www.ThePharmaJournal.com

The Pharma Innovation

Koppula Satya Sai Kumar
M.Sc., Ag., Division of Genetics and
Plant Breeding, IARI – Indian
Institute of Agricultural
Biotechnology, Ranchi, Jharkhand,
India

Shikha Jain
Ph.D. Research Scholar, Division of
Fruits and Horticultural Technology,
ICAR-Indian Agricultural Research
Institute, New Delhi, India

Ankit Raj
M.Sc., Ag., Division of Molecular
Biology and Biotechnology, IARI –
Indian Institute of Agricultural
Biotechnology, Ranchi, Jharkhand,
India

Sudheer Bishnoi
M.Sc., Ag., Division of Molecular
Biology and Biotechnology, IARI –
Indian Institute of Agricultural
Biotechnology, Ranchi, Jharkhand,
India

Pratik Prasad Singh
M.Sc., Ag., Division of Molecular
Biology and Biotechnology, IARI –
Indian Institute of Agricultural
Biotechnology, Ranchi, Jharkhand,
India

Eshwar Sai Prasad
M.Sc., Ag., Division of Genetics and
Plant Breeding, IARI – National
Institute of Biotic Stress
Management, Raipur, Chhattisgarh,
India

Shikha Saini
Ph.D. Research Scholar, Division of
Fruits and Horticultural Technology,
ICAR-Indian Agricultural Research
Institute, New Delhi, India

Poonam Maurya
Ph.D. Research Scholar, Division of
Fruits and Horticultural Technology,
ICAR-Indian Agricultural Research
Institute, New Delhi, India

Corresponding Author:
Shikha Saini
Ph.D. Research Scholar, Division of
Fruits and Horticultural Technology,
ICAR-Indian Agricultural Research
Institute, New Delhi, India

Circadian model of heterosis

Koppula Satya Sai Kumar, Shikha Jain, Ankit Raj, Sudheer Bishnoi, Pratik Prasad Singh, Eshwar Sai Prasad, Shikha Saini and Poonam Maurya

Abstract

Heterosis has been widely exploited in plants and animals, and also revolutionized agriculture by improving important agronomic traits. However, the molecular mechanism is still remaining elusive. Circadian clocks are endogenous timers that enable plants to synchronize biological processes with daily and seasonal environmental conditions in order to allocate resources during the most beneficial times of day and year. The circadian clock integrates external signals such as temperature with internal temporal processes to generate robust rhythms. The circadian clock regulates a number of central plant activities, including growth, development, and reproduction, primarily through controlling a substantial proportion of transcriptional activity and protein function. Hybrids had higher growth and vigor under altered amplitude of expression peaks of circadian clock genes but maintaining the clock period. Down regulation of clock repressor genes, such as CCA1 during day time, up regulate the output pathways of photosynthesis and carbohydrate metabolism, which results in higher accumulation of chlorophylls, starch and sugars. Emerging insights into circadian clock regulation of fundamental plant processes, including responses to abiotic and biotic stresses, are discussed to highlight promising avenues for further crop improvement. Expression of some stress-responsive genes is induced in hybrids under stress conditions which helps in balancing the tradeoff between stress responses and heterosis. Hybrids generated from parents with greater expression differences in stress-responsive genes most often expressed higher vigor than parents with smaller expression differences. The evolving epigenetic and genomic field put forward the role of interaction of alleles from different parental genomes in reprogramming the genes involved in stress tolerance, fitness and growth of hybrids. The level of methylation of genomic DNA in hybrids was found to downregulate the circadian-regulated stress-responsive genes. Furthermore, epigenetic modifications of the circadian clock genes and their reciprocal regulators were reported to regulate the expression of downstream genes and pathways leading to more product accumulation in hybrids.

Keywords: Circadian clock, heterosis, hybrids, methylation, regulation

Introduction

Crops are the primary source of energy and food for humans and animals. Human evolution is associated with crop domestication and improvement. With the ever-increasing population in the world coupled with climate change, it is challenging to serve the people with food produced in the available arable land from existing varieties. It is predicted that crop yield has to increase 100–110% between 2005 and 2050 to meet the global demand. The food crisis can be tackled by increasing the productivity per unit of land. The most successful and widely used tool to overcome this challenge is the deployment of hybrids. Hybrids are benefitted from a phenomenon called "Heterosis," wherein the hybrid progeny has superior performance compared to parental lines. Heterosis is agronomically significant because the superior performance can appear as grain yield in food crops, and biomass heterosis in vegetable and energy crops, besides biotic and abiotic stress tolerance. However, the molecular basis for heterosis stays a mystery, and traditional dominance and overdominance genetic models cannot adequately explain heterosis in yeast and many crop plants. One possible mechanism that promotes heterosis is the circadian clock, that influences almost all aspects of plant biology and confers a significant selective advantage (Das *et al.,* 2021) [4]. Many metabolic genes are under circadian control, indicating that clock significantly affects plant biochemistry. The clock also controls physiological processes such as leaf gas exchange, with stomata being more open during the subjective day than subjective night when plants are grown under continuous light.

To satisfy human requirements, breeders should develop crop cultivars that withstand adverse climatic conditions, fully utilize the light and temperature resources in the planting zones and achieve higher grain yields. There are several major cereal crops with varying photoperiod requirements in the world. Rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are short-day crops, while wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are long-day crops. They have different responses to environmental changes in day length and temperature; hence, photoperiod and vernalization have diverse impacts on flowering time for long- and short-day crops. Plants integrate the circadian clock and external signals such as temperature and photoperiod to synchronize flowering with seasonal environmental changes. This process makes cereal crops adapt to varied growth zones from temperate to tropical regions with better performance and stress tolerance (Liu *et al.,* 2020) [9].

