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Effect of Light Quality on In Vitro Growth and Flowering in *Perilla frutescens*

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Summary

This study investigated the effects of light quality on in vitro growth and flowering in *Perilla frutescens* (Crispa type). In this experiment, three cultivars, 'Hoko Ao-shiso', 'Hoko Aka-shiso' and 'Hoko Uraaka-shiso' were used, and each seedling of them was raised in a test tube dispensed the medium (1/2 MS medium, 30g/L sucrose, 8g/L agar, pH 5.8) from the seed. Then they were cultured under different light quality conditions (Mixed White (W): Red + Green + Blue, Blue (B): λ = 470 nm, Green (G): λ = 525 nm, Red (R): λ = 660 nm). Seeds were sterilized with 70 % ethanol for 5 minutes followed by 5% sodium hypochlorite (NaClO) for 5 minutes. Four light treatments (W, B, G, R, PPFD = 100μmol $m^{-2}s^{-1}$, 16 hr-Light/8 hr-Dark) started after appearing two unfolded true-leaves under the cool-white fluorescent lamps (PPFD = 130μmol $m^{-2}s^{-1}$, 16 hr-Light/8 hr-Dark). After the 3 months of culture, measured the growth and flowering responses. 'Hoko Ao-shiso' were flowered only under the G light treatment. 'Hoko Aka-shiso' and 'Hoko Uraaka-shiso' were flowered under the W, G and R light treatments respectively. Flowering was not occurred under the B light treatment. This is the first report, that *Perilla frutescens* in vitro flowering depending on light quality under non-inductive photoperiod (long day conditions). Hence, it is possible to analyze the flowering reaction of perilla by

light quality, excluding stress-induced flowering by in vitro experimental system.

INTRODUCTION

Flowering is a critical event in the plant life cycle, referring the transition from vegetative to reproductive growth. The process is regulated by a complex mechanism, which varies between plant species (Amaki and Watanabe, 2016). Research on the control of flowering has important implications for the horticulture industry, including improving yield and achieving early flowering.

Perilla frutescens L. is an annual herb in the Lamiaceae family, with its leaves, flowers, and seeds commonly used for food, medicine, and dyeing. It is a qualitative short-day plant, meaning that it grows vegetatively under long day condition and transitions to reproductive growth under short day condition. The critical day length of the perilla is 14 hours and 15 minutes (Takimoto and Ikeda, 1961). Due to its high responsiveness, perilla plant has been used to investigate the florigen and the control mechanisms of flowering.

Light quality is an important factor in plant morphogenesis, serving as one of the environmental signals. Previous studies have shown that blue light inhibits perilla flowering, regardless of the cultivars, while flowering occurs under green light in long day conditions (Kawana, 2010; Tadokoro, 2014; Ayata, 2021). However, perilla has been observed the flowering under long day condition by various environmental stresses, including nitrogen deficiency (Wada and Totsuka, 1982) and low light intensity (Wada et al., 2010). Therefore, it was necessary to confirm the flowering response to light quality by constructing an experimental system that excluded those environmental stress factors.

In vitro experiments provide a stable experimental system, which has been used to study the gene functions in various plants by clarifying the effects of changes in gene expression under constant cultivation conditions. In vitro flowering has been confirmed in perilla by adding plant growth regulators to the tissue culture medium (Zhang, 2007). However, there is no report on the effects of light quality on flowering under the in vitro experimental system. Hence, the purpose of this study was to investigate the effects of light quality on the growth and flowering of perilla under the in vitro experimental system.

