Journal of the Korean Society of Grassland and Forage Science 43(3): 177~182, 2023
https://doi.org/10.5333/KGFS.2023.43.3.177

ISSN 2287-5824 (Print)
ISSN 2287-5832 (Online)

Research Article

LED Light Quality Protects Iron Deficiency and Improves Photosynthesis and Biomass Yield in Alfalfa (*Medicago sativa* L.)

Ki-Won Lee, Sang-Hoon Lee, Yowook Song, Hyung Soo Park,
Jae Hoon Woo, Bo Ram Choi and Md Atikur Rahman*
Grassland and Forage Division, National Institute of Animal Science, RDA, Cheonan 31000, Republic of Korea

ABSTRACT

Iron (Fe) is a vital element for plants and other organisms, involving in several physiological processes including respiration, chlorophyll biosynthesis, and photosynthesis. Unfortunately, how Fe accumulation regulates in response to light quality has not been well established in plants. Therefore, the aim of the study was to explore the mechanism of Fe homeostasis by light quality. In this study, we found morpho-physiological attributes were significantly improved in response to blue (λmax: 450) compared to white (λ max: 500) and red (λmax: 660) light. The root-shoot length, plant biomass, photosynthesis efficiency (Fv/Fm) and leafgreen (SPAD) significantly declined in response to white and red light. However, these parameters were improved and iron deficiency was substantially alleviated by blue light exposure in alfalfa seedlings. This study might be useful to the forage breeders and farmers for improving alfalfa yield and nutritional benefits.

(Key words: Alfalfa yield, Essential element, Iron deficiency, LED light, Nutritional benefit)

Ⅰ. INTRODUCTION

Iron (Fe) is an essential micronutrient for plants because it significant role in metabolic processes such as nucleic acid (DNA) synthesis, photosynthesis and respiration (Kabir et al., 2023). Primary indication of Fe deficiency causes leaf chlorosis, plant growth retardation, and agronomic yield loss Rahman et al., 2020b; Rahman et al., 2021b; Kabir et al., 2022). This problem is most common in calcareous soil (Rahman et al., 2021a), even though overwatered plants growing in alkaline soils can trigger or aggravate Fe deficiency (Haque et al., 2022). Moreover, different toxic metals cause a deficiency of essential elements including Fe in plants (Rahman et al., 2020a; El-Shehawi et al., 2022). It is not well known whether the Fe absorption and utilization can be modified by light quality, intensity, and photoperiod. Therefore, study on the role of light quality in regulating Fe homeostasis and physiological and molecular responses would be exciting in Strategy-I and II plants.

Plants have evolved different type of Fe acquisition pattern from soils (Rahman et al., 2022a). Reduction based Fe uptake mechanism is one of the strategies of dicots and non-graminaceous monocots, which belong to Strategy-I plants (Rahman et al., 2021a). Alfalfa is a Strategy-I plant, this group of plants reduces Fe^{3+} into Fe^{2+} by plasma membrane ferric reductase enzyme encoded by the ferric reduction oxidase (FRO) gene. Under Fe deficiency, Strategy-I plants undertake several physiological and molecular alterations due to nutrient acquisition and their transportation. For instance, significant upregulation of Fe transporter gene1 (*IRT1*) triggers the Fe acquisition in plants (Rahman et al., 2021b). Other nutrient transporters including zinc (Zn) and sulfur (S) transporters such as *ZIP1* and *SULTR1;2* coordinate the acquisition, transport, signaling, and interacting pathways for Fe, Zn, S, N, and P at molecular level (Kumar et al., 2021). Responses of these key nutrient transporters at molecular level have not been well studied in plants exposure to light. Therefore, molecular responses of these transporter genes would be effective disclosing acquisition, transport and homeostasis of Fe and other nutrients in alfalfa.

