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Genome-wide analysis of G-type lectin genes in *Fragaria vesca* and functional characterization of *FaMBL1* gene in defense response of *F.* × *ananassa* to fungal pathogens

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Abstract

Strawberry (*Fragaria* × *ananassa*) is an important soft fruit but easily to be infected by pathogens. Anthracnose and gray mold are two of the most destructive diseases of strawberry which lead to serious fruit rot. The first chapter introduced strawberry anthracnose caused by *Colletotrichum acutatum*. The infection strategy, disease cycle and management of *C. acutatum* on strawberry were reported. Likewise, the second chapter summarized the infection strategy of *Botrytis cinerea* and the defense responses of strawberry. As we already know white unripe strawberry fruits are more resistant to *C. acutatum* than red ripe fruits. During the interaction between strawberry white/red fruit and *C. acutaum*, a mannose binding lectin gene, *FaMBL1*, was found to be the most up-regulated gene and induced exclusively in white fruit. *FaMBL1* belongs to the G-type lectin family which has important roles in plant development and defense process. To get insight into the role of *FaMBL1*, genome-wide identification was carried out on G-type lectin gene family in *Fragaria vesca* and the results were showed in chapter 3. G-type lectin genes make up a large family in *F. vesca.* Active expression upon biotic/abiotic stresses suggested a potential role of G-lectin genes in strawberry defenses. Hence, stable transgenic strawberry plants with *FaMBL1* gene overexpressed were generated. Transformed strawberry plants were screened and identified. The results were showed in chapter 4, content of disease-related phytohormone, jasmonic acid, was found decreased in overexpressing lines compared with wild type (WT). Petioles inoculated by *C. fioriniae* of overexpressing lines had lower disease incidence than WT. Leaves of overexpressing lines challenged by *B. cinerea* showed remarkably smaller lesion diameters compared with WT. The chitinase 2-1 (*FaChi2-1*) showed higher expression in overexpressing lines than in WT during the interaction with *B. cinerea*, which could be related with the lower susceptibility of overexpressing lines.

Contents

Chapter 1

Strawberry anthracnose caused by *Colletotrichum acutatum*

Abstract

Strawberry is an important soft fruit but easily infected by pathogens. Anthracnose caused by *Colletotrichum acutatum sensu lato* is one of the most destructive diseases of strawberry. *C. acutatum sensu lato* is actually a species complex which is composed of distinct subspecies. In this chapter the morphology, infection process and disease cycle of *C. acutatum* on strawberry were described. The management of *C. acutatum* on strawberry was addressed through three aspects: cultural methods, resistance breeding, and biocontrol. Updated information about cultural methods, resistance genes and new biocontrol agents was reported.

Keywords: *Colletotrichum* lifestyle, disease cycle, defense-related gene, disease management

1. Identified species of *Colletotrichum* on strawberry

Anthracnose is one of the most important diseases of strawberry (*Fragaria* × *ananassa*), which is caused by fungal pathogens *Colletotrichum acutatum* J.H. Simmonds, *C. fragariae* Brooks and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (Freeman & Katan, 1997). Of which *C. acutatum* and *C. gloeosporioides* are widely spread in Europe, especially *C. acutatum* (Denoyes & Baudry, 1995; Martinez-Culebras *et al.*, 2002). *C. acutatum* is quite destructive since they can infect all parts of the plant, including the leaf, petiole, stolon, crown, root, flower and fruit during nursery and production stages (Freeman & Katan, 1997). *C. acutatum* was originally described as an independent species by Simmonds (Simmonds, 1966). *C. acutatum*

and *C. gloeosporioides* have been traditionally distinguished by *C. acutatum*'s fusiform conidia with acute ends (Dowling *et al.*, 2020). However, this morphology character became soon insufficient for distinguishing the two species. For example, strains with only one acute end are frequently encountered (Damm *et al.*, 2012). Later, with the advent of sequencing and molecular identification, it was found that *C. acutatum* and *C. gloeosporioides*, along with other species of *Colletotrichum*, are actually "species complexes", composed of numerous diverse species (Damm *et al.*, 2012; Weir *et al.*, 2012; Dowling *et al.*, 2020). Using multilocus molecular phylogenetic analysis, 22 and 29 species were found in *C. gloeosporioides* and *C. acutatum* species complexes, respectively, in 2012 (Damm *et al.*, 2012; Weir *et al.*, 2012). Among the 29 species of *C. acutatum* species complexes, at least 6 species were associated with strawberry anthracnose, namely *C. simmondsii*, *C. nymphaeae*, *C. fioriniae*, *C. godetiae*, *C. acutatum s. str.* and *C. salicis* (Damm *et al.*, 2012). Studying the diversity of *C. acutatum* species complexes helps the understanding the dissemination and managing of this disease. The *C. acutatum* populations were characterized phenotypically and genetically in the UK and the United States strawberry production (Baroncelli *et al.*, 2015; Wang *et al.*, 2019). These studies revealed that the species *C. nymphaeae*, *C. godetiae* and *C. fioriniae* are associated with strawberry anthracnose in the UK, and that *C. nymphaeae* and *C. fioriniae* appeared to be more aggressive compared to *C. godetiae* (Baroncelli *et al.*, 2015). While two species, *C. nymphaeae* and *C. fioriniae*, were identified as responsible for the anthracnose fruit rot of strawberry in the United States (Wang *et al.*, 2019).

2. Morphology of *C. acutatum*

Colonies of *C. acutatum* usually show white mycelium at the early stage and become covered with pink to orange conidial masses later (Peres *et al.*, 2005). The primary production of conidia is in acervuli; however, *C. acutatum* is also capable of forming secondary conidia on the surface of living strawberry leaves and the symptomless leaves could be significant sources of inoculum for fruit infection (Leandro *et al.*, 2001). Conidia usually have at least one acute end, in contrast to *C. gloeosporioides* having both ends rounded (Simmonds, 1966). Germinated conidia typically develop one or two transverse septa. The germ tubes originate from either end of a conidium and occasionally from both ends. Germ tubes generally span one plant cell length or less, but occasionally grow across several cells (Curry *et al.*, 2002). The appressoria are developed from the swollen tip of germ tube, and a septum exists between the germ tube and appressorium, near the base of the appressorium. The appressoria are initially unmelanized, and become fully pigmented when mature (Curry *et al.*, 2002; Arroyo *et al.*,

2005). The sexual morphs of *C. acutatum* has been designated as *Glomerella acutata*. Perithecia of *G. acutata* were brown to black color (Guerber & Correll, 2001). Among the subspecies related with strawberry, *C. salicis* was proved having sexual morphs (Damm *et al.*, 2012).

3. Infection process of *C. acutatum*

The infection process of *C. acutatum* was studied on leaves, stolons and petioles of strawberry using light and electron microscopy (Curry *et al.*, 2002; Arroyo *et al.*, 2005)(Fig. 1). The invasion processes on different tissues were similar, however, each invasion event occurred more rapidly in stolons than in petioles and the production of secondary conidia (microcyclic conidiation) was detected only on leaves (Curry *et al.*, 2002; Arroyo *et al.*, 2005).

Once the conidia of *C. acutatum* adhere to the surface of strawberry tissues, they start to geminate by forming a germ tube from either end of the conidium, followed by the differentiation of swollen tip of germ tube into globose and subglobose appressoria. The appressoria take some time to be mature and their structures are modified during the maturation. The young appressoria have a bilayer cell wall and their plasma membrane shows a wavy appearance. With the development, a third layer occurred between the cell wall and the plasma membrane of the appressorium. Then the plasma membrane of appressorium became smooth. Afterwards, a penetration peg emerged, and it passed through the cuticle and generated a small infection vesicle when it reached the upper epidermal wall. Then the small infection vesicle enlarged to form an intramural infection vesicle. Once the infection was well established, abundant subcuticular and intramural hyphae are produced, causing severe degradation of the host cell walls. Once stroma within the cuticle of epidermal cells developed, the cuticle expanded considerably to accommodate the growing acervuli. The maturing acervuli erupt through the cuticle and eventually release conidia. This infection process proves that *C. acutatum* is a subcuticular intramural pathogen (Curry *et al.*, 2002; Arroyo *et al.*, 2005)(Fig. 1).

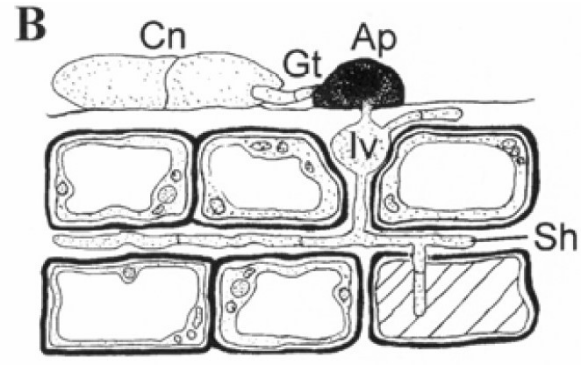

Fig. 1 Schematic drawings of strawberry-*C. acutatum* interactions. Infection strategy: Subcuticular, intramural necrotrophy with hyphal development within periclinal and anticlinal walls of epidermal host cells. Note that host cell walls are swollen and wider apart. Cn = conidium; Gt = germ tube; Ap = appressorium; Iv = infection vesicle; Sh = secondary hyphae; Dead host cells are indicated with diagonal lines. Illustrations by J. E. Adaskaveg. (Peres *et al.*, 2005)

4. Suitable environment condition for *C. acutatum*

The physiology of seven *C. acutatum* strains isolated from strawberries was studied (Es-Soufi *et al.*, 2018). Their development is maximal at 25˚C and 27˚C for all the isolates studied. They cannot growth at 5˚C or 37˚C (Es-Soufi *et al.*, 2018). Germination, secondary conidiation, and appressorial development were significantly affected by temperature and wetness treatments. Increasing wetness duration was favorable for all of these processes. Under continuous wetness, the optimum temperature range for conidial germination and appressoria development was 23.0 to 27.7 ˚C and 17.6 to 26.5 ˚C, respectively. The most suitable temperature range for secondary conidiation was 21.3 to 32.7°C (Leandro *et al.*, 2003). In addition, through monitoring of meteorological conditions, it was found a temperature of 20-25 ˚C is optimal for *C. acutatum* development in the field (Morkeliūnė *et al.*, 2021).

5. Disease Cycles

C. acutatum affects all parts of strawberry, including roots, while the root necrosis is highly unusual for this fungus (Peres *et al.*, 2005). Actually, roots are not easy to be infected when strawberry are planted as a perennial. While the production system nowadays (treating strawberry as an annual) affects the cycle of the pathogen. The transplants are produced in nursery and then transported to the production fields. Transplants may harbor inoculum in lesions, in symptomless leaves with quiescent infection, or in infested soil attached to roots (Leandro *et al.*, 2003). Moreover, Roots of transplants are probably contaminated during digging, trimming, and packing operations in the nursery (Peres *et al.*, 2005). The

transportation of transplants largely contributes to the long-distance dissemination of *C. acutatum*. The *C. acutatum* may undergo a period of quiescence in order to overcome resistance mechanisms in unripe fruit such as pre-formed toxic compounds and deficiency of nutrition or energy for the pathogen (Wharton & Diéguez-Uribeondo, 2004). With the maturation of fruits, the conditions become favorable to pathogen, lesions start to show on the fruit surface and numerous spores are released. The secondary conidiation process can occur on the surface of strawberry leaves, which contributes to a higher level of inoculum for flower and fruit infection (Leandro *et al.*, 2001; Peres *et al.*, 2005). Rainfall or overhead irrigation accelerate the spread of fungus, vegetative tissues could also show disease symptoms, especially the senescent materials. Handling of plants such as harvest process could also cause fungal dissemination (Leandro *et al.*, 2003). The fungi surviving in plant debris, soil and in the nearby crops and weeds are important inoculum sources (Freeman *et al.*, 2001) (Fig. 2).

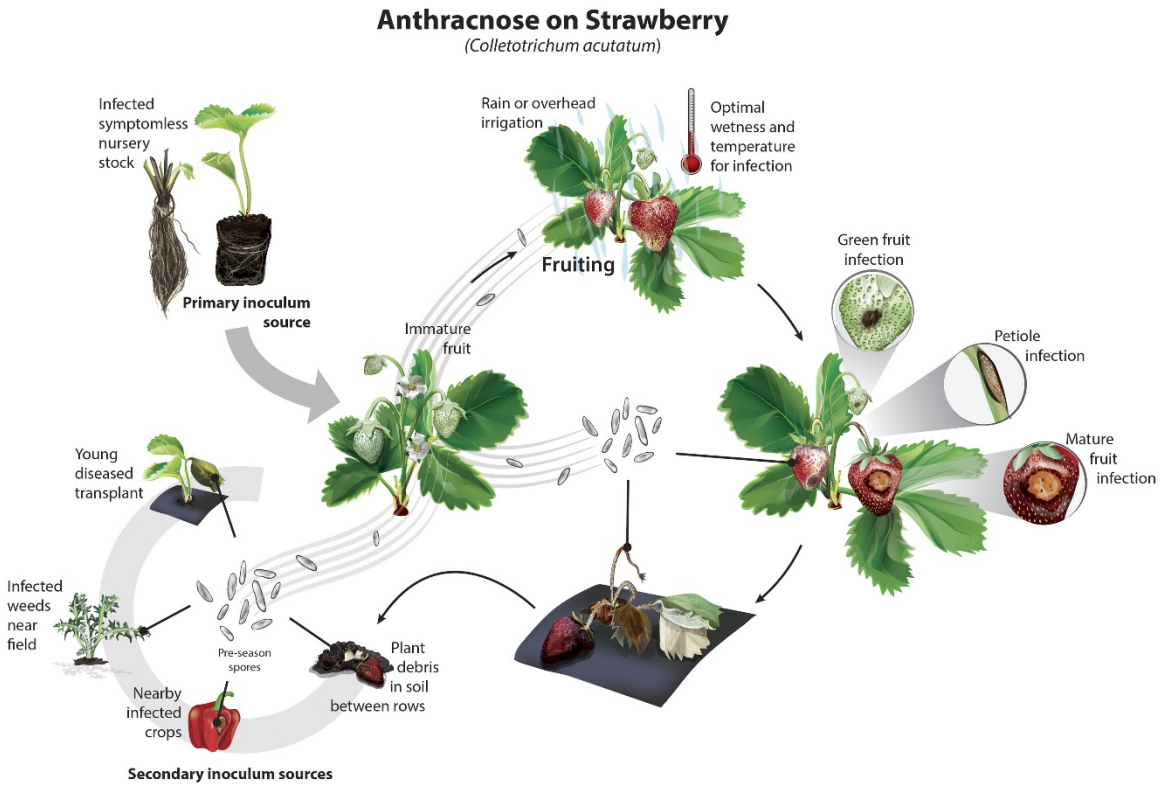

Fig. 2 Disease cycle of strawberry anthracnose caused by *C. acutatum* (Dowling *et al.*, 2020).

6. Management of *C. acutatum* on strawberry

Resistance breeding, cultural methods, chemical control and biological control are general methods for management of *C. acutatum*, usually, one or several methods are used in

strawberry production (Dinler & Benlioğlu, 2019). The details about chemical control of *C. acutatum* had been clearly described by Dowling et al. (Dowling *et al.*, 2020).

6.1. Cultural methods

Various cultural practices are routinely employed to manage anthracnose caused by *C. acutatum* according to the disease cycle. Generally, the principle is suppressing inoculum production and spread by adjusting the growth conditions (Dowling *et al.*, 2020). Healthy transplant is a prerequisite for disease control but difficult since the symptomless infection is common in strawberry (Freeman, 2008). Quantitative PCR are capable for detection of asymptomatic infection in strawberry leaves (Debode *et al.*, 2009). In addition, freezing or paraquat treatment of symptomless tissues are two quick methods to detect the latent infection (Mertely & Legard, 2004). The tissues are killed by freezing or paraquat treatment, then cultured in moist petri dishes, the latent infection could be reported by acervuli showed on the dead tissues. Dipping treatments of transplants with fludioxonil-cyprodinil or azoxystrobin before planting are industry standards for managing the disease in the US, which could decrease the infection of *C. acutatum* (Haack *et al.*, 2018). Soil disinfestation (solarization, fumigants and steaming) is also helpful, since the pathogen could survive in the soil (Freeman *et al.*, 2002). Overhead irrigation caused splash dispersal could be solved by drip irrigation in strawberry cultivation at both nursery and production fields (Daugovish *et al.*, 2012). In addition, rainfall caused splash dispersal has been minimized by growing the crop under cloches or walk-in greenhouses in Israel (Freeman, 2008). Furthermore, the annual hill plasticulture system is commonly used in strawberry production and can be highly profitable (Rahman *et al.*, 2013). For example, two major cultivars ‘Chandler’ and ‘Camarosa’, which have desirable horticultural traits but high susceptibility to anthracnose, are using the plasticulture system and beneficial from it (Rahman *et al.*, 2013). Importantly, all infected materials (petioles, stolons, leaves and fruits) should be removed from the cultivation area and not left in rows because it serves as an additional inoculum source (Freeman, 2008).

6.2. Resistance breeding

6.2.1. Susceptibility of commercial strawberry cultivars to *C. acutatum*

Given the increased fungicide tolerance of *C. acutatum* strains and public concern about food security, planting resistant cultivars becomes one of the most efficient and sustainable method for controlling *C. acutatum* of strawberry. A series of studies have been carried out to evaluate the susceptibility of different commercial cultivars to *C. acutatum*. Simpson et al.

(Simpson *et al.*, 1994) studied the susceptibility of strawberry cultivars from Northern Europe to a UK isolate of *C. acutatum*. Among the tested varieties, 'Elvira' and 'Honeoye' had a high level of resistance; 'Cambridge Favourite', 'Gorella', 'Pandora' and 'Pantagruella' showed a lower level of resistance; while 'Elsanta', 'Redgauntlet' and 'Tamella' were highly susceptible (Simpson *et al.*, 1994). The resistance of different varieties of strawberry harvested from west-central Florida was evaluated by comparing fruit rot incidence caused by *C. acutatum* (Seijo *et al.*, 2008). 'Sweet Charlie', 'Ruby Gem', 'Florida Elyana', and 'Florida Radiance' were the most resistant cultivars tested; 'Strawberry Festival' was intermediate in susceptibility; and 'Albion', 'Camarosa', 'Camino Real', 'Ventana', 'Candonga', and 'Treasure' were susceptible/highly susceptible (Seijo *et al.*, 2008). A screening system using detached strawberry leaves from in vitro propagated plants was adopted for resistance evaluation to *C. acutatum*. The susceptibility increased sequentially in cultivars 'Pelican', 'Sweet Charlie', 'Honeoye', 'Chandler', 'Latestar' and 'Delmarvel' according to the necrosis lesion size (Hammerschlag *et al.*, 2006). According to disease index, mycological analysis and obtained yield, susceptibility of seven popular varieties, 'Honeoye', 'Camarosa', 'Elsanta', 'Darselect', 'Florence', 'Senga Sengana' and 'Alfa Centauri' were reported. All tested cultivars were susceptible to *C. acutatum* but in different degree. Among which cv. 'Camarosa' was the most susceptible because of the highest disease index and the lowest yield of healthy fruit, while cv. 'Senga Sengana' was the most resistant (Wagner & Hetman, 2016). The resistance evaluation using *C. acutatum* subspecies, *C. nymphaeae*, under in vivo and greenhouse conditions showed that the common commercial cultivars grown in Iran including 'Camarosa', 'Paros', 'Pajaro', and 'Queen Eliza' were categorized to susceptible and highly susceptible cultivars (Bahrami Kamangar *et al.*, 2021). In general, 'Camarosa' is proved to be a susceptible cultivar in different countries using different evaluation methods (Seijo *et al.*, 2008; Wagner & Hetman, 2016; Bahrami Kamangar *et al.*, 2021). While 'Sweet Charlie' and 'Honeoye' are the relative resistant cultivars (Simpson *et al.*, 1994; Hammerschlag *et al.*, 2006; Seijo *et al.*, 2008; Wagner & Hetman, 2016). Unfortunately, there is no report about a completely resistant species of strawberry to anthracnose. However, these studies provide useful information for producers in choosing species with higher resistance. Moreover, the species with distinct resistance to *C. acutatum* are valuable materials for transcriptome analysis. For example, new defense mechanism or defense-related genes could be revealed by comparative transcriptome analysis using two strawberry species with different susceptibility (Amil-Ruiz *et al.*, 2012; Wang *et al.*, 2017; Min *et al.*, 2020). Most of the varieties mentioned above have been generated by traditional breeding, which is a time-consuming process, additionally, some undesirable traits

could occur with the disease-resistance trait. In addition, the narrow germplasm bases has caused deleterious effects of inbreeding (Amil-Ruiz *et al.*, 2011). Therefore, an alternative breeding method is in need. The high-quality genome sequencing (Edger *et al.*, 2019) and annotation (Liu *et al.*, 2021) of octoploid strawberry published recently provided bases for understanding the interaction between strawberry and *C. acutatum* at molecular level, which makes it possible to improve strawberry resistance by using biotechnological strategy introducing the resistance genes (Casado-Díaz *et al.*, 2006).

6.2.2. Screening and characterization of strawberry resistance genes

Disease resistance in plants commonly requires two complementary genes: an *avirulence* gene in the pathogen and a matching *resistance* gene in the host (Van Der Biezen & Jones, 1998). Several defense-related genes of strawberry to *C. acutatum* have been revealed and characterized, although the resistance mechanisms of these genes need to be further revealed. The inheritance of strawberry resistances to *C. acutatum* was also investigated (Denoyes-Rothan *et al.*, 2005; Jacobs *et al.*, 2019). A single dominant gene, *Rca2*, was found in control of the high-level resistance against *C. acutatum*, pathogenicity group 2, while the intermediate level of resistance was quantitative and controlled by minor genes (Denoyes-Rothan *et al.*, 2005). Recently, another major resistance locus, *FaRCa1*, was revealed by the University of Florida strawberry breeding program (Salinas *et al.*, 2019). *FaRCa1* located on linkage group 6B, different from *Rca2*, which mapped to linkage group 7B. In addition, *FaRCa1* conferred resistance to *C. acutatum*, pathogenicity group 1, probably mainly isolates of *C. nymphaeae* (Salinas *et al.*, 2019). These results have important implications that will lead to more efficient selection for anthracnose resistance in strawberry breeding (Salinas *et al.*, 2019). Study on the inheritance of resistance to *C. acutatum* and *C. gloeosporioides* in strawberry revealed a high genetic correlation between resistance to *C. acutatum* and *C. gloeosporioides*, implying that resistance genes against *C. gloeosporioides* probably work on resistance to *C. acutatum* (Jacobs *et al.*, 2019). This result provided more possibilities of finding resistance genes against both *C. acutatum* and *C. gloeosporioides.* Higuera et al. elucidated a negative regulatory role of *FaWRKY1* in resistance to *C. acutatum* in strawberry fruit (Higuera *et al.*, 2019). A strawberry mannose binding lectin gene, *FaMBL1*, was found to be related with the higher resistance of unripe fruits (Guidarelli *et al.*, 2011, 2014). Although strawberry fruits at both unripe and ripe stages can be infected by *C. acutatum*, the ripe fruits are more susceptible than unripe fruit and show disease symptoms, while in white fruits the pathogen becomes quiescent (Guidarelli *et al.*, 2011). *FaMBL1* was found to be the most up-

regulated gene in unripe fruit challenged by *C. acutatum*. Transient transformation of strawberry fruits with *FaMBL1* silenced and overexpressed was carried out, *FaMBL1* was proved involved in the resistance of unripe fruit to *C. acutatum* (Guidarelli *et al.*, 2014). Resistance genes from other organisms could be introduced to strawberry as well. *Trichoderma* is a fungus commonly used as a biocontrol agent under field conditions thanks to its expression of hydrolytic enzymes such as chitinases and glucanases. Susceptible strawberry cultivar 'Camarosa' transformed with either a chitinase, *chit-42*, or a *β-1,3-glucanase* gene, both from *Trichoderma*, showed less susceptibility to *C. acutatum* (Mercado *et al.*, 2007).

6.3. Biological control

Nowadays, there is a strong need for safe alternatives of fungicides used for disease control. Synthetic fungicides impose selective pressure on pathogen populations and may result in fungicide-resistant strains (Arroyo *et al.*, 2007). The natural products with antifungal property and low risk for developing pathogen resistance could be a good substitute. Exogenous applications of plant essential compounds Brassinosteroids (BRs) could be used to activate the innate immunity of strawberry to *C. acutatum* (Furio *et al.*, 2019). Strawberry plants treated with BRs increased the production of H_2O_2, $O_2^{\cdot-}$, NO, calcium oxalate crystals and higher callose and lignin deposition as compared to the control plants (Furio *et al.*, 2019). Volatile compound, (E)-Hex-2-enal, from strawberry fruit was capable of reducing the susceptibility of strawberry to *C. acutatum* (Arroyo *et al.*, 2007). (E)-Hex-2-enal could inhibit the mycelium growth and spore germination of *C. acutatum* by altering the structures of the cell wall and plasma membrane of the conidia, causing disorganization and lysis of organelles and, eventually, cell death (Arroyo *et al.*, 2007). The essential oil (EO) of *Lippia sidoides* was proved having antifungal activity to *C. acutatum in vitro* (Oliveira *et al.*, 2019). The major compound of this EO is thymol which was able to cause dehydration and rupture of the pathogen hyphae. A more efficient way to apply this EO is by direct contact than by volatilization (Oliveira *et al.*, 2019).

