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Review

The Strawberry Plant Defense Mechanism: A Molecular Review

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Strawberry, a small fruit crop of great importance throughout the world, has been considered a model plant system for Rosaceae, and is susceptible to a large variety of phytopathogenic organisms. Most components and mechanisms of the strawberry defense network remain poorly understood. However, from current knowledge, it seems clear that the ability of a strawberry plant to respond efficiently to pathogens relies first on the physiological status of injured tissue (pre-formed mechanisms of defense) and secondly on the general ability to recognize and identify the invaders by surface plant receptors, followed by a broad range of induced mechanisms, which include cell wall reinforcement, production of reactive oxygen species, phytoalexin generation and pathogenesis-related protein accumulation. Dissection of these physiological responses at a molecular level will provide valuable information to improve future breeding strategies for new strawberry varieties and to engineer strawberry plants for durable and broad-spectrum disease resistance. In turn, this will lead to a reduction in use of chemicals and in environmental risks. Advances in the understanding of the molecular interplay between plant (mainly those considered model systems) and various classes of microbial pathogens have been made in the last two decades. However, major progress in the genetics and molecular biology of strawberry is still needed to uncover fully the way in which this elaborate plant innate immune system works. These fundamental insights will provide a conceptual framework for rational human intervention through new strawberry research approaches. In this review, we will provide a comprehensive overview and discuss recent advances in molecular research on strawberry defense mechanisms against pathogens.

Keywords: *Fragaria* × *ananassa* • *Fragaria vesca*, Strawberry • Strawberry pathogen defense • Strawberry biotic stress defense • Strawberry defense response.

Abbreviations: BR, brassinosteroid; BTH, benzothiadiazole; EST, expressed sequence tag; ET, ethylene; ETI, effector-triggered immunity; FHT, flavanone 3-hydroxylase; Fra a, *Fragaria* × *ananassa* alergen; HR, hypersensitive response; JA, jasmonate; LOX, lipoxygenase; LTP, lipid transfer protein; MAMP, microbe-associated molecular pattern; MeSA, methylsalicylate; NBS-LRR, nucleotide binding-leucine-rich repeat; OGA, oligogalacturonide; PA, proanthocyanidin; PAL, phenylalanine ammonia lyase; PAMP, pathogen-associated molecular pattern; PG, polygalacturonase; PGIP, polygalacturonase-inhibiting protein; POX, peroxidase; PPO, polyphenoloxidase; PR, pathogenesis-related; PRR, pattern recognition receptor; PTI, PAMP-triggered immunity; RGA, resistance gene analog; SA, salicylic acid; STK, serine-threonine kinase; TIR, Toll/interleukin-1 receptor-like; TLC, thin-layer chromatography.

The nucleotide sequences reported in this paper have been submitted to GenBank under accession numbers, JN415652 (FaPR10-4) and JN415653 (FaCHI4-2).

Introduction

Importance and benefits of strawberry as a fruit crop

Strawberry is a small fruit crop of great importance throughout the world. The strawberry belongs to the family Rosaceae in the genus *Fragaria*, containing 23 species (Folta and Davis 2006, Shulaev et al. 2008). In *Fragaria*, four basic fertility groups exist which are associated primarily with their ploidy level or chromosome number (Hancock 1999). The most common native species, *F. vesca* L., has 14 chromosomes and is considered to be a diploid (Oosumi et al. 2006). Other remarkable *Fragaria* species include the diploid *F. viridis* Duchesne ($2n = 2x = 1$ 4 chromosomes) (Hodgson 2007), the hexaploid *F. moschata* Duchesne (musk strawberry, $2n = 6x = 42$ chromosomes) (Hancock 1999) and the octoploid *Fragaria* × *ananassa* Duchesne ($2n = 8x = 56$) (Davis et al. 2007), the main cultivated species, that stems from the cross of the octoploids *F. virginiana* Duchesne from eastern North America, which was noted for its fine flavor, and *F. chiloensis* (L.) Mill. from Chile, noted for its large size (Hancock 1999). Numerous varieties of strawberries

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have been developed in the temperate zones of the world by different breeding programs.

In 2009, the world strawberry production reached approximately 4.2 Mt, with projected increases for subsequent years [FAOSTAT Agriculture Data (http://faostat.fao.org/, updated 17 May 2011)]. Due to its broad horticultural importance, this crop has been proposed as an interesting model for the development of basic genomics and recombinant DNA studies among Rosaceae (Mezzetti 2009). Indeed, strawberry is unique within the Rosaceae, with a small basic ($x = 7$) genome size (~240 Mb), and a short generation time for a perennial (Folta and Davis 2006), the availability of a robust and facile in vitro regeneration and transformation system (Alsheikh et al. 2002) and the recently reported genome sequence (Shulaev et al. 2011).

The hybrid octoploid (*F.×ananassa* Duch.), a clonally propagated perennial and herbaceous plant, accouns for almost 60% of the world production, due to the organoleptic properties and health benefits of the fruit regarded as significant quality factors for both consumers and the food industry. Indeed, the benefits of consumption of strawberry fruit on cardiovascular, neurodegenerative and other human diseases such as aging, obesity and cancer are documented (Maas et al. 1991, Zhang et al. 2008, da Silva Pinto et al. 2010).

The resistance to pathogens in strawberry

Strawberry cultivars exhibit wide phenotypic diversity in terms of their susceptibility to a large variety of phytopathogenic organisms which limit both strawberry fruit quality and plant yield production (Simpson 1991, Maas 1998).

Natural sources of strawberry resistance to diseases have been reported among wild species (Harland and King 1957, Gooding et al. 1981, Maas 1998), and also in some varieties of cultivated *F.×ananassa* (Maas and Smith 1978, Melville et al. 1980, Wing et al. 1995, Nelson et al. 1996, Bell et al. 1997, Shaw and Gordon 2003, Mori et al. 2005, Particka and Hancock 2005, Zebrowska et al. 2006, Masny and Żurawicz 2009), but strawberry resistance to a variety of pathogens has been reported to be mostly polygenic and quantitatively inherited (Maclachlan 1978, Barritt 1980, Denoyes-Rothan and Baudry 1995, Shaw et al. 1996, Lewers et al. 2003, Zebrowska et al. 2006), making it difficult to associate molecular markers with disease resistance genes. This is further complicated by the octoploid genome structure of the main cultivated strawberry species, *F.×ananassa*. However, high levels of conserved macrosynteny and colinearity have been observed between the octoploid and diploid *Fragaria* genomes (Rousseau-Gueutin et al. 2008), and molecular markers linked to a single dominant strawberry disease resistance gene that segregates in a disomic fashion have been reported (Denoyes-Rothan and Baudry 1995, Takahashi et al. 1997, van de Weg 1997a, van de Weg 1997b, Denoyes-Rothan et al. 2005).

Traditional breeding for resistance is time consuming and, importantly, has not been shown to be durable in many plants (Quirino and Bent 2003). Moreover, due to the intensified focus on resistance, other substantial deficiencies for horticultural or productivity traits are usually co-selected (Shaw et al. 2005). Also, classical strawberry breeding is rather conservative due to difficulties in introgression of the resistance sources (Hancock and Bringhurst 1980, Hancock and Luby 1993). In addition, the development of 'a narrow germplasm base' (i.e. cultivars introduced from North American breeding programs from 1960 to 1990 are descendants of 53 founding clones with only 17 cytoplasm sources) (Dale and Sjulin 1990) has caused deleterious effects of inbreeding and genetic vulnerability to diseases, pests and environmental stresses.

Ultimately the control of pathogens and pests of strawberry requires a combination of chemical and cultural methods. The effectiveness of chemicals for controlling diseases in fruiting fields is unclear. It may be that the incubation time between infection and disease is so long that most chemicals are ineffective in controlling diseases. Regular pesticide applications are also environmental contaminants and have harmful effects on human health. Thus, their use is not yet considered an appropriate cultivation practice (González-León and Valenzuela-Quintanar 2007, Fernandes et al. 2011). In addition, plants make vitamins, polyphenolics and other antioxidants to protect themselves from dangers such as pests and drought. Many of these compounds are also healthy compounds for human consumption as they can act as antioxidants and may protect human cells against damage that can lead to heart disease, cancer and other diseases (Törrönen and Määttä 2002, Zhang et al. 2008, da Silva Pinto et al. 2010). Unlike wild plants and organically grown crops, it has been suggested that these healthy molecules are reduced in plants treated with pesticides, as they need to make less of these compounds (Asami et al. 2003).

Therefore, there is a growing need to develop alternative approaches for control of strawberry diseases. Advances in the last two decades in the understanding of the molecular interplay between plants (mainly those considered model systems) and various classes of microbial pathogens have provided a conceptual framework for rational human intervention through new strawberry research approaches, including the use of natural plant elicitors (Terry and Joyce 2000, Babalar et al. 2007, Hukkanen et al. 2007, Shafiee et al. 2010), and biocontrol agents to enhance natural defense responses (Adikaram et al. 2002, Forster et al. 2004, Sesan 2006, Oliveira et al. 2007, H. Zhang et al. 2010, Huang et al. 2011, Tortora et al. 2011). Studies in strawberry providing molecular information to engineer strawberry plants for durable and broad-spectrum disease resistance are still scarce, and most components and mechanisms of the strawberry defense network remain completely unknown. Therefore, major progress in the genetics and molecular biology of strawberry is still needed in order to uncover fully its elaborate plant innate immune system.

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Plant innate immunity in strawberry: what is, and what is not known?

Plant innate immunity is a term including all the molecular and cellular mechanisms that plants can display to prevent potential pathogen infection and pest attack, from pre-formed mechanical and chemical defenses to the expression of induced resistance responses after detection of a great variety of microbial pathogen such as viruses, bacteria, fungi, oomycetes, nematodes and insects. A schematic view of known strawberry defense mechanisms is shown in **Supplementary Fig. S1**.

Strawberry pathogens rely on a wide range of strategies for their survival (Maas 1998). Bacteria are able to enter through biological cell structures such as stomata and hydathodes (gas or water pores, respectively), or even gain access via wounds, and further proliferate in the intercellular spaces. Fungi can directly enter plant epidermal cells, or extend hyphae on top of, between or through plant cells. Pathogenic and symbiotic fungi and oomycetes eventually invaginate feeding structures (haustaria) into the host cell plasma membrane. In a different complex way, nematodes and aphids feed by inserting a stylet directly into a plant cell. Viruses need a direct transfer of sap through wounded plant tissues, and a biological vector such as an insect or nematode to spread and to infect healthy plants.

Similar to animals, plants are able to recognize pathogens and swiftly activate defense. However, the plant defense system differs notably from that of mammals (Nürnberger et al. 2004). Plants do not have mobile defender cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell, and on systemic signals produced and dispersed from infection sites (Chisholm et al. 2006).

It may be assumed that the strawberry plant must recognize pathogens and respond to diseases in a manner comparable with that known in other plants. Thus, the existence of structural and chemical barriers such as the cell wall and the cuticle shield should prevent strawberry from most invading organisms and it should conform to a pre-existent passive defense mechanism that would include pre-synthesized toxins, toxic chemicals, antifungal proteins and enzymatic inhibitors (Dixon 2001, Nürnberger and Lipka 2005). In addition, plants have developed induced defense systems to respond to microbes that manage to circumvent these pre-formed barriers. Generally, such challenged organisms are not able to invade a plant because of the activation of a primary defense response resulting in non-host resistance (Nürnberger and Lipka 2005). This primary active response [so-called pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI)] is initiated within the plant cell after pathogen interaction and perception of PAMPs or microbe-associated molecular patterns (MAMPs) through pattern recognition receptors (PRRs) at the plant's cell surface. In turn, these events induce a molecular reprogramming of the cell and facilitate complex compound deposition in the plant cell wall including callose, suberin, lignin and proteins, in addition to other metabolic changes leading to mounting of the plant's immune response. Most of the microbes able to evade or suppress the primary defense response are recognized by the plant via effector proteins which are secreted (avirulent factors or race-specific elicitors) to inhibit PTI. Additional plant receptors—called R proteins—can perceive such effectors to mount a second layer of defense called effector-triggered immunity (ETI), which in most cases involves a hypersensitive response (HR), and a systemic activation of plant defenses from the site of signal perception. All these events include the induction of specific signaling pathways and transcription of specific genes, and the activation and production of proteins and chemicals with a clear defensive role, including pathogenesis-related (PR) proteins, phytoalexins and reactive oxygen species. Therefore, primary and secondary defense responses in plants that lead to resistance rely on a complex system of receptor-mediated pathogen perception and subsequent downstream signal transduction cascades, whereby cellular changes caused by the secondary defense response are generally most pronounced (Jones and Dangl 2006, Stulemeijer and Joosten 2008).

In cultivated strawberry (*F.* × *ananassa*, Duch.), breeders have tended to share their good quality fruit selections with others, including those bred for resistance, in the last two centuries. Thus, the selection and maintenance within the strawberry cultivars of essential components for the primary and the secondary defense system should have been expected. However, knowledge of these molecular components and associated breeding markers in strawberry has been very limited, so far.

Strawberry passive defenses

Pre-formed structural/mechanical barrier

Strawberry fruit is considered a 'soft fruit' due to its delicate texture, coated by a very thin cuticle and presenting high susceptibility to physical damage. Fruit firmness also relies on the composition and structure of the cell wall, a rigid, cellulose-based support surrounding every cell. Heterogeneity of strawberry fruit in terms of firmness and response to physical damage has been reported among cultivars (Gooding 1976, Ferreira et al. 2008), and a clear relationship between skin strength or fruit firmness and susceptibility to pathogen infection has also been described (Gooding 1976, Barritt 1980). Indeed, pathogens use mechanical force or release cell wall-degrading enzymes to break down these barriers, to access cellular nutrients. **Table 1** shows a survey of known strawberry physical defense responses.

