NITROGEN METABOLISM IN A GRAPEVINE *IN VITRO* SYSTEM

MÉTABOLISME DE L'AZOTE D'UNE VIGNE CULTIVÉE DANS UN SYSTÈME *IN VITRO*

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Abstract: Ammonium, nitrate, nitrite, protein and individual and total amino acid contents were determined in grapevine (cv Sauvignon) cultured *in vitro*. The enzyme activities of nitrate and nitrite reductases, glutamine synthetase, glutamate synthetase and dehydrogenase were also determined. The nitrogen taken up by the plants was 70 % of the total nitrogen in the medium after 75 days of *in vitro* culture. Most of the nitrogen taken up was recovered in the leaves, yet only ammonia and amino acid concentrations were significantly higher in leaves. In roots, glutamine was the most abundant amino acid. In leaves, the most abundant amino acids were aspartate, glutamate, glutamine, alanine, arginine and g-aminobutirate. All enzyme activities were higher in roots than in leaves. These results suggest that both roots and leaves incorporate inorganic nitrogen into organic forms.

Résumé: Les teneurs en ammonium, nitrate, nitrite, protéines et acides aminés ont été déterminées dans la vigne (cv Sauvignon) cultivée *in vitro*. Les activités enzymatiques des nitrates et nitrites réductases, glutamine synthétase, glutamate synthétase et deshydrogénase ont également été déterminées. Après 75 jours de culture *in vitro,* 70 % de l'azote total initial du milieu a été prélevé. L'essentiel de cet azote a été retrouvé dans les feuilles. La concentration en ammonium et en acides aminés est significativement plus élevée dans les feuilles. Dans les racines, la glutamine est l'acide aminé le plus important. Dans les feuilles, les acides aminés les plus présents sont l'aspartate, le glutamate, la glutamine, l'arginine et le γ-aminobutirate. Les activités enzymatiques ont été plus importantes dans les racines que dans les feuilles. Les résultats suggèrent que les racines et les feuilles absorbent l'azote minéral et le transforment en azote organique.

Mots clés : vigne, azote, acides aminés, activités enzymatiques

Key words : vigne, azote, acides aminés, enzyme activities

INTRODUCTION

Nitrogen is generally accepted to be the nutrient which plays the most important role in plant growth. Recognition of this fact means that nitrogen fertilisation has become one of the most extensive agricultural practices the world over. In grapevine, the effect of nitrogen fertilisation and nitrogen demand during the vegetative period has been thoroughly analysed in field studies. Nevertheless, there are considerable difficulties in determining nitrogen metabolism in perennial plants such as grapevine. In fact, despite the studies into nitrogen fertilisation, knowledge of the nitrogen metabolism in grapevine is considerably limited, and there is no clear explanation of the differences in handling nitrogen soil resources, variety yield and partitioning of nitrogen compounds (SPONHOLZ, 1991). Nitrogen status and metabolism in grapevines have a considerable effect on grape production and quality and nitrogen status in musts affects the fermentation process. The amount of available nitrogen has been related to the fermentation rate.

Some of the difficulties of studying the nitrogen metabolism can be overcome through *in vitro* studies. The aim of the present study was to analyse the primary grapevine nitrogen metabolism in a closed system (*in vitro* explants). The concentration of major organic (protein and amino acids) and inorganic (nitrate and ammonium) nitrogen containing compounds was determined in leaves and roots. The activities of main enzymes that regulate the organification and metabolism of nitrogen have been quantified.

MATERIALS AND METHODS

Meristems of *Vitis vinifera* (cv Sauvignon) grapevines were cultured as in LLORENS *et al.* (2000). The nitrogen sources of the medium were 4.5 mM of NH_4NO_3 and 9.4 mM of KNO_3. The vines were cul-

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tured for up to 3 months. Roots (ca. 0.2 g) and leaves (mean number 5-6, 0.25 g) from in vitro plantlets were processed as in LLORENS *et al.* (2000).

Total nitrogen was determined by gas chromatography after oxidation and combustion of the whole plant. The nitrate and ammonium contents were quantified by enzymatic kits (Boehringer Mannheim). The protein content was estimated by the Bradford method. Total amino acids were measured by the ninhydrin test with glycine as a standard. Individual amino acids were determined by HPLC (Gilson Asted System) using the OPA (orto-phthaldialdehyde) and FMOC (9-fluorenyl methoxy carbonyl chloride) derivatives, as in LLORENS *et al.* (2000).