Although antagonists living on surfaces of plants have a potential effect in biological control, there is a limited number of studies on the biological control of *Colletotrichum* species (Dinler & Benlioğlu, 2019). *Trichoderma* strains, *T. harzianum* (T-39), *T. atroviride* (T-161) and *T. longibrachiatum* (T-166), were effective in controlling strawberry anthracnose caused by *C. acutatum* (Freeman *et al.*, 2004). These strains could be used independently or combined with each other at 0.4% or 0.8% concentrations, and at 7- or 10-day intervals for anthracnose control. *Azospirillum brasilense* REC3 is a plant growth-promoting bacterium isolated from

strawberry which could reduce the disease symptoms caused by *C. acutatum*. The induction of defense related genes and accumulation of SA indicated their participation in the defense response of strawberry to *C. acutatum*. The increased phenolic compounds and callous deposition implied the enhanced resistance was related with the structural cell wall modification (Tortora *et al.*, 2012).

7. Conclusions

The basis of disease management includes three aspects, making the environment unfavorable for pathogen growth and dissemination, increase the resistance of host plant as well as decreasing the number or virulence of pathogen. The lifestyle and disease cycle of *C. acutatum* on strawberry were revealed, according to which the cultural methods are made to decrease the number of inoculum and depress the dispersal. Molecular tools have been effectively used to distinguish *Colletotrichum* spp. and identify genetically distinct subgroups within *C. acutatum*. Despite a great effort has been made, the cultivated strawberry with completely resistance to *C. acutatum* is not generated yet. Resistance breeding using biotechnology is a promising way for strawberry anthracnose or other pathogen management. Therefore, the defense mechanisms and resistance genes of strawberry need to be revealed in-depth.

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Chapter 2

Strawberry gray mold caused by *Botrytis cinerea*

1. Introduction

Gray mold caused by *Botrytis cinerea* is a primary disease of strawberry all over the world resulting in enormous economic losses to the strawberry industry. *B. cinerea* causes disease symptoms not only on fruits, but also on flowers and vegetative tissues (Asch *et al.*, 2019) (Fig. 3). Under wet conditions, more than 80% of strawberry flowers and fruits can be lost if plants are not sprayed with fungicides (Asch *et al.*, 2019).

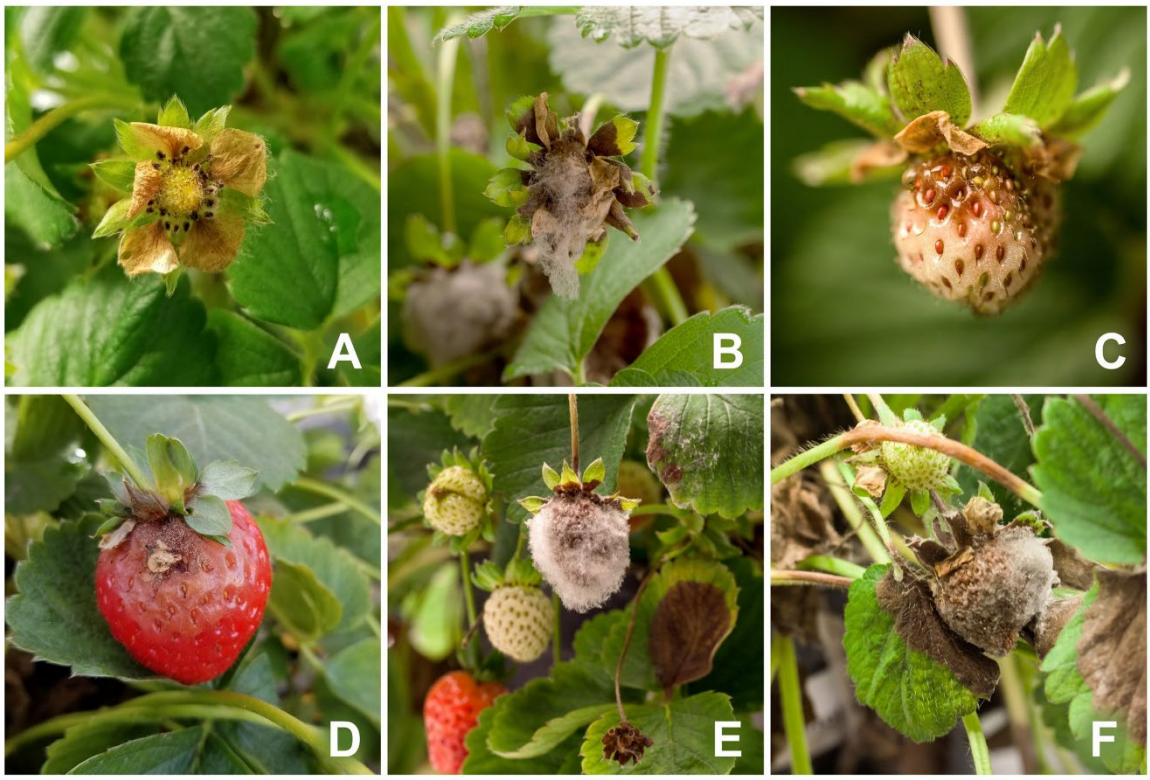

Fig. 3 Symptoms of *Botrytis cinerea* infections in strawberry. A) senesced flower with *B. cinerea* mycelium growth. B) advanced floral infection. C) and D) infections of fruit at different stages. An infected petal can be seen as the source of fruit infection in D. E) and F) browning of leaves due to *B. cinerea* infections (Asch *et al.*, 2019).

2. Infection process of *Botrytis cinerea*

B. cinerea is a ubiquitous pathogen with more than 1000 plant hosts. Geographically, it has an extremely wide distribution from tropical area, temperate area to frigid area (Elad *et al.*, 2016). The infection process of *B. cinerea* started with a conidium landing on the plant surface, followed by the process of adhesion to the tissues surface, germination of conidia, generation and differentiation of germ tube, penetration of appressoria, killing of host cell, establishment of the primary lesion (Kan, 2005).

Generally, moist condition is helpful for the attachment and germination of conidia of *B. cinerea* at the beginning of infection, although it was reported dry-inoculated conidia could germinate as well (Cole *et al.*, 1996). For example, the incidence of strawberry flowers infected by *B. cinerea* was reported closely related with the weather condition, infection was favored when day vapor pressure was high (Xu *et al.*, 2000). The adhesive forces keeping conidia attached are resulting from the hydrophobic interactions between host and conidial surfaces (Doss *et al.*, 1993). In several hours, conidia germinate and the germ tubes are enclosed by an

extensive sheath of fibrillar-like materials, also known as extracellular matrix (Cole *et al.*, 1996), consisting of carbohydrates and proteins, such as cutinases, cellulases, laccases and pectinolytic enzymes (Doss, 1999). Differently, dry-inoculated conidia germinate rapidly and produce short germ tube and the extracellular matrix was only detected around the penetration area of the germ tube (Cole *et al.*, 1996; Williamson *et al.*, 2007).

The penetration of *B. cinerea* relies on appressorium generated from swollen tip of germ tube. However, it is distinct from the classical types of appressoria found in *Colletotrichum* or other fungal pathogens. *B. cinerea* germlings do contain melanin in the extracellular matrix which is loosely associated with the fungal cell wall (Doss *et al.*, 2003) but they do not contain a wall that seals the appressorium from the germ tube, as would be required to enable generating extremely high osmotic pressures (Williamson *et al.*, 2007). The extracellular matrix may contribute to the swelling by retaining water, since its major polysaccharide component, cinerean, is extremely hygroscopic (Kan, 2005). Besides penetration using appressoria, *B. cinerea* can infect the host through wound sites or opening stoma (Mehli *et al.*, 2005). Given the simplicity of passing through wound sites or stoma, this process is regarded as an expansion rather than penetration (Kan, 2005). The penetration process of *B. cinerea* directly through intact cuticle of host was reported mediated by enzymatic activity of cutinase (Salinas & Verhoeff, 1995). Cutinase-encoding gene *cutA* was cloned and expressed together with the reporter gene GUS, then high GUS activity was detected from the onset of conidial germination and during penetration into epidermal cells (Van Kan *et al.*, 1997). While the penetration goes on, *B. cinerea* secretes numerous cell wall-degrading enzymes, including pectinase (Clark & Lorbeer, 1976; Collmer & Keen, 1986) and polygalacturonases (van den Heuvel & Waterreus, 1985). Enzymatic depolymerization of major cell wall components by these enzymes damages the plant cell wall and exposes the nutrients to pathogens (Nakajima & Akutsu, 2014).

After cuticle penetration, *B. cinerea* kills host cells in advance of invasion of infection hyphae (Clark & Lorbeer, 1976). A number of metabolites and proteins secreted by the fungus have been shown to cause cell death when applied to plant tissues, and some also induce symptoms of programmed cell death (Nakajima & Akutsu, 2014). Some secondary metabolites of *B. cinerea* have been found to be phytotoxins and important for the pathogenesis, including botcinolide (Cutler *et al.*, 1993), botrydial (Colmenares *et al.*, 2002), botcinic acid (Reino *et al.*, 2004) and related botcinins (Tani *et al.*, 2006). The oxalic acid (OA) and reactive oxygen species (ROS) are also related with the virulence of *B. cinerea*. OA is produced by *B. cinerea* on colonized plant tissues and the production of OA is regulated by ambient pH (Manteau *et*

al., 2003), while ROS produced by plants during their interaction with pathogens could induce defense genes expression and programmed cell death. However, the cell death benefits the invasion of necrotrophy such as *B. cinerea* (Govrin & Levine, 2000). In addition, *B. cinerea* itself also generates ROS (Nakajima & Akutsu, 2014). With the establishment of *B. cinerea*, necrotic lesion will show on the host surface.

3. Defense responses of strawberry to *B. cinerea*

Host surface penetration and the rupture of plant cell walls by enzymes of *B. cinerea* triggers a cascade of processes in the fungus as well as the host (Kan, 2005). Defense mechanisms can be divided into preformed and induced defenses. In strawberries, preformed defense compounds are especially abundant in the unripe stage (Asch *et al.*, 2019). Proanthocyanins (PAs) are able to restrict the activity of fungal enzymes like polygalacturonases (PGs) that are necessary for aggressive infection of hosts and induce *B. cinerea* quiescence in unripe fruit (Jersch *et al.*, 1989). Similarly, anthocyanins might delay *B. cinerea* infections or cause quiescence as well (Asch *et al.*, 2019). For instance, strawberries illuminated with white fluorescent light showed increased anthocyanin content and delayed development of gray mold (Saks *et al.*, 1996).

Induced defense includes accumulation of secondary metabolites and expression of defense-related genes of strawberry. Demethylated oligogalacturonides produced by degradation of pectin of host cell wall during the invasion of *B. cinerea* can trigger basal immune responses (Amil-Ruiz *et al.*, 2011). Expression of the *F.* × *ananassa* pectin methylesterase 1, *FaPE1*, in *F. vesca* partially demethylated oligogalacturonides in ripe fruit, which led to a higher resistance to *B. cinerea* (Osorio *et al.*, 2011). Expression of polygalacturonase-inhibiting protein gene, *FaPGIP*, was significantly increased in strawberry upon infection of *B. cinerea* (Mehli *et al.*, 2004; Schaart *et al.*, 2005). Overexpression of *FaPGIP1a* and *FaPGIP2a* in cisgenic plants conferred enhanced resistance to gray mold (Schaart, 2004). Other enzymes induced by *B. cinerea* infections are chitinases. Maximum expression of the chitinases *FaChi2-1* and *FaChi2-2* occurred at 16 h post inoculation of strawberries challenged by *B. cinerea* (Mehli *et al.*, 2005). Transgenic strawberry plants expressing a thaumatin II gene showed enhanced resistance to *B. cinerea* (Schestibratov & Dolgov, 2005).

Phytohormones are closely related to strawberry plant resistance to *B. cinerea*, although the mechanisms are yet to be revealed. Down-regulation of the ABA biosynthetic gene β-glucosidase 3, *FaBG3*, resulted in fruit with limited ripening and higher *B. cinerea* resistance

(Li *et al.*, 2013). The fungal elicitor, *Acremonium strictum* Elicitor Subtilisin (AsES), could induce strawberry innate immunity through a functional ethylene (ET) pathway and lead to a higher resistance to *B. cinerea* (Perato *et al.*, 2020). AsES induced a marked increase in local and systemic biosynthesis of ET. However, blocking of ET perception by 1-MCP prior to AsES induction prevented AsES from eliciting defense against *B. cinerea* and other fungi (Perato *et al.*, 2020). In addition, JA appears to be involved in defense responses against *B. cinerea*. For example, strawberries treated with MeJA had a delayed and much slower progression of *B. cinerea* infections (Saavedra *et al.*, 2017). The resistance of strawberry against *B. cinerea* was negatively regulated by transcription factor, *FaWRKY25* (Jia *et al.*, 2021). This regulation possibly dependents on JA-signaling pathways, since *FaWRKY25* expression level substantially affects the expression levels of genes related to JA biosynthesis and metabolism. Moreover, increasing JA content in strawberries by external JA treatment significantly reduced *FaWRKY25* expression and enhanced the fruits' resistance against *B. cinerea* (Jia *et al.*, 2021). On the contrary, transcription factor, *FaWRKY11*, was reported to be a positive regulator of strawberry resistance to *B. cinerea*, the mechanisms of regulation is yet to be revealed (Wang *et al.*, 2021).

4. Conclusions

B. cinerea is a necrotrophic pathogen with versatile secreted enzymes which assist the invasion and the pathogenicity of *B. cinerea*. Both reproductive and vegetative tissues of strawberry could be infected by *B. cinerea*, however, natural resistance material of cultivated strawberry is not present. In the future, it necessary to further explore the defense mechanisms of strawberry against *B. cinerea* and develop resistant species which is an effective way for strawberry gray mold management.

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Chapter 3

Genome-wide identification and characterization of G-type lectin in *Fragaria vesca*

Abstract

Lectins make up a large and diverse group of proteins in plants. G-type lectins are important type of lectins involved in plant development and defense process. However, studies about strawberry G-type lectins are limited. In this study, genome-wide identification was carried out on G-type lectin gene family in *Fragaria vesca*. A total of 133 genes were found belonging to this family and they were classified into four groups: G-type lectin receptor kinases, G-type lectin kinases, G-type lectin receptor proteins and G-type lectin proteins, according to their domain organizations. Their chromosome localization, phylogenetic and evolutionary relationship were also analyzed. The results showed that tandem and dispersed duplication occurred frequently, which led to the expansion of G-type lectin gene family in *F. vesca* and may have increased the types of domain arrangement. The expression profile of G-type lectin genes at different developmental stages of *F. vesca* and under various biotic/abiotic stresses was inferred from the available databases. G-type lectin genes are actively expressed during *F. vesca* development and respond to multiple biotic/abiotic stresses.

Keywords: bulb type lectin, diploid strawberry, GNA, Ser/Thr kinase domain

1. Introduction

Lectins are proteins containing one or more lectin domains that could specifically and reversibly bind carbohydrate or glycan structures (Van Holle & Van Damme, 2018). They

consist of a large and diverse class of proteins and exist in all kingdoms of life (Van Holle & Van Damme, 2019). Plant lectins can be classified into 12 distinct subfamilies based on their conserved carbohydrate-binding domains: *Agaricus bisporus* agglutinin family, Amaranthins, homologs of class V Chitinases (CRA), Cyanovirin, *Euonymus europaeus* lectin (EUL), *Galanthus nivalis* agglutinin (GNA), Hevein, Jacalin-related lectin (JRL), legume Lectin, Lysin Motif (LysM), *Nicotiana tabacum* agglutinin (Nictaba), and Ricin B lectin family (Van Damme *et al.*, 2008). Such abundance and diverse carbohydrate-binding ability imply an important function of these proteins in plants. Many lectins have already been shown to be involved in plant biotic and abiotic stress regulations (Chen *et al.*, 2006; Singh *et al.*, 2013; Sun *et al.*, 2013; Guidarelli *et al.*, 2014; Kim *et al.*, 2015; Liu *et al.*, 2015; Van Holle *et al.*, 2016). In particular, due to the capability of carbohydrate recognition and binding, lectin receptor-like kinases (LecRKs), are candidate proteins for pathogen-/damage-associated molecular pattern (P/DAMP) recognition. LecRKs usually consist of Ser/Thr kinase domain and lectin domains from CRA, GNA, Jacalin, Nictaba, LysM, and legume lectin family (Van Holle *et al.*, 2017).

GNA-related lectins, also known as G-type lectin or G-lectin, contribute to a large part of the whole plant lectins that have an affinity for mannose or mannose complex (Hogervorst *et al.*, 2006; Hwang & Hwang, 2011; Ghahremani *et al.*, 2019). Since the first GNA-related lectin was isolated in the bulbs of *Galanthus nivalis,* this type of lectin is also named bulb-type lectin or B-lectin, and the domain is named B-lectin or GNA domain (*Galanthus nivalis* agglutinin-related lectin domain) (Van Damme *et al.*, 1987).

Besides GNA domain, G-type lectins also contain other domains, such as S-locus glycoprotein domain (SLG), PAN/Apple domain (PAN), transmembrane domain (TM), and protein kinase domain (PK) (Vaid *et al.*, 2013). Concerning their role, G-type lectins are predicted to have important functions in plant development and resistance. A large group of G-type lectins has shown insecticidal properties, particularly against aphids of wheat, maize, potato, and sugarcane, by affecting their development and fecundity (Down *et al.*, 1996; Luo *et al.*, 2005; Wang *et al.*, 2005; Miao *et al.*, 2011). *CaMBL1* and *CaGLP1* are pepper G-type lectin genes involved in signaling and plant cell death that were shown to play a role in defense against *Xanthomonas campestris* pv *vesicatoria* (Hwang & Hwang, 2011; Kim *et al.*, 2015). Similarly, G-type LecRK gene of *Arabidopsis* takes part in defense signaling by recognizing lipopolysaccharides of *Xanthomonas* and *Pseudomonas* (Ranf *et al.*, 2015). Lipopolysaccharides are well-described PAMPs that trigger plant innate immunity (Zeidler *et al.*, 2004; Silipo *et al.*, 2005). The transfer of a G-type LecRK gene *Pi-d2* to rice conferred

race-specific resistance to *Magnaporthe grisea* (Chen *et al.*, 2006), and knocking down of *OslecRK* gene, also a G-type LecRK, reduced the resistance of rice plant to *X. oryzae pv. oryzae* and brown planthopper (Cheng *et al.*, 2013). In strawberry, *FaMBL1* was found involved in the resistance of unripe fruits to *Colletotrichum acutatum* (Guidarelli *et al.*, 2014).

Besides their role in resistance to biotic stress, G-type lectins play a role in plant adaption to abiotic stress. *OsSIK2* enhanced rice tolerance to salt and drought stresses, also delayed dark-induced leaf senescence (Chen *et al.*, 2013). Transgenic *Arabidopsis* plant expressing *GsSRK* exhibited enhanced salt tolerance and higher yields under salt stress (Sun *et al.*, 2013). Both *OsSIK2* and *GsSRK* could be induced by abscisic acid, salt, and drought stresses (Chen *et al.*, 2013; Sun *et al.*, 2013).

Interestingly, G-type lectins also have potential medical applications. Some G-type lectins could recognize some of the high-mannose N-glycans exposed at the surface of gp120 of HIV-1 (Houlès Astoul *et al.*, 2000; Hoorelbeke *et al.*, 2011), acting as inhibitors of the entry of HIV-1 into CD4+ T-lymphocytes. Besides, there are also G-type lectins that could specifically bind altered hypermannosylation N-glycans on the surface of cancer cells and cause programmed cell death of tumor cells (Wu & Bao, 2013).

Functional analysis and genome-wide studies of G-type lectin have been performed in different plants, such as Arabidopsis (Jiang et al., 2010; Vaid et al., 2012; Teixeira et al., 2018), soybean (Jiang et al., 2010), rice (Vaid et al., 2012), tomato (Teixeira et al., 2018), mulberry (Saeed et al., 2016), and cucumber (Dang & Van Damme, 2016). Strawberry is a good model plant for the study of Rosaceae plants and the study of G-type lectin family in this species can provide information for other Rosaceae plants as well. Recently, with the updated genome annotation and comprehensive gene expression atlas of *F. vesca* (https://www.rosaceae.org/species/fragaria_vesca/genome_v4.0.a2) (Li et al., 2019), reliable data are available for genome-wide analysis of G-type lectin genes in strawberry. Moreover, most studies on plant G-type lectins focused on the G-type LecRKs, lacking insights on potential biological functions of G-type lectins without kinase domain. In this study, using the newly released *F. vesca* genome annotation (v4.0.a2), we identified the woodland strawberry lectin gene family members and characterized their genomic organization and phylogenic relationship. To get insights into their functions, we further analyzed the variation in domain composition and their expression profile at different stages.

2. Methods

2.1. Identification and characterization of G-type lectin genes

2.1.1. Identification and domain organization

To identify G-type lectin genes, BLASTp search was performed first using GNA domain of FvH4_3g18380 (homolog of *FaMBL1*) as the query in the Genome Database for Rosaceae (GDR) (https://www.rosaceae.org/) (Jung *et al.*, 2019) website and using the database Fragaria_vesca_v4.0.a2 proteins. Results with E-value < 1E-6 were considered as candidate G-type lectin proteins. With the same setting, a second BLASTp was conducted using GNA domains of G-lectin proteins with higher variety found in the first BLASTp (FvH4_1g23370, FvH4_2g12390, FvH4_2g14250, FvH4_2g26490, FvH4_2g29050, FvH4_2g33830, FvH4_3g03230, FvH4_3g03301, FvH4_3g03410, FvH4_3g03430, FvH4_3g06140, FvH4_3g15930, FvH4_3g18370, FvH4_3g21270, FvH4_3g43440, FvH4_4g02170, FvH4_5g31680, FvH4_6g00300, FvH4_6g12870, FvH4_6g44106). The domains of each candidate gene were checked manually by InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/) (Quevillon *et al.*, 2005) website. The transmembrane domain was checked by using TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Krogh *et al.*, 2001).

2.1.2. Phylogeny analysis

To build a phylogenetic tree, full-length protein sequences were obtained by running Blastx on GDR website, using mRNA sequences from GDR as a query to score protein database. Protein sequences were aligned using MUSCLE mode by MEGA-X (Kumar *et al.*, 2018). Aligned sequences were analyzed via maximum likelihood bootstrapping (ML-BS) using IQ-TREE 1.6.12 (http://www.iqtree.org/) (Nguyen *et al.*, 2015). Once the best-fit model (WAG+F+I+G4) of molecular evolution was determined for G-type lectin genes, based on the Bayesian information criterion (BIC) scores (Chernomor *et al.*, 2016), ML-BS analysis was conducted with IQ-TREE 1.6.12. Statistical support for the branches was evaluated by conducting a ML-BS bootstrap analysis of 5000 replicates. The tree was annotated by iTOL (https://itol.embl.de/) (Letunic & Bork, 2019).

2.1.3. Chromosome location

The visualization of chromosome location of G-lectin genes was accomplished through MapGene2Chromosome V2 (http://mg2c.iask.in/mg2c_v2.0/) (Chao *et al.*, 2015). The location

coordinates of G-lectin genes on the strawberry genome (*F. vesca* v4.0.a2) were obtained from GDR website.

2.2. Expansion and evolution of G-lectin genes

Gene duplication was investigated in *F. vesca* genome. Gene tandem duplication was explored using the PTGBase plant tandem duplicated gene database (http://ocri-genomics.org/PTGBase/) (Yu et al., 2015), and dispersed duplication was investigated on the plant duplicate gene (plantDGD) database (http://pdgd.njau.edu.cn:8080/) (Qiao et al., 2019). The amount of tandem and dispersed duplication was showed by Venn diagram using R package VennDiagram (Chen & Boutros, 2011). The relationships of duplicated gene pairs were visualized by chord diagram using R package circlize (Gu *et al.*, 2014).

2.3. Expression analysis of G-type lectin genes

F. vesca G-lectin gene expression profiles were extracted from the database reported by Li and his colleagues (Li *et al.*, 2019). The expression levels of the genes in different tissues: flowers, fruit of different developmental stages, seedlings, leaves, meristems, and roots were used to draw a heatmap through R package, ComplexHeatmap (Gu *et al.*, 2016).

The transcriptome profiles of *F.* x *ananassa* strawberry infected by *Botrytis cinerea* (Xiong et al., 2018; Haile et al., 2019), *F. vesca* infected by *Phytophthora cactorum* (Toljamo et al., 2016), and by *Podosphaera aphanis* (Jambagi & Dunwell, 2015) were used to retrieve G-lectin gene expression. Besides this, transcriptome profile of *F.* x *ananassa* after cold stress (Zhang et al., 2019) and preharvest application of benzothiadiazole (BTH) and chitosan (Landi et al., 2017) were also used to obtain G-lectin gene expression profile. G-lectin genes from *F.* x *ananassa* transcriptome datasets were converted to their *F. vesca* orthologs.

