Industrial Crops and Products 99 (2017) 179–188

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Research paper

Effects of light deficiency on the accumulation of saikosaponins and the ecophysiological characteristics of wild *Bupleurum chinense* DC. in China

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 24 January 2017
Accepted 26 January 2017

Keywords:
Bupleurum chinense DC
Shade tolerance
Photosynthetic characteristics
Saikosaponin
Antioxidant system

ABSTRACT

Bupleurum chinense DC. is one of the most important traditional Chinese medicines. Among its primary pharmacologically active components, saikosaponins play a vital role. We investigated the ecophysiological characteristics, accumulation of secondary metabolites, and antioxidant system of wild plants at low light intensity in the forest understory at shady and sunny sites. Compared with plants at sunny sites, plants at the shady site were more abundant, had higher specific leaf area (SLA), a lower maximum electron transport rate (J_{max}), higher triose phosphate utilization (TPU), and higher chlorophyll *b* content (Chlb), but lower net photosynthesis (P_n), root and aboveground biomass, dark respiration rate (R_d), light compensation point (LCP), chlorophyll a to b ratio, carotenoid content, and nonphotochemical quenching (NPQ). These features suggest that under low light conditions, the species developed larger leaves to catch more light energy through light-harvesting complex II to increase its photosynthetic efficiency (its ability to use weak light) and photosynthetic electron transport, thereby improving carbon metabolism and survival. The maximum quantum efficiency of photosystem II was about 0.8 at both sites; thus, photoinhibition was not a major problem. Contents of saikosaponins a and d and the total saikosaponin content (SSa, SSd, and SS_{total}) were much higher at the shady site, accompanied by increased superoxide dismutase (SOD), catalase(CAT), and peroxidases (POD) activity and decreased malondialdehyde (MDA). This suggested that low light activates antioxidant stress-response pathways, leading to increased SSa, SSd, and SS_{total}. Our results suggest that *B. chinense* has strong shade tolerance and would be suitable for cultivation in an agroforestry system.

1. Introduction

Bupleurum L. is a genus in the Apiaceae. The species are perennial herbs that have been widely used in traditional Chinese medicine since ancient times. *Bupleurum chinense* DC. is a standard medicinal herb in the *Pharmacopoeia of the People's Republic of China* (Lee et al., 2011). Its dried root has been frequently used to treat the common cold and fever, influenza, inflammation, hepatitis, malaria, menstrual disorders, autoimmune diseases, and menopausal syndrome for 2000 years (Ushio et al., 1991; Pan, 2006; Saraçoğlu et al., 2012). These important pharmacological effects result mainly from the presence of many potentially pharmacologically active compounds from many classes of secondary metabolites (Ashour and Wink, 2011). Among them, the saikosaponins, and especially saikosaponins a (SSa) and d (SSd), are responsible for most of the pharmacological properties. Thus, saikosaponin content is one of the most important criteria for determining the quality of *Bupleurum* L. (Pan, 2006; Zhu et al., 2009a). Due to its medicinal importance, the demand for *Bupleurum* L. has increased steadily in recent years. About 8×10^6 kg of *Bupleurum* L. are used each year in China (Zhu et al., 2009a). The availability of wild *Bupleurum* species cannot meet the demand, so agriculture is becoming the main source for roots. Unfortunately, the root saikosaponin content is generally low. To meet the growing demand, it will be crucial to develop an efficient agricultural management strategy for the cultivation of plants with a high yield of saikosaponins. Agroforestry systems have been suggested as an effective cultivation method to improve the quality of many crops (Eibl et al., 2000). In Asia, and particularly in China, most *Bupleurum* species tend to grow in sunny areas, but some are also found in forests or on shady river

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http://dx.doi.org/10.1016/j.indcrop.2017.01.040
0926-6690/

banks (Wang et al., 2011). Thus, the species may be suitable for growing in an agroforestry system.

Plants can adjust their photosynthetic performance to compensate for deficiencies in their environment, but they can also adjust their morphological and physiological traits to maintain a homeostatic photosynthetic performance (Liang et al., 2010). These responses allow plants to survive and even to continue growing under adverse conditions. Studying their ecophysiological responses may therefore improve our understanding of how species adapt successfully to their environment, and will ultimately guide the selection of suitable habitats for maintenance or reintroduction of an endangered species. It will also provide a theoretical basis for the development of agricultural technologies.

The ecophysiological performance of plants results from the interaction of many complex processes that are influenced by a range of factors. These include micro-environmental factors such as solar radiation, temperature, and soil moisture, all of which greatly affect plant photosynthesis under field conditions (Johnson et al., 2004; Kayama et al., 2009; Zheng et al., 2012). Unfortunately, the effects of environmental conditions on the growth of wild *Bupleurum* species are unknown because there has been little research on these effects. In the past decade, research has focused on the pharmacological functions, taxonomy, chemical composition, and extraction and isolation of chemical components (Kusakari et al., 2011; Wang et al., 2011; Akın et al., 2012; Ashour et al., 2012; Benahmed et al., 2014).

