Indian J. Genet. Plant Breed., (2024); 84(2): 168-173
https://doi.org/10.31742/ISGPB.84.2.3
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Heat stress induced cytosine methylation in the coding region of Rubisco activase (Rca) reveals its genotype-specific expression in contrasting wheat genotypes

Monika Saroha[1], Aditi Arya[1], S. K. Singh[2], Gyanendra Singh and Pradeep Sharma[*]

Abstract

Rising environmental temperature has become a major concern in global wheat production since it has critical effects on plant growth, development and yield. Elevated temperatures have become. Further understanding of the processes behind the development of heat tolerance is necessary for significant agricultural crops. Cytosine methylation in DNA plays an essential function in epigenetic regulation of gene expression at various developmental stages and environmental stress in plants. In this study, we report the comparative analysis of cytosine methylation in the coding region of the Rubisco activase (Rca) gene in heat tolerant (RAJ3765) and heat susceptible (HUW510) genotypes of wheat in four growth stages under control and heat-stressed conditions in CG, CHG and CGG contexts. We found that the overall 5-mC increased due to heat stress in HUW510 during tillering (25%), boot stage (25%), heading (48%) and anthesis (50%) as compared to RAJ3765 during tillering (21%), boot stage (4%), heading (4%) and anthesis (11%). Additionally, gene expression profiling by using qPCR at the different plant growth stages also showed the decline in the gene expression in the leaf samples in both the genotypes due to heat stress, with a minimum in the susceptible genotype at the anthesis stage. The study will increase the knowledge on the molecular regulation of photosynthetic pathways by cytosine methylation which would assist in selection and manipulation of heat tolerance in wheat.

Keywords: Bisulfite sequencing, cytosine methylation, epigenetics, rubisco activase, heat stress, wheat, qPCR

Introduction

Plants' reaction to environmental stress is greatly influenced by epigenetic alterations, such as cytosine methylation in DNA, which plays a significant role in preserving the integrity of DNA structure, controlling transposable elements and regulating the plant gene expression (Lämke and Bäurle 2017). DNA methylation is a covalent alteration that results from the addition of a methyl group (-CH_3) to the nitrogenous bases (mostly cytosine, sometimes adenine) in the DNA strand, leading to the formation of 5-methylcytosine. In plants, DNA methylation can occur in three cytosine contexts- CG, CHG, CHH (H =A, C, T) and these pattern levels can be influenced and modified by abiotic stresses. These changes in DNA increase the rate of genetic mutations, thereby leading to adaptive phenotypes (Law and Jacobsen, 2007). DNA methylation in the promoter region of the genes suppresses its transcription and, hence its expression. However, there are conflicting reports indicating that gene body methylation can both suppress and enhance the gene expression (Regulski et al. 2013; Bewick and Schmitz 2017).

Bread wheat (*Triticum aestivum* L.) is among the world's most significant crops which is grown in 221 million hectares,

ICAR-Indian Institute of Wheat and Barley Research, Karnal 132 001, Haryana, India

[1]Deenbandhu Chhotu Ram University of Science and Technology, Murthal 131 039, Haryana, India

[2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

***Corresponding Author:** Pradeep Sharma, ICAR-Indian Institute of Wheat and Barley Research, Karnal 132 001, Haryana, India, E-Mail: neprads@gmail.com

How to cite this article: Saroha M., Arya A., Singh S.K., Singh G. and Sharma P. 2024. Heat stress induced cytosine methylation in the coding region of Rubisco activase (Rca) reveals its genotype-specific expression in contrasting wheat genotypes. Indian J. Genet. Plant Breed., **84**(2): 168-173.

Source of support: ICAR, New Delhi, Govt. of India, doe sanctioned LBSYOA award project

Conflict of interest: None.

