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Nitrogen compounds in the xylem sap of coffee

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Abstract

Some N containing compounds were analyzed and identified in the xylem sap collected from coffee seedlings. In order to facilitate sap bleeding, the seedlings were kept in a humidified chamber. Asparagine was the major amino acid present, representing 30.5% (257 μg N ml^{-1} sap) of the total nitrogen detected, while glutamine represented 6.9% (58 μg N ml^{-1} sap). Overall, amino acids accounted for 41.2% (347 μg N ml^{-1} sap) of the total sap nitrogen, whereas NO_3 was the most abundant N compound (51.9%, 437 μg N ml^{-1} sap). Caffeine (1.2%, 10.5 μg N ml^{-1} sap), and the ureides alantoin (4.2%, 35.6 μg N ml^{-1} sap) and allantoic acid (1.4%, 11.9 μg N ml^{-1} sap) were also detected in the xylem exudate.

Keywords: Allantoic acid; Allantoin; Amino acids; Caffeine; *Coffea arabica*; Rubiaceae; Coffee; Nitrogen transport; Ureides

1. Introduction

In higher plants, N is transported from the roots to the shoot, mainly as nitrate, amino acids, amides and ureides (Pate, 1973). Bollard (1957a) demonstrated that aspartic acid, asparagine and glutamine were the most abundant N compounds in the xylem sap of apple trees. This probably prompted Bollard to investigate several woody plants on this matter (Bollard, 1957b,c). In a comprehensive study, Bollard (1957c) examined xylem fluid extracted from shoots of several dicotyledons, monocotyledons and gymnnosperms, using paper chromatography. In nearly all species, glutamine and asparagine were quantitatively the most important N compounds. Allantoin and/or allantoic acid were observed in members of twenty-three families. A few species had considerable amounts of nitrate in the xylem fluid but in most cases it was present either in trace amounts or was absent.

Allantoin and allantoic acid were detected in plants almost a century ago (reviewed in Schubert & Boland, 1990). Probably the most extensive studies were carried out by the groups of Fosse, Brunel and Thomas (1931) and Mothes (1961) (and references therein). Later, results obtained by Japanese scientists suggested that these compounds might play an important role in the transport of N in nodulated soybean plants and that N fertilization (NO_3^- and urea) might interfere in the process, leading to a decrease in concentration of these compounds (Schubert & Boland, 1990; Thomas, Ross, Chastain, Koomanoff, Hendrix & Volkenburgh, 1981). Experiments carried out by the same Japanese group showed that ^{15}N from $^{15}N_2$ was significantly incorporated into allantoin and allantoic acid in nodules on soybean plants. Subsequently, ureides were detected in several nitrogen fixing plants (reviewed in Schubert, 1986).

Kalberer (1965) demonstrated in coffee that allantoin and allantoic acid were products of caffeine catabolism. Subsequent studies showed significant accumulation of radioactivity in these compounds when leaves and coffee fruits were fed with ^{14}C-labeled caffeine (Ashihara, Monteiro, Moritz, Gillies & Crozier, 1996; Suzuki & Waller, 1984a,b). Determination of the endogenous content of ureides in leaves and fruits of two coffee species showed appreciable amounts of these compounds (Vitória, 1998).

It was the aim of this study to determine the presence and the concentration of some N compounds in the xylem sap of coffee.

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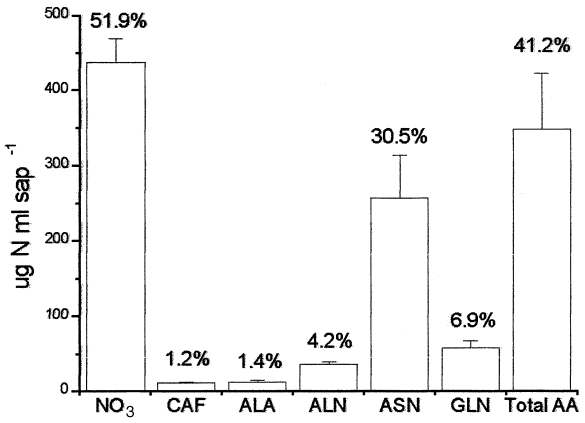

Fig. 1. Concentration of N compounds in the xylem sap of coffee. The percentages indicate the participation of each compound in the total N detected (NO_3^- + ureides + caffeine + total amino acids).

