

Review

The cuticle as a key factor in the quality of horticultural crops

Julio C. Tafolla-Arellano, Reginaldo Báez-Sañudo, Martín Ernesto Tiznado-Hernández*

Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C. Hermosillo, Sonora, 83304, México



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ABSTRACT

In recent years, there has been a major increase in the commercialization of horticultural crops in response to population increase. However, several important factors associated with pre- and post-harvest conditions, including drought stress, pathogen infection, water loss and climate change are limiting the commercialization potential of many horticultural crops, thereby compromising agricultural sustainability. The cuticle, which is composed of cutin and waxes, provides a critical structural barrier between a plant parts and its environment. Cuticle biosynthesis is influenced by many factors during plant development and contributes to the pre- and post-harvest quality of many horticultural crops. However, many aspects of the structure-function relationships of many aspects of the plant cuticle are not well understood. Studies focusing on cuticles are increasingly important due to the major challenges of crop production, such as diseases and abiotic stresses. In this review, we focus on the cuticle as a key factor in the quality of horticultural crops and provide an overview of the recent research and conclusions in the field.

1. Introduction

The aerial organs of terrestrial plants synthesize a hydrophobic cuticle as a protective barrier to avoid uncontrolled water loss. The cuticle is synthesized by the epidermal cell layer and is composed mainly of cutin and wax lipids (Kunst and Samuels, 2003). Cuticle biosynthesis comprises three processes: a) biosynthesis of lipids and elongation to form monomers; b) transport and export; and c) cuticle assembly. These processes are different among species and organs, including the patterns of cutin and wax deposition, suggesting that they are regulated by different mechanisms (Wang et al., 2016). The regulation of cuticle biosynthesis is complex and involves interacting signaling networks associated with the response to biotic and abiotic stresses, issues that are discussed in several reviews by Kunst and Samuels (2003), Samuels et al. (2008), Yeats and Rose (2013), Hen-Avivi et al. (2014), Martin and Rose (2014) and Fich et al. (2016).

Some cuticle properties, such as its flexibility and ability to self-repair, are of considerable economic significance and the variation in fruit cuticle composition may underlie differences in several traits such as resistance to biotic and abiotic stress (Riederer and Schreiber, 2001; Shi et al., 2013a). Despite the critical roles of the cuticle, its importance has been underappreciated and the structural bases of many of its roles are not well understood. Current knowledge of cuticle structure and composition have benefited from studies of *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) fruit, as well as transgenic lines with abnormal cuticles. This information has been useful for understanding

the role of the cuticle in stress responses (Shi et al., 2013a).

The cuticle has several biological roles, which are important for fruit quality and post-harvest shelf-life, such as desiccation control, limiting microbial infection, and physiological disorders (Martin and Rose, 2014). It also influences fruit softening and postharvest fruit quality (Saladié et al., 2007). Some of the roles of the cuticle that are discussed in this review are shown in Fig. 1. In addition, we have included sections discussing the importance of the cuticle in resistance to disease, biotic and abiotic stress.

2. The role of the cuticle in disease, biotic and abiotic stresses: challenges for maintaining crop quality

Biotic and abiotic stresses influence the crop life cycle and compromise agricultural sustainability, with consequent effects on plant resources, biodiversity and global food security (Ahuja et al., 2017). To resist adverse environmental conditions, plants have developed adaptive stress responses, such as the protective barrier known as the cuticle, whose biosynthesis and properties are influenced by many environmental factors such as temperature, UV-B radiation, light, humidity, ozone, and elevated carbon dioxide levels (Baker, 1974; Giese, 1975; McQuattie and Rebbeck, 1994; Shepherd et al., 1995; Matas et al., 2005). Variations in cuticular wax load depending upon growing conditions have been observed (Hunsche and Noga, 2011), as have changes in the wax chemical composition, morphology and surface wettability following changes in humidity. For instance, *Brassica oleracea*,

* Corresponding author.

E-mail address: tiznado@ciad.mx (M.E. Tiznado-Hernández).

