Osmotic stress alters circadian cytosolic Ca\(^{2+}\) oscillations and OSCA1 is required in circadian gated stress adaptation

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ABSTRACT
The circadian clock is a universal timing system that involved in plant physical responses to abiotic stresses. Moreover, OSCA1 is an osmosensor responsible for \([\text{Ca}^{2+}]\), increases induced by osmotic stress in plants. However, there is little information on osmosensor involved osmotic stress-triggered circadian clock responses. Using an aequorin-based Ca\(^{2+}\) imaging assay, we found the gradient (0 mM, 200 mM, 500 mM) osmotic stress (induced by sorbitol) both altered the primary circadian parameter WT of and osc1 mutant. This means the plant switch to a fast day/night model to avoid energy consumption. In contrast, the period of WT and osc1 mutant became short since the sorbitol concentration increased from 0 mM to 500 mM. As the sorbitol concentration increased, the phase of the WT becomes more extensive compared with osc1 mutant, which means WT is more capable of coping with the environmental change. Moreover, the amplitude of WT also becomes broader than osc1 mutant, especially in high (500 mM) sorbitol concentration, indicate the WT shows more responses in high osmotic stress. In a word, the WT has much more flexibility to cope with the osmotic stress than osc1 mutant. It implies the OSCA1 might be involved in the circadian gated plant adaptation to the environmental osmotic stress, which opens an avenue to study Ca\(^{2+}\) processes with other circadian signaling pathways.

Introduction
Drought is one of the most devastating of natural hazards, which cause tremendous agricultural yield loss every year.\(^1\) It is around 40% of the global land area situated in arid or semiarid climates which threatened the lives and livelihoods of millions of people.\(^1,2\) There is an urgent need for the development of plants with improved tolerance to abiotic stress, but the development of such improved varieties will require a thorough understanding of the mechanisms by which plants are affected by these conditions, and how they can tolerate them.

Plants have a remarkable ability to cope with the environmental osmotic stresses, which caused by drought, salinity, and cold. The circadian clock is a vital timing system that controls physiological responses to abiotic stresses in plants. The synchronization of the endogenous physiological and metabolic activities with the rhythmic environmental cycles is crucial for plant fitness and their ability to adapt to environmental challenges.\(^3,4\) The clock controls the amplitude of the basal expression of stress-responsive genes and their responses to environmental signals in a process called gating.\(^5\) Drought stress triggers a complex signal transduction pathway that ultimately leads to protection against water deficit. In Arabidopsis, drought responses could be classified into ABA-dependent and ABA-independent pathways (Shinozaki and Yamaguchi-Shinozaki 2007). In the ABA-dependent pathway, ABA accumulation increases under drought stress will reduce water-loss by accelerating stomata closure.

Calcium, \([\text{Ca}^{2+}]\), is a ubiquitous ion found in all living systems. It is a versatile second messenger in both animals and plants. Recent years, cytosolic calcium is also used as an indicator to measure circadian oscillation in the model plant Arabidopsis.\(^6\) In order to detect the in vivo \([\text{Ca}^{2+}]\) change, a variety of fluorimetric calcium indicators were used to measure \([\text{Ca}^{2+}]\) in plants.\(^7,8\) Most of the current calcium in vivo indicators are invasive, so it is not suited for the long-term in vivo measurement for circadian research. Till now, the most used protocol to measure circadian clock is use aequorin as an indicator.\(^6\) Aequorin, a photoprotein derived from the luminous jellyfish Aequoria victoria, reacts specifically with \text{Ca}^{2+}\ and emits blue light peak at \(\sim 470\) nm. The functional aequorin protein has three EF-hands, which have a high affinity and low capacity to calcium ion. Use genetic engineering, and the apoaequorin cDNA is expressed under the control of the 35S promoter from Cauliflower Mosaic Virus (35S: AEO) to give high constitutive expression in whole plants.\(^9\) Abiotic stimuli like the osmotic stress increases in the cytosolic/intracellular-free calcium concentration ([\text{Ca}^{2+}]\) in plants.\(^10,11\) The concept “calcium signature”: information, and thus the flow of calcium

