Functional characterization of MANNOSE-BINDING LECTIN 1, a G-type lectin gene family member, in response to fungal pathogens of strawberry

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Abstract

The mannos-binding lectin gene MANNOSE-BINDING LECTIN 1 (MBL1) is a member of the G-type lectin family and is involved in defense in strawberry (Fragaria × ananassa). Genome-wide identification of the G-type lectin family was carried out in woodland strawberry, F. vesca, and 133 G-lectin genes were found. Their expression profiles were retrieved from available databases and indicated that many are actively expressed during plant development or interaction with pathogens. We selected MBL1 for further investigation and generated stable transgenic FaMBL1-overexpressing plants of F. ×ananassa to examine the role of this gene in defense. Plants were selected and evaluated for their contents of disease-related phytohormones and their reaction to biotic stresses, and this revealed that jasmonic acid decreased in the overexpressing lines compared with the wild-type (WT). Petioles of the overexpressing lines inoculated with Colletotrichum fioriniae had lower disease incidence than the WT, and leaves of these lines challenged by Botrytis cinerea showed significantly smaller lesion diameters than the WT and higher expression of CLASS II CHITINASE 2-1. Our results indicate that FaMBL1 plays important roles in strawberry response to fungal diseases caused by C. fioriniae and B. cinerea.

Keywords: Anthracnose, fungal disease resistance, B-lectin, Botrytis cinerea, Colletotrichum fioriniae, Fragaria × ananassa, G-type lectin, grey mould, phytohormones, strawberry.

Introduction

Strawberry (Fragaria × ananassa) is an economically important fruit worldwide and is considered a model plant system for the Rosaceae. It is susceptible to a large number of pathogens including the Colletotrichum acutatum species complex (anthracnose) and Botrytis cinerea (grey mould), which cause enormous economic losses (Guidarelli et al., 2011; Petrasch et al., 2019). Although both fungi can infect the fruits at both unripe and ripe stages, the symptoms appear only on red ripe fruits whilst...
on white unripe fruits the pathogens become quiescent. Transcriptome analysis of white and red fruits inoculated with *C. acutatum* have shown that a mannose-binding lectin gene, *FaMBL1* (GenBank accession no. KF962716) is the most up-regulated gene in resistant white fruit (Guidarelli et al., 2011). Transient transformation to silence *FaMBL1* results in white fruit with an increased susceptibility to *C. acutatum* (Guidarelli et al., 2014).

The protein encoded by *FaMBL1* is composed of an N-terminal signal peptide, a *Galanthus nivalis* agglutinin-related lectin (GNA) domain, and a Pan-apple (PAN) domain. The GNA domain is the characteristic domain of G-type lectin, which is an important family of plant lectins that have affinity to mannose or mannose-containing N-glycans (Barre et al., 2001). Due to their ability to recognize and bind mannose, G-type lectins have important functions in plant growth and defense. The roles of G-type lectins in resistance to insects, fungi, and bacteria have been described; for example, the expression of G-type lectin genes in potato (Down et al., 1996), maize (Wang et al., 2005), and wheat (Miao et al., 2011) increases resistance to aphids by inhibiting their development and decreasing fecundity. The pepper G-type lectin genes *CaMBL1* and *CaGLP1* have also been found to be involved in defense against *Xanthomonas campestris pv vesicatoria* and are required for plant cell death and defense signaling (Hwang and Hwang, 2011; Kim et al., 2015). The G-type lectin gene *Pi-d2* from rice cv. Digu provides resistance to *Magnaporthe grisea*. The transfer of *Pi-d2* from the resistant Digu to the susceptible cv. TP309 confers the latter with resistance to *Magnaporthe grisea* (Chen et al., 2006). Interestingly, the difference between the native Pi-d2 protein forms in Digu versus TP309 is only a single amino acid change (Ile441Met) in the transmembrane (TM) domain. This suggests that the TM domain plays an important role in the ligand recognition and signal transduction (Chen et al., 2006). Another G-type lectin, LORE, from Arabidopsis is composed of GNA, S-locus glycoprotein, PAN, transmembrane, and kinase domains and plays a role in plant innate immunity via sensing the lipopolysaccharide (LPS) of *Pseudomonas* and *Xanthomonas* (Ranf et al., 2015). Accordingly, Arabidopsis Col-0 plants pre-treated with *Pseudomonas* LPS are more resistant to subsequent infection with *Pseudomonas*. To determine the role of different domains, Ranf et al. (2015) produced the mutants *lore-1* and *lore-2* by protein truncation after the S-locus glycoprotein domain and substitution of an amino acid (Gly391Glu) in the PAN domain, respectively, and found that LPS-induced resistance was lost in both.