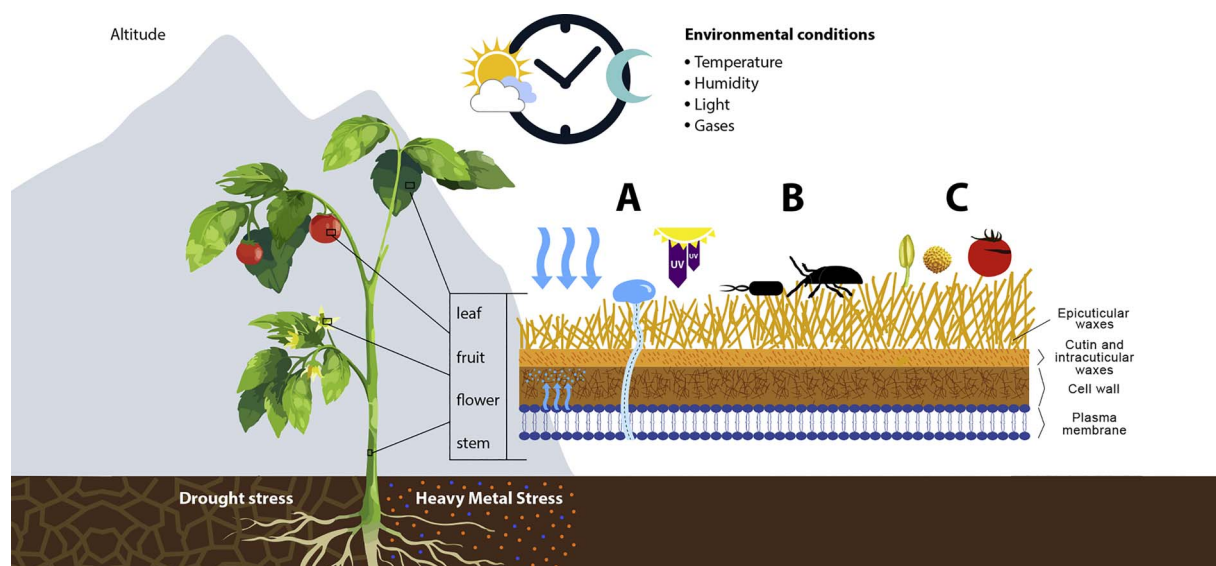


Fig. 1. The physiological roles of the cuticle in crop plants. The cuticle covers leaves, stems, flowers and fruits, protecting them against biotic and abiotic stress and diseases. Many environmental conditions influence cuticle biosynthesis. A, the cuticle protects against abiotic stress by reducing water loss, restricts disease via the “lotus effect” and reduces UV radiation damage. B, the cuticle protects against biotic stresses, such as those caused by bacteria, viruses and insects. C, the cuticle roles in diseases, such as in anther and exine formation as well as fruit russeting.

Eucalyptus gunnii and *Tropaeolum majus* grown under 98% relative humidity (RH) showed a decrease in total wax and wax crystal density, but a significant increase in leaf surface wettability. Conversely, under 20–30% RH, increased total wax amounts and reduced surface wettability were observed (Koch et al., 2006). In the Compositae family, the amount of total wax was significantly higher and levels of *n*-alkanes were the largest component in plants growing at high altitudes, while primary alcohols were the main wax components at low altitudes, suggesting that variations in cuticle components might be an adaptive response to altitude (Guo et al., 2016a). Recent studies of the role of the cuticle in responses to climate changes have shown that the presence of low oxygen levels (hypoxia) correlate with lower total wax and cutin levels and a downregulation of cuticle associated genes. This induced change in cuticle ultrastructure leads to increased permeability, allowing the plant to cope with oxygen deficiency (Kim et al., 2017).

The cuticle wax profile and ultrastructure may also vary by season and even with fruit position on trees such as orange (*Citrus sinensis* (L.) Osbeck) and these changes are associated with changes in environmental conditions and the effects of both biotic and abiotic stresses during each season (El-Otmani et al., 1989; Gülz et al., 1991; Jenks et al., 2002).