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could define the nature and magnitude of the response by a series of parameters, like time, phase, amplitude, and frequency. Dalchau et al. developed a mathematical model that provides insight into the control of $\text{Ca}^{2+}$ fluxes and exposed underlying control principles of circadian rhythms. This model, which was verified experimentally, demonstrated that the dynamics of $[\text{Ca}^{2+}]_{\text{cyt}}$ were best described by a network that consists of light signaling pathways that operate over rapid timescales directly regulating $[\text{Ca}^{2+}]_{\text{cyt}}$, in addition to regulation by TTLs (transcription translation loops).

A sensor for hyperosmotic stress is the Arabidopsis OSCA1 (reduced osmosmolality-induced $[\text{Ca}^{2+}]$ increase 1), which encodes a plasma membrane protein that forms hyperosmolality-gated calcium-permeable channels. Using calcium-imaging-based unbiased forward genetic screens, our lab isolated Arabidopsis mutants that exhibit low hyperosmolality-induced $[\text{Ca}^{2+}]$ increases. Aradiopsis oscal loss-of-function mutants display a reduced calcium elevation compared to wild-type plants when treated with osmotic stress agents like sorbitol. The previous study shows that many genes responsive to abiotic stress were found to be under the control of the circadian clock. In osmotic stress, the OSCA1 protein quickly transduces the stress signal and make a rapid increase in the cytosolic $\text{Ca}^{2+}$ concentration.

One of the most studied mechanisms involved in these adjustments is the circadian clock. Circadian clocks appear almost ubiquitous in higher organisms. It generates an internal approximation of external time. The internal time enables plants anticipation of environmental changes that occur at specific times during the 24 h cycle and imparts a degree of physiological optimization that might confer evolutionary fitness. In order to synchronize with the environmental change, the external time cues entrain circadian clocks, which called “time-givers” (or zeitgebers). An essential characteristic of the circadian clock is temperature compensation, which enables the circadian clock to maintain the period over a wide range of physiological temperature range. By restricting the time of maximal responsiveness, the circadian gating defines an efficient way to increase resistance to stress without substantially decreasing plant growth.

The circadian clocks are endogenous timekeeper that control rhythm a close to 24 h period via a network of multiple feedback loops. In Arabidopsis, the basic architecture of central oscillator consists of an interlocking transcriptional feedback loop by CCA1 (CIRCADIAN CLOCK ASSOCIATED1)/LHY (LATE ELONGATED HYPOCOTYL) and TOC1 (TIMING OF CAB EXPRESSION 1) (PRR1) (PSEUDO-RESPONSE REGULATOR 1). Experimental and mathematical studies suggested, the CCA1–LHY–TOC1 feedback loop has been proposed to function as follows: TOC1 is an evening-phased, clock-regulated gene. The nuclear-localized TOC1 protein indirectly promotes the two dawn-phased, MYB-domain transcription factors CCA1 and LHY as CCA1 and LHY are expressed rhythmically with a peak in transcript abundance at subjective dusk. The related proteins are produced within 2–3 h and inhibit TOC1 expression by binding an evening element (EE) in the TOC1 promoter. This process causes a gradual decline in TOC1 levels, and consequently a decline in CCA1 and LHY production. As CCA1 and LHY levels decline, the repression of TOC1 expression is lifted, and the cycle is re-initiated. Other members of the PRR family, like PRR5, PRR7, and PRR9, bind to the promoters of CCA1 and LHY and repress their expression. CCA1 and LHY, in turn, promote the expression of PRR7 and PRR9 by direct association with their promoters (Farré, Harmer et al.). The evening complex (EC) composed of EARLY FLOWERING3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) represses the expression of the day-phased clock gene PRR9. Further connections between the different modules are established by the pervasive repressing function of TOC1 regulating nearly all of the core clock components.

