

EFFECTS OF ULTRAVIOLET RADIATION AND HIGH TEMPERATURE ON TOTAL FLAVONOIDS CONTENT IN *GNETUM PARVIFOLIUM* WARB.

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Abstract

Gnetum parvifolium is one of important economic plants used as traditional medicine, and little is known about the flavonoid production in *G. parvifolium* in response to stress. The content of flavonoid in different tissues of *G. parvifolium* when exposed to treatments of high-temperature (40°C) and ultraviolet-C was investigated. The total flavonoid content of leaves, stems and roots reached 149.1, 53.9 and 52.79 mg/g DW, respectively. The concentrations of flavonoid in different tissues significantly decreased under the high-temperature condition. In contrast, the ultraviolet-C treatment had no significant impacts on the total flavonoid concentration in leaves while the content initially decreased and then increased in stems and roots under the treatment of ultraviolet-C. The present results showed that leaves of young seedlings were rich in flavonoids. The treatment of high temperature and ultraviolet-C did not significantly promote the accumulation of total flavonoids. The present study would help to understand how *Gnetum* gets adapted to tropical and subtropical biomes and habitats for the perspective of plant evolution. Besides, it can be beneficial for breeding flavonoids-rich gymnospermous plants for the purpose of industrial usages.

Introduction

Gnetum L., together with *Ephedra* L. and *Welwitschia*. Hook.f., comprises a small but unique genus of Gnetophyta. The genus comprises a monophyletic group and is considered to be morphologically and ecologically diverse in gymnosperms, and united based on special features of their cytology (Hou *et al.* 2015, Ickert and Renner 2016). Despite the striking evolutionary divergence, *Gnetum* is an important economic crops which is used as traditional medicine in many countries, such as Malaysia (Jørgensen and Rydin 2016, Langenberger *et al.* 2009, Moise *et al.* 2012). Biochemical obtained from several Asian species of *Gnetum* is able to cure chronic bronchitis, relieve swelling, and treat acute respiratory infections (Chang *et al.* 2018). Some *Gnetum* species, such as *G. africanum* and *G. gnemon* were sold as healthy vegetables and fruits to Europe, USA, countries in Southeast Asia and Central Africa (Ali *et al.* 2011, Bhat and Binti 2014, Kongkachuichai *et al.* 2015). The recent studies show that *Gnetum* is rich in secondary metabolites such as flavonoids, alkaloid and stilbenoids (Deng *et al.* 2014, Ikuta *et al.* 2015, Iliya *et al.* 2015), which are considered to be of important values in medical usages.

Flavonoids comprise a large group of chemical compounds ubiquitously occurred in plants for food. They usually occur as glycosides and contain several phenolic hydroxyl groups on their ring structures (Chu *et al.* 2000). Besides, they have been reported to act as scavengers of various oxidizing species, and reduce risks of cancer and heart diseases (Deng *et al.* 2016). Some of flavonoids (Bhat and Binti 2014, Deng *et al.* 2014) have been identified in various *Gnetum* species, such as 5,7,4'-trihydroxy-3'-methoxyflavone in *G. montanum* and 5,7,2'-trihydroxy-5'-methoxyflavone in *G. macrostachyum* (Saffar *et al.* 2009, Xiang *et al.* 2002). Hence secondary

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metabolites of *Gnetum* have attracted the attention because of the medical properties of their chemical production and roles in response to stress conditions. For example, some studies demonstrated that the content of flavonoids increased after stress treatments (González-Aguilar *et al.* 2007, Wang *et al.* 2009, Fini *et al.* 2011). Besides, seedlings of *Gnetum* have higher flavonoids and stilbene content than the mature plant (Deng *et al.* 2016), and it is more vulnerable to the stress, so the seedlings could be used as the ideal source of special ingredients.