It is well known that the balance of hormonal crosstalk strongly influences the outcome of plant–pathogen interactions (Robert-Seilaniantz et al., 2011), and the contribution of lectins to plant resistance seems to be consistently displayed in a phytohormone-dependent manner (Bonaventure, 2011; Gildroni et al., 2011; Hwang and Hwang, 2011).

Despite the clear importance of G-type lectins in plant defense responses, there have been few studies examining their expression strawberry (Martinez Zamora et al., 2008; Ma et al., 2021). Recently, with the release of the updated genome annotation and comprehensive gene expression atlas of woodland strawberry, *F. vesca* (Li et al., 2019), reliable data have become available for genome-wide analysis of G-type lectin genes in strawberry. In this study, we used this genome annotation together with the available transcriptome data to identify the G-lectin gene family members in this species and to analyse their domain arrangements and expression profiles. The results implied the great potential of many G-lectin members in strawberry defense responses and provided the basis for functional characterization of *FaMBL1* in cultivated strawberry. To gain insights into the effects of this gene on plant defense, we generated and characterized genetically transformed plants overexpressing *FaMBL1*.

### Materials and methods

#### Genome-wide analysis of G-type lectin genes in *F. vesca*

**Identification and domain organization of G-type lectins.** To identify G-type lectin genes, the GNA domain of *FvH4_3g18380* (homolog of *FaMBL1*) was used as the query for a BLASTp search in the Genome Database for Rosaceae (GDR; https://www.rosaceae.org/) (Jung et al., 2019). The *Fragaria vesca* Whole Genome v4.0.a2 database was also used (https://www.rosaceae.org/species/fragaria_vesca/genome_v4.0.a2). Results with E-values <1 x 10^-6 were considered as G-type lectin protein candidates. With the same settings, a second BLASTp was conducted using a number of variable GNA domains from G-lectin proteins found in the first BLASTp search: *FvH4_1g23370, FvH4_2g12390, FvH4_2g14250, FvH4_2g26490, FvH4_2g29050, FvH4_2g33830, FvH4_3g03230, FvH4_3g03301, FvH4_3g03410, FvH4_3g03430, FvH4_3g036140, FvH4_3g15930, FvH4_3g18370, FvH4_3g21270, FvH4_3g3440, FvH4_4g02170, FvH4_5g31680, FvH4_6g00300, FvH4_6g12870, FvH4_6g41406*. The domains of each candidate gene were checked manually using the InterProScan website (https://www.ebi.ac.uk/interpro/search/sequence/) (Quevillon et al., 2005). The transmembrane domain was checked using TMHMM Server v. 2.0 (https://dtu.biolib.com/DeepTMHMM) (Krogh et al., 2001).

**Chromosome location of G-type lectins.** Visualization of the chromosome locations of the G-lectin genes was accomplished using MapGene2Chromosome v2.0 (http://mg2c.iask.in/mg2c_v2.0/) (Chao et al., 2015). The coordinates of the G-lectin genes on the strawberry genome were obtained from the GDR website (*F. vesca* v4.0.a2).

**Expression profiles of G-type lectin genes.** Expression profiles for the *F. vesca* G-lectin genes were retrieved from the supplementary material provided by Li et al. (2019). The expression levels of the genes in different tissues (flowers, fruits at different developmental stages, seedlings, leaves, meristems, and roots) were used to construct a heatmap using the R package, ComplexHeatmap (Gu et al., 2016).