Received: Feb. 2024 **Revised:** April 2024 **Accepted:** May 2024

accounting for 15% of worldwide harvested agricultural land (FAOSTAT 2019) and delivering nearly 20% of the food energy intake by humans (Ray et al. 2013). The rise in global temperatures due to climate change has negative implications on food security by decreasing the overall crop growth, development and hence the yield (Pais et al. 2020). The optimum growth temperature for wheat is 22°C and temperatures above 25°C pose a deleterious influence on its yield (Porter and Gawith 1999) as it directly affects photosynthesis, thus limiting the plant growth and crop productivity (Singh et al. 2014). Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) (EC 4.1.1.39) plays a crucial function in CO_2 fixation in photosynthesis and also acts as an oxygenase in photorespiration. Under optimal environmental conditions, the Rubisco activity increases as the leaf expands in size. Its activity is maximum when the leaf is fully expanded and gradually decreases as senescence begins (Mae et al, 1983; Makino et al, 1984). Rubisco activase (Rca) ensures plants' adaptation to mild heat stress by preserving Rubisco in its active form to ensure ongoing photosynthetic efficiency (Shan et al. 2011; Tcherkez et al. 2013; Yin et al. 2014; Scafaro et al. 2016). However, Rca is highly thermolabile beyond optimal temperatures, which of makes it an undesirable participant in photosynthesis.

Keeping in sight the forecasts of future heat waves, a complete knowledge of how Rubisco expression is regulated via epigenetic modifications will become increasingly relevant (Slattery and Ort 2019). Therefore, it is necessary to carefully evaluate the underlying complicated process controlling Rubisco and Rca concentrations in wheat so as to enhance the photosynthetic potential as well as the yield. Hence, this study was carried out to examine the extent and patterns of cytosine methylation and how it affects the Rca gene expression levels in the leaf tissue of contrasting genotypes, *i.e.,* heat-tolerant (RAJ3765) and heat-susceptible (HUW510) at tillering, booting, heading and anthesis stages.

Material and methods

Sowing of seeds and growth conditions for sample collection

Seeds of the two contrasting wheat genotypes, RAJ3765 (heat tolerant) and HUW510 (heat susceptible) were obtained from the Germplasm Section of the Indian Institute of Wheat and Barley Research, Karnal, India. Three seeds were sown in 20cm plastic pots under standard growth conditions. A total of 48 pots (24 for each genotype) were used. At the tillering stage (Zadoks scale 24), one set (3 pots/replication) of the plants was kept as control under normal conditions while the other set (3 pots) was shifted to plant growth chambers, where a pre-treatment at 37°C was given for 2 hours, after which heat stress treatment at 45°C was given for 3 hours. Leaves were harvested from these heat-treated and control pots of the particular set, flash-frozen using liquid nitrogen and stored at -80°C till further use. Similar treatments were given at booting (Z 49), heading (Z 55) and anthesis (Z 65) stages and samples were collected.

RNA isolation, cDNA synthesis and qPCR

Using Trizol (Invitrogen), the total RNA was isolated as indicated by the manufacturer's instructions. SuperScript® First-Strand Synthesis System (Invitrogen) was used to prepare cDNA following the user's manual. The RNA and cDNA quality and quantity were determined by measuring $A_{260/280}$ in a NanoDrop-1000 spectrophotometer. The gene sequences were searched and downloaded from NCBI (https://ncbi.nlm.nih.gov/) and primers were designed using Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=Blast Home). The primer sequences are provided in Table 1. The real-time expression levels of the gene were done on BioRad CFX 96 (Biorad, UK) using the specific primers. Each reaction contained 5 µL of 10 ng/µL cDNA, mixed in 10 µL SYBR green PCR master mix, and 10 µM forward and reverse primers. A final volume of 20 µL was made by adding 4 µL of diethylpyrocarbonate (DEPC)-treated ddH2O. The PCR amplification program consisted of an initial denaturation for 5 minutes at 95°C, followed by 40 cycles of denaturation for 15 seconds at 95°C and annealing for 15 seconds at 55°C for 40 cycles. This was followed by melting at a temperature between 65 to 95°C with an increase of 0.5°C for 10 seconds. All PCR reactions were carried out using three biological replicates. The PCR data from different samples was normalized to the mean Ct values of the internal reference- wheat actin. The comparative Ct ($2^{-\Delta\Delta Ct}$) was used for calculating the cDNA expression level changes between experimental and control samples (Pffafl, 2001). Graphs were plotted for the control samples against the treated ones.