Changes in the cell wall composition and structure also occur naturally in strawberry at different developmental stages, and they involve both enzymatic and non-enzymatic processes (Rose et al. 2004). For instance, during strawberry fruit ripening, modification of the primary cell wall is required and, consequently, large variation in fruit firmness can take place during this physiological event. In other plants, it is accepted that cell wall disassembly is a key component of susceptibility to pathogens (Cantu et al. 2008), and it is known that the strawberry

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Table 1 Strawberry physical defenses

Plant structure	Strawberry cultivar	Tissue	Pathogen/pest	Defensive activity	References
Cuticle and cell wall	*F.* × *ananassa*	Fruit	*Botrytis cinerea*	Clear relationship between skin strength or fruit firmness and susceptibility to pathogen infection	Gooding (1976); Barritt (1980)
Cell wall	Alba	Fruit	*Colletotrichum acutatum*	Natural modification in strawberry fruit cell wall during ripening process makes the fruit more susceptible to the action of polygalacturonase enzymes from *C. acutatum*	Guidarelli et al. (2011)
	Apollo, Sequoia, Surecrop	Petiole	*Colletotrichum fragariae*	Thickening of the cell walls and a deposition of pectic material associated with fungal restriction in resistant cultivar	Milholland (1982)
	Pájaro	Leaf, petiole	*Colletotrichum fragariae*	Thickening of the cell wall of leaflets exposed to *C. fragariae*	Salazar et al. (2007)
	F. vesca	Fruit	*Botrytis cinerea*	Partial demethylation of strawberry cell wall oligogalacturonides is required for eliciting defense responses	Osorio et al. (2008, 2011)
Trichomes	Totem, Zephyr, Venta, Tenira, Induka, Bogota, Senga Sengana, Kokinskaja Pozdnaja, Korona, *F. chiloensis*	Leaf	*Tetranychus urticae* Koch	Relationship between the oviposition and survival of the two-spotted spider mite *Tetranychus urticae* Koch, and the number and density of glandular and non-glandular trichomes	Kishaba et al. (1972); Luczynski et al. (1990a, 1990b); Steinite and Levinsh (2003)
Leaf veins	64 *F.*×*ananassa* cultivars and clones	Leaf	*Xanthomonas fragariae*	The spreading of *Xanthomonas fragariae* is effectively blocked by strawberry leaf veins	Kennedy and King (1962a, 1962b)

fruit (*F.*×*ananassa* Duch.) varies in its inherent natural disease resistance according to its physiological status (Gilles 1959). Indeed, natural modifications in the strawberry fruit cell wall during ripening have been reported to make the fruit cell wall more susceptible to the action of polygalacturonase (PG) enzymes from *Colletotrichum acutatum* (Guidarelli et al. 2011). Also, the timing of the ripening process may vary among strawberry genotypes, causing different softening rates (Rosli et al. 2004), and, thus, different fruit susceptibility to pathogens has also been described among strawberry genotypes (Daugaard 1999, Casado-Díaz et al. 2006, Chandler 2006).

Changes in cell wall composition and structure are mainly due to the concerted action of a set of enzymes acting on the different cell wall polymers, and many of these enzymes have already been cloned in strawberry fruit (**Supplementary Table S1**). It is also predicted that microorganisms must secrete a similar set of counterpart hydrolytic enzymes to degrade the cuticles and disorganize the cell walls to allow the nutrient uptake and spread through the plant. Usually, plant cells respond to such attempts at entry by using several defense responses including de novo cell wall biosynthesis and deposition of the glucan polymer callose at the site of pathogen contact (Aist 1976, Kwon et al. 2008).

The dynamic changes in the structure and composition of the strawberry plant cell wall challenged with pathogens together with a functional analysis of strawberry cell wall-modifying genes and enzymes have not yet been well studied at the molecular level, but knowledge of these is expected to be useful for the understanding of the complex process of defense response in this crop. Nonetheless, functional characterization of some of the strawberry cell wall genes mentioned in **Supplementary Table S1** has been performed either by ectopic expression or by antisense down-regulation technology. Thus, biological roles have been reported for the endo-β-1,4-glucanase genes *Cel1* and *Cel2* (Woolley et al. 2001, Palomer et al. 2006, Mercado et al. 2010), the pectate lyase gene (*FaPLC*) (Jimenez-Bermudez et al. 2002, Sesmero et al. 2007, Santiago-Doménech et al. 2008, Youssef et al. 2009) and the PG gene *FaPG1* (García-Gago et al. 2009, Quesada et al. 2009). Also, a direct correlation between mRNA expression levels or enzyme activity and fruit firmness has been found in different cultivars for *FaExp1–7* genes (Dotto et al. 2006), *FaXyl1* (Martínez et al. 2004, Bustamante et al. 2006, Bustamante et al. 2009), *FcPL1* (Figueroa et al. 2008), *PME* (Lefever et al. 2004), PGs (Salentijn et al. 2003, Lefever et al. 2004, Villarreal et al. 2007, Figueroa et al. 2008, Villarreal et al. 2009), arabinofuranosidases (*FaAra1*, *FaAra2* and *FaAra3*) (Rosli et al. 2009) and the endo-β-1,4-glucanases (Trainotti et al. 1999a). So far, no further studies have been carried out with these strawberry genes, lines and cultivars, exploring their implication in the defense response to pathogens, but a partial demethylation of strawberry cell wall

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oligogalacturonides by the strawberry pectin methyl esterase 1 gene (*FaPE1*) required for eliciting defense responses in wild *F. vesca* has been reported (Osorio et al. 2008, Osorio et al. 2011) (see further below).

Proteins with fundamental roles in plants can also have additional functions in defense. Thus, structural cell wall proteins such as extensins and proline-rich proteins (hydroxyproline-rich glycoproteins, HyRGPs) play a role in cross-linking other components of the plant cell wall, and strengthening this protective layer against attack by pathogens (Showalter 1993, Wei and Shirsat 2006, Deepak et al. 2010). It is known that these proteins are actively synthesized after wounding (Cheong et al. 2002) and pathogen infection (Maleck et al. 2000, Schenk et al. 2000) but the dynamic composition of the cell wall during different stages of plant development is thought also to lead to differences in susceptibility to pathogens. In strawberry fruit, synthesis of extensins seems to be independent of auxin control (Aharoni et al. 2002a), although Blanco-Portales et al. (2004) reported a strawberry *FaHyPRP* gene (hybrid proline-rich protein) whose expression was regulated by auxins. DNA microarray studies have revealed differences in the level of expression of strawberry *HyPRP* genes between soft and firm strawberry cultivars (cv. Gorella and cv. Holiday, respectively) (Salentijn et al. 2003). These results clearly support the role of these proteins in strawberry cell wall reinforcement, but their involvement in the mechanism of resistance to pathogens in strawberry needs to be further assessed.

Morphological features of strawberry plant leaves are also thought to affect herbivores as in other plants (Peters and Berry 1980). In many plants, the presence of trichomes, hairs or spines has been shown to be a very efficient mechanism of defense against herbivores and some pathogens and thus more pubescent leaves (containing a major number of non-glandular trichomes) are more resistant to herbivores due to mechanical restrictions (Levin 1973, Dai et al. 2010). In strawberry, a negative relationship between the oviposition and survival of the two-spotted spider mite *Tetranychus urticae* Koch, and the number and density of glandular and non-glandular trichomes in leaves has been reported (Luczynski et al. 1990b). However, Kishaba et al. (1972) proposed that foliar pubescence might be related to spider mite susceptibility, and Steinite and Levinsh (2003) have reported that the density of non-glandular trichomes is not the key factor for the resistance of strawberry cultivars but, rather, the presence of pre-formed glandular trichomes containing oxidative enzymes.

In strawberry green tissues, leaf veins also seem to have a preventive function in defense, effectively block the spread of some pathogens. Thus, it has been reported that spreading of *Xanthomonas fragariae*, which causes angular leaf spots, is restricted by leaf veins in strawberry (Kennedy and King 1962a, Kennedy and King 1962b).

Pre-formed strawberry biochemical barriers

Pre-formed chemical barriers (phytoanticipins) appear to be decisive in the plant passive defense mechanism. Plants produce a broad range of secondary metabolites, either as part of their normal program of growth and development or in response to stress, many of which have a proven toxic effect against pathogens and pests (Dixon 2001). Phenolics, sulfur compounds, saponins, cyanogenic glycosides and glucosinolates make up this biological chemical barrier and act locally at the very early stages of pathogen attack. Most are derived from the isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide pathways (Kliebenstein 2004). The central phenylpropanoid pathway leads to a major group of these valuable natural products, and flavonoids represent one of the largest classes within this group, which are also known to be involved in a multitude of other physiological functions (Winkel-Shirley 2001). Important products of the main phenylpropanoid branches in plants also include lignin, chlorogenic acid, salicylic acid (SA) and catecholamines, many of which have been proved to act as antimicrobials (Kliebenstein 2004). **Table 2** shows a set of known compounds putatively related to strawberry defense.

In strawberry fruit, the phenylpropanoid pathway is switched on during the ripening process (see Singh et al. 2010 for more comprehensive details). Proanthocyanidins (PAs) and many other compounds of the flavonoid pathway are actively synthesized and accumulate to high levels in the strawberry fruit receptacle at early stages (green and white) of strawberry fruit ripening, thereby giving immature fruit an astringent flavour (Cheng and Breen 1991, Aharoni et al. 2002b, Almeida et al. 2007), contributing to plant defense (Terry et al. 2004, Halbwirth et al. 2006, Hukkanen et al. 2007). PAs in the strawberry consist of catechin units, which is a main flavonoid in strawberries (Ishimaru et al. 1995, Törrönen and Määttä 2002, Puhl and Treutter 2008, Wulf et al. 2008), and it is known to possess antimicrobial properties (Scalbert 1991, Yamamoto et al. 2000). Other compounds, such as euscaphic acid, tormentic acid and myrianthic acid, have also been identified through thin-layer chromatography (TLC) bioassays and nuclear magnetic resonance (NMR) spectral analysis in green-stage strawberry fruit and flowers (Hirai et al. 2000, Terry et al. 2004). It is known that *Botrytis cinerea*, the causal agent of strawberry fruit rot, penetrates floral parts (petals, stigmas, styles or stamens) of strawberries, raspberries and grapes, and remains quiescent until the fruit ripens (Jarvis 1977, Elad and Evensen 1995). Terry et al. (2004) reported that extracts of strawberry flowers at post-anthesis showed greater antifungal activity than at white bud and full bloom stages, and proposed that antifungal compounds in strawberry flowers may play a role in initiating *B. cinerea* quiescence.

Several other authors have also found a positive correlation between resistance to *B. cinerea* and the concentration of PAs in strawberry. Hébert et al. (2001, 2002) found that cultivars with higher concentrations of PAs (mainly free and bound catechin and epicatechin) were more resistant to fungal infection. Jersch et al. (1989) also found that aqueous extracts of immature strawberry cv. Chandler fruit also had

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Table 2 Metabolites related to strawberry defense

Chemical family	Compounds	Cultivar	Tissue	Pathogen/pest	Defensive activity	References
	Not identified	Deutch Evern	Green fruit	*Botrytis cinerea*	Pre-formed defense compound against *Botrytis cinerea*	Gilles (1959)
	Not identified[a]	Surecrop, Stelemaster, Blakemore	Root	*Phytophthora fragariae*, *Cladosporium cucumerinum*	Partially inhibitory to mycelial growth in bioassay	Mussell and Staples (1971)
	Not identified	Elsanta	Green fruit	*Botrytis cinerea*, *Cladosporium cladosporioides*	Antifungal activity against gray mold rot	Adikaram et al. (2002)
	Not identified[b]	Chandler, Sweet Charlie	Leaves	*Colletotrichum fragariae*	These compounds conferred resistance to *C. fragariae*	Vincent et al. (1999)
	Not identified	Chandler	Achenes	*Botrytis cinerea*	These compounds inhibited radial growth of *B. cinerea*	El Ghaouth et al. (1991)
	Fragarin	Chandler	Leaves	*Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*	Had a broad antibiotic spectrum, high activity against bacteria and fungal plant pathogens	Filippone et al. (1999, 2001)
	Cyanogenic glycosides (source for HCN)	Elsanta	Achenes		Increase in transcript abundance of genes putatively involved in the metabolism of cyanogenic glycosides in the achenes	Aharoni and O'Connell (2002)
Triterpenes	Euscaphic acid, tormentic acid, myrianthic acid	Houkouwase	Unripe fruit	*Colletotrichum musae*, *C. fragariae*	Effective against infections with the fungus *Colletotrichum*	Hirai et al. (2000)
	Not identified[a]	Elsanta	Achenes, fruit and flower	*Botrytis cinerea*	Pre-formed antifungal activity against the pathogen, *Botrytis cinerea*, and the bioassay organism, *Cladosporium cladosporioides*	Terry et al. (2004)
Lipoxygenase pathway	(E)-hex-2-enal (3)			*C. acutatum*, *Penicillium expansum*, *Botrytis cinerea*	Inhibited spore germination and fungus growth, and altered the structures of the cell wall and plasma membrane, causing disorganization and lysis of organelles and, eventually, cell death of the pathogen	Archbold et al. (1997); Fallik et al. (1998); Neri et al. (2006); Arroyo et al. (2007)
Phenolics	Ellagitannin, ellagic acid, quercetin, kaempferol and others	Jonsok	Leaves		Production has been shown to be stimulated by foliar application of benzothiadiazole and glycine betaine	Karjalainen et al. (2002); Hukkanen et al. (2007)
	Cathecol-based	Korona, Senga Sengana, Zephyr and others	Leaves	*Tetranychus urticae* Koch	Higher resistance is associated with a trichome-localized inducible increase of catechol-based phenolics	Steinite and Ievinsh (2002, 2003)
		Totem	Leaves	*Tetranychus urticae* Koch	Development of the two-spotted spider mite *T. urticae* Koch was negatively correlated to foliar concentrations of phenolics	Luczynski et al. (1990a, 1990b)