Gnetum parvifolium is mainly distributed in tropical and subtropical regions of southern China (Hou *et al.* 2016). The species has been used broadly as a traditional medicinal plant for 1500 years (Deng *et al.* 2016). In previous study, it was found that *G. parvifolium* is rich in flavonoids and stilbene, and the content of stilbene increased significantly under high temperature and UV-C treatments (Deng *et al.* 2016). In this study, different tissues of young *G. parvifolium* seedlings were analyzed using the same treatments, and focused on the total content of flavonoids in leaves, stems and roots, which would contribute to the breeding of flavonoids-rich plants.

Materials and Methods

One-year-old seedlings of *Gnetum parvifolium* were used for experimental materials. The seedlings cultivated in the greenhouse were transferred to a growth chamber for several-day acclimation, and then were divided into two groups. One group was exposed to UV-C irradiation (the power and wavelength were 20W and 200 - 275 nm); another group was exposed to high temperature (40°C). The treating time of these two groups were 0, 3, 6, 12, 24 and 48 hrs, respectively, the 0 was set as the control group, and each treatment was repeated with four biological repetition. The samples of leaf, stem and root were collected and stored in liquid nitrogen (-80°C). In total, 40 seedling samples were used in this experiment.

All tissues (leaf, stem and root) of two treatments were dried in the oven before ground into powders. The equal amounts of sample powders (10 mg) were immersed in methanol solution (the concentration was 80%, 500 µl) in centrifuge tube (1.5 ml), and then processed with ultrasonic to accelerate dissolution (30 min) followed by overnight storage at 4°C. The homogenates were centrifuged at 12000 rpm for 10 min and the supernatants were collected and stored at 4°C for further analysis. The NaNO₂-Al(NO₃)₃-NaOH spectrophotometric method was used to the quantification of total flavonoids (Deng *et al.* 2016). The absorbances of the samples were measured at 510 nm, the contents of the total flavonoids were calculated with quercetin (Tongtian Biotech. Co., Shanghai, China) as standard.

All data were obtained from four biological replications, and the results were presented as the mean ± standard deviation. The means were compared using the Tukey's honestly significant difference test (Tukey's HSD), and significance was accepted at ≤ 0.05. The above analysis was conducted by R software (3.4.1).

Results and Discussion

Since the content of total flavonoids in tender leaves is important for the health care effect the tender leaf of *Gnetum* is often used as healthy vegetables in Africa and Southeast Asia. In the study of other plants, the high temperature and UV treatment had certain influence on content of flavonoids (Julkunen-Tiitto *et al.* 2015, Mori *et al.* 2007). In the present results, the total flavonoids content of tender leaf reached up 149.11 mg/g DW, the content had a small increase at 3 hrs after high temperature treatment, but it was not significant (Fig. 1A), then the content significantly decreased at following stages (3 hrs compared to 6, 12, 24 and 48 hrs), the content at 48 hrs decreased by 36.95% (94.02 mg/g DW). The result indicated that high temperature reduced the accumulation of total flavonoids; correspondingly, the content had no significant changes between

6 stages (Fig. 1B), indicated that UV-C had no influence on total flavonoids accumulation in *G. parvifolium*.

The stem of *Gnetum* is often used as traditional medicine in China. In the present investigation, the content of total flavonoids in stems was 53.93 mg/g DW, then the content decreased continuously after 48 hrs under high temperature treatment, the content at 48 hrs decreased by 78.14% (only 11.79 mg/g DW) (Fig. 2A). This results showed that high temperature restrained the synthesis of flavonoids and the UV-C had the similar influence on the accumulation of total flavonoids, which significantly decreased at 6 hrs (19.51 mg/g DW,), then reached 42.63 mg/g DW at 12 hrs, at last had a sharp decrease. This suggested UV-C also restrained the synthesis of flavonoids in stems.