To infer the expression of G-lectin genes upon pathogen infection, we used the transcriptome profiles of *F. ×ananassa* infected by *Botrytis*.
In order to investigate the contents of defense-related phytohormones in the FaMBL1-overexpressing lines and the WT, phytohormones were extracted as described by Glauser et al. (2014) from leaves propagated from stolons. A young and fully expanded leaf was sampled from each of three plants per line and pooled together as one replicate, and a total of three replicates were used for each line. The samples were frozen immediately in liquid nitrogen and ground into fine powder. Jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), salicylic acid (SA), abscisic acid (ABA), and indole-3-acetic acid (IAA) were measured using ultra-high-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), according to the protocol described by Glauser et al. (2014), with 50 ng of tissue powder being used for IAA and 100 ng being used for the others. The hormone concentrations were calculated based on calibration curves. Five calibration points were set at 0.1, 0.5, 2, 20, and 100 ng ml⁻¹, and the solutions of calibration points and leaf samples contained each of the labeled internal standards at concentrations of 10 ng ml⁻¹ for d5-JA, d6-ABA, d6-SA, 13C6-JA-Ile, and 1 μg ml⁻¹ for d5-IAA.

Evaluation of susceptibility of FaMBL1-overexpressing plants to fungal diseases.

The susceptibility of FaMBL1-overexpressing lines to anthracnose was evaluated using Colletotrichum fioriniae, which belongs to the C. acutatum species complexes (Damm et al., 2012), and it had previously been found to be the most aggressive species to strawberry stocked in our lab. It was cultured on potato dextrose agar (PDA, in Petri dishes). After 10 d, conidia were harvested in distilled water and filtered through three layers of medical gauze. Using a hemocytometer the concentration was adjusted to 2 × 10⁶ conidia ml⁻¹ for use as the inoculum. Plants propagated from stolons were used for C. fioriniae inoculation. Five petioles of similar growth stage were used for each overexpressing line and the WT. Leaves were removed, and both ends of the petioles were then embedded in moist tissue paper to reduce desiccation. A tiny wound was made on each petiole using a sterilized needle and a 10-μl droplet of inoculum was placed on top of the wound. All inoculated petioles were put in a large plastic tray with moist tissue paper on the bottom. The tray was kept on a bench in the laboratory at room temperature. The disease incidence was recorded at 6 days post-inoculation (dpi) and calculated as the number of petioles with anthracnose symptoms divided by the total number of petioles treated with C. fioriniae suspension. The disease incidence was determined as the mean value of three repeated trials.

The susceptibility of FaMBL1-overexpressing lines to Botrytis cinerea (isolate B05.10) was determined using conidia harvested from 1-week-old B. cinerea cultured on PDA. To stimulate spore germination, a buffer containing 0.5% KH₂PO₄ and 1% PDA was prepared and the conidia suspension was mixed with buffer at a ratio of 1:1 (v:v) to get 1 × 10⁶ conidia ml⁻¹. The plants used for B. cinerea inoculation were the same as those used for C. fioriniae inoculation, and young, healthy, and fully expanded leaves of similar growth stages were sampled. One leaf (three leaflets) from each of five different plants per line was sampled and surface-sterilized using 1% sodium hypochlorite for 1 min, and then rinsed in sterilized distilled water for 2 min. The leaves were then separated into leaflets and put in Petri dishes (150 × 15 mm) with moist tissue paper on the bottom. Two 7.5-μl droplets of inoculum were placed on the adaxial side of each leaflet, one on each side of the midrib. Distilled water was sprayed into each Petri dishes to keep the humidity high and the dishes were kept on a laboratory bench at room temperature. The lesion diameters on the leaflets were recorded daily from 3–5 dpi. The rate of increase of lesion diameter was used to indicate the rate of disease progression, and was calculated as (diameter₅dpi − diameter₃dpi)/2. The inoculation trial was repeated three times.
Determination of gene expression levels in leaves inoculated with *B. cinerea*.

To investigate the molecular responses of the *FaMBL1*-overexpressing lines upon *B. cinerea* inoculation, we used qPCR to determine the expression levels of pathogenesis-related and hormone-synthesis genes. Six leaves of each transgenic line and the WT were subjected to inoculation with either *B. cinerea* as described above or a mock solution using the same volume of the buffer alone (0.25% KH₂PO₄ and 0.5% PDA). Individual leaflets were inoculated, then at 1 dpi they were pooled back together as leaves and frozen immediately in liquid nitrogen, and stored at –80°C until use. The expression of *FaMBL1*, *FaPGIP* (EU117215.1), *THAU-MATIN-LIKE PROTEIN 1b* (*FaTLP1b*, XM_004287756.2), PHENYLALANINE AMMONIA-LYASE 1 (*FaPAL1*, KX450226.1), CLASS II CHITINASE (*FaChi2-1*, MK301536.1; *FaChi2-2*, MF804503.1), ALLENE OXIDE SYNTHASE 1 (*FaAOS1*, XM_004291875.2), and 1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE (*FaACO*, AY706156.1) was measured by qPCR as described above. The primers used for are listed in Supplementary Table S1.