(continued)

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Table 2 Continued

Chemical family	Compounds	Cultivar	Tissue	Pathogen/pest	Defensive activity	References
	Methyl salicylate	Sunrise, Red Chief, Scott	Leaves	*Tetranychus urticae* Koch	Increased approximately 10-fold after fruit harvest in plants more resistant to the two-spotted spider mite	Hamilton-Kemp et al. (1988)
		Korona	Flowers	*Anthonomus rubi*	Induced in higher amounts by weevil feeding	Bichão et al. (2005)
		Polka, Honeoye	Leaves	*Phytonemus pallidus*	Emissions of methyl salicylate were greater from cyclamen mite-damaged strawberry plants than from intact plants	Himanen et al. (2005)
	Methyl salicylate[c]			Aphididae, Thripidae, Cicadellidae and others	Enhanced natural enemy attraction but did not increase nor decrease pest abundance	Lee (2010)
	Quercetin	Howard and Surecrop type	Root	*Phythium irregulare*, *Rhizoctonia solani*, *Alternaria alternata*	Decisive to confer moderate resistance to root rot diseases	Nemec (1973, 1976)
	Ellagitannins, flavonoids	Herut	Achenes		Ellagitannins and flavonoids accumulate particularly in the achene during early and late development, respectively	Fait et al. (2008)
	Gallic acid	Chandler, Seascape, Sweet Charlie, Annapolis	Fruit	*Botrytis Cinerea*		Hébert et al. (2001, 2002)
Flavonoids	Proanthocyanins, catechin, epicatechins	Chandler, Seascape, Sweet Charlie, Annapolis	Fruit	*Botrytis Cinerea*	Positive correlation between resistance to *B. cinerea* and the concentration of proanthocyanidins in strawberry fruit	Hébert et al. (2001, 2002)
	Proanthocyanins, catechins	Clea, Pájaro	Fruit	*Botrytis cinerea*	Positive correlation between resistance to *B. cinerea* and the concentration of proanthocyanidins in strawberry fruit	Di Venere et al. (1998)
	Proanthocyanidins	Senga Sengana	Fruit	*Botrytis cinerea*	Inhibition of *Botrytis cinerea* growth (quiescence) in green fruit	Jersch et al. (1989)
	Flavonols	Jonsok	Leaves	*Botrytis cinerea*	Strawberry leaves with increased susceptibility to gray mold had decreased their contents in flavonols	Hanhineva et al. (2009)
	(+)-Catechin	Morioka-16, Hokowase	Leaves	*Alternaria alternata*	Protective agent during induced resistance against *Alternaria* black spot	Yamamoto et al. (2000)
Flavanols	Proanthocyanidins catechins	Hybride	Leaves	*Mycosphaerella fragariae*	Precise localization of flavanols around fungal infections	Feucht et al. (1992)
	Catechin derived procyanidins, luteoliflavan, eriodictyol 7-glucoside	Elsanta	Fruit	*Botrytis cinerea*	Inhibit growth of *B. cinerea* in immature strawberry fruits (quiescence)	Puhl and Treutter (2008)

[a] Some of these compounds probably correspond to the same phytoalexins found in strawberry cv. Houkouwase unripe fruit and reported by Hirai et al. (2000).
[b] These compounds might be similar to the pre-formed antifungal compounds found in strawberry green-stage I fruit by Terry et al. (2004).
[c] Commercialized high purity chemical tested in bioasays.

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direct antifungal activity against *B. cinerea* conidial germination and mycelial growth, and suggested that a decline in PA concentration during fruit development governs *B. cinerea* quiescence through removing inhibition of a pathogen-derived PG. They also observed that the PA concentration was higher in the less susceptible strawberry cultivars. These results agree with the previously reported inactivation of a PG enzyme from *B. cinerea* by strawberry phenolics (Harris and Dennis 1982). An inverse relationship between the PA content of immature strawberry fruits of various cultivars and the colonization of *B. cinerea* was also observed by Di Venere (1998).

More recently, Puhl and Treutter (2008) showed that the accumulation of catechin-derived procyanidins was fundamental to inhibit the growth of *B. cinerea* in immature strawberry fruits. In fact, gray mold symptoms occur only in ripe, red colored fruits. They modified the concentration of flavanols in developing strawberry fruits by inhibiting flavanone 3-hydroxylase (FHT), a prominent dioxygenase of the flavonoid pathway, which is involved in the biosynthesis of catechin precursors. The accumulation of novel flavonoids, identified as luteoliflavan and eriodictyol 7-glucoside, and enhanced levels of catechin were found when green fruits were treated with prohexadione-Ca, a bioregulator whose structure mimics that of 2-oxoglutarate and is able to inhibit dioxygenase enzymes which require 2-oxoglutarate as co-substrate (Rademacher 2000, Roemmelt et al. 2003). Although the increase in catechin concentration seems to be contradictory to the occurring FHT bottleneck, similar observations on apple (Fischer et al. 2006) and grapevine (Puhl et al. 2008) have been explained by an additional strong inhibition of the flavonol synthase, which also is a 2-oxoglutarate-dependent dioxygenase. Thus, an excess supply of substrates for the remaining FHT activity was assumed. The effect of the bioregulator was dependent of the fruit developmental stage, showing a higher increment of these compounds after flowering, during the stage of small green fruits, but having no effect thereafter. The increasing catechin and PA concentrations at the small green stage restricted fungal growth, and it became obvious that young fruits just at flowering do not accumulate flavanols to a sufficient level to prevent primary receptacle infection. Thus, the choice of the flowers as the favored tissue for fungal invasion as well as the latency of the pathogen in green fruits can be regarded as the critical points in *B. cinerea* development. Indeed, the ability to develop latent infections on immature fruits, becoming quiescent until fruit ripens, has also been reported for other strawberry pathogens such as *Colletotrichum* spp. (Prusky 1996, Guidarelli et al. 2011).

Methyl salicylate (MeSA) has also been suggested to be implicated in strawberry plant resistance. Thus, Hamilton-Kemp et al. (1988) detected a 10-fold increase in the relative amount of MeSA when compared at flowering and after fruit harvest in strawberry plants that were more resistant to the two-spotted spider mite, *T. urticae* Koch. Surprisingly, this compound did not seem to have an effect on spider mite behavior, under bioassay at low concentrations.

It is believed that unripe fruit is highly protected by chemical barriers from herbivore and pathogen attack, to prevent the extensive spreading of as yet immature seeds. When fruit ripens, this protective layer usually decreases, and changes in the main branches of the phenylpropanoid pathway are produced, allowing the synthesis of color-, taste- and aroma-related compounds used for the recruitment of seed dispersers. In contrast, the seeds possess some chemical toxins and proteins, although they are often well protected by physical structures, to ensure that the seed is not consumed along with the fruit (Terras et al. 1995). Thus, the strawberry achene, the true fruit, is heavily protected, not only by a sturdy and tough covering, the pericarp, but it also has a high concentration of toxic compounds that prevents it from being consumed by pathogens and pests (Aharoni and O'Connell 2002, Terry et al. 2004, Fait et al. 2008). Aharoni and O'Connell (2002) reported an increase in transcript abundance of genes putatively involved in the metabolism of cyanogenic glycosides, a source for HCN (hydrocyanic acid) which can render a plant toxic, in achenes, pointing to their biosynthesis in the achene tissue. Also, Fait et al. (2008) detected defense-related compounds of phenylpropanoids, ellagitannins and flavonoids, which accumulate particularly in the achene during early and late development, respectively. Terry et al. (2004) detected antifungal activity in all tissue types tested (i.e. pith, cortex and epidermis) from strawberry green fruit, but particularly and in large amounts in the achenes.

Pre-formed antifungal compounds are also found in strawberry leaves. Vincent et al. (1999) found a positive correlation between the presence of these compounds (the identity of these compounds was not determined) and strawberry resistance to *Colletotrichum fragariae*. They found that the amount of these pre-formed compounds varied between cultivars moderately resistant (Sweet Charlie) and susceptible (Chandler) to anthracnose, with approximately 15 times more antifungal activity present in the former, suggesting that the resistance to *C. fragariae* of different strawberry cultivars may be mediated by these pre-formed antimicrobials. Terry et al. (2004) suggested that these compounds might be similar to the pre-formed antifungal compounds they found in strawberry green stage I fruit. Yamamoto et al. (2000) reported that catechin pre-formed in strawberry leaves inhibited *Alternaria alternata*, and Hanhineva et al. (2009) observed that strawberry leaves with increased susceptibility to gray mold had decreased contents of flavonols, thus highlighting the role of flavonols in strawberry plant defense (Terry et al. 2004, Halbwirth et al. 2006, Hukkanen et al. 2007). Also, Luczynski et al. (1990a) observed that the development of the two-spotted spider mite *T. urticae* Koch was negatively correlated to foliar concentrations of phenolics, especially catechol-based, compounds.

Filippone et al. (1999) reported the isolation of a new type of antimicrobial compound constitutively present in strawberry

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leaves, called fragarin. This compound was isolated from a soluble fraction of this tissue and turned out to be an amphipathic molecule of 316 Da that had a broad antibiotic spectrum, with a high activity against bacteria and fungal plant pathogens isolated from strawberry (*Colletotrichum gloeosporioides*, *C. fragariae* and *C. acutatum*) and other plants (*Clavibacter michiganensis* subsp. *sepedonicus*, strain C5, and *Pseudomonas corrugata*, isolated from tomatoes; *Pseudomonas syringae* isolated from onion; and *Erwinia* spp. isolated from rose leaves). These authors showed that fragarin was active against *C. michiganensis* by dissipating its membrane potential, and suggested that its action precedes or is simultaneous with cell death by altering the permeability of the membrane and disrupting its function (Filippone et al. 2001).

Quantitative differences of several phenolics are also found in strawberry root, and this appears to be decisive in conferring moderate resistance to root rot diseases caused by *Pythium irregulare*, *Rhizoctonia solani* and *A. alternata* (Nemec 1973, Nemec 1976).

Volatiles have also been found to be related to defense in strawberry. Volatile aldehydes and alcohols are key compounds in the fresh and green sensorial notes of vegetables and fruits (Rabetafika et al. 2008). They are produced by plants in response to various stresses and therefore may play a major role in plant defense mechanisms (Blée 2002). It has been reported that (E)-hex-2-enal (trans-2-hexanal), a characteristic strawberry aroma volatile product, which is generated from the oxidative degradation of linolenic acid by a lipoxygenase (LOX) pathway, showed antifungal activity against *C. acutatum*. This volatile compound inhibited spore germination and fungus growth, and altered the structures of the cell wall and plasma membrane, causing disorganization and lysis of organelles and, eventually, cell death of the pathogen (Arroyo et al. 2007).

So far, the presence of a wide range of pre-formed defense compounds has been described in strawberry. Many of these pre-formed compounds are shared by different tissues such as roots (Mussell and Staples 1971), leaves (Vincent et al. 1999) and green fruit (Hirai et al. 2000, Terry et al. 2004), so a similar pre-formed defense barrier seems to work against pathogens within the complete strawberry plant. In plants, >100,000 low molecular mass compounds are produced as secondary metabolites (Dixon 2001). Such diversity makes it difficult to unravel specific products and pathways involved in defense (both passive and active defenses) within particular plant species. It is known that related plant families tend to use related chemical structures (e.g. isoflavonoids in the Leguminosae, sesquiterpenes in the Solanaceae), and some chemical classes are used across taxa (e.g. phenylpropanoid derivatives) (Dixon 2001). A great deal of work is clearly still needed in this area, including effort to define products and genes, in order to determine branches of these pathways directly involved in the response to pathogens in strawberry.

Strawberry plant receptors: the PTI and ETI responses

Strawberry non-specific basal resistance (PTI)

In strawberry, the presence of extracellular surface plant PRRs that recognize MAMPS or PAMPS, common to many classes of microbes, has been inferred from some indirect experiments, but the characterization of these receptors and the transduction pathways they elicit are as yet far from being fully elucidated. In this crop plant, the ability of chitosan to stimulate defense enzymes such as acidic chitinases has been reported on treated fruits (El Ghaouth et al. 1992), but close contact with tissue seems to be required for the elicitation. Strawberry receptors, which can presumably recognize chitin or chitin derivate compounds, were not able to detect the elicitor molecule through the non-porous strawberry cuticle, which acts as a physical barrier preventing intimate interaction between the elicitor and the tissue; therefore, direct application on freshly cut fruits is needed to develop the elicited plant response.

Adikaram et al. (2002) demonstrated enhanced disease resistance to gray mold rot (*B. cinerea*) in green strawberry fruit elicited both by *Aureobasidium pullulans* inoculation and by heat-killed cells of this yeast.

Some cell wall proteins with lectin domains have been described in strawberry (Trainotti et al. 2001, Martínez Zamora et al. 2008). Lectins are high affinity carbohydrate-binding proteins, which are able to recognize a wide variety of ligands and interact directly with the cell wall. In many plants, lectins are described as being involved in plant defense and thus as being implicated in facilitating PAMP recognition (De Hoff et al. 2009). Curry et al. 2002 have provided evidence that these classes of proteins are involved in the strawberry defense response, and pathogens such as *C. fragariae* are recognized by this class of proteins.