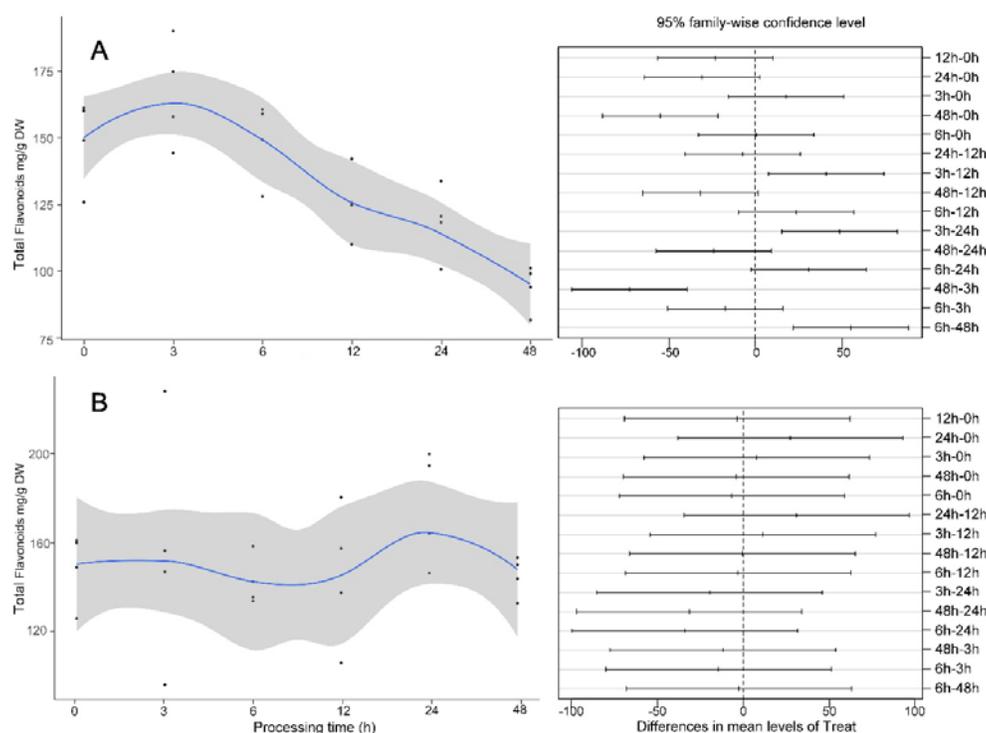


Fig. 1. Contents of total flavonoid in leaves of *Gnetum parvifolium* seedlings under stresses. X-axis represents the treatment time, Y-axis represents the content of total flavonoid [$\text{mg}\cdot\text{g}^{-1}\cdot\text{DW}$]. Total flavonoid in leaves under high temperature (A) and UV treatment (B). Means \pm SD, $n=4$. Pairwise comparison of 6 stages was on the right side, the line segment intersected with a vertical dotted line indicating no significant difference.

The root of *Gnetum* is also used as a traditional medicine. According to the present study, the content in roots was 52.79 mg/g DW, which was similar to that of stems. The high temperature had a same effect on content of flavonoids in roots, the content decreased continuously with processing time (Fig. 3A), reduced to 29.58 mg/g DW at 48 hrs. The effect of UV-C was similar to that of stems, and the content of total flavonoids decreased first and then increased, but it decreased overall significantly. The results showed that high temperature and UV-C treatment also restrained the synthesis of flavonoids in roots.