In recent years, it was found that ABA-induced the core circadian oscillator TOC1 expression. Furthermore, the ABA-signaling pathway is central to drought and salt stress responses. Moreover, because the calcium channel OSCA1 is an osmosensor that located at the upstream of the ABA-signaling pathway, therefore, the interaction between the circadian clock and OSCA1 as well as ABA-related responses could define how the clock associated with stress responses and keep fitness. Circadian clock-dependent gating appears to regulate the ABA signaling network at numerous points, including metabolism, transport, perception, and activity of the hormone.

The models of the circadian clock have been developed for many species to improve understanding of the clock mechanism. Some techniques and algorithms can analyze circadian data, all with different assumptions and with different levels of complexity. For most of the non-expert researcher, it is not easy to choose a suitable analyze method, and this always offers many pitfalls. BioDare2 (Biological Data Repository) online service was designed under the framework of the ROBuST project to solve such issue. BioDare2 provides six different algorithms under a variety of conditions. The pre-processing and pre-selection of the data will significantly help the system to return a reliable estimate of the critical circadian parameters. The repository, like BioDare, is hugely helping in coordinating the results of multiple analyses, including circadian data. The flexible software architecture of BioDare makes it possible to integrate different analytical methods, and this also gives us a powerful tool to understand the circadian data.

The objectives of this research in the model plant Arabidopsis were to understand better how osmotic stress applied in cotyledon stage affected the circadian clock, and how the osmosensor OSCA1 enhance the Arabidopsis osmotic tolerance under the gate of the circadian clock. For this, we tested (1) whether the gradient osmotic stress affected the critical parameters of the circadian clock in WT and (2) whether the gradient osmotic stress affected the critical parameters of the circadian clock in the oscal mutant. Our results showed the OSCA1 could enhance the plant tolerance to osmotic stress by adjusting the vital circadian parameters.

**Materials and methods**

*Plant material and growth conditions*

*Arabidopsis thaliana* ecotype Col-0 constitutively expressing intracellular $\text{Ca}^{2+}$ indicator aequorin (pMAQ2) (hereafter we...
use WT to represent) is a gift from M. Knight, and the principles of how the active aequorin is formed can be found in Knight et al.\(^9\) Arabidopsis seeds were surface-sterilized with 2.5% plant preservative mixture (PPM) (Caisson Laboratories) and stratified at 4°C for three days in the dark, then grew on 0.5 MS 0.8% (w/v) agar media, with pH adjusted to 6.0 with KOH; 2.15 g MS salts and 8 g bacto agar per liter sterile ddH\(_2\)O. Sucrose should not be added to the media in this experiment. Because 1–3% (w/v) sucrose abolishes circadian [Ca\(^{2+}\)]\(_{cyt}\) oscillations, through an unknown mechanism.\(^{25,26}\) Then plants were grown in the climate chamber at 20 ± 2°C with 12:12 light/dark cycles. The fluence rate of light was ~110 \(\mu\)mol m\(^{-2}\) s\(^{-1}\).

**Osmotic stress application**

Osmotic stress was applied after seedlings reached the two-leaf stage. To generate stable osmotic stress conditions in the roots of the plants, we moved 9-day-old seedlings from growth medium to gradient osmotic stress medium. In this experiment, we use three sorbitol concentrations, and they are 0 mM (control), 200 mM, 500 mM, respectively. Put the Petri dishes with osmotic stress back into the climate chamber for two days to let the seedlings accommodate the stress environment and recover from the mechanical stimuli suffered from transferring.

**Circadian rhythm bioluminescence measurements**

Dose 9-day-old plants with 40 \(\mu\)l 20 \(\mu\)M coelenterazine under-shaded conditions at the onset of the dark cycle. Moreover, repeat this on day 10. At the onset of the dark cycle of Day 10 put Petri dishes in the camera chamber until the next dawn (ZT0). The measurement consists of two parts, the first 2 days with neutral light (12 h light/12 h dark) condition, which called entrainment – moreover, the latter two days in constant darkness, which called free-run (or free-running). The LED arrays must be turned off during measurement, and a pulse inserted for 200 s to let the delayed fluorescence from the chloroplasts disperse.\(^{27}\) Then, record luminescence data for 1,500 s to increase the signal-to-noise ratio. The LED panel were commercial products which could output light intensities of 80 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). A total of four days were used to do this experiment, including two days’ entrainment and 2-days free running. The middle two days (one day of entrainment and one day of free running) were used to collect the image data.

