Initial Review: Exploiting Recent Advances in Plant Biology to Develop Drought-Resistant Crops

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1. Introduction

1.1 The Intensifying Threat of Climate Change

Climate change remains a significant threat to human, animal and plant life. In 2023 and 2024, both global sea surface and daily mean temperatures were significantly above average, with current trends suggesting that temperature records will be surpassed more frequently in the coming years (Ripple et al., 2024). Rising temperatures are driving more intense and frequent heatwaves and droughts, which contributes to wildfires that effect land and communities. At the same time, there has been a significant increase in the severity of flooding worldwide, with extreme floods causing significant damage and displacing many people. Methane gas concentrations are also at record highs, driven in part by a growing livestock population and thawing permafrost. Furthermore, the human population has also reached record levels, with the global food demand projected to increase by 56% by 2050 (Van Dijk et al., 2021). The escalating frequency of climate disasters across the world, coupled with the pressure of growing populations, has created a crisis that will worsen if current practices persist.

In response to these challenges, one key recommendation has urged the public to move away from carbon-intensive, animal-based diets in order to reduce greenhouse gas emissions and improve overall human health (Macdiarmid., 2022). A global transition to more plant-focused diets is predicted to reduce greenhouse gases by a third, potentially save millions of lives, and lead to significant healthcare savings by 2050 (Springmann et al., 2016). This has fuelled the growing popularity of vegetarianism and veganism worldwide, as seen in the surge of participation in initiatives like “Veganuary,” which has seen a 76.6% increase in participants since 2020, with approximately 20% citing environmental concerns for their involvement (Statista., 2025).

This has placed significant pressure on agriculture to increase both the quantity and quality of crop yields. However, the most recent Agriculture and Horticulture Development Board (AHDB) Harvest Progress Report, which provides an overview of the 2024 cereal and oilseed harvests across the UK, shows a disappointing outcome. The report highlights a decline in all yields—winter and spring barley, winter oilseed rape, oats, and wheat—compared to the five-year average, with an overall decrease of

6.9%. Winter barley was the most affected, experiencing a substantial decline of 13% (AHDB., 2025). The report references Met Office data, noting that a dry start to the year, followed by an exceptionally wet end and unpredictable weather patterns across different regions, caused significant challenges to harvests throughout the country. Therefore, as climate change intensifies and the global population continues to grow, the need for resilient, high-quality and plentiful crops have never been more crucial.

1.2 The Impact of Climate Change on Plant Health and Survival

1.2.1 Drought

Thermal stress and drought, driven by climate change, are key factors contributing to plant stress and increased mortality (Ripple et al., 2024). Drought refers to a prolonged period of water scarcity, which can manifest in various forms, including meteorological drought (below-average rainfall), agricultural drought (insufficient water for crop growth), ecological drought (water shortage impacting the local environment), and hydrological drought (low water supplies in streams and reservoirs) (Michra & Sign., 2010). As previously mentioned, rising temperatures have contributed to a rise in both the severity and frequency of drought, with 48% of the world's land experiencing at least one month of extreme drought in 2023 (Romanello et al., 2024).

Plants exposed to drought conditions show stunted growth, reduced leaf water content and decreased turgor pressure, with extended periods of drought disrupting cellular processes including protein synthesis (Zia et al., 2021). Additionally, water is essential for germination, therefore, in its absence, seedlings cannot develop. In addition to its morphological effects on plants, drought can also increase plants susceptibility to pathogen infections. For instance, prolonged and frequent drought conditions can make crops more vulnerable to diseases like root rot. Additionally, drought can lead to the development of new pathogens that are capable of surviving extreme environmental conditions and can therefore take advantage of changes in the plant that occur as a response to stress (Singh et al., 2023).