2. Results and discussion

Keeping the coffee seedlings inside the humidifier resulted in softer stems. This facilitated cutting, thus avoiding damage at the bleeding site. It was observed that sap collection was severely reduced if seedlings were removed from the humidifier 3–4 days before bleeding. The stems became harder offering greater resistance to cutting with a razor-blade.

Analysis of the sap revealed that NO_3^- was the main form of N transport, representing more than 50% of the total N of all compounds identified here (Fig. 1).

Asparagine and glutamine together accounted for almost 90% of the N of the amino acids fraction in the coffee sap (Table 1) and 37.5% of the total N (Fig. 1). Interestingly, the N content in asparagine was four times higher than that present in glutamine.

Table 1
Amino acid composition and concentration in coffee xylem sap.

Amino acid	μg N ml^{-1}	%N total amino acid
ASP	0.47 ± 0.08	0.14
GLU	0.56 ± 0.10	0.16
ASN	256.6 ± 55.8	73.31
SER	10.21 ± 1.65	2.98
GLN*	58.2 ± 9.10	16.23
GLY	0.38 ± 0.05	0.11
THR	4.57 ± 1.03	1.32
ARG	0.72 ± 0.14	0.21
ALA	3.67 ± 0.84	1.13
TYR	1.82 ± 0.26	0.53
MET	0.94 ± 0.20	0.27
VAL	2.03 ± 0.29	0.59
PHE	1.04 ± 0.23	0.30
ILE	0.95 ± 0.16	0.28
LEU	0.99 ± 0.16	0.29
LYS	3.97 ± 0.84	1.12

* HIS elutes just after GLN, however, due to the greater amount of the latter, HIS peak was overlapped

Serine contributed to 3% of the *N*-amino acids, followed by threonine (1.32%), alanine (1.13%) and lysine (1.12%) (Table 1). Our data are, therefore, in good agreement with those obtained by Bollard (1957a,b,c) for woody tree species. In general, he found that asparagine was the predominant amino acid followed by glutamine. However, depending on the species or sampling date (season of the year), glutamic acid sometimes became important, but was never higher than that of asparagine. Serine, threonine and alanine were almost always detected in the 1–2% range.

Allantoic acid and allantoin were also reported by Bollard (1957c) as present in certain saps. In *Persea americana* and *Acer pseudo-platanus*, allantoic acid was the major constituent. Unfortunately, Bollard (1957c) did not give exact values, only indicating that it was a major component representing more than 5 μg of N in a sample of 15 μg of total N applied to paper chromatography sheets, i.e. more than 30%.

Considering their ability to transport amides or ureides, nitrogen-fixing plants can be classified as amide exporters and ureide exporters, and asparagine and glutamine usually predominate in the xylem sap of amide exporters (Schubert, 1986). In non-nodulated tropical legumes receiving exogenous N, NO_3^- is the major form of N transport, followed by asparagine and glutamine (McClure & Israel, 1979). On the other hand, nodulated plants of tropical legumes are ureide exporters, since these compounds account for 60–90% of the total N in the xylem saps (Schubert, 1986), e.g., allantoic acid is the major component of the ureide fraction (70–90%) in the sap of nodulated soybean (Sodek & Silva, 1996).