The co-expression genes of *F. vesca* G-lectin genes were retrieved from the co-expression database (www.fv.rosaceaefruits.org) (Shahan et al., 2018) upon conversion of gene names from the previous genome annotations to the version 4.0.a2 (Li *et al.*, 2019), here used for lectin gene identification. Since different networks indicate varying correlation strengths, for G-lectin genes co-expression analysis, networks with the highest correlation were chosen: consensus100_hd_ltpm (consensus100 network of hand-dissected tissues), consensus100_lcm_ltpm (consensus100 network of laser captured tissues), and consensus100_fruit_ltpm (consensus100 network of ripening fruit tissue-only).

2.4. Subcellular localization prediction

For subcellular localization, amino acid sequences of strawberry G-lectin genes were submitted to two online predictors, TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP-1.1/index.php) (Emanuelsson *et al.*, 2000) and "subCELlular LOcalization predictor" CELLO v.2.5 (http://cello.life.nctu.edu.tw/) (Yu *et al.*, 2014). Using the default setting, the predicted positions of G-lectin genes were obtained.

3. Results

3.1. *F. vesca* G-lectin genes identification and characterization

3.1.1. G-lectin genes identification, classification, and domain organization

F. vesca protein sequences containing GNA domain were fished out by BLASTp from the *F. vesca* protein database included in the Genome Database for Rosaceae (GDR) (https://www.rosaceae.org/) (Jung *et al.*, 2019) website. The first BLASTp was carried out using the amino acid sequence (from 70 to 208 aa) of the GNA domain (domain ID: IPR001480) encoded by FvH4_3g18380 gene. This gene is the homolog of *F.* x *ananassa FaMBL1*, that encodes for a protein containing GNA and PAN domains and was reported as overexpressed in white strawberry fruit in response to anthracnose disease (Guidarelli *et al.*, 2014). The search allowed to retrieve 77 different protein sequences. To find out more proteins and reduce redundancy, 20 of these sequences with relatively low similarity were chosen and used for a second BLASTp search, leading to a total of 133 proteins with GNA domains found in *F. vesca*. Among these, 102 proteins containing PK and TM domain were classified into G-LecRK; 23 proteins lacking both domains were classified into G-LecP, and finally 4 proteins lacking PK but retaining the TM domain, were grouped G-LecRP. In addition, four genes (FvH4_3g03241; FvH4_3g03300; FvH4_3g15980; FvH4_6g44240) missing the TM domain but containing both GNA and PK domain were found, and they were classified into G-LecK.

Besides GNA domain, most of G-type lectins of *F. vesca* also contain other domains like S-locus glycoprotein domain (SLG), PAN/Apple domain (PAN), and Epidermal Growth Factor domain (EGF) (Fig. 4). SLG is involved in self-incompatibility reaction during flower fertilization (Kachroo *et al.*, 2001) and the PAN domain is believed to mediate protein-protein and protein-carbohydrate interactions (Tordai *et al.*, 1999). In some cases, G-type lectins have an EGF domain which may take part in the formation of disulfide bonds (Vaid *et al.*, 2013). Multiple arrangements of these domains lead to various G-type lectins in *F. vesca* (Fig. 4).

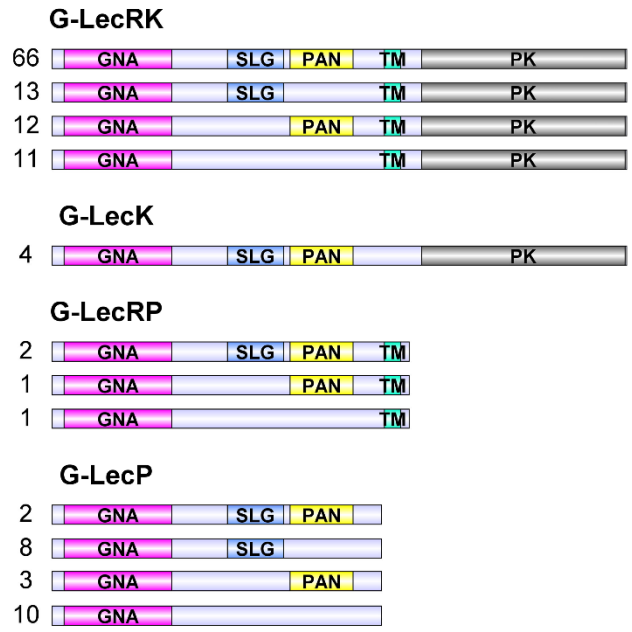

Fig. 4 Domain arrangements and classification of G-type lectins in Fragaria vesca. Numbers on the left indicate genes belonging to the type of domain organization. GNA: GNA domain; SLG: S-locus glycoprotein domain; PAN: PAN/Apple domain; PK: protein kinase domain; TM: transmembrane domain. Epidermal Growth Factor domain which overlaps with PAN domain and it is not shown in this figure.

3.1.2. Phylogenetic tree and nomenclature of G-type lectin genes

To highlight evolutionary differences, a phylogenetic tree of all 133 proteins was generated (Fig. 5). G-lectin genes are classified into six clades (I to VI). FvH4_1g03780 does not fall in any of these clades and it is designated singleton. All proteins in clade I have the same domain arrangement, GNA/PAN/TM/PK. In clade III, IV, and V, most of proteins are G-LecRKs with domain arrangement as GNA/SLG/PAN/TM/PK; while proteins of clade VI show the biggest diversity of domain arrangements, in total ten types of domain arrangements exist in this clade.

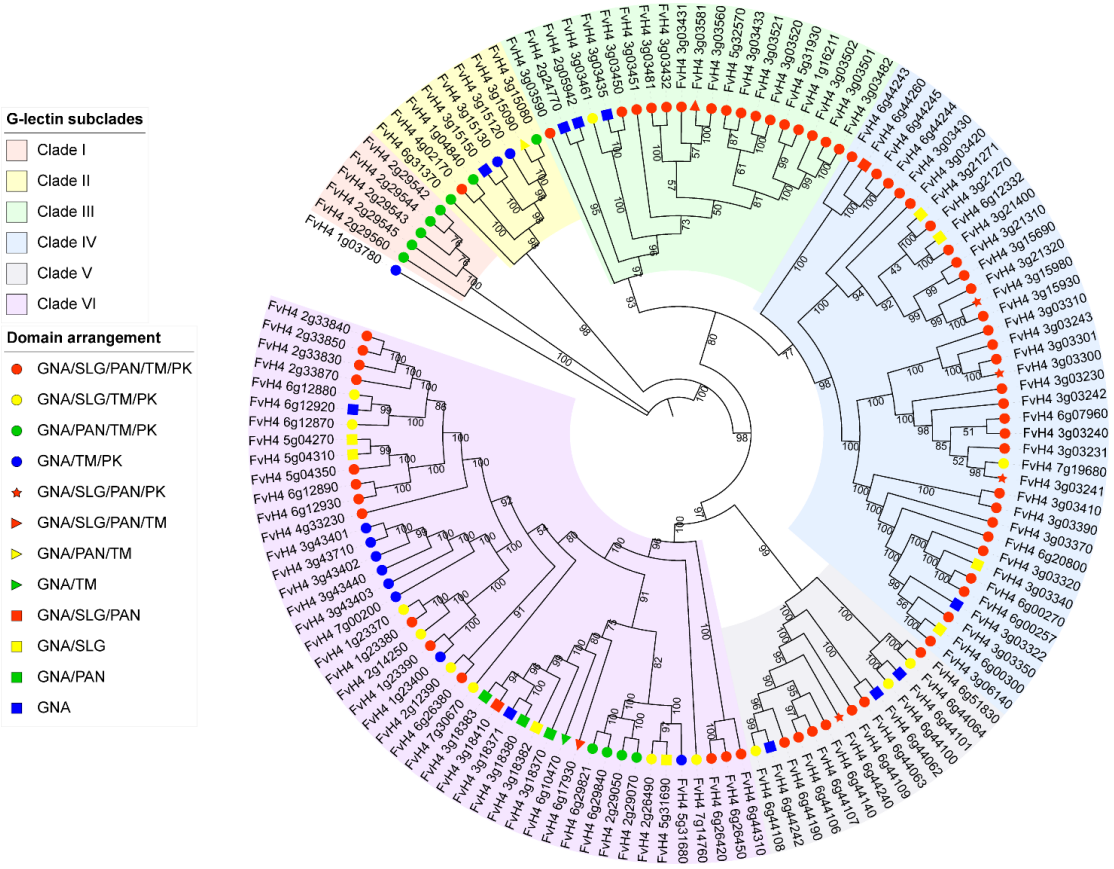

Fig. 5 Phylogenetic tree of *Fragaria vesca* G-type lectin genes. Different background colors indicate the six different clades. Symbols (circle, star, triangle, rectangular) at the end of each branch indicate the domain arrangement of each gene. Circle in different colors represent different domain arrangements of G-LecRKs; stars represent domain arrangement of G-LecKs; triangle in different colors represent different domain arrangement of G-LecRPs; rectangular in different colors represent different domain arrangements of G-LecPs. Numbers on each branch show the bootstrap.

To make it convenient to refer to the *F. vesca* G-lectin genes, we proposed a nomenclature based on the similarity shown in the phylogenetic tree (Table 1), where the genes included in each clade were named following a sequential numbering. In the name, letters "Fve" indicate the gene is from the organism *F. vesca* (Jung *et al.*, 2015); "GLRK", "GLRP", "GLP" and "GLK" represent G-LecRK, G-LecRP, G-LecP, and G-LecK, respectively. Since FvH4_1g03780 did not fall in any of the clades, it was named *FveGLRK7.1*, to distinguish it from genes in the six clades. These FveG-Lectins' names will also be used herein the following sections.

Table 1. Proposed nomenclature for G-type lectin genes in *Fragaria vesca*

Clade	Gene ID	New name	Clade	Gene ID	New name
Clade I	FvH4_2g29560	FveGLRK1.1	Clade IV	FvH4_6g00257	FveGLRK4.27
	FvH4_2g29545	FveGLRK1.2		FvH4_3g03322	FveGLP4.5

	FvH4_2g29543	FveGLRK1.3		FvH4_3g03350	FveGLRK4.28
	FvH4_2g29544	FveGLRK1.4		FvH4_6g00300	FveGLP4.6
	FvH4_2g29542	FveGLRK1.5		FvH4_3g06140	FveGLRK4.29
Clade II	FvH4_6g31370	FveGLRK2.1	Clade V	FvH4_6g51830	FveGLRK5.1
	FvH4_4g02170	FveGLRK2.2		FvH4_6g44064	FveGLRK5.2
	FvH4_1g04840	FveGLRK2.3		FvH4_6g44101	FveGLP5.1
	FvH4_3g15150	FveGLP2.1		FvH4_6g44100	FveGLRK5.3
	FvH4_3g15130	FveGLRK2.4		FvH4_6g44062	FveGLP5.2
	FvH4_3g15120	FveGLRK2.5		FvH4_6g44063	FveGLRK5.4
	FvH4_3g15090	FveGLRP2.1		FvH4_6g44109	FveGLRK5.5
	FvH4_3g15080	FveGLRK2.6		FvH4_6g44240	FveGLK5.1
Clade III	FvH4_3g03590	FveGLRK3.1		FvH4_6g44140	FveGLRK5.6
	FvH4_2g24770	FveGLP3.1		FvH4_6g44107	FveGLRK5.7
	FvH4_2g05942	FveGLP3.2		FvH4_6g44106	FveGLRK5.8
	FvH4_3g03461	FveGLRK3.2		FvH4_6g44190	FveGLRK5.9
	FvH4_3g03435	FveGLP3.3		FvH4_6g44242	FveGLP5.3
	FvH4_3g03450	FveGLRK3.3		FvH4_6g44108	FveGLRK5.10
	FvH4_3g03451	FveGLRK3.4	Clade VI	FvH4_6g44310	FveGLRK6.1
	FvH4_3g03481	FveGLRK3.5		FvH4_6g26450	FveGLRK6.2
	FvH4_3g03432	FveGLRK3.6		FvH4_6g26420	FveGLRK6.3
	FvH4_3g03431	FveGLRK3.7		FvH4_7g14760	FveGLRK6.4
	FvH4_3g03581	FveGLRP3.1	Clade VI	FvH4_5g31680	FveGLRK6.5
	FvH4_3g03560	FveGLRK3.8		FvH4_5g31690	FveGLP6.1
	FvH4_5g32570	FveGLRK3.9		FvH4_2g26490	FveGLRK6.6
	FvH4_3g03433	FveGLRK3.10		FvH4_2g29070	FveGLRK6.7
	FvH4_3g03521	FveGLRK3.11		FvH4_2g29050	FveGLRK6.8
	FvH4_3g03520	FveGLRK3.12		FvH4_6g29840	FveGLRK6.9
	FvH4_5g31930	FveGLRK3.13		FvH4_6g29821	FveGLRK6.10
	FvH4_1g16211	FveGLRK3.14		FvH4_6g17930	FveGLRP6.1
	FvH4_3g03502	FveGLRK3.15		FvH4_6g10470	FveGLRP6.2
	FvH4_3g03501	FveGLRK3.16		FvH4_3g18370	FveGLP6.2
	FvH4_3g03482	FveGLRK3.17		FvH4_3g18382	FveGLP6.3
Clade IV	FvH4_6g44243	FveGLRK4.1		FvH4_3g18380	FveGLP6.4
	FvH4_6g44260	FveGLP4.1		FvH4_3g18371	FveGLP6.5
	FvH4_6g44245	FveGLRK4.2		FvH4_3g18410	FveGLP6.6
	FvH4_6g44244	FveGLRK4.3		FvH4_3g18383	FveGLP6.7
	FvH4_3g03430	FveGLRK4.4		FvH4_7g30670	FveGLRK6.11
	FvH4_3g03420	FveGLRK4.5		FvH4_6g26380	FveGLRK6.12
	FvH4_3g21271	FveGLP4.2		FvH4_2g12390	FveGLRK6.13
	FvH4_3g21270	FveGLRK4.6		FvH4_1g23400	FveGLRK6.14
	FvH4_6g12332	FveGLP4.3		FvH4_1g23390	FveGLRK6.15
	FvH4_3g21400	FveGLRK4.7		FvH4_2g14250	FveGLRK6.16
	FvH4_3g21310	FveGLRK4.8		FvH4_1g23380	FveGLRK6.17
	FvH4_3g15690	FveGLRK4.9		FvH4_1g23370	FveGLRK6.18
	FvH4_3g21320	FveGLRK4.10		FvH4_7g00200	FveGLRK6.19
	FvH4_3g15980	FveGLK4.1		FvH4_3g43403	FveGLRK6.20
	FvH4_3g15930	FveGLRK4.11		FvH4_3g43440	FveGLRK6.21
	FvH4_3g03310	FveGLRK4.12		FvH4_3g43402	FveGLRK6.22
	FvH4_3g03243	FveGLRK4.13		FvH4_3g43710	FveGLRK6.23

	FvH4_3g03301	FveGLRK4.14		FvH4_3g43401	FveGLRK6.24
	FvH4_3g03300	FveGLK4.2		FvH4_4g33230	FveGLRK6.25
	FvH4_3g03230	FveGLRK4.15		FvH4_6g12930	FveGLRK6.26
	FvH4_3g03242	FveGLRK4.16		FvH4_6g12890	FveGLRK6.27
	FvH4_6g07960	FveGLRK4.17		FvH4_5g04350	FveGLRK6.28
	FvH4_3g03240	FveGLRK4.18		FvH4_5g04310	FveGLP6.8
	FvH4_3g03231	FveGLRK4.19		FvH4_5g04270	FveGLP6.9
	FvH4_7g19680	FveGLRK4.20		FvH4_6g12870	FveGLRK6.29
	FvH4_3g03241	FveGLK4.3		FvH4_6g12920	FveGLP6.10
	FvH4_3g03410	FveGLRK4.21		FvH4_6g12880	FveGLRK6.30
	FvH4_3g03390	FveGLRK4.22		FvH4_2g33870	FveGLRK6.31
	FvH4_3g03370	FveGLRK4.23		FvH4_2g33830	FveGLRK6.32
	FvH4_6g20800	FveGLRK4.24		FvH4_2g33850	FveGLRK6.33
	FvH4_3g03320	FveGLRK4.25		FvH4_2g33840	FveGLRK6.34
	FvH4_3g03340	FveGLP4.4	singleton	FvH4_1g03780	FveGLRK7.1
	FvH4_6g00270	FveGLRK4.26			

3.1.3. Chromosome location and duplication of G-lectin genes

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

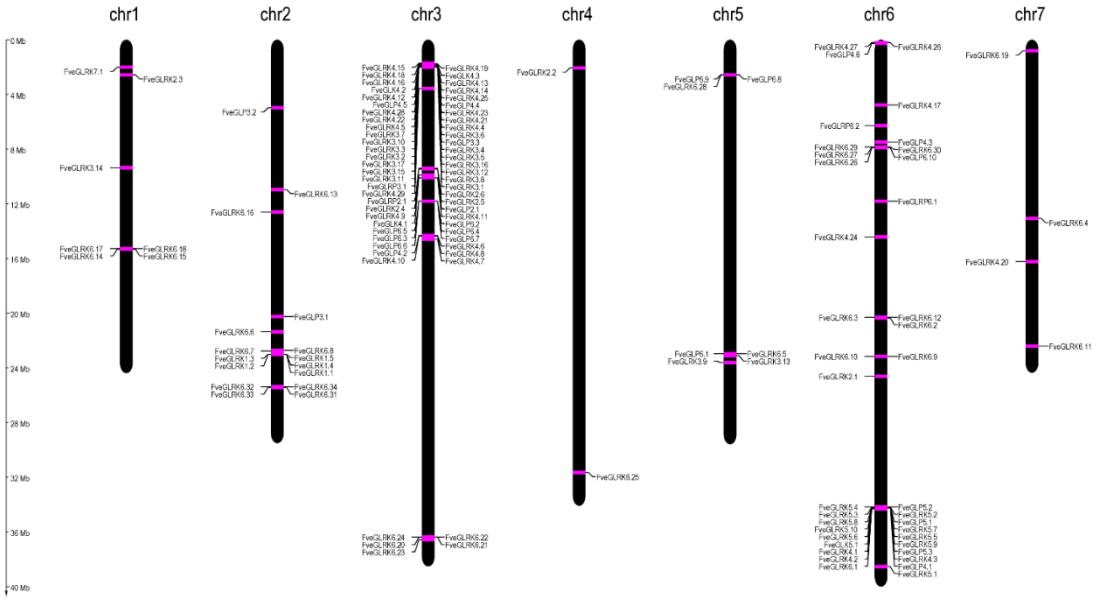

Fig. 6 Chromosome localization of G-LecRKs, G-LecKs, G-LecRPs, and G-LecPs in *Fragaria vesca*

Tandem and dispersed duplication are two modes of single gene duplication: i) tandem duplication, which generates consecutive gene copies in the genome and is believed to originate from unequal chromosomal crossing over (Wang *et al.*, 2012); ii) dispersed duplication, which

occurs with un-predictable and random patterns by still unclear mechanisms, resulting in two gene copies that are neither neighbors nor colinear (Qiao *et al.*, 2019). Out of the 133 G-lectin genes, 86 of them appear as duplicated genes, with either tandem duplication (55 genes) or dispersed duplication (51 genes) or both (20 genes), showing that duplication events are common in G-lectin family of strawberry, thus leading to the expansion of G-lectin family in strawberry (Fig. 7). Genes from the same tandem duplicated cluster (Supplemental file S1) are usually close on the phylogenetic tree, dispersed duplicated genes are also close on the tree (Supplemental file S2). The duplication events of *F. vesca* G-lectin genes were visualized by chord diagram (Fig. 8). These duplications are not evenly distributed on seven chromosomes of *F. vesca*, chromosome 3 and chromosome 6 show more duplications compared with the other chromosomes. No tandem duplication of G-type lectin genes on chromosome 4 or chromosome 7 is found.

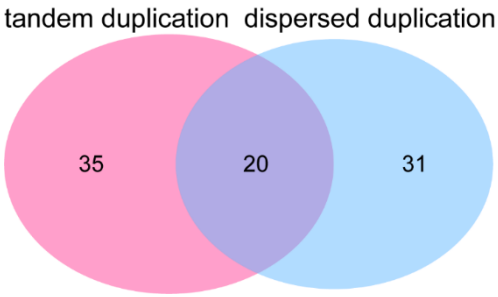

Fig. 7 Tandem and dispersed duplication of the G-lectin genes of *Fragaria vesca*.

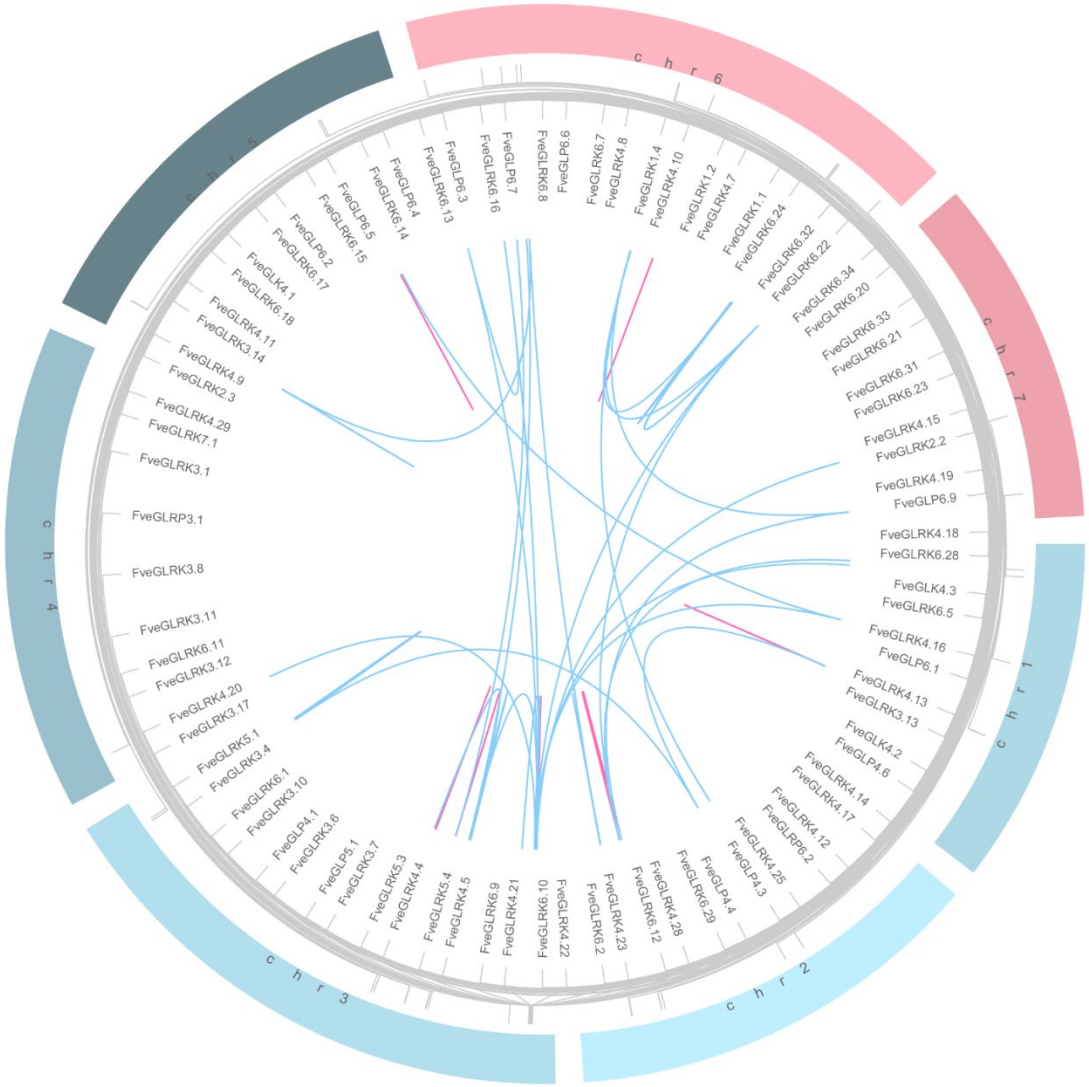

Fig. 8 Tandem and dispersed duplication events on G-type lectin genes among 7 chromosomes of *Fragaria vesca*. The different chromosomes are indicated in different colors. Genes connected by pink lines are tandem duplicates, while those lines connected by blue lines are dispersed duplicates.

3.2. Gene expression

3.2.1. G-lectin gene expression during development and under stress conditions

The expression profile of G-lectin genes was analyzed in different tissues and at different developmental stages, based on available strawberry RNA-seq datasets. As shown in the heatmap (Fig. 9), G-type Lectin genes display a wide range of transcription levels among the different tissues, some of them are highly expressed in various tissues, while others completely silenced (Fig. 9). Among the highly expressed genes, many of them belong to the G-LecRK group. Few genes appear specifically expression only in one or two tissues, as, for instance, *FveGLRP2.1* which is highly expressed in pollen and anthers, but not in the other

tissues. On the contrary, *FveGLP6.4*, the homolog of a known *F.* x *ananassa* G-type lectin, *FaMBL1*, showed active expression in many tissues during development. In general, G-lectin genes are more expressed in the ovary wall, seedling, style, root, and leaf, while they are hardly expressed in cortex and embryo (Fig. 9).

