme with endo-PG activity which potentially could produce oligogalacturonic acid (OGA) upon pectin degradation at the later stages of strawberry ripening. It would be expected that downregulation of FaPG1 results in a modified pattern of OGAs in transgenic fruits that could be related to altered susceptibility to several stresses, either of biotic or abiotic origin, but this assumption requires further investigation.

As a first step, the aim of this work was to inspect whether the downregulation of the *FaPG1* gene influences the fruit shelf life and fungal decay during the postharvest period.

2. Materials and Methods

Postharvest Behaviour of Transgenic FaPG1 and Control Fruits

Ripe fruits from FaPG1 and non-transgenic lines were harvested and stored for 4 days at 4 °C followed by 3 days at room temperature, to reproduce a usual postharvest period. Fruits from transgenic and wild type were weighted at day 0 and day 7 of the postharvest experiments to analyse weight loss. Firmness of transgenic and control fruits was analysed with a TA-XTplus texturometer using a puncture test. The bioyield point (N), defined as the first maximum peak shortly after the end of the elastic zone, related to the start of cell disruption at the local level, was used to compare fruit firmness between lines at day 7. Lastly, quality assessment of fruits was also evaluated at day 7, using the percentage of infected surface per fruit as an indicator of spoiling due to fungal decay. Postharvest assays were performed in triplicate with at least 5 fruits per experiment; data correspond to the mean ± SD. Statistical analysis was carried out by Student's t-test for the analysis of weight loss and fruit firmness, and Wilcoxon rank-sum test for the infected area data, for $\alpha = 0.05$.

3. Results

FaPG1 Transgenic Lines Performance during Postharvest Assays

In general, fruits from the FaPG1 line showed better quality in all the parameters tested at the end of the postharvest storage, including less weight loss and higher bioyield point (Figure 1). Less weight loss is indicative of a major water content in FaPG1 fruits, which showed a more turgid and luminous aspect than wild-type fruits. The increased bioyield point values of transgenic fruits are related to a firmer texture of FaPG1 than control fruits, at the end of the postharvest period.

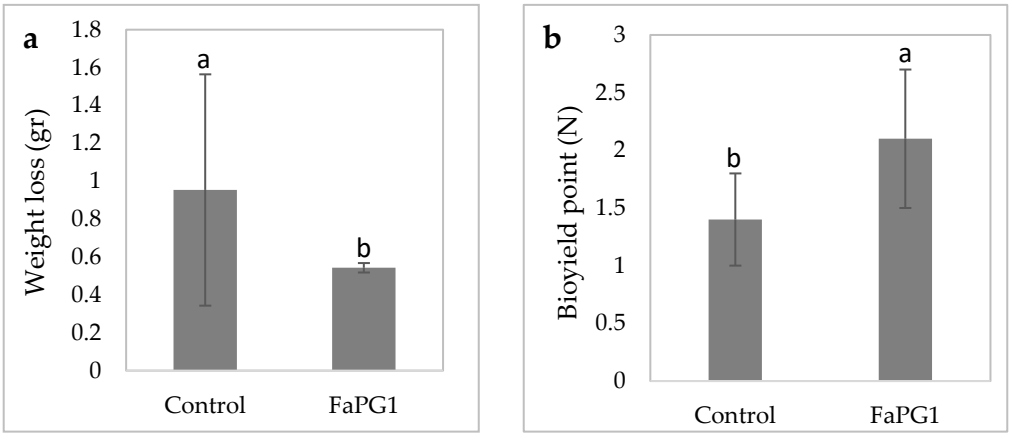

Figure 1. Postharvest behaviour of transgenic *FaPG1* and control fruits. Bar graphs correspond with different quality parameters of postharvest assay at final time point (day 7). (**a**) Fruit weight loss at day 7 of postharvest experiment of FaPG1 and control fruits. (**b**) Bioyield point representative of fruit firmness obtained with a puncture test by texturometer. Postharvest assays were done in triplicate with at least 5 fruits per experiment; data correspond to the mean ± SD. Different letters indicate significant differences by Student's *t*-test at $\alpha = 0.05$.

Additionally, transgenic fruits showed a lower rate of fungal decay after postharvest. The reduced incidence of infection in transgenic fruits is reflected in the lower percentage of infected area per fruit after 7 days of postharvest, which can be appreciated in the images at day 7 (Figure 2).

