New insights on flowering of *Cannabis sativa*

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Abstract

The main product of medical cannabis is the female inflorescence with thousands of glandular trichomes that produce and accumulate bioactive specialized metabolites. Although non-scientific public knowledge is largely available, in-depth research of cannabis flowering is limited. Growth under long photoperiod is considered "vegetative", however, the development of solitary flowers in the shoot internodes during that stage clearly indicates that the plant is not vegetative nor non-inductive in the classical sense. Nevertheless, a short photoperiod is required for the development of an inflorescence structure. This structure consists of the same basic phytomers that develop under a long day stage. Inflorescence development is characterized by a reduction in branchlets length, an increase in internode density and a clustering of solitary flowers. We demonstrate that the photoperiod signal induces inflorescence development within the first five days of short day exposure. Future understanding of the genetic and physiological mechanisms governing inflorescence development will lay the foundations for horticultural and biotechnological applications to modify the architecture and maximize plant productivity and uniformity in medical cannabis.

Keywords: florogenesis, inflorescence, photoperiod, cannabis, branching

INTRODUCTION

Global medical cannabis production is increasing every year. At the end of 2017 the value of the global cannabis industry output was $7.7 billion (https://www.prnewswire.com/news-releases). Cannabis taxonomy is not clear, and it is commonly accepted that it is a single species genus: *Cannabis sativa* L., consisting of three sub-species – *sativa*, *indica* and *ruderalis* (Small et al., 1976; Small, 2015; McPartland, 2018; Zhang et al., 2018). Each sub-species has its own typical characteristics, yet high levels of heterozygosity, inter-subspecies hybridization and long cultivation under specific conditions have resulted in the masking of the genetic origin of each sub-species or cultivar (McPartland, 2018). Therefore, clonal propagation of specific genotypes and chemotypes is employed in horticultural practice (Chandra et al., 2017). Cannabis is considered a dioecious plant, with the exception of a number of monoecious cultivars. Unfavorable growth conditions, stress and plant growth regulator application may result in sex inversion or in the development of hermaphroditic flowers (Hall et al., 2012). Since the main product of medical cannabis is unpollinated female inflorescence, research that focuses on florogenesis of female plants is of major horticulture and economical value.

Although non-scientific public knowledge on cannabis production is largely available, in-depth research on its flowering biology is limited. Cannabis is considered an obligatory short-day plant, since inflorescences develop following plant exposure to short photoperiod, of less than 14 h of light. Long day contributes to "vegetative" development (Cervantes, 2006; Hall et al., 2012). Recently we have shown that solitary flowers that develop in the leaf axes during the "vegetative stage" clearly indicate that the plant is already reproductive and that flowers are not initiated by day length (Spitzer-Rimon et al., 2019). However, a short photoperiod is required for the development of inflorescence. This process includes the intense branching and reduction of branch length and results in an increase in the density of

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the internodes and solitary flowers in the leaf axes. In addition, cultivar variability has been recorded in meristem differentiation and timing of inflorescence development (Spitzer-Rimon et al., 2019). The presented research demonstrates that, despite the variation between cultivars in their response to a short day, all examined cultivars react to the restriction of photoperiod to 12 h within the first five days. Our results will direct further analysis to determine the effects of a limited short photoperiod on quality traits of the cannabis inflorescence.

MATERIALS AND METHODS

Plant material and growth conditions

Three medical cultivars of *Cannabis sativa* L., NB130, NB140, and NB150 (Canndoc Ltd., Israel), were used in this study. Long photoperiod (LP) regime of 16/8 h (light/dark) was applied using MH bulbs (1,000 W) with a light intensity of 600 µmol m^{-2} s^{-1} (GrowLite Tru Blue, GrowLite Inc., Glendale, AZ, USA). Short photoperiod (SP) of 12/12 h (light/dark) was applied using 1000 W HPS bulbs with a light intensity of 1,000 µmol m^{-2} s^{-1} (Grow lite Real Red HPS). The plants were propagated from cuttings of female mother plants in a coconut fiber mixture. Mother plants and cuttings were grown under continuous LP. Rooted cuttings were transferred to 200-mL pots for 14 days and then to 2-L plastic pots, one cutting per pot, in a coconut/perlite growing mixture (Tuff Merom Golan, Israel).