1.2.2 Temperature

High Temperatures

High temperatures leading to heat stress significantly disrupt plant growth and development, with seed germination being one of the first stages impacted. Heat stress reduces germination rates across a wide range of plant species, and in extreme cases, can completely inhibit germination (Hasanuzzaman et al., 2013). Additionally, heat stress results in the overproduction and accumulation of reactive oxygen species (ROS) leading to oxidative stress which further harms the plant. The reproductive stage of plant development is particularly affected, as heat stress can inhibit flowering

and, consequently, seed production. High temperatures also cause leaf scorching, and fruit discolouration, all of which contribute to significant reduction in both yield quantity and quality (Hasanuzzaman et al., 2013). Moreover, the rise in global temperatures, coupled with the increased frequency of heatwaves, poses challenges for crops that require cold periods to develop, such as wheat, which depend on vernalisation (0-7°C) to induce flowering (AHDB., 2023). With fewer cold phases, these crops will likely struggle to achieve optimal yields.

Cold Temperatures

Cold temperatures also pose a significant threat to plant growth and development by inducing ice crystal formation in plant tissues. Additionally, chilling injuries can manifest as discoloured leaves, wilting and delayed blooming, all contributing to stunted growth and plant death (Pennisi et al., 2022). Similar to high temperatures, cold stress disrupts plant growth by hindering seed germination and establishment, increasing the accumulation of ROS, impairing photosynthesis, and impeding the reproductive phase, causing sterile pollen and aborted ovules (Soualiou et al., 2022). These effects contribute to severe reductions in crop yields and significant economic losses, with Japan having experienced over 55 billion yen in crop losses due to cold temperatures (Ambroise et al., 2020; Soualiou et al., 2022).

1.3 Plant Stress Responses

1.3.1 Drought Stress Response

Drought stress can occur suddenly or gradually, requiring plants to have effective stress response mechanisms in place in order to react appropriately. Many drought stress responses are mediated by abscisic acid (ABA), a plant hormone that controls stomatal closure and gene expression under drought conditions (Lata & Prasad., 2011). In response to drought, ABA accumulates in vascular leaf tissue and spreads throughout the plant, signalling the stomata to close and triggering changes in gene expression that enhance drought tolerance (Lata & Prasad., 2011). Similarly, ROS also accumulate during drought through the activation of respiratory burst oxidase homolog (RBOH) proteins, which produce ROS under these conditions. ROS play a crucial role in signal transduction, a process that is also observed during cold stress responses. A key component in the drought stress response are dehydration responsive element-binding (DREB) proteins (Figure 1). These transcription factors (TFs) bind to *cis*-elements in the promoter regions of target genes, modulating their expression to promote drought tolerance. Specifically, DREBs interact with dehydration-responsive elements (DREs), a mechanism also involved in the cold stress response pathway.

