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Short Research Article

Production and use of mycotoxins uniformly enriched with stable isotopes for their dosage in biological samples: (1) Production of uniformly enriched biomass†

MICHEL PÉAN[1,*], SÉVERINE BOIRY[1], JEAN-CLAUDE FERRANDI[1], FRÉDÉRIC GIBIAT[1], OLIVIER PUEL[2] and MARCEL DELAFORGE[3]

[1]CEA Cadarache, DEVM, Groupe de Recherches Appliquées en Phytotechnologie, Saint-Paul-les-Durance F-13108, France
[2]INRA, UR 66, Laboratoire de Pharmacologie-Toxicologie, Toulouse F-31931, France
[3]CEA, DSV, DBJC, SBFM, LTMD, Laboratoire de Transport Membranaire et Detoxication, and URA CNRS 2096, Gif-sur-Yvette F-91191, France

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Introduction

Enrichment of plants with stable isotopes is a powerful tool for structural,[1] nutritional[2] or metabolic[3] studies. In this work, in order to prepare ^{13}C- and ^{15}N-enriched (10%) natural mixture of mycotoxins (see poster 3), we developed a new strategy where the fungus were grown (see poster 2) on seeds or plants (wheat or maize) uniformly enriched with ^{13}C and ^{15}N.

To perform uniform biomass enrichment with ^{13}C and ^{15}N, it is necessary to provide ^{13}C-enriched carbon dioxide (which will be fixed by photosynthesis), and ^{15}N-enriched nitrate and ammonium (which will be absorbed by the roots) to the plants during their complete growth cycles, i.e. for several months. Whereas it is easy to supply enriched nitrogen via the nutrient solution, ^{13}C-enriched carbon dioxide input requires to overcome the production of plants in hermetically closed chambers for long times.

We present here the facilities we used to produce doubly (^{13}C and ^{15}N) enriched matter of wheat and maize.

Results and discussion

A platform dedicated to plant production in controlled conditions

Our multi-field team (technologists and biologists) manages, develops and maintains a platform dedicated to the production of plants in controlled conditions. It consists of air-tight chambers of various volumes (from 10 to 1500 L), phytotrons for GMO production and for experiments with toxic and radioactive elements.

Production of uniformly enriched wheat and maize plants

Wheat (*Triticum aestivum* cv. Ardente) and maize (*Zea mays* cv. Antares) are cultivated in air-tight chambers in which atmospheric gas composition and environmental parameters (photoperiods, photons flux rates, night and day temperatures and relative humidity) are closely regulated. Carbon dioxide concentrations into the chambers (both $^{12}CO_2$ and $^{13}CO_2$) are continuously monitored with an infra-red gases analyser. During light period, they are maintained at $360\,\mu L\,L^{-1}$ by automatic injections of concentrated ^{13}C-enriched CO_2, to compensate for photosynthetic assimilation. Plants are automatically watered with nutrient solution containing ^{15}N-enriched nitrate and ammonium salts. Perlite (mineral amorphous granules of volcanic origin) is used as culture substrate to avoid soil respiration that would release carbon dioxide with natural ^{13}C abundance (about 1%) and would lead to decrease the ^{13}C enrichment level. Volatile organic compounds

*Correspondence to: Michel Péan, CEA Cadarache, DEVM, Group de Recherches Appliquées en Phytotechnologie, Saint-Paul-les-Durance F-13108, France. E-mail; michel.pean@cea.fr

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which can be released by plants and disturb plant development are continuously trapped during the cultures.

Follow-up of plant growth and enrichment

The monitoring of the experiments is ensured by a control-command system piloted by the human–machine-interface software 'In Touch^{TM}'. It not only allows to permanently measure and record environmental parameters, but also to measure physiological parameters such as net photosynthesis, dark respiration, and plant transpiration. This permits the follow-up of plant growth and development during all the growth cycle of the plants and thus to continuously know the quantity of biomass produced.

Conclusions

We produced several hundred grams of doubly enriched dry matter of wheat and maize (10% ^{13}C and ^{15}N). This matter has been used to produce doubly enriched mycotoxins (see posters 2 and 3). Different levels of plant enrichment will be done in the framework of this program.

The facilities managed by our team are a useful and flexible tool to produce plants in controlled conditions for varied purposes. The platform is opened to exterior researchers for contracts or collaborations. Experiments are led there in the framework of a quality policy based on the ISO9001v2000 standard.

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