Alfalfa (*Medicago sativa* L.) is an excellent forage legume, widely cultivated as hay and fodder crops (Das et al., 2021;

*Corresponding author: Md Atikur Rahman, Grassland and Forage Division, National Institute of Animal Science, RDA, Cheonan 31000, Republic of Korea.
Tel: +82-41-580-6746, E-mail: atikbt@korea.kr

Rahman et al., 2016). Alfalfa provides nitrogen benefits to the soils that reduce the cost and commercial fertilizer dependency during agricultural production (Rahman et al., 2015). As a fodder crop, it is highly desirable to make sure nutrient content in forage crop. However, quality of light would be excellent alternative of nutrient homeostasis in plants. Therefore, the aim of this study was to explore light quality mediated Fe deficiency homeostasis in alfalfa plant. This study might be useful to alfalfa breeders and farmer for improving alfalfa through breeding program and metabolite engineering.

Ⅱ. MATERIALS AND METHODS

1. Alfalfa growth in response to LED light

Alfalfa (*Medicago sativa* L.) were obtained from the Grassland and Forages Division, National Institute of Animal Science, Cheonan, Korea. Seeds were sterilized with 70% ethanol for 1 min then rinsed with sterilized properly. The seeds were moved to alfalfa germination box for 2-3 days, then seedlings were grown with ¼ strength Hoagland nutrient solution with different type of LED-light quality for two-weeks (Hoagland and Arnon, 1950). Alfalfa seeding were grown with exposure of total four different combinations such as LED white light λmax: 400 nm (W400), red light λmax:660 nm (R660), blue light λmax:660 nm (R660), and 1:1 combination of red light λmax:660 nm (R660) and blue light λmax:660 nm (R660+B450). Hence, only trace amount of growing nutrient (¼ strength) used in order screen the proper light effects on nutrient update and regulation process in alfalfa. Following two weeks of treatments were terminated. The alfalfa samples were collected in small in size polybag, whereas roots were properly with Milli-Q water to remove excess growing nutrients on root surface. The alfalfa samples were freezed with liquid N_2, and kept at -80 °C for further molecular analyses.

2. Determination of photosynthesis, chlorophyll content and morphological parameters

The maximum florescence of quantum efficiency of photosystem II (Fv/Fm) was determined using a portable fluorometer system (LI-600 Porometer/ Fluorometer, Germany), and alfalfa chlorophyll was measured as leaf greenness by SPAD measuring machine (SPAD-502, Minolta, Japan). The root-shoot length (cm) of alfalfa was determined using a by caliper, and plant dry weight (g) was determined by digital balance.

3. Gene expression analysis

Iron and other nutrient transporter genes were conducted by the q-RT PCR following the protocol used previously (Rahman et al., 2021a; Haque et al., 2022). The gene-specific primers for *IRT1* (F: TTTACCCTTGGCGACACGTT; R: CATGAACCCGGTC CCAAGAA), ZIP1 (F: ATGGGAATCGCATTGCTAAG; R: C TGCGGTTTGAAGCCTT TAG), *SULTR1;2* (F: CAGATAAG CAAGGAGTAGAA; R: TCATAGAACCAACGACAT) were used for gene expression analyses. Total RNA was extracted from alfalfa root tissue using an RNA extraction kit (QIAGEN, USA) following the protocol used previously (Rahman et al., 2022b), and then RNA was quantity was checked using a Nanodrop Spectrophotometer. In the next step, RNA was calculated based on RNA yield and then converted to first-strand cDNA. The expression of transporter genes at the mRNA level was amplified using a thermal-cycler (CFX96 Dx, Biorad-USA). The targeted candidate gene amplification was completed using the q-RT-PCR program at 95°C for 3 min, 40 cycles at 95°C for 5 s, 60°C for 30 s. Candidate gene expressions were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), where *Actin* was used as internal control. Total of three individual replications were taken for each treatment.

4. Statistical analysis

Total data were analyzed by analysis of variance (ANOVA). The significant level of the analyzed data was considered at $p \leq 0.05$. The graphical data were prepared using GraphPad Prism software (V.8.0.2). At least three biological replicates were considered for each treatment.