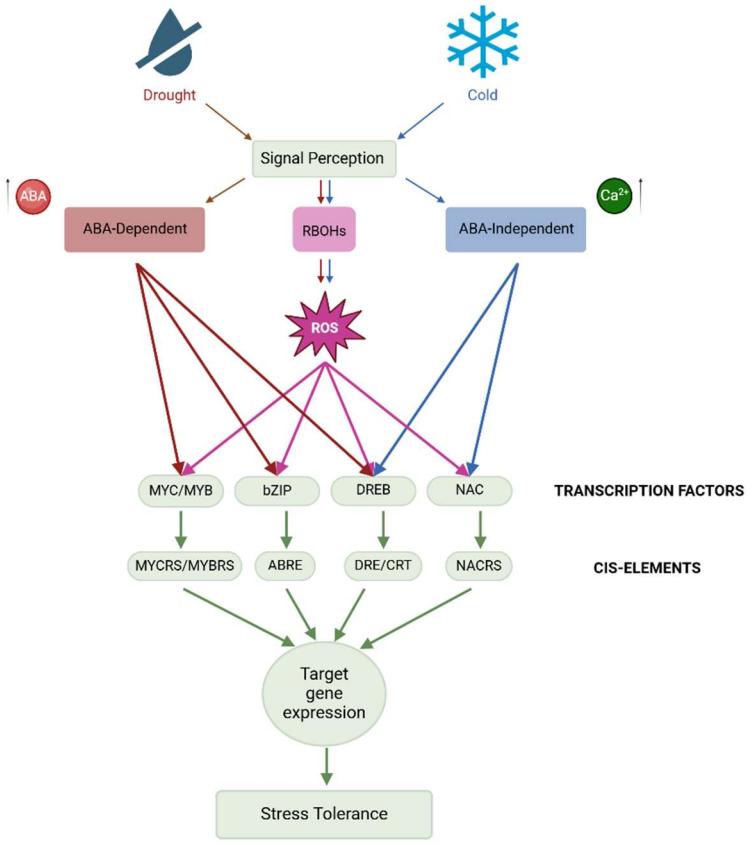

Figure 1. Shared Components in Plant Drought and Cold Stress Response Pathways. A schematic representation of stress signal perception and downstream responses in plants, leading to changes in gene expression and stress tolerance. The pathways are categorised into abscisic acid (ABA)-dependent and ABA-independent processes. While ABA plays a central role in the drought stress responses, calcium (Ca^{2+}) undertakes a similarly crucial role in the cold stress response. Both pathways involve respiratory burst oxidase homolog (RBHO) proteins, which generate reactive oxygen species (ROS) under stress conditions. Key transcription factors (TFs) involved include myelocytomatosis (**MYC**), myeloblastosis (**MYB**), basic leucine zipper (**bZIP**), dehydration-responsive element-binding proteins (**DREB**), and NAM/ATAF/CUC (**NAC**). These TFs recognise specific cis-regulatory elements in target genes, such as MYC recognition sequences (**MYCRS**), MYB recognition sequences (**MYBRS**), abscisic acid-responsive elements (**ABRE**), dehydration responsive elements (**DRE**)/C-repeat elements (**CRT**), and **NAC** recognition sequences (**NACRS**) (Adapted from Lata & Prasad., 2011).

1.3.2 Cold Stress Response

The cold stress response in plants shares similarities with the drought response, as both freezing and water-deficient soils trigger comparable effects. Cold stress is detected through a range of receptors that sense changes in membrane rigidity, metabolite levels, protein conformation, and the accumulation of ROS (Jahed et al., 2023). These changes result in the activation of calcium (Ca^{2+}) channels, leading to the influx of Ca^{2+} into the cytoplasm, where it acts as a secondary messenger to relay the stress signal, initiating a phosphorylation cascade (Jahed et al., 2023). As in the drought

stress response, the perception of low temperatures leads to the activation of RBOH and the subsequent production of ROS (Jahed et al., 2023; Miller et al., 2010). The crosstalk between Ca^{2+} and ROS ultimately regulates the expression of TFs, which control the activation of cold stress-related genes, helping the plant cope with cold conditions (Jahed et al., 2023). Similar to drought stress, DREBs and DREs play a crucial role in the cold stress response (Figure 1), highlighting the importance of DREBs as key regulators of plant responses to abiotic stresses.

As climate change intensifies and the frequency of extreme temperature and drought events rises, understanding how plants respond to these stressors is essential for improving their tolerance and reducing the risk of crop failure.

1.4 The Plant Circadian Clock

An important aspect of a plant's biology which must be considered when studying its response to stress is the circadian clock. Many environmental stresses, such as changes in temperature and light intensity, follow predictable patterns tied to day-night cycles. The circadian clock , an endogenous timekeeper operating in a repeating 24-hour cycle, enables plants to anticipate and synchronise their biological processes with these rhythmic environmental changes. This is particularly advantageous for sessile organisms like plants as it allows them to optimise resources by employing defence mechanisms at the most appropriate times (Creux & Harmer., 2019). The central oscillator of the clock consists of gene repressors and activators which operate to coordinate the expression of specific genes throughout the day.

It is widely understood that circadian gene expression varies throughout the day, with TFs sequentially accumulating to regulate the expression of target genes. Recent studies show that circadian proteins often form different complexes at specific times of day, with each complex having a unique role in influencing gene expression and, consequently, plant behaviour. These complexes can be grouped into distinct 'hubs,' each characterized by a primary circadian transcription factor that plays a key role (Figure 2).

