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Research review paper

Transcriptional regulation of cuticle biosynthesis

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ABSTRACT

Plant cuticle is the hydrophobic protection layer that covers aerial plant organs and plays a pivotal role during plant development and interactions of plants with the environment. The mechanical structure and chemical composition of cuticle lipids and other secondary metabolites vary considerably between plant species, and in response to environmental stimuli and stresses. As the cuticle plays an important role in responses of plants to major abiotic stresses such as drought and high salinity, close attention has been paid to molecular processes underlying the stress-induced biosynthesis of cuticle components. This review addresses the genetic networks responsible for cuticle formation and in particular highlights the role of transcription factors that regulate cuticle formation in response to abiotic stresses.

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1. Introduction

The plant cuticle provides a protective hydrophobic coating for aerial plant organs. The cuticle is composed of a cutin polyester layer impregnated and overlaid with free waxes (Beisson et al., 2012). It has been suggested that the primary functions of the cuticle, and in particular of the cuticle waxes, are in protection against excessive solar radiation and preservation of internal plant water (Goodwin and Jenks, 2005; Shepherd and Wynne, 2006). Protection against ultraviolet (UV) radiation has been attributed to the light-scattering properties of the cuticle (Long et al., 2003). Several studies have indicated that drought can induce increased wax depositions on the leaf surfaces of different plant species, including *Arabidopsis thaliana* (Kosma et al., 2009; Yang et al., 2011), cotton (*Gossypium hirsutum*; Bondada et al., 1996), rose (*Rosa hybrid*; Jenks et al., 2001), peanut (*Arachis hypogaea*; Samdur et al., 2003) and tree tobacco (*Nicotiana glauca*; Cameron et al., 2006). The importance of cuticle function is highlighted by studies using mutants defective in cuticle biosynthesis, which often do not survive when germinating under normal conditions but can be rescued by high humidity (Yang et al., 2008).

Increased amounts of cuticular waxes have been associated with improved drought tolerance in oat (*Avena sativa*; Bengtson et al., 1978), rice (*Oriza sativa*; Islam et al., 2009), sorghum (*Sorghum bicolor*; Jordan et al., 1984), alfalfa (*Medicago sativa*) and crested wheatgrass (*Agropyron cristatum*; Jefferson et al., 1989). On the other hand, a naturally occurring mutant of wild barley (*Hordeum spontaneum*), *eibi1*, with a very thin cutin layer, was found to be hypersensitive to drought (Chen et al., 2011). Breeding for greater tolerance and yield under drought conditions led to increased amounts of cuticle waxes, further confirming the connection between drought tolerance and cuticle properties (González and Ayerbe, 2010). Thus, the activated biosynthesis of cuticle waxes appears to be a near-universal plant response to dry and hot conditions.

Mutants defective in synthesis and accumulation of cuticular components have been found in many plant species, and have been intensively studied in members of the *Bracicaceae* family and in cereal species such as rice (*Oryza sativa*), barley (*Hordeum vulgare*) and maize (*Zea mays*). The accumulated biochemical, physiological and genetic evidence for a role of the cuticle in plant protection against biotic and abiotic stresses has stimulated searches for gene networks underlying such protective responses. A number of genes encoding enzymes involved in the biosynthesis of cuticle components have been isolated and characterised, mostly in the model plant *Arabidopsis* (Jetter and Kunst, 2008; Jetter et al., 2006; Samuels et al., 2008a). To date more than 190 genes have been demonstrated to be involved in cuticular wax biosynthesis and transportation in *Arabidopsis* (Beisson et al., 2003; Li-Beisson et al., 2013).

It has been shown that a number of transcription factors (TFs) are involved in the regulation of biosynthesis and accumulation of cuticle components. Most of them belong to one of the three different families: ethylene responsive factors (ERFs), myeloblastosis family (MYB) TFs and homeodomain-leucine zipper class IV (HD-Zip IV) factors (Aharoni et al., 2004; Broun et al., 2004; Cominelli et al., 2008; Javelle et al., 2010; Kannangara et al., 2007; Seo et al., 2011; Zhang et al., 2005). Over-expression of these TFs leads to changes in cuticle accumulation and/or composition, and often to increased stress tolerance in a variety of transgenic plants (Aharoni et al., 2004; Broun et al., 2004; Javelle et al., 2010; Seo et al., 2011; Seo and Park, 2011; Zhang et al., 2005). However, despite obvious positive effects on plant protection, in many cases ectopic expression of TFs negatively affects plant growth and yield (Aharoni et al., 2004; Zhang et al., 2005).

This review will briefly summarise the existing knowledge on the chemical structure, biosynthesis and protective functions of the plant cuticle. It will focus on transcriptional regulation of genes involved in cuticle biosynthesis, and cutin and wax deposition. This includes summarising data on spatial and temporal gene expression and induction by stresses, and on effects that up- and/or down-regulate genes in transgenic plants, and pointing at the significance of mutants in explaining cuticle size and composition, which collectively impact on plant stress tolerance. This review will also report on target genes that have been demonstrated or predicted to play roles in cuticle biosynthesis. Existing and potential applications of genes that are responsible for regulation of cuticle biosynthesis in plant biotechnology will be discussed. Whenever possible, data obtained in the model plant *A. thaliana* will be complemented with the information that has been obtained using economically and commercially important plant species.

2. Cuticle structure, biosynthesis and functions

2.1. Cuticle composition and structural dynamics

The plant cuticle, a continuous protective sheet that covers aerial surfaces of plant organs has evolved as an exterior extension of the epidermal cell walls. Related plant protective lipid barriers include suberin, located in the root endodermal and exodermal cell layers, and in the cork layer of the stem of woody plants, and sporopollenin, localised in the outer wall (exine) of pollen grains (Pollard et al., 2008; Samuels et al., 2008b).

One of the main cuticle components, cutin, consists mostly of C16 and C18 fatty acids and glycerol molecules, which are covalently linked with one another. Cutin and the related polymer suberin are the most abundant lipidic polyesters in nature (Beisson et al., 2012). Cutin is resistant to mechanical damage and provides structural support for a second cuticle component, cuticular wax. The waxes, subdivided to intra- and epi-cuticular waxes, are typically a complex mixture of derivatives of Very-Long-Chain (C20–C36) Saturated Fatty Acids (VLCFAs), including alkanes, aldehydes, ketones, primary alcohols and secondary alcohols. Free fatty alcohols and/or wax esters often form the largest fraction of wax compounds (Jetter and Kunst, 2008; Jetter et al., 2006; Samuels et al., 2008b).

Cuticle synthesis begins in the early stages of plant development. In *Arabidopsis*, constant wax and cutin deposition starts at the late globular stage of embryo development and is tightly coordinated with plant growth (Suh et al., 2005; Tanaka et al., 2002). Along with vegetative organs, the cuticle also develops on flowers, fruits and seeds (Jessen et al., 2011).

Cuticle composition varies considerably between plant species. It may also differ between organs of the same plant, and further depend on developmental stage and environmental conditions. For example, waxes of young maize seedling leaf contain up to 63% free C32:0 primary alcohol, while the main fraction of cuticular waxes from mature leaves (42%) consists of wax esters (Bianchi et al., 1985). Although cutin and waxes are the main components of cuticle, chemical and structural variability is generated by modifications of these primary elements and by incorporation of additional compounds such as terpenoids, flavonoids and phenolic lipids. In addition, cuticles of particular groups of plants may contain specific compounds. For example, grasses contain significant amounts of aliphatic β-diketones and related compounds (Adamski et al., 2013; Mikkelsen, 1979; von Wettstein-Knowles, 2012).