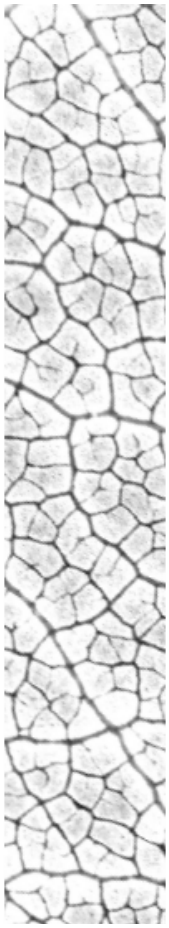

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 287-291 (2019)

Use of cryptochrom for the expression of succinate dehydrogenase genes in corn leaf

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Abstract
Corn is one of the most important cereals in Iran. Which has a high importance in the quality of nutrition and high consumption of industrial products, and is a worldwide strategic product. This research was carried out in 2018 in the laboratory of Samarra university to investigate the expression of suction dehydrogenase genes in corn leaf using copiquorum. The results indicated that blue light prevents the activity of succinate dehydrogenase and thus prevents the gene expression of green leafy corn. Irradiation with light blue to corn plants leads to a reduction in the unstable transcription of the encoded genes of Sdh1-2 and Sdh2-3 and mutually reduces the encoded protein of the fluoroprotein and coded protein of iron-sulfur. The effect of light blue is probably due to the transcription factors COP1 and HY5, and the second is increased by light blue treatment. This has been associated with a decrease in the expression of COP1, and probably contributes to HY5 protease decomposition. It also proves that calcium ions do not participate in this process.

Keywords: corn grain, dehydrogenase dehydrogenase, cryptochrom, blue light, gene expression

Jasim ME, Fadhil KB, Jawad AS (2019) Use of cryptochrom for the expression of succinate dehydrogenase genes in corn leaf. Eurasia J Biosci 13: 287-291.

INTRODUCTION

With the world population increasing, demand for food production has increased (Sai and Erdogan 2011). Corn is one of the most important cereals in Iran (Seyed Sharifi and Khavavazi 2011), which has a high importance in the quality of nutrition and high consumption of industrial products and is considered as a strategic product worldwide (Khajehpour 2010). In 2013 corn In terms of cultivating area, wheat was the second largest crop in the world (FAO 2013). But in Iran, corn after wheat, barley and rice has been harvested and ranked fourth. In the cultivation year of 2013-14, the cultivated area of this product in Kurdestan province has been equal to 3034 hectares and its production has been 22754 tons (Moradi-Toche et al. 2012, Orhan 2018, Yuca et al. 2015).

One of the most important mechanisms for regulating metabolism in green tissues of plants during active photosynthesis is the inhibition of plant respiration in light (Gardstrom and IGAMBUDIO 2016). This mechanism, through inhibition of the combination of pyruvate dehydrogenase (Gemm and Randall 1992), regulation of NAD- and NADP-dependent diphtheria isocitate-dependent (IGAMBIRO and GARDSTROOM, 2003) and inhibition of suction dehydrogenase (SDH), Which can be done at the stage of enzyme activity regulation (Dalloso et al. 2015) And also through the modulation of the expression of the gene expression from phytokromase A, mediated by calcium ions (Davoodabadi and Aghajani 2013, Opensio et al. 2016).

Another mechanism for regulating respiratory tract can be cited by the effects of light blue absorbed by cryptochromes (Fox et al. 2015)This absorption was initially observed in Arabidopsis using cortisol mutations for the formation of SDH (suction catecholamines) (Izinsto et al. 2015). Blue light has a positive photomorphogenic regulator and the uvequitin ligases act as negative photomorphogenic regulators. COP1 is transmitted in the dark to the nucleus and subjected to decomposition under the interaction with HY5 in the nucleus, and COP is regulated by blue light towards the cytosol, which regulates the subcellular position of COP1 (Oesterland et al. 2000b).The transcription factor of HY5 is a transcription factor inherent in the nucleus (Ang et al. 1998) and the promoter responding to the light contained within the box G, and ensures the desired expression of the genes involved.HY5 decomposition in the dark also provides a mechanism in which the HY5 activity and the centralized HY5-expression of the activity can be regulated in light (Yazdani et al. 2018, Zhang et al. 2011). Oprinsto et al. (2015) demonstrated the effect of cryptochrom on susiton dehydrogenase in Arabidopsis. Now, in this study, we examine the mechanism of corpuscular-dependent sucrozinate dehydrogenase-dependent cryptochrom mechanism, which reveals the mechanism of intracellular

Received: November 2018
Accepted: April 2019
Printed: May 2019

transferability, transmitting the light-receptor signal to the nuclei, A mechanism that induces changes in the expression of the subunit of encoded genes of succinate dehydrogenase gene.