DNA isolation and bisulfite treatment

Genomic DNA from leaf samples was extracted using CTAB-based method as given by Doyle and Doyle, 1987. The quality as well as the concentration, was measured in NanoDrop-1000 spectrophotometer. The bisulfite treatment of the DNA samples was performed using EZ DNA Methylation- Gold TM Kit (Zymo Research) by following the manufacturer's instructions. In brief, 2 µg total DNA and 130 µL of the CT conversion reagent were mixed. The samples were then provided with incubation of 98°C for 10 minutes, followed by incubation of 64°C for 2.5 hours, and by holding at 4°C. After this, 600 µL M-binding buffer was put in the columns provided in the methylation- Gold TM kit and the converted sample was added to it. The converted samples and binding buffer were mixed thoroughly and centrifuged at 10,000 X g for 30 seconds. Again 100 µL M-Wash buffer was added and centrifuged. This was followed by adding 200 µL of M-Desulphonation buffer, waiting for 20 minutes, followed by centrifugation for 30 seconds. The column was

Table 1. Primers used for qPCR, and bisulfite sequencing analyses of the Rubisco gene in wheat

S. No.	Particulars	Primer sequence	Product size(bp)
1.	Rubisco activase gene	F 5′-CGGGGAGAGAACAATCGACA-3′ R 5′-GTTTGAGTGGATTCGAGTCCAA-3′	~1350
2.	Bisulfite sequencing PCR	F 5′-TGGGTATTAATTTTATTATGATGAG-3′ R 5′-AATTCTCCTCCTTATTATACATCCC-3′	282
3.	qPCR	F 5′-GAGGCTGCCGACATTATCAA-3′ R 5′-TGGTTGTTCACCGTGTACTG-3′	110
4.	Wheat Actin	F 5′-CAAATCATGTTTGAGACC-3′ R 5′-ACCAGAATCCAACACGAT-3′	108

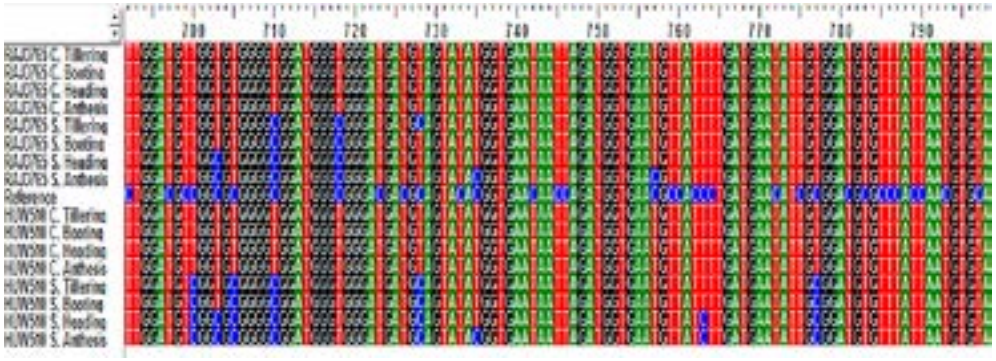

Fig. 1. Alignment of bisulfite sequences of Rubisco gene along with the reference (unmodified) DNA sequence in leaf tissues of RAJ3765 and HUW510 genotypes grown under control (C) and heat stress (S) conditions at different growth stages

washed and spun again with 100 µl M-Wash buffer. This washing step was repeated again. Final elution of the DNA was done by adding 10 µL of M-Elution buffer. This bisulfite-treated DNA was visualized on 1% agarose gel.

Gene-specific primers were designed using MethPrimer software as listed in Table 1.

Amplification and sequencing of bisulfite-treated DNA

PCR amplification of the bisulfite-modified genomic DNA was done using ExTaq DNA polymerase and these gene-specific primers. The PCR reaction contained 5 µL of 5X GC buffer, 0.5µL of 10mM dNTP, 2.50 µL of 10 µM of forward and reverse primers, 0.25 µL of Phusion polymerase (NEB) and 3 µL of 15 ng/µL of the bisulfite-treated DNA. The total volume was made to 25 µL by adding 13.75 µL of sterile water. The PCR program used consisted of initial denaturation at 98°C for 60 seconds., 45 cycles of 98°C for 30 seconds., primer annealing at 55°C for 60 seconds., DNA extension at 72°C for 75 seconds., and a final extension of 10 minutes. After PCR amplification, the amplified DNA was run on 1.5% agarose gel so as to substantiate the product size. The PCR-amplified and purified products were outsourced for sequencing.

