SHORT COMMUNICATION

Aporphine alkaloid contents increase with moderate nitrogen supply in *Annona diversifolia* Saff. (Annonaceae) seedlings during diurnal periods

José Agustín Orozco-Castillo\(^a,b\), Rocío Cruz-Ortega\(^c\), Mariano Martinez-Vázquez\(^d\) and Alma Rosa González-Esquinca\(^a\)

\(^{a}\)Laboratorio de Fisiología y Química Vegetal, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutiérrez, Mexico; \(^{b}\)Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Coyoacán, Mexico; \(^{c}\)Instituto de Ecología, Universidad Nacional Autónoma de México, Delegación Coyoacán, Mexico; \(^{d}\)Instituto de Química, Universidad Nacional Autónoma de México, Delegación Coyoacán, Mexico

**ABSTRACT**

Aporphine alkaloids are secondary metabolites that are obtained in low levels from species of the Annonaceae family. Nitrogen addition may increase the alkaloid content in plants. However, previous studies published did not consider that nitrogen could change the alkaloid content throughout the day. We conducted this short-term study to determine the effects of nitrogen applied throughout the diurnal period on the aporphine alkaloids via measurements conducted on the roots, stems and leaves of *Annona diversifolia* seedlings. The 60-day-old seedlings were cultured with the addition of three levels of nitrogen (0, 30 and 60 mM), and alkaloid extracts were analysed using high-performance liquid chromatography. The highest total alkaloid content was measured in the treatment with moderate nitrogen supply. Further, the levels of aporphine alkaloids changed significantly in the first few hours of the diurnal period. We conclude that aporphine alkaloid content increased with moderate nitrogen supply and exhibited diurnal variation.

**KEYWORDS**

liriodenine; atherospermidine; lyciscamine; diurnal variation; hydroponic

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**CONTACT**

Alma Rosa González-Esquinca aesquinca@unicach.mx

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1. Introduction

Annona diversifolia belongs to the Annonaceae family, which has been widely studied for the presence of biological active molecules (Chavan et al. 2012; Costa et al. 2013a; Pimenta et al. 2014) such as alkaloids (Leboeuf et al. 1980; Pimenta et al. 2014), acetogenins (Cavé et al. 1997; Miao et al. 2015), essential oils (Fournier et al. 1999; Costa et al. 2013b; Thang et al. 2013) among other secondary metabolites (Rout & Kar 2014).

The benzylisoquinoline alkaloids (BIAs) are a large and diverse group of alkaloids that include the aporphine alkaloids such as liriodenine, lycamine and atherospermidine (Lúcio et al. 2015) (Figure 1). BIAs are known to be important for their pharmacological activity (Facchini 2001; Pimenta et al. 2014; Lúcio et al. 2015) and are biosynthesised from two tyrosine molecules in a common biosynthesis pathway (Stadler et al. 1989). Previous studies examining the effect of nitrogen on BIAs have been limited to Papaver somniferum because of its importance in morphine production. Long-term studies conducted on P. somniferum show an increase in morphine content in response to a moderate supply of nitrogen (≤100 kg ha⁻¹); however, the morphine content decreased when this concentration was exceeded (Yadav et al. 1984; Losak & Richter 2004). The short-term effects of nitrogen on alkaloid biosynthesis were not considered in these studies, and no studies have been conducted on other BIAs such as the aporphine alkaloids. These results show that there are insufficient studies on BIAs to clearly explain the effect of nitrogen on aporphine alkaloids. Therefore, we conducted this short-term study to determine the effect of three nitrogen levels applied throughout the diurnal period on the levels of aporphine alkaloids (liriodenine, lycamine and atherospermidine) in the whole plant based on measurements of these compounds in the roots, stems and leaves of A. diversifolia seedlings.

2. Results and discussion

2.1. Effect of nitrogen levels on total alkaloid content

The total alkaloid content differed significantly among the 0 and 30 and 60 mM N treatments (p < 0.05) during the diurnal period. The highest total alkaloid content was measured in the 30 mM N treatment, with the lowest in the 60 mM N treatment, although the presence of alkaloids was not completely eliminated by the addition of 60 mM N (Figure 2). One possible explanation is that a high nitrogen supply exceeds the plant’s capacity to assimilate the absorbed nitrogen because there are insufficient carbon skeletons for nitrogen assimilation (Rasmussen et al. 2008). Consequently, the nitrogen accumulation causes the decrease of

![Figure 1. Aporphine alkaloids from A. diversifolia Saff.](image)
amino acid precursors for alkaloid biosynthesis because the shikimate pathway and alkaloid biosynthesis are repressed (Scheible et al. 2004; Fritz et al. 2006).