MATERIALS AND METHODS

Seeds of *Perilla frutescens* L. 'Hoko Ao-shiso' (“Ao”), 'Hoko Aka-shiso' (“Aka”) and 'Hoko Uraaka-shiso' (“Ura”) obtained from Nakahara Seed Corporation. The perilla seeds were sterilized with 70% ethanol for 5 minutes, followed by 5% sodium hypochlorite (NaClO) for 5 minutes. The sterilized seeds were then washed three times for 1 minute with sterilized water. The half strength of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), which contained 30 g/L sucrose, 8 g/L agar, and was adjusted to pH 5.8. Twenty mL of the medium was dispensed into each of a flat-bottomed test tube (ø 40×130 mm) and sealed with aluminum foil (8×8cm). The test tubes were then autoclaved at 121℃ for 15 minutes. The sterilized seeds were sowed on the surface of medium and cultured under fluorescent lamps (FLR40S･EX-N/M-H, TOSHIBA Lighting & Technology Corp.) provided a photosynthetic photon flux density (PPFD) of 130 μmol $m^{-2}s^{-1}$ for 16 hours of light and 8 hours of darkness at 24 ± 1℃ until the seedling stage with two unfolded true leaves. The seedlings were cultured under different light qualities using light emitting diode (LED) panel light source

(ISL series, CCS Inc.) including Mixed White (W: Red + Green + Blue), and the 3 types of monochromatic LED, Blue (B) (λ = 470 nm), Green (G) (λ = 525 nm) and Red (R) (λ = 660 nm). Each LED provided 100 μmol $m^{-2}s^{-1}$ PPFD. After three months of culture, the growth and flowering responses were measured. All the data were analyzed for the statistical significance using analysis of variance (ANOVA) with mean separation by Tukey's multiple range test. Statistical analyses were performed using RStudio (R ver.4.1.2).

RESULT AND DISCUSSION

The results of this experiment were shown in **Figure 1**. Plant height tended to be higher under blue light (B) treatment regardless of cultivars. This tendency for B treatment to promote stem elongation was consistent with the results of previous studies (Kawana, 2010; Tadokoro, 2014; Ayata, 2021). The numbers of stem node and alive leaf showed the highest value in "Aka" under B treatment. No significant differences were observed between other treatments. The reason for the cultivar difference was thought to be that the growth rate of "Aka" was accelerated by B treatment compared to other cultivars (**Fig. 1**).

