Sweet immunity in the plant circadian regulatory network

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Abstract
All organisms have an internal timing mechanism, termed the circadian clock, to anticipate the light/dark cycle. The clock, with an oscillating rhythm that approximates 24 h, is a rather robust system persisting to a great extent in continuous light and dark. It is widely accepted that plant growth and development are regulated by the clock, hormones, and sugar signals. On the one hand, sugar signalling can affect circadian rhythms by altering the expression pattern of clock-regulated genes. More in particular, the clock seems to be particularly sensitive to sucrose-mediated signalling which is also associated with immunity and abiotic stress responses. Also, hormonal interaction with the clock can contribute to appropriate plant immune responses. Recent data show a prominent role for the clock in growth and stress responses. On the other hand, the clock seems to be essential in controlling the gene expression and activity of an array of carbohydrate-metabolizing enzymes, suggesting a complex reciprocal relationship between the clock and metabolic signalling processes. Therefore, the clock fulfils a crucial role at the heart of cellular networks. The players involved in the complex plant circadian network and their possible contribution to the novel 'sweet immunity' concept are discussed.

Key words: Circadian clock, defence, immunity, pathogen, sucrose, sugar signalling.

Introduction
Today, sugars can be considered as signalling molecules rather than just providers of carbon and energy in plants (Rolland et al., 2006; Bolouri Moghaddam et al., 2010). Similar to plant defence hormones, they participate in various mechanisms including immune and defence responses. Although plant cell-wall-derived and pathogen-derived oligosaccharides are widely accepted players in plant innate immunity, recent data strongly suggest that sweet, endogenous saccharides (or saccharide-likes) such as sucrose, raffinose family oligosaccharides, and galactinol might also play important roles in such processes (Bolouri Moghaddam and Van den Ende, 2012 and references therein). This novel concept, termed 'sweet immunity' (Fig. 1) or 'sugar-enhanced defence' (Sonnewald et al., 2012), is not yet widely accepted and awaits further exploration (Bolouri Moghaddam and Van den Ende, 2012). Focusing on the dual role of sucrose as a metabolizable transport sugar and a potential 'priming' molecule in plant defence reactions, it is clear that sudden increases in apoplastic sucrose levels would indicate cellular rupture (e.g. wounding by herbivores), increasing the risk on microbial infections. Generally, increased overall leaf sucrose levels (e.g. reduced sugar export from the leaf under mild abiotic stress) might attract certain herbivores. Thus, it would be beneficial for plants to use (so far unidentified) extracellular or intracellular sucrose sensors (Fig. 1) to detect such increased sucrose levels and use these signals to strengthen their immune responses (Fig. 1). According to this view, exogenous application of sucrose and ATP to slightly wounded lima bean leaves stimulated jasmonate (JA)-mediated defence responses (Heil et al., 2012). Interestingly, Arabidopsis leaf sucrose levels follow diurnal patterns reaching a maximum at dusk, when leaf growth processes reach a minimum (Ruts et al., 2012). Furthermore, many sugar-metabolizing enzymes, such as for instance cell-wall invertases (CWINVs), vacuolar invertases (VINVs) and sucrose synthases are under control by the circadian clock (Bläsing et al., 2005).
In general, the circadian clock (McClung, 2011a, b, c; O’Neill et al., 2011; Troein et al., 2011; Farré and Weise, 2012) synchronizes biological processes with environmental triggers, both in plants (Khan et al., 2010; Filichkin et al., 2011; Wang and Wang, 2011; Xu et al., 2011; Troncoso-Ponce and Mas, 2012) and in other eukaryotic organisms including cyanobacteria (Liu et al., 1995; Golden and Canales, 2003). Overall, prokaryotic and eukaryotic clocks are significantly different, but peroxiredoxins seem to be crucial players in all circadian rhythms (Edgar et al., 2012). The clock is a relatively robust system, with a period of circa 24 h, for anticipating day/night cycles. It persists under continuous light or dark and is buffered relatively well under temperature changes (Espinoza et al., 2010; Haydon et al., 2011). In plants, metabolites, second messengers, and light signals are incorporated to entrain the oscillator through altering the expression of circadian clock genes and modulation of related functions (Schultz and Kay, 2003; Dalchau et al., 2010, 2011).

