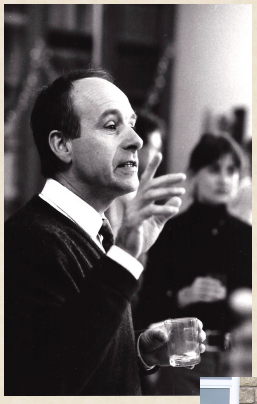
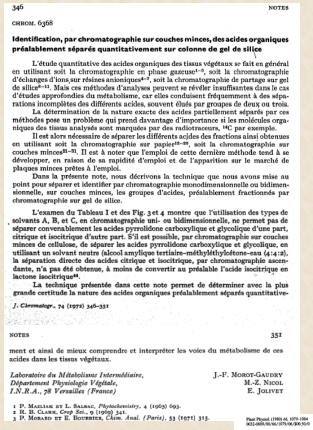
# Jean-François MOROT-GAUDRY





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CHROM. 6368

**Identification, par chromatographie sur couches minces, des acides organiques préalablement séparés quantitativement sur colonne de gel de silice**

L'étude quantitative des acides organiques des tissus végétaux se fait en général en utilisant soit la chromatographie en phase gazeuse[1-3], soit la chromatographie d'échanges d'ions sur résines anioniques[4-7], soit la chromatographie de partage sur gel de silice[8-11]. Mais ces méthodes d'analyses peuvent se révéler insuffisantes dans le cas d'études approfondies du métabolisme, car elles conduisent fréquemment à des séparations incomplètes des différents acides, souvent dûs par groupes de deux ou trois.

La détermination de la nature exacte des acides partiellement séparés par ces méthodes pose un problème qui prend davantage d'importance si les molécules organiques des tissus analysés sont marquées par des radiotraceurs, $^{14}C$ par exemple.

Il est alors nécessaire de séparer les différents acides des fractions ainsi obtenues en utilisant soit la chromatographie sur papier[12-16], soit la chromatographie sur couches minces[8-11]. Il est à noter que l'emploi de cette dernière méthode tend à se développer, en raison de sa rapidité d'emploi et de l'apparition sur le marché de plaques minces prêtes à l'emploi.

Dans la présente note, nous décrivons la technique que nous avons mise au point pour séparer et identifier par chromatographie monodimensionnelle ou bidimensionnelle, sur couches minces, les groupes d'acides, préalablement fractionnés par chromatographie sur gel de silice.

L'examen du Tableau I et des Fig. 3 et 4 montre que l'utilisation des types de solvants A, B, et C, en chromatographie uni- ou bidimensionnelle, ne permet pas de séparer convenablement les acides pyrrolidone carboxylique et glycolique d'une part, citrique et isocitrique d'autre part. S'il est possible, par chromatographie sur couches minces de cellulose, de séparer les acides pyrrolidone carboxylique et glycolique, en utilisant un solvant neutre (alcool amylique tertiaire–méthyléthylcétone–eau (4:4:2), la séparation directe des acides citrique et isocitrique, par chromatographie ascendante, n'a pas été obtenue, à moins de convertir au préalable l'acide isocitrique en lactone isocitrique[6].

La technique présentée dans cette note permet de déterminer avec la plus grande certitude la nature des acides organiques préalablement séparés quantitative-

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ment et ainsi de mieux comprendre et interpréter les voies du métabolisme de ces acides dans les tissus végétaux.

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**Oxygen Effect on Photosynthetic and Glycolate Pathways in Young Maize Leaves**

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ABSTRACT

To study the effect of $O_2$ on the photosynthetic and glycolate pathways, young maize leaves were fed $^{14}CO_2$ in the light. Under normal conditions (21 to 25 %), in the two $O_2$ concentrations after a $^{14}CO_2$ pulse of seconds followed by a $^{12}CO_2$ chase, there was a slight difference in $CO_2$ uptake and in the distribution of $^{14}C$ in the photosynthetic products. The distribution especially in phosphoglycerate, sugar bisphosphates, glycine, and serine. The kinetics of $^{14}C$ incorporation into glycine and serine showed a transitory accumulation of glycine. Although in normal conditions (21 to 25 %), labeling of glycine was reduced by 50% and that of serine was reduced by 70%, relative to the control in 20% $O_2$, a similar effect was observed in the two $O_2$ concentrations when the leaves were fed in the dark. In the case $^{14}C$ feed under 20% $O_2$, was found in glycine pulses increase dates after 60 seconds chase. This figure is 50% in C$_3$ plants. Isotoxical feed in the dark, the amount of radioactivity incorporated in glycine is 25 %, and the first carbon atom of serine was preferentially labeled during the first seconds of the chase. These results suggest the hypothesis that photorespiration, which is known to be inhibited by high $O_2$ concentrations, is inhibited in the first carbon atom during the first seconds of photosynthesis.