MATERIALS AND METHODS

This research was conducted in 2018 in the laboratory of Samarra University to investigate the expression of the suction-dehydrogenase genes in corn leaf using cryptochrom. For this purpose, green leaves of 15-day corn, which were exposed to light and 22 ° C for 12 hours a day, were used. The plants were exposed to a dark room for 24 hours and have been irradiated with light blue, produced by light diodes, at 0.044 Wm^{-2} and wavelength 470-465 nm for 15 minutes. Succinate dehydrogenase gene activity (EC 1.3.99.1) was measured on the UV-vis metering spectrum. A portion of the enzyme can convert one micromole of substrate to the product within one minute at 25 ° C. Using an absorption coefficient cm^{-1} $21mM^{-1}$ Dichlorophenol-indophenyl oxidized, which was obtained by irradiation with a wavelength of 600 nm, this led to a reduction in the adsorption of dichlorhenol-indophenol, an electron receptor, which was measured by this SDH absorption reduction (Cooper and Beaver 1969). The environment of this reaction contains 50mM potassium phosphate buffer, pH 7.8, containing "1mM Na" N_3 (sodium azide), 10mM of mask, "0.1Mm" of phenazine Meta bisulfite, and 0.008mM of dichlorhenol-indophenol. The core components were separated according to the theory of Lee and Lin (2008), and the amount of free calcium was measured by spectrophotometric method using a color reaction with a colored reaction of moroxide in the presence of glycerol (Scarpa 1972).

According to Chomsky and Sachi (1987), with the guanidine extraction method of thiouzanate phenol chlorophyram, all RNA of corn leaves have been separated. Polymerase chain reaction with specific gene primers was performed using amplifier-reactive reactors (Oprinsto et al. 2017). For parsing the PCR, primers have the following nucleotide sequences: Sdh1-2-5'CGAATGGGTCATTGCCAACT-3 'to be analyzed, _5'-ACCTTTGAAAGGGTACAAA-3' translated, for Sdh2-3_ 5'GAGAGGCTACAGGCAATAACTGAG3 'decomposition Send and translate _5'GGATTTTGACTTGACTGGGATTG-3 ', translate COP1_5'TCTGCGTCCACAGATAGCAC-3' for parsing and translate _ 5'GTCTGGCGATCCAAATCTGT-3 ', send it to HY5_5'-ATTGAGTTGCAGGGATGGAG-3', and _ 5 -CCCTCTGTAGCCTGTTGAGC-3 'have been translated. Polymerase chain reaction has been carried out in Tercik amplifier (amplifier). Immediate polymerase chain reaction (RT-PCR) has been performed on LightCycler 96 (using light blue SYBR). The amplification parameters were initialized at 95 ° C for a duration of 5 minutes, followed by 40 cycles are achieved in 20 seconds at 95 degrees, 30 seconds at 58 degrees, 40 seconds at 72 degrees, and at the end of 4 minutes at 72 degrees. All tests repeated 3 to 4 times and have been statistically analyzed. The data presented on the tables means three times the biological repeatability of ± SD. Statistical measures and significant statistical differences have been investigated at $P < 0.05$.

RESULTS AND DISCUSSION

The results showed that (**Fig. 1**), during the first three hours of irradiation with light blue, there was no change in the activity of the succinate dehydrogenase gene, however, after 5 hours of activity, the succinate dehydrogenase gene is reduced 2 to 3 times and close to the activity level of the suction dehydrogenase gene at daylight, as well as the activity of the succinate dehydrogenase gene in the dark is three times that of its activity in brightness. The results of the activity of the succinate dehydrogenase gene are consistent with the expression pattern of Sdh1-2, and according to this, the activity of the succinate dehydrogenase gene in the dark is twice as high as its activity in the brightness and after 5 hours of irradiation with blue light activity levels are reduced to the same level of activity that we saw in the light. The expression of Sdh2-3 has shown a similar pattern to that of Sdh1-2, with the difference that the exposure to blue light is more visible than the Sdh1-2 pattern within three hours after the irradiation.