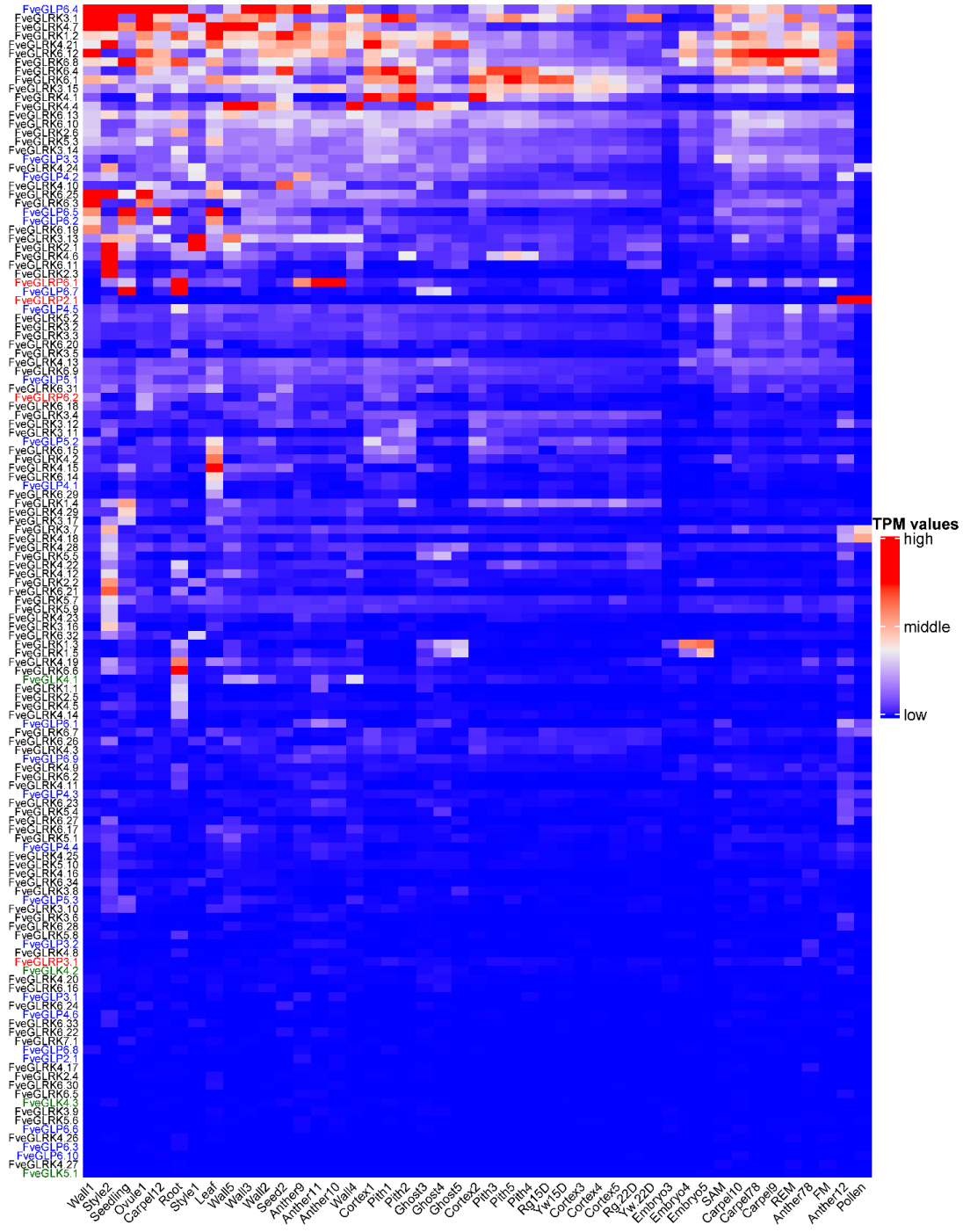

Fig. 9 Expression profiles of G-type lectin genes in *Fragaria vesca*. The expression profiles of different types of G-lectin genes are indicated in different colors: G-LecRKs in black, G-LecPs in blue, G-LecRPs in red, and G-LecKs in green. The expression levels are indicated by TPM (transcript per

million reads) and the TPM value for each gene is scaled before constructing the heatmap. Yw.22D: white fruit stage of Yellow Wonder 5AF7 (receptacle tissues at 22 days post-anthesis), Rg.22D: white fruit stage of Ruegen, Yw.15D: green fruit stage of Yellow Wonder 5AF7 (receptacle tissues at 15 days post-anthesis), Rg.15D: green fruit stage of Ruegen, Wall: ovary wall, Ghost: entire seed with its embryo removed (seed coat), REM: receptacle meristem, FM: flower meristem, SAM: shoot apical meristem

The log2 fold changes of G-lectin genes' expression under stresses were visualized in a heatmap. G-lectin genes were also found differently expressed during the interaction with pathogenic fungi, *Botrytis cinerea*, *Podosphaera aphanis* as well as Oomycete*, Phytophthora cactorum* (Fig. 10). Around 50 G-lectin genes were differentially expressed during the interaction with *P. cactorum* in strawberry root and most of them were upregulated. Several G-lectin genes were also found upregulated in response to *P. aphanis* infection at 8 days post-infection. Compared to these two pathogens, few G-lectin genes were transcriptionally altered upon *B. cinerea* infection (Fig. 10). Moreover, some G-lectin genes appear to be regulated by plant resistance elicitor, benzothiadiazole, and chitosan. Interestingly, genes like *FveGLP6.4* (homolog of *FaMBL1*) were upregulated upon *B. cinera*, *P. cactorum*, and *P. aphanis* infection as well as by the inducers, benzothiadiazole, and chitosan. With regard to abiotic stress, cold stress caused both upregulation and downregulation of several G-lectin genes (Fig. 10).

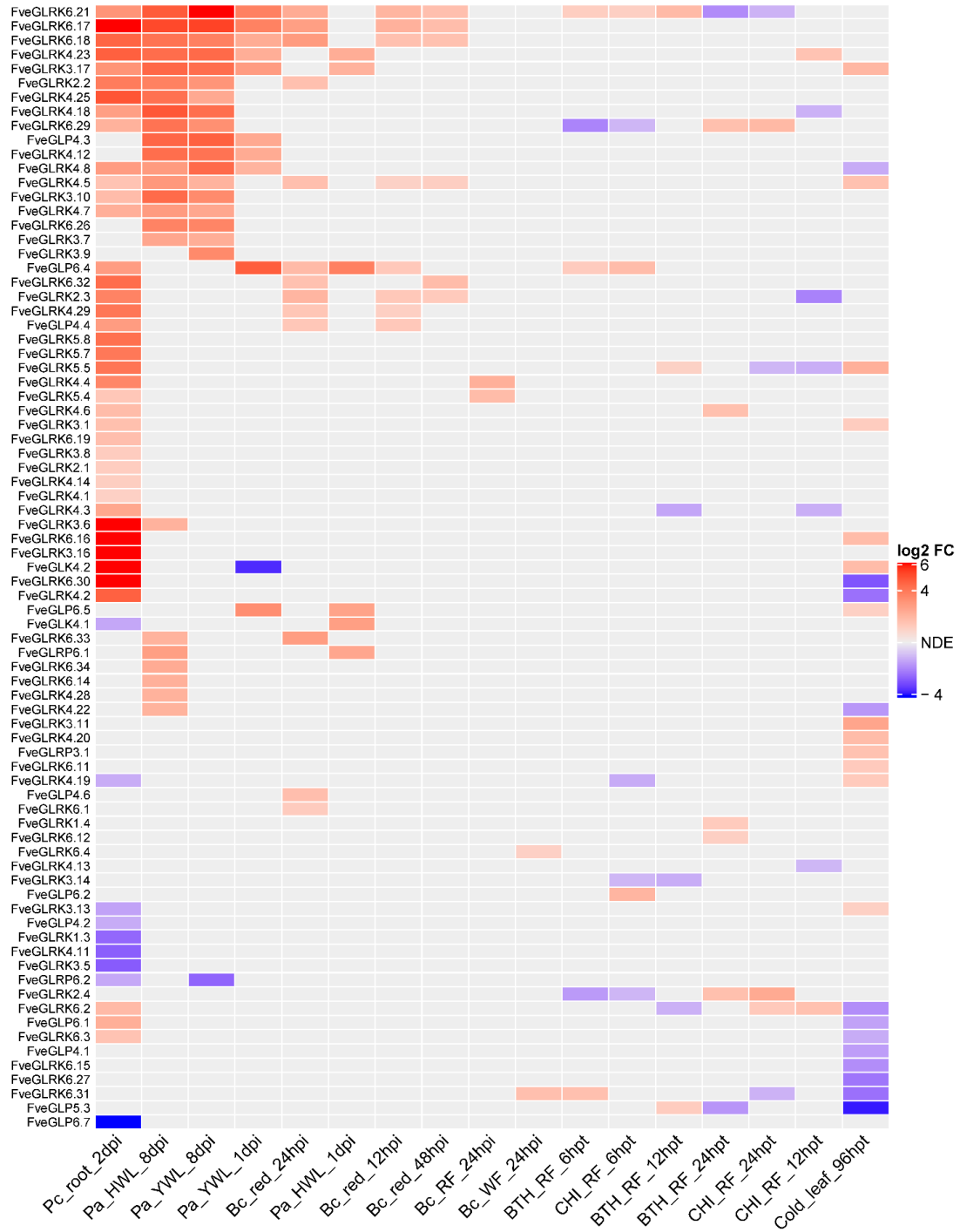

Fig. 10 Differently expressed strawberry G-lectin genes after challenged by pathogens and treated with inducers and cold. Data are expressed using log2 FC; negative value represents downregulation; positive value represents upregulation; and NDE represents no different expression. WF: white fruit; RF and red: red fruit; HWL: leaves of strawberry cv. Hawaii 4; YWL: leaves of Yellow Wonder 5AF7; Bc: Botrytis cinerea; Pc: Phytophthora cactorum; Pa: Podosphaera aphanis; BTH: benzothiadiazole; CHI: chitosan; Cold_leaf: cold stressed leaf of seedlings; h/dpi: hours/days post-inoculation; hpt: hours post-treatment.

3.2.2. G-lectin gene co-expression prediction

To further get insights into the function of strawberry G-type lectins, the genes co-expressed with FveG-lectin genes were retrieved from the co-expression database (www.fv.rosaceaefruits.org) (Shahan et al., 2018). Genes with functions in different plant defense pathways were predicted to co-express with G-lectin genes (Supplemental file S3). Some G-lectin genes, for instance, *FveGLRK2.3* and *FveGLRK4.18*, were predicted to co-express with other receptor-like kinases including cysteine-rich RLK, Leucine-rich receptor-like protein kinase, receptor-like protein 1, receptor-like protein 7. G-type lectins such as *FveGLRK1.1*, *FveGLRK4.13*, and *FveGLRK3.4* were predicted to co-express with disease resistance proteins, including NB-ARC domain-containing disease resistance protein, CC-NBS-LRR class family, TIR-NBS-LRR class family. *FveGLRK6.24*, *FveGLRK6.22*, and *FveGLRK2.3,* were predicted to co-express with many genes to make up a big defense co-expression network. For example, *FveGLRK2.3* was predicted to co-express with genes coding cysteine-rich RLK, Leucine-rich RLK, glutamate receptor 2.7, beta-glucosidase 13, and many more (Supplemental file S3).

3.3. Subcellular location

The subcellular localization of strawberry G-lectin proteins was predicted by CELLO and TargetP (Supplemental file S4). CELLO uses a reliable index to compare the possibility of different subcellular locations. Based on the CELLO prediction, all G-LecRKs had a higher reliable index of being located at the plasma membrane except for *FveGLRK6.31* and *FveGLRK4.13*, which were predicted to be located on the extracellular compartment. Conversely, almost all G-LecPs were located on the extracellular compartment. TargetP localization prediction is based on the presence of a signal peptide which drives proteins into the secretory pathway. According to this prediction, most *F. vesca* G-lectin proteins are driven to the secretory pathway, which is consistent with the prediction of CELLO, for which most of *F. vesca* G-lectin genes encode for proteins that are located on the plasma membrane or extracellular compartments.

4. Discussion

In plants, G-type lectin is a big gene family that is believed to play roles in biotic and abiotic stresses (Ghequire *et al.*, 2012; Siripipatthana *et al.*, 2015). Their role in defense was also reported in strawberry. For instance, 34 G-type LecRK genes were found upregulated in *F. vesca* root after *P. cactorum* inoculation (Toljamo *et al.*, 2016), and the G-type lectin gene

FaMBL1 was found involved in *F.* x *ananassa* resistance against *C. acutatum* (Guidarelli *et al.*, 2014). A study about strawberry Serine/Threonine Kinase disease resistance gene family showed that many Serine/Threonine Kinase genes belong to G-type LecRK (Martínez Zamora *et al.*, 2008), but insights about the genomic organization of G-lectin proteins in strawberry was still limited. Recently, high-quality *F. vesca* genome annotation provided a good chance for the genome-wide study of G-lectin genes in *F. vesca*.

To identify G-lectin encoding genes, we used only sequences of the GNA domain as a query rather than the whole sequence. This choice was made to avoid using the kinase domain sequence as a query, which would lead to much ambiguity in G-lectin identification. Eventually, 133 proteins were found belonging to the G-lectin family in *F. vesca* and the majority (102 out of 133) of G-lectins contained kinase domain belonging to the G-LecRK class. Four genes containing both GNA and kinase domain, but lacking TM domain, were classified into G-LecK. The lack of TM domain may lead to function alteration of these G-lectins.

TM domains are required for the plasma membrane localization of G-LecRKs (Chen *et al.*, 2006; Sun *et al.*, 2020). In rice, a single amino acid substitution (Ile144Met) in the TM domain of *Pi-d2,* a rice G-LecRK conferring resistance to *M. grisea* strain ZB15, made the plant susceptible to the strain ZB15, suggesting that the TM domain of *Pi-d2* may participate in the ligand recognition and signal transduction (Chen *et al.*, 2006). Indeed, the substitution did not change the plasma membrane location of *Pi-d2*, so the altered structure of the mutated TM may have lost or modified its ligand-binding function and signal transduction from the extracellular domain to the intercellular kinase catalytic domain (Chen *et al.*, 2006). This fact implies that the TM domain of G-lectin has a role in both membrane localization and signal transduction. However, most of G-LecPs in *F. vesca,* despite lacking TM domains, were also predicted to anchor to the plasma membrane. In this regard, a pepper G-LecP, *CaMBL1*, consisting of GNA domain and PAN domain and regulating plant defense to bacterial *X. campestris pv vesicatoria*, was reported to be located on plasma membrane (Hwang & Hwang, 2011). Moreover, the transient expression of *CaMBL1* induces the accumulation of salicylic acid and the activation of defense-related genes, which indicates a role in defense signaling, although without TM and kinase domain (Hwang & Hwang, 2011). These data show that despite most of the previous studies on G-lectins focused on G-LecRKs, studies on G-LecPs could also cover important functions in plant.

Except for GNA, TM, and kinase domains, G-Lectins also contain some of SLG, PAN, and EGF domains. The various domain arrangements of G-lectins create an enormous degree of protein diversity. Proteins consisting of arrangements with PAN and SLG domains have GO

functions related to the recognition of pollen, protein phosphorylation, and cell recognition which make these proteins important in reproduction and in general in signal perception or/and transduction (Kersting *et al.*, 2012). Multiple domain proteins are more species-specific compared with single-domain proteins, which are commonly shared among many plant species (Kersting *et al.*, 2012). In *F. vesca*, more than 90% of G-type lectins were found to belong to multiple domain proteins. These species-specific domain arrangements might be a consequence of frequent duplication events followed by lineage-specific retention (Paterson *et al.*, 2010). This is consistent with our result where a big portion of *F. vesca* G-lectin genes appear to originate from duplication and various domain arrangements. The various domain arrangements of G-type lectins could be considered as a kind of flexible genetic mechanism to produce species-specific adaptation to changing environments (Kersting *et al.*, 2012).

Tandem and dispersed duplication significantly contribute to the expansion of the G-lectin gene family in *F. vesca.* More than half of G-type lectin genes of *F. vesca* originate from duplication events. Chromosome 3, where the highest number of G-type lectin genes is located, showed a big number of duplication events of G-type lectin genes. Conversely, no tandem duplication event on G-type lectin genes on chromosome 4 and chromosome 7 was found and these two chromosomes also contain fewer G-type lectin genes than other chromosomes. Species-specific expansion of the G-type lectin gene family was also reported in a study about lectins in soybean, rice, and *Arabidopsis* where tandem and segmental duplications have been regarded as the major mechanisms to drive lectin expansion (Jiang *et al.*, 2010). Consistently, a study about lectin genes in cucumber also revealed that 106 out of 146 genes (76.8%) were involved in the tandem duplication events (Dang & Van Damme, 2016).

According to the transcriptome data, many G-lectin genes, no matter G-LecRKs or G-LecPs, are actively expressed on different tissues at different developmental stages of strawberries. G-lectins in *F. vesca* actively respond to pathogens, abiotic stress, and elicitors; and some G-lectin genes appear to respond to both biotic and abiotic stress. Up to now, only one G-lectin gene, *FaMBL1* (homolog of *FveGLP6.4*) was studied for its involvement in resistance against pathogens in strawberries (Guidarelli *et al.*, 2014); however, the molecular mechanism underneath is not yet elucidated. *FveGLP6.4* appears to be not only expressed in several tissues of strawberry during its development but also found upregulated after challenges by *B. cinerea*, *P. aphanis*, and *P. cactorum* pathogens (Fig. 9 and Fig. 10), implying the involvement of *FveGLP6.4* in *F. vesca* (or *FaMBL1* in *F.* x *ananassa*) in plant defense.

The molecular features of some G-type lectins from other plant species are better known: *Pi-d2* (Chen *et al.*, 2006), LORE (Ranf *et al.*, 2015), *OsSIK2* (Chen *et al.*, 2013), and

CaMBL1 (Hwang & Hwang, 2011), which could regulate plant defense responses, were proved to be located at the plasma membrane by using confocal microscopy. For *CaMBL1*, its ability of mannose affinity and the importance of GNA domain for its localization are known (Hwang & Hwang, 2011). According to the study, *CaMBL1* has affinity toward Manα and/or Manβ and GalNAc residues, and GNA domain is essential for its binding to D-mannose. A preliminary working model of *OslecRK* was also proposed by Cheng *et al.* (Cheng *et al.*, 2013). Here sensing of biotic stress first stimulates *OslecRK* expression, followed by the interaction of its kinase domain with *OsADF* (actin-depolymerizing factor) to transduce the signals. Following these events, the expression of defense-related genes (*PR1a*, *LOX* and *CHS*) was induced to strengthen the plant's immune response.

To further predict the function of G-lectins in *F. vesca*, we retrieved the genes predicted to co-express. G-lectin genes could co-express with other G-lectin genes, receptor kinase, and disease resistance genes which provides clues for uncovering their function.

5. Conclusions

In conclusion, G-type lectin is a big gene family in *F. vesca* with various domain arrangements and great potential in strawberry defense to biotic/abiotic stresses. G-lectin genes deserve additional studies to understand their role in-depth and their functioning mechanisms. Studying mannose-binding ability and identifying downstream interacting proteins of G-type lectins are important to uncover their role in strawberry.

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Chapter 4

Functional characterization of *FaMBL1* gene in defense response of *Fragaria* × *ananassa* to fungal pathogens

Abstract

Strawberry (*Fragaria* × *ananassa*) is an important soft fruit but easily get infected by pathogens. Anthracnose and gray mold are two of the most destructive diseases of strawberry which lead to serious fruit rot. As it is known, white unripe strawberry fruits are more resistant to *Colletotrichum acutatum* than red ripe fruits. During the interaction between unripe and ripe strawberry fruits and *C. acutatum*, a mannose-binding lectin gene *FaMBL1* was found to be the most upregulated gene and induced exclusively in white fruit. *FaMBL1* belongs to the G-type lectin family, which has important roles in plant development and defense process. Hence, stable transgenic strawberry plants overexpressing the *FaMBL1* gene were generated. Transformed strawberry plants were selected and molecularly characterized through droplet digital PCR and RT-PCR analysis. In total three overexpressing lines with different copy numbers were obtained and used for the subsequent studies, including the evaluation of disease-related phytohormones content and their reaction to biotic stresses. Accordingly, jasmonic acid (JA) content was found decreased in overexpressing lines compared to wild type (WT). Petioles of overexpressing lines inoculated by *C. fioriniae* had lower disease incidence than WT, and leaves of overexpressing lines challenged by *B. cinerea* showed remarkably smaller lesion diameters compared to WT. The chitinase 2-1 (*FaChi2-1*) gene showed higher expression in overexpressing lines than in WT during *B. cinerea* infection development. Our results show that *FaMBL1* gene plays a role in inducing disease resistance, probably JA-dependent.

Key words: anthracnose, gray mold, phytohormones, strawberry

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an economically important fruit worldwide and is considered as a model plant system for Rosaceae. It is susceptible to a large number of pathogens including *Colletotrichum acutatum* species complex and *Botrytis cinerea*, which cause enormous economic losses (Guidarelli *et al.*, 2011; Petrasch *et al.*, 2019).

Although both fungi can infect strawberry fruits at both unripe and ripe stages, the symptoms appear only on red ripe fruits since on white unripe fruits, the pathogens become quiescent. Transcriptome analysis of white and red fruits, inoculated with *C. acutatum*, revealed that a mannose-binding lectin gene, *FaMBL1* (GenBank accession number: KF962716), was the most up-regulated gene in resistant white fruit (Guidarelli *et al.*, 2011). With transient transformation of *FaMBL1* gene, *FaMBL1*-silenced white fruit showed an increased susceptibility to *C. acutatum* (Guidarelli *et al.*, 2014).

Proteins encoded by *FaMBL1* are composed of a N-terminal signal peptide, Galanthus nivalis agglutinin-related lectin (GNA) domain and Pan-apple domain. GNA domain is the characteristic domain of G-type lectin which is an important family of plant lectin having affinity to mannose or mannose-containing N-glycans (Barre *et al.*, 2001). Due to their ability of mannose recognizing and binding, G-type lectins have important functions in plant growth and defenses. Resistance role of G-type lectins against insects, fungi, and bacteria have already been described. For example, introducing G-type lectin genes in potato (Down *et al.*, 1996), maize (Wang *et al.*, 2005) and wheat (Miao *et al.*, 2011) plants increased their resistance to aphids by inhibiting development and decreasing their fecundity. The pepper G-type lectin genes *CaMBL1* and *CaGLP1* were also found involved in defense against *Xanthomonas campestris* pv *vesicatoria* and required for plant cell death and defense signaling (Hwang & Hwang, 2011; Kim *et al.*, 2015). The transfer of a G-type lectin, *Pi-d2*, to rice conferred resistance to a *Magnaporthe grisea* (Chen *et al.*, 2006). The contribution of lectins to plant resistance seems to be displayed in a phytohormone-dependent manner (Bonaventure, 2011; Gilardoni *et al.*, 2011). In fact, it is well known that the balance of hormonal crosstalk strongly influences the outcome of plant-pathogen interactions (Robert-Seilaniantz *et al.*, 2011).

The genome-wide analysis of G-type lectin genes implied great potential of these genes in strawberry defense and provided bases for functional characterization of *FaMBL1* gene. To get insight into the effect of *FaMBL1* on strawberry plant defense, genetically transformed strawberry plants overexpressing *FaMBL1* were generated and characterized.

2. Materials and methods

2.1. *Agrobacterium*-mediated genetic transformation of strawberry plants

The plasmid vector pK7WG2 (https://gatewayvectors.vib.be/), was chosen for overexpression of the *FaMBL1* gene in strawberry. The full length sequence of the *FaMBL1* gene was cloned into the pK7WG2 vector and the resulting construct overexpressing *FaMBL1* was checked by PCR, restriction enzyme digestion, and sequencing of the PCR product. The resulting vector, 35S:FaMBL1, was introduced into *Agrobacterium tumefaciens* strain EHA105 using the freeze–thaw shock method (Weigel & Glazebrook, 2006) to generate transgenic plants.

Expanded leaves from 3-week old *in vitro* elongated strawberry shoots (*Fragaria* × *ananassa* cv Sveva) were used as starting explant for the genetic transformation trial, following the protocol developed by Cappelletti et al. (Cappelletti *et al.*, 2015), with slight modifications. Briefly, single colonies of *A.tumefaciens* strain EHA105, harbouring the plasmid pK7WG2 expressing the gene construct 35S:FaMBL1, were grown overnight in liquid LB medium supplemented with kanamycin, on a shaker (150 rpm) at 28°C. The inoculum suspension was stopped as the OD_{600} reached a value between 0.5-1.

Strawberry leaves were cut perpendicular to the leaf mid vein and placed in contact with the infection solution for 15 minutes, then co-coltured for 48 hours in the dark. Strawberry explants were then transferred to regeneration and selection medium (4.4 mg L^{-1} MS salts and vitamins, 30 g L^{-1} sucrose, 7.5 g L^{-1} plant agar, 3 mg L^{-1} N6-benzyladenine, 0.2 mg L^{-1} indole-3-butyric acid, 200 mg L^{-1} Cefotaxime, 30 mg L^{-1} Kanamycin, Duchefa Biochemie) and maintained in dark condition for three weeks at 24 ± 2°C. Eventually, the explants were exposed to light (16h photoperiod at a light intensity of 70 mol m^{-2} s^{-1}) in the growth chamber at 24 °C, and sub-cultured every three weeks on fresh media. Putative transgenic lines of Sveva regenerated on selective medium were in vitro proliferated and moved to rooting medium for one month. In vitro rooted shoots were finally acclimatized in pots containing commercial peat, and grown in the greenhouse at 20°C with 16h light/ 8h dark.