Plant damage sensing is involved in the basal defense response against pathogens and pests (Steinite and Ievinsh 2002). The ability of strawberry plants to be damage elicited has been documented to confer resistance against pests (Kilkiewicz 1988, Steinite and Ievinsh 2002, Greco and Sanchez 2003) and pathogens (Terry et al. 2004, Myung et al. 2006). In other plants, during the process of plant–pathogen interaction, cell wall breakdown fragments of [1→4]-α-linked oligogalacturonides (OGAs), generated by either the plant or the microbe, have been shown to elicit various plant defense responses (Côté and Hahn 1994, Aziz et al. 2004). How these responses are activated in strawberry needs to be further studied. Recent evidence suggests partially demethylated cell wall pectin-derived OGAs as true elicitor molecules capable of activating strawberry plant basal defenses (Osorio et al. 2008). The ectopic expression of the fruit-specific *F. ananassa* pectin methyl esterase (FaPE1) in wild strawberry *F. vesca* induced a reduced degree of esterification of cell wall OGAs compared with those from wild-type fruits, and the transgenic *F. vesca*

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lines had a constitutively activated SA signaling pathway and higher resistance to the necrotrophic fungus *B. cinerea*.

Oligomeric particles (10–15 monomers) are also induced by plant proteins with PG-inhibiting activity [polygalacturonase-inhibiting proteins (PGIPs)], which are included among the microbe-detecting molecules that are employed by the plant immune system to activate PTI (De Lorenzo and Ferrari 2002). PGIPs are thought to interfere with pathogen PG activity, and to interrupt the degradation of cell wall components to monomers. A PGIP has also been isolated in strawberry and will be discussed further below, in the section 'Strawberry proteins with a role in defense'.

Strawberry plant R-proteins: effector-triggered immunity (ETI)

Recogniction of pathogen avirulent effectors has been reported in strawberry. A small cysteine-rich protein, PcF, identified in *Phytophthora cactorum*, was able to trigger necrosis in strawberry plants and also in tomato (Orsomando et al. 2001). This protein elicited the activity of the enzyme phenylalanine ammonia lyase (PAL), but its exact mode of action remains unclear (Orsomando et al. 2003). It has been reported that an avirulent isolate of *C. fragariae* has the ability to protect the strawberry *F.×ananassa* cv. Pájaro against the development of anthracnose (Salazar et al. 2007). Thus, culture supernatant derived from that strain was able to induce HR, oxidative burst, accumulation of SA and callose deposition in strawberry cv. Pájaro. This elicitor was later identified as a 37 kDa protein, which belongs to the family of the subtilisin-like serine proteases. It conferred resistance to different degrees to other strawberry cultivars, and it also induced the accumulation of hydrogen peroxide (H_2O_2) and superoxide anion ($O2\cdot^-$) and callose deposition in *Arabidopsis thaliana* (Salazar et al. 2007, Chalfoun et al. 2009).

Martínez-Zamora et al. (2004) reported for the first time on resistance gene analogs (RGAs) in strawberry. Seven distinct families of RGAs of the NBS-LRR (nucleotide binding-leucine-rich repeat) type, the most prevalent family of plant receptors (McHale et al. 2006), were identified from wild species *F. vesca* and *F. chiloensis*, and six different *F.×ananassa* cultivars, by genomic DNA amplification using degenerate primers. Fifty-one clones presented significant homology to R gene sequences and RGAs from other species in the GenBank NR Database. All strawberry RGAs isolated were grouped into the TIR (Toll/interleukin-1 receptor-like) class of R genes, except one of them, which fell on the non-TIR branch. More recently, Jung et al. (2010) reported a cluster of four RGAs, contained in a strawberry (*F. vesca*) fosmid (34E24), with NBS and LRR domains, and conserved in all the rosid genomes with which they compared them. They also have found that none of the genes have the TIR domain, so they may belong to the non-TIR class. Although no experimental evidence about a correlation between the degree of resistance/susceptibility to a particular pathogen and the presence or absence of any particular class of RGAs has yet been shown, all the strawberry RGAs detected are closely related to R genes from other species; thus, some (if not all) of them may have pathogenesis response implications in strawberry resistance.

More recently, Martínez-Zamora et al. (2008) have also reported on the presence of serine-threonine kinase (STK) domain R gene receptors in strawberry. By using degenerate oligonucleotides to amplify conserved regions of the interspecific STK domain, they performed a broad screening on three related strawberry wild species (*F. vesca*, *F. chiloensis* and *Potentilla tucumanensis*), and seven different *F.× ananassa* cultivars [Camarosa, Gaviota, Oso Grande, Sweet Charlie, Pájaro, Milsei Tudla and the breeding line US159 from Galleta et al. (1993)]. They reported 31 putative strawberry STK clones (11 not redundant), and identified seven groups of STK genes out of the 11 non-redundant genes. Five of them (containing seven unique sequences) were classified as Pto-like kinases. The two unique sequences corresponding to group 6 were classified as B-lectin receptor kinases, a novel class of plant R genes also involved in plant defense (De Hoff et al. 2009), and the other two sequences, making up the seventh group, were closely related to the S-receptor-like protein kinases, involved in the mechanism by which hermaphrodite flowering plants avoid self-fertilization (Cui et al. 2000).

The first reported evidence of the synthesis of strawberry R proteins being regulated in response to pathogens can be found in Casado et al. (2006). They performed gene expression profiling and quantitative analysis of some strawberry genes coding for LRR receptor-like proteins (Falrrp1, Falrrk1, and Falrrk2), after *C. acutatum* infection. The genes analyzed showed a wide range of responses to the pathogen, which were tissue and cultivar dependent. Thus, the transcript level of *Falrrp1* and *Falrrk1* genes was higher in infected than in uninfected control fruit from cv. Camarosa, indicating a clear up-regulation of this gene after *C. acutatum* infection. In crown tissue, the expression of *Falrrk1* was modulated differently in the two cultivars analyzed, cv. Andana and cv. Camarosa, and varied from up- to down-regulation during the time of pathogen interaction. These results highlight the importance of considering spatial–temporal molecular studies in addition to the genotype in order to understand fully the mechanism of strawberry defense.

In the last decades, advances in the understanding of molecular aspects leading to host genotype-specific resistance have been made in Arabidopsis and other model plants, and they have been mainly focused on the identification and functional characterization of plant resistance (R) proteins and their cognate pathogen effectors (Bent and Mackey 2007, Lukasik and Takken 2009). However, disease resistance based on a single race-specific resistance (R) gene has not been shown to be durable in many crop species, as members of the pathogen population emerge that avoid recognition by the plant immune system, requiring the introduction of new resistance traits (Quirino and Bent 2003). Therefore, unraveling all of the

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strawberry-associated molecular components of the signaling pathways and genes they control related to active defense is necessary to understand this process fully in this crop plant.

Strawberry active defenses

Cell wall fortification and HR

Milholland et al. (1982) first reported that strawberry cultivars with different susceptibility to anthracnose produced by *C. fragariae* (Apollo and Sequoia as resistant cultivars, and Surecrop as the susceptible cultivae) presented clear histological differences after pathogen attack. While the most susceptible cultivars showed plant cellular collapse and necrosis, and successful fungal invasion, the less susceptible cultivars showed a thickening of the cell walls and a deposition of pectic material filling the intercellular spaces of the cortex. In addition, accumulation of tannins in the surrounding parenchyma cells was also found. All together, these changes were associated with fungal restriction to a few cells beneath the infection site. Although pre-formed structural and chemical components of the cell contribute to these mechanisms, actively synthesized de novo compounds are also implicated.

Salazar et al. (2007) also reported on morphological changes occurring on the strawberry plant cv. Pájaro challenged with *C. fragariae*. The plant response started with an early oxidative burst within 4 h after the inoculation with the fungus. The authors detected thickening of the cell wall of leaflets exposed to the microorganism, mainly due to the enlargement of the parenchyma cells and the intercellular space rather than to an increase in the number of layers of the mesophyll. They also describe the accumulation of pigments and of a new type of amorphous brown crystals in the intracellular mesophyll cells.

Cell wall fortification during infection, achieved by callose deposition (an amorphous, high molecular weight β-1,3-glucan polymer) in cell wall appositions (papillae), just below penetration sites, is a common defense response in plants (Luna et al. 2011).

Recently, a novel endo-β-1,3-glucanase gene (*Faβgln1*) from *F.×ananassa* cv. Chandler has been isolated upon infection with *C. acutatum* (Casado-Díaz et al. 2006). It encodes an unusual type of β-1,3-glucanase whose sequence structure contains a glycosylphosphatidylinositol (GPI) membrane anchor domain (J. L. Caballero, unpublished results). Nucleotide and protein sequence analyses identified this strawberry Faβgln1 as an acidic β-1,3-glucanase homologous to plant glycosyl hydrolases family 17. Although the (1→3)-β-D-glucanases are related to callose metabolism and plant defense, the exact biological role of these enzymes in relation to callose has not yet been clearly established (Minic and Jouanin 2006). Currently, the gene encoding strawberry Faβgln1 is being fully characterized and, curiously, its expression seems to be repressed in strawberry plants after challenge with *C. acutatum* (Casado-Díaz et al. 2006, J. L. Caballero, unpublished results).

Production of phytoalexins and other new antifungals

Evidence has been reported that strawberry has the capacity and ability to induce many of the genes encoding proteins with antifungal and antimicrobial activities, and enzymes that catalyze the new production of defense metabolites (phytoalexins), including chemical volatiles and those needed for the reinforcement of the cell wall, after detection of pathogen or cell damage by plant cell receptors.

Mussell and Staples (1971) detected production of phytoalexins in two strawberry cultivars, Surecrop and Stelemaster, with increased resistance to *Phytophthora fragariae*, challenged with the pathogen. Between 48 and 72 h after inoculation, the only discernible symptom was a browning of root epidermal cells, which contained two undetectable compounds in healthy roots. On TLC assays, these compounds showed a partially inhibitory effect on mycelial growth of *P. fragariae* but they were strong inhibitors of the growth of *Cladosporium cucumerinum*, a fungal pathogen that affects cucumbers. When a susceptible strawberry cultivar was tested (Blakemore), only one of these two compounds was produced after a longer period (5–8 d) of *P. fragariae* inoculation. Apparently, the activity of PAL, which increases during the synthesis of many phytoalexins (Hadwiger et al. 1970), was not essential for the synthesis of these inhibitors in root tissue after infection of these two strawberry cultivars. Vincent et al. (1999) also reported detection of a phytoalexin compound after *C. fragariae* infection that was solely induced in the strawberry cv. Sweet Charlie, a cultivar with reported increased resistance to this pathogen.

Hirai et al. (2000) identified three triterpene antifungal compounds from unripe strawberry fruit wounded and inoculated with *Colletotrichum musae* as euscaphic acid, tormentic acid and myrianthic acid. These triterpene phytoalexins were effective against infections with the fungus *C. fragariae*. The authors pointed out that these compounds probably correspond to the same phytoalexins found in strawberry cv. Surecrop roots and reported by Mussell et al. (1971). This observation suggests that strawberry fruit may produce similar antifungal compounds to those in the roots.

Adikaram et al. (2002) showed that skin tissue from strawberry green fruit inoculated with *A. pullulans* had greater antifungal activity against gray mold rot than the control non-inoculated tissue.

Yamamoto et al. (2000) proposed that induced catechin synthesis in response to strawberry leaf inoculation with a non-pathogenic strain of *A. alternata* was needed to inhibit penetration of the hyphae of this fungus into the leaf tissues. They concluded that the accumulation of (+)-catechin correlated with the time of spore inoculation of this non-pathogenic fungus, causing most of the resistance response in the strawberry leaf.

Ellagitannins and ellagic acid conjugates are present in many berries, including strawberry (Aaby et al. 2005, Aaby et al. 2007, Gasperotti et al. 2010, Hager et al. 2010). Production of

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ellagitannin, ellagic acid and gallic acid derivatives, and quercetin and kaempferol conjugates has also been shown to be stimulated by foliar application of benzothiadiazole (BTH), a synthetic plant systemic acquired resistance (SAR) activator, and glycine betaine, an amino acid derivate from sugar beet (Gorlach et al. 1996, Karjalainen et al. 2002), and this suggests a contribution of these phenolic compounds in strawberry active defense.

Increased strawberry resistance to *T. urticae* Koch has been described to be dependent on the presence and higher activity of wound-induced enzymes such as polyphenol oxidase and peroxidase (Steinite and Ievinsh 2002, Steinite and Levinsh 2003). As mentioned before, these authors suggest that the higher resistance of some strawberry cultivars to this pest is associated with a trichome-localized inducible increase of catechol-based phenolics produced by the activity of these oxidative enzymes.

Also, induced volatiles are known to be important for the strawberry plant to respond to attack by herbivore predators, as in many other plants (Maffei 2010). More than 360 volatiles are produced by strawberry (Schwab et al. 2009). From them, only six have been identified so far as key flavor compounds in the typical strawberry-like odor, and they are also species-specific significant volatiles: (Z)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, methyl butanoate, ethyl butanoate, methyl 2-methylpropanoate and 2,3-butanedione (Larsen et al. 1992). Also, linalool, nerolidol, α-pinene and limonene are quantitatively predominant in strawberry, reaching up to 20% of the total fruit volatiles (Loughrin and Kasperbauer 2001). It has been suggested that many of the strawberry volatile compounds might serve a dual role as attractants for animals, insects and humans and as protectants against pests and pathogens (Aharoni et al. 2003, Kappers et al. 2005). Although these pre-formed molecules can be considered to be phytoanticipins, the synthesis of many of them and other new compounds can be increased 'de novo' after pathogen attack and thus be part of the induced arsenal of the strawberry defense against pathogens.

