Industrial Crops and Products 86 (2016) 210–218

Contents lists available at ScienceDirect

Industrial Crops and Products

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

The bioactive monoterpene indole alkaloid *N*,β-D-glucopyranosyl vincosamide is regulated by irradiance quality and development in *Psychotria leiocarpa*

Hélio Nitta Matsuura[a,1], Variluska Fragoso[a,1,2], Juçara Terezinha Paranhos[b], Mariana Ritter Rau[a], Arthur Germano Fett-Neto[a,*]

[a] Plant Physiology Laboratory, Center for Biotechnology and Department of Botany, Federal University of Rio Grande do Sul (UFRGS), CP 15005, Porto Alegre, RS 91501-970, Brazil
[b] Department of Botany, Federal University of Santa Maria, Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 24 February 2016
Accepted 24 March 2016
Available online 2 April 2016

Keywords:
Alkaloid
Antioxidant
UV-B
Blue light
Far-red light
Stress tolerance

ABSTRACT

Leaves of *Psychotria leiocarpa* accumulate a major shoot-specific indole alkaloid, *N*,β-D-glucopyranosyl vincosamide (GPV), which has antioxidant, but no apparent antifeedant or allelopathic activity. The species is tolerant to high doses of UV-B, possibly due to constitutive GPV accumulation in leaves. In seedlings, GPV accumulation is induced by white light in a photosynthesis independent fashion. To better understand the regulation of the *in planta* GPV pool, detailed alkaloid profiles were examined. Light shift tests confirmed that GPV accumulation is light dependent. GPV was tightly regulated in its organ distribution, with highest concentrations in reproductive structures. Far-red and blue wavelengths promoted GPV accumulation without clear correlation with carbohydrate or protein concentrations or dry biomass in seedlings, thus indicating direct effects of light. Since light conditions are often associated with higher oxidative stress, light-induced accumulation of GPV may contribute to maintain redox balance. *In vitro*, GPV was an overall more effective antioxidant compared to closely related alkaloids. Indirect evidence of potential antioxidant properties of GPV *in vivo* was obtained by application of GPV on leaves of UV sensitive species exposed to high doses of UV-B, which significantly improved tolerance to this stress. In contrast to the known constitutive accumulation of GPV in leaves, the alkaloid concentration proved to be highly dynamic, changing during development, reproductive organogenesis and seedling irradiance treatments. Data further support a role for GPV as an oxidative stress protectant and provide a means to improve alkaloid yields in plant biomass for pharmacological applications.

1. Introduction

Plant alkaloid metabolism is often influenced by development and environmental factors, including light quantity and quality (Vazquez-Flota and De Luca, 1998; Liu et al., 2015). *N*,β-D-glucopyranosyl vincosamide (GPV) is a major shoot specific *N*-glycosylated monoterpene indole alkaloid (MIA) accumulating in *Psychotria leiocarpa* (Henriques et al., 2004). GPV has ROS quenching activity, which may protect the plant against oxidative burst caused by stressful conditions, possibly contributing to the significant tolerance of the species to acute high dose UV-B treatment (Matsuura and Fett-Neto, 2013). In vegetative adult plants, GPV content is relatively high and stable; alkaloid steady-state does not seem to respond to unfavorable environmental conditions such as high UV-B or mechanical wounding. Besides, no antifeedant (Matsuura and Fett-Neto, 2013) or allelopathic effects (Correa et al., 2008) have been observed for GPV. In contrast, GPV accumulation in etiolated *versus* photomorphogenic seedlings is higher in the latter, apparently without a direct involvement of photosynthetic energy supply (Henriques et al., 2004).

Abbreviations: CRY, cryptochrome; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DW, dry weight; FW, fresh weight; GPV, *N*,β-D-glucopyranosyl vincosamide; HPLC, high performance liquid chromatography; MIA, monoterpene indole alkaloid; NBT, nitro blue tetrazolium; PAR, photosynthetically active radiation; PHYA, phytochrome A; PHYB, phytochrome B; ROS, reactive oxygen species; TFA, trifluoroacetic acid; UV, ultraviolet radiation; UV-A, ultraviolet A radiation; UV-B, ultraviolet B radiation; VLFR, very low fluence response.

