biology and life sciences forum

MDPI

Proceeding Paper

Silencing of *FaPG1*, a Fruit Specific Polygalacturonase Gene, Increased Strawberry Fruit Resistance to *Botrytis cinerea* †

Candelas Paniagua [1], Cristina Sánchez-Raya [1], Rosario Blanco-Portales [2], Jose A. Mercado [1], Elena Palomo-Ríos [1] and Sara Posé [1,*]

[1] Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM-UMA-CSIC). Departamento de Botánica y Fisiología Vegetal, Universidad de Málaga, 29071, Málaga, Spain; candelaspc@uma.es (C.P.); csr196@gmail.com (C.S.-R.); mercado@uma.es (J.A.M.); epalomorios@uma.es (E.P.-R.)

[2] Departamento de Bioquímica y Biología Molecular, Campus de Rabanales, Universidad de Córdoba, 14071, Córdoba, Spain; bb2blpor@uco.es

* Correspondence: sarapose@uma.es

† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: Plant health is a major target in breading programs because crops are under constant biotic stress, and climate change is exacerbating pests and disease that have negative impacts in agriculture. Obtaining crop varieties armed with better defences is a potential strategy to reduce losses from biotic attacks. Plant cell walls perform crucial roles on many physiological processes, and under biotic stress, play crucial defensive roles as protecting barrier, 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 alter the release of oligogalacturonides, could have a role on 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 infections by *Botrytis cinerea*, 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), *Botrytis cinerea*; oligogalacturonic acid (OGA), postharvest shelf life; strawberry; Fragaria x ananassa

Citation: Lastname, F.; Lastname, F.; Lastname, F. Silencing of *FaPG1*, a Fruit Specific Polygalacturonase Gene, Increased Strawberry Fruit Resistance to *Botrytis cinerea*. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Feibo Wu

Published: 7 December 2021

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 negative impacts of pests in agriculture [1]. Plant immunity has evolved a complex multilayered system which 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 the 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 abiotic stresses [2].

Plant cell walls are carbohydrate rich extracellular matrices with crucial roles on many physiological processes. Their complexity at chemical and structural level, and its highly dynamic metabolism prevent the complete understanding of how they perform all

its functions. Accumulating evidences 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 from species with agronomic interest 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 down-regulated (>99% by qPCR), because FaPG1 encodes an endo polygalacturonase enzyme which hydrolyse deesterified domains of polygalacturonic acid, the major pectic component in 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 an 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 their main characteristics are a less polygalacturonase activity, fruit cell walls enriched in pectins, and pectin fractions with longer and more branched structure at 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 ripe stage [6–8]. Recently, the transcriptomic analysis of FaPG1 fruits by RNAseq expression profiles showed that 15 genes were differentially expressed (DEGs) relative to the wild type, including another cell wall related genes [9]. As previously stated, *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 down-regulation of FaPG1 resulted 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 required further investigation.

As a first step, the aim of this work was to inspect whether the downregulation of *FaPG1* gene influences the fruit shelf life and resistance to *Botrytis cinerea*, a necrothropic fungi which is one of the most important disease in strawberry.

2. Materials and Methods

2.1. Postharvest Behaviour of Transgenic FaPG1 and Control Fruits.

Ripe fruits from FaPG1 and non transgenic lines were harvested and stored during 4 days at 4ºC followed by 3 days at room temperature, to reproduce a usual postharvest period. Fruits from transgenic and wild type were weighted at day 4 and day 7 of the postharvest experiments to analyse water loss. Additionally, 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 maximun peak shortly after the end of the elastic zone, related to the start of cell disruption at local level, was used to compare fruit firmness between lines. Lastly, quality assessment of fruits was also evaluated at day 7, using the percentage of infected surface per fruit as a indicator of spoiling due to fungal decay. Posthavest assays were done in triplicate with at least 5 fruits per experiment; data correspond to the mean±SD.

2.2. Bioassays of Fruit Resistance to Botrytis cinerea.

Botrytis cinerea (B05.10 strain) was grown for 10 days on potato dextrose agar at 25º C. Spores were resuspended in saline solution (NaCl 0.9%) and adjusted to 2.10^5 conidia/mL using a hemacytometer. For inoculation, control and transgenic fruits were injected with 7 μl of sterilized water (control) or conidia suspension (*Botrytis*), and later stored in plastic boxes at high humidity during 7 days. All fruits were evaluated at days 3 and 7 dpi for surface symptoms of Botrytis infection.