Oxylipins are known to be synthesized 'de novo' in response to various stresses, including wound injury (Andreou et al. 2009). Their substrates, polyunsaturated fatty acids, are liberated from membrane lipids and converted into various oxylipins via several enzymatic steps. In strawberry leaves and fruit, (E)-hex-2-enal is a major volatile oxylipin produced upon wounding and it is not detectable on intact strawberry tissue (Hamilton-Kemp et al. 2003). Thus, after bruising, strawberry fruit emitted (E)-hex-2-enal and its precursor *cis*-3-hexenal, and the activities of the key enzymes, LOX and hydroperoxide lyase were also increased (Myung et al. 2006). (E)-hex-2-enal is a good inhibitor of conidial germination of *Penicillium expansum*, one of the main fungus pathogens causing post-harvest diseases in pears fruit (Neri et al. 2006), and it also has been reported to influence, either by inhibiting or by promoting, the development of the fungal pathogen *B. cinerea* Pers in strawberry fruit (Archbold et al. 1997, Fallik et al. 1998). Pérez et al. (1999) found that (E)-hex-2-enal is the most represented endogenous aldehyde on strawberry fruit during most developmental stages. Decreases in its content during the process of fruit ripening were related to the appearance of anthracnose symptoms. In addition, Arroyo et al. (2007) evaluated the effect of eight of the volatile products characterizing the strawberry aroma, and generated by the oxidative degradation of linoleic and linolenic acids through the LOX pathway, on the mycelial growth and conidia development (spore germination) of *C. acutatum* on strawberry fruit. A positive correlation between an increased content of (E)-hex-2-enal and an enhanced resistance of strawberry fruits to *C. acutatum* was found. The authors showed that this volatile compound altered the structures of the fungal cell wall and plasma membrane, causing disorganization and lysis of organelles and, eventually, cell death, and concluded that (E)-hex-2-enal was the most efficient of the volatile products in the control of *C. acutatum* infection. These results coincide with those reported by Fallik et al. (1998) and by Neri et al. (2006), in which (E)-hex-2-enal was related to enhanced resistance to *B. cinerea* and to *P. expansum*, respectively, and open up new perspectives on the biological control of pathogens by plant volatile compounds.

Also MeSA is naturally produced by plants, including strawberry, in response to herbivores. Thus, an increase in MeSA release and that of other volatiles has been detected in strawberry plants after injury (Hamilton-Kemp et al. 2003, see **Table 2**), and infection with cyclamen mite (Himanen et al. 2005) and strawberry blossom weevil (Bichão et al. 2005). In other plants, it is well documented that MeSA and other volatiles are attractive to natural enemies, a plant defense strategy called 'indirect defense', so being beneficial for pest control (Kessler and Baldwin 2002, James and Price 2004). For instance, MeSA reduced the aphid *Phorodon humili* Schrank in hop yard (Lösel et al. 1996), and it delayed the establishment of bird cherry-oat aphid, *Rhopalosiphum padi* (L.), in barley (Ninkovic et al. 2003). Alternatively, MeSA may also repel pests, and it seems to inhibit development of gray mold, *B. cinerea* Pers. ex Pers, on the fruit (Archbold et al. 1997). In strawberry, Lee (2010) reported that MeSA enhanced natural enemy attraction but did not increase, or decrease pest abundance. However, natural enemies of major strawberry pests responded to MeSA in the laboratory, including *Anaphes iole* Girault, an egg parasitoid of *Lygus hesperus* Knight (Williams et al. 2008), and *Phytoseiulus persimilis* Athias-Henriot, a predator mass released for control of the two-spotted spider mite, *T. urticae* Koch (de Boer and Dicke 2004).

Fadini et al. (2007, 2010) also demonstrated a positive communication through such strawberry volatiles and *P. macropilis*, a predator of *T. urticae* Koch. This phenomenon remains to be studied further, but there is evidences that strawberry have the capacity and ability to perform such a defense strategy. Thus, Aharoni et al. (2003) demonstrated that ectopic overexpression of a strawberry dual linalool/nerolidol synthase gene (*FaNES1*) in chloroplasts of *A. thaliana* significantly increased the amount of volatile terpenes such as linalool and its

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derivatives in leaves, and these transgenic plants were able to repel the attack of the aphid *Myzus persicae*. The recombinant FaNES1 enzyme generated (S)-linalool and *trans*-(S)-nerolidol from geranyl diphosphate (GDP) and farnesyl diphosphate (FDP), respectively. The authors demonstrated that unwounded transgenic plants were able to attract the aphid predatory mite *P. persimilis* easily. Kappers et al. (2005) targeted FaNES1, a strawberry linalool/nerolidol synthase, especifically to the mitochondria, and found that the majority of the predatory mites made their first visit to the transgenic plants, which demonstrates a clear preference for the undamaged transgenic plants.

These results suggest the possibility to protect strawberry plants from insect pests by stimulating the emission of volatile organic compounds produced upon feeding, which eventually attract 'bodyguard' predators as suggested by Kappers. Curiously, although similar genes have been found in wild and cultivated strawberry species, only FaNES1 is exclusively present and highly expressed during fruit ripening in cultivated octoploid varieties (Aharoni et al. 2003, Aharoni et al. 2004).

Strawberry proteins with a role in defense

An outstanding role in plant defense response to pathogen and pests is assigned to an important group of plant proteins regulated under biotic stress conditions. Components of this group, the so-called PR proteins, have been categorized into 18 families (van Loon et al. 2006). It is accepted that the term 'pathogenesis-related protein' includes all microbe-induced proteins and their homologs, even though some of them are generally constitutively present in the plant, and only increase during most infections. Among others, this is the case with enzymes such as PAL, peroxidase (POX) and polyphenoloxidase (PPO), which are often also referred to as PR proteins (van Loon et al. 2006).

Table 3 shows an update on recognized components of known families of PR proteins in strawberry. As shown, members of almost all known plant PR protein families have been reported in strawberry. However, the implications in strawberry defense have been mostly inferred from their induction pattern after pathogen attack, and their exact functional role remains to be determined or experimentally proven for the majority of them.

Glucanases and chitinases are the most abundant classes of strawberry PR genes with hydrolytic activity identified so far. Thus, three strawberry members (FaBG2-1, FaBG2-2 and FaBG2-3) of class II β-1,3-glucanases of the plant PR2 family have been cloned and partially characterized (Khan et al. 2003, Shi 2005, Shi et al. 2006). The genes *FaBG2-1* and *FaBG2-3* were shown to be induced after infection of strawberry leaves with either *C. fragariae* or *C. acutatum*. A higher level of induction was detected when the former pathogen was tested (Shi et al. 2006). Previous studies have also shown that a gradual increase in total β-1,3-glucanase activity occurred in strawberry from 2 to 48 h post-infection in response to either of the two fungi (Shi 2005). Similarly, a gradual increase in total chitinase activity during the first 24 h post-infection was also detected in strawberry challenged with either of these two pathogens. In addition, the overall chitinase activity was also induced to a significant level when strawberry plants were injured or treated with either SA or ethephon (Khan 2002). These results highlight the importance of chitinases in strawberry in response to both biotic and abiotic stresses. So far, three strawberry chitinase genes have been cloned, a class III chitinase from the PR8 family (*FaChit3-1*) (Khan et al. 1999), and two class II chitinases from the PR3 family (*FaChi2-1* and *FaChi2-2*) (Khan and Shih 2004). Similarly to the *FaBG2-1* and *FaBG2-3* genes, the *FaChi2-1* and *FaChi2-2* genes were induced upon *C. fragariae* or *C. acutatum* infection within 2–6 or 24–48 h post-inoculation, respectively (Khan and Shih 2004). More recently, the cloning and sequencing of two *FaChi2-1* alleles from Toyonaka and Akihime strawberry cultivars has also been reported, but no information other than sequence comparison with pea (L37876), Kentucky bluegrass (AF000966), pepper (AY775335), parsley (AF141372), Norway spruce (AY544781) and muskmelon (AF241538) orthologous genes is described (Zhang et al. 2009).

Two strawberry osmotin-like-coding genes, *FaOLP1* and *FaOLP2*, belonging to the plant PR5 family have been cloned (Wu et al. 2001, Zhang and Shih 2007). The expression of *FaOLP1* has been examined upon fungal infection (Zhang 2006). Thus, both *C. fragariae* and *C. acutatum* triggered a substantial induction of FaOLP1 in strawberry leaves at 24–48 h post-inoculation, suggesting the involvement of FaOLP1 in strawberry defense against these fungi. The spatial expression pattern of FaOLP2 has also been studied in strawberry plant (Zhang and Shih 2007). Thus, high level of FaOLP2 transcripts was detected in the crown and leaf while a relatively low level was detected in root and ripe red fruit, and a very low level in green fruit. Interestingly, *FaOLP2* was up-regulated by ABA, SA and mechanical wounding within 2–6 h post-treatment, and was more prominently induced by SA than by the other abiotic stimuli, indicating that this strawberry gene responds to abiotic stresses (Zhang and Shih 2007). Surprisingly, no expression studies to support the involvement of this strawberry *FaOLP2* gene in response to biotic stresses have been reported to date.

A strawberry member of the PR6 family has been cloned and characterized (Martinez et al. 2005). This strawberry *Cyf1* gene (*FaCPI-1* gene) encodes a phytocystatin, a protein with proteinase inhibitor activity. Plant phytocystatins have been implicated in the endogenous regulation of protein turnover (Arai et al. 2002, Corre-Menguy et al. 2002), programmed cell death (Solomon et al. 1999, Belenghi et al. 2003) and also in defense mechanisms against insects and pathogens (Vain et al. 1998, Gutierrez-Campos et al. 1999). It has been speculated that alterations in the fungal membrane permeability could be the origin of the antifungal properties of this family of plant defense proteins (Giudici et al. 2000, van der Vyver et al. 2003). Curiously, the strawberry *Cyf1* gene was originally obtained from a developing fruit of *F.* × *ananassa* cv. Elsanta

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Table 3 Strawberry pathogenesis-related proteins

PR protein family[a]	Family properties	Strawberry gene ID	Standarized gene name[b]	Accession No.[c]	Characterization		References
					Protein activity	Gene expression regulation	
PR1	Unknown	FaPR1	–	DV440399	NAD	Up-regulated by aplication of UV-C treatment. Related to *Botrytis* resistance.	Pombo et al. (2011b)
PR2	β-1, 3-Glucanase	FaBG2-1	–	AY170375	NAD	Up-regulated upon *Colletotrichum fragariae* or *Colletotrichum acutatum* infection, and by UV-C treatment.	Khan et al. (2003); Pombo et al. (2011b)
		FaBG2-3	–	AY989819			Shi et al. (2006); Pombo et al. (2011b)
		FaBG2-2	–	AY989818		NAD	Shi et al. (2006); Pombo et al. (2011b)
		Fagln1	–	AJ871767		Reduced level of transcripts after fruit infection by *Colletotrichum acutatum*.	Casado-Díaz et al. (2006)
PR3	Chitinase type I, II, IV, V, VI, VII	Fachit-1	FaCHI1-1	AJ871765	NAD	Down-regulated on *Colletotrichum acutatum*-infected fruits.	Casado-Díaz et al. (2006)
		FaChi2-1 FaChi2-2	FaCHI2-1 FaCHI2-2	AF147091 AF320111		Induced upon *Colletotrichum fragariae*, *Colletotrichum acutatum* or *Botrytis cinerea* infection. FaChi2-2 increased in UV-C-treated fruit immediately after the treatment.	Khan and Shih (2004); Mehli et al. (2005); Zhang (2009); Pombo et al. (2011b)
		Class IV chitinase	FaCHIT4-1	TA9333_57918		Up-regulated in red and white strawberry fruits 24 h after *Colletotrichum acutatum* infection.	Guidarelli et al. (2011)
		M16D12	FaCHI4-2	JN415653		Strong up-regulation under biotic (*Coletotrichum acutatum*) and abiotic (JA) stress.	Unpublished results
PR5	Thaumatin-like	FaOLP1	FaOLP-1	AAF13707	NAD	Induction triggered by *Colletotrichum fragariae* and *Colletotrichum acutatum*.	Wu et al. (2001); Zhang (2006)
		FaOLP2	FaOLP-2	DQ325524		Expressed at different levels in leaves, crowns, roots, green fruits and ripe red fruits. Up-regulated by the signal molecules ABA and SA, and by mechanical wounding, more prominently induced by SA. Not regulated by aplication of UV-C treatment.	Zhang and Shih (2007); Pombo et al. (2011b)
		PR5	FaPR5-3	EU289405		Constitutive higher gene expression in strawberry trangenic (FaPE1) lines more resistant to Botrytis cinerea.	Osorio et al. (2008)
		Fapr5-1	FaPR5-1	AJ871764		Up-regulated in fruit and crown tisues by *Colletotrichum acutatum* infection.	Casado-Díaz et al. (2006)
		Fapr5-2	FaPR5-2	AJ871763		Up-regulated in fruits by *Colletotrichum acutatum* infection.	Casado-Díaz et al. (2006)
PR6	Proteinase-inhibitor	FaCPI-1	–	AJ862660	Recombinant FaCPI-1 protein expressed in *E. coli* was a good inhibitor of papain and other cysteine proteinases and showed in vitro antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum*	Expressed in fully expanded leaves, in roots and in achenes, but surprisingly not in the receptacle (pseudocarp) during fruit development.	Martinez et al. (2005)

(continued)