It is important to consider the cuticle anti-adhesive properties and their role in pesticide retention and/or absorption, since the persistence of pesticide residues may be a potential risk to consumers. Riccio et al. (2006) reported that epicuticular waxes influence the penetration of the insecticide, chlorpyrifos-methyl, into the pulp of some fruits. It has also been reported that higher cuticular wax load and lower contact angles are related to the uptake of leaf-applied agrochemicals (Hunsche and Noga, 2011). The hydrophobic properties of epicuticular waxes confer water-repellent properties, resulting in a self-cleaning surface and “the lotus-effect” (Neinhuis and Barthlott, 1997), where water droplets roll off plant surfaces, carrying with them contaminating particles such as dust and fungal spores.

The cuticle has been associated with several horticultural diseases in both pre- and postharvest contexts. It also prevents organ fusion, as exemplified by the tomato recessive *positional sterile* (*ps*) mutation, where cuticle composition is altered by an almost complete absence of *n*-alkanes and aldehydes, resulting in floral organ fusion and positional sterility (Leide et al., 2007). Furthermore, overexpression of the *CFLAP1* and *CFLAP2* transcription factors has been shown to lead to

changes in the expression of genes involved in the fatty acid, cutin and wax biosynthetic pathways, and to cause multiple cuticle defective phenotypes, such as organ fusion, breakage of the cuticular layer and decreased epicuticular wax crystal load (Li et al., 2016). Interestingly, it has been proposed that epicuticular wax structure and chemical composition, rather than total wax load, influences rind staining and peel pitting of Fortune mandarin (*Citrus reticulata*) fruits (Sala, 2000). It has also been reported that cuticle microcracking can generate pathways for pathogen invasion in cucumber fruit (Martínez and Fernández-Trujillo, 2007). In cactus pear (*Opuntia ficus-indica* Mill. (L.) cv. Gialla), heat treatment caused a partial melting of the epicuticular wax layers, leading to a modified cuticle structure and sealing of micro-wounds and cracks. This was considered a contributing factor in the protection against pathogen wounding that reduced decay during storage, retention of visual quality and prevention of chilling injury (Schirra et al., 1999).

The cuticle plays a role during ripening and storage, and the epicuticular wax composition and morphology, in particular, are considered to be an integral part of fruit ripening. These changes can reduce non-chilling peel pitting and disease incidence caused by *Penicillium digitatum* in orange fruit (*Citrus sinensis*), suggesting that the formation of new waxes in fruit treated with ethylene may partially cover stomata, cracks or areas lacking wax (Cajuste et al., 2010). Finally, during fruit storage, changes in wax compounds and wax morphology lead to skin greasiness in apples (*Malus domestica*) (Yang et al., 2017).

2.1. Drought stress

Drought stress (DS) is one of the main limitations to global agricultural production, and future water-limiting environments caused by climate changes will further exacerbate a reduction in the production and quality of horticultural crops. Plants have evolved diverse adaptive strategies and mechanisms to avoid such stress, including stomatal closure, promotion of root growth and alteration of the water potential components to allow water uptake, among others. Cuticular wax biosynthesis has also been shown to contribute to drought resistance (Seo and Park, 2011), and plant species that grow in naturally dry conditions provide an opportunity to understand the role of the cuticle under DS. For example, transcriptomic analysis of the desert plant, *Reaumuria*

soongorica, which has an extremely thick cuticle and effective physiological mechanisms to avoid DS, such as a reduced transpiration rate and increased water use efficiency, identified genes that regulate the biogenesis and development of the cuticle to be involved in drought avoidance (Shi et al., 2013b). Similarly, in the desert plant *Cynanchum komarovii*, which possesses strong resistance to abiotic stresses, gene expression analysis indicated that under DS, biochemical pathways involved in “cutin, suberin and wax biosynthesis” were induced (Ma et al., 2015). Furthermore, genes involved in biosynthesis, export, and regulation of the plant cuticle were differentially expressed and cuticle accumulation increased, consistent with the cuticle protecting plants against DS.

