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Research article

High temperature and UV-C treatments affect stilbenoid accumulation and related gene expression levels in *Gnetum parvifolium*

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ABSTRACT

Background: *Gnetum parvifolium* stems and roots have been used for a long time in traditional Chinese medicines. Stilbenes are bioactive compounds present in *G. parvifolium* plants, and they possess antioxidative and anticancer properties. However, little is known about the responses of *G. parvifolium* stilbene biosynthetic pathways to stress conditions. Therefore, we investigated stilbene biosynthesis, including the expression of relevant genes, in *G. parvifolium* exposed to high-temperature and ultraviolet-C treatments.

Results: High temperatures did not influence the accumulation of total stilbenes in stems but decreased stilbene concentrations in roots at 3 h, with a subsequent restoration to control levels. In contrast, ultraviolet irradiation induced the accumulation of total stilbenes in stems but not in roots. We also observed that high temperatures inhibited the production of resveratrol and piceatannol in *G. parvifolium* stems and roots, whereas ultraviolet treatments initially inhibited their accumulation (up to 6 h) but induced their production at later time points. Analyses of specific genes (i.e., *PAL*, *C4H*, *4CL*, *STS*, and *CYP*) revealed that their expression levels generally increased in stress-treated stems and roots, although there was some variability in the expression profiles during treatments.

Conclusions: Our results indicated that high temperatures and ultraviolet irradiation differentially affect the biosynthesis of specific stilbenes in *G. parvifolium* stems and roots. Therefore, cultivating *G. parvifolium* seedlings under optimal stress conditions may increase the biosynthesis of specific stilbene compounds.

1. Introduction

The genus *Gnetum* consists of 35–40 species and is one of three unique groups of the phylum Gnetophyta [1]. This genus forms a morphologically and ecologically diverse monophyletic group with special cytological features [2,3,4]. There are some controversies regarding its phylogenetic position among seed plants, and resolving this issue may provide important insights into the evolution and origin of flowers [5]. Several *Gnetum* spp. are edible or used as paper pulp, which makes them economically valuable in many regions of Africa and Asia [6]. Moreover, these *Gnetum* spp. are important sources of raw materials for traditional medicines in many countries [1]. Stem and root extracts have been used to cure ocular complications, relieve swelling, treat acute respiratory infections, and cure chronic bronchitis [7]. In addition, numerous studies have indicated that these extracts contain a wide range of natural bioactive compounds, including stilbenoids [8,9,10]. Together with plants from the families Pinaceae, Cyperaceae, Vitaceae, Dipterocarpaceae, and Fabaceae, *Gnetum* spp. are considered one of the best sources of stilbenoids among plants [11].

Stilbenoids are a family of polyphenols known for their diverse biological activities [11,12]. Some of these compounds exhibit hypotensive, antioxidant, anticancer, and antibacterial effects [13]. About 100 different types of stilbenoids have been identified in at least 15 *Gnetum* spp., representing almost the full spectrum of natural stilbenoids [11,14]. Some stilbenoids, such as resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), have attracted considerable attention. Resveratrol is believed to be involved in the health benefits associated with the moderate consumption of red wine [15] and is currently one of the most extensively studied natural products [16]. Numerous studies have revealed that it can inhibit the progression of diverse illnesses, including cancer, HIV disease, and cardiovascular

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disease. Resveratrol can also prolong the lifespan of various organisms by activating sirtuin deacetylases [17,18,19]. Furthermore, its hydroxylated analog, piceatannol, exhibits considerable antitumorigenic and antileukemic activities [20,21].

Stilbenes are synthesized by stilbene synthases (STSs) together with chalcone synthases that share a common upstream pathway [22,23]. Key enzymes [i.e., phenylalanine ammonia-lyase (PAL) and cinnamic acid 4-hydroxylase (C4H)] catalyze the deamination of L-phenylalanine to *trans*-cinnamic acid during the first step of phenylpropanoid biosynthesis. The resulting *p*-coumaric acid is converted to *p*-coumaroyl-CoA through a reaction catalyzed by 4-coumarate: CoA ligase (4CL). Three acetate extender units (derived from malonyl-CoA) are added to produce a linear tetraketide intermediate. The subsequent folding and cyclization of the generated intermediate lead to the production of a chalcone or stilbene ring structure depending on the polyketide synthase activity [22]. Resveratrol may be further hydroxylated by some members of the CYP gene family (e.g., cytochrome P450) to produce piceatannol (Fig. 1). We previously analyze *Gnetum parvifolium* transcriptome and determine that these genes are primarily involved in stilbenoid biosynthesis [1].

