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What degree of light deficiency is suitable for saikosaponin accumulation by *Bupleurum chinense* DC.?

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ABSTRACT

Total saikosaponin yield in *Bupleurum chinense* DC. roots depends on the root biomass production and the saikosaponin content in roots, which are both affected by abiotic factors. *Bupleurum chinense* is relatively shade-tolerant. To test how total saikosaponin yield responds to light conditions, different light intensities (500, 200, and 50 μmol $m^{-2} s^{-1}$) were established in artificial climate incubators. Responses of plant morphological and physiological characteristics and of saikosaponin synthesis to different light intensities were measured to find suitable light conditions for improving total saikosaponin yield. Among these light conditions, moderately low light intensity (MLI, 200 μmol $m^{-2} s^{-1}$) produced plants with the highest leaf area, chlorophyll content, effective quantum yield of PSII (Φ_{PSII}), photochemical quenching coefficient (q_P), electron transport rate (ETR), maximum carboxylation efficiency (V_{cmax}), maximum electron transport rate (J_{max}), and triose phosphate utilization rate (V_{TPU}), which suggests a well-functioning photosynthetic apparatus capable of fully utilizing the limited light energy. Moreover, the plants had the highest root–shoot ratio, saikosaponin content, and total saikosaponin yield, which indicates that they allocated more resources to storage and defensive adaptations. Under extremely low light intensity (ELI, 50 μmol $m^{-2} s^{-1}$), more energy was partitioned to photoprotection, and strong oxidation resistance developed, but saikosaponin accumulation and root biomass were lowest due to greatly reduced carbon assimilation. The MLI improved both the quantity and the quality of herbal extracts from *B. chinense*: the total saikosaponin yield was 1.3 times the corresponding value of the control (500 μmol $m^{-2} s^{-1}$), and lower illumination levels were counterproductive. Our results suggest that *B. chinense* would be suitable for introducing to an agroforestry system.

1. Introduction

Chai hu (also known as *bupleuri radix*) is one of the most important raw materials in traditional Chinese medicine, and has been used for at least 2000 years (Chen et al., 2011). According to the Pharmacopeia of the People's Republic China, dried roots of *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. are the only two authentic sources of *chai hu*, and are used to treat the common cold, fever, influenza, menstrual disorders, alternating chills and fever, and rectocele (Chinese Pharmacopoeia Commission, 2010). The pharmacological activity of *chai hu* depends mainly on the content of saikosaponins, which are the principal bioactive components isolated from *chai hu* (Tian et al., 2009). In recent years, interest in saikosaponins has been increasing because of the anti-tumor potential of these compounds. In addition, recent research suggests that saikosaponins, especially saikosaponins a and d, exhibit significant immune-regulation, neuro-regulation, anti-inflammatory, anti-allergic, and anti-viral activity. Because of the pharmacological importance of saikosaponins, demand for *chai hu* remains high in Asia (Yuan et al., 2017). In China alone, more than 8×10^6 kg of *chai hu* are sold domestically or exported each year (Zhu et al., 2009a). The growing demand for high-quality *chai hu* is leading to overexploitation of the resource and destruction of wild habitats. As a result, the supply of wild *B. chinense* has decreased rapidly (Szakiel et al., 2011). However, efforts to produce saikosaponins at high yields through tissue culture have failed; the biomass growth and saikosaponin production are generally low, and it would be difficult to scale up this approach (Aoyagi et al., 2001; Murthy et al., 2014). This has created an urgent need to apply biological and agricultural technologies to replace the wild plant resources with managed agricultural resources.

Field-grown *B. chinense* seems likely to remain the main source of saikosaponins. Unfortunately, the quality of cultivated field-grown *B. chinense* is unsatisfactory (Tan et al., 2008). Thus, agronomists are seeking a new planting pattern that will improve the quantitative and qualitative yield. To date, studies of *B. chinense* have mainly focused on

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the composition, type, structure, pharmacology, and toxicological properties of saikosaponins (Ebata et al., 1996; Yuan et al., 2017). However, there has been little research on the influence of external and intrinsic factors on saikosaponin accumulation. The level of saikosaponins can be influenced by internal factors, such as plant age, growth and development stage, and the part of the plant, and by external factors, such as light intensity, water availability, soil nutrient contents, temperature, herbivory, and competition with neighboring plants. These factors influence not only the quantity, but also the composition (quality) of the saikosaponins, and ultimately affect the pharmaceutical properties. Saikosaponins are secondary metabolites that play an important role in defense against stresses and in interactions with the plant's environment, so proper levels of environmental stress can stimulate saikosaponin metabolism (Agrelli et al., 2003; Golawska et al., 2006; Szakiel et al., 2011). Understanding the physiological and biochemical mechanisms of how saikosaponin metabolism and accumulation respond to environmental stresses is important for developing more effective methods of cultivation capable of increasing the quantity and enhancing the quality of the saikosaponins produced under cultivation (Zhu et al., 2009a).