In experiment 1 (Figure 1), cuttings were cultivated at LP for one week, and then moved to additional four weeks under SP. In experiment 2 (Figure 2), rooted cuttings were cultivated at LP for seven weeks. Thereafter, five plants from each cultivar were transferred to growth chambers under SP growth condition for five days and then transferred back to the original LP conditions, while the control plants remained in SP as in experiment 1.

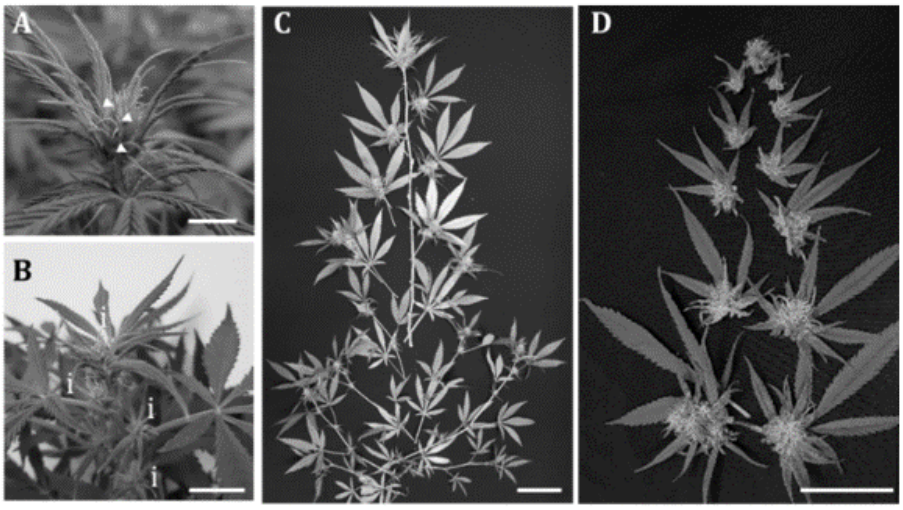

Figure 1. Simultaneous differentiation of the main and axillary inflorescences in medical cannabis female plants. (A) and (B) Shoot apex of 'NB150', grown under SP for two weeks. Visible inflorescence is defined by three couples of visible stigmata (arrows). i – primary and secondary inflorescences. Bars = 1 and 3 cm, respectively. (C) Architecture of the adult plant 'NB150', grown under SP for four weeks. Bar = 10 cm. (D) Close-up to the apical and secondary inflorescences of 'NB150', harvested at the same time as in C. Bar = 5 cm.