Statistical analysis.

Student’s *t*-test or one-way ANOVA followed by Fisher’s LSD test were conducted using the OriginPro 2018 statistical software (https://www.originlab.com/2018).

Results

**Genome-wide analysis of G-type lectin genes in *F. vesca***

**Identification and classification.**

A total of 133 proteins with *Galanthus nivalis* agglutinin (GNA)-related lectin domains were found in *F. vesca*. Among these, 102 containing protein kinase (PK) and transmembrane (TM) domains were classified as G-type lectin receptor kinases (G-LecRKs); 23 lacking both domains were classified as G-type lectin proteins (G-LecPs); four lacking PK but retaining the TM domain were grouped as G-type lectin receptor proteins (G-LecRPs); and four missing the TM domain but containing the PK domain were classified as G-type lectin kinases (G-LecKs). Besides the TM and PK domains, most of the G-type lectins in *F. vesca* also contained domains such as S-locus glycoprotein and/or Pan-apple (PAN) that are involved in self-incompatibility and protein–protein interactions, respectively. Thus, *F. vesca* G-lectins showed multiple domain arrangements (Fig. 1).

For convenience and clarity with regards to the *F. vesca* G-lectin genes, we propose a nomenclature based on the classification of G-lectin genes and their chromosome locations (Supplementary Table S2). According to this scheme, ‘Fve’ at the beginning of the name indicates that the gene is from *F. vesca* (Jung et al., 2015), and then ‘GLRK’, ‘GLRP’, ‘GLP’, and ‘GLK’ represent G-LecRK, G-LecRP, G-LecP, and G-LecK, respectively. The number of the chromosome where the gene is located is then given, followed by a sequential number according to the chromosome location of each type of G-lectin gene on this chromosome.

Thus, FvH4_3g18380 (the homolog of *FaMBL1*) becomes FveGLP3.7, i.e. it encodes a G-LecP, it is located on chromosome 3, and it is the seventh G-LecP on this chromosome. This nomenclature for *F. vesca* G-type lectins is used hereafter in this paper.

**Chromosome locations of G-type lectin genes.**

In order to visualize the chromosome locations, the G-lectin genes were mapped to the *F. vesca* genome (Fig. 2). G-LecRKs were found to be distributed on all the chromosomes, whereas the majority of G-LecRKs were mapped on chromosomes 3 and 6, G-LecPs were mapped on chromosomes 2, 3, 5, and 6, and G-LecRPs were found only on chromosomes 3 and 6.

**Expression profiles of G-lectin genes based on RNA-seq datasets.**

The expression profiles of G-lectin genes were analysed in different tissues and at different developmental stages based on an available strawberry RNA-seq dataset (Li et al., 2019). This showed that G-type lectin genes display a wide range of transcription levels, with some genes highly expressed in various tissues including the style, ovary wall, leaves, and roots, while others are completely silenced (Fig. 3; Supplementary Fig. S1). A few genes appeared to be specifically expressed...
only in one or two tissues, such as *FveGLRP3.2* for example, which was highly expressed in pollen and anthers, but not in the other tissues. In contrast, *FveGLP3.7* (the homolog of *FaMBL1*) showed active expression in many tissues during development, such as in seedlings, roots, and the ovary wall (Fig. 3).

Differences in expression of G-lectin genes were found in the interactions of strawberry with *B. cinerea*, *Podosphaera aphanis*, and *Phytophthora cactorum* (Fig. 4). Around 50 G-lectin genes appeared differentially expressed when the roots were infected with *P. cactorum*, with most of them being up-regulated. Several were also found to be up-regulated in response to *Pa. aphanis* infection at 8 dpi. Compared to these two pathogens, few G-lectin genes were transcriptionally altered upon *B. cinerea* infection. In addition, some G-lectin genes appeared to be regulated by the plant resistance elicitors benzothiadiazole and chitosan. Interestingly, the homolog of *FaMBL1*, *FveGLP3.7*, was up-regulated upon infection by *B. cinerea*, *Ph. cactorum*, and *Pa. aphanis* as well as by benzothiadiazole and chitosan. In terms of abiotic stress, cold stress caused both up- and down-regulation of several G-lectin genes (Fig. 4).