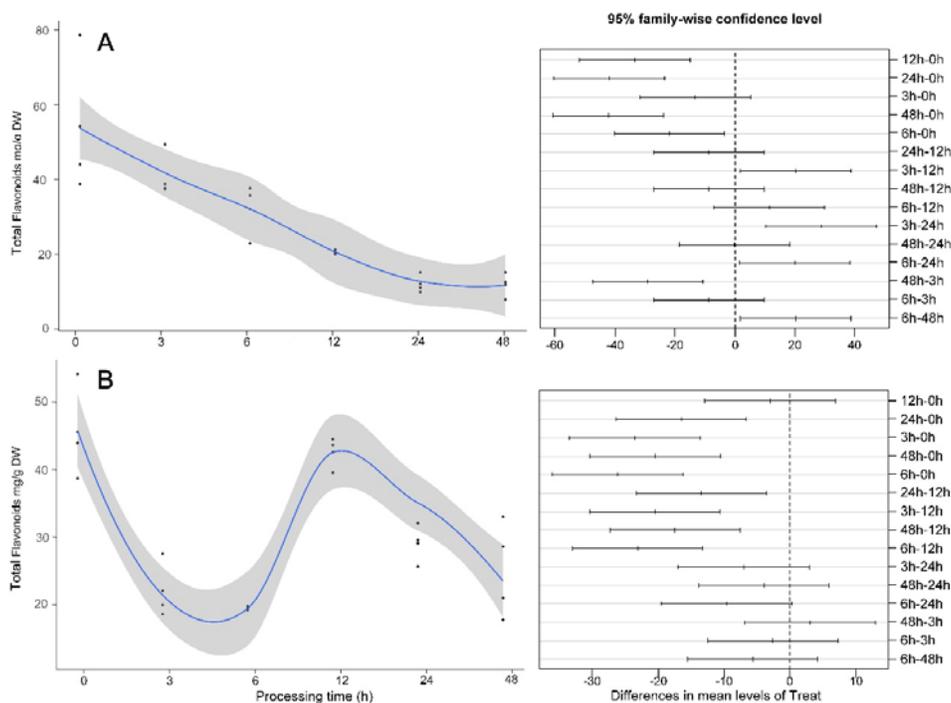


Fig. 2. Contents of total flavonoid in stem of *Gnetum parvifolium* seedlings under stresses. X-axis represents the treatment time, Y-axis represents the content of total flavonoid [mg/g/DW]. Total flavonoid in stem under high temperature (A) and UV treatment (B). Means \pm SD, n = 4. Pairwise comparison of 6 stages was on the right side, the line segment intersected with a vertical dotted line indicating no significant difference.

The results showed that total flavonoids was mainly distributed in leaves of young seedlings, which was different from the distribution of total stilbene (Deng *et al.* 2017), this indicated that the distribution is tissue-specific. The high temperature and UV-C treatment didn't promote the accumulation of total flavonoids, and the content of three tissues decreased significantly after treatments, which was quite different from other plants such as mango (González *et al.* 2007) and blueberries (Wang *et al.* 2009), and the levels of flavonoids in these plants are found to increase after illumination with UV-C. One possible reasons of this situation is that the samples are too weak to suffer the severe damage, especially the longtime treatment. So the detailed investigation of flavonoids would be performed in the different dosage ranges of stress treatments in the next work.

Flavonoids are synthesized by a common pathway together with stilbenes, using the same substrate, p-coumaroyl-CoA via chalcone synthases (CHSs) and stilbene synthases (STSs) as key branch enzymes, respectively, in the phenylpropanoid pathway (Katsuyama *et al.* 2007, Watts *et al.* 2006). The consumption of the common substrate may cause the competition between synthesis of flavonoid and stilbene. To understand the relationship between total flavonoid and stilbene in *G. parvifolium*, the correlation analysis of this two component was conducted, and combined the data of total stilbenes from previous study. The result in Fig. 4 showed that they were significantly positively correlated with each other. This results suggested that there are other secondary metabolites which consume the common substrate causing the decrease of flavonoids, and need further research.