Heterosis
Hybrids are formed by cross-fertilization between different strains, races or species of plants or animals. Joseph Koelreuter (1733–1806) recorded that some tobacco hybrids exceeded their parents in growth vigour. A century later, in 1876,

Charles Darwin systematically characterized growth patterns and concluded that "the crossed plants when fully grown were plainly taller and more vigorous than the self- fertilised ones." George Harrison Shull introduced the term "heterosis" in 1914. It is now well known that hybrid plants, such as maize, or hybrid animals, such as some dogs, grow more vigorously and adaptively than their parent (Hochholdinger and Baldauf, 2018) [7] (Figure 1).
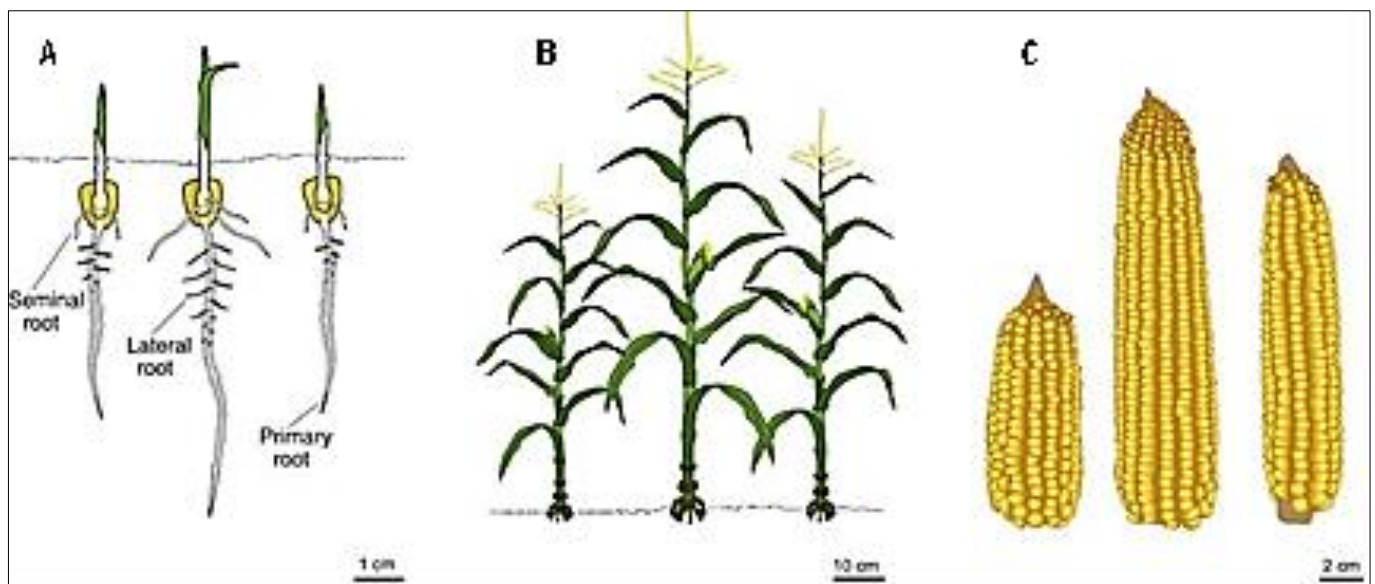

Fig 1: Manifestation of heterosis with respect to different phenotypic traits of maize. Heterosis is observed for (A) seedling root traits, such as lateral- root density, primary-root length and seminal-root number, (B) plant height and (C) cob size of the F1-hybrid offspring (center) in comparison to both parental inbred lines (left and right).

Heterosis is defined as "The increased vigour, size, fruitfulness, speed of development, resistance to disease and pests manifested in cross-bred organisms as compared to corresponding inbred as the specific result of unlikeliness in the constitution of uniting parental gametes (Shull, 1948) [14]."

Circadian rhythm
Any biological process be it metabolic, physiological, or behavioral process that repeats itself over a period of approximately 24 hours and maintains this rhythm even in the absence of external stimuli is called a Circadian rhythm.

History of plant circadian biology
The first ever report of biological rhythm was documented by French biologist Jean-Jacques d'Ortous de Mairan in his paper 'Observation botanique.' He mentioned that leaves and peduncles of heliotropic plant *Mimosa pudica* (Touch me not plant) fold and open in response to sunlight in the same way it responds to touch. Later in 1880 Charles Darwin in his book, "The power of movement in plants" discussed about sleep movements in Medicago. He mentioned that leaves of *Medicago marina* are awake during day and asleep at night (Hubbard and Antony, 2016) [8] (Figure 2).
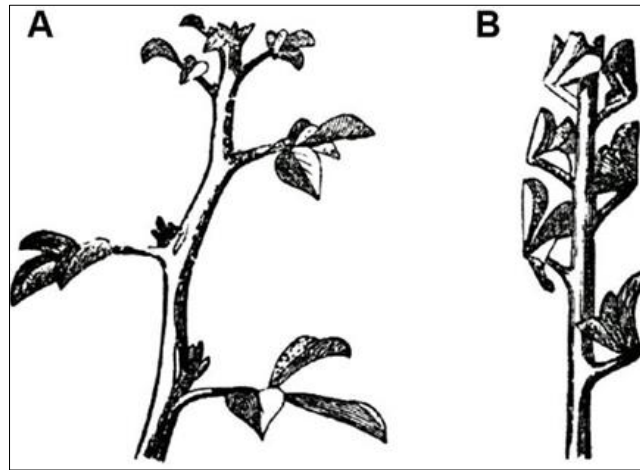
Fig 2: *Medicago marina*: (A) Leaves during the day, (B) Leaves asleep at night

Measurable properties of circadian rhythms
The circadian rhythm attributes will vary depending on the activity of the circadian clock, so monitoring them is useful. Circadian rhythms may adopt a period that is longer or shorter than 24 h, for instance, if genes encoding certain components of the circadian oscillator are altered. Similar to this, modifications to other aspects of the circadian clock can alter its amplitude or phase. The identification of the circadian clock's fundamental elements, the knowledge of their interactions, and the knowledge of which aspects of plant physiology are governed by each component have all benefited significantly from measuring these features (McClung, 2006) [10].
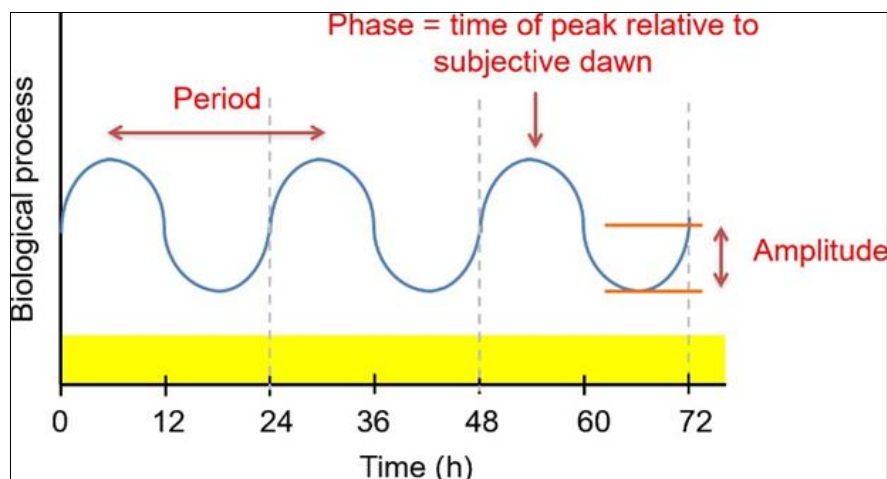