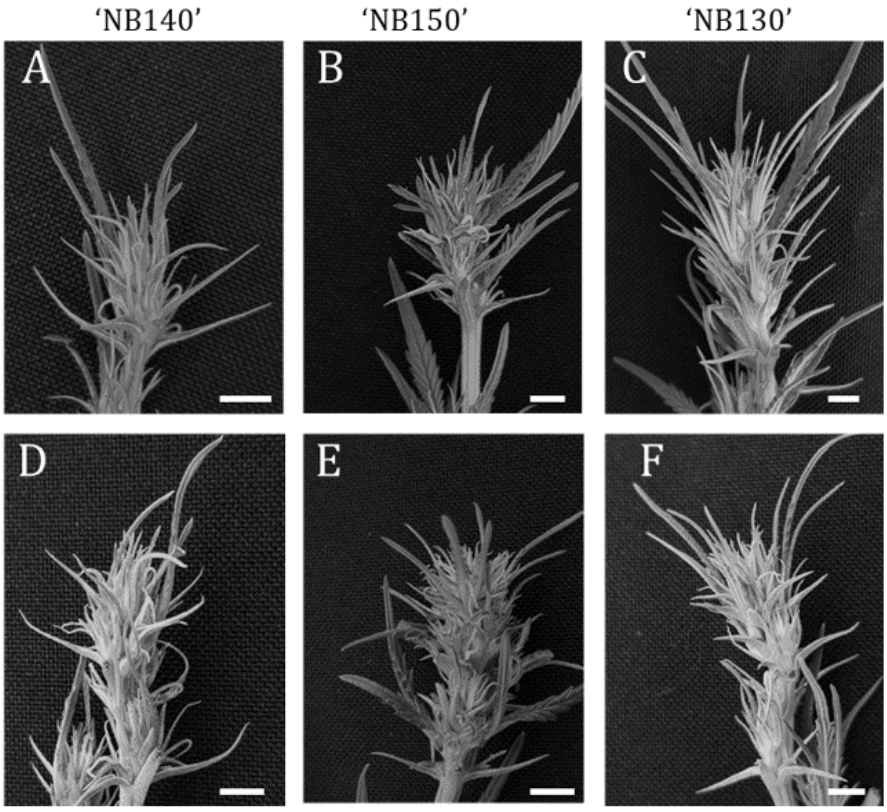

Figure 2. Short photoperiod for five days satisfies cannabis requirements for inflorescence development. (A, B, C) Plants grown under LP, transferred to SP for five days and then returned to LP. Pictures were taken nine days after the beginning of photoperiod manipulations. (D, E, F) Control plants that grown in LP and moved permanently to SP. Pictures were taken nine days after the beginning of photoperiod manipulation. Bars = 1 cm.

Inflorescence appearance was defined as a stage when at least three couples of stigmata at the apical part of the shoot became visible (Figure 1A-B; Spitzer-Rimon et al., 2019). Irrigation was supplied via 1 L h^{-1} discharge-regulated drippers (Plastro-Gvat, Kibbutz Gvat, Israel), one dripper per pot (Bernstein et al., 2019). The volume of irrigation was 500-800 mL pot^{-1} day^{-1}.

RESULTS AND DISCUSSION

Cannabis plants, propagated from the vegetative cuttings, develop solitary flowers in the leaf axes, and therefore can be defined as reproductive even when grown under LP (Spitzer-Rimon et al., 2019). Yet, inflorescence development is induced only under SP, with the appearance of flowers clustered in apical and axillary branches. In different cultivars, visible inflorescence appeared in the apical part after 8-12 days of cultivation at SP (Figure 1A-B, Spitzer-Rimon et al., 2019). Later on, during plant maturation, the flower clusters become visible in the branches up to the 3rd orders as well (Figure 1C-D). Each inflorescence section consists of condensed branchlets of higher orders (Spitzer-Rimon et al., 2019). Thus, the developed composed inflorescence assembles the branchlets and solitary flowers of several orders.

To evaluate the timing and dynamics of SP signaling perception by the apical meristems, we exposed plants to a LP for seven weeks, followed by exposure to SP for five days, and then plants were moved back to a LP. As a control, plants were grown at LP for seven weeks, and

then exposed to SP until the end of the experiment. Under all the experimental conditions, all plants went through the typical morphogenesis and developed an inflorescence (Figure 2A-F). Cannabis plant is considered a short-day plant (Chandra et al., 2017). As expected, mother plants of the same varieties under continuous LP did not develop an inflorescence.

Our results clearly indicate that signal perception for inflorescence development occurs within the first five days of exposure to a SP. In addition, it seems that all cultivars have a similar sensitivity to photoperiod. Despite the fact that SP application for only five days satisfied branching and inflorescence development, it may also be required for maintaining the intensive branching for longer periods. For a better understanding of the differences between cultivars in photoperiod perception, future research with additional time points is required.

CONCLUSIONS

Photoperiod affects various traits of cannabis growth, including accumulation of plant mass, branching, inflorescence differentiation and maturation. Morphogenetic changes, including branching and inflorescence initiation, occur simultaneously in both the apical and axillary shoots. While the critical day length and the dynamics of signaling and perception of short day may varies between cultivars' application of SP only for five days satisfied requirements for intense branching and inflorescence differentiation of all cannabis cultivars tested.

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