The plant circadian clock consists of at least three auto-regulatory interlocked transcriptional feedback loops involving positive and negative regulators that contribute to the control of gene transcription, as well as to the activity of some effectors (Harmer, 2009; Troncoso-Ponce and Mas, 2012). Although the involvement of the clock in modulation of responses to abiotic stress is widely accepted (Fowler et al., 2005; Espinoza et al., 2010), its effect on biotic stress responses remained a matter of debate for a long time (McClung, 2006). It would be beneficial for plants to evolve a circadian regulation to maximize the level of defence compounds (toxins, defence hormones) and/or sweet immunostimulators at the time of the day when the encounter with the pathogen or herbivore is more likely to occur. For example, circadian JA accumulation in Arabidopsis synchronizes with insect feeding behaviour, providing evidence for the emerging concept of ‘clock-enhanced herbivory resistance’ (Goodspeed et al., 2012). Similarly, Arabidopsis shows a circadian clock-mediated variation in resistance to the virulent bacterial pathogen Pseudomonas syringae pv. tomato DC3000 (Bhardwaj et al., 2011). According with this view, a large number of defence-related genes are regulated by diurnal and circadian rhythms in this species (Bhardwaj et al., 2011). Taken together with other data (McClung, 2011b; Wang et al., 2011a, b; Desclos-Theveniau et al., 2012), it seems that the clock also fulfils an important role in biotic stress responses.

In this review, the focus is on the emerging connections between sugar metabolism, sugar signalling, hormone...
signalling, and the circadian clock, with repercussions on plant innate immunity, uncovering the possible integrated network among these components.

Light effects on the circadian clock and invertases

The central feedback loop of the clock consists of CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), and TIMING OF CAB EXPRESSION 1 (TOC1) (Alabadi et al., 2001; Ding et al., 2007). TOC1 acts as a negative regulator of GIGANTEA (GI). Light and temperature are the two best-characterized stimuli for the circadian clock, acting on components in the clock’s central loop to synchronize with periodic changes in the environment (McWatters and Devlin, 2011). Light signals are perceived and transduced by phytochromes and cryptochromes (Lopez et al., 2012). In Arabidopsis leaves, three photoreceptors are known to be involved in the light entrainment of the clock: PHYA-E, a R/FR sensing phytochrome; CRY1 and CRY2, the blue-light sensing cytochromes; and a family of three F-box proteins including ZEITLUPE (ZTL) (Hotta et al., 2007; Li et al., 2011a; Wenden et al., 2011). The interactions of three positive regulators of the PHYA-signalling pathway, FAR-RED ELONGATED HYPOCOTYL 3 (FHY3), FAR-RED-IMPARED RESPONSE 1 (FAR1), and ELONGATED HYPOCOTYL5 (HY5) with clock components were recently established (Li et al., 2011a). The dawn cues induce CCA1 and LHY interacting in a putative complex with FHY3, FAR1, and HY5 to suppress EARLY FLOWERING 4 (ELF4), the central rhythmic component of the clock. Loss of CCA1 and LHY expression from this complex throughout the following day activates ELF4 expression (Li et al., 2011a). Also, the dusk signal involves relief from light repression of TOC1 degradation by ZTL (McClung, 2006).

Phytochromes were also reported to enhance CWINV activity in wheat coleoptiles and radish hypocotyls (Krishnan et al., 1985). The increased activity of the Arabidopsis soluble VINVs and the increased gene expression of one CWINV (Atfruct1/Atcv1INV1) and the two VINV genes of Arabidopsis (Atfruct3 and Atfruct4) were shown to be induced by a phytochrome-related machinery mediating the de novo synthesis of active gibberellic acid (GA), stimulating germination (Mitsuhashi et al., 2004). Similar findings were reported on watermelon seedlings (Ranwala et al., 2002). The expression of a VINV gene in rose buds also seems to be controlled by light as well as by sucrose and fructose (Rabot et al., 2012). It can be hypothesized that light can regulate GA and invertase (INV) activities to generate sugar signals that might entrain the clock, as will be discussed. It can be further speculated that plants that perceived a light stimulus in the dawn can anticipate further events in coming dusk, mediated by INV activities.

Sugar-signalling interactions with the circadian clock

Sucrose is the main product of photosynthesis as well as the main transport sugar in most, but not all plants (Koch, 2004). It contributes to various plant regulatory and signalling mechanisms including growth, development, and stress-related responses (Wind et al., 2010). INV activity on sucrose leads to the formation of hexoses. Glucose-, sucrose-, and fructose-mediated sugar-signalling pathways have been described (Moore et al., 2003; Wind et al., 2010; Li et al., 2011b).