**Circadian rhythm data analysis**

The vital circadian parameters, including period, phase and the relative amplitude error of rhythmicity, were calculated using BioDare2 online analysis platform (https://biodare2.ed.ac.uk/welcome).

**Statistical analysis**

Independent experiments were performed at least three times, and the statistical analysis was performed using EXCEL2019. Data were presented as mean ± s.d. To analyze the difference between genotypes two-way analysis of variance (ANOVA) was carried out using SAS 9.3 software (SAS Institute). For the Figures listed in this paper, the error bars represent s.d., and \(P\) values < .05 were considered statistically significant.

**Results**

**Monitoring of osmotic stress in Arabidopsis cotyledon using bioluminescence imaging**

To study the relationship between the circadian clock and osmotic stress in Arabidopsis, we developed a simple but reliable method based on the other protocol.\(^6\) The Arabidopsis seedlings initially were cultivated vertical on ½ MS medium for 8 days. On the morning of day nine, we transferred WT and osca1 seedlings to the top of agar medium of 8 mm petri dish (with or without sorbitol stress treatment) in parallel lines. Each line with 20 seedlings which were put side by side (Figure 1a). a set of four Petri dishes (could be a group of treatment) (Figure 1b) were put into the dark box of ChemiPro HT system which was installed two commercial LED panel (bought from Taobao) (Figure 1c). Since the seedlings cultivated on ½ MS medium need to accommodate the osmotic stress and the entrainment environment (unusually light), which may be a little bit vary from the climate chamber. The Princeton Instruments WinView/32 software package provides full image acquisition, display, processing, and archiving functions. However, one disadvantage of the camera control function is that it cannot set a series of the period to turn on and switch off the camera automatically. In that case, the CCD array would expose to high photon environment, and this will shorten the lifetime of CCD electronics and leads to a higher signal/noise ratio. To solve this problem, we installed a screen control software, named “Anjian ghost” (www.anjian.com). Then, we write a command to turn on and turn off the CCD camera at the specific time of day.

-Model illustrating how WT and osca1 mutant seedlings were put on the Petri dishes. Plants expressing aequorin were put on ½ MS medium (without sucrose) with or without sorbitol stress treatment. The WT and osca1 mutants were put side by side, and each group consists of 20 seedlings. b. A group of four Petri dishes were put into ChemiPro light-tight dark box, the location of WT and osca1 mutant were put randomly to avoid location effect. Each group of samples includes two groups of 0 mM sorbitol treatment samples, one group 200 mM and one group 500 mM sorbitol treatment samples.

-Model is illustrating the improved LN-cooled End-on Camera ChemiPro system. Two commercial LED panels were installed on the opposite side in the dark box.

**Osmotic stress affects calcium oscillation of WT and osca1 mutant**

To examine whether osmotic stress affects long-term calcium oscillation which is an indicator of the changes of the circadian clock. We studied WT and osca1 diurnal changes of calcium rhythm under 0 mM (control), 200 mM, 500 mM sorbitol stress (Figure 2, Figure 3). For WT on 0 mM and 200 mM sorbitol medium (Figure 2a, b), the peak time of entrainment is
Figure 1. The diagram of sample arrangement and improved imaging system.

Figure 2. Aequorin luminescence measured from WT and osca1 mutant under gradient sorbitol stress treatment.