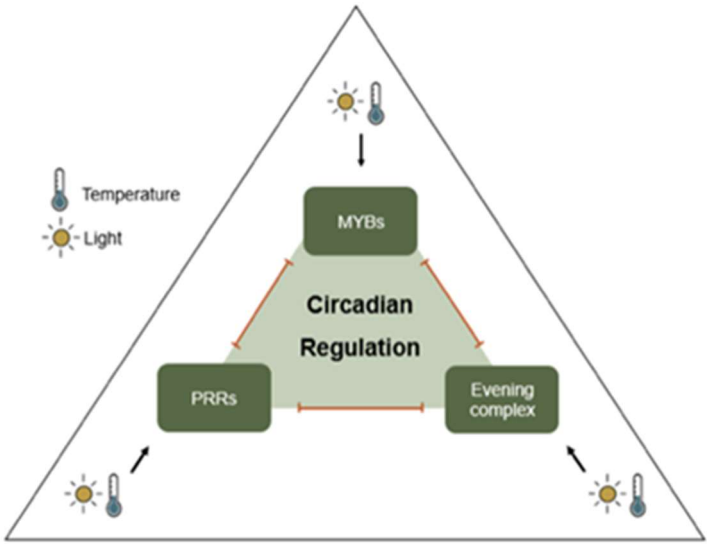

Figure 2. Environmental Signals Influence the Plant Circadian Clock. The Arabidopsis circadian clock can be organised into three main hubs: REVEILLE (RVE), PEUSDORESPONCE REGULATOR (PRRs), and the Evening Complex. These hubs consist of transcription factors that regulate promoter activity throughout the day, working together to maintain the 24-hour cycle of the clock. The activity of these hubs is modulated by environmental factors, such as temperature and light, ensuring synchronisation with external cues (Sharples et al., submitted).

1.4.1 The REVEILLE Hub

The REVEILLE (RVE) hub is composed of RVE TFs, which belong to the MYB-family, named after their conserved MYB DNA-binding motif (Pratyusha & Sarada., 2022). The well described CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) are the founding members, with the discovery of RVE1-8 coming later (Wang and Tobin, 1998; Green and Tobin, 1999; Alabadí et al., 2002; Gray et al., 2017). RVE TFs are predominantly expressed either shortly before or at dawn: *CCA1* and *LHY* have peak expression in the early morning, where they repress evening-expressed genes, while *RVE8* activity peaks in the evening, promoting expression of evening-expressed genes. Members of the RVE family have shared gene targets including *TIMING OF CAB EXPRESSION1/PSEUDORESPONSE REGULATOR1* (*TOC1/PRR1*) (Farinas and Mas., 2011; Alabadi et al., 2001; Rawat et al., 2011). The DNA-binding specificity of MYB proteins is characterised by the dimerisation of two recognition helices (Feller et al., 2011), and MYB family members can consequently function as either homodimers or heterodimers, allowing them to form complexes that can perform specific roles at specific times of day (Pireyre and Burow, 2015). Indeed, CCA1 and LHY have been shown to heterodimerise making it plausible that other RVEs could heterodimerise and too form complexes (Lu et al., 2009). While most RVE genes are well characterized, *RVE2* is less explored. However, a recent study by James et al. (2024) has provided new insights into how *RVE2* contributes to the cold response in *Arabidopsis Thaliana* (*Arabidopsis*) through cold-induced alternative splicing (AS).

1.4.2 Alternative Splicing

Splicing is the process by which non-coding regions of pre-mRNA, known as introns, are removed and coding regions, or exons, are joined together. AS occurs when these exons are spliced together in different combinations, producing multiple distinct mature mRNA isoforms from a single pre-mRNA transcript (Clancy, 2008). This process is regulated by the spliceosome, a large protein complex that recognises splice sites within the pre-mRNA, with the RNA structure itself influencing splicing by affecting the accessibility of the spliceosome to these splice sites (Warf & Berglund., 2010). Interestingly, RNA structure can be influenced by temperature, suggesting that temperature could impact splicing, a theory proposed by James et al. (2024).