The starchless phosphoglucomutase (pgm) mutants, with their strongly altered diurnal sugar changes, already pinpointed the essential role of sugars in modifying gene expression of up to about half of the clock-regulated genes in Arabidopsis (Bläsing et al., 2005; Dodd et al., 2005). These data hinted the putative existence of a sugar-mediated entraining of the clock.

Upon exogenous sucrose addition, Johnson et al. (1995) recorded the abolishment of circadian oscillations in cytosolic-free Ca2+ concentrations while James et al. (2008) described a differential perturbation of diurnal gene expression oscillations in roots compared to aerial tissues. Recently, it was described that sucrose stimulates the clock’s core central oscillator genes GI, TOC1, and CCA1 (Fig. 2) (Knight et al., 2008; Dalchau et al., 2011). More in particular, experimental and mathematical studies showed that GI is needed for a full response of the circadian clock to sucrose (Dalchau et al., 2011).

The possible cooperation of sugars and the clock highlights the putative importance of enzymes involved in plant sugar partitioning. Besides the enzymes of sucrose metabolism, especially the INVs, also the enzymes of starch and raffinose family oligosaccharide metabolism come into the picture (Espinoza et al., 2010; Valluru and Van den Ende, 2011; Baroja-Fernandez et al., 2012; Stitt and Zeeman, 2012). Differential INV activities (producing hexoses) and sucrose synthases (producing UDP-glucose instead of glucose) might modulate sucrose/hexose ratios affecting sugar-specific signalling pathways (Xiang et al., 2011 and references therein) as well as photoperiodic flowering (Seo et al., 2011). Therefore, it is proposed here that such altered sugar balances might also contribute to the proper entraining of the circadian clock (Fig. 2).

Vice versa, it is well known that the clock regulates the expression of many genes involved in carbohydrate metabolism (Harmer et al., 2000; Rolland et al., 2002; Fig. 2). Intriguingly, both the expression of a vacuolar INV (VINV) gene in sugar beet petioles (González et al., 2005) and the expression of the CWINV LIN6 gene of tomato have been shown to be regulated by a diurnal rhythm (González and Roitsch, 2009), but their rhythm seems to be different. Interestingly, CCA1 and LHY impose a synergistic effect on the induction and activity of the LIN6 promoter (González and Roitsch, 2009). Therefore, it seems likely that the clock can regulate sucrose partitioning enzymes (Fig. 2) to shift sucrose/hexose ratios, differentially affecting sucrose and hexose-signalling pathways. Besides the circadian regulation of genes encoding sucrose-splitting enzymes, also some sucrose phosphate synthase genes (Okamura et al., 2011) and genes encoding starch-branching enzymes are regulated by the circadian clock (Ral et al., 2006; Mutisya et al., 2009; Stitt and
In addition to regulation at the transcriptional level, a clock-mediated regulation at the posttranscriptional and/or posttranslational level was also demonstrated or suggested for some sugar-metabolizing enzymes (Jones and Ort, 1997; Smith et al., 2004).

The diurnal pattern of starch synthesis during the day and breakdown during the night in source leaves is widely documented. On the part of starch synthesis, it was reported that both the clock and sucrose control the expression of a starch synthase (Wang et al., 2001). Recent evidence shows that the clock plays an important role in controlling starch degradation during the night (Graf et al., 2010; Graf and Smith, 2011), as derived from growth studies on lhy/cel1 double knockouts under well-chosen photoperiods. Therefore, the authors concluded that plant production greatly depends on the clock since overall growth rate depends on the supply of carbon and that the clock orchestrates diurnal carbon allocation and growth (Yazdanbakhsh et al., 2011).