2.2. Genomic DNA extraction, PCR, and droplet digital PCR

Genomic DNA (gDNA) was extracted from strawberry leaves (40 mg) of putative transgenic and WT lines using Wizard® Genomic DNA Purification Kit (Promega, Madison, USA) following the product manual. The gDNA was used for PCR and droplet digital PCR (ddPCR) analysis.

PCR was performed using gDNA to confirm the insertion of the 35S:FaMBL1 gene construct in the genome of the putative transformed lines. All primers, designed through Primer3 software (http://bioinfo.ut.ee/primer3/), used in this study are listed in Table 2. Secondary structures of the primers designed were checked by Beacon Designer™ (http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1). Primers designed on expressing vector, 35S:FaMBL1, are indicated in Fig. 11, P35S2 (forward primer) and attB2 (reverse primer) were used in overexpressing plants. The transgenic lines which could generate specific amplicons were used for the following test.

Table 2 List of primer name and primer sequences with their descriptions

Primer description	**Primer names**	**Primer sequences from 5' to 3'**
overexpressing vector	P35S2	ATTACAATTTACTATTCTAGTCG
	attB2	ACCACTTTGTACAAGAAA
ddPCR_overexpressing vector	ddPCRlec	ACCTTGACCAAAGTTGCCAACTCC
	T35S	TTTTGCGGACTCTAGCATGGCCG
ddPCR_reference gene	ddPCR-FaPGIP-3	GTCCTATACAACCATCCATCGC
	ddPCR-FaPGIP-4	GAGTTGGCTGATGAAGTCAGG
RT-PCR *FaMBL1* gene	FaMBL1_CUR fw	AAACCAACACGGCCAATAAG
	FaMBL1_CUR rev	GTCTGTCGGGTAGTCGAAGC
RT-PCR reference gene	FaEF1a fw	TGGATTTGAGGGTGACAACATGA
	FaEF1a rev	GTATACATCCTGAAGTGGTAGTCGGAGG
FaPAL1	FaPAL1 fw	TGGACTACGGCTTCAAAGGT
	FaPAL1 rev	GACATCTTGGTTGTGTTGCTC
FaPGIP	FaPGIP fw	ATCTCACAGGTCCCATCCAG
	FaPGIP rev	GCTGAGGAAGTCAGGGACTG
FaAOS1	FaAOS1 fw	AGGTTAAAGAAGGCGAGGTGTT
	FaAOS1 rev	GAGGACCGTTAGACCAAAGCA
FaChi2-1	FaChi2-1 fw	AAGCCCTCTTGTCACGATGT
	FaChi2-1 rev	TCGAGTCCACCGTTGATGAT
FaChi2-2	FaChi2-2 fw	ATGTG GGCGT GGACA AGATA
	FaChi2-2 rev	AACAG TCCAA GTTGT CCCCA
FaACO	FaACO fw	AGGTTAAAGAAGGCGAGGTGTT
	FaACO rev	GAGGACCGTTAGACCAAAGCA
FaTLP1b	FaTLP1b fw	GTGCATCACTTCTTCCAAGT
	FaTLP1b rev	CAAACCTGCTAGCAGTGAAG

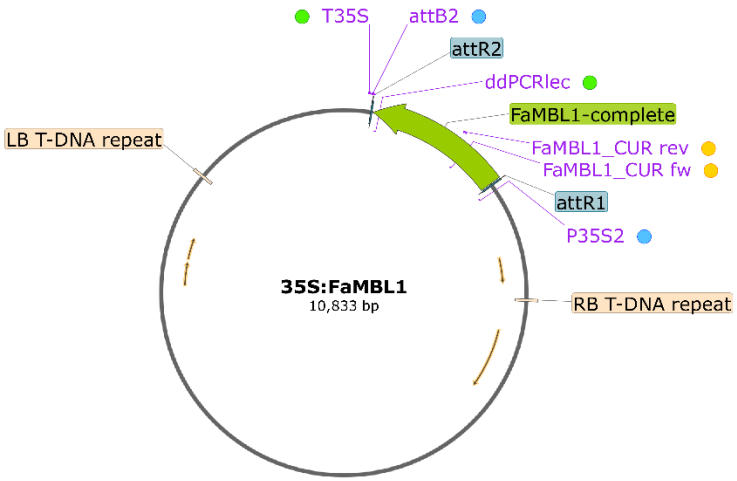

Fig. 11 The plasmid vector 35S:FaMBL1, generated by insertion of *FaMBL1* gene into the pK7WG2 plasmid. The positions of primers used for overexpressing vector are displayed.

ddPCR was performed to measure the copy number of the target gene in each transgenic line using Bio-Rad's QX100™ Droplet Digital PCR system, following the application guide of copy number variation analysis. To separate tandem gene copies and make proper random partitioning into droplets, gDNA was digested by EcoRI and HindIII restriction enzymes. After digestion, gDNA was quantified using a Qubit fluorometer and the dsDNA quantification kit (Invitrogen, California, USA), before diluting to a final concentration. Primer pairs of ddPCR were designed following the criteria specified by the instrument manufacturer. ddPCRlec (forward primer) and T35S (reverse primer) were used for overexpressing lines (Table 2, Fig. 11). *F.* × *ananassa* polygalacturonase-inhibiting protein (*FaPGIP*, accession number: EU117215.1) (Schaart, 2004) was used as a reference gene for ddPCR. *FaPGIP* was reported having a low copy number (Mehli *et al.*, 2004) which is good for the proper random partitioning of this gene into droplets and increasing accuracy of copy number measurement. The primer sets used for *FaPGIP* were ddPCR-FaPGIP-3 and ddPCR-FaPGIP-4 (Table 2). To identify a suitable annealing temperature that separates the positive and negative droplets, the ddPCR was designed to run across a thermal gradient (from 54 to 63°C) for overexpressing and reference genes. Accordingly, 60°C and 62°C were found to be optimal temperatures for amplifications of overexpressing vector and reference gene. At the optimal annealing temperature, formal ddPCR was conducted, followed by droplet reading using QX-200 droplet reader. The number of positive droplets was counted and the content of positive droplets in the whole reaction solution (20 µL) was calculated and shown as droplet μL^{-1}. Through ddPCR, the positive droplet content of overexpressing and reference gene were obtained, then the copy

number of overexpressing vector was calculated according to the formula: $CN_T = PC_T/PC_R \times CN_R$ (where CN_T: Copy Number of Target gene; CN_R: Copy Number of Reference gene; PC_T: Positive droplet Content of Target gene; PC_R: Positive droplet Content of Reference gene). Since the copy number of the reference gene *FaPGIP* is unknown, we used 1 to represent the copy number of *FaPGIP*. The copy number of overexpressing vector obtained in this study is, therefore, a relative value. The overexpressing lines with different copy numbers were subsequently propagated through stolons in the greenhouse, to obtain 30 plants for each transformed line, then subjected to the subsequent trials.

2.3. RNA extraction, cDNA synthesis, and quantitative PCR

Young leaves (150 mg) of transgenic and WT plants, of similar growth stage, were used for RNA extraction following a rapid CTAB method (Gambino *et al.*, 2008). The obtained RNA was treated with TURBO DNA-free™ Kit (Invitrogen, California, USA) for residual DNA removal. Purified RNA was quantified by NanoDrop 1000 Spectrophotometer (Thermo scientific, Waltham, USA) and RNA integrity was checked on an agarose gel. Then RNA was used for first-strand cDNA synthesis through ImProm-II™ reverse transcription system (Promega, Madison, USA).

Quantitative PCR (qPCR) was conducted to evaluate the expression level of the *FaMBL1* gene of overexpressed lines and WT strawberry plants. The housekeeping gene, *elongation factor 1a* (*FaEF1a*, accession number: BK009992.1) was used as a reference gene (Guidarelli *et al.*, 2011). The primers *FaMBL1*_CUR fw and *FaMBL1*_CUR rev were used to amplify *FaMBL1* gene. qPCR was performed using three biological replicates and two technical replicates, using SYBR™ Green PCR Master Mix (Thermo Fisher, Waltham, USA) and Mx3000P qPCR Systems (Agilent, California, USA). The gene expression was calculated using a standard curve and normalized by *FaEF1a*.

2.4. Hormone Profiling of *FaMBL1* overexpressing plants

In order to investigate the contents of defense-related phytohormones in *FaMBL1* overexpressing lines and WT, phytohormones were extracted (Gaetan *et al.*, 2014) from strawberry leaves of transgenic and WT plants propagated from stolons. Three young and fully expanded leaves from each line were pooled together as one replicate, and three replicates were set for each line. Leaves were frozen immediately in liquid nitrogen and ground into fine powder under liquid nitrogen. 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) analysis, according to the protocol of Gaetan and his co-workers (Gaetan *et al.*, 2014) with the following specific steps: one hundred mg tissue powder of each sample was used for hormone extraction and the extract was used for SA, ABA, JA and JA-Ile measurement. Fifty mg tissue was used for IAA extraction. Hormone concentrations of tested samples were calculated based on a calibration curve. Five calibration points were set at 0.1, 0.5, 2, 20 and 100 ng mL^{-1}, separately. Solution 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, $^{13}C6$-JA-Ile, and 1 µg mL^{-1} for d5-IAA. Hormone contents were indicated as ng g^{-1} fresh weight (ng g^{-1} FW).

2.5. Resistance evaluation of *FaMBL1* overexpressing plants

The resistance of *FaMBL1* overexpressing plants to anthracnose was evaluated. *C. fioriniae* was used for the inoculation trials which belongs to *C. acutatum* species complexes (Damm *et al.*, 2012) and was the most aggressive species to strawberry stocked in our lab. *C. fioriniae* was cultured on potato dextrose agar (PDA, in Petri dishes). After 10 days, conidia were harvested in distilled water and filtered through 3 layers of gauze. The concentration was determined using a hemocytometer and adjusted to 2×10^4 conidia mL^{-1}. Plants propagated from stolons were used for *C. fioriniae* inoculation. 5 petioles of similar growth stage were used for each overexpressing line (18F6G1, 19F2G1 and 27F8G1) and WT plants. Leaves were removed from petioles and both ends of petioles were embedded in moist tissue paper, to reduce desiccation. A tiny wound was made through a sterilized needle on each petiole and a droplet of inoculum (10µL of 2×10^4 conidia mL^{-1} spore suspension) was put on top of the wound. All inoculated petioles were put in plastic tray with moist tissue paper on the bottom. The tray was kept on the laboratory bench at room temperature. This inoculation trial was repeated three times. Disease progress was followed for six days.

For checking the resistance level of *FaMBL1* overexpressing lines against *B. cinerea* (isolate B05.10), conidia were harvested and the concentration was determined as mentioned above from a one-week-old *B. cinerea* cultured on PDA. To stimulate spore germination, a buffer containing 0.5% KH_2PO_4 and 1% PDA were prepared. Then the conidia suspension was mixed with buffer at the ratio of 1:1 (V:V) to get 1×10^4 conidia mL^{-1}. Plants used for *B. cinerea* inoculation were the same as those used for *C. fioriniae* inoculation. Young, healthy, and fully expanded leaves of similar growth stage were used. Five leaves (15 leaflets), from 5 different pots for each line, were surface sterilized, using 1% sodium hypochlorite for 1 min and rinsed in sterilized distilled water for 2 min. Then leaves were separated into leaflets and

put in Petri dishes (150mm × 15mm) with moist tissue paper on the bottom. Two droplets (7.5 µL droplet^{-1}) of inoculum were put on the adaxial side of the leaflet, on each side of the midrib. Distilled water was sprayed in each Petri dishes to keep high humidity. The Petri dishes with infected leaflets were kept on the laboratory bench at room temperature. Disease progress was followed for five days. This inoculation trial was repeated three times.

2.6. qPCR of *B. cinerea* inoculated leaves

To investigate *FaMBL1* overexpressing lines' responses at molecular level upon *B. cinerea* inoculation, expression levels of pathogenesis-related genes and hormone synthesis genes were measured. Six leaves (18 leaflets) of each transgenic line (18F6G1, 19F2G1 and 27F8G1) and WT were subjected to *B. cinerea* and mock inoculation. Conidia suspension and concentration was as mentioned above, while mock inoculation was done using the same volume of the buffer (0.25% KH_2PO_4 and 0.5% PDA). At 1 day post-inoculation, leaves were sampled and frozen immediately in liquid nitrogen, and stored at -80°C until use. *FaMBL1*, *FaPGIP* (EU117215.1), thaumatin-like protein 1b (*FaTLP1b*, XM_004287756.2), phenylalanine ammonia-lyase 1 (*FaPAL1*, KX450226.1), class II chitinases (*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) expression were measured by qPCR using the protocol mentioned above. The primers used for qPCR are listed in Table 2.

2.7. Statistical analysis

One-way ANOVA and Fisher test were used for the statistical analysis, using OriginPro 2018 statistical software, OriginLab Corporation, USA.

3. Results

3.1. Screening of genetically transformed plants

The above results suggest the importance of G-type lectin gene family in strawberry defense, especially *FveGLP6.4* gene (homolog of *FaMBL1*) showed great potential. Therefore, transformation experiments overexpressing *FaMBL1* gene were carried out. From the transformation experiments three lines (18F6G1, 19F2G1, and 27F8G1) were selected that were able to proliferate and root on MS medium supplemented with high concentrations of kanamycin. To confirm the correct insertion of the *FaMBL1* transgene the three lines were

assessed by PCR on gDNA. Specific amplicons were obtained from all three lines confirming that the target gene was successfully inserted into their genome (gel was shown in Fig. 12).

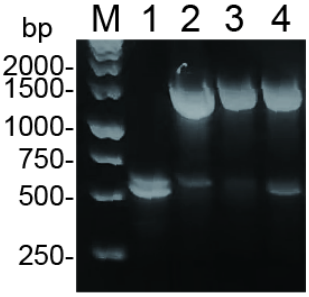

Fig. 12 Gel showing specific amplicons (1430bp) of *FaMBL1* overexpressing lines by PCR. Lane M, 1kb DNA ladder; lane 1, wild type; lane 2, 18F6G1; lane 3, 19F2G1; lane 4, 27F8G1.

The copy number of the target gene of each overexpressing line was detected by ddPCR and used to verify the clonal diversity between the different transgenic lines. The copy number of 18F6G1, 19F2G1, and 27F8G1 resulted 6, 2.5 and 1, respectively.

3.2. *FaMBL1* expression in overexpressing lines

The *FaMBL1* expression level of overexpressing lines (18F6G1, 19F2G1, and 27F8G1) was tested. All tested lines showed significantly higher *FaMBL1* expression compared to WT, both in leaves and stolons (Fig. 13). 19F2G1 and 27F8G1 had similar expression levels of *FaMBL1*, while 18F6G1 had a lower expression level among overexpressing lines.

These data indicated that there was no correlation between *FaMBL1* expression level and copy numbers. For instance, 27F8G1 had a higher *FaMBL1* expression level than 18F6G1, whereas it contained a lower copy number than 18F6G1 (1 versus 6). 19F2G1 and 27F8G1 harbored different copy numbers of the target gene (2.5 and 1, respectively), while they showed a similar level of *FaMBL1* expression (Fig. 13). This suggests that suppression of gene expression may occur in those lines that contain more *FaMBL1* copy numbers (Butaye *et al.*, 2005).

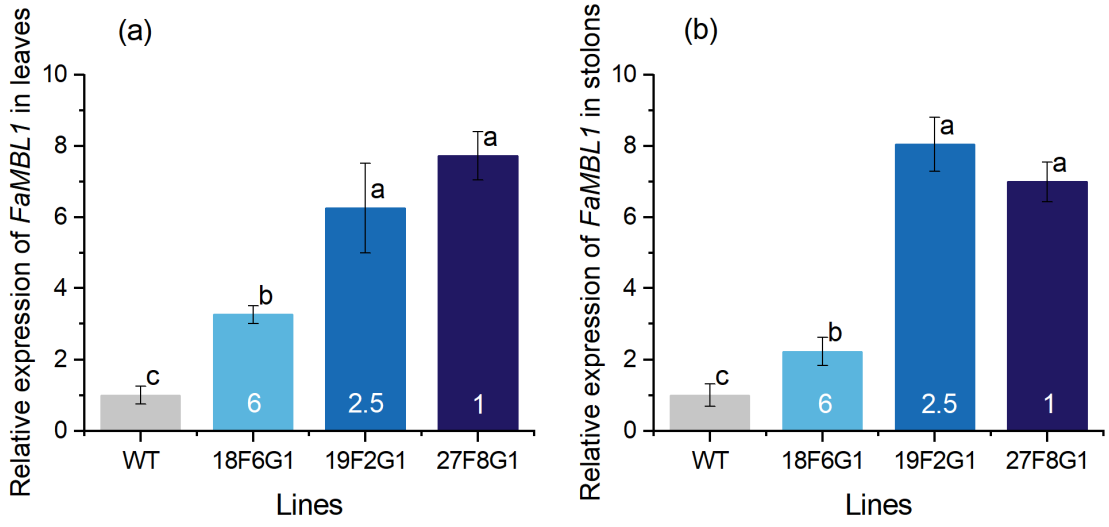

Fig. 13 Relative expression of *mannose-binding lectin* (*FaMBL1*) gene in (**a**) leaves and (**b**) stolons of strawberry plants. All values were normalized to the expression level of the *elongation factor 1α* housekeeping gene. Each value is an average of three biological replicates with its standard error. Means with the same letter are not significantly different at $p < 0.05$. Numbers on the column indicate the copy number of *FaMBL1* in each transgenic line.

3.3. Phytohormone contents in *FaMBL1* overexpressing lines

The amount of phytohormones were measured in overexpressing lines to get insight into the influence of *FaMBL1* on their production. The content of JA was lower in overexpressing lines compared to WT, though the effect was a bit less in conjugated JA (Fig. **14a, b**). On the other hand, the contents of IAA, ABA, and SA in transgenic lines were more or less similar to that of the WT (Fig. **14c, d, e**), suggesting that *FaMBL1* could probably participate more in the modulation of JA than the other classical hormones in strawberry.

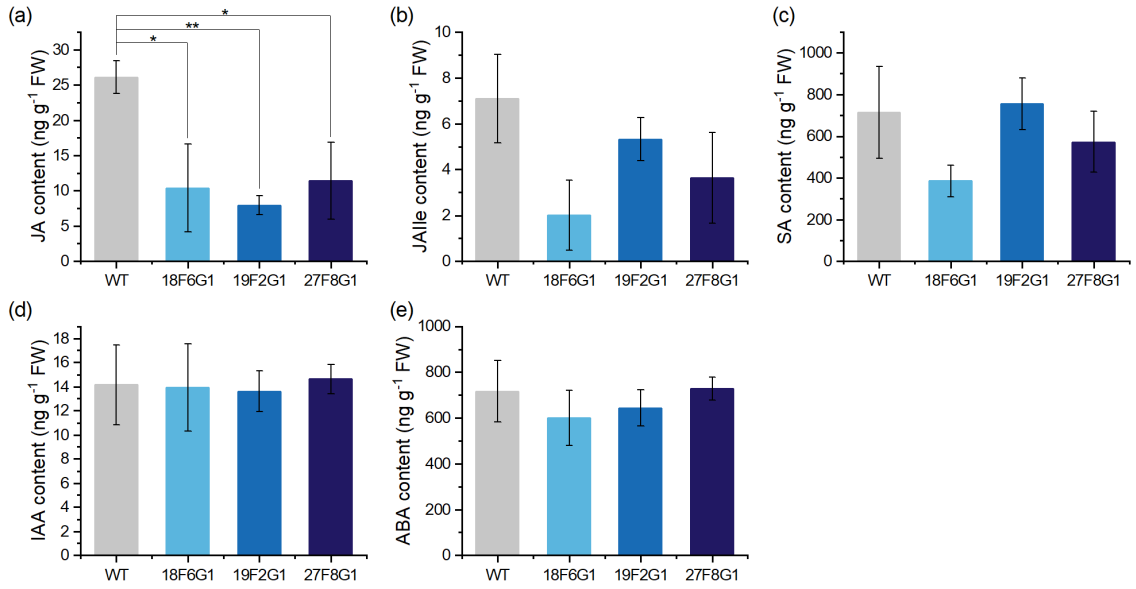

Fig. 14 Phytohormones content in strawberry leaves of *mannose-binding lectin* (*FaMBL1*) gene overexpressing lines and the corresponding wild type. (**a**) jasmonic acid (JA) content, (**b**) jasmonoyl-isoleucine (JA-Ile) content, (**c**) salicylic acid (SA) content, (**d**) indole-3-acetic acid (IAA) content, (**e**) abscisic acid (ABA) content. The content was expressed as ng g^{-1} fresh weight (FW).

3.4. Response of *FaMBL1* overexpressing lines to fungal inoculation

The response of the overexpressing lines to *C. fioriniae* inoculation was evaluated. The disease incidence was significantly lower in overexpressing lines as compared with the wild type (Fig. **15a**). As depicted in Fig. **15b**, infected petioles showed anthracnose symptoms at 4 dpi and followed by rapid disease progress at 5 and 6 dpi, when the symptom was more apparent. The inoculation trials were repeated three times and, as general rule, some petioles from overexpressing lines showed smaller lesion size compared to WT. These results thus suggest that the overexpression of *FaMBL1* gene decrease the incidence of strawberry anthracnose infection.

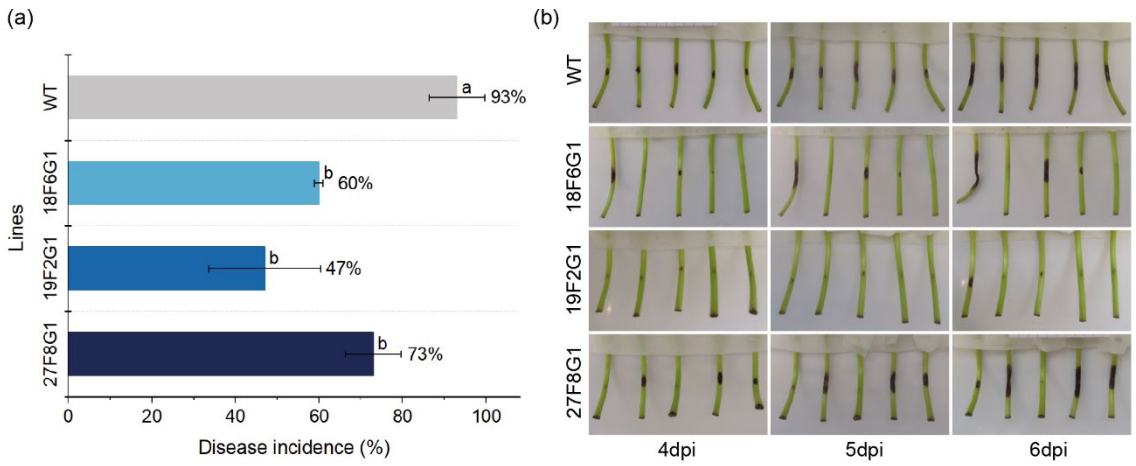

Fig. 15 Anthracnose progress on strawberry petioles. (**a**) Disease incidence of *Colletotrichum fioriniae* on strawberry petioles, at 6dpi. (**b**) *Colletotrichum fioriniae* inoculated strawberry petioles of *FaMBL1* overexpressing lines and wild type. For disease incidence, petioles with anthracnose lesions longer than 3mm were regarded as infected by *C. fioriniae* successfully. Disease incidence = number of petioles with anthracnose symptom/ number of petioles treated with *C. fioriniae* × 100%

Usually for strawberry, concerns are given on the resistance of fruits more than other parts of the plant due to their direct economic value, even though *B. cinerea* can infect both vegetative and reproductive tissues of strawberry. However, the infected vegetative tissues are important source of inoculum, thus improving the resistance of vegetative tissue is indispensable for the management of *B. cinerea*. Moreover, infection trials using leaves show uniform disease symptom and repeatable data which is ideal for resistance evaluation of transgenic lines. For this reason, the response to *B. cinerea* necrotrophic fungus of *FaMBL1* overexpressing lines was tested on detached leaves. From this experiment, the necrotic lesions started to show at 3 dpi in both transgenic lines and WT, however, the disease progress was higher in the WT than in the overexpressing lines (Fig. **16a**). The rate of lesion size increament was significantly higher in the WT than in the overexpressing lines (Fig. **16b**), indicating *FaMBL1* overexpressing lines were less susceptible to *B. cinerea* compared with the WT.