The activities of nitrate reductase, nitrite reductase, glutamate synthase Ferredoxin dependent, glutamine synthetase and glutamate dehydrogenase were determined as in LLORENS *et al.* (2000).

RESULTS AND DISCUSSION

The uptake of nitrogen from the medium was very fast after shoot development, starting when rooting (15 days, 5 % of total nitrogen in the medium), reaching 50 % after 60 days of culture and peaked 2 months after rooting, when 70 % of the total nitrogen in the medium was already in the plantlet. Most of the nitrogen (table I) was recovered in the leaves (2/3 of the total plant nitrogen). Considerable nitrogen translocation from roots to leaves was also observed in field experiments (CONRADIE, 1991, ROUBELAKIS-ANGELAKIS and KLIEWER, 1991). Nitrate, ammonium, protein and amino acids accounted for 50 % and 70 % of total nitrogen in the leaves and roots respectively. However, a bulk of nitrogen compounds were not determined and presumably they were in the form of nucleic acids, alkaloids, polyamines and chlorophyll (ROUBELAKIS-ANGELAKIS and KLIEWER, 1992). The ratio between root and leaf nitrogen concentrations was very similar to that reported in field systems (CONRADIE, 1991), although the actual numbers may be different due to the obvious differences between field and *in vitro* plants. After 75 days, the final concentration of nitrogen in the medium was 0.3 mg N/g fresh weight, a value that was 10 times lower than the root value. This may be due to the presence of an active system of uptake in this organ and influence of *in vitro* culure. The root level of nitrate and ammonia were in the assumed normal range for other plants and field or potted grapes (GU *et al.*, 1996). However, the leaf levels of ammonia and nitrate were in the upper part of the range. In plants, nitrate and ammonia are transported *via* xylem from roots to leaves, although the total amount of ammonium is low; so the high ammonium levels observed in leaves could be related to other ammonium sources such as photorespiration, which is considered to be the highest ammonium producing pathway. It may also be due to urea cycle or deamination activities (ROUBELAKIS-ANGELAKIS and KLIEWER, 1991). In roots, nitrate still accounted for a large amount of the total nitrogen, whereas ammonium concentration was low.

Nitrogen, in the form of protein and free amino acids (table I), accounted for similar percentages in

Table I - Nitrogen, nitrate, ammonium, protein and amino acid concentrations in roots and leaves.

Results are the mean ± SE of 5 different experiments.

Concentration en azote, nitrate, ammonium, protéines et acides aminés dans les racines et dans les feuilles.

	Leaves	Roots
Nitrogen (mg/g fresh weight)	6.1±0.3	3.3±0.2
Nitrate (mg/g fresh weight)	1.4±0.2	1.8±0.3
Ammonia (µg/g fresh weight)	562±78	94±5
Protein (mg/g fresh weight)	11.9±1.1	8.3±0.6
Amino acids (µmols/g fresh weight)	29.7±1.7	23.2±1.9

Table II - Free amino acid concentrations (nmols/g of fresh weight) in roots and leaves.

Results are the mean ± SE of 5 different experiments.

Concentrations en acides aminés libres dans les racines et dans les feuilles

	Leaves	Roots
Aspartate	1150±71	493±12
Glutamate	2209±278	1459±23
Asparagine	920±10	1326±71
Glutamine	2091±272	11923±418
Alanine	1427±91	1476±64
Serine	750±26	846±23
Glycine	87±12	188±15
Threonine	401±28	<dl
Proline	93±4	<dl
Histidine	<dl	<dl
Lysine	135±12	94±6
Arginine	1003±14	2642±111
Tyrosine	<dl	<dl
Tryptophan	89±4	47±1
Phenylalanine	288±16	93±7
Leucine	130±2	136±10
Isoleucine	99±3	100±7
Valine	119±8	174±12
Cysteine	<dl	<dl
Methionine	<dl	<dl
γ-aminobutirate	1113±77	1086±42
α-aminobutirate	<dl	<dl

<dl: below detection limit (10 nmols/g fresh weight).

