Functional Analysis of Fvcry1 Reveals the Mechanism of Blue Light in Regulating Vegetative-Reproductive Growth Balance of Strawberry

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Abstract. Strawberry is an ideal crop for vertical farming due to its tiny size and high commercial value of fruit. Even though the LED has been widely used as artificial light resource for cultivation of strawberry in vertical farm. The molecular mechanism of blue light spectrums regulating development of strawberry remain to be elucidated. There are two light receptors, including FvCRY1 and FvCRY2, initiate blue light signal in strawberry. To reveal the mechanism of blue light in regulating vegetative-reproductive growth balance of strawberry. We utilized the RNAi method to knock down the expression level of FvCRY1 in wild strawberry (Fragaria vesca 'Fin56'). The phenotype of RNAi lines comparing the wild type lines was performed. The vegetative growth was significantly promoted displaying an increasing numbers and lengths of stolons in RNAi lines. The B-box transcription factors (BBX) play important roles in light signal transduction and regulation of flowering time. We further analyzed the gene expression of FvBBXs in RNAi lines using qRT-PCR. The FvCO, which has been demonstrated to be a critical regulator in vegetative-reproductive growth balance, was significant repressed in gene expression level. Besides, two new BBX genes, including FvBBX19a and FvBBX28c, were promoted in RNA lines. Collectively, our results shed light on the role of blue light signaling mediated by FvCRY1 in regulating the balance of vegetative-reproductive growth in strawberry.

Keywords: Blue light, B-box proteins, Cryptochromes, Flowering time, Photobiology, Strawberry, Vegetative-Reproductive Growth Balance.

1. Introduction

Strawberry is an ideal crop for vertical farming due to its tiny size and high commercial value of fruit. Various cultivation methods, such as hydroponics and substrate cultivation, have been developed for strawberry production in plant factory. LED lighting is widely applied in plant factory as artificial light resource. In strawberry cultivation practices, the spectral composition of artificial light plays an important role in fruit quality, photosynthetic activity and transition from vegetative-reproductive growth. Blue light has been reported to regulated the from vegetative-reproductive growth balance by promoting flowering time in both wild strawberry (Fragaria vesca) and cultivated strawberry (Fragaria ananassa) [1]. Blue light is also particularly effective in inducing flowering under long daylight conditions, which can improve the productivity of strawberry cultivation in vertical farm. Yet, the molecular mechanisms by which blue light spectra regulates strawberry vegetative-reproductive growth balance remain to be elucidated.

Blue light signal is perceived by multiple light receptors (Fig 1), including CRYs (Cryptochromes), PHOTs (Phototropins) and ZLFs (ZTL/FKF1/LKP2). Two CRY proteins in Arabidopsis perform redundant but distinct functions in blue light signaling [2]. FvCRY1 and FvCRY2 are corresponding CRY proteins in wild strawberry. Here we utilized the RNAi method to knock down the expression level of FvCRY1 in wild strawberry (Fragaria vesca 'Fin56'). The phenotype of RNAi lines comparing the wild type lines was performed. The expression levels of downstream genes involved in light signal transduction were analyzed. These result regarding function of FvCRY1 can contribute to our understanding of blue light signaling and enhance the application of blue light in plant factory.

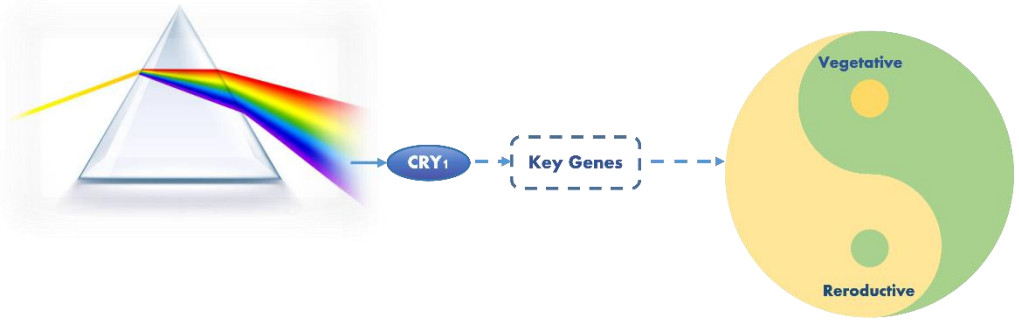

Figure 1. Research objective in present work

In present research work, we investigate the function of *FvCRY1* involved in regulating the vegetative-reproductive growth balance in wild strawberry.Besides, A further Identification of the key genes involved in blue light signaling initiated by FvCRY1 was performed to reveal a mechanism of affecting developmental balance in wild strawberry by blue light signaling (Figure 1).

