Industrial Crops and Products 30 (2009) 220–226

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Growth, photosynthesis and root reserpine concentrations of two *Rauvolfia* species in response to a light gradient

Z.Q. Cai[a,*], W.H. Wang[b], J. Yang[b], C.T. Cai[a]

[a] Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
[b] Analytic and Testing Research Center of Yunnan, Kunming University of Science and Technology, Kunming, China

ARTICLE INFO

Article history:
Received 18 February 2009
Received in revised form 27 March 2009
Accepted 28 March 2009

Keywords:
Biomass allocation
Growth analysis
Rauvolfia
Reserpine
Photosynthesis

ABSTRACT

Growth and secondary metabolites responses to light environment can be useful measurements to determine favourable habitat conditions for the cultivation and conservation of medicinal plants. We analyzed the growth, photosynthesis and root reserpine concentrations in seedlings of *Rauvolfia vomitoria* Afzel and *Rauvolfia verticillatae* (Lour.) Baill, two important medicinal plants yielding anti-hypertension alkaloids, at four different light levels (20%, 52%, 75% and 100% of full sunlight) in a shade house. Across all light intensities, seedlings of *R. vomitoria* grew faster with higher relative growth rate (RGR) than *R. verticillatae*, attributed to its higher photosynthetic capacities (light-saturated photosynthetic rate, A_{max}) and leaf area ratio (LAR). Typical shade-sun morphological responses to increasing light levels included decreased specific leaf area and LAR, whereas RGR and A_{max} was highest at median light levels for both species. *R. vomitoria* allocated higher leaf mass fraction and fine-root mass fraction, similar coarse root mass fraction, but lower stem biomass fraction than *R. verticillatae*. For fine roots, *R. vomitoria* had greater specific root length and small diameter than *R. verticillatae,* indicating its higher resource (water and nutrition) capture abilities. Both species had higher nitrogen concentration and lower reserpine concentration in fine roots than those of coarse roots. Neither reserpine concentration nor nitrogen was affected by light intensities. The reserpine concentration in coarse roots of *R. vomitoria* increased, whereas that of *R. verticillatae* decreased with increasing irradiance. Although not significantly, reserpine concentrations in coarse roots were positively correlated with A_{max} and RGR for both species across all light intensities. Shade or photosynthesis inhibition apparently did not increase alkaloid synthesis, which contrasted with the carbon/nutrient balance theory of plant defense. These results suggested that *R. vomitoria* and *R. verticillatae* could attain high biomass and yield of reserpine in high-light habitats (with max. in 75% sunlight), and intermediate-light habitats (with max. in 25–52% sunlight), respectively.

1. Introduction

Light is one of the major environmental factors for plant growth, distribution and yield (Boardman, 1977; Poorter, 2005; Coelho et al., 2007; Wang et al., 2007). At the low end of the light gradient, plant relative growth rate (RGR) steeply increases with an increase in light and it attains its maximum at intermediate light levels, above which it declines again (Poorter, 1999). In addition, the ability of plants to capture and utilize resources (i.e., light, nutrients and water) is an important determinant of growth potential and fitness. Generally, inherently fast-growing species have a higher net assimilation rate (in leaf level, higher light-saturated photosynthetic rate, A_{max}) and/or higher leaf area per unit plant biomass (leaf area ratio, LAR) to harvest and use light efficiently than slow-growing species (Poorter, 2005). Moreover, among tree species, a faster growth rate is also associated with higher specific fine root length (SRL) and smaller root diameter which enable the tree to acquire more water and nutrients (Reich et al., 1998; Comas and Eissenstat, 2004).

Variation in environmental resources (e.g., light) could represent a significant factor on secondary metabolism in plant materials besides the growth response (Nina and Lerdau, 2003). These secondary metabolites (SM) are organic compounds that are not directly involved in the normal growth and development of organisms. They play a major role in defenses against herbivores (Ehrlich and Raven, 1964; Coley and Barone, 1996; Nina and Lerdau, 2003), and are frequently utilized in medicine as therapeutic agents.