The clock and stress responses

Arabidopsis seedlings show rhythmic growth under diurnal conditions (short day) with an associated circadian clock regulation of phytochrome-interacting factors (PIF) 4 and 5 (Soy et al., 2012). In contrast, PIF3 does not show such circadian regulation but emerges as an important growth regulator, in conjunction with PIF4 and PIF5 (Soy et al., 2012). The clock also plays a prominent role in stress responses (Fig. 2) such as cold (Nakamichi et al., 2009; Espinoza et al., 2010). Recent work uncovered the regulation of CCA1 by temperature-responsive differential splicing into two variants CCA1α and CCA1β (Seo et al., 2012). Plants overexpressing CCA1β showed increased freezing tolerance compared with the wild type, cca1 mutants, and CCA1β overexpressers (Seo et al., 2012). Therefore, it seems of great importance to study the clock’s components and alternative splicing mechanisms in other types of plants particularly those adapted to other types of stresses such as dry environments. Crassulacean acid metabolism plants are such a case, in which many players are already known to be regulated by the clock. This is reviewed elsewhere (Nimmo, 2000). Notably, it was reported that the circadian control of phosphoenolpyruvate carboxylase kinase and carbon dioxide fixation can be overridden by metabolic signals (Borland et al., 1999; Borland and Taybi, 2004). Recent findings also stimulate deeper research on the clock’s responses under oxidative stresses (e.g. heavy metal stress as described in Maistri et al., 2011). Indeed, many genes responding to reactive oxygen species also show a circadian pattern (Covington et al., 2008).

In conclusion, it becomes increasingly evident that the clock is taking a central and important position in cellular networks (Fukushima et al., 2009; Kooke and Keurentjes, 2012; Krasensky and Jonak, 2012), acting as an important integrator of environmental signals (Fig. 2).

Involvement of sugar signalling in plant innate immunity

Plants protect themselves from dangerous pathogens and herbivores through a combination of constitutive and induced defences (Chisholm et al., 2006). The plant innate immunity involves two kinds of responses: (i) a response to slowly emerging pathogen-associated molecular patterns (PAMPs), better defined as microbial-associated molecular patterns (MAMPs), such as flagellin, through transmembrane pattern recognition receptors (PRRs) leading to PAMP-triggered immunity and (ii) a response inside the cell, known as effector-triggered immunity (Chisholm et al., 2006;
Jones and Dangl, 2006). Damage-associated molecular patterns (DAMPs), which are endogenous molecules that are produced by the plant after infection, are also recognized by PRRs to trigger defensive reactions. Insect-derived compounds could be designated as herbivore-associated molecular patterns (HAMPs). These are also presumably recognized by PRRs but so far HAMP receptors have not been identified (Erb et al., 2012).

As mentioned in the introduction, sugar-signalling events might fulfil prominent roles in plant defence responses under biotic and abiotic stresses (Bolouri Moghaddam and Van den Ende, 2012). Among sugars involved in defence mechanisms (Bolouri Moghaddam and Van den Ende, 2012), extracellular sucrose is emerging as a candidate signalling molecule in plant innate immunity (Gómez-Ariza et al., 2007; Birch et al., 2009; Heil et al., 2012). However, so far it is not clear whether extracellular sucrose is sensed at the plasma membrane or internalized and recognized by an intracellular sucrose sensor (Fig. 1). Alternatively, sucrose may first be processed by CWINV activity, generating glucose signals that might be sensed at the plasma membrane by putative glucose sensors (e.g. Urano et al., 2012) or internally (e.g. hexokinases; Moore et al., 2008), presumably through mitogen-activated protein kinases (MAPKs)-signalling cascades (Fig. 1). It has been shown that glucose can activate multiple MAPKs in Chenopodium rubrum cells (Ehness and Roitsch, 1997). However, this glucose specificity was recently challenged by the discovery that sucrose-specific MAPKs show the same sucrose specificity as described for the CisAPK, a MAPK in Cephalostachyum fuchsianum Gamble cells (Li et al., 2012). This suggests that sucrose-specific MAPK activation, perhaps acting in parallel with glucose-mediated MAPK activation (Fig. 1), can function as an early signal to regulate various sugar-specific responses including defence reactions in plant cells (Li et al., 2012). It would be interesting to find out whether some Arabidopsis MAPKs show the same sucrose specificity as described for the CisAPK.

CWINVs are themselves considered as pathogenesis-related proteins (Roitsch et al., 2003) that can be induced by endogenous saccharides (e.g. poly- or oligogalacturonides), pathogen-derived elicitors (Sturm and Chrispeels, 1990; Berger et al., 2004; Hyun et al., 2009) and JA (González and Roitsch, 2009; Hyun et al., 2011; Landgraf et al., 2012) (Figs. 1 and 2). It was recently established that the wall-associated kinase (WAK)-signalling pathway controls the expression of many defence genes and alters cellular sugar metabolism through sensing of extracellular oligogalacturonides that are released in response to pathogen invasion. WAKs probably act as PRRs or oligogalacturonide receptors in this case (Brutus et al., 2010), transferring the signals across the plasma membrane. The WAK genes are induced by salicylic acid (SA), wounding, and bacterial infection (He et al., 1998; Wagner and Kohorn, 2001). The link between WAKs and transcriptional/enzyme regulation is established by MAPKs such as MPK3 and MPK6 in Arabidopsis (Kohorn et al., 2012). MAPKs are known to be involved in the process of elicitor-mediated induction of CWINV gene expression during plant–pathogen interactions (Hyun et al., 2009).