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Table 3 Continued

PR protein family[a]	Family properties	Strawberry gene ID	Standarized gene name[b]	Accession No.[c]	Characterization		References
					Protein activity	Gene expression regulation	
PR8	Chitinase type III	FaChi3-1	FaCHI3-1	AF134347	NAD	Expressed constitutively at low levels in strawberry leaves, and increased expression in UV-C-treated fruit immediately after the treatment.	Khan et al. (1999); Khan (2002); Pombo et al. 2011b
PR9	Peroxidase	Faprox-1	FaPOX-1	AJ871771	NAD	Down-regulated on *Colletotrichum acutatum*-infected fruits.	Casado-Díaz et al. (2006)
		362ACC04	FaPOX-2	AJ871760		Represented in mock portion of a sustractive strawberry (Fa cv. Andana) mock *Colletotrichum*-infected library.	Casado-Díaz et al. (2006)
		Peroxidase	FaPOX-3	DV439771		Increase expression in white fruit Fa cv. Alba upon *Colletotrichum acutatum* atack.	Guidarelli et al. (2011)
PR10	Ribonuclease-like	Fra a 1a	FaPR10-1.1	DQ385511, AM084674, Q3T923	Demonstrated allergenic properties. Essential biological function in pigment formation in strawberry fruit (might be involved in processes leading to the formation and/or the accumulation of anthocyanins).	Fra a 1 protein is >7-fold more abundant in the red compared with white strawberry varieties. Fra a 1e mainly expressed in roots and the transcript levels decrease from the open flower stage to the ripe fruit. More transcipt level of an *Fra a 1E* gene in white non-matured fruit when compared with red ripe fruit of Fxa cv. Camarosa and in cv. Alba where is also up-regulated in red strawberry fruits 24 h after *Colletotrichum acutatum* infection.	Karlsson et al. (2004);Hjernø et al. (2006); Musidlowska-Persson et al. (2007); Muñoz et al. (2010); Guidarelli et al. (2011)
		Fra a 1b	FaPR10-1.2	AM236313, AM236314, AM236315, Q256S7			
		Fra a 1c	FaPR10-1.3	AM236317, AM236318, Q256S6			
		Fra a 1d	FaPR10-1.4	AM236316, Q256S2			
		Fra a 1e	FaPR10-1.5	AM236319, AM236320, TA487_3747, Q256S4			
		DY673343	FaPR10-1.6	DY673343			
		Fra a 2	FaPR10-2	GQ148818	An essential biological function in pigment formation in strawberry fruit (might be involved in processes leading to the formation and/or the accumulation of anthocyanins).	Highly transcribed in the late stages of fruit ripening, might be a hyperallergenic isoform.	Muñoz et al. (2010)
		Fra a 3	FaPR10-3	GQ148819, EU289406		Uniform expression pattern between strawberry tisues. Substantially higher (2- to 5-fold) in ripe fruits of the white-fruited *F. chiloensis* cultivar than in the red variety. Similarly, in *F. vesca* mRNA level higher in white than in red fruits. Gene expression non-afected in strawberry trangenic (FaPE1) lines more resistant to *Botrytis cinerea*.	Osorio et al. (2008); Muñoz et al. (2010)
		M23D11	FaPR10-4	JN415652	NAD	Strong up-regulation under biotic (*Coletotrichum acutatum*) and abiotic (JA) stress.	Unpublished results
		EX672442	FaPR10-5	EX672442		Increase in expression in infected red and white fruits of Fa cv. Alba by *Colletotrichum acutatum*.	Guidarelli et al. (2011)
		DY671909	FaPR10-6	DY671909			
		DY676200	FaPR10-7	DY676200			
		TA11697_57918	FaPR10-8	TA11697_57918			

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Table 3 Continued

PR protein family[a]	Family properties	Strawberry gene ID	Standarized gene name[b]	Accession No.[c]	Characterization		References
					Protein activity	Gene expression regulation	
PR13	Thionin	Faγthio-1	FaTHIO-1	AJ871768	NAD	Down-regulated on *Colletotrichum acutatum*-infected fruits.	Casado-Díaz et al. (2006)
PR14	Lipid-transfer protein	LTP1	FaLTP-1.1	DQ066727	Alergenic properties. Induced histamine release at a 100-fold higher concentration than peach LTP. Has less allergenic potency than peach and apple LTP, and therefore is aninteresting tool for future immunotherapy.	Expression of LTP genes was observed in white and ripe fruit (including seeds) and leaves of strawberry cultivar Elsanta. Responds to abiotic treatments such as ABA and SA, but not to salt and heat stresses; also reported that the expression of the *Fxaltp* gene is stimulated by wounding and repressed by cold stress, and negatively regulated in strawberry crown tissue infected by *Colletotrichum acutatum*.	Yubero-Serrano et al. (2003); Zuidmeer et al. (2006)
		LTP2	FaLTP-1.2	DQ066728			
		LTP3	FaLTP-1.3	DQ066729			
		LTP4	FaLTP-1.4	DQ066730			
		LTP5	FaLTP-1.5	DQ066731			
		Fxaltp	FaLTP-1.6	AJ315844			
		LTP6	FaLTP-2	DQ066732			
		LTP	FaLTP-3	TA11085_57918	NAD	More highly expressed in white fruits than in red ones in cv. Alba.	Guidarelli et al. (2011)

NAD, no available data.
[a] Only PR families with recognized members in strawberry are shown.
[b] A recomendation of a standarized gene name is sugested for some of the strawberry genes [i.e. FaCHI2-1.1: Fa (species), CHI (gene type), 2 (class of gene type), -1 (order it was discovered), .1 (alele)].
[c] Institute for Genomic Reasearch (TIGR) and National Center for Biotechnology Information (NCBI) codes of transcript sequences.

(Martinez et al. 2005). Northern blot and in situ hybridization analyses indicated that the *Cyf1* gene is expressed in fully expanded leaves, in roots and in achenes, but surprisingly not in the receptacle (pseudocarp) during fruit development. However, the recombinant FaCPI-1 protein expressed in *Escherichia coli* was a good inhibitor of papain and other cysteine proteinases and showed in vitro antifungal activity against *B. cinerea* and *Fusarium oxysporum*. Previous studies have shown that the ectopic expression of a peptidase inhibitor from cowpea (CpTi, cowpea trypsin inhibitor) in strawberry was effective against insects (Graham et al. 1997, Graham et al. 2002). Therefore, the inhibitory properties shown by the strawberry FaCPI-1 protein highlight the importance of this endogenous *FaCyf1* gene as a valuable tool for control of fungal strawberry diseases.

Members of the PR10 family have also been described in strawberry. Thus, seven strawberry proteins homologous to proteins from the PR10 group, called Fra a 1 (five isoforms: a–e), Fra a 2 and Fra a 3, have been reported (Hjernø et al. 2006, Musidlowska-Persson et al. 2007, Muñoz et al. 2010). Apart from their known allergenic properties (Karlsson et al. 2004, Musidlowska-Persson et al. 2007), an essential biological function in pigment formation in strawberry fruit has been recently proposed for some members of this strawberry family (Muñoz et al. 2010). By transient expression analysis in strawberry fruit, Muñoz et al. (2010) directly linked the genes *Fra a 1e*, *Fra a 2* and *Fra a 3* to flavonoid biosynthesis. It was also suggested that these genes could function either as carriers of flavonoid pathway intermediates or as (co-) transporters of anthocyanins into the plant vacuole. However, more recently, some *Fra a* alleles have also been shown to be induced in strawberry plants upon pathogen attack. Thus, *Fra a 1* (gene DY673343) and *Fra a 1E* (gene TA487_3747) were up-regulated in red ripe fruit of *F.* × *ananassa* cv. Alba 24 h after *C. acutatum* infection (Guidarelli et al. 2011) (see also below). Also a new member of the PR10 family (FaPR10-4), strongly up-regulated under biotic (*C. acutatum*) and abiotic [jasmonate (JA)] stress, has been cloned from strawberry crown tissue and is currently being characterized (J. L. Caballero, personal communication).

Yubero-Serrano et al. (2003) described the cloning and characterization of a strawberry *Fxaltp* gene (PR14 family), which responds to abiotic treatments such as ABA and SA, but not to salt and heat stresses. It was also reported that the expression of the *Fxaltp* gene is stimulated by wounding and repressed by cold stress. The *Fxaltp* gene showed a tissue-dependent regulatory mechanism, and responded differently to these abiotic treatments in fruit and leaves, highlighting the importance of spatial expression studies to understand fully the role of this and other strawberry genes in defense. The *Fxaltp* gene, now renamed *FaLTP1.6* (J. L. Caballero, personal communication), belongs to type 1 of extracellular plant non-specific lipid transfer proteins (nsLTPs). Curiously, allergenic properties have also been proved for this class of strawberry genes (Zuidmeer et al. 2006). Thus, FaLTP1 (alleles *LTP1–5* and *Fxaltp1*) and FaLTP2 (allele *LTP6*) proteins induced histamine release at a 100-fold

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higher concentration than peach LTP, and have less allergenic potency than peach and apple LTP; they therefore are proposed as an interesting tool for future immunotherapy. A wide range of extracellular roles has been suggested for members of this family of plant proteins, including a specific defensive function against bacterial and fungal pathogens (García-Olmedo et al. 1995, Molina et al. 1996, Kirubakaran et al. 2008, Sarowar et al. 2009), as well as a putative role in the early recognition of plant intruders and in systemic resistance signaling (Buhot et al. 2001, Blein et al. 2002, Maldonado et al. 2002, Sarowar et al. 2009). However, the exact in vivo role remains unclear for most of them. Interestingly, the *Faltp1* gene is negatively regulated in strawberry crown tissue infected by *C. acutatum* (J. L. Caballero personal communication).

In a recent study, Pombo et al. (2011a, b) directly related the enhancement of gene expression and enzymatic activity of a set of strawberry genes to the increase of strawberry resistance against *B. cinerea*. They studied the effect of UV-C treatment on the growth of *B. cinerea* during strawberry fruit post-harvest decay and analyzed the activity of enzymes such as PAL, PPO, POX and β-1,3-glucanase, and as well as the level of gene expression of *FaPAL6* (Pombo et al. 2011a) and PR genes such as *FaChi2-2*, *FaChi3*, *FaBG2-1*, *FaBG2-3* and *FaPR1* (**Tables 3**, **4**). An improvement in fruit resistance against this pathogen was observed in collected fruit after this physical treatment. In addition, except for genes *FaChi2-1* and *FaOLP2*, both the expression level and the enzymatic activity increased for all these genes and enzymes, supporting a defensive role for all of them against this fungal pathogen.

A cell wall-related strawberry (*F.× ananassa*) fruit gene coding for a PGIP protein (FaPGIP) has been cloned and described to play a role in strawberry defense (Mehli et al. 2004). It is known that PGIPs are bound by ionic interactions to the extracellular matrix of plant cells (Shanmugam 2005). These plant proteins display LRR domains and have a high affinity for fungal endo-PGs, which are important pathogenicity factors (O'Connell et al. 1990). In fact, PGs are among the first enzymes secreted by *B. cinerea* upon infection (van der Cruyssen et al. 1994, Rha et al. 2001). Seven different variants of FaPGIP from five strawberry cultivars (Elsanta, Korona, Polka, Senga sengana and Tenira) were identified, and divided into three major groups (FaPGIP1a, FaPGIP1b, FaPGIP1c, FaPGIP2a, FaPGIP2b, FaPGIP2c and FaPGIP3) (Mehli et al. 2004, Schaart et al. 2005, **Table 4**). After inoculation of fruit with *B. cinerea*, all five strawberry cultivars studied displayed a significant induction in the overall *FaPGIP* gene expression. Specific analysis showed that all the *FaPGIP* variants studied were up-regulated when white-stage fruits were inoculated with the pathogen. In addition, by using either of the two *FaPGIP* allelic sequences *FaPGIP1a* or *FaPGIP2a*, these authors produced genetically modified strawberry lines with expression of this *FaPGIP* gene regulated by the strong and constitutive *Cauliflower mosiac virus* (CaMV) *35S* promoter (Schaart 2004). The strawberry transgenic lines expressed a less susceptible phenotype against *B. cinerea* than the control untransformed line. These results strongly support a defensive role for this strawberry *FaPGIP* gene. From the analysis of other plant PGIPs, the strawberry *FaPGIP* gene also showed spatial and fruit developmental regulation. Curiously, in crops such as pear (Abu-Goukh et al. 1983), raspberry (Johnston et al. 1993), apple (Yao et al. 1999) and cantaloupe (Fish and Davis 2004), the expression of PGIP genes is higher in immature than in mature fruit, but the opposite is true for strawberry where the *FaPGIP* gene presents the highest level of expression in healthy mature fruit (Mehli et al. 2004). This fact may reflect a strawberry plant-specific strategy focused on enhancing fruit protection during the most helpless and soft stages.

Hormonal and signaling pathways involved in the strawberry defense response

Molecules such as SA, JA and ethylene (ET) are well known as plant response regulators of biotic stresses. The SA-dependent signaling pathway is critical in establishing the HR and systemic pathogen resistance, and prevents progression of pathogens mainly with biotrophic and hemibiotrophic lifestyles, while JA- and ET-dependent signaling pathways are mainly induced in response to necrotrophic pathogens, mechanical wounding and herbivore predation (Glazebrook 2005). ABA, auxin, gibberellic acid, cytokinin, brassinosteroids (BRs) and peptide hormones are also part of the hormonal arsenal used by plants in defense signaling pathways (Bari and Jones 2009). Extensive cross-talk between these hormone-dependent signaling pathways fine-tunes the regulatioin of the plant defense response.