Several theories have been proposed to explain potential trade-offs between growth and SM synthesis. The resource availability theory suggests that the way a plant defends itself ultimately depends on resource availability and its intrinsic growth rate (Coley et al., 1985; Strauss and Agrawal, 1999). This theory predicts that

* *Corresponding author*. Tel.: +86 6918715070; fax: +86 6918715070.
E-mail address: czq@xtbg.org.cn (Z.Q. Cai).

0926-6690/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2009.03.010

rapidly growing plants in resource-rich habitats contain low levels of highly mobile SM (e.g., alkaloids and cyanogenic glycosides). These plants exhibit biochemical and morphological plasticity to allow them to take advantage of pulses in resource availability. Nitrogen is taken up early in the growing season in excess of the plant's needs for growth. Excess N is available to be synthesized into N-based SM, which can be rapidly induced upon injury to protect the plant from herbivory (Mooney et al., 1983). The carbon/nutrient balance theory suggests if light becomes limiting (i.e., shade) in nutrient-rich environments, the decline in photosynthesis may limit carbohydrates for growth and C-based defenses (Bryant et al., 1983). Nutrient uptake continues, leaving excess N that could be shunted to N-based defense compounds, such as alkaloids. For example, some carbon-based SM (such as phenols or tannins, terpenes, etc.) decreased (Briskin and Gawienowski, 2001; Wang et al., 2007) and N-containing SM increased (Coelho et al., 2007) in leaves of medicinal plants with the decrease in light. Nevertheless, Kurata et al. (1997) found high irradiance enhanced N-containing SM production in non-photosynthetic tissues in *Coffea arabica*. On the other hand, the negative correlation is coherent with the thesis of a trade-off between growth and defense (Herms and Mattson, 1992). However, it is much less clear if potential RGR is necessarily correlated with chemical defense (Herms and Mattson, 1992; Almeida-Cortez et al., 1999). Differences in the growth trajectories of plants may also lead to both positive and negative correlations between intrinsic growth rate and the ability to compensate for herbivory (Stowe et al., 2000; Weis et al., 2000). Understanding how different growth rates and environmental factors affect the production of SM will be of great importance for the conservation of medicinal plants and optimizing field growth conditions for maximal recovery of phytomedicinal chemicals.

The aim of the present study was to evaluate the light response in seedlings of two *Rauvolfia* species, in terms of growth traits and alkaloid contents. The genus *Rauvolfia* consists of one hundred species found in many tropical and sub-tropical regions of the world. *Rauvolfia* is important from a medicinal point of view because of the presence of N-containing indole alkaloids, which are localized in the roots (Iwu and Court, 1977; Srivastava et al., 2006; Hu et al., 2008). Reserpine is the most prominent of these alkaloids, and is useful in the treatment of hypertension, cardiovascular diseases, nervous disorders and as a tranquilizing agent that is in great demand by modern pharmaceutical industries (Graham and Maxwell, 1954; Weiss and Fintelmann, 2000). The study focused on *R. vomitoria* and *R. verticillatae*, widely cultivated treelets in SW China (Feng et al., 1965). *R. verticillatae* is an endangered native species of China, usually found under the canopy of shade-tolerant species. *R. vomitoria*, introduced from Ghana, Africa, was cultivated in large scale plantations in SW China (Liu and Xiao, 1995). These species present high research priorities because of their valuable medicinal properties. Harvesting of wild medicinal plants can be problematic in terms of biodiversity loss and resources depletion. Therefore, the knowledge of their physiological and growth performance, especially their responses to light, is important for optimal cultivation and conservation programs.