Light is the major environmental component that affects the survival rate, morphological traits, photosynthetic characteristics, and primary and secondary metabolism of plants (Niinemets et al., 2015). In China, wild plants of *Bupleurum* L. species tend to grow in sunny areas, but some are also found in forest understories or on shady river banks (Wang et al., 2011). However, research in the Dongling Mountains, one of the natural habitats of *B. chinense*, has shown that shaded plants develop larger leaves and improved photosynthetic performance, thereby increasing survival rates despite the limited light. In addition, these results indicate that light deficiency can stimulate saikosaponin synthesis (Gong et al., 2017). Traditional field planting does not provide sufficiently shady conditions for optimal saikosaponin production. Agroforestry offers a potential alternative, since it combines the planting of trees or shrubs (which can provide shade) with the planting of herbaceous species to improve the crop's quality, increase carbon sequestration, increase biodiversity, and conserve soils and water; as a result, this approach is increasingly widely used in agriculture and reforestation (Montagnini and Nair, 2004). To improve the quality of *B. chinense*, application of agroforestry systems may therefore narrow the quality gap between plants produced by cultivation and wild plants. Unfortunately, inappropriate application of agroforestry systems can decrease the crop quality. If the canopy layer blocks too much light, the growth and development of understory plants are inhibited (Gao et al., 2013). Low light intensity directly controls the efficiency of energy acquisition, and therefore constrains carbon accumulation and the primary and secondary metabolism of plants (Zavala and Ravetta, 2001; Hazrati et al., 2016). Managing *B. chinense* within an agroforestry system that offers suitable light levels could enhance the crop's pharmaceutical value, lower the cost of refining the plants to extract the active ingredients, and increase the economic benefits, while also reducing the pressure on wild populations (Kørup et al., 2017). However, the most appropriate light intensity to optimize saikosaponin accumulation is not clear.

Synthesis of saikosaponins plays a vital role in defending plants and protecting them against the oxidative stresses that accompany many kinds of biotic and abiotic stresses (Shohael et al., 2006). In plant cells, photosynthesis and aerobic metabolism unavoidably produce reactive oxygen species (ROS) as a byproduct, so plants have evolved antioxidant systems, including antioxidant enzymes and non-enzymatic antioxidants, that eliminate ROS and create homeostasis (Apel and Hirt, 2004; Corpas and Barroso, 2017). Under stress, the equilibrium between producing and scavenging ROS is perturbed; ROS production increases, leading to damage to biological macromolecules (e.g., DNA, lipids, proteins) and plant cells; in response, production of antioxidants increases to combat the increased oxidative stress. The content of malondialdehyde (MDA), which is the end-product of lipid peroxidation, can be used as a biomarker of oxidative stress (Mittler, 2002; Shohael et al., 2006). Light deficiency can activate the antioxidant stress-response pathways, leading to increased saikosaponin content, but creates a trade-off between defense (increased synthesis of antioxidants and saikosaponins) and growth (decreased biomass accumulation), leading to decreased total saikosaponin yields (Gong et al., 2017).

In our previous field research in China's Dongling Mountains, we found pharmacological differences between *B. chinense* growing in the forest understory at the sunny and shady slopes, where the integrated value of photosynthetic photon flux density (PPFD) and photoperiod are 13 and 2.3 mol m^{-2} day^{-1} daily photosynthetic irradiance (DPI), respectively (Gong et al., 2017). In the present study, our goal was to build on this previous result by focusing on the effects of low light intensities on the responses of the morphological and physiological characteristics and on the responses of saikosaponin synthesis by *B. chinense* to different levels of low light, with the ultimate goal of finding the most suitable light intensity for saikosaponin accumulation in an agricultural or agroforestry production system. To accomplish those goals, we created a range of light intensities in artificial climate incubators based on the field measured DPI values of farmland in mountainous regions and natural habitats (forest understory at the sunny and shady slopes) of *B. chinense*. We then cultivated *B. chinense* under these light intensities and measured the morphological characteristics, physiological characteristics, characteristics of the antioxidant system, and saikosaponin yield. Our goals were to (1) describe the response of plant ecophysiological characteristics to the different light intensities and the plant's adaption mechanisms, (2) determine a suitable light intensity to promote saikosaponin accumulation, and (3) guide the development of an appropriate agroforestry system for commercial production of *B. chinense*.

2. Materials and methods

2.1. Plant materials and light intensities

A pot experiment was conducted from October 2016 to June 2017 at the State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, Beijing, China. *Bupleurum chinense* seeds harvested in September 2016 were obtained from Seed Administration of the Department of Agriculture of the People's Republic of China. The seeds were soaked in deionized water at 20 °C for 24 h, followed by surface disinfection with 0.01% mercuric chloride solution for 3 min, followed by rinsing 3 times with deionized water. The seeds were then placed on double filter paper beds in petri dishes for germination at a constant temperature of 20 °C in the dark. Fourteen days later, germinated seeds were sown in plastic pots (12 cm in depth, 13 cm in diameter) that contained 400 g of sand, vermiculite, and natural soil (9:5:5 v/v/v). Each pot was then sown with 10 germinated seeds. Undisturbed natural soil was collected at the study site used in our previous research (40°2′20″N, 115°29′27″E), at an elevation of 1580 m in the Dongling Mountains, Mentougou District, Beijing City, China. Fresh soil (soil pH 6.56 ± 0.12) was sieved through a 0.1-mm mesh, disinfected for 3 days with 3% formalin solution, and then air-dried (soil pH 6.50 ± 0.28). Seedlings were grown in artificial climate incubators with constant spectral quality. The environmental conditions were a light/dark regime of 13/11 h, day/night temperatures of 25/18 °C, and a relative humidity of 50%, under a photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}. The pots were watered regularly with quantitative Hoagland solution and deionized water (1:3 v/v) to maintain the soil water content at approximately 22% g/g (soil pH 6.43 ± 0.28). We established a total of 48 pots. After 30 days, we randomly divided the pots into three groups with different light intensities in artificial climate incubators (i.e., $n = 16$ per light intensity). The light/dark regime, day/night temperatures, light quality and relative humidity were remained unchanged. The light condition of