Studies have also been performed of agricultural crops, such as rice (*Oryza sativa* L.), whose production demand large amounts of water and is limited by water deficit and DS. Some key genes, such as *OsGLI* (Islam et al., 2009), *OsWR1* (Wang et al., 2012), *OsGLI-6* (Zhou et al., 2013), *OsGLI-3* (Zhou et al., 2015), *OsWS1* (Xia et al., 2015), have been shown to be involved in cuticular wax accumulation in response to DS. Their expression influences wax structure and composition, thereby regulating cuticle permeability. In both soybean (*Glycine max* L.) and sesame (*Sesamum indicum* L.), DS caused a 30% increase in the total wax levels, with 59% and 34%, respectively, corresponding to alkanes (Kim et al., 2007a, 2007b). It was also reported that glaucous wheat, is more drought-tolerant and showed an increase in the cuticular wax content of ~150% after DS, compared with ~114% in non-glaucous wheat (Guo et al., 2016b). Finally, although total wax load increased by 1.5–2.5-fold in tobacco (*Nicotiana glauca* L. Graham) following DS, the wax composition was reported not to change (Cameron et al., 2006).

In tomato, expression analysis in transgenic lines over-expressing the *SISHN1* transcription factor indicated that several wax-related biosynthetic genes were induced and that the plants showed both higher cuticular wax deposition and drought tolerance when compared with wild-type plants (Al-Abdallat et al., 2014). Other transcription factors, such as wheat *TaMYB31* and *TaMYB74*, have been reported to be involved in the regulation of cuticle biosynthesis under DS in wheat (Bi et al., 2016). Additionally, the *ECERIFERUM1* (*CER1*) gene, which plays a key role in very long chain alkane biosynthesis, affects cuticle properties and drought tolerance in cucumber (*Cucumis sativus* L.) and wheat (*Triticum aestivum* L.), as well as in other species (Wang et al., 2015; Liu et al., 2015a). It has been reported that wax composition and accumulation as well as the expression levels of cuticle-associated genes are significantly induced by DS and by soil moisture content (Mofatto et al., 2016; Lee et al., 2015; Xu et al., 2016) in several crops, such as coffee (*Coffea arabica*) and broccoli (*Brassica oleracea*).

2.2. Heavy metal stress

Heavy metal-contaminated soil, caused by different industrial activities, is an increasing problem in agriculture affecting growth, development and water relations of plants. It has been reported that uptake of metals from both the soil and foliage influence the cuticle structure and permeability. In sugar beet (*Beta vulgaris*), a positive correlation between transpiration rate and cadmium (Cd) concentrations were reported, which may influence cuticle biosynthesis and composition leading to a higher water loss through the cuticle due to changes in permeability (Greger and Johansson, 1992). Similar results were obtained in (*Hordeum vulgare*), in where Cd-induced a ~50% wax increase leading to changes in wax components such as acyl alcohols, esters, alkanes and aldehydes. Moreover, the size and density of the wax flakes increased in exposed plants (Hollenbach et al., 1997). In one of the most important natural fiber crops, ramie (*Boehmeria nivea* L.), high levels of Cd can inhibit stem growth reducing the crops economic value (Liu et al., 2015b). A pathway enrichment analysis revealed that the expression of 155 genes were assigned into 50 pathways, among them, cutin, suberin and wax biosynthesis was markedly increased suggesting that the cuticle plays an important role in Cd-tolerance.

Stress imposed by iron (Fe) deficiency chlorosis induced by calcareous and alkaline soils affects plants in many areas of the world. In pear (*Pyrus communis*) and peach (*Prunus persica*) leaves, Fe deficiency was shown to reduce the amount of cuticular lipids and the leaves had a less smooth surface, which could in turn influence water relations, solute permeability and pest and disease resistance (Fernández et al., 2008).

2.3. Anther cuticle and pollen exine formation affects reproduction

Anthers and pollen are covered by the anther cuticle and pollen exine, respectively, which provide protection against environmental and biological stresses and influence reproductive development. Accordingly, alteration or defects in their production can lead to male sterility. In rice, the ABC transporters, *OsABCG26* and *OsABCG15*, together regulate rice male reproduction and formation of the anther cuticle and the pollen exine. The *osabcg26 osabcg15* double mutant displays an almost complete absence of these two structures, similar to the phenotype of the *osabcg15* single mutant (Zhao et al., 2015). *OsABCG26* is specifically expressed in the anther and pistil, and studies of the *osabcg26* cuticle deficient mutant revealed that *OsABCG26* is involved in anther cuticle and pollen exine formation by regulating lipid transport from the tapetum. It also plays a role in pollen-pistil interactions, affecting pollen tube growth in the pistil (Chang et al., 2016).