In order to determine the possible role of COP1 and HY5 in transmitting cryptochromes messages, the level of transcription of the genes was determined (**Fig. 2**). The results indicated that in the dark, the level of transcription of the COP1 gene significantly increased, however, the level of transcription of the HY5 gene was much less than COP1 in brightness. After exposure to light blue, the expression of COP1 decreased, and after 1 hour of irradiation, its activity level decreased by a large amount compared to the activity level observed in the light and remained at the same level in the next hour. Contrary to the expression of COP1, HY5 levels increased after irradiation with light blue light, and after 3 to 5 hours of exposure to blue light treatment peaked, however, the sustainability of this activity was less than the sustainability of its activity under daylight. The level of calcium in the nucleus of corn cells in the dark was not twice as high as its surface at significant brightness (**Fig. 3**). The light obstructed the SDH activity (**Figs. 1** and **2**), which is consistent with the results of Apirinto et al in 2016.Blue light can be effective in preventing SDH activity, and cryptochromes may also be involved. After several hours of exposure to light-blue light, its effect is considerable, and this effect refers to a mechanism that is obtained due to relatively slow changes in the expression of genes. The effect of light blue is fast (within an hour), while the transcriptional level of the HY5

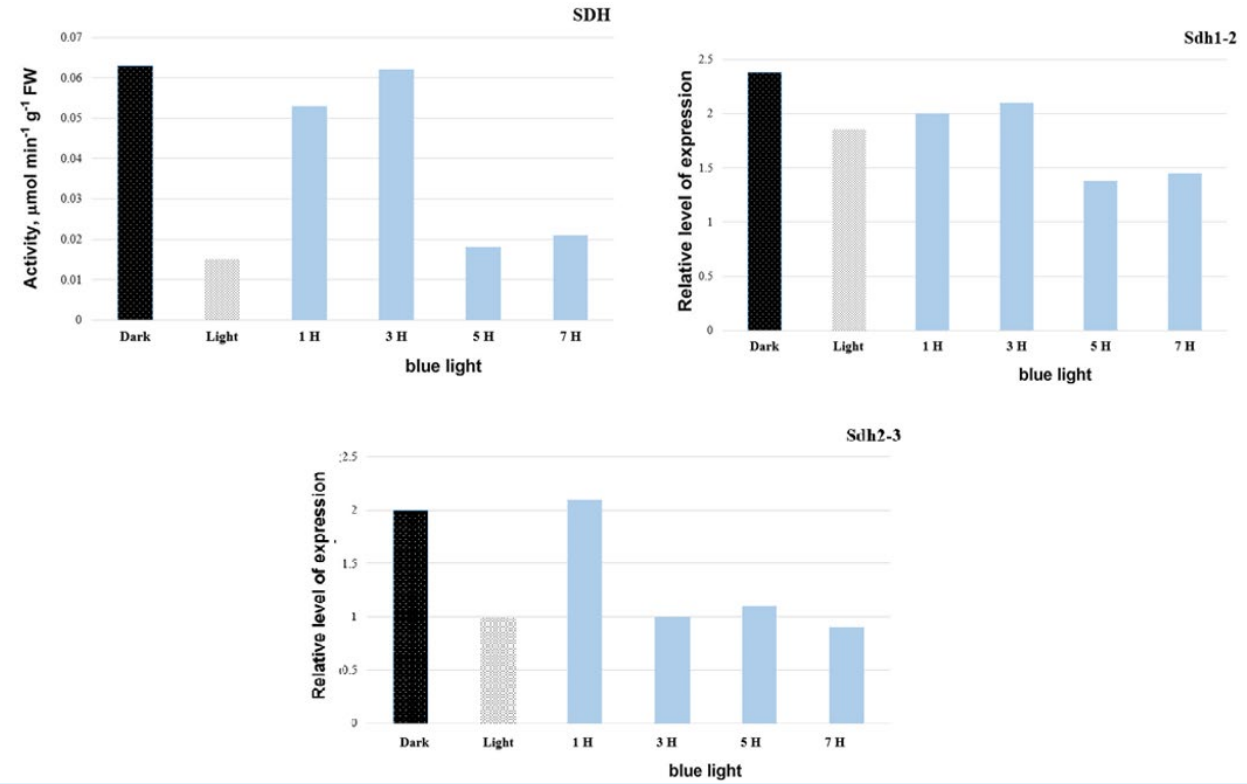

Fig. 1. The effect of light regimen on dexygenase activity and expression of Sdh1 and Sdh2 genes