1.4.3 REVEILLE2

James et al. (2024) revealed through *Arabidopsis* mutant experiments that *RVE2* undergoes rapid splicing in response to chilling, making it a potential early actor in the *Arabidopsis* cold response. Within just 20 minutes of cold exposure, *RVE2* is alternatively spliced, producing seven transcripts, with just one, FS(.1), encoding a protein. Notably, this isoform is expressed at low levels under ambient temperatures, with its expression increasing substantially following chilling. Interestingly, the extent of *RVE2* AS correlates with temperature changes, highlighting AS as a key mechanism in plant stress responses to environmental factors (James et al., 2024). Furthermore, a *rve2-2* mutant exhibited increased expression of cold-response genes, such as C-repeat binding factors (CBFs) (also known as DREBs), and reduced photosynthetic capacity. These findings suggest that the non-mutant function of RVE2 is to repress the cold response, and potentially the drought response. This highlighted RVE2 as a repressor of the cold response at night, with its role being to conserve energy by preventing unnecessary activation of the cold stress response before dawn (James et al., 2024).

2. PhD Plan

2.1 Overall Aim

Building on the work of James et al. (2024), my goal is to further investigate the molecular mechanism behind the alternative splicing of *RVE2* and its impact on plant stress response pathways, specifically in relation to cold and drought stress. In doing so, I aim to contribute to the development of strategies for optimising plant stress responses, potentially leading to the creation of more resilient crops, easing the pressures on agriculture and helping to ensure healthy, abundant yields despite the challenges posed by climate change.

2.2 RVE2 Natural Variants

Aim: Investigate whether natural variants of RVE2 obtained from different areas of the world exhibit distinct responses to cold and drought stress

Hypothesis: I hypothesise that RVE2 variants originating from warmer climates, such as Spain, will exhibit greater responsiveness to drought stress, as these variants may have adapted to frequent exposure to drought conditions. On the other hand, I hypothesise that these same variants will have a weaker response to cold stress as they are less likely to have evolved mechanisms for coping with lower temperatures.

The lab has obtained various *Arabidopsis* variants and isolated *RVE2* from each. Subsequent experiments with these variants revealed that when compared to the lab's Col-0 line, IP-Pds-1, a variant from Spain, exhibited reduced splicing strength at 12°C, making it a key focus of interest. Additionally, a synthetic RVE2 has been constructed in the lab, closely resembling that of Col-0, but with the inclusion of the 3' end of intron 1 from IP-Pds-1, which is hypothesised to interact with the spliceosome. These two variants will be used to generate stable transgenic lines which will allow us to assess whether the different RVE2 variants cause any behavioural and/or physiological changes to the plant when compared to Col-0.

To do so, restriction enzymes (EcoRI and XbaI) will be used to cut the pEZRLN 35S-FLAG-phot1 plasmid to remove phot1, producing a space in which *RVE2* variants can be inserted via Gibson Assembly, producing three new plasmids:

1. pEZRLN 35S-FLAG-IP-Pds-1RVE2
2. pEZRLN 35S-FLAG-SYNRVE2
3. pEZRLN 35S-FLAG-Col-0RVE2

Following successful Gibson Assembly, each plasmid will be transformed into *Arabidopsis* via floral dipping. Two *Arabidopsis* lines in the rve2-2 mutant background will be used for floral dipping: the CCA1::LUC line, which allows us to observe the effect of RVE2 on the circadian clock, as *CCA1* is a core clock gene, and the CBF3::LUC line, which allows us to assess the effect of RVE2 on cold- and drought-responsive genes.

2.2.1 CBF3::LUC/CCA1::LUC Reporter System

Aim: Investigate the role of RVE2 in regulating *CBF3* and *CCA1* expression during drought stress.