Computational analysis of sequencing results

Comparative analysis was done by aligning the sequences using the ClustalW feature of BioEdit v7.2 software. The comparison consisted of all the bisulfite sequences for the contrasting genotypes under control as well as heat-stressed conditions. The methylation data was analyzed by Quma tool (http://quma.cdb.riken.jp/; http://quma.cdb.riken.jp/) as per the default parameters.

Results

Analysis of methylation levels in Rca gene

The quantitative analysis of 5-mC in the exon region of the Rca gene (from 573-806 nt of the gene body) was found to contain 15 (57.6%) of the cytosines as per the CG sequence context, 2 (7.7%) cytosine as per the CHG sequence context, and 9 (34.6%) cytosine as per the CHH sequence context. The changes in the methylation levels among the samples from the various stages of growth of both the genotypes under control and heat-stress conditions were clearly visible in the alignment of the bisulfite sequences (Fig. 1).

High temperature led to an increase in the cytosine methylation levels in the CG sequence context in both the tolerant as well as the susceptible genotypes. In the tolerant genotype, the cytosine methylation level in the CG sequence context was 2 (13%) at all the stages under study. But this increase was comparatively higher in the susceptible genotype, where it was 4 (26.7%) during tillering and booting stages, while 3 (33%) at the heading stage and 6 (40%) methylated during the anthesis stage, respectively (Fig. 2).

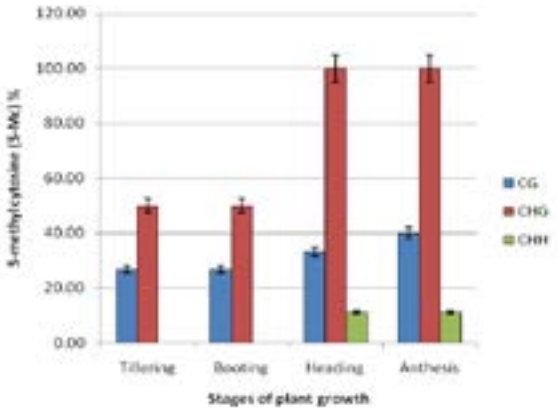

Fig. 2. Cytosine methylation (5-mC) levels in Rubisco gene in different contexts (CG, CHG, and CHH) in the heat- susceptible wheat genotype, HUW510 at different growth stages under heat stress

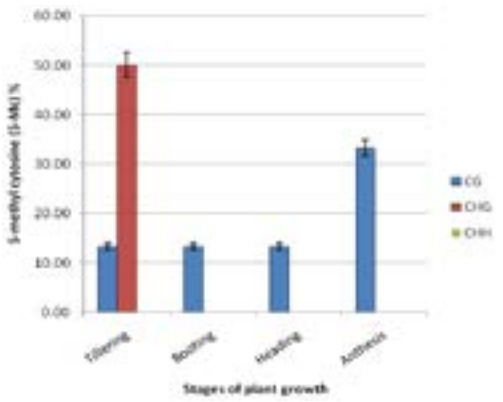

Fig. 3. Cytosine methylation (5-mC) levels in Rubisco gene in different contexts (CG, CHG, and CHH) in the heat- tolerant wheat genotype, RAJ3765 at different growth stages under heat stress

In the susceptible genotype, the cytosine methylation levels in the CHG sequence context were observed to be 2 (100% methylated) during heading and anthesis stages (Fig. 2), whereas tillering and booting stages showed 50% methylation in the CHG sequence context. The tolerant genotype showed 50% methylation in the CHG sequence context during the tillering stage, whereas zero methylation was observed in the other growth stages in the CHG sequence context (Fig. 3).

There was no increase in cytosine methylation in CHH sequence context in the tolerant genotype; however, the susceptible genotype showed 11.11% methylation in the CHH sequence context at the heading and anthesis stages.

Amplification and expression analysis of Rca gene in the two genotypes

Gene-specific primers led to the amplification of the Rca gene from the leaves of RAJ3765 and HUW510 during the different growth stages, i.e., booting, tillering, heading and anthesis at control and heat-stressed conditions (Fig. 4).

The qPCR expression of the gene showed that under heat stress, its expression decreased by 1.7-fold in the RAJ3765 genotype, whereas it decreased by 1.9-fold in the HUW510 genotype during the tillering stage. At a booting stage, the expression of the gene decreased by 2.0-fold in the leaves of the RAJ3765 genotype, whereas it decreased by 2.7-fold in the HUW510 genotype. Similarly, at heading and anthesis stages, the gene expression levels decreased by 2.2- and 2.8-fold in the RAJ3765 genotype during heading and anthesis, whereas in the HUW510 genotype, the gene expression levels decreased by 2.5- and 3.3-fold during heading and anthesis stages, respectively.