3. Results

3.1. FaPG1 Transgenic Lines Performed Better during Postharvest Assays.

In general, fruits from 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 aspects than wild type fruits. The increased bioyield point values of transgenic fruits is related with a firmer texture of FaPG1 than control fruits, at the end of the postharvest period.

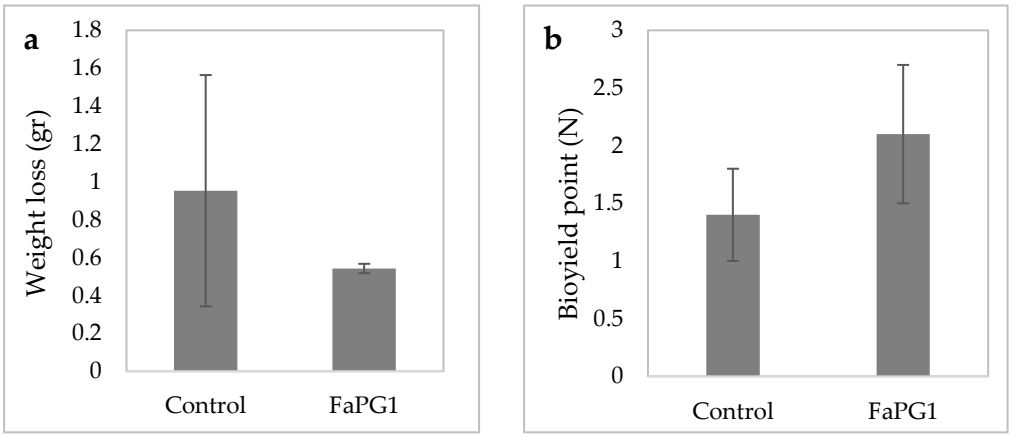

Figure 1. Postharvest behaviour of transgenic *FaPG1* and control fruits. Bar graphs correspond with different quality parameters of postharvest assay at final time point (day 7) **(a)** Fruit weight loss at day 7 of postharvest experiment of FaPG1 and control fruits. **(b)** Bioyield point representative of fruit firmness obtained with a puncture test by texturometer. Posthavest assays were done in triplicate with at least 5 fruits per experiment; data correspond to the mean±SD.

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 pictures at day 7 (Figure 2).

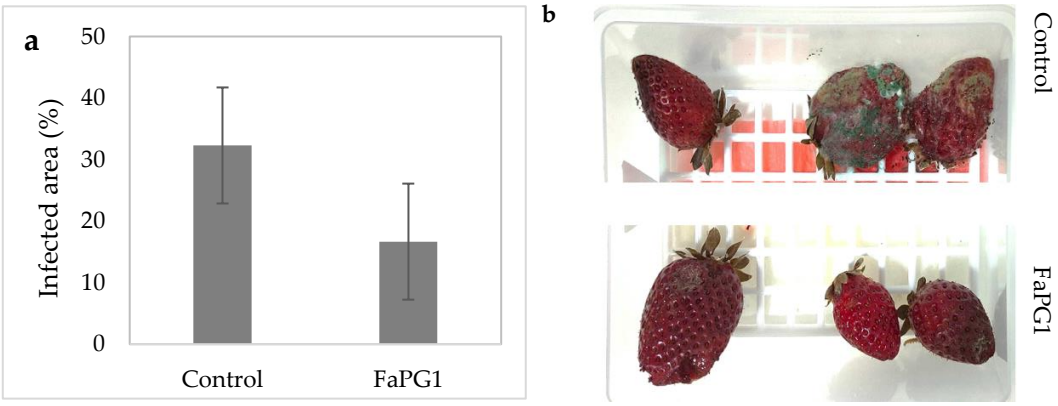

Figure 2. Assessment of fruit spoilage by fungal decay in FaPG1 and control fruits during postharvest assay. (A) Percentage of fruit surface with symptoms of fungal decay. (B) Representative image of fruits at postharvest day 7. Posthavest assays were done in triplicate with at least 5 fruits per experiment; data correspond to the mean±SD.

3.2. FaPG1 Transgenic Fruits Were Less Susceptible to Botrytis cinerea Infection

Transgenic FaPG1 fruits showed a lower incidence of Botrytis infection than control after inoculation with Botrytis conidia (Figure 3). After 3 dpi, the growth of Botrytis mycelia was clearly observed in the surface of all control fruits around the inoculation point;

by contrast, most FaPG1 fruits were symptomless and preserve better their integrity and luminosity.