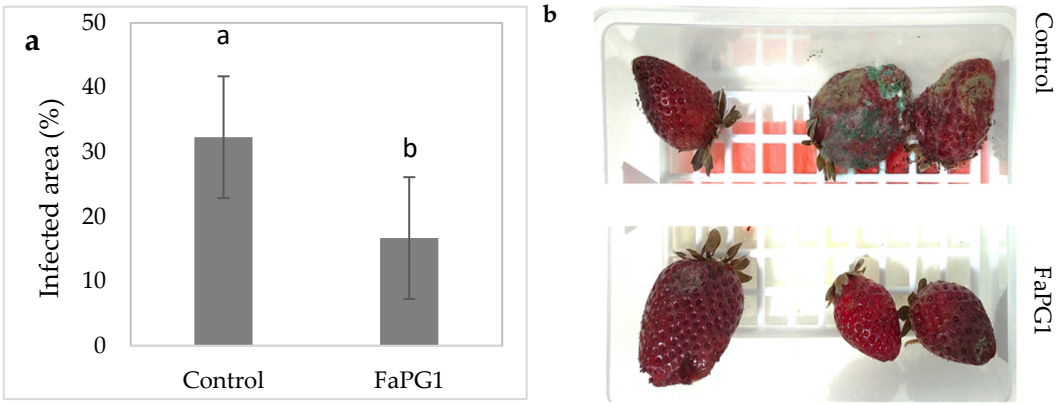

Figure 2. Assessment of fruit spoilage by fungal decay in FaPG1 and control fruits during postharvest assay. (**a**) Percentage of fruit surface with symptoms of fungal decay. (**b**) Representative image of fruits at postharvest day 7. Postharvest assays were done in triplicate with at least 5 fruits per experiment; data correspond to the mean ± SD. Different letters indicate significant differences by Wilcoxon test at $\alpha = 0.05$.

4. Discussion

As expected, transgenic fruits with an endopolygalacturonase gene silenced (FaPG1) resulted in fruits with enhanced quality during postharvest analysis, including a better resilience against pest infections. Transgenic FaPG1 fruits are characterized by having less degraded pectin polymers, which translate into less degraded cell wall matrix and firmer fruit phenotype at the tissue/organ level [6–8]. This aspect would explain the better shelf life of these transgenic fruits due to the enhanced integrity of their cell walls, because they are the outermost barrier which any pathogen must break to successfully invade and establish inside their plant hosts.

Cell wall roles are not only limited to being a physical fence, but they also serve as a source of molecular patterns released during pathogen invasion, implicated in the regulation of the host–pathogen relationship during their first contact. Thus, fragments of their own plant cell walls are released due to the action of pathogen-degrading enzymes, known as DAMPs, acting as signalling molecules able to induce plant innate responses to restrict pathogen infiltration. Among them, OGAs are well-known molecules with DAMP activity [4,5]. Besides the higher integrity of cell walls in FaPG1 ripe fruits, the increased resistance to *fungal decay during postharvest* in FaPG1 fruits could be related to the alteration of quantity and/or structure of OGAs that are released after fungal infection, due to the better preservation of cell wall components in these fruits. This hypothesis needs further work, and more experiments are underway including specific resistance evaluation against fungal pathogens such as *Botrytis cinerea*, which will help to determine if the enhanced biotic resistance of this transgenic strawberry line is due to less-degraded cell walls acting as stronger barriers, and/or if DAMPs release alterations upon infection are also activating plant natural resistance mechanisms. Obtaining crop varieties armed with better defences (stronger physical barriers and/or active chemical signals) is a potential strategy to enhance plant resistance and reduce losses from biotic attacks on strawberry crops. Furthermore, the use of DAMP-based products is actively researched; their use as foliar spray treatments could represent a green alternative solution to fight against pest diseases of crops [3,10,11]. Thus, better knowledge of potential DAMPs is a promising asset to widen the availability of biostimulants as more sustainable substances than chemical pesticides to enhance plant resistance in strawberry crops.

5. Conclusions

In conclusion, the presented results in this work showed an enhanced fruit shelf life together with increased resistance to fungal decay of *FaPG1* fruits in comparison with non-transgenic control fruits. Future analysis will determine if this enhanced resistance is related to plant innate responses induced by DAMPs.

Supplementary Materials: The poster presentation can be downloaded at: https://www.mdpi.com/article/10.3390/IECPS2021-12049/s1.

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