Allantoin and allantoic acid were detected in the xylem sap of coffee by HPLC and colorimetric determinations. However, higher values were obtained for total ureides with HPLC. In general, the colorimetric determinations were 30% lower than the HPLC values (data not shown). Although contaminants were present, it was possible to separate allantoin and allantoic acid by HPLC under the conditions used here (Fig. 2). Moreover, since the integrator of the chromatography system was adjusted to process integration of peaks on tails, as observed for allantoic acid, we assume that the HPLC determinations were more accurate than the colorimetric method. Thus, considered together, ureides represented 5.6% of the total N determined (Fig. 1).

Caffeine was also detected but at a low concentration (Fig. 1), this being distributed throughout the coffee plant (Hamidi & Wanner, 1964; Herndlhofer, 1933; Raju & Gopal, 1979). However studies carried out with labeled caffeine showed that it is not translocated among leaves of the same branch and very little is transported from leaves to fruits of the same node

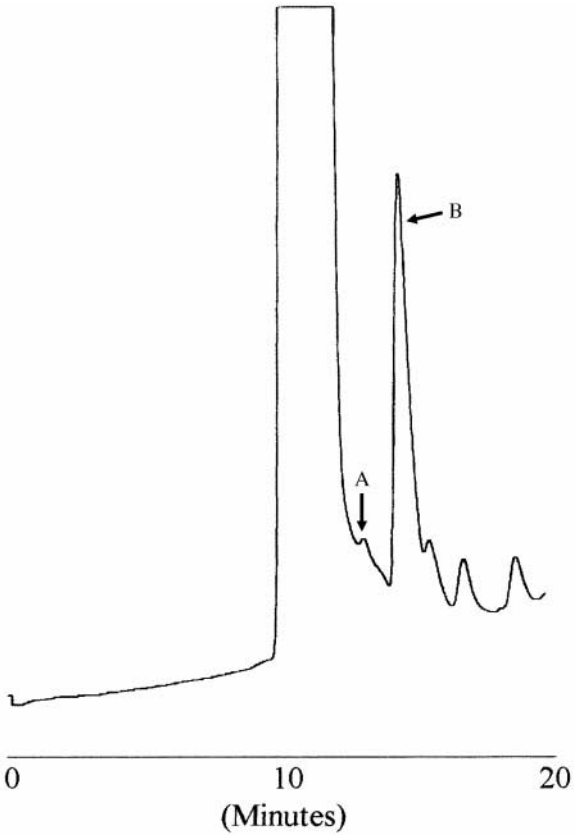

Fig. 2. HPLC profile obtained at 235 nm for detection of (a) allantoic acid and (b) allantoin.

(Baumann & Wanner, 1972; Vitória, 1998). In addition, radioactivity was not detected in phloem sap collected in EDTA solutions of coffee branches which had leaves infiltrated with labeled caffeine (Vitória, 1998). This suggests that caffeine is not or poorly translocated in the phloem and, therefore, it seems probable that it would be synthesized in the roots and other tissues of coffee. However, investigations of enzymes of caffeine biosynthesis have been carried out only for fruits and leaves of coffee (Mazzafera, Crozier & Sandberg, 1994; Roberts & Waller, 1979).

Interestingly, caffeine levels decrease in aging leaves (Hamidi & Wanner, 1964). In addition, this alkaloid is found in the intercellular space of coffee leaves (Baumann & Röhrig, 1989), especially old leaves, where it represents 16% of the total leaf caffeine compared to 4% in young leaves (Vitória, 1998). Leakage of caffeine to the intercellular space in leaves has been associated with chlorogenic acid, which retains the alkaloid inside the cell through the formation of a complex (Baumann & Röhrig, 1989). Therefore, the presence of caffeine in the xylem sap could be related to the concentration of chlorogenic acid in the roots. It has been shown that compared to cotyledons of developing seedlings, the concentration of this phenolic in the roots was much lower (Aerts & Baumann, 1994).