Fig 3: Critical terms used to describe circadian rhythms

- Phase = Time of day at any given event.
- Period = Time taken to complete one full cycle (Crest to Crest /Trough to Trough)
- Amplitude = One-half the Crest-to-trough distance.
- By convention, when sun rises in dawn, it is ZT0 and when sun sets at dusk, it is ZT12

Characteristics of circadian rhythms
- Circadian rhythms are subset of biological rhythms with a period of approximately 24 h.
- The rhythm is endogenously generated and self-sustaining.
- The rhythms are entrainable.
- The rhythms exhibit temperature compensation.

Subset of biological rhythm
A biological rhythm is a periodic variation in organism's function whose period varies from seconds as seen in heartrate or pulse rate to months or years as seen in annual breeding cycles, mating cycles. Circadian rhythm is also biological rhythms with a period of approximately 24 hrs. This period is maintained by the genes and proteins of the "circadian oscillator".

Endogenous, self-sustaining
Circadian rhythms are self-sustaining in the absence of signals from the environment. The rhythms are repeated in anticipation of environmental input, rather than simply reacting to those inputs. The plant is typically grown for a period of time under cycles of day and night and is then transferred to constant conditions. Under constant conditions, the circadian clock is said to "free run," and the experimental conditions are sometimes called "free-running conditions." The plant's leaves open and close at the exact times of the day regardless of external inputs, even if the rhythmic inputs from the environment are removed (Figure 4). The fact that rhythms continue in conditions of constant temperature and light (or darkness) indicates that an intrinsic biological process generates these rhythms (Hubbard and Antony, 2016) [8].
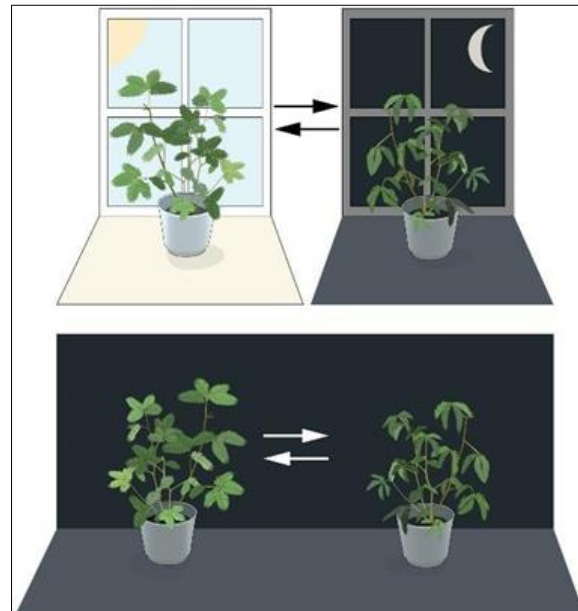
Fig 4: An internal biological clock.

Entrainable

The time of sunrise and sunset vary throughout the year. In order to efficiently utilize the light period, plants have to modify their internal clock such that internal metabolic/physiological processes are in sync with the sun rise or sunset. If the circadian clock is not resynchronized with the environment every day, it would not accurately anticipate time for the plant because circadian clocks never have a period of exactly 24 hours.

A rhythm can be reset/adjusted to external environment by a signal. The property of synchronising internal clock to external environmental cue is called "Entrainment." The signal that brings this synchronization is called Zeitgeber. A zeitgeber can be red light/ blue light/ sugars/ temperature fluctuations. The two chief entraining stimuli that synchronize the endogenous clock with the exogenous temporal environment are light and temperature (McClung, 2006) [10].

Temperature compensation

It is well known that rate of reaction varies with temperatures.

But, unlike other chemical reactions, the output of the clock system remains relatively constant despite fluctuations in ambient temperature, this phenomenon is called temperature compensation (Figure 5 A). Temperature compensation does not mean that the clock is temperature independent instead, it exhibits less than expected temperature dependency.

The gene FBH1 has role in temperature compensation. Despite changes in temperature from 22 -28°C, wild type maintains relatively same period. However, when FBH1-overexpressing lines are transferred to 28°C, it was found that the period of *CCA1* shortens by ∼1h (Figure 5 B). These results suggest that FBH1 overexpression at elevated temperature affects *CCA1* expression to a degree that alters the ability of the clock to compensate at warm temperatures, thus changing the pace of the clock by causing a significant shortening of the *CCA1* period (Nagel *et al.,* 2014) [12]. This proves that circadian clock buffers against temperature fluctuations.
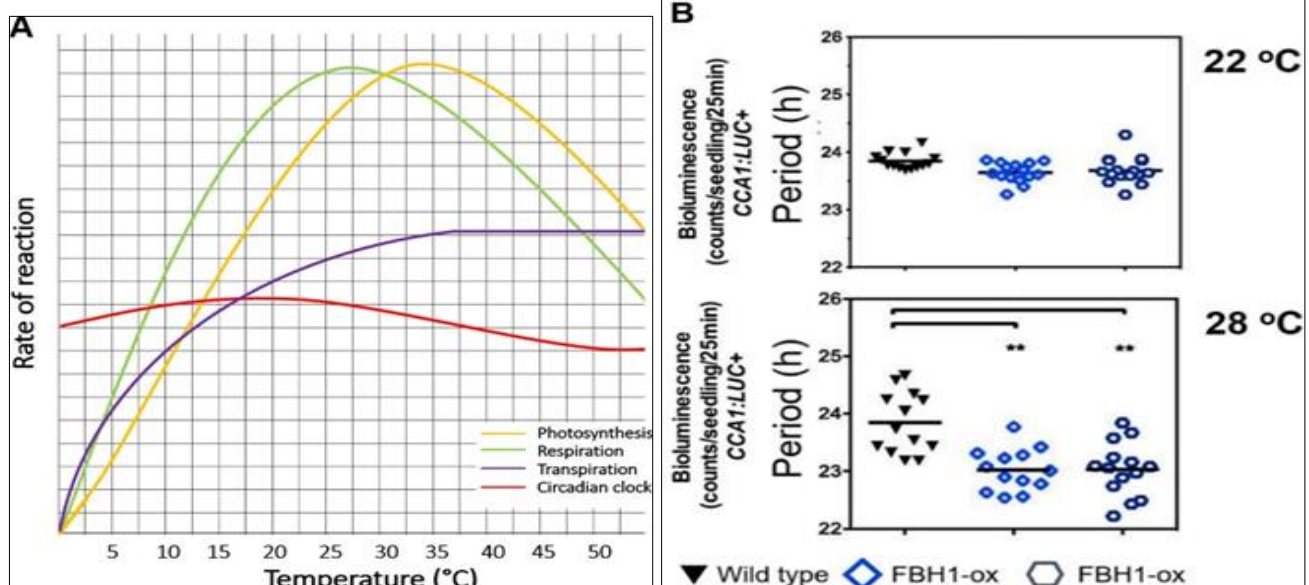