The main objectives of this study were to determine: (1) the differences between the two species with respect to the responses to a light gradient in terms of biomass allocation, growth, and photosynthesis, (2) whether the introduced *R. vomitoria* exhibited higher resource capture ability and efficiency (i.e., higher SRL, LAR, A_{max}) and thus higher RGR than the native *R. verticillatae*, and (3) how root reserpine concentrations of two species varied with irradiance levels, in order to provide basic information for the conservation and improving yield of cultivated *Rauvolfia*.

2. Materials and methods

2.1. Study site and plant materials

The study was carried out in Xishuangbanna Tropical Botanical Garden (21°56′N, 101°15′E, 600 m asl), Chinese Academy of Sciences, Yunnan, SW China. The climate of Xishuangbanna is dominated by the southwest monsoon with a distinct dry season from November to April. The mean annual temperature is 22.9 °C and mean precipitation is 1500 mm, 80% of which falls in the wet season.

Seeds of *R. vomitoria* Afzel and *R. verticillatae* (Lour.) Baill. (Apocynaceae) were collected from the nursery of economic plants in Xishuangbanna Botanical Garden and were sown in June 2007, at the middle of the wet season. When the seedlings of the two species were approximately 8 cm tall, uniform seedlings were selected and each of them was transplanted to pottery pots (25 l in volume) containing topsoil from the nearby forest and then moved into a shadehouse. After 1 mo adaptation in 20% light, 15–25 randomly selected pots for each treatments were moved to each of four irradiance treatment: 20%, 52%, and 75% sunlight, and open site (100% sunlight), respectively. These irradiance levels were created using layers of neutral-density screen on a steel frame. To characterize light availability in the shadehouse, photosynthetic photon flux density (PPFD) was measured on clear days using a LI-190 SB quantum sensor (Li-Cor Lincoln, NE) positioned 10 cm above the ground and the relative light intensity was calculated. Seedlings were watered on days without rain to maintain the soil near field capacity, and fertilized monthly with 20 g NPK slow-release compound fertilizer (Osmocote, Scotts, Marysville, OH, USA) through the experiment. Four to five plants per species in each treatment were harvested and over dried at 70 °C for 2 d to determine the initial biomass (dry biomass ranged from 2.6 to 3.3 g) at the beginning of the experiment (October 2007). At the end of the experiment (after approx. 14 mo), physiological measurements were made on three or four plants per treatment. Morphological and biomass measurements were made for five to seven plants per treatment.

2.2. Leaf photosynthesis, seedling morphology and growth analysis

At the end of the experiment, gas exchange measurements were made on the uppermost, fully expanded, young sun leaves using a portable Li-6400XT photosynthesis system (Li-Cor, Lincoln, NE, USA) in open system mode at mid-morning, between 9 h and 11 h. Three to four plants per treatment were selected for photosynthetic measurements. Photosynthetic light-response curves (A-PPFD) were determined at irradiances between 2000 and 0 μmol m^{-2} s^{-1} using a built-in LED-B light source. During measurements, leaf temperature was kept at 25–27 °C; the CO_2 concentration (C_a) in the cuvette was held at 400 μmol mol^{-1} and the vapor pressure deficit (VPD) at less than 1 kPa, respectively. Photosynthetic light response curves were fitted using a nonrectangular hyperbola (Thornley, 1976). The non-linear regression coefficients of determination for each curve explained >95% of the variation. The asymptotic light-saturated rate of net photosynthesis (A_{max}), apparent quantum efficiency (AQE), dark respiration rate (R_d), light compensation point (LCP) and near-light saturation point (LSP) were calculated from these curves. After photosynthetic measurements, leaves were harvested and weighed after drying at 70 °C for 48 h. Total fine- and coarse-root nitrogen concentrations were measured on dry samples by semi-micro Kjeldahl using a wet digestion procedure.

In each treatment, five to seven plants were harvested and washed free of soil particles with tap water. The plants were separated into leaves, stems, coarse roots (diameter ≥ 1 mm) and fine roots (diameter < 1 mm). Subsamples of leaf and fine roots were