Under normal conditions, the observed differential labeling of phosphoglycerate and sugar bisphosphates. Phosphoglycerate is more labeled than sugar bisphosphate in all the cases. Changes in the distribution of $^{14}C$ in the photosynthetic products, induced by the $O_2$ effect, are not due to a change in the activity of the enzymes involved in the Calvin cycle. To understand the effect of $O_2$ on the distribution of $^{14}C$ in these metabolites, it was postulated that, in air, there remains an oxygenase function which produces additional phosphoglycerate at the expense of ribulose bisphosphate.



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ORIGINAL ARTICLE

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**Nitrite accumulation and nitric oxide emission in relation to cellular signaling in nitrite reductase antisense tobacco**

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**Abstract** An antisense nitrite reductase (NiR, EC 1.7.7.1) tobacco (*Nicotiana tabacum* L.) transformant (NiaAS) with 10% of wild type NiR activity has been correlated to between nitrate reductase (NR, EC 1.6.1.1)-dependent nitrite accumulation and nitric oxide (NO) production, and to assess the regulation of signal transduction in response to stress conditions. Nitrite accumulation in NiaAS leaves was 10-fold higher than in wild type leaves. Increased protein tyrosine nitration in clone 251 suggests that high NO production resulted in decreased peroxynitrite (ONOO$^-$) formation. Tyrosine nitration was also observed in the presence of peroxynitrite in leaf extracts. As in nitrosomonas cells, NO and derivatives also increased synthesis of proteins like (A-L) and cyclophilin, which are both involved in regulation of activity and stability of enzymes.

**Keywords** Cyclophilin · Nitrite · Nitric oxide · Peroxynitrite · 14-3-3 protein · Tyrosine nitration

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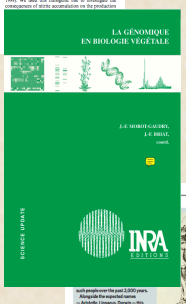
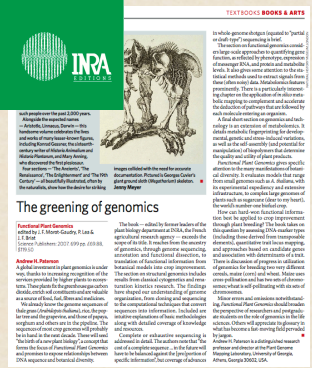
**Introduction**

Nitrate and its downstream reduction products like nitrite, nitrogen oxides and ammonium are important signaling compounds in plants, but may also induce toxicity syndromes. In particular, nitric oxide (NO) is recognized not only as a signal molecule regulating ion fluxes and gene expression in mammalian cells (Lamattina et al. 2002, 2004). NO also appears to be involved in signal transduction in plant-pathogen interactions (Durner and Klessig 1999; Wendehenne et al. 2001). The cytotoxic effects of NO may be ascribed to nitrosylation of Fe-S centers, to the formation and reaction of NO$^-$ and superoxide (Yamasaki and Sakihama 2000; Radi et al. 2001). Peroxynitrite is a potent oxidant that reacts with a variety of biological molecule (Pryor and Squadrito 1995). With tyrosine residues it generates 3-nitrotyrosine derivatives (Beckman and Koppenol 1996).

Previously, it was observed that the tobacco NiaAS mutant, which has been transformed with antisense RNA under the control of the GAPA-1/IR promoter grows normally on ammonium, but displays drastically reduced development and chlorotic leaves when grown on nitrate as the sole source of nitrogen. This plant accumulates nitrite in the leaves to a level 10-fold higher level (Vaucheret et al. 1992) and produced NiR with increasing nitrite levels in plant tissues (Crouhma et al. 1995). We used this transgenic line to investigate the consequences of nitrite accumulation on the production

**Abbreviations** NiR: nitrite reductase · NO: nitric oxide · NiR: nitrate reductase · ONOO$^-$: peroxynitrite · WT: wild type

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