ZT4, and the trough time is ZT16. When it comes to the free-running period, the peak time and the trough time of WT are ZT32 and ZT40, respectively. When WT is grown on 500 mM sorbitol medium, the peak time of entrainment shows in ZT8 and the trough time moves to ZT20 (Figure 2c). While on 500 mM sorbitol medium, the peak time of the free-running period switch to ZT32, and the trough time is ZT48 and ZT40, respectively. Interestingly, the range of amplitude is between 610 and 636 for WT on 0 mM and 200 mM sorbitol medium, but when the sorbitol concentration increased to 500 mM, the range of amplitude is raised between 649 and 669.

a, WT and osca1 mutant \([\text{Ca}^{2+}]\), circadian oscillation on 0 mM sorbitol stress medium. b, WT and osca1 mutant \([\text{Ca}^{2+}]\), circadian oscillation on 200 mM sorbitol stress medium. c, WT and osca1 mutant \([\text{Ca}^{2+}]\), circadian oscillation on 500 mM sorbitol stress medium. Data were collected for two days, one day in entrainment condition (12:12 light and dark cycle), represented by white and black bars; and one day in free-running condition, represented by gray and black bars. The image was taken every two hours by the integration of 1,500 s. Data for three separate experiments are shown (mean ± standard deviation (s.d.); n = 30; two-way ANOVA, P < .05).

Because OSCA1 is a putative osmosensor, which we thought might be involved in crosstalk between the circadian clock and osmotic stress, so we also studied osca1 diurnal changes of calcium rhythm under the gradient sorbitol medium. For osca1 on 0 mM sorbitol medium (control) (Figure 2a), the peak time of entrainment is ZT4 while the trough time is ZT16. The peak time of entrainment on 200 mM and 500 mM sorbitol medium is ZT8 (Figure 2b,c). The trough time of entrainment for each concentration is ZT20. In the free-running period, the peak time for all osca1 samples is ZT28. The trough time of free-running period for osca1 is distinct in different sorbitol concentrations. The trough time on 0 mM sorbitol is ZT40. In contrast, the trough time shifted to ZT44, when on 200 mM sorbitol medium. The trough time shifted to ZT40 when put on 500 mM sorbitol medium. Like the WT, the amplitude range of osca1 mutant on 500 mM
sorbitol medium also shifted to a higher oscillation level of 628 to 644, compared to osca1 mutant grow on 0 mM and 200 mM sorbitol medium of 605 to 636.

**Osmotic stress affects parameters of circadian rhythm both in WT and osca1 mutant**

The circadian rhythms often take the form of a sinusoidal wave that can be described by mathematical terms like period, phase, and amplitude. In this experiment, the online circadian data analyze system BioDare2 is used to assume the variation of circadian parameters. BioDare2 gives the six most popular and accurate algorithms for calculating. Among the six algorithms, the MFourFit gives the best fit. For WT, the period is 23.65 h and 23.50 h on 0 mM and 200 mM sorbitol stress treatment, respectively. However, when the sorbitol concentration comes to 500 mM, the period of WT had a decrease to 19.92 h (Figure 3). For osca1 mutant, the period is 23.36 h and 23.53 h on 0 mM and 200 mM sorbitol stress, respectively. Moreover, the osca1 mutant on 500 mM sorbitol stress is 21.28 h (Figure 3).

The WT and osca1 period estimation under gradient sorbitol stress treatment. Datasets under gradient sorbitol stress treatment were analyzed using the MFourFit algorithm. Data collected from WT with different sorbitol concentrations represented by blue bars; and data from the osca1 mutant with different sorbitol concentrations represented by red bars. Data for three separate experiments are shown (mean ± standard deviation (s.d.); n = 30; two-way ANOVA, P < .05).

In circadian rhythm research, the parameter phase is always used to illustrate the relationship between the chronobiotic’s time of administration and the magnitude of the treatment effects. In our case, the phase of the WT is 11.75 and 12.1 on 0 mM and 200 mM sorbitol stress treatment, respectively. When the sorbitol stress raised to 500 mM, the phase of WT shifted to 13.26 (Figure 4). For osca1, the phase is 11.40 and 11.70 on 0 mM and 200 mM sorbitol concentration, respectively. When the sorbitol concentration raised to 500 mM, the phase of osca1 shifted to 12.40 (Figure 4). Interestingly, the previous study showed that the phasing of expression of the majority of genes is found to be conserved across monocots and dicots.