Fatty alcohols and their derivatives are major components of the anther cuticle and pollen wall. The male sterile mutant, *defective pollen wall* (*dpw*: encoding a fatty acyl carrier protein reductase), which has defective anther development and degenerated pollen grains with an irregular exine, exhibits a major reduction in cutin monomers and an altered composition of cuticular waxes (Shi et al., 2011). Similarly, the mutant, *defective pollen wall 2* (*dpw2*: encoding a BAHD acyltransferase), has an abnormal anther cuticle with increased amounts of cutin and waxes and is male sterile (Xu et al., 2017). Genes from other large families, such as the cytochrome P450 fatty acid hydroxylase encoded by the *ZmCYP703A2* gene in maize (Somaratne et al., 2017) and *OsCYP703A3* in rice (Yang et al., 2014), influence male reproduction and formation of the anther cuticle and pollen exine. The tomato mutant *glycerol-3-phosphate acyltransferase 6* (*gpat6-a*) exhibits altered cuticle thickness, composition, and properties and has perturbed pollen formation, although it is not male-sterile (Petit et al., 2016).

2.4. UV radiation

The cuticle provides a barrier between the plant and its environment, and is the first obstacle for radiation hitting the plant surface. It has been shown that the cuticle protects the plant from the deleterious effects of UV-B radiation. Irradiation with enhanced UV-B (280–320 nm) levels was reported to cause changes in cuticular wax composition, predominantly in the alkane fraction, and the total amount of wax increased by about 25% in cucumber (*Cucumis sativus* L.), bean (*Phaseolus vulgaris* L.) and barley (*Hordeum vulgare* L.), suggesting that epicuticular waxes are involved in attenuating enhanced UV radiation via UV reflectance and scattering (Steinmüller and Tevini, 1985; Steinmüller and Tevini, 1986). UV-B treatment was also reported to result in altered the wax composition of the pea (*Pisum sativum*) adaxial leaf surface, such as the conversion of alcohols to esters and other hydrocarbons, and the ratio of short to long chain alkyl ester homologs increased (Gonzalez et al., 1996). In *Sorghum bicolor*, epicuticular wax was shown to be associated with the scattering of radiation in the canopy environment by increasing the transmittance of photosynthetically active radiation, while reducing the transmittance of UV-B (Grant et al., 1995). In contrast, an increase in UV-B led to higher wax alkane content in sugar maple (*Acer saccharum* [Marsh.]), suggesting that a decarbonylation pathway was induced rather than a reductive pathway (Gordon et al., 1998).

UV-B (280–320 nm) irradiation was also reported to cause an

increase (~ 40%) in the epicuticular wax content of *Simarouba glauca*, and it was suggested that a thicker waxy cuticle might help to reflect the UV-B radiation and protect internal tissues from UV-B induced damage (Patil and Gaikwad, 2016). A similar response was found in apple (*Malus domestica* Borkh.) seedlings and cotton (*Gossypium hirsutum* L.), where exposure to UV-B radiation increased the total wax content and altered the chemical composition of the cuticle (Kakani et al., 2003; Bringe et al., 2007). Interestingly, this cuticle property has been proposed as a way of reducing UV-B radiation effects on humans by developing green belts of *S. glauca* (Patil and Gaikwad, 2016).

2.5. Pathogen attack

The cuticle represents the first barrier against pathogen invasion. In one study, two *Brassica napus* genotypes were used to evaluate the role of epicuticular wax during infection with *Sclerotinia sclerotiorum*, which attacks more than 400 plant species, including some economically important crops. The total wax and wax composition were significantly higher in the susceptible cultivar than in the resistant cultivar, with the main difference between cultivars being the higher level of alkanes and the up-regulation of the transcription of wax-related genes in the resistant cultivar (Ni et al., 2014). It was concluded that resistance to *S. sclerotiorum* correlated more with the composition than with the total amount of wax (Ni et al., 2014). This was in agreement with previous studies investigating the physical role of epicuticular wax in *B. napus* and *B. campestris* in the defense against *Alternaria brassicae*, which causes blackspot disease (Tewari and Skoropad, 1976). The findings in this study led to the conclusion that the surface bloom in rapeseed, due to the epicuticular waxes, confers partial resistance to *A. brassicae* by providing a water-repellent surface. It has also been reported that leaf epicuticular waxes decrease the susceptibility to *A. brassicae* by creating a hydrophobic surface that reduces the retention of water-borne fungal spores, thereby reducing germination of conidia, and reducing the production of germ tubes (Conn and Tewari, 1989).