Most likely, extracellular (sugar) signals of endogenous or pathogenic origin, CWINV activities, and WAK-mediated signalling cascades as well as hormonal (JA) signalling may cooperate to mediate appropriate immune responses (Fig. 1). Other intriguing players in this story are the CWINV inhibitors (indicated as INH in Fig. 1; Hothorn et al., 2010). The fact that some pathogens are able to inhibit the expression of these inhibitors indicates that CWINV/inhibitor complexes probably fulfil a prominent role in pathogen responses (Essmann et al., 2008; Kocal et al., 2008; Bonfig et al., 2010).

Interaction of immunity-related factors with the circadian clock

The production of anthocyanins should be considered as part of the plant’s defence response (Karageorgou and Manetas, 2006). Intriguingly, anthocyanin biosynthesis is controlled by a sucrose-specific signalling pathway (Teng et al., 2005) indicating that specific sucrose sensors or sucrose-specific MAPKs (cfr Fig. 1) might be involved. The synthesis of anthocyanins, for instance, is controlled by a set of closely related MYB transcription factors (PAP1/MYB75, PAP2/MYB90, MYB113, and MYB114) that stimulate the transcription of both general phenylpropanoid and anthocyanin-specific genes (Gonzalez et al., 2008). Importantly, the MYB75-dependent PAL1 expression is described to be regulated by the circadian clock (Harmar et al., 2000; Rogers et al., 2005) indicating an intimate cooperation of sucrose involved in immunity under a clock-regulated mechanism.

The expression of some defence genes is also regulated by CCA1, the MYB-related transcription factor acting in the heart of the clock (Wang et al., 2011b). CK2 phosphorylates the CCA1 and LHY proteins (Sugano et al., 1999), controlling the pace of the clock (Lu et al., 2011) and triggering clock-regulated defence responses. The defence hormone SA is known to activate nuclear protein kinase CK2 in tobacco (Hidalgo et al., 2001).

Hormone/sugar-signalling-related immunity and clock relations

The cross-talk between hormone- and sugar-signalling pathways in plant immune responses has been reviewed before (Leon and Sheen, 2003). Whenever a plant encounters a pathogen attack, phytohormones can act as signalling molecules to activate defence responses through the stimulation of plant innate immunity (Pieterse et al., 2009). Stomata are suitable natural openings for pathogens to enter plant cells. The stomatal opening follows a diurnal pattern (Tallman, 2004) and the circadian clock may play a considerable role in the regulation of stomatal aperture (Hubbard and Webb, 2011; Kinoshi and Hayashi, 2011) allowing anticipation of dawn and dusk signals (Hotta et al., 2007) (Fig. 2). Besides abscisic acid (ABA), JA is emerging as an important player
in stomatal closure as well (Hossain et al., 2011) (Fig. 2). Interestingly, the sensitivity of stomata to ABA seems to be higher in the afternoon than in the morning (Correia et al., 1995). Not unexpectedly, MAMPs such as chitosan act as inducers of stomatal closure (Salam et al., 2012 and references therein), as exemplified in Figs. 1 and 2.

At the first layer of defence against MAMP, ABA has a recognized stimulatory role in stomatal closure with a fine-tuned cross-talk among other hormones, PAMP-triggered signals, and HSP90 (Ton et al., 2009). ABA can be an essential signal in plant resistance to pathogens by activating defence genes and affecting JA biosynthesis (Adie et al., 2011). The diurnal expression of ABA-related genes (ABARICHLHL/HIGUN5) has been shown to be regulated by TOC1 (Castells et al., 2010) (Fig. 2). Moreover, ABI3 physically interacts with TOC1 (Kurup et al., 2000; Dekkers et al., 2008).

It seems that circadian clock-dependent gating regulates the signalling network of ABA at numerous points of its function and activity (Seung et al., 2012). The transcription of HSP90, an important player in innate immunity responses (Ting et al., 2008), is also increased by ABA (Leng, 2008). HSP90, in association with the STG1 chaperone, stabilizes the nucleotide-binding sites and leucine-rich repeat proteins (Wilmanski et al., 2008) mediating plant defence responses (Shirasu, 2009). Interestingly, it was shown that the Arabidopsis circadian clock-associated ZTL is a unique client for cytoplasmic HSP90 (Kim et al., 2011). This suggests a close relation between ABA, the clock, and ZTL in innate immunity.