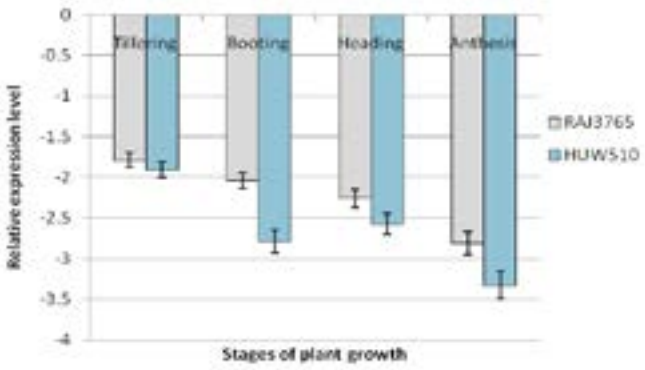

Fig. 4. Quantitative expression analysis of Rubisco gene in the leaves of the contrasting wheat genotypes, RAJ3765 and HUW510, during different growth stages. The results are presented as a mean fold change in relative expression over the control with three biological and three technical replicates, normalized with respect to the actin (reference) gene expression. Bars represent standard deviation

Discussion

Plants are inevitably affected by environmental challenges at various phases of growth throughout their life cycle. In order to respond to the abiotic challenges, epigenetic controls play an important role. DNA methylation is an essential epigenetic modification that leads to the subsequent modulation of gene expression in plants under various abiotic stresses. High environmental temperature is detrimental to plant growth as well as development. As a result, plants have developed signaling pathways that detect changes in environmental temperatures and modify various processes at metabolic and cellular levels to avoid high-temperature damage. Epigenetic regulations play an important role in this regard. Photosynthesis, the prime event for a plant's overall growth, is highly sensitive to elevated temperatures. Rubisco, found in chloroplasts, is a heat-sensitive protein and becomes inactive under heat stress. In this study, we used Rca gene coding region (from 573-806 nt) for the quantitative study of methylation levels due to heat stress. In rice, 31% of the genes showed methylation in the CpG islands of the DNA coding regions, whereas 8% of genes showed methylation in the DNA promoter sequences (Yan et al. 2010). Hohn et al. (1996)

also have demonstrated that cytosine methylation of the exon in the gene leads to gene silencing. Heat stress leads to demethylation in the DNA of *Arabidopsis thaliana* genic regions, according to studies (Korotko et al. 2021). According to earlier research, there can be significant differences in the levels of cytosine methylation in genotypes with different tolerance levels to environmental stress (Wang et al. 2016; Xia et al. 2017). We observed that as compared to the control plants, the heat-stressed plants showed increased levels of methylation in different contexts in both the tolerant as well as susceptible genotypes. Also, the heat-susceptible genotype, HUW510 exhibited a rise in cytosine methylation levels of the Rca gene region under study as compared to the tolerant genotype. Similar outcomes have also been reported in rapeseed seedlings (Gao et al. 2014), Arabidopsis (Malabarba et al. 2021), wheat (Kaur et al. 2018), rice (Li et al. 2023; Xia et al. 2017). Following heat stress, the methylation levels increased in the CHG sequence context but decreased in the CHH sequence context. Similar observations have been stated by Quinton et al. (2015) in wheat.

To validate the association between the methylation changes in the Rca gene and its effect on the gene expression levels during heat stress, we performed qRT-PCR during the different growth stages. The quantitative expression analysis indicated an overall lower level in the expression of the transcript in the heat stressed samples as compared to the control samples. Many previous studies (Vicente et al. 2015; Pérez et al. 2005; Crafts and Salvucci 2000; Law et al. 1999; Vu et al. 1997) support this finding. Also the susceptible genotype showed more decrease in the expression levels as compared to the tolerant genotype. The expression level decreased with the growth of both the genotypes, i.e., expression levels decreased more at the anthesis stage, followed by heading, booting and tillering stages. The findings indicated that the gene showed increased methylation levels, thereby decreasing gene expression levels at high temperatures as earlier reported (Hohn et al. 1996; Schmitz et al. 2013; Baek et al. 2011; Wang et al. 2014; Kumar et al. 2017). However, it is still unknown how gene expression and DNA methylation relate to one another in various sequence contexts. In plants, certain genes experience dynamic variations in their methylation levels as they grow and develop in response to environmental disturbances. Thus, epigenetic changes must be studied and used in agricultural breeding programs to increase plant tolerance to changing climatic circumstances.