G. parvifolium is mainly distributed in tropical and subtropical regions of southern China. Its stems and roots have been used in traditional Chinese medicines for more than 1500 years [14]. Our previous studies indicate that *Gnetum* spp. are rich in stilbenes [1,24,25]. We also determine that stilbene accumulation differs among *G. parvifolium* tissues, with concentrations mediated by the expression of relevant biosynthetic genes [1]. In addition, several stilbene compounds have been detected in *G. africanum* stems [10,26,27] or *G. parvifolium* stems and roots [1]. Stilbene production is induced by high temperature and ultraviolet (UV) treatments [1], implying that stress factors can stimulate the accumulation of stilbenes in leaves. In this study, we analyzed young *G. parvifolium* stems and roots treated with high temperature or UV light. We focused on the accumulation of total stilbenes, including resveratrol and piceatannol, and the expression of genes responsible for stilbenoid biosynthesis. A more comprehensive characterization of the responses of *G. parvifolium* stilbene biosynthetic pathways to environmental stresses may contribute to the breeding of stilbene-rich plants.

2. Materials and methods

2.1. Materials and treatments

All experiments were conducted using 1-year-old *G. parvifolium* (Warb.) C.Y. Cheng ex Chun seedlings cultivated in the greenhouse. The seedlings were acclimated in a climate chamber for several days and then divided into two equal groups. One group was exposed to high temperature (40°C), whereas the other group was treated with UV-C irradiation (20 W; wavelength: 200–275 nm). Both the groups were treated for 3, 6, 12, 24, and 48 h. We did not use the within 1-h UV-C treatment applied in many earlier studies [28,29]. We applied relatively long irradiation times in our experiments because of the thick leathery leaves in this *Gnetum* plant. Our earlier results of photosynthetic experiments suggested that *Gnetum* was not sensitive to strong irradiation and was able to withstand several hours of UV-C treatment without any noticeable loss of viability, except for withering after the extreme 48-h treatment (data not shown). In addition, long UV processing time (9 h) has been applied to *Arabidopsis thaliana* [30]. These observations provided us with an opportunity to check much longer range of UV-C treatments than are commonly applied in plants. The experiments were completed using four biological replicates. All

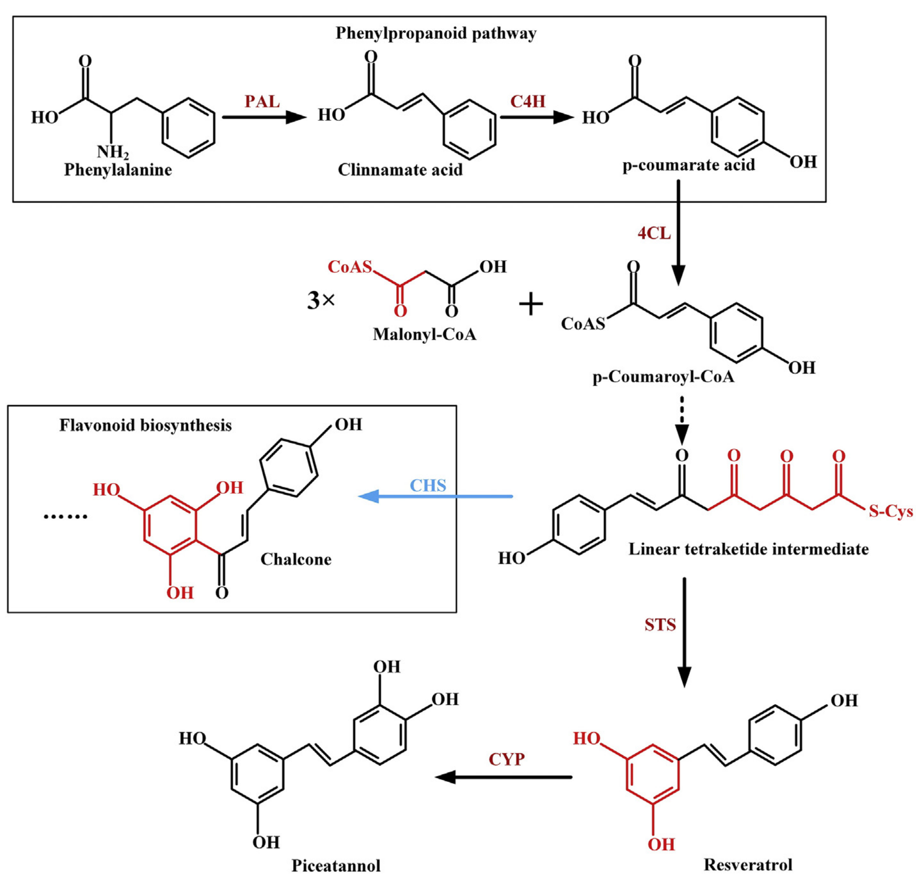

Fig. 1. The biosynthesis pathway of stilbenes and candidate genes involved in stilbenoids biosynthesis in *G. parvifolium*. PAL: phenylalanine ammonia-lyase, C4H: cinnamic acid 4-hydroxylase, 4CL: 4-coumarate: CoA ligase, CYP: cytochrome P450 gene, STS: stilbene synthase.