Considering the close relationship between caffeine metabolism and ureide formation in coffee (Ashihara et al., 1996; Suzuki & Waller, 1984a,b) and that considerable amounts of ureides are found in leaves (0.28–0.44 mg g^{-1}) and fruits (0.56–1.05 mg g^{-1}) (Vitória, 1998), it is reasonable to suggest that ureides present in xylem fluid could originate from caffeine degradation in the roots.

Ureides have been extensively investigated in N-fixing plants, mainly in nodulating tropical legumes. Their contribution to the N economy of the plant is well recognized and established. On the other hand, although several reports have shown transport of alkaloids in the phloem and xylem (Kitamura, Yamashita, Miura & Watanabe, 1993; Nowacki & Waller, 1973; Waller & Nowacki, 1978), their role as N storage compounds is still controversial. Several studies have suggested that caffeine is not a N storing molecule in coffee (Mazzafera, 1990) and tea (*Camellia sinensis*) (Cloughley, 1982; Suzuki & Waller, 1986). Petermann and Baumann (1983) suggested that methyluric acids, formed particularly in some coffee species, might also be related to N economy of these plants. On the other hand, we have been unable to detect uric acid or its derivative in the coffee species used here.

Although ureides have been found in several woody plants, only few species contain significant amounts of these compounds in the xylem sap. Contrary to woody plants, the role of ureides in nodulating legume species has been clearly established in N transport for the nutrition of seeds in particular. However, since most of the N required by the coffee plants throughout the year is concentrated during seed filling (Catani & Moraes, 1958), when nitrate reductase activity and nitrate content of the leaves is high (Carelli, Fahl & Magalhães, 1989), the composition of N compounds in xylem sap might be different.

Therefore, additional information given by studies on xylem composition throughout the growing season is required to reach any conclusion concerning the importance of ureides as N transporters in the coffee plant.

3. Experimental

3.1. Plant material and sap collection

Seeds of *Coffea arabica* cv. Catuaí Vermelho were germinated in vermiculite (2 seeds per 200 ml pot) in greenhouse and after emergence the seedlings were transferred to a humidifier chamber (80–90.5% RH), situated inside the greenhouse. When they had produced 2 pairs of leaves they were bled for sap collec-

tion: coffee is a woody plant which makes xylem sap collection very difficult as opposed to herbaceous plants, but this problem can be overcome by keeping the stem soft by growth in a humidified chamber. At this stage, the leaves were 20–30 mm in length, and a third leaflet (5 mm) was emerging. During this period, each pot received 100 ml nutritive solution every other day (Hoagland & Arnon, 1950). The mean minimum and maximum temperatures inside the greenhouse were 20° and 32°, respectively, and the light day length was 13 h. The plants were cut with a razor-blade just below the cotyledonary internode and the cut surface thoroughly washed with distilled water. After blotting excess moisture, the exudation sap was collected with a micro-capillary for 45–60 min (McClure & Israel, 1979). The sap was kept on ice during collection and stored at −20° until analysis. All analyses were carried out using four samples, each sample representing sap from 8 seedlings.

3.2. Analyses

Nitrate in the xylem sap was determined according to Cataldo, Haroon, Schrader and Youngs (1975). Amino acid composition was obtained by HPLC and fluorimetric detection of the *o*-phthaldialdehyde derivatives (Jarret, Coosky, Ellis & Anderson, 1986). Caffeine concentration was determined by HPLC with UV detection (Mazzafera, Silvarolla, Lima & Medina Filho, 1997). Allantoin and allantoic acid were analysed by HPLC using a reversed-phase C_{18} column (Hypersil ODS 5 μm, 250 mm × 4.6 mm, Supelco) with UV detection at 235 nm. The compounds were eluted from the column with 0.5% HOAc at a flow rate of 0.2 ml min^{-1}. Allantoic acid and allantoin concentrations in the sap were also obtained with a colorimetric assay (Vogels & van der Drift, 1970). Pure standards were used to calculate their concentrations in the coffee xylem sap.

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