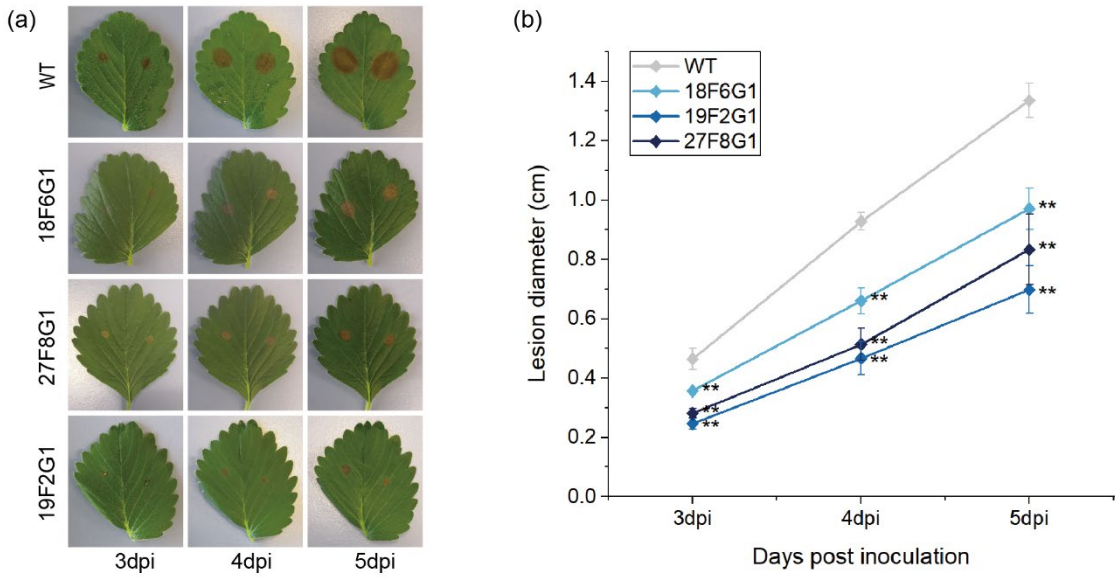

Fig. 16 Botrytis cinerea progress in detached strawberry leaves. (**a**) Appearance of B. cinerea lesions on detached strawberry leaflets. (**b**) Lesion size of detached strawberry (Fragaria × ananassa) leaves inoculated by B. cinerea. (**: P<0.01)

3.5. Defense-related genes expression after *B. cinerea* inoculation

To reveal the contribution of *FaMBL1* gene in strawberry defense against *B. cinerea*, relative expression of *FaMBL1* gene together with genes previously reported expressing upon *B. cinerea* infection and contributing to resistance of strawberry, including *class II chitinases* (*FaCHI2-1* and *FaCHI2-2*), *polygalacturonase-inhibiting protein* (*FaPGIP*), *phenylalanine ammonia-lyase 1* (*FaPAL1*), *thaumatin-like protein 1b* (*FaTLP1b*), *1-aminocyclopropane-1-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), were investigated in leaves of wild type strawberry and overexpressing lines at 1d after *B. cinerea* inoculation. The relative expression of *FaMBL1* gene in leaves with mock and *B. cinerea* inoculation is shown in Fig. **17a**. Despite that *FaMBL1* expression in both WT and overexpressing lines was depressed upon *B. cinerea* infection, the overexpressing lines still kept significantly higher level of *FaMBL1* than WT. The gene *chitinase2-1* (*FaChi2-1*) expression of overexpressing lines and WT were not statistically different (Fig. **17b**). On the other hand, after *B. cinerea* infection, *FaChi2-1* expression in overexpressing lines was significantly higher than in WT. The expression of the other tested genes didn't show any difference between overexpressing lines and WT. To reveal genes responses upon *B. cinerea* inoculation, their expression upon mock inoculation was used as control. Surprisingly, up-regulation of defense-related genes was not observed upon *B.*

cinerea inoculation. In contrast, expressions of *FaPAL1*, *FaPGIP*, *FaAOS1* (involved in JA synthesis), and *FaACO* (involved in ET synthesis) genes were decreased in both WT and overexpressing plants after inoculation (Fig. 17).

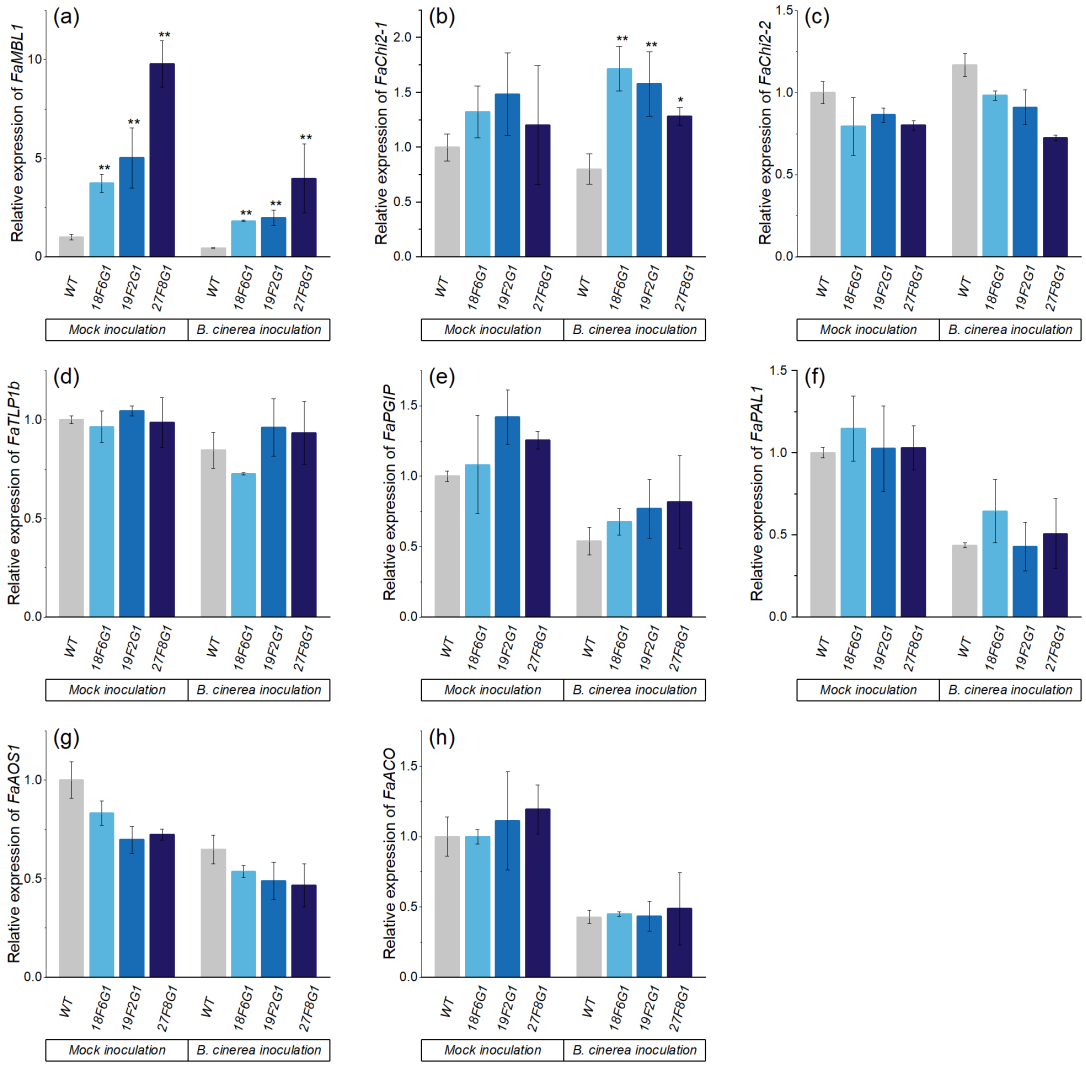

Fig. 17 Relative expression of defense-response genes in leaves of transgenic lines and wild type challenged with *Botrytis cinerea* at 1 day post inoculation. (**a**) relative expression of *mannose-binding lectin* (*FaMBL1*) gene, (**b**) relative expression of *class II chitinase 1* (*FaChi2-1*) gene, (**c**) relative expression of *class II chitinase 2* (*FaChi2-2*) gene, (**d**) relative expression of *thaumatin-like protein 1b* (*FaTLP1b*) gene, (**e**) relative expression of *polygalacturonase-inhibiting protein* (*FaPGIP*) gene, (**f**) relative expression of *phenylalanine ammonia-lyase 1* (*FaPAL1*) gene, (**g**) relative expression of *allene oxide synthase 1* (*FaAOS1*) gene, (**h**) relative expression of *1-aminocyclopropane-1-carboxylate oxidase* (*FaACO*) gene. All values were normalized to the expression level of the *elongation factor 1α* housekeeping gene. Each value is an average of three biological replicates with its standard error. *: P<0.05, **: P<0.01

4. Discussion

Anthracnose and gray mold are two of the most destructive strawberry fungal diseases. Increasing plant resistance is one of the most sustainable and effective strategies of strawberry disease management. However, the high level of heterozygosity and polyploidy of strawberries make it difficult to get improved through the traditional breeding methods (Nehra *et al.*, 1990; Limera *et al.*, 2017), and genetic transformation becomes an especially suitable way for studying strawberry resistance genes.

Given the potential of *FaMBL1* gene in strawberry defenses, we produced genetically transformed strawberry plants overexpressing *FaMBL1* to get insight into the role of *FaMBL1* on strawberry defenses. We obtained three overexpressing lines with different copy number and higher *FaMBL1* expression compared to WT plant. Their content of the major phytohormones, SA, JA, JAIle, IAA and ABA, were measured together with WT. Plant hormones play important roles in modulating plant resistance and susceptibility to pathogens (Robert-Seilaniantz *et al.*, 2011). In this study, JA content was found decreased in *FaMBL1* overexpressing lines compared to WT. This result implied that *FaMBL1* could possibly participate in the modulation of JA, which may contribute to the lower susceptibility of *FaMBL1* overexpressing lines to *B. cinerea* and *C. fioriniae*. SA signaling was believed serving as the core defense signaling mechanism against *Colletotrichum* spp.. SA content was increased and genes in SA pathway were activated after *C. fructicola* infection to induce defense responses in strawberry. Simultaneously, JA pathway was suppressed to assist the defense response of host plants due to the antagonism between SA and JA (He *et al.*, 2019). Consistently, transcriptome analysis revealed that strawberry genes related to SA were widely up-regulated, while genes related to JA and ET were largely inhibited after *C. fructicola* infection (Zhang *et al.*, 2018). Nevertheless, plant innate immunity relies also on pre-formed and induced defensive compounds (Amil-Ruiz *et al.*, 2011). Therefore, the strawberry defense responses were not only related to pre-formed phytohormones, but also the induced phytohormone responses depending on the species of pathogens. The phytohormone profile after pathogens infection should be measured in future study to decode the response of JA and other plant hormones upon pathogens. To date, limited studies reported G-type lectins being involved in phytohormone-related defense pathways. For example, *Nicotiana attenuata* G-type lectin receptor kinase1 has been identified to be used by the plant to suppress the *Manduca sexta*-triggered accumulation of SA, which allows for the induction of JA-mediated defense responses against *M. sexta* herbivory (Bonaventure, 2011; Gilardoni *et al.*, 2011). Additionally,

hydrophobicity plots showed that the rice G-type lectin, *Pi-d2*, contains a hydrophobic pocket that has the potential to bind hydrophobic ligands, such as plant hormones (Chen *et al.*, 2006), suggesting that hormones could be involved in the functioning of G-type lectins.

The less susceptibility of *FaMBL1* overexpressing lines to *B. cinerea* implies the possible role of *FaMBL1* in strawberry defense responses against pathogens. Interpreting the working mechanism of *FaMBL1* will help to improve the understanding of strawberry defense mechanism againts *B. cinerea* and the role of G-type lectins in strawberry. To date, genes like *class II chitinases* (*FaChi2-1* and *FaChi2-2*), *polygalacturonase-inhibiting protein* (*FaPGIP*), *phenylalanine ammonia-lyase 1* (*FaPAL1*), *thaumatin-like protein 1b* (*FaTLP1b*), *1-aminocyclopropane-1-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), have been reported as induced in expression upon *B. cinerea* infection and contribute to resistance of strawberry. Their expression together with *FaMBL1* were investigated in leaves of wild type strawberry and overexpressing lines at 1d after *B. cinerea* challenge. Despite the expression of *FaMBL1* being suppressed by *B. cinerea* inoculation, transgenic lines showed a higher level of *FaMBL1* than WT, which was probably related to the lower susceptibility of overexpressing lines. During interaction with *B. cinerea*, overexpressing lines also showed higher expression of *FaChi2-1* than WT. *FaChi* is one of the most abundant classes of strawberry PR genes with hydrolytic activity (Amil-Ruiz *et al.*, 2011). *FaChi2-1* was involved in the defense responses against both anthracnose (Tortora *et al.*, 2012) and gray mold (Mehli *et al.*, 2005). Overall, the higher expression of *FaMBL1* in overexpressing lines at the early infection of *B. cinerea* can be associated with the higher expression of *FaChi2-1*. Consistently, rice G-type lectin gene, *OslecRK*, which showed resistance to *M. grisea*, *X. oryzae* and brown planthoppers affected the expression of defense related genes such as *PR1a*, lipoxygenase and chalcone synthase (Cheng *et al.*, 2013). With the exception of *FaChi2-1*, up-regulation of defense related genes was not found in *FaMBL1* overexpressing lines at 24h post *B. cinerea* infection, to decipher if these genes were induced by *B. cinerea* inoculation before 24h, genes expression at later post infection timepoints should be measured in the future.

5. Conclusions

Transgenic strawberries overexpressing *FaMBL1* gene were less susceptible to fungal diseases, anthracnose and gray mold. *FaMBL1* could probably contribute to strawberry resistance by participating in a JA-dependent defense signaling pathway and inducing defense-related genes like *FaChi2-1* expression. Hence, this gene could be used in future trials for

improving resistance in economically important strawberry cultivars by overexpressing it, even by using a full cisgenic approach. In this way, the development of new high-quality cultivars will be speed up with a more sustainable approach, reduced needs of risk evaluation and higher acceptance by growers and consumers.

6. References

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Supplemental files

Supplemental file S1.

Tandem duplication of G-lectin gene in *Fragaria vesca*

Cluster	Gene ID	New gene name
Cluster_1	FvH4_1g23370	FveGLRK6.18
	FvH4_1g23380	FveGLRK6.17
Cluster_2	FvH4_1g23390	FveGLRK6.15
	FvH4_1g23400	FveGLRK6.14
Cluster_3	FvH4_2g29050	FveGLRK6.8
	FvH4_2g29070	FveGLRK6.7
Cluster_4	FvH4_2g29544	FveGLRK1.4
	FvH4_2g29545	FveGLRK1.2
	FvH4_2g29560	FveGLRK1.1
Cluster_5	FvH4_2g33830	FveGLRK6.32
	FvH4_2g33840	FveGLRK6.34
Cluster_6	FvH4_3g03230	FveGLRK4.15
	FvH4_3g03231	FveGLRK4.19
	FvH4_3g03240	FveGLRK4.18
	FvH4_3g03241	FveGLK4.3
	FvH4_3g03242	FveGLRK4.16
	FvH4_3g03243	FveGLRK4.13
Cluster_7	FvH4_3g03300	FveGLK4.2
	FvH4_3g03301	FveGLRK4.14
	FvH4_3g03310	FveGLRK4.12
	FvH4_3g03320	FveGLRK4.25
Cluster_8	FvH4_3g03340	FveGLP4.4
	FvH4_3g03350	FveGLRK4.28
	FvH4_3g03370	FveGLRK4.23
	FvH4_3g03390	FveGLRK4.22
Cluster_9	FvH4_3g03410	FveGLRK4.21
	FvH4_3g03420	FveGLRK4.5
	FvH4_3g03430	FveGLRK4.4
	FvH4_3g03431	FveGLRK3.7
	FvH4_3g03432	FveGLRK3.6
	FvH4_3g03433	FveGLRK3.10
	FvH4_3g03451	FveGLRK3.4
	FvH4_3g03482	FveGLRK3.17
Cluster_10	FvH4_3g03520	FveGLRK3.12
	FvH4_3g03521	FveGLRK3.11
Cluster_11	FvH4_3g03581	FveGLRP3.1
	FvH4_3g03590	FveGLRK3.1

Cluster_12	FvH4_3g21310	FveGLRK4.8
	FvH4_3g21320	FveGLRK4.10
Cluster_13	FvH4_3g43401	FveGLRK6.24
	FvH4_3g43402	FveGLRK6.22
	FvH4_3g43403	FveGLRK6.20
Cluster_14	FvH4_5g31680	FveGLRK6.5
	FvH4_5g31690	FveGLP6.1
Cluster_15	FvH4_6g29821	FveGLRK6.10
	FvH4_6g29840	FveGLRK6.9
Cluster_16	FvH4_6g44063	FveGLRK5.4
	FvH4_6g44100	FveGLRK5.3
	FvH4_6g44101	FveGLP5.1
Cluster_17	FvH4_3g18370	FveGLP6.2
	FvH4_3g18371	FveGLP6.5
	FvH4_3g18380	FveGLP6.4
	FvH4_3g18382	FveGLP6.3
Cluster_18	FvH4_3g18383	FveGLP6.7
	FvH4_3g18410	FveGLP6.6

Supplemental file S2.

Dispersed duplicated gene pairs in G-type lectin family of *Fragaria vesca* (dispersed duplicate 1 and dispersed duplicate 2 are a pair of duplicated gene)

Dispersed duplicate 1		**Dispersed duplicate 2**	
Gene ID	New gene name	Gene ID	New gene name
FvH4_1g03780	FveGLRK7.1	FvH4_2g29560	FveGLRK1.1
FvH4_1g04840	FveGLRK2.3	FvH4_3g03590	FveGLRK3.1
FvH4_1g16211	FveGLRK3.14	FvH4_3g03560	FveGLRK3.8
FvH4_1g23370	FveGLRK6.18	FvH4_2g14250	FveGLRK6.16
FvH4_2g12390	FveGLRK6.13	FvH4_6g26380	FveGLRK6.12
FvH4_2g14250	FveGLRK6.16	FvH4_3g43402	FveGLRK6.22
FvH4_2g29050	FveGLRK6.8	FvH4_7g30670	FveGLRK6.11
FvH4_2g29560	FveGLRK1.1	FvH4_6g51830	FveGLRK5.1
FvH4_2g33830	FveGLRK6.32	FvH4_2g33850	FveGLRK6.33
FvH4_2g33840	FveGLRK6.34	FvH4_2g33850	FveGLRK6.33
FvH4_2g33850	FveGLRK6.33	FvH4_2g33870	FveGLRK6.31
FvH4_2g33870	FveGLRK6.31	FvH4_6g12870	FveGLRK6.29
FvH4_3g03242	FveGLRK4.16	FvH4_7g19680	FveGLRK4.20
FvH4_3g03340	FveGLP4.4	FvH4_6g00300	FveGLP4.6
FvH4_3g03350	FveGLRK4.28	FvH4_3g06140	FveGLRK4.29
FvH4_3g03420	FveGLRK4.5	FvH4_3g15980	FveGLK4.1
FvH4_3g03430	FveGLRK4.4	FvH4_3g15980	FveGLK4.1
FvH4_3g03432	FveGLRK3.6	FvH4_3g03560	FveGLRK3.8
FvH4_3g03451	FveGLRK3.4	FvH4_3g03560	FveGLRK3.8
FvH4_3g03560	FveGLRK3.8	FvH4_1g16211/FvH4_3g03432/FvH4_3g03451	FveGLRK3.4/FveGLRK3.6/FveGLRK3.14
FvH4_3g03590	FveGLRK3.1	FvH4_6g51830	FveGLRK5.1

FvH4_3g06140	FveGLRK4.29	FvH4_3g03350	FveGLRK4.28
FvH4_3g15690	FveGLRK4.9	FvH4_3g15980	FveGLK4.1
FvH4_3g15930	FveGLRK4.11	FvH4_3g15690	FveGLRK4.9
FvH4_3g15980	FveGLK4.1	FvH4_6g12332	FveGLP4.3
FvH4_3g18383	FveGLP6.7	FvH4_6g10470	FveGLRP6.2
FvH4_3g21400	FveGLRK4.7	FvH4_3g15980	FveGLK4.1
FvH4_3g43401	FveGLRK6.24	FvH4_3g43710	FveGLRK6.23
FvH4_3g43402	FveGLRK6.22	FvH4_3g43710	FveGLRK6.23
FvH4_3g43403	FveGLRK6.20	FvH4_3g43710	FveGLRK6.23
FvH4_3g43440	FveGLRK6.21	FvH4_3g43710	FveGLRK6.23
FvH4_3g43710	FveGLRK6.23	FvH4_3g43401/FvH4_3g43402/FvH4_3g43403/FvH4_3g43440	FveGLRK6.24/FveGLRK6.22/FveGLRK6.20/FveGLRK6.21
FvH4_4g02170	FveGLRK2.2	FvH4_3g03590	FveGLRK3.1
FvH4_5g04270	FveGLP6.9	FvH4_5g04350	FveGLRK6.28
FvH4_5g04350	FveGLRK6.28	FvH4_6g12870	FveGLRK6.29
FvH4_5g31930	FveGLRK3.13	FvH4_1g16211	FveGLRK3.14
FvH4_6g00300	FveGLP4.6	FvH4_6g07960	FveGLRK4.17
FvH4_6g07960	FveGLRK4.17	FvH4_3g03242	FveGLRK4.16
FvH4_6g10470	FveGLRP6.2	FvH4_3g18383	FveGLP6.7
FvH4_6g12332	FveGLP4.3	FvH4_3g15980	FveGLK4.1
FvH4_6g12870	FveGLRK6.29	FvH4_2g33870/FvH4_5g04350	FveGLRK6.31/FveGLRK6.28
FvH4_6g26380	FveGLRK6.12	FvH4_7g30670/FvH4_2g12390	FveGLRK6.11/FveGLRK6.13
FvH4_6g26450	FveGLRK6.2	FvH4_6g51830	FveGLRK5.1
FvH4_6g44063	FveGLRK5.4	FvH4_6g51830	FveGLRK5.1
FvH4_6g44100	FveGLRK5.3	FvH4_6g51830	FveGLRK5.1
FvH4_6g44101	FveGLP5.1	FvH4_6g44310	FveGLRK6.1
FvH4_6g44260	FveGLP4.1	FvH4_6g51830	FveGLRK5.1
FvH4_6g44310	FveGLRK6.1	FvH4_6g26450	FveGLRK6.2
FvH4_6g51830	FveGLRK5.1	FvH4_3g03590	FveGLRK3.1
FvH4_7g19680	FveGLRK4.20	FvH4_3g03242	FveGLRK4.16
FvH4_7g30670	FveGLRK6.11	FvH4_6g26380	FveGLRK6.12

Supplemental file S3.