**Functional characterization of MBL1 in F. ×ananassa**

**Transgenic lines and their copy numbers.**

The data reported above together with previously published results (Guidarelli *et al.*, 2014) suggest the importance of the G-type lectin gene family in strawberry defense against pathogens, especially *FveGLP3.7* (homolog of *FaMBL1*). Hence, we used *Agrobacterium*-mediated transformation of octoploid strawberry (*F. ×ananassa*, cv. Sveva) (Ma *et al.*, 2021) in order to obtain plants stably overexpressing *FaMBL1*. Three lines (18F6G1, 19F2G1, and 27F8G1) were selected and used for this study. Using ddPCR (Ma *et al.*, 2021), and we determined that the relative copy numbers for 18F6G1, 19F2G1, and 27F8G1 were 6, 2.5, and 1, respectively.

**FaMBL1 expression levels in the transgenic lines.**

All three of the selected transgenic lines showed significantly higher *FaMBL1* expression compared to the WT in leaves, stolons, and petioles (Fig. 5). The lowest expression levels were generally found in 18F6G1 and the highest in 27F8G1. *FaMBL1* was barely expressed in the petioles of WT plants (Supplementary Fig. S2) whereas expression was observed in the petioles of the three transgenic lines, thereby indicating the success of the transformation (Fig. 5C).

There was a lack of correlation between the transgene copy number and the *FaMBL1* expression level in the three over-expressing lines. For instance, 27F8G1 (copy number 1) had higher expression levels in all the tissues tested than 18F6G1 (copy number 6; Fig. 5). This suggested that RNAi-mediated suppression of transcript expression might have occurred in those lines containing higher *FaMBL1* copy numbers (Butaye *et al.*, 2005).
Phytohormone contents in FaMBL1-overexpressing lines.

The contents of JA were lower in the overexpressing lines compared to the WT (Fig. 6A), but the effect was reduced and less clear for conjugated JA (Fig. 6B). The contents of IAA, ABA, and SA in the transgenic lines were about the same as those in the WT (Fig. 6C–E) Thus, FaMBL1 could be directly or indirectly involved in the regulation of JA concentration in strawberry.

Response of FaMBL1-overexpressing lines to fungal inoculation.

We found that C. fioriniae became quiescent after infection on strawberry leaves and they appeared symptomless, hence making them unsuitable for evaluation of susceptibility. In contrast, petioles showed uniform and reproducible anthracnose symptoms after inoculation, and hence we used them to examine the responses of the FaMBL1-overexpressing lines. The disease incidence was significantly lower in overexpressing lines as compared to the WT at 6 dpi (Fig. 7A), suggesting that the overexpression of FaMBL1 decreases the susceptibility of strawberry to C. fioriniae. Where infection occurred, petioles showed anthracnose symptoms at 4 dpi and this was followed by rapid progress of disease at 5 dpi and 6 dpi, when the symptoms were more apparent (Fig. 7B).

Whilst B. cinerea can infect both vegetative and reproductive tissues of strawberry, the greatest economic loss due to this pathogen is the result of fruit infection. However, infected vegetative tissues are an important source of inoculum, and improving its resistance is indispensable for the management of B. cinerea. Moreover, infection trials using leaves show uniform disease symptoms and reproducible data, which is ideal for evaluation of resistance. Hence, we tested the responses of the FaMBL1-overexpressing lines to B. cinerea on detached leaflets. We found that necrotic lesions started to be apparent at 3 dpi in both the transgenic lines and the WT (Fig. 8A); however, the disease progression was greater in the WT. The rate increase of lesion size was significantly higher in the WT than in the FaMBL1-overexpressing lines (Fig. 8B), indicating that they were less susceptible to B. cinerea.

Expression of defense-related genes after B. cinerea inoculation.