The WT and osca1 phase comparison under gradient sorbitol stress treatment. Datasets under gradient sorbitol stress treatment were analyzed using the MFourFit algorithm. Data collected from WT with different sorbitol concentrations represented by blue bars; and data from the osca1 mutant with different sorbitol concentrations represented by red bars. Data for three separate experiments are shown (mean ± standard deviation (s.d.); n = 30; two-way ANOVA, P < .05).

The amplitude analysis of BioDare2 shows, compared with control, the amplitude of WT is 5.83 and 5.85 on 0 mM and 200 mM sorbitol stress treatment. Moreover, when the sorbitol concentration comes to 500 mM, the amplitude of WT increased to 6.61 (Figure 5). The amplitude of osca1 is 4.96 and 4.81 on 0 mM and 200 mM sorbitol stress treatment. When the sorbitol concentration comes to 500 mM, the amplitude of osca1 increased to 4.82 (Figure 5).

The WT and osca1 mutant amplitude comparison under gradient sorbitol stress treatment. Datasets under gradient sorbitol stress treatment were analyzed using the MFourFit algorithm.
Discussion

Calcium (Ca\(^{2+}\)) is among the essential, ubiquitous secondary messengers involving signal transduction in all eukaryotes, including plants.\(^{29,30}\) When plants face biotic and abiotic stresses or periodic environmental changes, there will be a short term or long-term cytosolic calcium concentration change with information loading on it to fine-tune each metabolic process to happen at the specific time of day. The circadian clock controls the expression of a significant fraction of abiotic stress-responsive genes, as well as biosynthesis and signaling pathway downstream of stress-responsive hormones.\(^5\) One recent study has shown that osmotic stress can induce cytosolic-free calcium increase, which is known as OICI (osmotic-induced calcium increase). Thus, OSC1 represents a channel responsible for increases of [Ca\(^{2+}\)], induced by osmotic stress in plants.\(^{14,31}\) Besides, [Ca\(^{2+}\)]\textsubscript{cyt} circadian oscillations can regulate circadian clock function through the Ca\(^{2+}\)-dependent action of CALMODULIN-LIKE24 (CML24). Genetic analyses demonstrate a linkage between CML24 and the circadian oscillator, through pathways involving the circadian oscillator gene TIMING OF CAB2 EXPRESSION1 (TOC1). The circadian clock involves daily variations in the transcription of a set of core genes. Based on the above opinions, we speculate that osmotic-induced [Ca\(^{2+}\)], signaling is mediated via osmosensor. Moreover, this is involving in circadian clock regulated stress responsiveness, which is an efficient way to increase resistance to stress without substantially decreasing plant growth.\(^{32}\) By contrast the critical circadian parameters (period, phase and amplitude) of WT and osca1 mutant, the osca1 mutant circadian oscillation shows less variation on period, phase shift, and amplitude (Figures 3, 4, 5). These results demonstrate that OSC1 may be a vital element for osmotic gated circadian clock regulation.

The effect of the circadian clock in plant daily growth regulation is well characterized, especially in light and temperature.\(^{19,33}\) However, there are few scientific research paper focus on osmotic stress-related circadian clock crosstalk. As sessile organisms, plants have developed a series of sophisticated mechanisms to cope with and respond to the environmental osmotic stresses. At the same time, circadian gating of response to changes in water status is among one of the hotspot issues. In Arabidopsis, drought stress triggers ABA-dependent and ABA-independent pathways.\(^{34}\) Cytosolic free calcium [Ca\(^{2+}\)]\textsubscript{cyt} as one of the primary regulators of drought-stress signaling, is regulated by the circadian clock and integrates oscillator time with the environmental signals.\(^{15}\) Besides, ABA accumulation increases under drought and reduces water-loss by stomata closure. The stomata aperture seems to be more responsive to ABA in the afternoon, coinciding with the timing of [Ca\(^{2+}\)] peak oscillation.\(^{32,35}\) The previous research found that the majority of drought-inducible genes peaked around dawn.\(^5\) In our research, we found with the increase of sorbitol stress, the timing of [Ca\(^{2+}\)] peak oscillation was postponed (Figure 2). Plants experienced a water deficit in the daytime comes with a prolonged opening of stomata for photosynthetic gas exchange, and this leads to a series of rhythmic expression of drought-inducible genes may allow plants to minimize water loss. The transcriptomic analysis also shows an intensive connecting between drought-responsive genes and circadian signaling. For instance, the timing of TOC1 functioning in ABA signaling also correlates with the higher sensitivity of stomatal opening to ABA in the afternoon.\(^{5,36}\) In a recent study, LKP2 was suggested to play a role in controlling responses to drought. Overexpressing the lkp2 gene showed a smaller stomatal opening and up-regulated several other drought-responsive genes.\(^3\) GI is a significant LKP2 partner who was found to regulate the expression of genes involved in drought and cold responses, including some EARLY RESPONSIVE TO DEHYDRATION genes and cold-regulated genes.\(^{38}\)