Fig 5: (A) Rate of reactions of plant growth processes and circadian clock (B) *CCA1* period at 22 °C and 28 °C

Circadian oscillator

The Circadian oscillators are the results of transcription-translation feedback loops. Consider two genes, A and B whose proteins make up a basic oscillator. In this case, the genes control one another cyclically by activating one another and repressing the other (Figure 6 A). Gene A is expressed throughout the day, and the protein it generates acts as an inducer for gene B. Therefore, gene B is activated and expressed later in the day once a specific quantity of protein A has accumulated. But protein B inhibits gene A. So when protein B increases, gene A is shut down and protein A levels fall at night. Since protein A is necessary for gene B activation, gene B becomes inactive as protein A levels drop. Consequently, protein B's inhibition of gene A is removed, and the next morning, gene A's expression shoots up yet again. The cycle is finished in 24 hours because of the biological kinetics of these reactions, which add rate constants and delays the oscillator. Thus, transcript abundance of both genes is ensured (Figure 6 B) (Hubbard and Antony, 2016) [8].
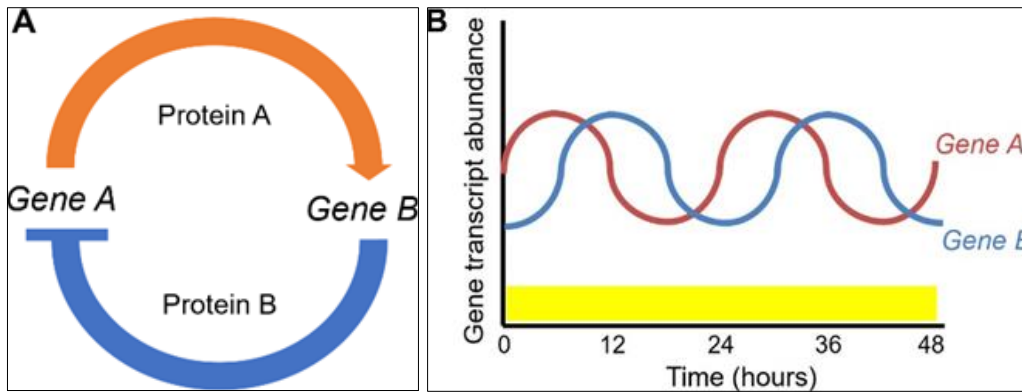

Fig 6: (A) Transcription-Translation feedback loop (B) Transcript abundance of gene A and gene B

Circadian gating

Circadian gating is the mechanism by which the clock acts as a valve on the plant's response to the environment so that the same environmental cue causes a different strength response depending on the time of day. In essence, this implies that the intensity of the circadian rhythm varies in response to an identical stimulus when applied at various times of the day. For a similar environmental cue, at some times of day, the gate is open, and the signal passes through, while other times, the gate is closed and cannot pass through (Figure 7). If the circadian clock responded identically to light at every time of day, it would be reset to dawn continuously and be unable to provide a measure of time. The clock regulates its sensitivity to light, so how it responds to light depends on the time of day. Adjusting sensitivity to light throughout the day helps plants in the natural environment since light intensity changes significantly during the day owing to cloud cover or shading by other plants (Hubbard and Antony, 2016) [8].
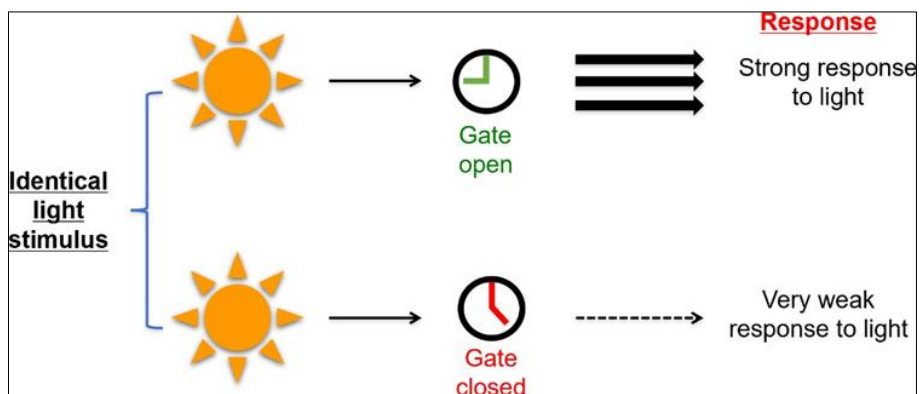