Ⅲ. RESULTS AND DISCUSSION

This study implies new insights of LED light quality mediated Fe deficiency protection in alfalfa plants. We found light Fe deficiency significantly caused leaf chlorosis, photosynthesis, and biomass yield. The leaf chlorosis induced and photosynthesis, and biomass yield were declined in alfalfa under white and red

lights. However, the optimum blue LED light quality significant protects alfalfa plant form Fe deficiency, and maintain required Fe demand during plant growing stage. The following stages we described the obtained results and their interpretation, and discussion.

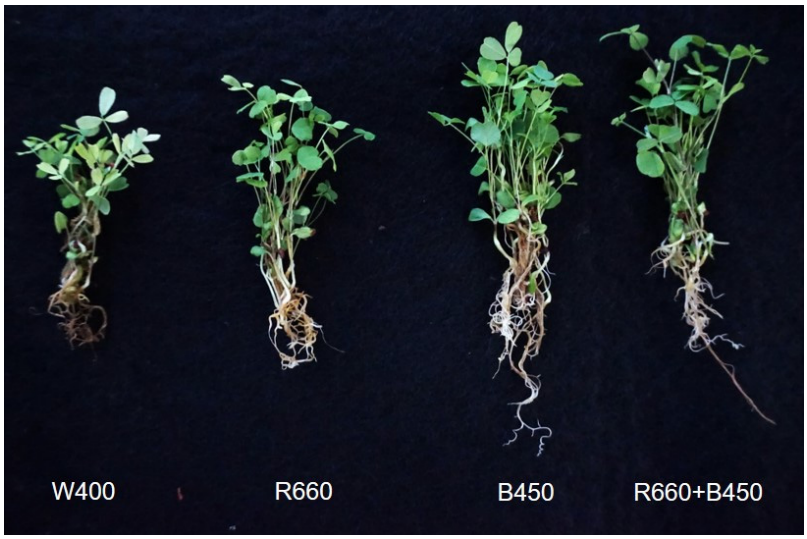

Fig. 1. Phenotypic alteration in alfalfa. The 2-week-old alfalfa plants show phenotypic differences after exposing to different LED light quality. Abbreviation, W400, white LED (λmax: 400); R660, red LED (λmax: 660); B450, blue LED (λmax: 450); R660+B450, combination (1:1) of red LED (λmax: 660) and blue LED (λmax: 450).

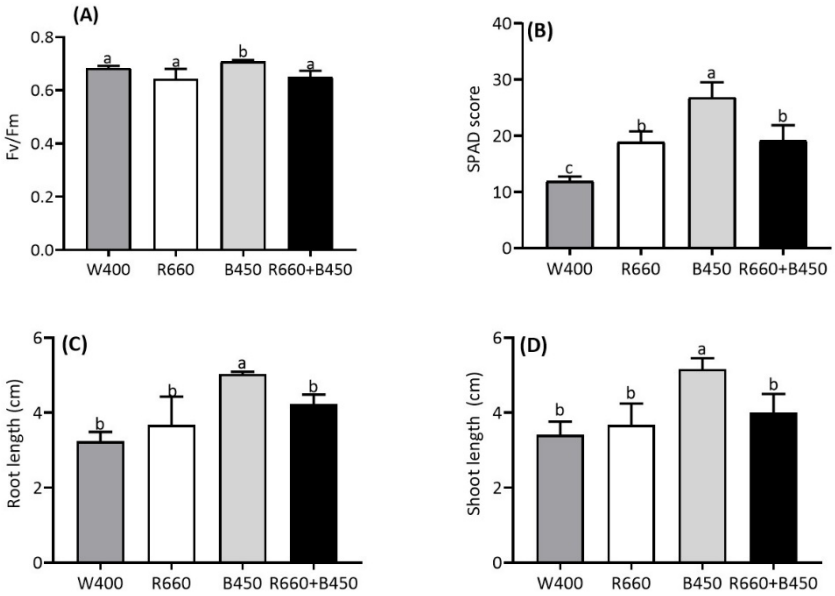

Fig. 2. Alteration of photosynthesis, chlorophyll content and root-shoot growth. The 2-week-old alfalfa plants show physiological and growth differences after 2 weeks exposure of different LED light quality. Fv/Fm (A), chlorophyll content present as leaf greenness (B), root length (C), and shoot length (D). Abbreviation, W400, white LED (λmax: 400); R660, red LED (λmax: 660); B450, blue LED (λmax: 450); R660+B450, combination (1:1) of red LED (λmax: 660) and blue LED (λmax: 450).