Hypothesis: Based on the findings of James et al (2024), I hypothesise that RVE2 activity will lead to reduced CBF3 expression and increased CCA1 expression under drought conditions, mirroring its effects observed during cold stress.

CBF3::LUC and CCA1::LUC reporter systems will be used to assess whether RVE2 influences *CBF3* and *CCA1* expression during drought stress.

To assess the role of RVE2 in regulating *CBF3* expression, I will grow wild-type (WT) and rve2 mutant *Arabidopsis* plants hydroponically. Both genotypes will contain the CBF3::LUC reporter construct, where luciferase expression is driven by the CBF3 promoter. Drought stress will be induced by adding mannitol to the hydroponic system, while well-watered WT and mutant plants will serve as controls. All plants will then be sprayed with luciferin, and luciferase activity will be measured using a luciferase camera. The level of luminescence will reflect the level of *CBF3* expression. Therefore, by comparing luminescence levels between WT and rve2 mutants under drought and control conditions, I will be able to assess whether, and to what extent, RVE2 influences *CBF3*.

The above protocol will also be performed in plants containing the CCA1::LUC reporter construct, to assess whether RVE2 also influences *CCA1* expression.

This experiment will be performed for all the RVE2 variants outlined in 2.2.

2.2.2 The Spliceosome and RVE2 Natural Variants

Aim: Characterise the *Arabidopsis* spliceosome and investigate how cold affects mRNA folding.

Hypothesis: I hypothesis that cold temperatures alter the folding of mRNA, which in turn affects the accessibility of the spliceosome to its target sites. This, in turn, promotes alternative splicing of mRNA transcripts.

Surprisingly, the structure of the *Arabidopsis* spliceosome is not yet known, with researchers generally assuming it resembles that of yeast. Through collaboration with Pennsylvania State University, I aim to characterise the *Arabidopsis* spliceosome and explore how it interacts with RVE2. The current

hypothesis suggests that, in response to cold, RVE2 mRNA undergoes structural changes, exposing different regions to the spliceosome, which leads to the production of a new isoform. Additionally, it would be interesting to explore whether the mRNA folding of each RVE2 variant differs at varying temperatures. Understanding the spliceosome structure would enable me to investigate this idea further.

2.3 Constitutively Splice Inducible RVE2

Aim: Directly assess the influence of RVE2 on gene expression.

Hypothesis: I hypothesise that constitutive splicing of *RVE2* will be sufficient to initiate RVE2-mediated signaling in the absence of chilling/drought.

A constitutively spliced version of RVE2 has been generated in the lab and inserted into the pER8 plasmid. This variant is designed to be induced upon exposure to estradiol, providing precise control over *RVE2* expression and enabling direct assessment of its effect on gene expression. While the effect of RVE2 on gene expression has primarily been studied in response to stress, as stress appears necessary for RVE2 activation via alternative splicing, the constitutively spliced RVE2 form allows us to have precise control over its activation, and therefore an accurate assessment of its effect on gene expression.

To identify homozygous lines, hygromycin screens will be conducted. Subsequently, qPCR will be performed on seedlings before estradiol application to confirm that RVE2 is not constitutively expressed. Once confirmed, all lines will be treated with 30 µM estradiol to induce *RVE2* expression. Samples will be collected from each line after 1 and 8 hours, and qPCR will be performed to verify *RVE2* expression. Following this, RNA sequencing (RNA-seq) will be conducted to determine which genes are upregulated or downregulated when RVE2 is active.

2.4 Transient Expression Systems

2.4.1 *Arabidopsis* RVE2

Aim: Investigate whether *RVE2* is alternative spliced in response to drought.

Hypothesis: Like in response to cold temperatures, *RVE2* is alternatively spliced in response to drought.

Following on from my previous project, where I demonstrated that *RVE2* is alternatively spliced in response to chilling using a dual-luciferase reporter system, I aim to assess whether the same alternative splicing event occurs in response to drought.