Fig 7: Circadian gating

Interconnected parts of the circadian system

Mechanisms must exist to link the circadian clock to aspects of the plant that have circadian rhythms. These are "output pathways" as they communicate temporal information from the oscillator to other cell parts. Entrainment pathways synchronize the oscillator with the external time of day so that the clock stays accurate. The circadian oscillator responds to external cues (Zeitgebers) through entrainment pathways and regulates transcription, physiology, and biochemistry rhythms through output pathways (Figure 8). Circadian gating regulates both the entrainment and output pathways. Output pathways impacts various physiological and biochemical activities, including chlorophyll synthesis, starch metabolism, sugar translocation, blooming date, and stress response genes (Hubbard and Antony, 2016) [8].
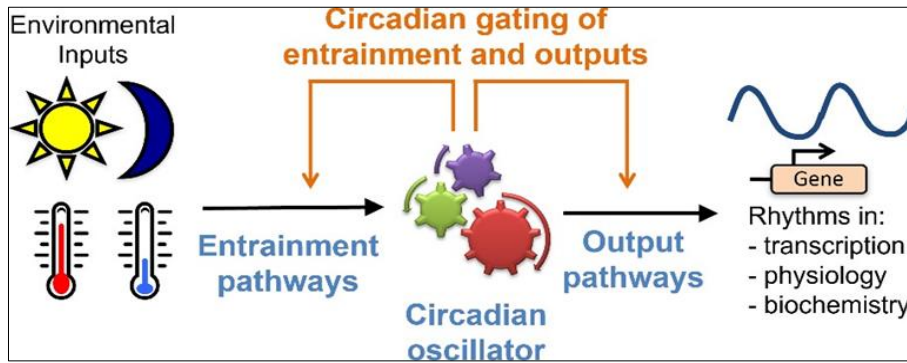
Fig 8: Components of Circadian clock

Plant clock mechanism
During the day, the circadian clock genes, *CCA1* (CIRCADIAN CLOCK-ASSOCIATED 1) and *LHY* (LATE ELONGATED HYPOCOTYL) are transcribed and translated into transcription factors. These transcription factors regulate genes containing EE to repress gene transcription. They bind to the EE (evening element) of the promoter region of the *TOC1* (TIMING OF CAB EXPRESSION 1) gene, a repressor of clock genes and other evening-phased genes in the nucleus, inhibiting their expression during the day. During the night,

the clock genes are not transcribed anymore. Consequently, the downstream genes are no longer repressed. *TOC1* gene is expressed during the night to produce *TOC1* protein that, in turn, repress clock genes. In the late night, the quantity of *TOC1* protein decreases gradually due to ubiquitination by ZTL, which leads to *TOC1* degradation. As a result, *LHY* and *CCA1* are no longer repressed by the end of the night and turned on by the beginning of the day. PHYB connects red-light signalling with the circadian clock (Figure 9) (McClung, 2006) [10].
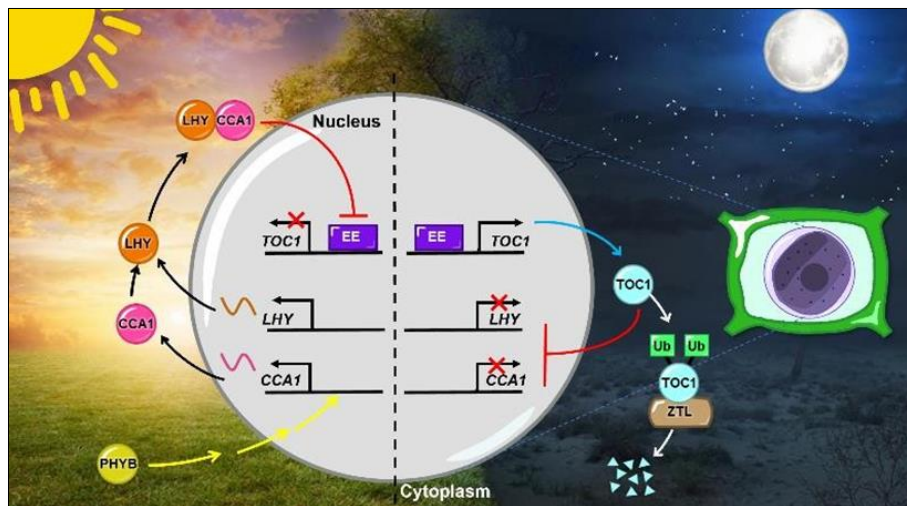

Fig 9: Plant clock mechanism

Models for altered clock gene expression
Altered amplitude of clock expression
Both the circadian period and the circadian amplitude affect growth vigour in plants. Maintaining the clock period is essential for overall metabolism, growth, and development. Altering the amplitude of expression peaks of circadian clock genes while maintaining the clock period leads to growth vigour in *Arabidopsis* hybrids (Figure 10 A). In hybrids, clock genes such as *CCA1* are downregulated during the day, which promotes the upregulation of output pathways in photosynthesis and carbohydrate metabolism and the accumulation of chlorophylls, starch and sugars. As a result, more sugar and starch accumulate during the day, so more can be used and degraded at night to promote growth. In the morning, the residual amount of starch that remains can correlate negatively with growth (Chen, 2013) [2]. The positive correlation of daytime starch accumulation and the negative correlation of morning residual starch levels with growth is not contradictory. Altered expression peaks of circadian clock genes in hybrids might also affect other

biological pathways including stress-responsive and phytohormone signalling genes.

Chromatin regulation of clock gene expression peaks
It is speculated that chromatin modifications of maternal and paternal alleles are altered in hybrids, thus leading to the repression or activation of clock regulators that in turn alter the expression of downstream genes (Chen, 2013) [2]. In *Arabidopsis* allotetraploids, the expression peaks of *CCA1* and *LHY* and of their regulators *TOC1* and GI are altered relative to the progenitors and are positively associated with levels of histone H3 lysine 9 acetylation (H3K9ac) and H3K4 dimethylation. In *A. thaliana*, the circadian oscillation in gene expression is paralleled by oscillation in H3 acetylation (H3K56ac and H3K9ac) and methylation (H3K4me3 and H3K4me2). These data suggest a crucial role for histone modifications in the transition from peak to trough in circadian oscillation. *CCA1* promoter- binding factors including *TOC1* could recruit chromatin factors including histone acetyltransferases (HATs), histone deacetylases

(HDACs), and DNA methylation (MET1, a DNA methyltransferase for maintenance methylation and DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) engages in de novo DNA methylation) or histone methylation factors (AXTR3, a histone methyltransferase for histone H3 lysine 4 trimethylation (H3K4me3)). Similarly, Small interfering