The concentration of saikosaponins depends on the growth location, the time of harvest, and the part of the root (Szakiel et al., 2011), but can be also affected by environmental factors: light, temperature, water, seasonal changes, water availability, and climate (Fournier et al., 2003; Jochum and Mudge, 2007; Peng et al., 2009; Zhu et al., 2009a, 2009b). Light is a particularly important factor because it affects the formation of most plant products, including both primary and secondary metabolites, and regulates the secretion mechanisms of secondary products (Shohael et al., 2006). Low light intensity decreases plant productivity by affecting gas exchange, but it also limits the energy available for all metabolic activities. When carbon gain was limited relative to nutrient availability, the accumulation of secondary metabolites may increase to cope with the stress (Coelho et al., 2007; Hou et al., 2010). The synthesis of secondary metabolites by plants is a common part of the defense responses against various kinds of stress. For example, oxidative stress is associated with damage to stressed plants and plays an important role in the synthesis of secondary metabolites (Shohael et al., 2006; Zhu et al., 2009a). The antioxidant capacity of saikosaponins, which scavenge reactive oxygen species and inhibit lipid peroxidation, has been observed in pharmacological experiments (Wang et al., 2004; Liu et al., 2005). However, much less information is available concerning the regulatory mechanisms responsible for saponin biosynthesis in response to changing environmental conditions, mainly due to the difficult and laborious nature of such research.

Given these contexts, our goal in the present study was to fill in some of the gaps in our knowledge through field experiments. To provide this knowledge, we studied the ecophysiological characteristics, antioxidant system, and saikosaponin contents of wild *B. chinense* in the forest understory at the sunny and shady slope sites. Our goal was to provide an in-depth understanding of the ecophysiological characteristics and saikosaponin content of this species and provide a theoretical basis for domestication of the species and intensification of plant production in an agroforestry system. Specifically, our goals were to (1) identify the morphological and physiological adaptions of the species to low light, (2) clarify the relationships between saikosaponin content and biomass and their response to shade, and (3) explore whether the regulation of saikosaponin biosynthesis is related to the species' photosynthetic characteristics and antioxidant system.

Table 1
Environment characteristics of July and September in which *Bupleurum Chinese* DC. was studies.

Month	Temperature (°C)	Relative humidity(%)	PARphoton flux (mmol m^{-2} s^{-1})
July	32	54.47	1412
September	22.26	45.82	1345

2. Materials and methods

2.1. Study site and plant materials

The study site is located in the Dongling Mountains (40°00′N–40°05′, 115°26′E–115°40′E), near Beijing, China. It lies in the western part of Beijing (a provincial-scale administrative division) and is far from the city center. The mountains and forest provide important ecological services for Beijing and its citizens. The region has a typical warm temperate continental monsoon climate, with an average annual precipitation of 500–650 mm, with more than 70% falling during the growing season from June to August. The mean annual temperature is 7 °C, but the mean monthly temperature ranges between −7.8 °C in January and 21.1 °C in July (Zhang et al., 2012). The weather conditions on both July and September are shown in Table 1.

We chose two different sites (sunny and shady slopes) at the study site. Both locations were above 1100 m asl. The plant material was wild *B. chinense* growing in the forest understory.

2.2. Morphological traits and biomass

At both the study sites, we investigated the plant morphological traits in five randomly selected 10 m × 10 m quadrats at each site in July and September 2014. For all *B. chinense* in each quadrat, we measured plant height, the number of plants, and leaf area. In addition, we randomly selected and then excavated 10 individuals per quadrat to collect the roots. We divided the plants into leaves, shoots, and roots. Leaf area was determined with a leaf area meter (LI-3000A, LI-COR, Lincoln, USA). All of the samples were oven-dried to constant weight at 65 °C. Specific leaf area (SLA) was calculated as the ratio of leaf area to dry weight. The roots were used to determine the saikosaponin content.

2.3. Gas exchange and chlorophyll fluorescence

Measurements of gas exchange were conducted in the two plots in July and September 2014. Five plants were randomly chosen in each plot for each measurement period. The measurements were conducted on clear, cloudless days between 08:00 and 18:00 using an LI–6400 portable photosynthesis system (LI-COR). For these individuals, we measured the net photosynthetic rate ($P_{n,}$), transpiration (E), the intercellular CO_2 concentration (C_i), and stomatal conductance (g_s). We calculated the water-use efficiency (WUE) of each plant using the following equation:

$$WUE = P_n/E$$

We obtained photosynthetic light-response curves at 2-h intervals from 09:00 to 11:00 at a range of light intensities (from 0 to 2500 μmol m^{-2} s^{-1}) using the LI–6400 instrument. The irradiance response was measured at 25 °C and a relative humidity of 50%, with the CO_2 concentration at 400 μmol mol^{-1}. The photosynthetic photon flux density (PPFD) was set at 2500, 2000, 1500, 1000, 500, 200, 150, 100, 50, 20, and 0 μmol m^{-2} s^{-1}. CO_2 response curves were