To gain insights into the role of FaMBL1 in strawberry defense against B. cinerea, we examined its relative expression together with those of other defense-related genes in the leaves of the overexpressing lines and the WT at 24 h after inoculation. We selected genes that have previously been reported to be induced upon B. cinerea infection, namely those encoding CLASS II CHITINASE (FaChi2-1 and FaChi2-2), POLYGALACTURONASE-INHIBITING PROTEIN (FaPGIP), PHENYLALANINE AMMONIA-LYASE 1 (FaPAL1), THAUMATIN-LIKE PROTEIN 1b (FaTLP1b), 1-AMINOCYCLOPROPANE-CARBOXYLATE OXIDASE (FaACO), AND ALLENE OXIDE SYNTHASE 1 (FaAOS1) (Mehli et al., 2005; Nagpala et al., 2016; Jia et al., 2021; Lee et al., 2021). Whilst FaMBL1 expression was reduced in both the overexpressing lines and the WT upon B. cinerea infection, it remained significantly higher in the overexpressing lines (Fig. 9A). The expression of FaChi2-1 did not differ among the overexpressing lines and the WT in
the absence of inoculation (Fig. 9B); however, after *B. cinerea* infection, its expression was significantly induced only in the overexpressing lines (Fig. 9B). The overexpressing lines 19F2G1 and 27F8G1 showed significant down-regulation of *FaChi2-2* compared with the WT in infected plants (Fig. 9C) and significant down-regulation of *FaAOS1* compared with the WT.
in the absence of infection (Fig. 9G). No other genes showed any significant differences in expression between the FaMBL1-overexpressing lines and the WT in the absence of infection. In contrast, in the presence of *B. cinerea* the expression of the defense-related genes *FaPGIP*, *FaPAL1*, *FaAOS1* (involved in JA synthesis), and *FaACO* (involved in ET synthesis) was downregulated in both the overexpressing and WT plants (Fig. 9E–H).

**Discussion**

Anthracnose (*Colletotrichum acutatum* species complex) and grey mould (*Botrytis cinerea*) are two of the most destructive strawberry fungal diseases. Increasing plant resistance is one of the most sustainable and effective strategies for management; however, the high levels of heterozygosity and polyploidy in strawberry
increase the complexity of applying traditional breeding methods (Nehra et al., 1990; Limera et al., 2017; Mezzetti et al., 2018), and hence the importance of genetic transformation techniques for investigating resistance genes.

G-type lectins are reported to play roles in plant defense against biotic and abiotic stresses (Ghequire et al., 2012; Siripipatthana et al., 2015). In woodland strawberry, _F. vesca_, we found 133 genes belonging to the G-lectin family (Fig. 1).
FveG-lectin proteins encoded by these genes showed multiple domain arrangements, which creates a fairly high degree of protein diversity and provides flexible adaptation to changing environments (Kersting et al., 2012). According to the transcriptome data, many G-lectin genes are actively expressed in different tissues at different developmental stages of strawberries (Fig. 3; Supplementary Fig. S1). Moreover, many G-lectins in strawberry actively respond to pathogens and elicitors, and some appear to respond to biotic stresses. Notably, red fruits infected with *B. cinerea* have been found to show different...
expression profiles of G-lectin genes at 24 h post-infection (hpi; Fig. 4). In particular, FveGLP3.7, the FaMBL1 homolog, is up-regulated in response to B. cinerea in the octoploid red fruit of F. × ananassa cv. Benihoppe (BH in Fig. 4) but not in diploid fruit of cv. Alpine (AP). On the other hand, we found that expression of FaMBL1 was down-regulated in leaves of octoploid cv. Sveva infected with B. cinerea at 24 hpi (Fig. 9A), suggesting that the regulation of this gene in response to infection is not only cultivar-specific but also dependent on the tissue type.

The resistance to B. cinerea that we observed in the leaves of transgenic plants overexpressing FaMBL1 (Fig. 8) could be due to signaling pathways downstream of FaMBL1 that we observed in the B. cinerea tissue type. The resistance to infection is not only cultivar-specific but also dependent on the modulation of this gene in response to infection strategies to infection by necrotrophic fungi (see review by Antico et al., 2012). However, there are also several studies that have shown that some mutations causing deficiency in JA biosynthesis display increased resistance to some necrotrophic fungi (Antico et al., 2012). Taken together, these observations reveal the complexity of the JA-dependent regulation of plant responses.

The decreased JA production that we observed in the FaMBL1-overexpressing lines might ultimately be related to the resistance to fungal pathogens that we observed; however, the data that we obtained are not sufficient to confirm this hypothesis. Further experiments are needed to establish a relationship between FaMBL1 expression and JA-mediated defense responses, using exogenous applications of inducers or inhibitors and quantification of the hormones in infected tissues.