The tomato mutant *delayed fruit deterioration* (*dfd*) was reported to have compositional and ultrastructural characteristics of its fruit cuticle that enhance resistance to infection by opportunistic fungal pathogens, even following prolonged storage under high humidity conditions (Saladié et al., 2007). In addition, spores of the fungus *Botrytis cinerea* were applied to the surface of ripe fruit from both *dfd* and a normally softening tomato cultivar, Alisa Craig (AC), and while AC fruit were consistently infected by both ectopic application and application through a wound, DFD fruits were only infected when the cuticle was damaged (Saladié et al., 2007). Isaacson et al. (2009) identified three tomato mutants, *cutin deficient 1,2 and 3* (*cd1*, *cd2* and *cd3*), which exhibited extremely thin cuticles with a reduction of up to ~95% in cutin content and abnormal cuticle ultrastructures. This condition resulted in an increased susceptibility to opportunistic saprophytes, and to direct *B. cinerea* inoculation suggesting that cutin plays an important role in protecting tissues from microbial infection.

Studying a tomato mutant (*sitiens*) with reduced abscisic acid (ABA) production, Curvers et al. (2010) demonstrated that ABA deficiency leads to increased cuticle permeability, influencing the cuticle composition and structure, and increased resistance to *B. cinerea* infection. *SISHN3* silenced tomato lines and the *cyp86a69* mutant showed an increased susceptibility to the fungus *C. coccodes*, which was associated with differences in cuticle composition in both waxes and cutin. In this study, a substantial reduction in cuticle thickness was observed, which may facilitate the ability of *C. coccodes* to penetrate the fruit surface (Shi et al., 2013a). Changes in the cutin monomer composition have also been shown to inhibit the germination of powdery mildew (*Erysiphe polygoni*) spores (Wang et al., 2000). The free fatty acids, linoleic and palmitic acid, are deposited as cutin monomers and oligomers to reinforce the barley cuticle, which acts as a barrier against one of the most devastating crop diseases: fusarium head blight, caused by *Fusarium graminearum* (Kumar et al., 2016). In Asian pear (*Pyrus pyrifolia*)

fruit, the inhibition of spore germination and mycelial growth of *A. alternata*, which caused rot, were associated with the composition of cuticular wax *n*-alkanes, triterpenoids and fatty acids (Yin et al., 2011). Pear fruit cuticular wax composition and hydrophobicity were reported to influence fungal invasion by regulating the growth and differentiation of *A. alternata* during the pre-penetration phase (Tang et al., 2017). This highlights the importance of the cuticle in plant-pathogen interaction to consider as a criterion for breeding horticultural crops to face the pathogen attack (Conn and Tewari, 1989).

2.6. Plant-insect interactions

Cuticular waxes can also physically hinder the movement and reduce the attachment of insects to the plant surface. Gorb et al. (2017) provided evidence that a more three-dimensional deposition of epicuticular waxes, in general, reduce adhesion by adsorption of insects, which rely on wet adhesion. Glossy phenotype crops are often less susceptible to insect attack due to a variation in wax chemical composition and morphology. In *Eucalyptus globulus*, psyllid adhesion was reported to decrease when wax from juvenile leaves was transferred to glossy adult leaves (Brennan and Weinbaum, 2001).

