

# Analysis of Isoquinoline Alkaloid Composition and Wound-Induced Variation in *Nelumbo* Using HPLC-MS/MS

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## Supporting Information

**ABSTRACT:** Alkaloids are the most relevant bioactive components in lotus, a traditional herb in Asia, but little is known about their qualitative and quantitative distributions. Here, we report on the alkaloid composition in various lotus organs. Lotus laminae and embryos are rich in isoquinoline alkaloids, whereas petioles and rhizomes contain trace amounts of alkaloids. Wide variation of alkaloid accumulation in lamina and embryo was observed among screened genotypes. In laminae, alkaloid accumulation increases during early developmental stages, reaches the highest level at full size stage, and then decreases slightly during senescence. Vegetative and embryogenic tissues accumulate mainly aporphine-type and bisbenzylisoquinoline-type alkaloids, respectively. Bisbenzylisoquinoline-type alkaloids may be synthesized mainly in lamina and then transported into embryo via latex through phloem translocation. In addition, mechanical wounding was shown to induce significant accumulation of specific alkaloids in lotus leaves.

**KEYWORDS:** *Nelumbo*, alkaloids, bioactive components, mechanical wounding

## INTRODUCTION

Lotus, also known as sacred lotus or Chinese water lily, belongs to the genus *Nelumbo*, the single member of the Nelumbonaceae family.<sup>1</sup> The genus *Nelumbo* consists of two species, Asian lotus (*Nelumbo nucifera* Gaertn.), distributed in Asia and the northern parts of Oceania, and American lotus (*Nelumbo lutea* Wild.), distributed in eastern North America.<sup>2</sup> Lotus has received considerable attention in the recent past, due to its high economic value in food, ornamental, and medicinal uses. All parts of the lotus are used in traditional Chinese and Indian medicine to treat various diseases, such as fever, sweating, strangury, bleeding, pectoralgia, hepatopathy, and obesity.<sup>3</sup> Modern pharmacological studies have confirmed its activities against cancer, diabetes, obesity, hypertension, and HIV.<sup>4–10</sup>

Alkaloids are among the first important active components in lotus.<sup>3</sup> Since the first isolation of alkaloids from lotus leaves and embryos in 1960s, more than 20 alkaloids have been identified.<sup>11–15</sup> On the basis of their structures, alkaloids in lotus can be divided into three categories: monobenzylisoquinolines, aporphines, and bisbenzylisoquinolines (Figure 1). The aporphine-type alkaloids, such as nuciferine, *O*-nornuciferine, *N*-nornuciferine, anonaine, roemerine, dehydronuciferine, and pronuciferine, are mainly distributed in lotus lamina, whereas the bisbenzylisoquinoline-type alkaloids, including liensinine, isoliensinine, and neferine, predominantly accumulate in the embryo of the seeds.<sup>11,12,16–21</sup> Monobenzylisoquinoline-type alkaloids, the intermediate products in the biosynthesis of both

aporphine- and bisbenzylisoquinoline-type alkaloids, occur in trace amounts in several lotus organs.<sup>11,12,17,21</sup>

With the improvement of extraction and separation techniques, several new alkaloids have been isolated, including *N*-methylasimilobine *N*-oxide and three bisbenzylisoquinoline alkaloids, nelumboferine and nelumborines A and B.<sup>11,12</sup> Meanwhile, continuous efforts are being made to elucidate pharmacological effects of the pure individual alkaloids, instead of crude extracts.<sup>19,22,23</sup> Such pharmacological studies require the production of individual alkaloids in a high state of purity. To facilitate the production of high-purity alkaloids, it is crucial to identify varieties that contain high levels of alkaloids and to investigate individual alkaloid distribution throughout the lotus plant.

In this study, we conducted both qualitative and quantitative analyses of alkaloids in different parts of *N. nucifera*. In addition, variation of alkaloid concentration was screened among rhizome-, flower-, and seed-producing genotypes, and the effect of wounding on alkaloid accumulation was investigated as well. To our knowledge, this study represents the first report on spatial and temporal variations of alkaloid accumulation in lotus. It will be helpful for understanding alkaloid biosynthesis and regulation at the whole plant level.

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