Infection with B. cinerea has been reported to induce the expression of CLASS II CHITINASE (FaChi2-1 and FaChi2-2), POLYGALACTURONASE-INHIBITING PROTEIN (FaPGIP), PHENYLALANINE AMMONIA-LYASE 1 (FaPAL1), THAUMATIN-LIKE PROTEIN 1b (FaTLp1b), 1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE 1 (FaACO), and ALLEN OXIDE SYNTHASE 1 (FaAOS1) and to contribute to strawberry resistance against this pathogen (Mehli et al., 2005; Nappala et al., 2016; Jia et al., 2021; Lee et al., 2021). After strawberry infection with C. acutatum, uncoupling between SA and JA accumulation and the induction of important SA- and JA-related genes has also been reported, including PATHOGENESIS-RELATED PROTEIN 1 (FaPR1-1), LIPOXYGENASE-2 (FaLOX2), JASMONATE RESISTANT-1 (FaJAR1), and PLANT DEFENSIN-1 (FaPDF1) (Amil-Ruiz et al., 2016). We assessed the expression of some of these genes in the leaves of the FaMBL-overexpressing lines at 24 h after B. cinerea inoculation and found that they showed higher expression of FaChi2-1 than the WT (Fig. 9B). FaChi is one of the most abundant classes of strawberry pathogenesis-related genes and has hydrolytic activity against fungal cell walls (Amil-Ruiz et al., 2011). FaChi2-1 has been found to be involved in defense responses against both anthracnose (Tortora et al., 2012) and grey mould (Mehli et al., 2005). Overall, the higher expression of FaMBL1 in the overexpressing lines can be associated with the higher expression of FaChi2-1 at the early stage of infection by B. cinerea. The rice G-type lectin gene, OslecRK, which is consistently associated with resistance to M. oryzae, Xanthomonas oryzae, and brown plant hoppers, influences the expression of defense-related genes such as PR1a, lipoxygenase, and chalcone synthase (Cheng et al., 2013). Interestingly, we found that the expression of FaAOS1, a key enzyme in JA biosynthesis, was significantly lower in the 19F2G1 and 27F8G1 transgenic lines compared to the WT in the absence of infection (Fig. 9C), and this could be related to the lower JA contents in the overexpressing lines.
compared to the WT (Fig. 6A). In contrast, in 18F6G1, where the level of expression of FaMBL1 was lower with respect to the other lines (Fig. 5), the expression of FaAOS1 was not significantly different to that the WT (Fig. 9G), supporting the hypothesis of an involvement of FaMBL1 in downstream regulation. Similarly, the expression of FaChi2-2 was not significantly different in 18F6G1 (Fig. 9C). More studies are needed to clarify the involvement of FaMBL1 in the regulation of downstream expression of defense genes.

In conclusion, G-type lectins form a large gene family in *F. vesca* and have exploitable potential for strawberry defense against biotic stresses. We found that transgenic octoploid strawberries overexpressing FaMBL1 were less susceptible to the fungal diseases anthracnose and grey mould. The association that we observed between FaMBL1 and JA-dependent signaling pathways should be further investigated in order to obtain more evidence of the defense role of FaMBL1. This gene could be used in future trials for improving resistance in economically important strawberry cultivars through full transgenic, cisgenic, or genome-editing approaches.

**Supplementary data**

The following supplementary data are available at [*JXB*](https://doi.org/10.1093/jxb/erz492) online.

Fig. S1. Expression profiles of G-type lectin genes in different tissues of *Fragaria vesca* and at different developmental stages.

Fig. S2. Relative expression of *FaMBL1* in different tissues of wild-type *Fragaria × ananassa*.

Table S1. List of primers used in this study.

Table S2. Proposed nomenclature for G-type lectin genes in *Fragaria vesca*.

**Author contributions**

EB, FN, and LM conceived and designed the research; LM and FN conducted the bioinformatic analysis; SS conducted the genetic transformation experiments; ZHM supervised the resistance evaluation and data analysis; LM performed the other experiments and wrote the original manuscript; all authors revised and approved the manuscript; EB and BM obtained the funding for this research.

**Conflict of interest**

The authors declare that they have no conflicts of interest in relation to this work.

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**Data availability**

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

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