Figure 2. Nitrogen treatments effect on total alkaloids from *A. diversifolia* Saff. Values are means ± SD of three determinations and the different letters above columns indicate significant differences (HSD Tukey-test; *p* < 0.05).

Figure 3. Nitrogen levels influence on liriodenine (LYR), lysicamine (LYS) and atherospermidine (ATHER) in roots (A) stems (B) and leaves (C). Values are means ± SD of three determinations and the different letters above columns indicate significant differences (HSD Tukey-test; *p* < 0.05).
2.2. The effect of nitrogen levels on organ-specific aporphine alkaloid contents in the first part of the diurnal period

The concentration of alkaloids varied among the three treatments during the seven periods evaluated (Figures S1–S4). However, the largest effects of nitrogen effect on alkaloid content in the roots and stems were measured at 2 and 6 h following the application of nitrogen (09:00 and 13:00 h). In leaves, the nitrogen effect was exhibited at 4 and 6 h (11:00 and 13:00 h). The marked diurnal variation observed in the aporphine alkaloids maybe caused by changes in photosynthesis and carbon-nitrogen metabolism, and this effect is likely to be most noticeable during nitrogen assimilation because photosynthesis is strongly affected by nitrogen supply (Scheible et al. 2000). In addition, Scheible et al. (2004) reported that reprogramming of the expression and activity of enzymes related to nitrogen assimilation occurred between 30 min and 6 h after the addition of nitrogen.

The highest levels of liriodenine, lysicamine and atherospermidine in the roots were measured at 2 and 6 h in response to 0 and 30 mM N. The liriodenine, lysicamine and atherospermidine levels in the stems exhibited similar response patterns to 0 and 30 mM N at these times. The lowest stem content of the three alkaloids was measured in the 60 mM N treatment, similar to the roots. High levels of liriodenine and atherospermidine in leaves were detected at 4 h in the 0 and 30 mM N treatment, respectively. However, the highest lysicamine content at this time period was measured in the 60 mM N treatment. Surprisingly, the 60 mM N treatment significantly increased the levels of liriodenine, lysicamine and atherospermidine at 6 h (Figure 3).

The increase in leaf alkaloid content under high nitrogen supply may be due to nitrogen being exported from the roots to the photosynthetic tissues for assimilation and the availability of tyrosine for aporphine alkaloid biosynthesis. Lee and Facchini (2011) suggest that the shikimate pathway and aromatic amino acid metabolism (tyrosine in particular) can affect the ability of a plant to biosynthesise BIAs. Fritz et al. (2006) showed that the levels of minor amino acids (including tyrosine) in tobacco leaves increased under high nitrogen supply during the first 6 h of the diurnal period and they suggested that amino acids are biosynthesised in light more quickly than they are utilized or exported.

The liriodenine, lysicamine and atherospermidine contents differed significantly between treatments and the evaluated time periods. However, in roots and stems, the relative abundance of these alkaloids was maintained, i.e. liriodenine was the most abundant alkaloid, followed by atherospermidine and lysicamine. A similar response was not observed in the leaves because the abundance changed in response to the high nitrogen supply, resulting in lysicamine being the most abundant and liriodenine being the least abundant alkaloid. Because of their similar structure, these alkaloids maybe interconverted due to stress conditions such as nutrient deficiency or nutrient excess. Therefore, we believe that alkaloid interconversion overlapped with the response to nitrogen, thereby preventing us from determining the effect of nitrogen on each alkaloid. The interconversion between BIAs was first reported by Winterstein and Trier in 1910 for opium alkaloids and has subsequently been reported for BIAs. Alkaloids can be interconverted as a result of abiotic stress conditions, including the absence of nitrogen, excessive nitrogen supply or osmotic stress (Misra & Gupta 2006). This study is the first to provide evidence of the effect of nitrogen on aporphine alkaloid content.
3. Experimental
See supplementary material.

4. Conclusion
In conclusion, our results clearly demonstrate that the total content of aporphine alkaloids increased with moderate nitrogen supply (30 mM N) and decreased when the plants were exposed to high nitrogen concentrations (60 mM N). However, the main effect of nitrogen on aporphine alkaloids was observed at 2 and 6 h in roots and stems, and at 4 and 6 h in leaves. The leaf was the organ most strongly influenced by the high level of nitrogen supplied, and this increased the aporphine alkaloid content. Finally, there are factors interacting with the effect of nitrogen on aporphine alkaloids, making it difficult to discern the specific effect of nitrogen on individual alkaloids.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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