1. Fe deficiency caused phenotypic and physiological changes in alfalfa

Iron (Fe) deficiency significantly changed the morphological alteration in alfalfa in response to different LED light intensity (Fig. 1). The leaf chlorosis was higher at W400, and R660 compared to B450 and combined treatment. The highest leaf chlorosis occurred at W400, while it was protected by B450 (Fig. 1). As a consequence, the photosynthesis performance and chlorophyll content were significantly declined during W400 and R660 light exposure, while these traits were significantly improved by B450 (Fig. 2A and B). Fe has significant role in regulating of plant photosynthesis, respiration and DNA biosynthesis processes in plants (Kabir et al., 2023). In our study, we clamed leaf chlorosis at W400 and R660 due to these light intensities, which are not suitable for Fe acquisition in plant during growth stages.

2. Fe deficiency declined alfalfa growth and biomass yield

Fe deficiency with unfavorable LED light quality significantly effects on alfalfa growth and biomass yield. As we found the root-shoot length was significantly declined due to plant exposure at W400 and R660, while B450 significantly maintained normal growth of alfalfa (Fig. 2C and D). As a consequence, the plant

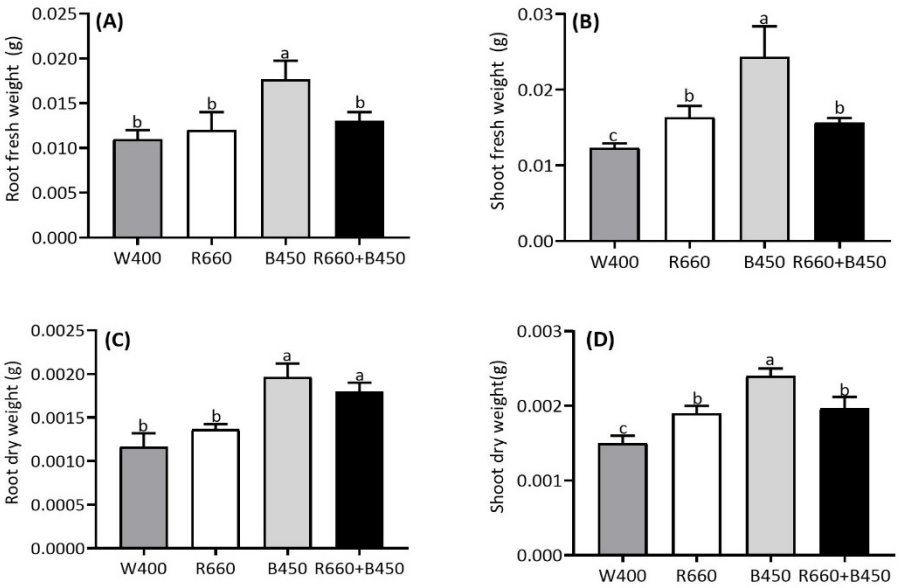

Fig. 3. Changes of biomass yield in alfalfa seedlings. The 2-week-old alfalfa plants show biomass yield differences after 2 weeks' exposure of different LED light quality. Root fresh weight (A), shoot fresh weight (B), root dry weight (C), and shoot dry weight (D). Abbreviation, W400, white LED (λmax: 400); R660, red LED (λmax: 660); B450, blue LED (λmax: 450); R660+B450, combination (1:1) of red LED (λmax: 660) and blue LED (λmax: 450).