To do this, *RVE2* has been inserted via Gibson Assembly into the pGREAT2 plasmid, which contains both red and green luciferase. Green luciferase is expressed only when *RVE2* is alternatively spliced, while red luciferase serves as a control. This plasmid, containing *RVE2*, will be transformed into Agrobacterium for infiltration into the leaves of *Nicotiana benthamiana* plants. A luciferase camera will be used to monitor luciferase activity over a 4-day period, during which the leaves are exposed to drought conditions. The detection of a strong luciferase signal in response to drought would indicate that *RVE2* is alternatively spliced in response to this stress.

2.4.2 Barley RVE2 Homologs

Aim: Investigate whether *RVE2* is alternatively spliced in a RVE2 barley homolog in response to cold and drought stress.

Hypothesis: Barley *RVE2* is alternatively spliced in response to cold and drought stress

Through collaboration with the James Hutton Institute, potential homologs of the RVE family have been identified in barley. My goal is to identify a barley RVE2 homolog in which the experiment described in section 2.4.1 can be conducted, to determine whether barley RVE2 behaves similarly to its *Arabidopsis* counterpart.

3. Additional Experiments

3.1 Intelligent Growth Solutions (IGS)

I will continue to work with IGS on a distinct project to translate my findings into the indoor farming sector.

References

Agriculture and Horticulture Development Board (AHDB), 2025. *AHDB harvest progress*. Available at: https://ahdb.org.uk/cereals-oilseeds/gb-harvest-progress [Accessed 06/01/25].

AHDB Cereals & Oilseeds (2023). Wheat Growth Guide [online]. Agriculture and Horticulture Development Board. Available at: Wheat growth guide (2023).pdf. [Accessed 09.10.23].

Alabadí, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Más, P. and Kay, S.A., 2001. Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science*, *293*(5531), pp.880-883.

Ambroise, V., Legay, S., Guerriero, G., Hausman, J.F., Cuypers, A. and Sergeant, K., 2020. The roots of plant frost hardiness and tolerance. *Plant and Cell Physiology*, *61*(1), pp.3-20.

Clancy, S., 2008. RNA splicing: introns, exons and spliceosome. *Nature Education*, *1*(1), p.31.

Creux, N. and Harmer, S., 2019. Circadian rhythms in plants. *Cold Spring Harbor Perspectives in Biology*, *11*(9), p.a034611.

Farinas, B. and Mas, P., 2011. Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. *The Plant Journal*, *66*(2), pp.318-329.

Feller, A., Machemer, K., Braun, E.L. and Grotewold, E., 2011. Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *The plant journal*, *66*(1), pp.94-116.

Gray, J.A., Shalit-Kaneh, A., Chu, D.N., Hsu, P.Y. and Harmer, S.L., 2017. The REVEILLE clock genes inhibit growth of juvenile and adult plants by control of cell size. *Plant Physiology*, *173*(4), pp.2308-2322.

Green, R.M. and Tobin, E.M., 1999. Loss of the circadian clock-associated protein 1 in Arabidopsis results in altered clock-regulated gene expression. *Proceedings of the National Academy of Sciences*, *96*(7), pp.4176-4179.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Roychowdhury, R. and Fujita, M., 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences*, *14*(5), pp.9643-9684.

Jahed, K.R., Saini, A.K. and Sherif, S.M., 2023. Coping with the cold: unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. *Frontiers in Plant Science*, *14*, p.1246093.

James, A.B., Sharples, C., Laird, J., Armstrong, E.M., Guo, W., Tzioutziou, N., Zhang, R., Brown, J.W., Nimmo, H.G. and Jones, M.A., 2024. REVEILLE2 Thermosensitive Splicing: A Molecular Basis for the Integration of Nocturnal Temperature Information by the Arabidopsis Circadian Clock. bioRxiv, pp.2023-04.

Lata, C. and Prasad, M., 2011. Role of DREBs in regulation of abiotic stress responses in plants. *Journal of experimental botany*, *62*(14), pp.4731-4748.