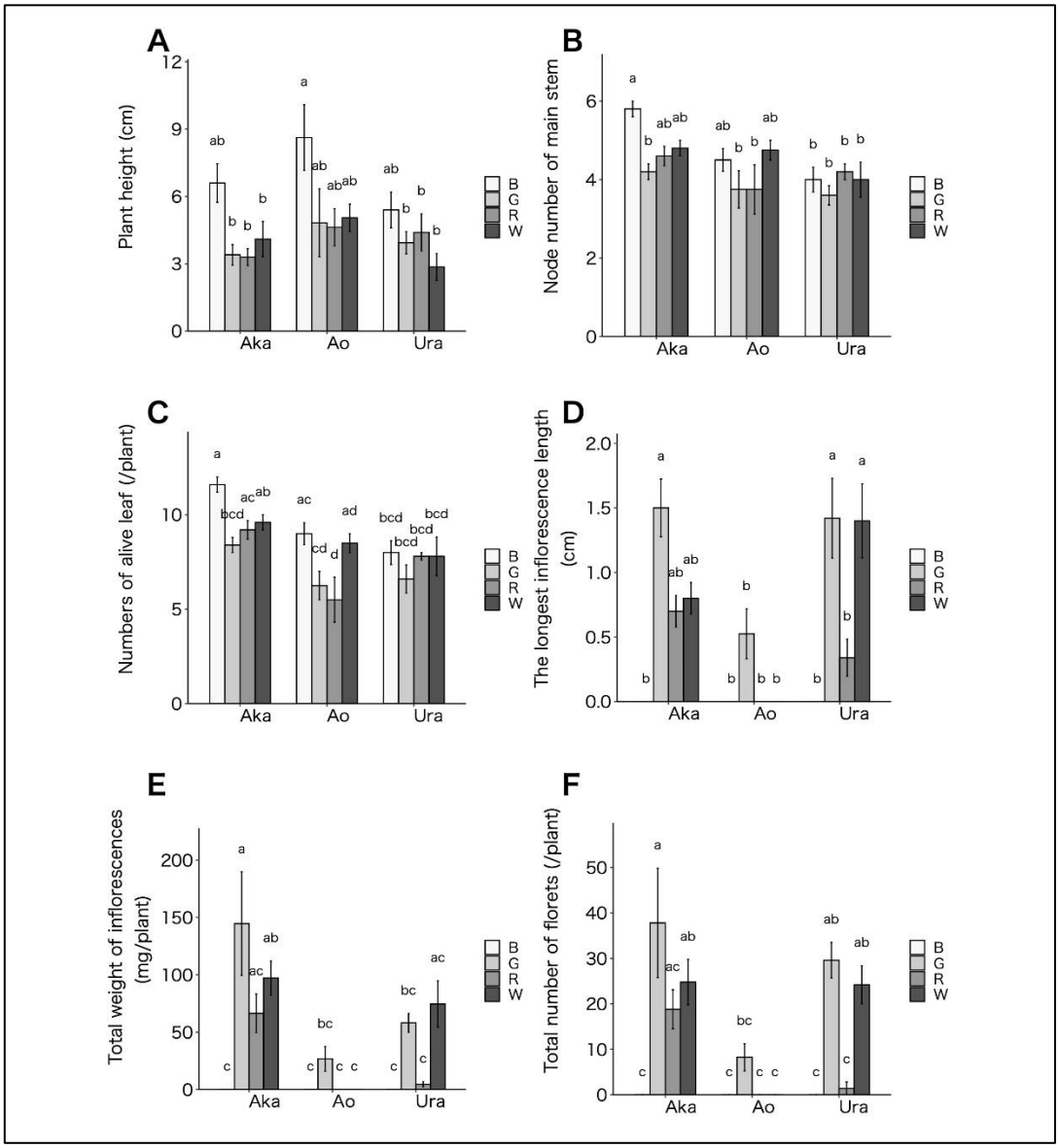

Figure 1. Effect of light quality on the growth and flowering of perilla under in vitro environment. Plant height (A), number of stem nodes (B), number of alive leaves (C), inflorescence length (D), total

inflorescence weight (E), total number of floletss (F). “Aka” indicates 'Hoko Akashiso' (n=5), “Ao” indicates 'Hoko Aoshiso' (n=4), “Ura” indicates 'Hoko Uraakashiso' (n=5). Tukey's multiple range test showed a significant difference at the 5% level between different alphabets (error bars are standard error).

All perilla cultivars used in this experiment showed a tendency to increase under green light (G) treatment for inflorescence length, total weight of inflorescence and total number of florets. Flowering reactions were observed in “Aka” and “Ura” under mixed white light (W), G and red light (R) treatments, but flowering reactions were observed in “Ao” only under G treatment. From these results, in cultivar “Ao”, the lowering was strongly suppressed under all light quality conditions under long-day (non-inductive flowering) conditions compared to the other two cultivars. Therefore, it was revealed that cultivar “Ao” exhibits the strongest flowering suppression response among the three cultivars used under the long-day conditions. On the other hand, cultivar “Aka” exhibited a stronger flowering response than cultivar “Ura” in the G and R treatments, indicating that “Aka” is a cultivar that is less susceptible to flowering suppression due to the long day conditions.

Seed formation was observed in all flowering perilla plants. The size of the seeds was comparable to commercially available seeds, and it appeared possible to propagate them through auto-self-pollination under the in vitro conditions (**Fig. 2**).

Figure 2. Comparison of 'Hoko Aoshiso' seeds collected by in vitro auto-self-pollination and commercially available seeds. Left: Commercially available seeds. Right: Seeds collected by in vitro auto-self-pollination.

In this study, in line with findings from prior research (Ayata, 2021; Nguyen and Oh, 2021), blue light was found to promote vegetative growth and suppressing flowering responses, both in vitro and in potted cultivation. Furthermore, under the monochromatic green light, flowering responses were clearly promoted compared to other treatments of light quality. These findings suggest the possibility of replicating perilla's photomorphogenesis within an in vitro environment, with the potential to eliminate environmental stress-induced flowering reactions through precise control of the in vitro conditions. Additionally, since successful seed formation through auto-self-pollination in vitro was achieved, it is anticipated that differences in responses to among lots of perilla seed can be effectively mitigated.

In conclusion, the use of in vitro experimental system offers the opportunity to investigate perilla's flowering response in greater detail by eliminating environmental stress-induced flowering and reducing the influence of seed lot variations.

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