* Corresponding author.
E-mail address: fettneto@cbiot.ufrgs.br (A.G. Fett-Neto).

[1] These authors contributed equally to this work.

[2] Current address: Department of Molecular Ecology, Max-Planck-Institute for Chemical Ecology, Jena, Germany.

http://dx.doi.org/10.1016/j.indcrop.2016.03.050
0926-6690/

P. leiocarpa is highly abundant in its habitat, the understorey of the Atlantic Forest biome, distinguished from unrelated plants by having small white flowers and purple-blue berries when mature. *Psychotria* is the largest genus in Rubiaceae and since conspicuous distinguishing morphological features are not common among its species, indole alkaloids constitute an important tool in chemotaxonomy, given their high diversification (Van de Santos et al., 2001). Although present in high concentrations in some plants (*e.g.* psychollatine from *Psychotria umbellata* reaching 4% DW in inflorescences), the main role of MIAs in these species is not clear, but seems to involve the metabolic redox balance in relation to several environmental stresses (Matsuura et al., 2014).

In spite of being an understorey tree, *P. leiocarpa* is surprisingly tolerant to acute UV-B stress, which coupled to GPV *in vitro* antioxidant properties (Matsuura and Fett-Neto, 2013), suggests a role for the alkaloid in this phenotype. Detailed understanding of GPV metabolism in response to light and developmental signals may help not only to elucidate its main *in planta* role, but also allow the establishment of optimal protocols for harvesting GPV-rich biomass or eliciting its accumulation prior to extraction and isolation, with a view towards pharmaceutical uses of this alkaloid.

Brachycerine, a closely related leaf alkaloid from *Psychotria brachyceras* that has antioxidant and antimutagenic properties (Nascimento et al., 2007), is induced by approximately 10-fold compared to its basal level upon acute UV-B exposure (Gregianini et al., 2003), involving early changes at mRNA level (Nascimento et al., 2013). Unlike brachycerine and similar to GPV, psychollatine (another related alkaloid with similar properties from *P. umbellata*) is not induced by acute UV-B exposure (Fragoso et al., 2008; Paranhos et al., 2009). Therefore, it appears that GPV has a dual profile being induced by light in seedlings and remaining at stable concentrations upon high doses of UV-B exposure in leaves of adult cuttings.

To further understand the details of irradiance and developmental control of GPV accumulation, herein we characterize the influence of light shifts and irradiance quality on GPV biosynthesis in photomorphogenic and etiolated seedlings and the dynamics of GPV accumulation during reproductive growth. A detailed analysis was conducted on the accumulation dynamics of GPV during reproductive growth of adult plants and in different tissues of developing flowers and fruit. The structural basis of antioxidant potential in GPV was studied in an *in vitro* comparative test with similar indole alkaloids from closely related *Psychotria* species and with well-known antioxidants. The *in vivo* antioxidant and protective effect of GPV was indirectly examined by application of the alkaloid on leaves of two UV-B sensitive species (*Phaseolus vulgaris* and *Psychotria carthagenensis*) and evaluation of their tolerance to high doses of UV-B. Taking into account the overall results, potential implications of alkaloid dynamics for GPV *in planta* functions and improved alkaloid yields for pharmaceutical evaluation are discussed.