RNAs (siRNAs), bind to ARGONAUTE4 (AGO4, component in RNA- directed DNA methylation (RdDM) pathway), and guide DNA methylation and heterochromatin formation. This recruitment could remodel chromatin structure, thus leading to active chromatin (*CCA1* activation) or repressive chromatin (*CCA1* repression) (Figure 10 B).
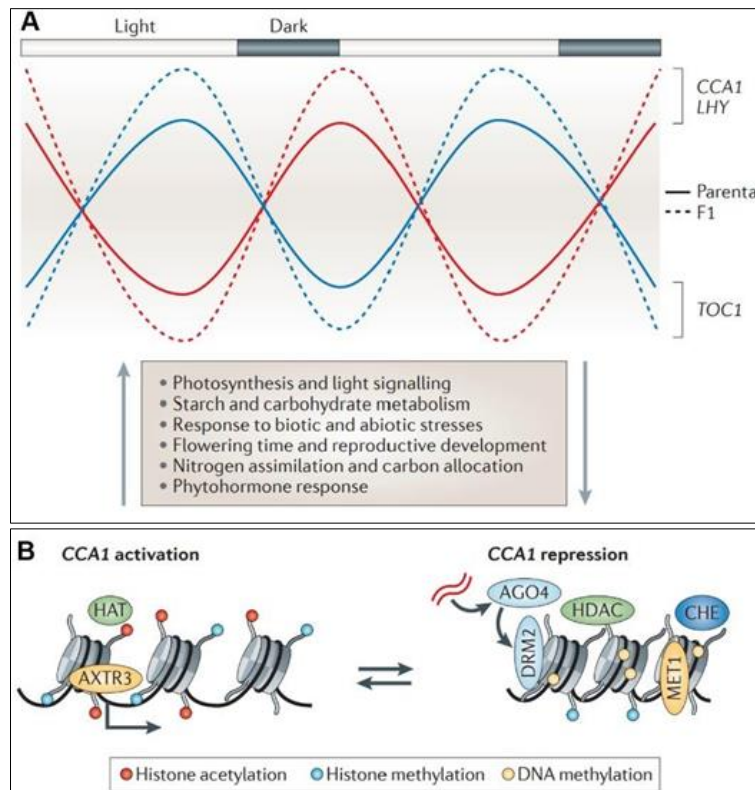

Fig 10: Models for altered clock gene expression. (A) Altered amplitudes of clock gene expression peaks in the hybrids relative to the parents, leading to activation or repression of downstream biological pathways or regulatory networks. (B) Histone modifications of clock genes.

Circadian clock control over plant metabolism

The first circadian transcriptome studies revealed extensive control of metabolism by the circadian oscillator. This oscillator regulates the transcription of a large number of enzymes of primary metabolism, including chlorophyll biosynthesis, photosynthetic electron transport, starch synthesis and degradation, and nitrogen and sulfur

assimilation (Figure 11) (Farre´ and Weise, 2012) [6]. In many cases, the peak in RNA abundance precedes that of the actual physiological process that the enzyme is involved in; for example, the expression of chlorophyll biosynthesis genes occurs at the end of the night, anticipating the onset of dawn. By contrast, the peak expression of genes associated with starch.
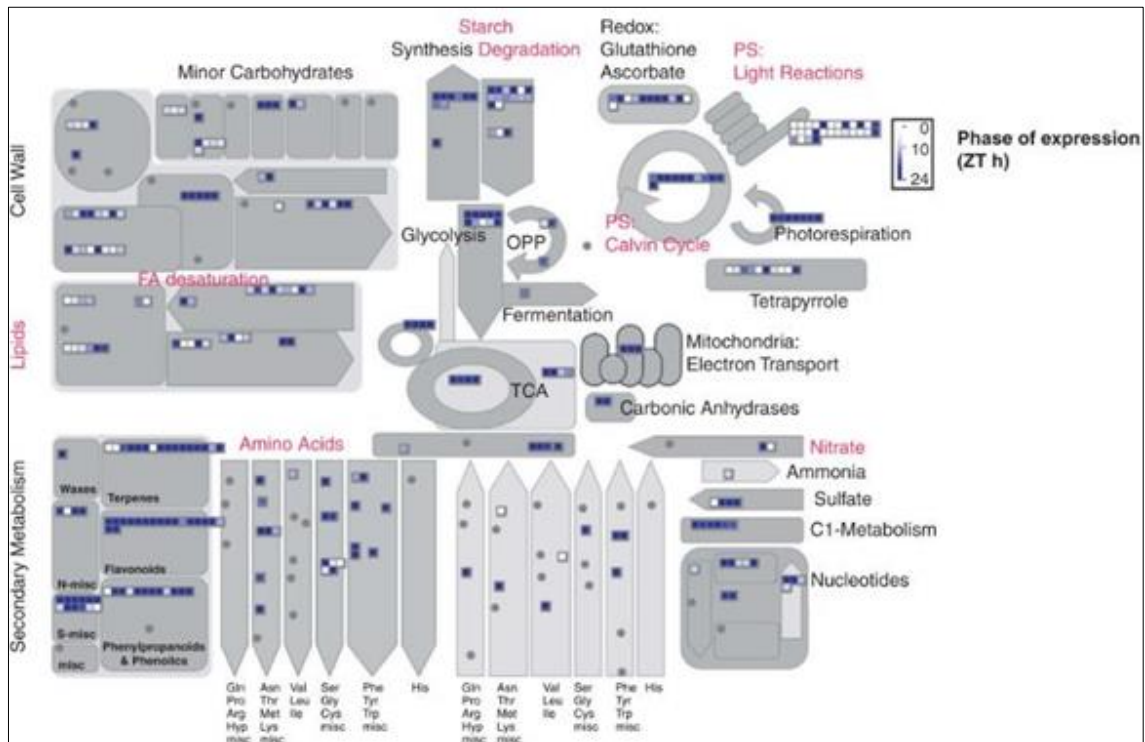
Fig 11: Circadian clock regulating transcript amounts of numerous enzymes involved in plant primary metabolism catabolism occurs around subjective dusk.