Similarly to other plants, SA seems to work as a defense inducer in strawberry. Treatments of strawberry plants with BTH, an SA analog, greatly increased the concentration of SA in leaves (Hukkanen et al. 2007). Strawberry plants treated with this hormonal compound improved the post-harvest quality of fruit (Babalar et al. 2007, Cao et al. 2010b, Shafiee et al. 2010), and exhibited changes in chemical composition, mainly of phenolic compounds such as ellagitannins (Cao et al. 2010a, Cao et al. 2011), enhancing the total antioxidant capacity of the fruit (Asghari and Babalar 2009) and the level of expression of specific genes related to defense, which led to a reduction in the microbial population (Zhang and Shih 2007, Hukkanen et al. 2007, Encinas-Villarejo et al. 2009, Cao et al. 2010b). Exogenous application of SA at non-toxic concentrations to strawberry fruits also enhanced resistance to pathogens such as *B. cinerea*, and effectively reduced fungal decay (Babalar et al. 2007, Asghari and Aghdam 2010).

Methyl jasmonate also increases the level of phenolic compounds such as chlorogenic acid and rutin, and induces strawberry resistance to the two-spotted spider mite (*T. urticae* Koch) (Warabieda et al. 2005).

It has been described that repression of auxin-responsive genes is part of the SA-mediated disease resistance mechanism (Wang et al. 2007). In strawberry, auxins have mainly been implicated in developmental processes, acting as key regulators for growth and fruit ripening (Aharoni et al. 2002a,

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Table 4 Other strawberry defense-related proteins

Protein family	Strawberry gene ID	Standarized gene name[a]	Accession No.[b]	Characterization		References
				Protein activity	Gene expression regulation	
Phenylalanine ammonia-lyase	FaPAL1	FaPAL-1.1	AB360390	NAD	NAD	Unpublished (GenBank sequences)
	FaPAL3	FaPAL-1.2	AB360392			
	FaPAL4	FaPAL-1.3	AB360393			
	FaPAL5	FaPAL-1.4	AB360394			
	FaPAL2	FaPAL-2.1	AB360391			
	FaPAL6	FaPAL-2.2	AJ871757, HM641823	Correlation between gene expression and accumulation of higher anthocyanin amount in Camarosa cultivar.	Represented in infected portion of a sustractive strawberry (Fa cv. Andana) mock *Colletotrichum*-infected library. Up-regulated by aplication of UV-C treatment.	Casado-Díaz et al. (2006); Pombo et al. (2011a, 2011b)
Polygalacturonase-inhibiting protein	FaPGIP1.a	FaPGIP-1.1		Genetically modified strawberry overexpressing FaPGIP which is less susceptible to gray mold.	Mature fruit showed the highest constitutive gene expression levels (the gene is developmentally regulated). After inoculation with *B. cinerea*, all five cultivars studied ('Elsanta', 'Korona', 'Polka', 'Senga sengana', 'Tenira') displayed a significant induction of gene expression, this up-regulation was accompanied by a significant change in *FaPGIP* allele frequencies when compared with non-treated fruits. Gene expression not afected in strawberry trangenic (FaPE1) lines more resistant to *Botrytis cinerea*.	Mehli et al. (2004, 2005); Schaart (2004); Schaart et al. (2005); Osorio et al. (2008)
	FaPGIP1.b	FaPGIP-1.2				
	FaPGIP1.c	FaPGIP-1.3				
	FaPGIP2.a	FaPGIP-1.4				
	FaPGIP2.b	FaPGIP-1.5				
	FaPGIP2.c	FaPGIP-1.6				
	FaPGIP3	FaPGIP-1.7				
	FaPGIP	FaPGIP-1.8	EU117213	NAD	NAD	Y. Zhang et al. (2010)
	FrveA7 PGIP	FaPGIP-1.9	AF196892	NAD	NAD	Osorio et al. (2008)
	FrveA6 PGIP		AF196891	NAD	NAD	Mehli et al. (2004)
	PGIP	FaPGIP-1.10	AY534684	NAD	Up-regulated in red and white strawberry fruits 24 h after *Colletotrichum acutatum* infection	Guidarelli et al. (2011)
	ABV04088	FaPGIP-1.11	ABV04088	NAD	NAD	Y. Zhang et al. (2010)
	gene20317	FvPGIP-2				Strawberry genome release; Shulaev et al. (2011)
WRKY transcription factor	FaWRKY1	–	AJ871772	*FaWRKY1 can play a role as important element mediating defence response to C. acutatum in strawberry.*	*Induced by Colletotrichum acutatum in fruit and crown tissues from very susceptible (cv. Camarosa) and moderately susceptible (cv. Andana) cultivars, treatments with elicitors, and wounding.*	Casado-Díaz et al. (2006)
Hypersensitive-induced response protein	Fahir-1	–	AJ871769	NAD	Induced by *Colletotrichum acutatum* in fruit and crown tissues from very susceptible (cv. Camarosa) and moderately susceptible (cv. Andana) cultivars.	Casado-Díaz et al. (2006)
LRR receptor-like proteins	Falrrk-1	–	AJ871784	NAD	Down-regulated in fruits and crown tisues infected by *C. acutatum*.	Casado-Díaz et al. (2006)
	Falrrk-2	–	AJ871783		Down-regulated in infected fruits by *C. acutatum*.	Casado-Díaz et al. (2006)
Xyloglucanase-inhibiting protein	TA10709_57918	–	TA10709_57918	NAD	Significantly up-regulated after inoculation with *C. acutatum* in both white and red fruit stages.	Guidarelli et al. (2011)
Cytochrome p450 monoxygenases	TA9078_57918	–	TA9078_57918			

(continued)

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Table 4 Continued

Protein family	Strawberry gene ID	Standarized gene name[a]	Accession No.[b]	Characterization		References
				Protein activity	Gene expression regulation	
Aldehyde dehydrogenase	TA12321_57918	–	TA12321_57918		Significantly up-regulated after inoculation with *C. acutatum* in both white and red fruit stages.	Guidarelli et al. (2011)
Flavonol synthase	TA9432_57918	–	TA9432_57918			
Tropine reductase	DY673561	–	DY673561			
Alpha/beta amyrin synthase	TA11548_57918	–	TA11548_57918			
3-Hydroxy-3-methylglutaryl (HMG) coenzyme-A synthase	CO381295	–	CO381295			
Lectin family	TA10594_57918	–	TA10594_57918		Enhanced expression in white stage fruit upon *C. acutatum* infection.	
Glutatione S-transferase	CO79212	–	CO79212		Up-regulated in red fruits challenged with *C. acutatum*.	
Snaking-1	CO378568	–	CO378568			

NAD, no available data.
[a] A recomendation of a standarized gene name is sugested for some of the strawberry genes [i.e. FaCHI2-1.1: Fa (species), CHI (gene type), 2 (class of gene type), -1 (order it was discovered), .1 (alele)].
[b] Institute for Genomic Reasearch (TIGR) and National Center for Biotechnology Information (NCBI) codes of transcript sequences.

Mezzetti et al. 2004). However, recent evidence provided by Osorio et al. (2011) also associates auxins with plant defense response in strawberry. Thus, resistance of *F. vesca* transgenic FaPE1 lines to *B. cinerea* was correlated to a significant decrease in the auxin content as well as an enhanced expression of some auxin-repressed genes in transgenic fruit.

ET has been considered a ripening hormone in other plants, but its role in strawberry fruit ripening has been thought to be negligible, and strawberry is considered to be a non-climacteric fruit. However, it has been reported that the achenes of red strawberry fruit produce ET at low concentrations (Iannetta et al. 2006). Interestingly, SA-treated strawberries effectively reduced fruit ET production (Babalar et al. 2007), a physiological mechanism resembling that of auxin genes.

Positive or negative cross-talk between SA and JA/ET signaling pathways is dependent on the specific pathogen, and protein factors such as NPR1 (non-expressor of PR1) or WRKY play important roles in this antagonistic interaction (Spoel et al. 2007). Thus, WRKY70 proteins have been shown to act as a positive regulator of SA-dependent defenses and a negative regulator of JA-dependent defenses (Li et al. 2004). Recently, two *F.×ananassa* WRKY70 gene analogs has been cloned (J. L. Caballero, unpublished). Preliminary expression analyses indicate that both strawberry genes are induced in cv. Andana plants infected with *C. acutatum*, and also respond to SA treatments, which suggests that these *FaWRKY70* genes may take part in the SA signaling network of strawberry defense. Also, another strawberry gene *FaWRKY707* is strongly induced on *C. acutatum*-infected fruits (J. L. Caballero, unpublished). *FaWRKY707* has high similarity to *AtWRKY33*, which is rapidly and strongly induced by fungal and bacterial PAMPs in Arabidopsis (Lippok et al. 2007), and acts as a positive regulator of JA- and ET-mediated defense signaling but as a negative regulator of SA-mediated responses (Zheng et al. 2006). The identification of these WRKY orthologous factors in strawberry indicates that key regulatory members of defense mechanisms are also presents in strawberry, and suggest that an antagonistic relationship between the known plant defense-related signaling pathways might also be working in strawberry in response to pathogens; however, this needs to be analyzed further.

Emerging evidence suggests that gibberellin signaling components play major roles in the control of plant immune responses [i.e. by modulating SA- and JA-dependent defense responses (Navarro et al. 2008, Tanaka et al. 2006)]. In addition, BRs, which are plant hormones structurally related to the animal steroid hormones (Bajguz 2007), enhance resistance to pathogens in tobacco, rice (Nakashita et al. 2003), tomato and potato (Krishna 2003), and may be involved in cross-talk with other hormone signaling in mediating defense responses in plants, as such with ABA and ET (Krishna 2003). Although some of the genes involved in hormone-regulated processes of gibberellin, auxin, ET and BR signaling have been reported in strawberry (Bombarely et al. 2010, Csukasi et al. 2011), no detailed information is available to date about their putative implications in the strawberry plant defense response.

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Transcriptomic approaches for defense-related gene discovery in strawberry

So far, few studies in strawberry have been published focused on pursuing high-throughput gene discovery related to the mechanism of defense. Casado et al. (2006) reported the first study aimed to identify strawberry genes with altered expression in response to *C. acutatum* infection. Using a subtractive hybridization approach, a large number of strawberry genes involved in signaling, transcriptional control and defense, and many genes with unknown function were isolated. Spatial and temporal gene expression profiles after *C. acutatum* infection yielded a first insight into some of the genes responding to this pathogen, and showed that the strawberry response was dependent on the tissue and cultivar analyzed. Thus, strawberry genes belonging to PR5 (*Falpr5-1* and *Falpr5-2*, encoding two thaumatin-like proteins) and PR10 (*Falpr10-1*, an RNase-like gene) families, as well as the genes *Fahir-1* (encoding a hypersensitive-induced response protein) and *Fawrky1* (encoding a protein with similarity to WRKY transcription factors) were found to be induced in fruit and crown tissues from very susceptible (cv. Camarosa) and moderately susceptible (cv. Andana) cultivars, but their expression pattern was found to be different in both cultivars, being either stronger and/or quicker in the less susceptible cultivar. Interestingly, strawberry members of PR2 (*Fagln-1*, encoding a β-1,3-glucanase), PR3 (*Fachit-1*, encoding a class 1 chitinase), PR9 (*Faprox-1*, encoding a peroxidase) and PR13 (*Faγthio-1*, encoding a γ-thionin) families, as well as the genes *Falrrk-1* and *Falrrk-2*, encoding two LRR receptor-like proteins, were clearly down-regulated in infected fruits. The genes *Fachit-1* and *Falrrk-1* were also significantly inhibited in cv. Camarosa-infected crown tissues. Chitinases and related β-glucanases are known to be rapidly induced in plants upon pathogen infection or treatment with elicitors (Leubner-Metzger and Meins 1999, Khan et al. 2003, Khan and Shih 2004, Mehli et al. 2005, Shi et al. 2006, Zhang et al. 2009, Pombo et al. 2011b), and down-regulation of β-1-,3-glucanase genes has only been reported for tobacco (class I) genes by treatment with ABA (Leubner-Metzger et al. 1995, Rezzonico et al. 1998) and by combination of auxin and cytokinin (Vögeli-Lange et al. 1994) (a wider dynamic range of gene expression information can be obtained in Casado et al. 2006). Thus, the results described by Casado et al. suggest that *C. acutatum* progression can be dependent upon a reduction of the active defenses of strawberry, and highlight the importance of further studies on these strawberry genes to understand fully the process of infection and strawberry plant defense against this pathogen.

Recently, the strawberry *Fawrky1* gene has been further characterized (Encinas-Villarejo et al. 2009). The *Fawrky1* gene is up-regulated in strawberry following *C. acutatum* infection, treatments with elicitors and wounding. A *Fawrky1* full-length cDNA was cloned which encodes a IIc WRKY transcription factor (FaWRKY1). The ectopic expression of FaWRKY1 in Arabidopsis mutants in its orthologous gene *Atwrky75* has provided some positive clues to its function in plant defense. Thus, the overexpression of this strawberry gene in *Atwrky75* mutants and the wild type reverted the enhanced susceptibility, and even increased resistance to avirulent strains of *P. syringae*, demonstrating an active role for this FaWRKY1 protein in the activation of basal and R-mediated resistance in Arabidopsis. Further experimental results provided by these authors strongly suggest that FaWRKY1 can play a role as an important element mediating defense response to *C. acutatum* in strawberry (Encinas-Villarejo et al. 2009). Currently, new experiments to unravel the exact function of this *Fawrky1* gene are in progress (J. L. Caballero, unpublished).