OSCA1 was reported to involve in osmotic-stress-induced fast signaling events, intermediate cellular processes and prolonged growth and development responses, and was also activated by hyperosmolality, revealing OSC1 to be an osmosensor.\(^{14}\) In our research, we found that OSC1 also participate in osmotic-stress-induced long-term signaling events, especially in high osmotic stress. Compared with WT, the osca1 mutant showed less increase in circadian oscillation amplitude (Figure 5). This result implies OSC1 is an essential element in both circadian gated osmotic-stress-induced long-term signaling and [Ca\(^{2+}\)], homeostasis. Besides, there are significant and exciting examples reported in the literature describe the enhancement of responses after experiencing stress, also named as “priming” or “memory”.\(^{39–43}\) In constant hyperosmotic stimulation, the Ca\(^{2+}\) response for many sensory systems tends to return to baseline. This mechanism ensures that the sensory system remains the capability to respond to further change in stimulus intensity.\(^{43}\) In our case, WT shows a broader range of oscillation to osca1 mutant (Figure 2), implies OSC1 is an important osmosensor helps the “sensory adaptation”.

An essential step in the analysis of circadian data is to make an accurate estimate of the core circadian parameters (period, phase, and amplitude). There are many different platforms and algorithms with different complexities for determining these parameters. The algorithm and implementation which the non-experts choose can offer many pitfalls. Here, we referenced the advantages and disadvantages of these algorithms based on many research paper.\(^{24,44–50}\) Combine the data type and the criteria we choose for this research; finally, the MFourFit algorithm was determined to use for the data analysis. MFourFit is one of the curve-fitting methods, which developed for data under-entrained conditions. Our data consists of two parts, one day of entrainment period and one day of a free-running period. The calcium oscillation is always dampening quickly, especially in the free-running period. So the data collected during the entrainment period become a necessary correction and quality control for the whole dataset.

Water is one of the most crucial factors for plant growth and development. Circadian clock can fine-tune the water potential...
in the plant in the specific time of day, to use the minimum biological input to keep fit. The circadian clock and osmotic stress crosstalk are one of the hotspot issues in recent years. Before the osmosensor discovered, most of the previous research focuses on the circadian modulated stress response pathways at multiple levels and through a range of mechanisms, including the transcriptional regulation of core regulators of the stress response, the direct regulation of stress-responsive genes and signaling by stress response hormones. The past few years of research have established a web of regulatory connections between abiotic stress response pathways and the plant circadian clock. But the mechanisms of how circadian clock manage plant water usage still needs to be elucidated in the future.

Conclusion

Our study demonstrated that gradient osmotic stress altered the circadian calcium oscillation in the Arabidopsis cotyledon stage and thus acted as a spatial-temporal input signal into the clock. Besides, OSCA1 is not only an osmosensor involving in osmotic stress signaling cascade and osmotic triggered Ca$^{2+}$ activity but also a potential shortcut to bridge with the circadian clock system. Thus, deep into the research about the OSCA1 and circadian clock interaction, especially in whole-genome RNA and protein level might help to understand the drought-resistant and circadian clock mutual beneficial mechanism.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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