

OFFICIAL ABSTRACT and CERTIFICATION

The Effect of Light on the Epitranscriptome of Plants

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It is important for land plants to properly perceive light, since light perception controls plant functions through cues and is also essential for photosynthesis, which is the main mechanism for harvesting energy in plants. In order to study the biomolecular mechanisms of light perception, plant models must be studied under the wavelengths of light that photoexcite photoreceptors. The cryptochromes photoreceptors are of interest as they are responsible for seedling de-etiolation (the inhibition of hypocotyl lengthening), flowering, and clock functions. It has been proposed that the protein ECT2, evolutionarily conserved C-terminal region 2, produces a similar phenotype in plants to cryptochrome 2 (CRY2). ECT2 is part of an RNA-protein complex, N6-methyladenosine (m6A), that is potentially responsible for many critical RNA modifications and functions. It was therefore hypothesized that ECT2 and CRY2 may interact to induce the de-etiolation response in plants through RNA modification. In order to test this, different Arabidopsis thaliana genotypes, wildtype, cry1, cry2, cry1cry2, and ect2, were grown in darkness (control) and blue light (blue light photoexcites cryptochromes), where cry2 and ect2 were expected to demonstrate etiolation. Hypocotyl assays were evaluated through ImageJ, showing that cry2 and ect2 mutants induce significant amounts of etiolation ($p < 0.05$) as expected. In the eleventh trial, the average hypocotyl lengths were 1.54370968 cm, 1.82241026 cm, and 1.88347059 cm for the wildtype, cry2, and ect2 genotypes respectively, and t-tests between the wildtype and cry2 and between the wildtype and ect2 genotypes produced p-values less than 0.05. A bimolecular fluorescence complementation assay to show interaction between ECT2 and CRY2 in tobacco plants produced false positive results. The m6A system contains several molecules, referred to as writers, that aid in methylation, and gateway cloning has been used to prepare the writers, MTA, MTB, and FIP37 for future assays. In the future, BLC assays with luciferase can be conducted instead to determine interactions between ECT2 and CRY2 through analyzing the interactions of writers. The hypocotyl assays can also be repeated with phytochromes under red light to confirm that the de-etiolation effect is not limited to blue light exposure.

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