Epicuticular waxes are important factors in antixenotic resistance of cabbage (*Brassica oleracea* L.) to cabbage flea beetle (*Phyllotreta* spp.) and cabbage stink bug (*Eurydema* spp.). Although the levels of epicuticular waxes differ between cabbage genotypes, a pronounced negative correlation was observed between wax abundance and the extent of injuries caused by both groups of harmful pests (Bohinc et al., 2014). Furthermore, studies with *Brassica* mutants with low levels of cuticular waxes demonstrated that leaf epicuticular waxes influences the rate and pattern of flea beetle (*Phyllotreta cruciferae*) feeding (Bodnaryk, 1992). Similar results were reported for reduced-wax bloom *P. sativum* plants in that antixenosis contributed to the lower aphid populations (White and Eigenbrode, 2000).

In contrast, silkworms (*Bombyx mori*), the primary producer of silk, have a strong feeding preference for the most turgid and hydrated mulberry leaves. This behavior has been associated with the amount of leaf surface waxes, suggesting that the improvement of the leaf water retention capacity of mulberry leaves could enhance commercial silk quality and production (Mamrutha et al., 2017; Ni et al., 2015).

2.7. Water loss

In fruit crops, post-harvest desiccation leads to over softening, tissue collapse, and microbial infection (Martin and Rose, 2014), reducing the commodity quality and their availability in the markets. Maintenance of water content is, therefore, a crucial factor. In some cases, the cuticle is the only transpiration barrier controlling the water status, due to the absence of stomata at maturity (Riederer et al., 2015). Indeed, several studies have suggested a role for cuticle composition and physical properties in postharvest firmness, by regulating water status and maintaining fruit surface integrity.

In support of the role of the cuticle in controlling water loss, Saladié et al. (2007) reported that fruit of the *delayed fruit deterioration* (*dfd*) tomato genotype showed minimal transpirational water loss and substantially elevated cellular turgor, and maintained firmness for several months after ripening, in contrast to normally softening tomato fruit. This was associated with specific compositional or ultrastructural characteristics of the fruit cuticle, consistent with the cuticle provides protection against water loss. Leide et al. (2007) also reported a direct relationship between cuticular transpiration barrier properties and changes in cuticular wax composition during tomato fruit development. An alteration in cuticle composition in tomato fruit ripening mutants has also been reported in the *ripening inhibitor* (*rin*) and *non-ripening* (*nor*) mutants and the delayed-ripening landrace variety, Alcaboça, which provide a system to study the genetic and physiological basis of cuticle function (Kosma et al., 2010).

Parsons et al. (2013) analyzed the fruit cuticle composition of 50 pepper (*Capsicum* spp.) accessions from diverse sources worldwide, to investigate correlations postharvest water loss rate. Their results revealed a large amount of variation in cuticle composition and significant differences in water loss among the accessions, and further suggested that alkane content/composition is a significant determinant of water permeability. Based on a transcriptomic analysis of mango (*Mangifera indica* L.) fruit peels, Tafolla-Arellano et al. (2017) reported that the pathway leading to biosynthesis of cutin is up-regulated during over-ripening, which is the fruit developmental stage associated with an increased water loss. This analysis, together with gravimetric and microscopic studies of cuticle deposition, revealed a complex continuous pattern of cuticle deposition during ripening/over-ripening.

In the case of litchi (*Litchi chinensis*) and longan (*Dimocarpus longan*) fruits, water loss is biphasic and is controlled by a complex pericarp transpiration barrier. These fruits have two cuticles (endo- and exocarp) and two reservoirs of water (Riederer et al., 2015). Similarly, Matas et al. (2011) reported the presence in tomato of an inner cuticle facing the locular cavity, with a different cutin composition compared with the outer epidermal cuticle. This was consistent with the expression of large numbers of cuticle associated genes both in the outer and inner epidermis. The authors concluded that this inner cuticle could influence water movement in the pericarp, turgor pressure and fruit tissue biomechanical properties. These findings are important because the pericarp of most mature fruits has only one cuticle covering its outer surface.

2.8. Russetting

Russetting, or cracking, is a commercially important surface defect in many fruit crops, in the form of a brown, rough appearance, which reduces their quality and limits global distribution, causing postharvest and economic losses. For example, this physiological disorder is one of the major causes of economic loss in cherry production worldwide, affecting up to 90% of the harvested fruit (Rios et al., 2015).