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both organs. The reported levels of protein in systems similar to ours are in the same range (LLORENS *et al.*, 2000). The concentrations of individual amino acids (table II) were very different ranging from 11,900 to less than 10 nmols/g fresh weight. There was a clear difference between the amino acids which are important in the transport and metabolism of nitrogen and the ones that are basically used for protein synthesis. In roots, glutamine was the most abundant amino acid (an order of magnitude more than the others in roots or leaves). Other abundant amino acids were arginine, glutamate, asparagine, alanine and γ-aminobutirate. In leaves, the most abundant amino acids were aspartate, glutamate, glutamine, alanine, arginine and γ-aminobutirate. The high levels of glutamine and glutamate in roots and leaves may indicate that the organification of nitrogen was active and similar in both organs. However, the high levels of glutamine in the roots, along with very high glutamine synthetase activity, might suggest that this amino acid has an important role in the translocation of nitrogen from root to leaf (DEMBINSNKI *et al*., 1995), and is probably quantitatively more important than the translocation of inorganic nitrogen. Also important were the levels of arginine, which have been reported as indicators of the nitrogen status in plants (ROUBELAKIS-ANGELAKIS and KLIEWER, 1991). However, this is related to the role of arginine as nitrogen storage in perennial parts, which is not the case in our experiments.

All the enzyme activities (table III) were higher in roots than in leaves, except nitrite reductase for which the activity was similar. Thus, both organs were capable of incorporating inorganic nitrogen in organic forms, although most of this activity was in roots, as shown by the activity of nitrate reductase in roots which was twice that of the leaves (PEREZ and KLIEWER, 1982). This indicates that nitrate is basically reduced in roots. Glutamine synthetase levels were similar to those reported in field grapevines (KELLER and KOBLET, 1995), yet activity was ten times higher in roots. The Glutamate synthase activity was also higher in the roots, which may be related to the high requirement of glutamate for the synthesis of glutamine.

Table III - Activities (nmol/min.mg protein) of several enzymes of primary nitrogen metabolism in roots and leaves.

Results are the mean ± SE of 5 different experiments.

Activités de plusieurs enzymes du métabolisme primaire de l'azote dans les racines et dans les feuilles

	Leaves	Roots
Nitrate reductase	0.89±0.02	2.2±0.8
Nitrite reductase	2.7±0.7	2.2±0.3
Glutamine synthetase	57.2±5.8	590±28
Glutamate synthase -ferredoxine	102±4	149±4
Glutamate synthase -NADH	nd	1.2±0.2
Glutamate dehydrogenase	9.7±1.3	13.8±1.6

nd: not detected

The results presented here suggest that roots have a critical role in the organification of nitrogen. Most of the nitrogen is probably incorporated in organic forms in roots and translocated as glutamine to the rest of the plant. However, the pathway of nitrogen organification is also present in leaves, perhaps because of the limited transport of inorganic nitrogen as well as the use of ammonium produced by photorespiration.

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REFERENCES

CONRADIE W.J., 1991. Translocation and storage of nitrogen by grapevines as affected by time of application. *In: Proceedings of the International Symposium on Nitrogen in Grapes and Wine*. The American Society for Enology and Viticulture, Davis, p. 32-42.

DEMBINSKI E., BANY S. and RACZYNSKA-BOJANOWSKA K. R. 1995. Asparagine and glutamine in the leaves of high and low protein maize. *Acta Physiol. Plant.*, **17**, 361-365.

GU S., LOMBARD P. B. and PRICE S. F. 1996. Effect of shading and nitrogen source on growth, tissue ammonium and nitrate status, and inflorescence necrosis in Pinot noir grapevines. *Am. J. Enol. Vitic.*, **47**, 173-180.

KELLER M. and KOBLET W. 1995. Stress-induced development of inflorescence necrosis and bunch-stem necrosis in *Vitis vinifera* L. in response to environmental and nutritional effects. *Vitis*, **34**, 145-150.

LLORENS N., AROLA L., BLADÉ C. and MAS A. 2000. Effects of copper exposure upon nitrogen metabolism in tissue cultured *Vitis vinifera*. *Plant Sci*., **160**, 159-163.

PEREZ J. R. and KLIEWER W. M. 1982. Influence of light regime and nitrate fertilization on nitrate reductase activity and concentrations of nitrate and arginine in tissue of three cultivars of grapevines. *Am. J. Enol. Vitic*., **33**, 86-93.

ROUBELAKIS-ANGELAKIS K. A. and KLIEWER W. M. 1991. Synthesis and regulation of glutamate dehydrogenase in grapevine callus *In: Proc. of the Int. Symp. on Nitrogen in Grapes and Wine*. The American Society for Enology and Viticulture, Davis, p 306-311.

ROUBELAKIS-ANGELAKIS K. A. and KLIEWER W. M. 1992. Nitrogen metabolism in grapevine. *Horticult. Rev.*, **14**, 408-452.

SPONHOLZ W. R. 1991. Nitrogen compounds in grapes, musts and wine. *In: Proc. of the Int. Symp. on Nitrogen in Grapes and Wine*. The American Society for Enology and Viticulture, Davis, p 67-77.

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