2. Method and Materials

2.1. Construction of the transgenic lines

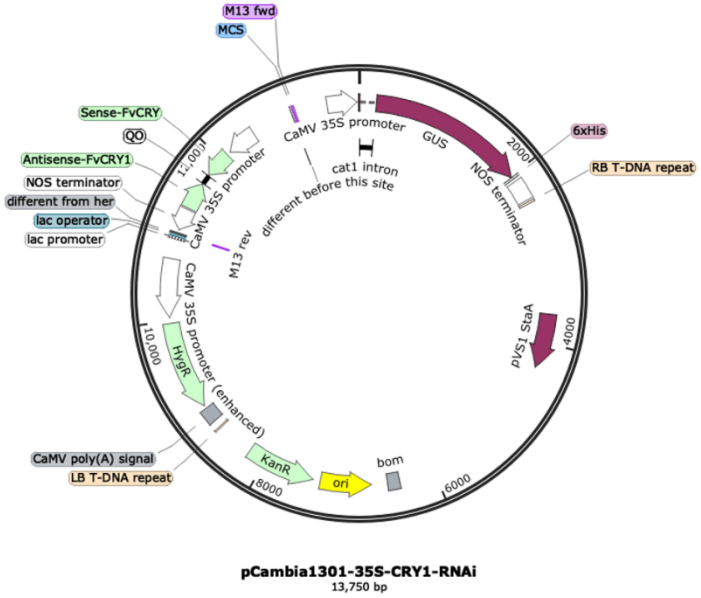

Figure 2. The RNAi plasmid vector used in present work

RNAi lines were obtained using agrobacterium-mediated transformation in wild strawberry (Fragaria vesca 'Fin56'). An RNAi plasmid vector (Figure 2) was constructed to knock down the gene expression level of *FvCRY1*. The qPCR evaluating the expression level of FvCRY1 and GUS stain was further performed to confirm the RNAi lines.

2.2. Analysis of phenotype of Transgenic lines

For the phenotype analysis, the number and length of runners formed from the RNAi lines and wild type lines are observed under short-day (day/night=12/12) photoperiod condition. The qRT-PCR was used to evaluate the gene expression of *FvBBX* genes which were shown to play roles in blue light signaling in cultivated strawberry [1].

3. Results and discussion

3.1. The Validation of RNAi Lines

Two transgenic strawberry lines were obtained by the agrobacterium-mediated transformation using Fin56 as genetic background line. As shown in Figure 3A, two lines were blue in GUS stain test, demnstrated tant that the lines were genetic transforemd (Fig 3A). To further explore the gene expression of FvCRY1, the semi-qPCR and qRT-PCR were performed. As shown in Figure 3B and Figure 3C, the gene expression of *FvCRY1* was significantly down-regulated in the obtained trangic lines. Those results demonstrated that two RNAi lines were constructed with lower expression of FvCRY1. The lines,thus, could be used in afterward works.

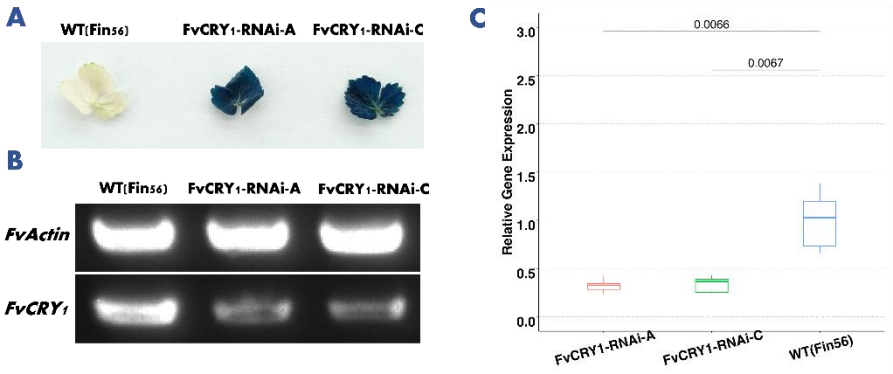

Figure 3. The construction of transgenic lines and gene expression of FvCRY1 in transgenic lines. GUS stain of transgenic lines (A); Gene expression level of FvCRY1 evaluated by semi-qPCR (B) and qRT-PCR (C)

3.2. Phenotype observation of RNAi Lines

For strawberry, runnering is a typical vegetative growth which is regulated by endogenous and environmental factor [3]. The phenotype observation of RNA lines were conducted. The resuluts showed that the RNAi lines have more runners than wild type lines (Fig 4A & Fig 4B). Besides, the length of runners also was promoted in RNAi lines (Fig 4C).

These result indicated that the blue light signal transduction mediated by *FvCRY1* have significant effect on the vegetative growth in wild strawberry.

Figure 4. The phenotype of two RNAi lines compared to wild type line. Phenotype of strawberry lines (A). Runner count of RNAi lines and wild type line (B). Runner length of RNAi lines and wild type line (C)

3.3. Expression level of Key Genes

In our previous work, we demonstrated the role of blue light in promoting cultivated strawberry flowering time. Here we suspected that the B-box transcription factors participate in both regulating the flowering time and the vegetative-reproductive growth balance [1, 4]. Then we further analyzed the expression level of *FvBBXs* in RNAi lines to identify the potential key genes. As shown in Figure 6, *FvBBX19a* and *FvBBX29c* were significantly promoted in RNAi lines. Interestingly, *FvCO* was reported to play a role in regulating vegetative-reproductive growth balance [5]. Whereas its gene expression level was unchanged in present result.