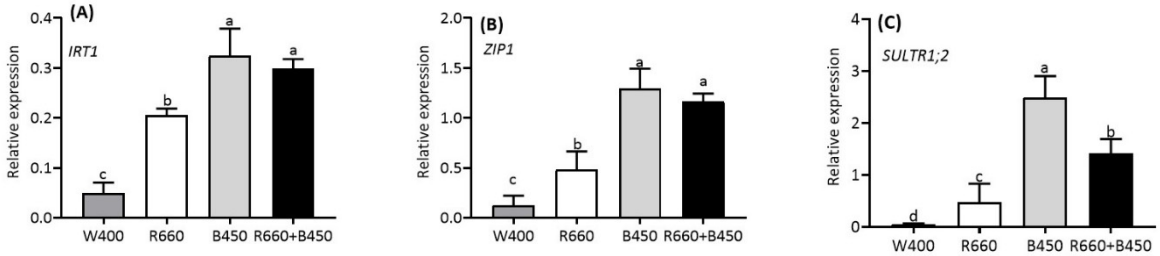

Fig. 4. Expression of iron responsive candidate genes in alfalfa roots. The 2-week-old alfalfa plants show biomass yield differences after 2 weeks' exposure of different LED light quality. *IRT1* (A), *ZIP1* (B), *SULTR1;2* (C). Abbreviation, W400, white LED (λmax: 400); R660, red LED (λmax: 660); B450, blue LED (λmax: 450); R660+B450, combination (1:1) of red LED (λmax: 660) and blue LED (λmax: 450).

biomass yields were significantly reduced by Fe deficiency with unfavorable LED light quality, while B450 and combined light quality significantly enhanced these attributes (Fig. 3A-D). The decline of plant biomass yield by multiple stress stimuli is a common phenomenon in legumes and other crop plants (Chauhan et al., 2022; Rahman et al., 2022c; Islam et al., 2023). However, the finding of plant biomass yield reduction due to unfavorable LED light quality is not extensively reported in plants. Thus this information with be helpful for LED-light-mediated plant improvement research.

3. Blue light enhanced Fe, Zn and S-transporter genes, which overcomed Fe-deficiency in alfalfa

The expression of Fe, Zn and S-transporter genes *IRT1, ZIP1* and *SULTR1* were significant declined due to Fe deficiency and unfavorable LED light quality of W400, and R660 (Fig. 4A-C). However, these candidate gene expressions were significantly enhanced in alfalfa plants exposure to B450 and combined light. It has been reported that nutrient transporters including zinc (Zn) and sulfur (S) transporters such as *ZIP1* and *SULTR1;2* coordinate the acquisition, transport, signaling, and interacting pathways for Fe, Zn, S, N, P at molecular level (Kumar et al., 2021). In our study, Fe, Zn and S genes (*IRT1, ZIP1* and *SULTR1;2*) were declined following Fe deficiency and unfavorable LED light quality of W400, and R660. These findings reveal that Fe deficiency with unfavorable LED light surely hinders the Fe, Zn and S, which may affect the Strategy I responses of alfalfa due to Fe-deficiency and low LED light quality.

Ⅳ. CONCLUSIONS

This current study adds to our new understanding of the mechanistic insight of Fe regulation and effect of different light quality in this process in alfalfa. The Fe deficiency and unfavorable LED light quality of W400 and R660 significantly declined alfalfa growth, photosynthesis, chlorophyll and gene expression of *IRT1, ZIP1* and *SULTR1;2*. However, the B450 light, and combined light quality (red and blue) significantly enhanced the expression of these genes in alfalfa roots to overcome Fe deficiency. The alfalfa improvement and Fe deficiency alleviation in alfalfa by B450 light quality will encourage us for further LED-mediated plant improvement research. This insight might be useful to forage breeders and farmers for alfalfa improvement through breeding program and metabolite engineering in other forage species.

Ⅴ. ACKNOWLEDGEMENTS

This study was supported by the RDA Fellowship Program of National Institute of Animal Science, and Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01592501), Rural Development Administration, Republic of Korea.

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(Received : September 18, 2023 | Revised : September 26, 2023 | Accepted : September 26, 2023)