Co-expression prediction of strawberry G-lectin genes

Consunses_100_HD			
G-lectin gene ID	**G-lectins (new name)**	**Co-expressed gene**	**Description of the co-expressed gene**
FvH4_5g04350	FveGLRK6.28	FvH4_1g02272	
FvH4_6g44310	FveGLRK6.1	FvH4_1g15390	Disease resistance protein (CC-NBS-LRR class) family
FvH4_5g04350	FveGLRK6.28	FvH4_2g00033	
FvH4_3g03310	FveGLRK4.12	FvH4_2g26151	MLP-like protein 423
FvH4_3g43440	FveGLRK6.21	FvH4_3g07830	Heavy metal transport/detoxification superfamily protein
FvH4_5g04350	FveGLRK6.28	FvH4_3g17090	F-box/RNI-like superfamily protein

FvH4_5g04350	FveGLRK6.28	FvH4_3g20611	
FvH4_3g15150	FveGLP2.1	FvH4_3g35900	Disease resistance protein (TIR-NBS-LRR class)
FvH4_2g29545	FveGLRK1.2	FvH4_3g42840	varicose-related
FvH4_2g29544	FveGLRK1.4	FvH4_5g00150	nitrate transporter 1.1
FvH4_5g31690	FveGLP6.1	FvH4_5g12970	tetraspanin11
FvH4_5g04350	FveGLRK6.28	FvH4_5g27412	coatomer gamma-2 subunit, putative / gamma-2 coat protein, putative / gamma-2 COP, putative
FvH4_5g04350	FveGLRK6.28	FvH4_5g27721	
FvH4_5g04350	FveGLRK6.28	FvH4_6g06760	F-box family protein
FvH4_3g43440	FveGLRK6.21	FvH4_6g27024	RING/U-box superfamily protein
FvH4_2g24770	FveGLP3.1	FvH4_6g43590	SMAD/FHA domain-containing protein

Consunses_100_LCM

G-lectin gene	G-lectins (new name)	Co-expressed gene	Description of co-expressed gene
FvH4_6g12930	FveGLRK6.26	FvH4_1g02410	Pentatricopeptide repeat (PPR) superfamily protein
FvH4_3g03310	FveGLRK4.12	FvH4_1g03430	Pentatricopeptide repeat (PPR) superfamily protein
FvH4_1g23400	FveGLRK6.14	FvH4_1g05900	Protein of unknown function (DUF295)
FvH4_1g23390	FveGLRK6.15	FvH4_1g16860	Protein kinase superfamily protein
FvH4_3g03420	FveGLRK4.5	FvH4_1g26280	ATP binding cassette subfamily B4
FvH4_3g03320	FveGLRK4.25	FvH4_2g13902	Ribonuclease H-like superfamily protein
FvH4_3g03432	FveGLRK3.6	FvH4_2g19520	C2H2 and C2HC zinc fingers superfamily protein
FvH4_3g03520	FveGLRK3.12	FvH4_2g29861	cysteine-rich RLK (RECEPTOR-like protein kinase) 29
FvH4_3g21310	FveGLRK4.8	FvH4_3g03390	S-locus lectin protein kinase family protein
FvH4_3g03390	FveGLRK4.22	FvH4_3g21310	S-locus lectin protein kinase family protein
FvH4_3g18382	FveGLP6.3	FvH4_4g09470	F-box/RNI-like superfamily protein
FvH4_5g04270	FveGLP6.9	FvH4_4g16282	
FvH4_3g18371	FveGLP6.5	FvH4_4g35560	glutathione S-transferase TAU 18
FvH4_5g04270	FveGLP6.9	FvH4_5g11820	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
FvH4_2g29050	FveGLRK6.8	FvH4_5g19390	Exostosin family protein
FvH4_3g43440	FveGLRK6.21	FvH4_5g24690	
FvH4_3g18380	FveGLP6.4	FvH4_6g19281	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
FvH4_5g31690	FveGLP6.1	FvH4_6g21351	eukaryotic translation initiation factor 3C
FvH4_3g03420	FveGLRK4.5	FvH4_6g40020	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein
FvH4_3g03590	FveGLRK3.1	FvH4_6g46420	K-box region and MADS-box transcription factor family protein
FvH4_3g03482	FveGLRK3.17	FvH4_7g33440	NB-ARC domain-containing disease resistance protein

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G-lectin gene	G-lectins (new name)	Co-expressed gene	Description of co-expressed gene
FvH4_1g04840	FveGLRK2.3	FvH4_1g09190	beta-galactosidase 7
FvH4_1g04840	FveGLRK2.3	FvH4_1g12210	ferulic acid 5-hydroxylase 1
FvH4_1g04840	FveGLRK2.3	FvH4_1g19240	elicitor-activated gene 3-1
FvH4_1g04840	FveGLRK2.3	FvH4_1g19540	U-box domain-containing protein kinase family protein
FvH4_1g04840	FveGLRK2.3	FvH4_2g05133	GroES-like zinc-binding alcohol dehydrogenase family protein
FvH4_1g04840	FveGLRK2.3	FvH4_2g20930	polygalacturonase abscission zone A. thaliana
FvH4_1g04840	FveGLRK2.3	FvH4_2g23301	
FvH4_1g04840	FveGLRK2.3	FvH4_3g02440	Protein kinase superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_3g03340	S-locus lectin protein kinase family protein

FvH4_1g04840	FveGLRK2.3	FvH4_3g05300	
FvH4_1g04840	FveGLRK2.3	FvH4_3g19870	cysteine-rich RLK (RECEPTOR-like protein kinase) 29
FvH4_1g04840	FveGLRK2.3	FvH4_3g24572	
FvH4_1g04840	FveGLRK2.3	FvH4_3g30030	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_3g32961	glutamate receptor 2.7
FvH4_1g04840	FveGLRK2.3	FvH4_3g44731	
FvH4_1g04840	FveGLRK2.3	FvH4_3g44880	Leucine-rich receptor-like protein kinase family protein
FvH4_1g04840	FveGLRK2.3	FvH4_3g44920	Leucine-rich receptor-like protein kinase family protein
FvH4_1g04840	FveGLRK2.3	FvH4_3g44961	receptor like protein 2
FvH4_1g04840	FveGLRK2.3	FvH4_3g44962	
FvH4_1g04840	FveGLRK2.3	FvH4_3g45880	
FvH4_1g04840	FveGLRK2.3	FvH4_4g18830	MLP-like protein 423
FvH4_1g04840	FveGLRK2.3	FvH4_4g19310	myb domain protein 111
FvH4_1g04840	FveGLRK2.3	FvH4_4g21600	ATP binding cassette subfamily B4
FvH4_1g04840	FveGLRK2.3	FvH4_4g22101	
FvH4_1g04840	FveGLRK2.3	FvH4_4g32811	Calcium-dependent lipid-binding (CaLB domain) family protein
FvH4_1g04840	FveGLRK2.3	FvH4_5g07570	cytochrome P450, family 71, subfamily A, polypeptide 25
FvH4_1g04840	FveGLRK2.3	FvH4_5g08370	F-box and associated interaction domains-containing protein
FvH4_1g04840	FveGLRK2.3	FvH4_5g19760	alpha/beta-Hydrolases superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_6g10730	TRICHOME BIREFRINGENCE-LIKE 34
FvH4_1g04840	FveGLRK2.3	FvH4_6g14650	exocyst subunit exo70 family protein H4
FvH4_1g04840	FveGLRK2.3	FvH4_6g19290	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_6g38750	NAC domain containing protein 42
FvH4_1g04840	FveGLRK2.3	FvH4_6g44750	cytochrome P450, family 71, subfamily B, polypeptide 14
FvH4_1g04840	FveGLRK2.3	FvH4_6g47230	glycyl-tRNA synthetase / glycine--tRNA ligase
FvH4_1g04840	FveGLRK2.3	FvH4_6g50850	NAD(P)-binding Rossmann-fold superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_6g53000	beta glucosidase 13
FvH4_1g04840	FveGLRK2.3	FvH4_7g18081	UDP-glucosyl transferase 89B1
FvH4_1g04840	FveGLRK2.3	FvH4_7g21683	
FvH4_1g04840	FveGLRK2.3	FvH4_7g24610	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
FvH4_1g04840	FveGLRK2.3	FvH4_7g27130	expansin B2
FvH4_1g04840	FveGLRK2.3	FvH4_7g34050	EF hand calcium-binding protein family
FvH4_1g23370	FveGLRK6.18	FvH4_4g26500	Protein kinase superfamily protein
FvH4_2g29545	FveGLRK1.2	FvH4_1g16060	
FvH4_2g29545	FveGLRK1.2	FvH4_2g02354	dynamin-like protein 6
FvH4_2g29545	FveGLRK1.2	FvH4_2g02432	dynamin-like protein 6
FvH4_2g29545	FveGLRK1.2	FvH4_2g11390	aldehyde dehydrogenase 6B2
FvH4_2g29545	FveGLRK1.2	FvH4_3g03410	S-locus lectin protein kinase family protein
FvH4_2g29545	FveGLRK1.2	FvH4_3g25020	K-box region and MADS-box transcription factor family protein
FvH4_2g29545	FveGLRK1.2	FvH4_3g33584	BED zinc finger ;hAT family dimerisation domain
FvH4_2g29545	FveGLRK1.2	FvH4_3g42840	varicose-related
FvH4_2g29545	FveGLRK1.2	FvH4_4g06200	cryptochrome 2
FvH4_2g29545	FveGLRK1.2	FvH4_4g08670	histone acetyltransferase of the GNAT family 1
FvH4_2g29545	FveGLRK1.2	FvH4_4g16180	
FvH4_2g29545	FveGLRK1.2	FvH4_4g16191	

FvH4_2g29545	FveGLRK1.2	FvH4_5g29320	
FvH4_2g29545	FveGLRK1.2	FvH4_6g02430	Peptidase C13 family
FvH4_2g29545	FveGLRK1.2	FvH4_6g06441	RECQ helicase SIM
FvH4_2g29545	FveGLRK1.2	FvH4_6g23770	
FvH4_2g29545	FveGLRK1.2	FvH4_6g37800	Integrin-linked protein kinase family
FvH4_2g29545	FveGLRK1.2	FvH4_7g04780	
FvH4_2g29545	FveGLRK1.2	FvH4_7g31430	D111/G-patch domain-containing protein
FvH4_2g29560	FveGLRK1.1	FvH4_1g00270	Pectin lyase-like superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_1g01364	Glycosyl hydrolase family protein with chitinase insertion domain
FvH4_2g29560	FveGLRK1.1	FvH4_1g04280	F-box/RNI-like superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_1g08454	oxophytodienoate-reductase 3
FvH4_2g29560	FveGLRK1.1	FvH4_1g19001	
FvH4_2g29560	FveGLRK1.1	FvH4_2g04010	
FvH4_2g29560	FveGLRK1.1	FvH4_2g10322	
FvH4_2g29560	FveGLRK1.1	FvH4_2g19760	Disease resistance protein (CC-NBS-LRR class) family
FvH4_2g29560	FveGLRK1.1	FvH4_2g29970	Peroxidase superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_3g07960	senescence-associated gene 12
FvH4_2g29560	FveGLRK1.1	FvH4_3g14411	Ribonuclease H-like superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_3g17000	HXXXD-type acyl-transferase family protein
FvH4_2g29560	FveGLRK1.1	FvH4_4g13620	no vein-like
FvH4_2g29560	FveGLRK1.1	FvH4_4g21070	ADP-ribosylation factor A1F
FvH4_2g29560	FveGLRK1.1	FvH4_5g06850	RING/U-box superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_5g19552	
FvH4_2g29560	FveGLRK1.1	FvH4_5g22901	hydroxyproline-rich glycoprotein family protein
FvH4_2g29560	FveGLRK1.1	FvH4_5g25480	Protein of unknown function (DUF1423)
FvH4_2g29560	FveGLRK1.1	FvH4_6g09550	F-box and associated interaction domains-containing protein
FvH4_2g29560	FveGLRK1.1	FvH4_6g38111	
FvH4_2g29560	FveGLRK1.1	FvH4_6g42530	putative mitochondrial RNA helicase 2
FvH4_2g29560	FveGLRK1.1	FvH4_6g45851	F-box/RNI-like superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_6g47161	Galactose oxidase/kelch repeat superfamily protein
FvH4_2g29560	FveGLRK1.1	FvH4_6g53651	F-box family protein
FvH4_2g29560	FveGLRK1.1	FvH4_7g21720	
FvH4_2g33840	FveGLRK6.34	FvH4_6g16380	SAUR-like auxin-responsive protein family
FvH4_2g33850	FveGLRK6.33	FvH4_1g02250	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
FvH4_3g03240	FveGLRK4.18	FvH4_2g35260	Protein kinase family protein with leucine-rich repeat domain
FvH4_3g03240	FveGLRK4.18	FvH4_2g37650	Transmembrane amino acid transporter family protein
FvH4_3g03240	FveGLRK4.18	FvH4_3g00340	nudix hydrolase homolog 2
FvH4_3g03240	FveGLRK4.18	FvH4_3g02870	cysteine-rich RLK (RECEPTOR-like protein kinase) 29
FvH4_3g03240	FveGLRK4.18	FvH4_3g11860	NAC domain containing protein 42
FvH4_3g03240	FveGLRK4.18	FvH4_3g23780	NAD(P)-binding Rossmann-fold superfamily protein
FvH4_3g03240	FveGLRK4.18	FvH4_4g16140	Acyl-CoA N-acyltransferases (NAT) superfamily protein
FvH4_3g03240	FveGLRK4.18	FvH4_4g18840	DNAJ heat shock N-terminal domain-containing protein
FvH4_3g03240	FveGLRK4.18	FvH4_5g04350	lectin protein kinase family protein
FvH4_3g03240	FveGLRK4.18	FvH4_6g16610	Protein of unknown function (DUF1624)
FvH4_3g03240	FveGLRK4.18	FvH4_6g20470	syntaxin of plants 121
FvH4_3g03240	FveGLRK4.18	FvH4_6g52190	Pectin lyase-like superfamily protein

FvH4_3g03240	FveGLRK4.18	FvH4_7g06350	Transmembrane amino acid transporter family protein
FvH4_3g03243	FveGLRK4.13	FvH4_6g48220	Disease resistance protein (CC-NBS-LRR class) family
FvH4_3g03430	FveGLRK4.4	FvH4_3g12710	copper-exporting ATPase / responsive-to-antagonist 1 / copper-transporting ATPase (RAN1)
FvH4_3g03430	FveGLRK4.4	FvH4_4g34900	Protein kinase superfamily protein
FvH4_3g03430	FveGLRK4.4	FvH4_5g14270	C2H2-like zinc finger protein
FvH4_3g03430	FveGLRK4.4	FvH4_6g35250	Plant protein of unknown function (DUF936)
FvH4_3g03451	FveGLRK3.4	FvH4_2g14321	disease resistance protein (TIR-NBS-LRR class) family
FvH4_3g03521	FveGLRK3.11	FvH4_3g04260	Protein of unknown function (DUF1191)
FvH4_3g03521	FveGLRK3.11	FvH4_3g04330	RING/U-box superfamily protein
FvH4_3g03521	FveGLRK3.11	FvH4_6g15920	Wall-associated kinase family protein
FvH4_3g03521	FveGLRK3.11	FvH4_6g27710	
FvH4_3g03521	FveGLRK3.11	FvH4_7g31950	cyclic nucleotide gated channel 1
FvH4_3g03521	FveGLRK3.11	FvH4_7g32760	disease resistance protein (TIR-NBS-LRR class), putative
FvH4_3g15930	FveGLRK4.11	FvH4_1g20540	FAD/NAD(P)-binding oxidoreductase family protein
FvH4_3g15930	FveGLRK4.11	FvH4_1g25140	Glycosyl hydrolase family 35 protein
FvH4_3g15930	FveGLRK4.11	FvH4_2g03870	
FvH4_3g15930	FveGLRK4.11	FvH4_2g25380	Predicted pyridoxal phosphate-dependent enzyme, YBL036C type
FvH4_3g15930	FveGLRK4.11	FvH4_3g25190	Protein of unknown function (DUF579)
FvH4_3g15930	FveGLRK4.11	FvH4_3g26315	
FvH4_3g15930	FveGLRK4.11	FvH4_3g26822	
FvH4_3g15930	FveGLRK4.11	FvH4_3g28523	Protein of unknown function (DUF604)
FvH4_3g15930	FveGLRK4.11	FvH4_3g34330	
FvH4_3g15930	FveGLRK4.11	FvH4_3g36820	pectinesterase 11
FvH4_3g15930	FveGLRK4.11	FvH4_3g39270	Esterase/lipase/thioesterase family protein
FvH4_3g15930	FveGLRK4.11	FvH4_4g15171	
FvH4_3g15930	FveGLRK4.11	FvH4_4g18133	Nucleotidylyl transferase superfamily protein
FvH4_3g15930	FveGLRK4.11	FvH4_5g08881	DEAD box RNA helicase family protein
FvH4_3g15930	FveGLRK4.11	FvH4_5g16010	Protein of unknown function, DUF593
FvH4_3g15930	FveGLRK4.11	FvH4_5g23751	
FvH4_3g15930	FveGLRK4.11	FvH4_6g04230	
FvH4_3g15930	FveGLRK4.11	FvH4_6g32281	
FvH4_3g15930	FveGLRK4.11	FvH4_6g34881	
FvH4_3g15930	FveGLRK4.11	FvH4_6g42672	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
FvH4_3g15930	FveGLRK4.11	FvH4_7g22362	heat shock protein 70
FvH4_3g15980	FveGLK4.1	FvH4_3g14040	Protein of unknown function (DUF620)
FvH4_3g18371	FveGLP6.5	FvH4_3g21420	S-locus lectin protein kinase family protein
FvH4_3g18371	FveGLP6.5	FvH4_3g30040	L-O-methylthreonine resistant 1
FvH4_3g18383	FveGLP6.7	FvH4_5g15962	
FvH4_3g18383	FveGLP6.7	FvH4_6g28890	H(+)-ATPase 9
FvH4_3g43401	FveGLRK6.24	FvH4_1g07570	
FvH4_3g43401	FveGLRK6.24	FvH4_1g11001	
FvH4_3g43401	FveGLRK6.24	FvH4_1g11002	
FvH4_3g43401	FveGLRK6.24	FvH4_1g11081	
FvH4_3g43401	FveGLRK6.24	FvH4_1g12500	Pectin lyase-like superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_1g17321	DNAJ heat shock N-terminal domain-containing protein
FvH4_3g43401	FveGLRK6.24	FvH4_1g19280	Matrixin family protein

FvH4_3g43401	FveGLRK6.24	FvH4_2g00980	
FvH4_3g43401	FveGLRK6.24	FvH4_2g02230	AGAMOUS-like 62
FvH4_3g43401	FveGLRK6.24	FvH4_2g02270	AGAMOUS-like 62
FvH4_3g43401	FveGLRK6.24	FvH4_2g02401	
FvH4_3g43401	FveGLRK6.24	FvH4_2g07500	mRNA capping enzyme family protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g13220	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g13390	Protein of unknown function (DUF1278)
FvH4_3g43401	FveGLRK6.24	FvH4_2g14700	cell wall / vacuolar inhibitor of fructosidase 2
FvH4_3g43401	FveGLRK6.24	FvH4_2g15120	Flavin-binding monooxygenase family protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g15281	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43401	FveGLRK6.24	FvH4_2g15970	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g16230	Arabidopsis protein of unknown function (DUF241)
FvH4_3g43401	FveGLRK6.24	FvH4_2g19880	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g20680	S-protein homologue 1
FvH4_3g43401	FveGLRK6.24	FvH4_2g21010	ubiquitin 7
FvH4_3g43401	FveGLRK6.24	FvH4_2g24200	cell wall / vacuolar inhibitor of fructosidase 1
FvH4_3g43401	FveGLRK6.24	FvH4_2g28680	Beta-1,3-N-Acetylglucosaminyltransferase family protein
FvH4_3g43401	FveGLRK6.24	FvH4_2g32980	nucleosome assembly protein1;1
FvH4_3g43401	FveGLRK6.24	FvH4_2g37971	
FvH4_3g43401	FveGLRK6.24	FvH4_3g01180	RPM1 interacting protein 4
FvH4_3g43401	FveGLRK6.24	FvH4_3g04620	serine carboxypeptidase-like 31
FvH4_3g43401	FveGLRK6.24	FvH4_3g05941	
FvH4_3g43401	FveGLRK6.24	FvH4_3g11042	FAD-binding Berberine family protein
FvH4_3g43401	FveGLRK6.24	FvH4_3g11043	FAD-binding Berberine family protein
FvH4_3g43401	FveGLRK6.24	FvH4_3g11044	FAD-binding Berberine family protein
FvH4_3g43401	FveGLRK6.24	FvH4_3g11060	Ribosomal protein L35Ae family protein
FvH4_3g43401	FveGLRK6.24	FvH4_3g14642	
FvH4_3g43401	FveGLRK6.24	FvH4_3g14643	
FvH4_3g43401	FveGLRK6.24	FvH4_3g15260	Peroxidase superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_3g15490	
FvH4_3g43401	FveGLRK6.24	FvH4_3g17670	
FvH4_3g43401	FveGLRK6.24	FvH4_3g20800	Late embryogenesis abundant (LEA) protein-related
FvH4_3g43401	FveGLRK6.24	FvH4_3g21860	Plant self-incompatibility protein S1 family
FvH4_3g43401	FveGLRK6.24	FvH4_3g21910	gibberellin 2-oxidase 8
FvH4_3g43401	FveGLRK6.24	FvH4_3g26240	
FvH4_3g43401	FveGLRK6.24	FvH4_3g31470	TCP family transcription factor
FvH4_3g43401	FveGLRK6.24	FvH4_3g40500	senescence-associated gene 12
FvH4_3g43401	FveGLRK6.24	FvH4_3g43402	receptor-like protein kinase 1
FvH4_3g43401	FveGLRK6.24	FvH4_3g44420	hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase
FvH4_3g43401	FveGLRK6.24	FvH4_4g03020	C2H2 and C2HC zinc fingers superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g03531	
FvH4_3g43401	FveGLRK6.24	FvH4_4g04231	eukaryotic translation initiation factor 3G2
FvH4_3g43401	FveGLRK6.24	FvH4_4g04232	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43401	FveGLRK6.24	FvH4_4g06751	
FvH4_3g43401	FveGLRK6.24	FvH4_4g07190	Pectin lyase-like superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g07480	Eukaryotic aspartyl protease family protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g13850	RNI-like superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g20391	
FvH4_3g43401	FveGLRK6.24	FvH4_4g20430	cell wall / vacuolar inhibitor of fructosidase 1

FvH4_3g43401	FveGLRK6.24	FvH4_4g20480	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g22310	Integrase-type DNA-binding superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_4g22390	
FvH4_3g43401	FveGLRK6.24	FvH4_4g23640	acyl-CoA synthetase 5
FvH4_3g43401	FveGLRK6.24	FvH4_4g28850	
FvH4_3g43401	FveGLRK6.24	FvH4_4g34320	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_5g02460	
FvH4_3g43401	FveGLRK6.24	FvH4_5g09381	
FvH4_3g43401	FveGLRK6.24	FvH4_5g13790	exocyst subunit exo70 family protein E2
FvH4_3g43401	FveGLRK6.24	FvH4_5g21500	AGAMOUS-like 36
FvH4_3g43401	FveGLRK6.24	FvH4_5g22960	UDP-glucosyl transferase 78D2
FvH4_3g43401	FveGLRK6.24	FvH4_5g24840	
FvH4_3g43401	FveGLRK6.24	FvH4_5g30180	plantacyanin
FvH4_3g43401	FveGLRK6.24	FvH4_5g33570	xylulose kinase-2
FvH4_3g43401	FveGLRK6.24	FvH4_5g35160	cytochrome P450, family 93, subfamily D, polypeptide 1
FvH4_3g43401	FveGLRK6.24	FvH4_6g03900	Cystatin/monellin superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g04820	XB3 ortholog 5 in Arabidopsis thaliana
FvH4_3g43401	FveGLRK6.24	FvH4_6g05851	Regulator of Vps4 activity in the MVB pathway protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g08020	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g08460	AGAMOUS-like 80
FvH4_3g43401	FveGLRK6.24	FvH4_6g13140	Protein of unknown function (DUF604)
FvH4_3g43401	FveGLRK6.24	FvH4_6g17310	pectin methylesterase 44
FvH4_3g43401	FveGLRK6.24	FvH4_6g17570	cytochrome P450, family 76, subfamily G, polypeptide 1
FvH4_3g43401	FveGLRK6.24	FvH4_6g17601	
FvH4_3g43401	FveGLRK6.24	FvH4_6g21972	
FvH4_3g43401	FveGLRK6.24	FvH4_6g23002	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43401	FveGLRK6.24	FvH4_6g26990	sodium/calcium exchanger family protein / calcium-binding EF hand family protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g29154	
FvH4_3g43401	FveGLRK6.24	FvH4_6g30501	
FvH4_3g43401	FveGLRK6.24	FvH4_6g32790	Protein of Unknown Function (DUF239)
FvH4_3g43401	FveGLRK6.24	FvH4_6g33790	cell wall / vacuolar inhibitor of fructosidase 1
FvH4_3g43401	FveGLRK6.24	FvH4_6g35584	GDSL lipase 1
FvH4_3g43401	FveGLRK6.24	FvH4_6g39940	uclacyanin 1
FvH4_3g43401	FveGLRK6.24	FvH4_6g45802	Late embryogenesis abundant protein (LEA) family protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g46771	Gag-Pol-related retrotransposon family protein
FvH4_3g43401	FveGLRK6.24	FvH4_6g47571	tRNA/rRNA methyltransferase (SpoU) family protein
FvH4_3g43401	FveGLRK6.24	FvH4_7g04251	zinc-finger protein 1
FvH4_3g43401	FveGLRK6.24	FvH4_7g10114	receptor like protein 7
FvH4_3g43401	FveGLRK6.24	FvH4_7g14820	Pentatricopeptide repeat (PPR-like) superfamily protein
FvH4_3g43401	FveGLRK6.24	FvH4_7g22363	heat shock protein 70
FvH4_3g43401	FveGLRK6.24	FvH4_7g24960	Glutaredoxin family protein
FvH4_3g43401	FveGLRK6.24	FvH4_7g26521	
FvH4_3g43401	FveGLRK6.24	FvH4_7g27774	glycosyl hydrolase 9B1
FvH4_3g43401	FveGLRK6.24	FvH4_7g31350	RWP-RK domain-containing protein
FvH4_4g02170	FveGLRK2.2	FvH4_3g20590	G protein coupled receptor