Epigenetic mechanisms like DNA cytosine methylation significantly contribute to how plants adapt to their changing environmental conditions. These mechanisms in the plants modify and control the gene expression so as to maintain survival under stress conditions. Heat stress leads to many epigenetic mechanisms (known or unknown) in wheat. Substantial efforts are required to study and discover the function of DNA cytosine methylation of various genes so as to develop new technologies which can be useful for altering gene expression as well as the generation of abiotic stress tolerant genotypes.

Authors' contribution

Conceptualization of research (PS); Designing of the experiments (PS); Contribution of experimental materials (MS, PS); Execution of field/lab experiments and data collection (MS); Analysis of data and interpretation (MS, PS); Preparation of the manuscript (MS, AA, SKS, GS, PS).

Acknowledgment

The authors are grateful to ICAR, New Delhi, doe sanctioned LBSYOA award project and providing funds for this research work.

References

Baek D., Jiang J., Chung J. S., Wang B., Chen J., Xin Z. and Shi H. 2011. Regulated AtHKT1 gene expression by a distal enhancer element and DNA methylation in the promoter plays an important role in salt tolerance. Plant Cell Physiol., **52**(1): 149-161.

Bewick A. J. and Schmitz R. J. 2017. Gene body DNA methylation in plants. Curr. Opin. Plant Biol., **36**: 103-110.

Crafts-Brandner S.J. and Salvucci M.E. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc. Natl. Acad. Sci., **97**: 13430–13435.

Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, **19**: 11-15.

Faostat F. 2019. Food and Agriculture Organization of the United Nations-Statistic Division https://www.fao.org/faostat/en/#data/QC

Gao G., Li J., Li H., Li F., Xu K., Yan G., Chen B, Qiao J, and Wu X. 2014. Comparison of the heat stress induced variations in DNA methylation between heat-tolerant and heat-sensitive rapeseed seedlings. Breed. Sci., **64**(2): 125-133

Hohn T., Corsten S., Rieke S., Müller M. and Rothnie H. 1996. Methylation of coding region alone inhibits gene expression in plant protoplasts. Proc. Natl. Acad. Sci., **93**(16): 8334-8339.

Kaur A., Grewal A., and Sharma P. 2018. Comparative analysis of DNA methylation changes in two contrasting wheat genotypes under water deficit. Biol. Plant., **62**: 471-478.

Korotko U., Chwiałkowska K., Sańko-Sawczenko I., and Kwasniewski M. 2021. DNA demethylation in response to heat stress in Arabidopsis thaliana. Int. J. Mol. Sci., **22**(4): 1555.

Kumar S., Beena A. S., Awana M. and Singh A. 2017. Salt-induced tissue-specific cytosine methylation downregulates expression of HKT genes in contrasting wheat (*Triticum aestivum* L.) genotypes. DNA Cell Biol., **36**(4): 283-294.

Lämke J. and Bäurle I. 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol., **18**(1): 1-11.

Law R. D. and Crafts-Brandner S. J. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1, 5-bisphosphate carboxylase/oxygenase. Plant Physiol., **120**(1): 173-182.

Li B., Cai H., Liu K., An B., Wang R., Yang F., Zeng C., Jiao C. and Xu Y. 2023. DNA methylation alterations and their association with

high temperature tolerance in rice anthesis. J. Plant Growth Regul., **42**(2): 780-794.

Mae T., Makino A. and Ohira K. 1983. Changes in the amounts of ribulose bisphosphate carboxylase synthesized and degraded during the life span of rice leaf (*Oryza sativa* L.). Plant Cell Physiol., **24**(6): 1079-1086.

Makino A., Mae T. and Ohira K. 1984. Relation between nitrogen and ribulose-1, 5-bisphosphate carboxylase in rice leaves from emergence through senescence. Plant Cell Physiol., **25**(3): 429-437.