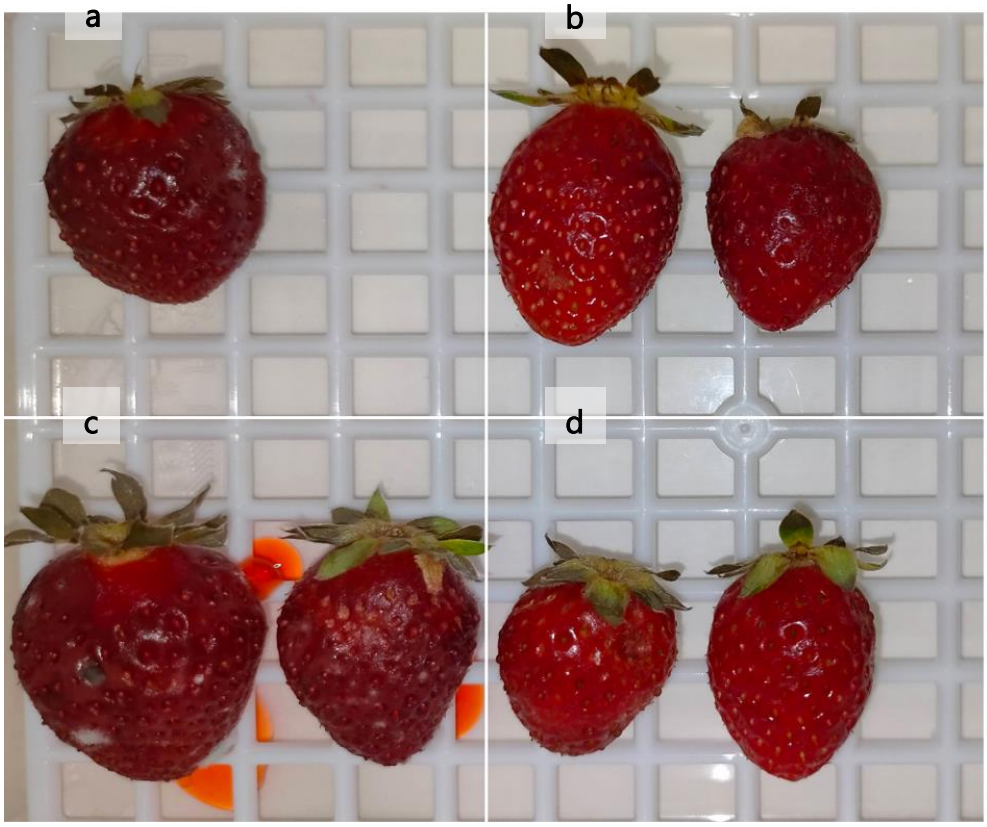

Figure 3. Representative images of *Botrytis cinerea* strawberry assay at day 3 post-injection. *Botrytis cinerea* (B05.10 strain) infection assays were done by injection with 7 μl of sterilized water (mock control) in wild type (a) and FaPG1 (b) fruits, or with 7 μl conidia suspension (Botrytis at 2.10^5 conidia/mL) in wild type (c) and FaPG1 (d) fruits.

4. Discussion

As expected, transgenic fruits with an endo-polygalacturonase gene silenced (FaPG1) resulted in fruits with enhanced quality during postharvest analysis, including a better resilience against pest infections. Transgenic FaPG1 fruits are characterized for having less degraded pectin polymers, which translate in less degraded cell wall matrix and firmer fruit phenotype at tissue/organ level [6–8]. This aspect would explain the better shelf life of this 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 walls roles are not only limited to be a physical fence, and 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 signaling 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 *B. cinerea* 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 next steps will be 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. To achieve

these, cell wall extracts from control and transgenic lines, before and after *Botrytis* inoculation, will be isolated and screened for their DAMP/OGAs composition, to determine whether the differential biotic resistance observed in this transgenic strawberry line is due to a modified DAMPs release. 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 in strawberry crops. Today, 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, a better knowledge of potential DAMPs are promising assets to widen the availability of biostimulants as more sustainable substances than chemical pesticides to enhance plant resistance in strawberry crops.

5. Conclusions

As a conclusion, presented results in this work showed an enhanced fruit shelf life together with an increased resistance to fungal infections by *Botrytis cinerea* 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.

Author Contributions: Conceptualization, S.P.; methodology, C.P. and C.S.R.; formal analysis, C.P. and C.S.R.; resources, S.P., E.P. and J.A.M.; writing—original draft preparation, S.P.; writing—review and editing, S.P. and E.P.; visualization, C.P. and C.S.R.; supervision, J.A.M.; project administration, S.P., and J.A.M.; funding acquisition, S.P., E.P., R.B.P. and J.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the project PID2020-118468RB-C21 funded by Ministerio de Ciencia e Innovación of Spain and FEDER EU funds, and project B1-2020_09 funded by Universidad de Málaga.