Altered metabolite levels of clock mutants in light-dark cycles

Metabolite concentrations in circadian mutants differ from those in wild-type plants, indicating a link between the circadian oscillator and metabolism. A detailed metabolomic analysis using a triple mutant, d975, clearly demonstrated that *PRR*9/7/5 is involved in maintaining mitochondrial homeostasis in *Arabidopsis*. Here, a robust link between the function of *PRR*9/7/5 and mitochondrial metabolism was detected by transcriptomic and metabolomic analyses. The mutant d975 was characterized by a dramatic increase in TCA cycle intermediates (e.g., malate, fumarate) and antioxidant vitamins in addition to osmolytes, whereas no drastic changes occurred in these metabolites in *CCA1*-ox. The triple mutants also have elevated levels of shikimate, which is a precursor molecule for many secondary metabolites. These results suggest the presence of a *PRR*9/7/5-mediated mechanism for cooperative control, through which appropriate metabolite levels may be maintained, especially those in the TCA cycle (Fukushima *et al.,* 2009) [5].
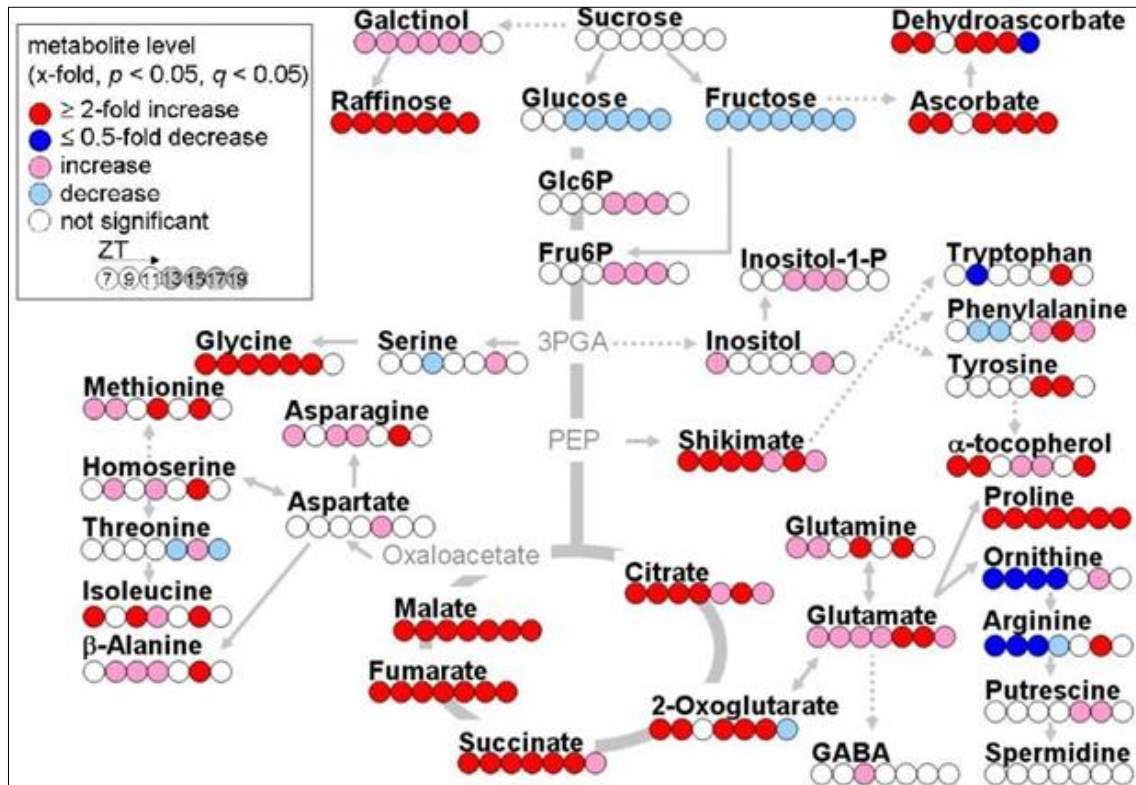
Fig 12: Changes in metabolite levels in triple mutant, d975

Circadian clock genes associated with key agronomic traits
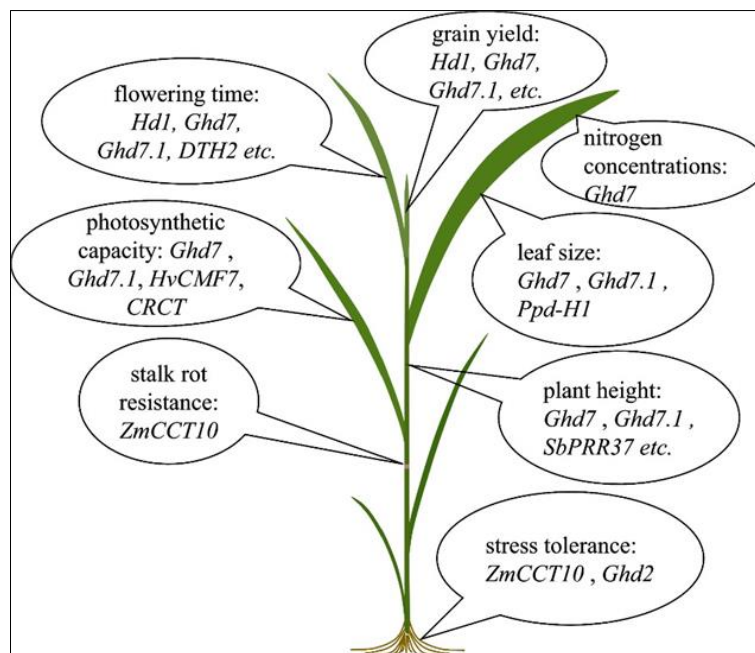

Table 1: Circadian clock genes associated with key agronomic traits (Bendix *et al.,* 2015) [1]

Species	QTL/Loc us	Gene in species	Arabidosis homolog(s)	Role/Trait
B. vulgaris ssp. *vulgaris*	b	BvBTC1	PRR7/PRR3	Biennial growth habit; CO function
	a	BvPRR7	PRR7	b
G. max	E2/FT2	GmGIa	GI	Flowering time regulation
P. sativum	a	LATE1	GI	Circadian clock function; flowering time regulation
	HR/QTL3	HR	ELF3	Circadian clock function; flowering time regulation; light response
	DNE	DNE	ELF4	Circadian clock function; flowering time regulation
	SN	SN	LUX	Circadian clock function; flowering time regulation