Lu, S.X., Knowles, S.M., Andronis, C., Ong, M.S. and Tobin, E.M., 2009. CIRCADIAN CLOCK ASSOCIATED1 and LATE ELONGATED HYPOCOTYL function synergistically in the circadian clock of Arabidopsis. *Plant physiology*, *150*(2), pp.834-843.

Macdiarmid, J.I., 2022. The food system and climate change: are plant-based diets becoming unhealthy and less environmentally sustainable?. *Proceedings of the Nutrition Society*, *81*(2), pp.162-167.

Mishra, A.K. and Singh, V.P., 2010. A review of drought concepts. *Journal of hydrology*, *391*(1-2), pp.202-216.

Pennisi, B., Thomas, P. and Stalknecht, E., 2022. *Effects of Low Temperature on Plants*. University of Georgia Cooperative Extension. Available at: B 1467_2.PDF [Accessed 06/01/25].

Pireyre, M. and Burow, M., 2015. Regulation of MYB and bHLH transcription factors: a glance at the protein level. *Molecular Plant*, *8*(3), pp.378-388.

Pratyusha, D.S. and Sarada, D.V., 2022. MYB transcription factors—master regulators of phenylpropanoid biosynthesis and diverse developmental and stress responses. *Plant Cell Reports*, *41*(12), pp.2245-2260.

Rawat, R., Takahashi, N., Hsu, P.Y., Jones, M.A., Schwartz, J., Salemi, M.R., Phinney, B.S. and Harmer, S.L., 2011. REVEILLE8 and PSEUDO-REPONSE REGULATOR5 form a negative feedback loop within the Arabidopsis circadian clock. *PLoS genetics*, *7*(3), p.e1001350.

Ripple, W.J., Wolf, C., Gregg, J.W., Rockström, J., Mann, M.E., Oreskes, N., Lenton, T.M., Rahmstorf, S., Newsome, T.M., Xu, C. and Svenning, J.C., 2024. The 2024 state of the climate report: Perilous times on planet Earth. *BioScience*, *74*(12), pp.812-824.

Romanello, M., Walawender, M., Hsu, S.C., Moskeland, A., Palmeiro-Silva, Y., Scamman, D., Ali, Z., Ameli, N., Angelova, D., Ayeb-Karlsson, S. and Basart, S., 2024. The 2024 report of the Lancet

Countdown on health and climate change: facing record-breaking threats from delayed action. *The Lancet*, *404*(10465), pp.1847-1896.

Sharples, C., McFarlane, Z.G., Fernander Pinheiro, M. and Jones, M.A., submitted. Temperature-Dependent Alternative Splicing Re-Configures the Arabidopsis Circadian System.

Singh, B.K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J.E., Liu, H. and Trivedi, P., 2023. Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, *21*(10), pp.640-656.

Soualiou, S., Duan, F., Li, X. and Zhou, W., 2022. Crop production under cold stress: An understanding of plant responses, acclimation processes, and management strategies. *Plant Physiology and Biochemistry*, *190*, pp.47-61.

Springmann, M., Godfray, H.C.J., Rayner, M. and Scarborough, P., 2016. Analysis and valuation of the health and climate change cobenefits of dietary change. *Proceedings of the National Academy of Sciences*, *113*(15), pp.4146-4151.

Statista, 2025. *Number of people participating in Veganuary worldwide*. Available at: Number of people participating in Veganuary 2023 | Statista [Accessed 06/01/25].

Statista, 2025. *Top motivation for Veganuary* 2023. Available at: Top motivations for Veganuary 2023 | Statista [Accessed 06/01/25].

Van Dijk, M., Morley, T., Rau, M.L. and Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*, *2*(7), pp.494-501.

Wang, Z.Y. and Tobin, E.M., 1998. Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. *Cell*, *93*(7), pp.1207-1217.

Warf, M.B. and Berglund, J.A., 2010. Role of RNA structure in regulating pre-mRNA splicing. *Trends in biochemical sciences*, *35*(3), pp.169-178.

Zia, R., Nawaz, M.S., Siddique, M.J., Hakim, S. and Imran, A., 2021. Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiological research*, *242*, p.126626.