As well as a recent report showing a positive interaction between ABA and fructose signalling through biosynthesis of this hormone (Cho and Yoo, 2011), other reports indicate that ABA provokes the expression of anthocyanin biosynthesis-related genes such as CHS, CHI, DFR, and UFGT, as well as the regulatory factor VvmybA1 in grape skin (Ban et al., 2003; Jeong et al., 2004).

ABA shows a complex and dual role in pathogen resistance as well as on the induction of INV activity (Trouverie et al., 2004; Hayes et al., 2010; Ji et al., 2011). However, it seems that responses to drought stress and pathogens partially overlap, with ABA as a positive stimulator (Bolouri Moghaddam and Van den Ende, 2012). Taken together, it seems that the clock, by regulating ABA gene expression as well as related transcription factors, can conduct sugar signals through different pathways leading to appropriate defence responses under abiotic stresses.

Besides their well-documented physiological roles in plant growth and development, cytokinins are also involved in defence mechanisms. They can induce resistance against different viruses, suppress the hypersensitive response, and induce SA in wounded responses (Sano et al., 1996; Pogany et al., 2004, Barna et al., 2008). Recently, cytokinins were described to enhance resistance against *P. syringae* in tobacco. The mechanism includes stimulation of two antimicrobial phytoalexins, independently of SA (Grosskinsky et al., 2011). Cytokinins are involved in controlling anthocyanin accumulation in Arabidopsis (Deikman and Hammer, 1995), but many other factors such as sucrose, ABA, and GA play a role as well (Shan et al., 2009; Bolouri Moghaddam and Van den Ende, 2012).

Typically, CWINV and hexose transporter expression levels are induced by cytokinins (Ehness and Roitsch, 1997) and leaf senescence is delayed by cytokinins, by overexpressing CWINV or by downregulating CWINV inhibitors (Balibrea et al., 2004; Jin et al., 2009). It has been shown that cytokinins in association with nitric oxide contribute to resistance responses against *Botrytis cinerea* in Arabidopsis through oligogalacturonide-triggered immunity reactions (Rasul et al., 2012). The interaction of cytokinins with clock components has also been demonstrated. The Arabidopsis expression of LHY and CCA1 are specifically induced by cytokinins whereas TOC1 is repressed in a light-dependent manner (Zheng et al., 2006) (Fig. 2). The data suggest a possible interaction between sugar signalling (probably mediated by CWINV), cytokinins, and plant defence responses downstream of the clock (Figs. 1 and 2).

**Conclusion and perspectives**

Sugar-signalling pathways contribute in various ways to plant defence responses. Plant innate immunity is a complicated network in which many signalling molecules and hormonal cross-talks are involved. The clock components have close and direct interactions with plant-hormone-related pathways. Sugar signals can have a reciprocal role with the clock; on the one hand delivering environmental cues to the clock, influencing its components, but on the other hand also delivering a signal from the clock to different downstream pathways. Therefore, it seems likely that the most effective response of plant innate immunity to MAMPs through sugar-signalling and/or hormonal pathways as well as priming events greatly depends on the actual status of the clock. It can be concluded that: (i) the exact components of the clock and their connections with sugar-signalling and innate immunity-related pathways should be further investigated; (ii) more studies should be undertaken to rigorously quantify a wider array of defence-related metabolites at frequent time intervals over the diurnal cycle; and (iii) deeper studies on the pathogen abundance and/or herbivore feeding behaviour would allow a better prediction of the time window during a natural day when crop plants would be most vulnerable. All these insights might allow the optimization of the exact diurnal timing of the application of cheap and biodegradable sugar(-like) compounds as priming agents and immunostimulators (Bolouri Moghaddam and Van den Ende, 2012). However, other remaining challenges include: (i) finding whether application of priming agents is already possible at the seedling stage or during later development stages; (ii) testing how many applications are necessary; and (iii) investigating whether or not the expected beneficial effect would remain intact throughout the entire life cycle of the plant. Last but not least, genetic manipulation of the clock’s components, especially those that are intimately linked with stress-tolerance mechanisms, might pave the way to the development of ‘all season crops’.
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