FvH4_4g02170	FveGLRK2.2	FvH4_4g16600	Transducin/WD40 repeat-like superfamily protein
FvH4_4g33230	FveGLRK6.25	FvH4_2g37640	OPC-8:0 CoA ligase1
FvH4_5g31690	FveGLP6.1	FvH4_1g26670	PLATZ transcription factor family protein
FvH4_5g31690	FveGLP6.1	FvH4_3g08210	glutamate receptor 2.7
FvH4_5g31690	FveGLP6.1	FvH4_3g16530	Homeodomain-like superfamily protein
FvH4_5g31690	FveGLP6.1	FvH4_4g18641	
FvH4_5g31690	FveGLP6.1	FvH4_5g12601	phytochrome and flowering time regulatory protein (PFT1)
FvH4_5g31690	FveGLP6.1	FvH4_6g39781	
FvH4_5g31690	FveGLP6.1	FvH4_6g44030	pinoid-binding protein 1
FvH4_6g26380	FveGLRK6.12	FvH4_1g21650	Eukaryotic aspartyl protease family protein
FvH4_6g44310	FveGLRK6.1	FvH4_3g03010	RING/U-box superfamily protein
FvH4_3g03410	FveGLRK4.21	FvH4_1g16060	
FvH4_3g03410	FveGLRK4.21	FvH4_2g02354	dynamin-like protein 6
FvH4_3g03410	FveGLRK4.21	FvH4_2g02432	dynamin-like protein 6
FvH4_3g03410	FveGLRK4.21	FvH4_2g11390	aldehyde dehydrogenase 6B2
FvH4_3g03410	FveGLRK4.21	FvH4_2g29545	receptor kinase 3
FvH4_3g03410	FveGLRK4.21	FvH4_3g25020	K-box region and MADS-box transcription factor family protein
FvH4_3g03410	FveGLRK4.21	FvH4_3g33584	BED zinc finger ;hAT family dimerisation domain
FvH4_3g03410	FveGLRK4.21	FvH4_3g42840	varicose-related
FvH4_3g03410	FveGLRK4.21	FvH4_4g06200	cryptochrome 2
FvH4_3g03410	FveGLRK4.21	FvH4_4g08670	histone acetyltransferase of the GNAT family 1
FvH4_3g03410	FveGLRK4.21	FvH4_4g16180	
FvH4_3g03410	FveGLRK4.21	FvH4_4g16191	
FvH4_3g03410	FveGLRK4.21	FvH4_5g29320	
FvH4_3g03410	FveGLRK4.21	FvH4_6g02430	Peptidase C13 family
FvH4_3g03410	FveGLRK4.21	FvH4_6g06441	RECQ helicase SIM
FvH4_3g03410	FveGLRK4.21	FvH4_6g23770	
FvH4_3g03410	FveGLRK4.21	FvH4_6g37800	Integrin-linked protein kinase family
FvH4_3g03410	FveGLRK4.21	FvH4_7g04780	
FvH4_3g03410	FveGLRK4.21	FvH4_7g31430	D111/G-patch domain-containing protein
FvH4_3g03340	FveGLP4.4	FvH4_1g04840	S-locus lectin protein kinase family protein
FvH4_3g03340	FveGLP4.4	FvH4_1g09190	beta-galactosidase 7
FvH4_3g03340	FveGLP4.4	FvH4_1g12210	ferulic acid 5-hydroxylase 1
FvH4_3g03340	FveGLP4.4	FvH4_1g19240	elicitor-activated gene 3-1
FvH4_3g03340	FveGLP4.4	FvH4_1g19540	U-box domain-containing protein kinase family protein
FvH4_3g03340	FveGLP4.4	FvH4_2g05133	GroES-like zinc-binding alcohol dehydrogenase family protein
FvH4_3g03340	FveGLP4.4	FvH4_2g20930	polygalacturonase abscission zone A. thaliana
FvH4_3g03340	FveGLP4.4	FvH4_2g23301	
FvH4_3g03340	FveGLP4.4	FvH4_3g02440	Protein kinase superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_3g05300	
FvH4_3g03340	FveGLP4.4	FvH4_3g19870	cysteine-rich RLK (RECEPTOR-like protein kinase) 29
FvH4_3g03340	FveGLP4.4	FvH4_3g24572	
FvH4_3g03340	FveGLP4.4	FvH4_3g30030	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_3g32961	glutamate receptor 2.7
FvH4_3g03340	FveGLP4.4	FvH4_3g44731	
FvH4_3g03340	FveGLP4.4	FvH4_3g44880	Leucine-rich receptor-like protein kinase family protein
FvH4_3g03340	FveGLP4.4	FvH4_3g44920	Leucine-rich receptor-like protein kinase family protein

FvH4_3g03340	FveGLP4.4	FvH4_3g44961	receptor like protein 2
FvH4_3g03340	FveGLP4.4	FvH4_3g44962	
FvH4_3g03340	FveGLP4.4	FvH4_3g45880	
FvH4_3g03340	FveGLP4.4	FvH4_4g18830	MLP-like protein 423
FvH4_3g03340	FveGLP4.4	FvH4_4g19310	myb domain protein 111
FvH4_3g03340	FveGLP4.4	FvH4_4g21600	ATP binding cassette subfamily B4
FvH4_3g03340	FveGLP4.4	FvH4_4g22101	
FvH4_3g03340	FveGLP4.4	FvH4_4g32811	Calcium-dependent lipid-binding (CaLB domain) family protein
FvH4_3g03340	FveGLP4.4	FvH4_5g07570	cytochrome P450, family 71, subfamily A, polypeptide 25
FvH4_3g03340	FveGLP4.4	FvH4_5g08370	F-box and associated interaction domains-containing protein
FvH4_3g03340	FveGLP4.4	FvH4_5g19760	alpha/beta-Hydrolases superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_6g10730	TRICHOME BIREFRINGENCE-LIKE 34
FvH4_3g03340	FveGLP4.4	FvH4_6g14650	exocyst subunit exo70 family protein H4
FvH4_3g03340	FveGLP4.4	FvH4_6g19290	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_6g38750	NAC domain containing protein 42
FvH4_3g03340	FveGLP4.4	FvH4_6g44750	cytochrome P450, family 71, subfamily B, polypeptide 14
FvH4_3g03340	FveGLP4.4	FvH4_6g47230	glycyl-tRNA synthetase / glycine--tRNA ligase
FvH4_3g03340	FveGLP4.4	FvH4_6g50850	NAD(P)-binding Rossmann-fold superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_6g53000	beta glucosidase 13
FvH4_3g03340	FveGLP4.4	FvH4_7g18081	UDP-glucosyl transferase 89B1
FvH4_3g03340	FveGLP4.4	FvH4_7g21683	
FvH4_3g03340	FveGLP4.4	FvH4_7g24610	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
FvH4_3g03340	FveGLP4.4	FvH4_7g27130	expansin B2
FvH4_3g03340	FveGLP4.4	FvH4_7g34050	EF hand calcium-binding protein family
FvH4_3g43402	FveGLRK6.22	FvH4_1g07570	
FvH4_3g43402	FveGLRK6.22	FvH4_1g11001	
FvH4_3g43402	FveGLRK6.22	FvH4_1g11002	
FvH4_3g43402	FveGLRK6.22	FvH4_1g11081	
FvH4_3g43402	FveGLRK6.22	FvH4_1g12500	Pectin lyase-like superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_1g17321	DNAJ heat shock N-terminal domain-containing protein
FvH4_3g43402	FveGLRK6.22	FvH4_1g19280	Matrixin family protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g00980	
FvH4_3g43402	FveGLRK6.22	FvH4_2g02230	AGAMOUS-like 62
FvH4_3g43402	FveGLRK6.22	FvH4_2g02270	AGAMOUS-like 62
FvH4_3g43402	FveGLRK6.22	FvH4_2g02401	
FvH4_3g43402	FveGLRK6.22	FvH4_2g07500	mRNA capping enzyme family protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g13220	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g13390	Protein of unknown function (DUF1278)
FvH4_3g43402	FveGLRK6.22	FvH4_2g14700	cell wall / vacuolar inhibitor of fructosidase 2
FvH4_3g43402	FveGLRK6.22	FvH4_2g15120	Flavin-binding monooxygenase family protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g15281	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43402	FveGLRK6.22	FvH4_2g15970	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g16230	Arabidopsis protein of unknown function (DUF241)
FvH4_3g43402	FveGLRK6.22	FvH4_2g19880	GDSL-like Lipase/Acylhydrolase superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g20680	S-protein homologue 1

FvH4_3g43402	FveGLRK6.22	FvH4_2g21010	ubiquitin 7
FvH4_3g43402	FveGLRK6.22	FvH4_2g24200	cell wall / vacuolar inhibitor of fructosidase 1
FvH4_3g43402	FveGLRK6.22	FvH4_2g28680	Beta-1,3-N-Acetylglucosaminyltransferase family protein
FvH4_3g43402	FveGLRK6.22	FvH4_2g32980	nucleosome assembly protein1;1
FvH4_3g43402	FveGLRK6.22	FvH4_2g37971	
FvH4_3g43402	FveGLRK6.22	FvH4_3g01180	RPM1 interacting protein 4
FvH4_3g43402	FveGLRK6.22	FvH4_3g04620	serine carboxypeptidase-like 31
FvH4_3g43402	FveGLRK6.22	FvH4_3g05941	
FvH4_3g43402	FveGLRK6.22	FvH4_3g11042	FAD-binding Berberine family protein
FvH4_3g43402	FveGLRK6.22	FvH4_3g11043	FAD-binding Berberine family protein
FvH4_3g43402	FveGLRK6.22	FvH4_3g11044	FAD-binding Berberine family protein
FvH4_3g43402	FveGLRK6.22	FvH4_3g11060	Ribosomal protein L35Ae family protein
FvH4_3g43402	FveGLRK6.22	FvH4_3g14642	
FvH4_3g43402	FveGLRK6.22	FvH4_3g14643	
FvH4_3g43402	FveGLRK6.22	FvH4_3g15260	Peroxidase superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_3g15490	
FvH4_3g43402	FveGLRK6.22	FvH4_3g17670	
FvH4_3g43402	FveGLRK6.22	FvH4_3g20800	Late embryogenesis abundant (LEA) protein-related
FvH4_3g43402	FveGLRK6.22	FvH4_3g21860	Plant self-incompatibility protein S1 family
FvH4_3g43402	FveGLRK6.22	FvH4_3g21910	gibberellin 2-oxidase 8
FvH4_3g43402	FveGLRK6.22	FvH4_3g26240	
FvH4_3g43402	FveGLRK6.22	FvH4_3g31470	TCP family transcription factor
FvH4_3g43402	FveGLRK6.22	FvH4_3g40500	senescence-associated gene 12
FvH4_3g43402	FveGLRK6.22	FvH4_3g43401	receptor-like protein kinase 1
FvH4_3g43402	FveGLRK6.22	FvH4_3g44420	hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase
FvH4_3g43402	FveGLRK6.22	FvH4_4g03020	C2H2 and C2HC zinc fingers superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g03531	
FvH4_3g43402	FveGLRK6.22	FvH4_4g04231	eukaryotic translation initiation factor 3G2
FvH4_3g43402	FveGLRK6.22	FvH4_4g04232	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43402	FveGLRK6.22	FvH4_4g06751	
FvH4_3g43402	FveGLRK6.22	FvH4_4g07190	Pectin lyase-like superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g07480	Eukaryotic aspartyl protease family protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g13850	RNI-like superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g20391	
FvH4_3g43402	FveGLRK6.22	FvH4_4g20430	cell wall / vacuolar inhibitor of fructosidase 1
FvH4_3g43402	FveGLRK6.22	FvH4_4g20480	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g22310	Integrase-type DNA-binding superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_4g22390	
FvH4_3g43402	FveGLRK6.22	FvH4_4g23640	acyl-CoA synthetase 5
FvH4_3g43402	FveGLRK6.22	FvH4_4g28850	
FvH4_3g43402	FveGLRK6.22	FvH4_4g34320	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_5g02460	
FvH4_3g43402	FveGLRK6.22	FvH4_5g09381	
FvH4_3g43402	FveGLRK6.22	FvH4_5g13790	exocyst subunit exo70 family protein E2
FvH4_3g43402	FveGLRK6.22	FvH4_5g21500	AGAMOUS-like 36
FvH4_3g43402	FveGLRK6.22	FvH4_5g22960	UDP-glucosyl transferase 78D2
FvH4_3g43402	FveGLRK6.22	FvH4_5g24840	
FvH4_3g43402	FveGLRK6.22	FvH4_5g30180	plantacyanin
FvH4_3g43402	FveGLRK6.22	FvH4_5g33570	xylulose kinase-2

FvH4_3g43402	FveGLRK6.22	FvH4_5g35160	cytochrome P450, family 93, subfamily D, polypeptide 1
FvH4_3g43402	FveGLRK6.22	FvH4_6g03900	Cystatin/monellin superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g04820	XB3 ortholog 5 in Arabidopsis thaliana
FvH4_3g43402	FveGLRK6.22	FvH4_6g05851	Regulator of Vps4 activity in the MVB pathway protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g08020	Plant invertase/pectin methylesterase inhibitor superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g08460	AGAMOUS-like 80
FvH4_3g43402	FveGLRK6.22	FvH4_6g13140	Protein of unknown function (DUF604)
FvH4_3g43402	FveGLRK6.22	FvH4_6g17310	pectin methylesterase 44
FvH4_3g43402	FveGLRK6.22	FvH4_6g17570	cytochrome P450, family 76, subfamily G, polypeptide 1
FvH4_3g43402	FveGLRK6.22	FvH4_6g17601	
FvH4_3g43402	FveGLRK6.22	FvH4_6g21972	
FvH4_3g43402	FveGLRK6.22	FvH4_6g23002	BED zinc finger ;hAT family dimerisation domain
FvH4_3g43402	FveGLRK6.22	FvH4_6g26990	sodium/calcium exchanger family protein / calcium-binding EF hand family protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g29154	
FvH4_3g43402	FveGLRK6.22	FvH4_6g30501	
FvH4_3g43402	FveGLRK6.22	FvH4_6g32790	Protein of Unknown Function (DUF239)
FvH4_3g43402	FveGLRK6.22	FvH4_6g33790	cell wall / vacuolar inhibitor of fructosidase 1
FvH4_3g43402	FveGLRK6.22	FvH4_6g35584	GDSL lipase 1
FvH4_3g43402	FveGLRK6.22	FvH4_6g39940	uclacyanin 1
FvH4_3g43402	FveGLRK6.22	FvH4_6g45802	Late embryogenesis abundant protein (LEA) family protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g46771	Gag-Pol-related retrotransposon family protein
FvH4_3g43402	FveGLRK6.22	FvH4_6g47571	tRNA/rRNA methyltransferase (SpoU) family protein
FvH4_3g43402	FveGLRK6.22	FvH4_7g04251	zinc-finger protein 1
FvH4_3g43402	FveGLRK6.22	FvH4_7g10114	receptor like protein 7
FvH4_3g43402	FveGLRK6.22	FvH4_7g14820	Pentatricopeptide repeat (PPR-like) superfamily protein
FvH4_3g43402	FveGLRK6.22	FvH4_7g22363	heat shock protein 70
FvH4_3g43402	FveGLRK6.22	FvH4_7g24960	Glutaredoxin family protein
FvH4_3g43402	FveGLRK6.22	FvH4_7g26521	
FvH4_3g43402	FveGLRK6.22	FvH4_7g27774	glycosyl hydrolase 9B1
FvH4_3g43402	FveGLRK6.22	FvH4_7g31350	RWP-RK domain-containing protein
FvH4_5g04350	FveGLRK6.28	FvH4_2g35260	Protein kinase family protein with leucine-rich repeat domain
FvH4_5g04350	FveGLRK6.28	FvH4_2g37650	Transmembrane amino acid transporter family protein
FvH4_5g04350	FveGLRK6.28	FvH4_3g00340	nudix hydrolase homolog 2
FvH4_5g04350	FveGLRK6.28	FvH4_3g02870	cysteine-rich RLK (RECEPTOR-like protein kinase) 29
FvH4_5g04350	FveGLRK6.28	FvH4_3g03240	S-locus lectin protein kinase family protein
FvH4_5g04350	FveGLRK6.28	FvH4_3g11860	NAC domain containing protein 42
FvH4_5g04350	FveGLRK6.28	FvH4_3g23780	NAD(P)-binding Rossmann-fold superfamily protein
FvH4_5g04350	FveGLRK6.28	FvH4_4g16140	Acyl-CoA N-acyltransferases (NAT) superfamily protein
FvH4_5g04350	FveGLRK6.28	FvH4_4g18840	DNAJ heat shock N-terminal domain-containing protein
FvH4_5g04350	FveGLRK6.28	FvH4_6g16610	Protein of unknown function (DUF1624)
FvH4_5g04350	FveGLRK6.28	FvH4_6g20470	syntaxin of plants 121
FvH4_5g04350	FveGLRK6.28	FvH4_6g52190	Pectin lyase-like superfamily protein

FvH4_5g04350	FveGLRK6.28	FvH4_7g06350	Transmembrane amino acid transporter family protein

Supplemental file S4.

Subcellular localization of strawberry G-lectin genes

Gene ID	New gene name	Subcellular location predicted by CELLO	Subcellular location predicted by TargetP-2.0
FvH4_1g03780	FveGLRK7.1	PM	Other
FvH4_1g04840	FveGLRK2.3	PM	Signal peptide
FvH4_1g16211	FveGLRK3.14	PM	Signal peptide
FvH4_1g23370	FveGLRK6.18	PM	Signal peptide
FvH4_1g23380	FveGLRK6.17	PM	Signal peptide
FvH4_1g23390	FveGLRK6.15	PM	Signal peptide
FvH4_1g23400	FveGLRK6.14	PM	Signal peptide
FvH4_2g12390	FveGLRK6.13	PM	Signal peptide
FvH4_2g14250	FveGLRK6.16	PM	Signal peptide
FvH4_2g26490	FveGLRK6.6	PM	Signal peptide
FvH4_2g29050	FveGLRK6.8	PM	Signal peptide
FvH4_2g29070	FveGLRK6.7	PM	Signal peptide
FvH4_2g29542	FveGLRK1.5	PM	Signal peptide
FvH4_2g29543	FveGLRK1.3	PM	Signal peptide
FvH4_2g29544	FveGLRK1.4	PM	Other
FvH4_2g29545	FveGLRK1.2	PM	Signal peptide
FvH4_2g29560	FveGLRK1.1	PM	Signal peptide
FvH4_2g33830	FveGLRK6.32	PM	Other
FvH4_2g33840	FveGLRK6.34	PM	Other
FvH4_2g33850	FveGLRK6.33	PM	Other
FvH4_2g33870	FveGLRK6.31	EX	Signal peptide
FvH4_3g03230	FveGLRK4.15	PM	Signal peptide
FvH4_3g03231	FveGLRK4.19	PM	Signal peptide
FvH4_3g03240	FveGLRK4.18	PM	Signal peptide
FvH4_3g03242	FveGLRK4.16	PM	Signal peptide
FvH4_3g03243	FveGLRK4.13	EX	Signal peptide
FvH4_3g03301	FveGLRK4.14	PM	Signal peptide
FvH4_3g03310	FveGLRK4.12	PM/EX	Signal peptide
FvH4_3g03320	FveGLRK4.25	PM	Signal peptide
FvH4_3g03350	FveGLRK4.28	PM	Other
FvH4_3g03370	FveGLRK4.23	PM	Signal peptide
FvH4_3g03390	FveGLRK4.22	PM	Other
FvH4_3g03410	FveGLRK4.21	PM	Signal peptide
FvH4_3g03420	FveGLRK4.5	PM	Signal peptide
FvH4_3g03430	FveGLRK4.4	PM	Signal peptide
FvH4_3g03431	FveGLRK3.7	PM	Signal peptide
FvH4_3g03432	FveGLRK3.6	PM	Other
FvH4_3g03433	FveGLRK3.10	PM	Signal peptide
FvH4_3g03450	FveGLRK3.3	PM	Signal peptide
FvH4_3g03451	FveGLRK3.4	EX/PM	Other
FvH4_3g03461	FveGLRK3.2	PM	Signal peptide

FvH4_3g03481	FveGLRK3.5	PM	Other
FvH4_3g03482	FveGLRK3.17	PM	Signal peptide
FvH4_3g03501	FveGLRK3.16	PM	Signal peptide
FvH4_3g03502	FveGLRK3.15	N/PM	Other
FvH4_3g03520	FveGLRK3.12	PM	Signal peptide
FvH4_3g03521	FveGLRK3.11	PM	Signal peptide
FvH4_3g03560	FveGLRK3.8	PM	Signal peptide
FvH4_3g03590	FveGLRK3.1	PM	Signal peptide
FvH4_3g06140	FveGLRK4.29	PM	Signal peptide
FvH4_3g15080	FveGLRK2.6	PM	Other
FvH4_3g15120	FveGLRK2.5	PM	Other
FvH4_3g15130	FveGLRK2.4	PM	Signal peptide
FvH4_3g15690	FveGLRK4.9	PM	Other
FvH4_3g15930	FveGLRK4.11	PM	Thylakoid luminal transfer peptide
FvH4_3g21270	FveGLRK4.6	C/PM/N	Other
FvH4_3g21310	FveGLRK4.8	PM	Signal peptide
FvH4_3g21320	FveGLRK4.10	PM	Signal peptide
FvH4_3g21400	FveGLRK4.7	PM	Signal peptide
FvH4_3g43401	FveGLRK6.24	C/PM	Other
FvH4_3g43402	FveGLRK6.22	PM	Signal peptide
FvH4_3g43403	FveGLRK6.20	PM	Other
FvH4_3g43440	FveGLRK6.21	PM	Signal peptide
FvH4_3g43710	FveGLRK6.23	PM	Signal peptide
FvH4_4g02170	FveGLRK2.2	PM	Signal peptide
FvH4_4g33230	FveGLRK6.25	PM	Signal peptide
FvH4_5g04350	FveGLRK6.28	PM	Signal peptide
FvH4_5g31680	FveGLRK6.5	PM	Other
FvH4_5g31930	FveGLRK3.13	PM	Signal peptide
FvH4_5g32570	FveGLRK3.9	PM	Other
FvH4_6g00257	FveGLRK4.27	PM	Signal peptide
FvH4_6g00270	FveGLRK4.26	PM	Signal peptide
FvH4_6g07960	FveGLRK4.17	PM	Signal peptide
FvH4_6g12870	FveGLRK6.29	PM	Signal peptide
FvH4_6g12880	FveGLRK6.30	PM	Other
FvH4_6g12890	FveGLRK6.27	PM	Signal peptide
FvH4_6g12930	FveGLRK6.26	PM	Signal peptide
FvH4_6g20800	FveGLRK4.24	PM	Signal peptide
FvH4_6g26380	FveGLRK6.12	PM	Signal peptide
FvH4_6g26420	FveGLRK6.3	PM	Signal peptide
FvH4_6g26450	FveGLRK6.2	PM	Other
FvH4_6g29821	FveGLRK6.10	PM	Signal peptide
FvH4_6g29840	FveGLRK6.9	PM	Signal peptide
FvH4_6g31370	FveGLRK2.1	PM	Signal peptide
FvH4_6g44063	FveGLRK5.4	PM/C	Other
FvH4_6g44064	FveGLRK5.2	PM	Signal peptide
FvH4_6g44100	FveGLRK5.3	PM	Other
FvH4_6g44106	FveGLRK5.8	EX/PM	Signal peptide
FvH4_6g44107	FveGLRK5.7	PM	Signal peptide
FvH4_6g44108	FveGLRK5.10	EX/PM	Other
FvH4_6g44109	FveGLRK5.5	PM/EX	Other
FvH4_6g44140	FveGLRK5.6	PM	Other
FvH4_6g44190	FveGLRK5.9	EX/PM	Signal peptide

FvH4_6g44243	FveGLRK4.1	PM	Other
FvH4_6g44244	FveGLRK4.3	PM	Signal peptide
FvH4_6g44245	FveGLRK4.2	PM	Signal peptide
FvH4_6g44310	FveGLRK6.1	PM/EX	Signal peptide
FvH4_6g51830	FveGLRK5.1	PM	Signal peptide
FvH4_7g00200	FveGLRK6.19	PM	Signal peptide
FvH4_7g14760	FveGLRK6.4	EX/PM	Signal peptide
FvH4_7g19680	FveGLRK4.20	PM	Signal peptide
FvH4_7g30670	FveGLRK6.11	PM	Other
FvH4_3g03581	FveGLRP3.1	*EX*	*Signal peptide*
FvH4_3g15090	FveGLRP2.1	*EX*	*Signal peptide*
FvH4_6g10470	FveGLRP6.2	*PM*	*Signal peptide*
FvH4_6g17930	FveGLRP6.1	*EX/PM*	*Signal peptide*
FvH4_3g03241	FveGLK4.3	EX	Signal peptide
FvH4_3g03300	FveGLK4.2	PM/N	Other
FvH4_3g15980	FveGLK4.1	EX	Signal peptide
FvH4_6g44240	FveGLK5.1	EX	Other
FvH4_2g05942	FveGLP3.2	EX	Other
FvH4_2g24770	FveGLP3.1	C/EX	Other
FvH4_3g03322	FveGLP4.5	EX/PM	Signal peptide
FvH4_3g03340	FveGLP4.4	EX	Signal peptide
FvH4_3g03435	FveGLP3.3	EX	Other
FvH4_3g15150	FveGLP2.1	EX	Other
FvH4_3g18370	FveGLP6.2	EX	Signal peptide
FvH4_3g18371	FveGLP6.5	EX/Vacuole	Signal peptide
FvH4_3g18380	FveGLP6.4	EX	Other
FvH4_3g18382	FveGLP6.3	EX	Other
FvH4_3g18383	FveGLP6.7	EX	Signal peptide
FvH4_3g18410	FveGLP6.6	EX	Other
FvH4_3g21271	FveGLP4.2	EX	Other
FvH4_5g04270	FveGLP6.9	EX	Other
FvH4_5g04310	FveGLP6.8	EX	Signal peptide
FvH4_5g31690	FveGLP6.1	PM/EX	Signal peptide
FvH4_6g00300	FveGLP4.6	EX	Signal peptide
FvH4_6g12332	FveGLP4.3	EX	Signal peptide
FvH4_6g12920	FveGLP6.10	EX	Signal peptide
FvH4_6g44062	FveGLP5.2	EX	Other
FvH4_6g44101	FveGLP5.1	EX/N	Other
FvH4_6g44242	FveGLP5.3	EX/PM	Signal peptide
FvH4_6g44260	FveGLP4.1	EX	Signal peptide

EX: Extracellular; PM: PlasmaMembrane; C: Cytoplasmic; N: Nuclear