2. Materials and methods

2.1. Alkaloid isolation and GPV analysis

N,β-D-glucopyranosyl vincosamide (GPV), brachycerine and psychollatine were purified from *P. leiocarpa* Cham. & Schltdl., *P. brachyceras* Mull. Arg. and *P. umbellata* Vell. leaves, respectively (harvest license by Federal Authority Sisbio/ICMBio 32855-1; authentication code: 58482685), following previously described methods with minor modifications (Henriques et al., 2004; Both et al., 2002; Kerber et al., 2001). High Performance Liquid Chromatography (HPLC) was used to evaluate compound purity in comparison to authentic alkaloids. For GPV analysis, methanolic extracts from samples were analyzed by HPLC as previously described (Henriques et al., 2004). Shortly, fresh tissue samples (250 mg each) were macerated in liquid nitrogen and 1 mL of cold methanol (HPLC grade, Merck) was added; the extract was then sonicated for 30 min at 4 °C and the extract centrifuged at 13,000*g* at 4 °C for 15 min. Supernatant was recovered and analyzed by HPLC. Extracted dry weights (DW) were obtained after drying pellets at 60 °C until constant weight. The samples were analyzed in a Thermo Scientific Surveyor HPLC with a C18 reverse phase column equipped with respective guard column (Shimadzu) using linear gradient (1 mL min^{-1} flow), starting with water: methanol (60:40), and ending with methanol. Trifluoroacetic acid (TFA) (Sigma) was added to a final concentration 0.05% in both eluents. An external standard curve was prepared with authentic GPV. Content of GPV was expressed on a dry weight basis. Voucher specimens are deposited at the ICN herbarium at UFRGS (138157-*P. leiocarpa*; 7899-*P. brachyceras*; and 98869-*P. umbellata*).

2.2. Psychotria leiocarpa *seed germination*

Fruits were harvested from field grown *P. leiocarpa* plants (Morro Santana, Porto Alegre, Brazil). After pulp extraction, seeds were stored at 10 °C for two weeks before surface disinfection with 70% ethanol (v/v) (1 min) and 1.5% NaClO (v/v) (10 min). For aseptic germination, seeds were maintained in 0.1× MS (Murashige and Skoog, 1962) nutrient medium salts, pH 5.8, containing 0.6% agar with or without 1.5% sucrose (w/v), and grown under aseptic conditions at 26 ± 2° C in light [35 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 16 h day^{-1} photoperiod] or dark. For non-aseptic germination, seeds were planted in an autoclaved substrate composed of top commercial soil and vermiculite (1:1, v/v) and kept in a growth chamber (16 h day^{-1} photoperiod, 60 μmol m^{-2} s^{-1} PAR and 28 ± 2° C).

2.3. Dark-light transitions and GPV accumulation

Seedlings from *P. leiocarpa* containing six to ten leaves grown in aseptic conditions without sucrose were used to investigate light influence on GPV biosynthesis. A group of light grown seedlings was transferred to dark (at least 5 seedlings per sample, and at least 5 samples per treatment) and a group of dark grown seedlings was transferred to light (same numbers as for the light to dark group). Both groups were kept in the respective condition for 14 days. For GPV analysis, aerial parts of seedlings were harvested after 7 and 14 days of the light shift, immediately frozen in liquid nitrogen, and stored at −20 °C until analysis. Transitions from light to dark in light grown seedlings and dark to light in etiolated seedlings (25 days in darkness after germination) were also carried out to evaluate the role of sucrose presence or absence in growth media. Aerial parts of seedlings were harvested after 14 days of transition.

2.4. Light quality effects on concentrations of GPV, proteins and carbohydrates, and on dry biomass of seedlings

To identify the possible photoreceptor system involved in GPV light regulation, different wavelength enrichment assays were performed. Seedlings containing six to ten leaves from non-aseptic germination were exposed to white light filtered through different cellophane sheets (transparent, blue, red or green) for light enrichment in different wavelengths (control, blue, red or far-red respectively) (Ruedell et al., 2013). The absorbance spectra were obtained by wavelength scans in a Cintra 5 spectrophotometer (GBC, Victoria, Australia) (see Supplementary Fig. S1 in the online version at DOI: 10.1016/j.indcrop.2016.03.050). Treatment PARs were normalized at 30 μmol m^{-2} s^{-1} in the first assay and 90 μmol m^{-2} s^{-1} in the second, for which previously etiolated plants were used (after 55 days in dark). Seedlings were harvested