Very recently, Guidarelli et al. (2011) have performed microarray analyses of white and red fruit strawberries after 24 h of their interaction with *C. acutatum*. These authors have provided new data on strawberry genes regulated upon *C. acutatum* infection. Thus, a DNA microarray of >93,300 oligo-probes was produced using expressed sequence tags (ESTs) from the TIGR Plant Transcript Asemblies database (http://plantta.jvci.org/) (4,197 of *F.×ananassa*, release 2; 13,366 of *Fragaria vesca*, release 3; 124 of *Malus domestica*, release 2). Many genes encoding PR proteins were found to be up-regulated in both white and red infected fruit upon infection. Thus, genes coding for a xyloglucanase-inhibiting protein (gene TA10709_57918), for several isoforms of the PR-10 protein family (genes TA11697_57918, EX672442, DY671909 and DY676200), as well as for cytochrome P450 monoxygenases (gene TA9078_57918), which are known to play important roles in plant detoxification pathways, were induced. In addition, several metabolism genes coding for toxic aldehyde scavengers, such as an aldehyde dehydrogenase (ALDH) (gene TA12321_57918), for enzymes involved in the synthesis of stress-related flavonol and alkaloid compounds, such as flavonol synthase (gene TA9432_57918) and tropin reductase (gene DY673561), respectively, and for enzymes involved in the biosynthesis of terpenoid defense compounds, such as the α/β amyrin synthase (gene TA11548_57918) and the 3-hydroxy-3-methylglutaryl (HMG)coenzyme-A synthase (gene CO381295), were also found to be significantly up-regulated after inoculation with the pathogen in both white and red fruit stages. The expression of many other strawberry genes related to biotic stress defense was increased only in one of the two fruit stages, and so the transcript level of genes coding for a peroxidase (PR-9 family, gene DV439771) and a member of the lectin family (gene TA10594_57918) was enhanced in white-stage fruit whereas genes coding for Fra a protein isoforms (PR10 family, genes DY673343 and TA487_3747), a glutathione *S*-transferase (gene CO79212), a snaking-1 a polygalacturonase-inhibiting protein (gene AY534684) and a class IV chitinase (PR-3 family, gene TA9333_57918) were up-regulated in red challenged fruits [see Guidarelli et al. (2011) for a more extensive list of differentially regulated strawberry genes].

Regardless of the availability of transcriptomic information from the strawberry plant–*C. acutatum* interaction, to date no

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direct evidence about the strawberry plant defense response or functional gene characterization has been reported for the majority of the identified genes.

Conclusions and perspectives

Despite the worldwide importance of strawberry and the lack of cultivars fully resistant to any disease in this crop, the molecular mechanism and components of the defense signaling pathways exhibited by this plant to face a diverse array of pathogen attack strategies is still scarce and very poorly understood. In response to both biotic and some forms of abiotic stress, it is clear that the strawberry can exhibit molecular mechanisms similar to those reported in other higher plants. Thus, strawberry is able to activate primary (PTI) and secondary (ETI) defense systems as members of both layers of plant defense have been identified. However, little is known yet about the exact function of these individual components, and many genes and factors still remain undiscovered. For this reason, several authors have directed their efforts towards proving the positive effect that the ectopic expression of known plant defense-related genes can have on increasing resistance in strawberry. It can be predicted that a similar counterpart gene with either the same or a similar role in defense could be present in the strawberry genome.

Many examples of strawberry transgene-mediated resistance against pathogens have been reported using the heterologous strategy. Thus, the expression of a variety of plant chitinases from tomato, rice or bean, the thaumatin II gene from *Thaumatococcus daniellii* Bennett and a PGIP gene from pear fruit has been shown to reduce the damage caused by some fungal pathogens in strawberry. Also the introduction of a cowpea protease inhibitor gene into strawberry improved protection against herbivores (see **Supplementary Table S2** for details).

New breeding strategies using the ectopic expression of heterologous genes in strawberry can indeed also help to obtain important varieties of this crop with increased resistance, but acceptance of a transgenic modification in a fresh fruit for human consumption is far from being achieved. Therefore, the discovery of the strawberry orthologous genes will not only help to unravel the molecular mechanisms underlying the activation of defense responses in this plant but, in addition, a cisgenic approach (Schaart et al. 2004) using these endogenous genes can be a useful tool to obtain strawberry varieties with increased resistance, which can provide consumer acceptance of a healthy fruit for human consumption.

Furthermore, the identification and characterization of specific and partial resistance traits, such as race-specific R genes responsible for the monogenic resistance found to *P. fragariae*, *C. acutatum* and *A. alternata* (Denoyes-Rothan and Baudry 1995, van de Weg 1997a, van de Weg 1997b, Takahashi et al. 1997, Denoyes-Rothan et al. 2005), together with studies on identification of genome regions containing sets of genes that control resistance or quantitative trait loci (QTL), which have been undertaken to determine polygenic quantitative inheritance of resistance (Maclachlan 1978, Barritt 1980, Denoyes-Rothan and Baudry 1995, Shaw et al. 1996, Lewers et al. 2003, Zebrowska et al. 2006), offer promising assistance in conventional breeding programs searching for disease resistance in this crop, and this has been very recently reviewed by Korbin (2011).

The strawberry ESTs and microarray data collection already available (Casado-Díaz et al. 2006, Bombarely et al. 2010, Guidarelli et al. 2011) constitute valuable information for searching candidate genes involved in strawberry defense. The recent publication of the complete sequence of the *F. vesca* genome represents an enormous scientific contribution to this aim (Shulaev et al. 2011). However, progress in the field of basic genomics in the diploid species *F. vesca* is still necessary and is of great interest. Currently, a second generation of 'in-house' microarrays has been developed using a set of selected strawberry unigenes from the EST information provided by Casado et al. (2006), and new transcriptomic analyses are being performed using infected and uninfected crown tissue from *F.×ananassa* cultivars with different susceptibility to *C. acutatum* (J. L. Caballero, unpublished). Certainly, the strawberry transcriptomic approaches will benefit from the *F. vesca* genome information as improved DNA chips, containing high-density arrays of short synthetic oligonucleotides, can be developed and used as a powerful tool to identify novel defense genes.

Proteomic and metabolomic approaches offer complementary methodologies that need to be applied in strawberry to help to understand the molecular mechanisms underlying the defense response of this plant. To this end, non-targeted analysis of metabolite composition in strawberry has recently been improved (Hanhineva et al. 2008), but the application of metabolomic technologies to obtain a description of the chemical defenses deployed by this plant against pathogens needs to be further implemented. Indeed, only an analysis of particular groups of secondary metabolites has been reported for each individual case (Hanhineva et al. 2010).

Combined results produced by the application in strawberry of these high-throughput technologies will also yield new insights into the role played by genes and compounds in strawberry plant defense, and this approach should be explored further. Indeed, very recently, analyses of metabolic and transcriptional changes in the receptacle of *FaPE1* transgenic *F. vesca* fruits have provided relevant new information on the molecular changes associated with the resistance to this pathogen (Osorio et al. 2011). *Fragraria vesca* transgenic lines overexpressing the *FaPE1* gene, an *F.×ananassa* gene encoding a pectin methyl esterase related to formation of the architecture of the strawberry plant cell wall, were previously shown to have increased resistance to *B. cinerea* (Osorio et al. 2008). The transcriptomic and metabolomic analyses of the ripe receptacle of these transgenic lines have shown an increased expression of genes related to plant defense such as genes encoding PR10 proteins, WRKY transcription factors and metallothioneins,

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which was in parallel to the channeling of metabolites to aspartate and aromatic amino acids as well as phenolics, flavanones and sequiterpenoids (for a more detailed description of genes and compounds, see Osorio et al. 2011). By taking these results together, a wider overview of changes in metabolites and transcripts is obtained, helping to assign important candidate genes to putative metabolic pathways.

In recent years, description of high efficiency transformation protocols for strawberry (Oosumi et al. 2006) has also allowed the use of new research strategies such as reverse genetics for functional genomic analyses in this crop (Oosumi et al. 2010). These authors report the development of efficient T-DNA tagging in *F. vesca* as a model for insertional mutagenesis in Rosaceae, and efficiently use the TAIL-PCR (thermal asymmetric interlaced-PCR) method (Liu et al. 1995, Liu and Chen 2007) to amplify the *F. vesca* genomic sequence flanking T-DNA insertion. About 60% of T-DNAs were integrated into genetic regions, with 154 of 213 (72%) of the T-DNA tagged genomic sequences showing homology to plant genes, proteins and ESTs. These authors have shown that the T-DNA integration process in strawberry is not random but is directed by sequence microsimilarities in the host genome. By using this T-DNA tagging technology, a wide range of strawberry mutagenic lines and phenotypes is anticipated. This certainly will help molecular studies in all the fields of interest regarding strawberry. Other new emerging technologies such as RNA sequencing (Ozsolak et al. 2009, Ozsolak and Milos 2011), which eliminates several challenges posed by microarray technologies and accurately offers a global view of the whole transcriptome changes, would certainly be beneficial for unraveling the complexity of the defense response in strawberry.

In summary, the use of high-throughput technologies will provide large amounts of molecular information relating to the defense response in strawberry in the very near future. In particular, a thorough characterization of strawberry control genes encoding important transcription factors and key enzymes, which translate recognition of pathogens into appropriate transcriptional outputs, is required. To accomplish this need, the efficient use of transient expression technology in strawberry (Hoffmann et al. 2006, Muñoz et al. 2010, Hoffmann et al. 2011) is expected to reduce the time needed to unravel the complex network of defense signaling pathways in this important crop. Simultaneously, as strawberry traits such as resistance are controlled by multiple genes (Faedi et al. 2002, Folta and Davis 2006), key regulatory genes offer the possibility to be used as important genetic markers for genetic diversity analysis and selective breeding, which might allow the engineering of new strawberry varieties with improved resistance and healthier qualities in a shorter period, leading to reduced use of chemicals and less environmental risk.

Supplementary data

Supplementary data are available at PCP online.

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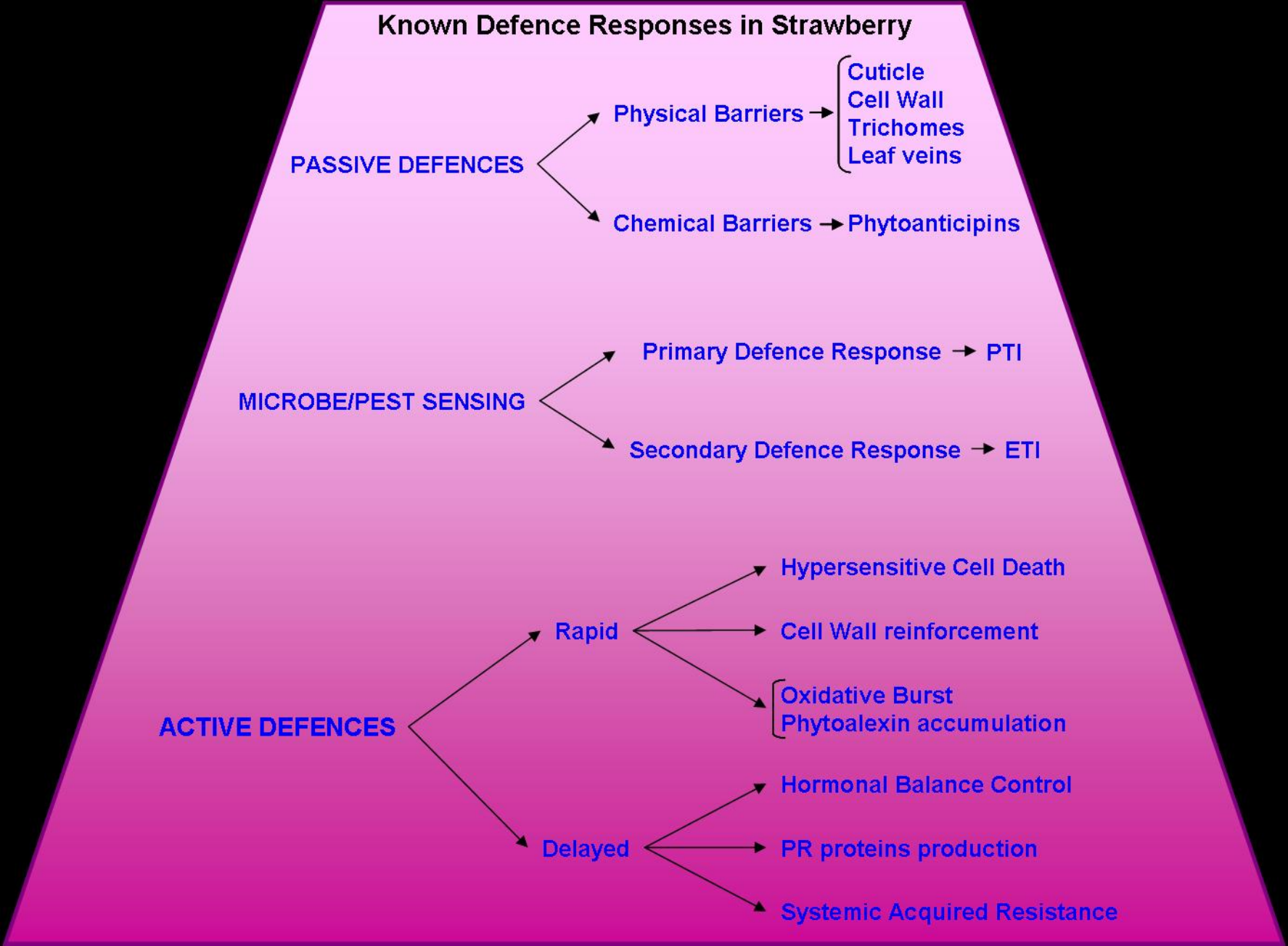
Legends to figures

Supplemental Figure 1. Schematic view of the already known physiological responses exposed by the strawberry plant challenged by pathogens and pests.

Known Defence Responses in Strawberry



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