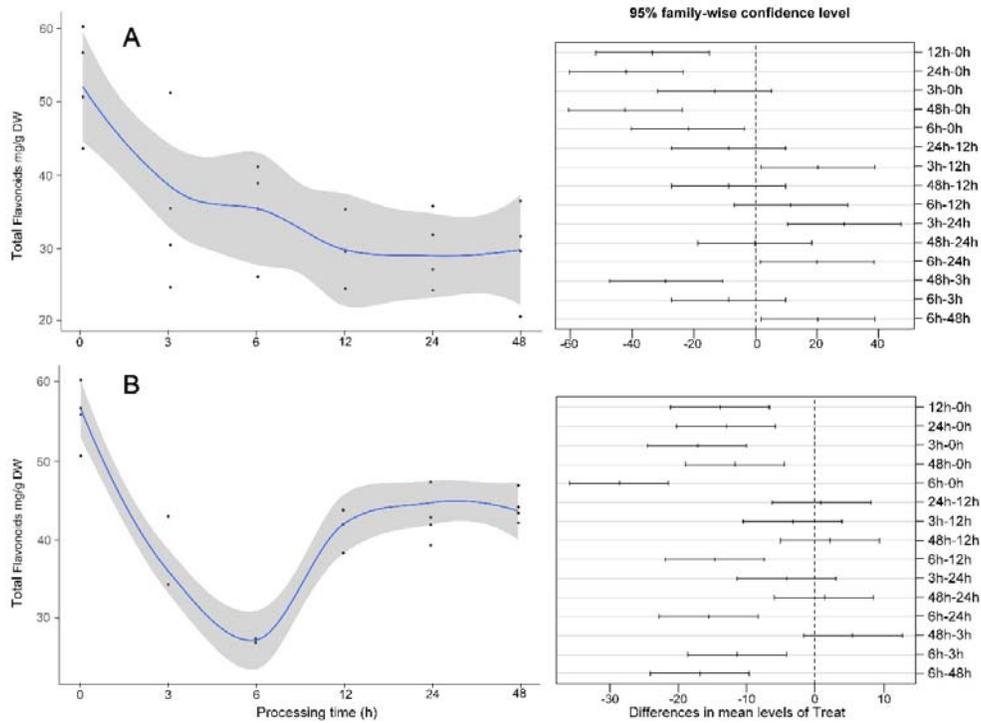


Fig. 3. Contents of total flavonoid in roots of *Gnetum parvifolium* seedlings under stresses. X-axis represents the treatment time, Y-axis represents the content of total flavonoid [mg/g/DW]. Total flavonoid in roots under high temperature (A) and UV treatment (B). Means \pm SD, n = 4. Pairwise comparison of 6 stages was on the right side, the line segment intersected with a vertical dotted line indicated no significant difference.

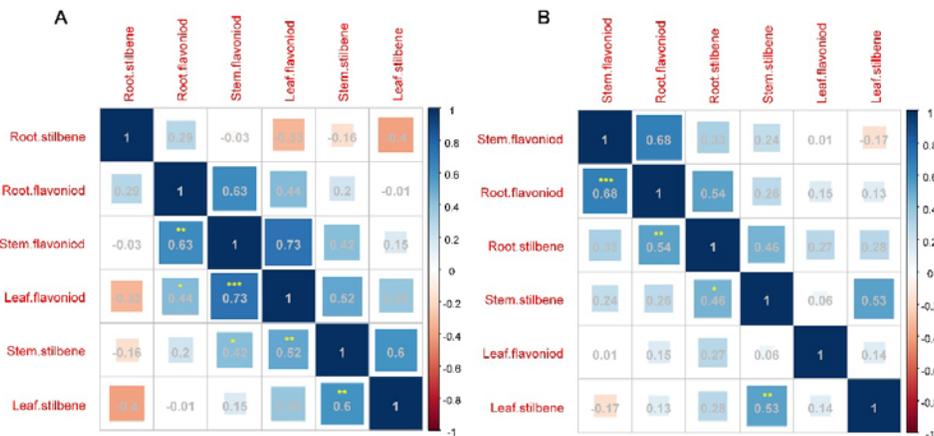


Fig. 4. Correlation analysis of total flavonoid and stilbene content under high temperature (A) and UV treatment (B). *: significant level = 0.05, **: significant level = 0.01, ***: significant level = 0.001.

The present study was the first attempt to clarify the flavonoids changes of *Gnetum* responses to stress factors. This results would help the scientists to understand how *Gnetum* adapts to tropical and subtropical habitats and ultimately will enable to breed flavonoids-rich *Gnetum* plants.

Acknowledgments

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