Informed Consent Statement: Not applicable

Acknowledgments: We would like to thank Dr. David Vela for kindly provide the *Botrytis cinerea* strain B05.10, as well as technical advice.

Conflicts of Interest: "The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results".

References

1. Newton, A.C.; Johnson, S.; Gregory, P. Implications of climate change for diseases, crop yields and food security. *Euphytica* **2011**, *179*, 3–18, doi:10.1007/s10681-011-0359-4.
2. Nejat, N.; Mantri, N. Plant Immune System: Crosstalk Between Responses to Biotic and Abiotic Stresses the Missing Link in Understanding Plant Defence. *Curr. Issues Mol. Biol.* **2017**, *23*, 1–16, doi:10.21775/cimb.023.001.
3. Bacete, L.; Mélida, H.; Miedes, E.; Molina, A. Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *Plant J.* **2018**, *93*, 614–636, doi:10.1111/tpj.13807.
4. Benedetti, M.; Pontiggia, D.; Raggi, S.; Cheng, Z.; Scaloni, F.; Ferrari, S.; Ausubel, F.M.; Cervone, F.; De Lorenzo, G. Plant immunity triggered by engineered in vivo release of oligogalacturonides, damage-associated molecular patterns. *Proc. Natl. Acad. Sci.* **2015**, *112*, 5533–5538, doi:10.1073/pnas.1504154112.
5. Gallego-Giraldo, L.; Liu, C.; Pose-Albacete, S.; Pattathil, S.; Peralta, A.G.; Young, J.; Westpheling, J.; Hahn, M.G.; Rao, X.; Knox, J.P.; et al. ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE 1 (ADPG1) releases latent defense signals in stems with reduced lignin content. *Proc. Natl. Acad. Sci.* **2020**, *117*, 3281–3290, doi:10.1073/pnas.1914422117.
6. Quesada, M.A.; Portales, R.B.; PoséS.; García-GagoJ.A.; Jiménez-BermúdezS.; Muñoz-SerranoA.; Caballero, J.L.; Pliego-Alfaro, F.; Mercado, J.A.; Blanco, J.M. Antisense Down-Regulation of the FaPG1 Gene Reveals an Unexpected Central Role for Polygalacturonase in Strawberry Fruit Softening. *Plant Physiol.* **2009**, *150*, 1022–1032, doi:10.1104/pp.109.138297.
7. Posé, S.; Paniagua, C.; Cifuentes, M.; Portales, R.B.; Quesada, M.A.; Mercado, J.A. Insights into the effects of polygalacturonase FaPG1 gene silencing on pectin matrix disassembly, enhanced tissue integrity, and firmness in ripe strawberry fruits. *J. Exp. Bot.* **2013**, *64*, 3803–3815, doi:10.1093/jxb/ert210.
8. Posé, S.; Kirby, A.R.; Paniagua, C.; Waldron, K.W.; Morris, V.J.; Quesada, M.A.; Mercado, J.A. The nanostructural characterization of strawberry pectins in pectate lyase or polygalacturonase silenced fruits elucidates their role in softening. *Carbohydr. Polym.* **2015**, *132*, 134–145, doi:10.1016/j.carbpol.2015.06.018.

9. Paniagua, C.; Ric-Varas, P.; A García-Gago, J.; López-Casado, G.; Blanco-Portales, R.; Muñoz-Blanco, J.; Schückel, J.; Knox, J.P.; Matas, A.J.; A Quesada, M.; et al. Elucidating the role of polygalacturonase genes in strawberry fruit softening. *J. Exp. Bot.* **2020**, *71*, 7103–7117, doi:10.1093/jxb/eraa398.
10. Mélida, H.; Bacete, L.; Ruprecht, C.; Rebaque, D.; Del Hierro, I.; López, G.; Brunner, F.; Pfrengle, F.; Molina, A. Arabinoxylan-Oligosaccharides Act as Damage Associated Molecular Patterns in Plants Regulating Disease Resistance. *Front. Plant Sci.* **2020**, *11*, 1210, doi:10.3389/fpls.2020.01210.
11. Molina, A.; Miedes, E.; Bacete, L.; Rodríguez, T.; Mélida, H.; Denancé, N.; Sánchez-Vallet, A.; Rivière, M.-P.; López, G.; Freydier, A.; et al. Arabidopsis cell wall composition determines disease resistance specificity and fitness. *Proc. Natl. Acad. Sci.* **2021**, *118*, doi:10.1073/pnas.2010243118.