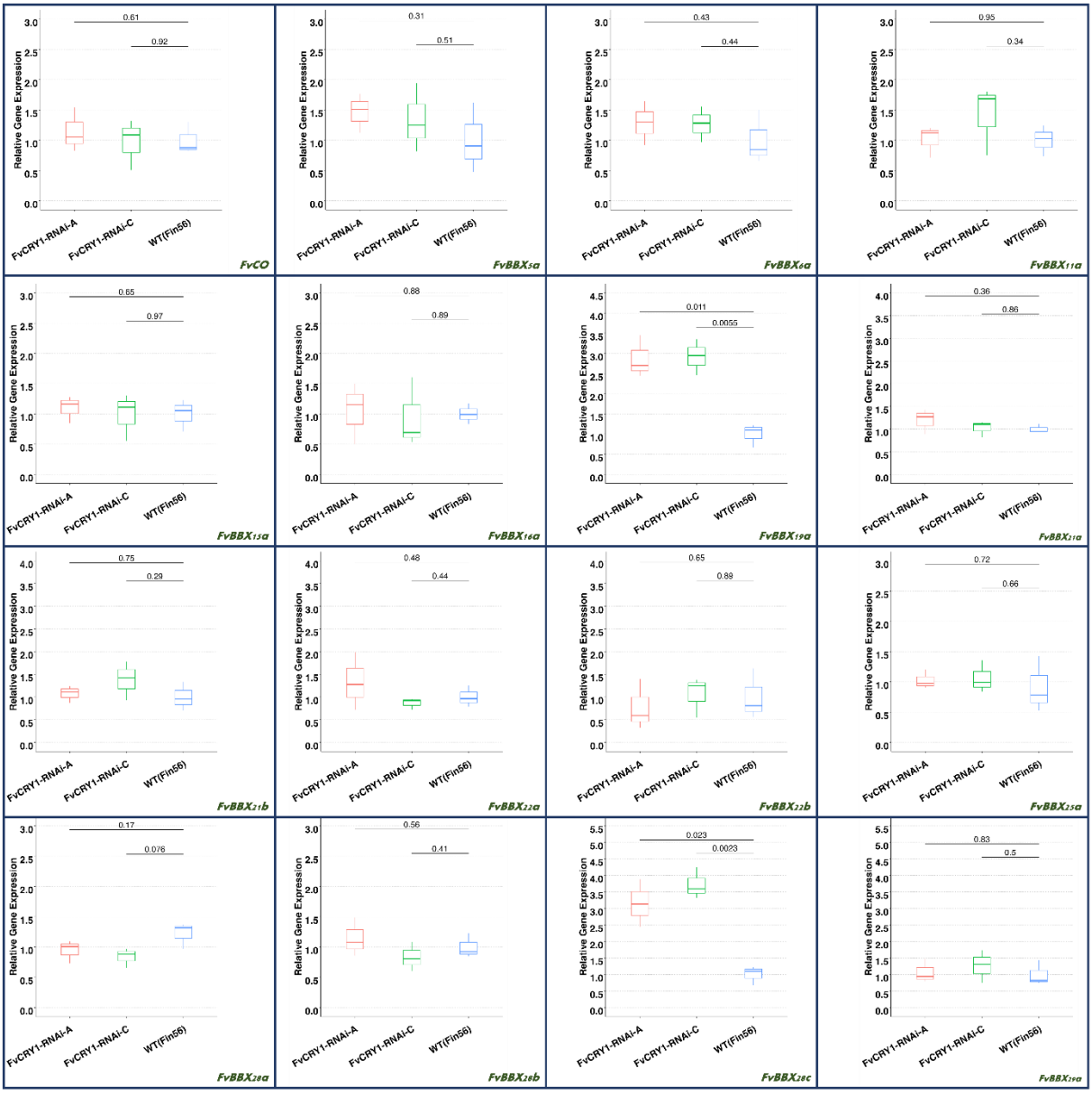

Figure 5. Gene expression of FvBBXs in strawberry lines

4. Conclusion

The vegetative-reproductive growth is crucial for product efficiency of crops. The LED was widly used as light resource in horticulture, such as vertical farm [6]. The different light quality has various effect on the growth of horticulture plant, of which the function of blue light is still unclear in molecular mechanism. In present work, we showed that the blue light mediated by *FvCRY1* has significant effect on vegetative-reproductive growth balance by promoting the number and length of

runners in wild strawberry. The gene expression level showed that *FvBBX19a* and *FvBBX28c* are promoted in RNAi lines which implied these genes likely involved in vegetative-reproductive growth balance in strawberry regulated by blue light. However, the further function details of *FvBBX19a* and *FvBBX28c* is needed in the future.

Acknowledgments

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