Russetting results from microscopic cracks in the cuticle, and the subsequent accumulation of suberin on the inner side of the cell wall is a response to control water loss (Khanal et al., 2013; Legay et al., 2015). Several factors are reported to contribute to russetting, including prolonged periods of surface wetness, irrigation regimes, high humidity and a discrepancy between surface expansion and cuticle deposition (Knoche and Peschel, 2006; Gibert et al., 2007; Knoche et al., 2011; Khanal et al., 2013). However, the genetic background may play a more significant factor than environmental variables, and it has been reported from studies of the mechanical properties of tomato fruit cuticles, that cracked tomato fruit have a less deformable cuticle than non-cracked fruit (Domínguez et al., 2012). The cessation of cutin deposition in post-veraison grape berries (*Vitis vinifera* L.) together with ongoing fruit expansion results in an increased strain in the cuticle, which in turn causes microcracking (Becker and Knoche, 2012). A correlation between a reduction in nighttime transpiration, which causes high turgor pressure and cracking in bell pepper has also been observed (Aloni et al., 1998). Finally, the tolerance to cracking in sweet cherry may be associated with cuticle composition such as *n*-alkanes content and the expression of certain cuticle associated genes, such as *PaWINB* and *PaKCS6* (Rios et al., 2015; Balbontín et al., 2014).

Recently, in apple (*Malus × domestica*), Legay et al. (2015) identified genes and pathways associated with russetting. Gene ontology enrichment showed a repression of cuticle biosynthesis genes in russeted exocarps, concomitant with an enhanced expression of suberin deposition, suggesting that russetting is induced by a decreased expression of cuticle biosynthetic genes. Additionally, a *MdMYB93* transcription factor has been identified to be a regulator of suberin deposition in russeted apple fruit (Legay et al., 2016), and the *MdSHN3* transcription factor promotes apple fruit cuticle formation and prevents russetting development (Lashbrooke et al., 2015).

2.9. Chilling injury

The use of low temperatures is considered an effective method for prolonging the postharvest shelf life of fruits and vegetables. However, many important horticultural crops are susceptible to chilling injury, a physiological disorder resulting from exposure to low, nonfreezing temperatures. The most apparent symptoms are surface pitting, necrotic areas, and external discoloration, causing economic and postharvest losses (Lyons, 1973; Wang, 1990). Chilling injury influences the stomatal control of transpiration and membrane permeability, and a relationship between epicuticular wax structure and composition and chilling injury has been proposed. For instance, chilling injury in grapefruit (*Citrus paradise*) is associated with changes in epicuticular wax levels and composition involving alkanes, squalene, and long-chain aldehydes (Nordby and McDonald, 1991). In addition, chilling injured carambola (*Averrhoa carambola* L.) fruit were reported to have a higher content of waxes and reduced water loss compared with fruit stored at 20 °C (Pérez-Tello et al., 2001). A similar response was reported in maize, which was attributed to wax composition (Vigh et al., 1981).

In grapefruit, the exterior sun-exposed surface was reported to be more susceptible to chilling injury (5 °C, 80%–92% relative humidity) and had larger wax platelets than the interior shaded surface of the same fruit (McDonald et al., 1993), and in 'Fortuné' mandarins, chilling injury causes a substantial increase in cuticle permeability (Vercher et al., 1994). Some epicuticular wax components were proposed as candidates for providing protection against chilling injury in grapefruit, such as squalene, a highly unsaturated C30 isoprene hydrocarbon (Nordby and McDonald, 1990). The absence of cracks in cuticles may be associated with chilling injury as waxes may affect the exchange of internal gas and it was suggested that low cuticle permeability due to wax profiles may be a factor in chilling pitting in mango (Han et al., 2006).

3. Concluding remarks

The plant cuticle is a key barrier for protecting plants against several biotic and abiotic stresses. This review summarizes the diverse roles of the cuticle in horticultural crops. Despite research into the physiological importance of the cuticle, many aspects remain poorly understood. Physiological analyses, together with studies of cuticle-associated genes using 'omics' approaches, will help the development of strategies to increase the production of horticultural crops through designing cuticles with specific characteristics to improve the post-harvest shelf life.

Author contributions

JCTA, RBS, and METH conceived and designed the review. All authors contributed to writing, critically reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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