Species	QTL/Loc us	Gene in species	Arabidopsis homolog (s)	Role/Trait
L. culinaris	HR	HR	ELF3	Flowering time regulation
O. sativa	a	OsPRR1	TOC1	b
	Hd2	OsPRR37	PRR3/PRR7	Flowering time regulation
	a	OsPRR73	PRR7/PRR3	b
	a	OsPRR59	PRR5/PRR9	b
	a	OsPRR95	PRR9/PRR5	b
	a	OsGI	GI	Flowering time and growth regulation; circadian clock function
O. sativa	ef7/hd17	OsELF3-1	ELF3	Light-dependent circadian clock regulation; flowering time regulation
Species	QTL/Loc us	Gene in species	Arabidopsis homolog (s)	Role/Trait
H. vulgare	Ppd-H1	HvPRR37	PRR3/PRR7	Flowering time regulation
	a	HvGI	GI	Limited flowering time regulation
	eam8/mat- a	EAM8	ELF3	Circadian clock function; flowering time regulation
	eam10	EAM10	LUX	Circadian clock function; flowering time regulation
T. aestivum	a	Ppd-D1	PRR3/PRR7	Flowering time regulation
	a	Ppd-A1	PRR3/PRR7	Flowering time regulation
	a	Ppd-B1	PRR3/PRR7	Flowering time regulation
S. bicolor	Ma1	SbPRR37	PRR3/PRR7	Flowering time regulation
Z. mays	a	ZmGI1	GI	Flowering time and growth regulation
	a	ZmGI2	GI	b
H. vulgare	Ppd-H1	HvPRR37	PRR3/PRR7	Flowering time regulation
	a	HvGI	GI	Limited flowering time regulation
	eam8/mat- a	EAM8	ELF3	Circadian clock function; flowering time regulation
	eam10	EAM10	LUX	Circadian clock function; flowering time regulation
T. aestivum	a	Ppd-D1	PRR3/PRR7	Flowering time regulation
	a	Ppd-A1	PRR3/PRR7	Flowering time regulation
	a	Ppd-B1	PRR3/PRR7	Flowering time regulation
S. bicolor	Ma1	SbPRR37	PRR3/PRR7	Flowering time regulation
Z. mays	a	ZmGI1	GI	Flowering time and growth regulation
	a	ZmGI2	GI	b

a - No known QTL or locus associated with gene. b - Function unknown or undefined.

Conclusion

Although different approaches have been used to prove the relationship with the molecular mechanism of heterosis, none of them can predict heterosis individually. Heterosis should be seen as the outcome of network interaction in the genomes, which in turn result in complex modifications of genetic, epigenetic, regulatory, and biochemical networks. It is now clear that the circadian clock influences almost all plant processes in one way or another. Altered expression of clock genes in hybrids compared to parents gives a scope to exploit heterosis. The complexity of the clock and the diversity of processes that it controls mean that we have to consider the circadian clock to be an extensive control network within plant cells. A number of circadian clock genes underlie quantitative trait loci (QTLs) associated with key agronomic traits, indicating that the circadian-related fitness advantages conferred to *Arabidopsis* by circadian regulation may also be relevant to crops. The circadian clock is broadly similar in crop plants, and represents a target for agronomic optimization

Future thrust

- The interface between circadian biology and downstream processes is still poorly understood in some cases. An increased understanding of plant circadian rhythms may result in the optimization of agriculturally important traits.
- The obvious target characteristics are flowering time and biomass accumulation, but given the oscillator's extensive influence, there is more characteristics of crop productivity that could be improved with a clearer appreciation of circadian rhythms.
- In the face of climate change and associated increases in abiotic stress, the gating of environmental signalling pathways may also become an area of interest.
- By altering the circadian clock's function in photoperiodism, we can increase the latitudinal range across which some crops can be cultivated.

References

1. Bendix C, Marshall CM, Harmon FG. Circadian clock genes universally control key agricultural traits. Molecular plant. 2015;8(8):1135-1152.
2. Chen ZJ. Genomic and epigenetic insights into the molecular bases of heterosis. Nature reviews. Genetics. 2013;14(7):471-482.
3. Darwin C, Darwin F. The Power of Movement in Plants. United States: D. Appleton; c1900.
4. Das AK, Choudhary M, Kumar P, Kr Y, Kumar R, Singh A, *et al*. Heterosis in Genomic Era: Advances in the Molecular Understanding and Techniques for Rapid Exploitat. Critical reviews in plant sciences, 2021, 26.
5. Fukushima A, Kusano M, Nakamichi N, Kobayashi M, Hayashi N, Sakakibara H, *et al*. Impact of clock-associated Arabidopsis pseudo-response regulators in metabolic coordination. Proceedings of the National Academy of Sciences. 2009;106(17):7251-7256.
6. Farré EM, Weise SE. The interactions between the circadian clock and primary metabolism. Current opinion in plant biology. 2012;15(3):293-300.
7. Hochholdinger F, Baldauf JA. Heterosis in plants. Current biology. 2018;28(18):R1089-R1092.
8. Hubbard K, Antony D. Rhythms of Life: The Plant Circadian Clock. The Plant cell. 2016;28(4):tpc.116.tt0416.
9. Liu H, Zhou X, Li Q, Wang L, Xing Y. CCT domain-containing genes in cereal crops: flowering time and beyond. Theoretical and Applied Genetics.

2020;133(5):1385-1396.

10. McClung CR. Plant circadian rhythms. The Plant cell. 2006;18(4):792-803.

11. Miller M, Song Q, Shi X, Juenger TE, Chen ZJ. Natural variation in timing of stress- responsive gene expression predicts heterosis in intraspecific hybrids of Arabidopsis. Nature communications. 2015;6(1):7453.

12. Nagel D, Pruneda-Paz J, Kay S. FBH1 affects warm temperature responses in the *Arabidopsis* circadian clock. Proceedings of the National Academy of Sciences of the United States of America, 2014 Oct 7;111(40):14595-600. 111. 10.1073/pnas.1416666111.

13. Shim JS, Kubota A, Imaizumi T. Circadian Clock and Photoperiodic Flowering in Arabidopsis: CONSTANS Is a Hub for Signal Integration. Plant physiology. 2017;173(1):5-15.

14. Shull GH. What Is Heterosis?. Genetics. 1948;33(5):439–446.