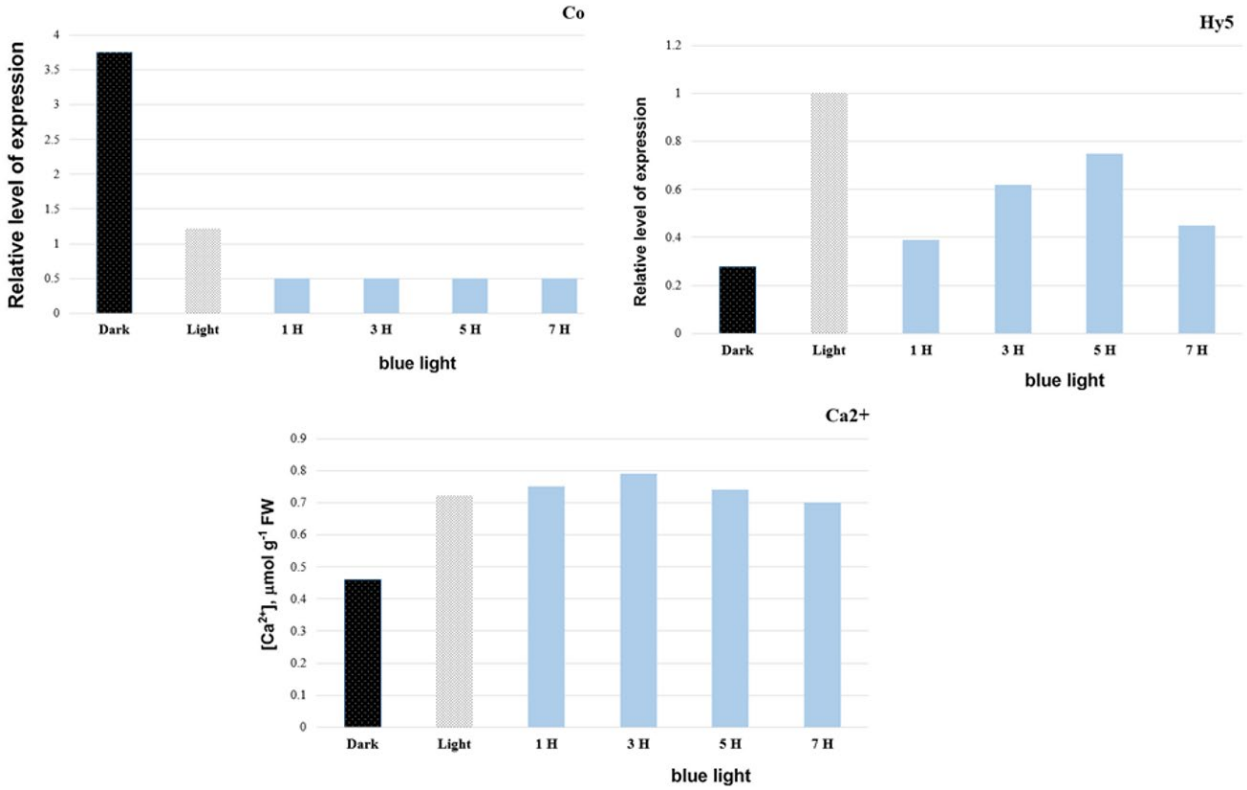

Fig. 2. The mean of optical treatment on the expression of COP1, HY5 and free calcium genes in the nucleus

gene increases very slowly (within 3 to 5 hours). In the dark, the HY5 protein breaks down due to the COP1 factor acting as a UG-IgA ligase and breaking the core protease, (Oströlvand et al. 2000b). The results indicate that the mechanism that incorporates COP1 and HY5 proteins can also be realized in the control of SDH

activity in light. The regulation of the phycocyan of the expression of SDH (Oprinsto et al., 2013) is due to changes in the level of calcium in the nucleus. Ca^{2+} Changes are due to activation of phycocyan-dependent anionic channels, also during irradiation with blue light plants (China et al. 2004). In our experiments, no change in Ca^{2+} has been observed, therefore, the possibility of Ca^{2+} interference in the regulation of SDH expression by cryptochrom has not been considered.

CONCLUSION

The results showed that the use of blue light due to changes in cryptochrom induced by the transcription of Sdh1-2, Sdh2-3, controlled the activity of the succinate dehydrogenase gene in corn leaves, and these changes were not caused by calcium, it may also be due to the participation of transcription factors COP1 and HY5.

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