Malabarba J., Windels D., Xu W. and Verdier J. 2021. Regulation of DNA (de) methylation positively impacts seed germination during seed development under heat stress. Genes, **12**(3): 457.

Pais I. P., Reboredo F. H., Ramalho J. C., Pessoa M. F., Lidon F. C., and Silva M. M. 2020. Potential impacts of climate change on agriculture-A review. Emir. J. Food Agric., **32**: 397-407.

Pérez P., Morcuende R., del Molino I. M. and Martínez-Carrasco R. 2005. Diurnal changes of Rubisco in response to elevated CO_2, temperature and nitrogen in wheat grown under temperature gradient tunnels. Environ. Exp. Bot., **53**(1): 13-27.

Porter J. R. and Gawith M. 1999. Temperatures and the growth and development of wheat: a review. Eur. J. Agron., **10**(1): 23-36.

Quinton-Tulloch M., Olohan L., Price J., Hall N. and Hall A. 2015. A genome-wide survey of DNA methylation in hexaploid wheat. Genome Biol., **16**: 1-15.

Ray D. K., Mueller N. D., West P. C. and Foley J. A. 2013. Yield trends are insufficient to double global crop production by 2050. PloS one, **8**(6): e66428.

Regulski M., Lu Z., Kendall J., Donoghue M. T., Reinders J., Llaca V., Deschamps S., Smith A., Levy D., McCombie W.R., Tingey S. 2013. The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA. Genome Res., **23**(10): 1651-1662.

Scafaro A. P., Gallé A., Van Rie J., Carmo-Silva E., Salvucci M. E. and Atwell B. J. 2016. Heat tolerance in a wild Oryza species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog. New Phytol., **211**(3): 899-911.

Shan X., Wang J., Chua L., Jiang D., Peng W. and Xie D. 2011. The role of arabidopsis Rubisco activase in jasmonate-induced leaf senescence. Plant Physiol., **155**(2): 751-764.

Singh J., Pandey P., James D., Chandrasekhar K., Achary V. M. M., Kaul T., Tripathy B.C. and Reddy M. K. 2014. Enhancing C3 photosynthesis: an outlook on feasible interventions for crop improvement. Plant Biotechnol. J., **12**(9): 1217-1230.

Slattery R. A. and Ort D. R. 2019. Carbon assimilation in crops at high temperatures. Plant Cell Environ., **42**(10): 2750-2758.

Tcherkez G. 2013. Modelling the reaction mechanism of ribulose-1, 5-bisphosphate carboxylase/oxygenase and consequences for kinetic parameters. Plant Cell Environ., **36**(9): 1586-1596.

Vicente R., Pérez P., Martínez-Carrasco R., Usadel B., Kostadinova S. and Morcuende R. 2015. Quantitative RT–PCR platform to measure transcript levels of C and N metabolism-related genes in durum wheat: transcript profiles in elevated [CO2] and high temperature at different levels of N supply. Plant Cell Physiol., **56**(8): 1556-1573.

Vu J. C. V., Allen Jr, L. H., Boote K. J. and Bowes G. 1997. Effects of elevated CO2 and temperature on photosynthesis and Rubisco in rice and soybean. Plant Cell Environ., **20**(1): 68-76.

Wang W., Qin Q., Sun F., Wang Y., Xu D., Li Z. and Fu B. 2016. Genome-wide differences in DNA methylation changes in two contrasting rice genotypes in response to drought conditions. Front. Plant Sci., **7**: 1675.

Xia H., Huang W., Xiong J., Yan S., Tao T., Li J., Wu J. and Luo L. 2017. Differentially methylated epiloci generated from numerous genotypes of contrasting tolerances are associated with osmotic-tolerance in rice seedlings. Front. Plant Sci., **8**: 11.

Yan H., Kikuchi S., Neumann P., Zhang W., Wu Y., Chen F. and Jiang J. 2010. Genome-wide mapping of cytosine methylation revealed dynamic DNA methylation patterns associated with genes and centromeres in rice. The Plant J., **63**(3): 353-365.

Yin Z., Zhang Z., Deng D., Chao M., Gao Q., Wang Y., Yang Z., Bian Y., Hao D. and Xu C. 2014. Characterization of RuBisCo activase genes in maize: an α-isoform gene functions alongside a β-isoform gene. Plant Physiol., **164**(4): 2096-2106.