Photosynthesis Research
https://doi.org/10.1007/s11120-019-00634-9

HISTORY AND BIOGRAPHY

Tribute Roland Douce, 1939–2018

Jacques Joyard[1] · **Hartmut K. Lichtenthaler**[2]

Received: 4 February 2019 / Accepted: 6 March 2019

Abstract
On November 4, 2018, Roland Douce, Professor Emeritus at the University of Grenoble, France, died at the age of 79. In Grenoble, where he spent most of his scientific career, Roland Douce created a world-renowned school of plant science, studying the structure, functions, and interactions of plant organelles involved in photosynthesis, respiration, and photorespiration. His main achievements concern the chemical and functional characterization of chloroplast envelope membranes, the demonstration of the uniqueness of plant mitochondria, and the integration of metabolism within the plant cell, among manifold activities. Roland Douce devoted his whole life to science and research with passion and enthusiasm: he was a true charismatic leader.

Keywords Chloroplast envelope · Glycerolipids · Lipidomics · Metabolomics · Mitochondria · Photorespiration

Life and career

Roland Douce (Fig. 1) was born on May 18, 1939 in Saint-Maur-des-Fossés (Val-de-Marne, France). He was the second of three sons of Robert Douce, a certified accountant, and his wife Marie. He attended high school at the Lycée Marcellin Berthelot, Saint-Maur-des-Fossés, and in 1958 started a college degree in physics, chemistry, and biology at the well-known University "La Sorbonne," in Paris. Having a passion for life sciences (he could recognize many bird songs), he developed a major interest in plant physiology and biochemistry. In 1961, Douce began his scientific career as an assistant at the Sorbonne, preparing his PhD. In 1966, just after his marriage to Danielle, his PhD work was interrupted for 16 months as he had to perform the compulsory military service. He presented his PhD thesis in 1970 on the "Structure, localization and metabolism of diphosphatidylglycerol, or cardiolipin, in plants."

Roland Douce started his scientific research with investigations on plant cell organelles and lipids, with a main focus on cardiolipin. At the time, nothing was known about this phospholipid in plants. Douce demonstrated that the compound analyzed by many groups was actually phosphatidylmethanol, an artifact produced during incubation of cells with methanol. This paved the way for the first characterization of diphosphatidylglycerol as cardiolipin in plants (Coulon-Morelec and Douce 1968)—the topic of Douce's PhD dissertation.

Just after completing his Ph.D., Roland Douce went from 1970 to 1972 to the Johnson Research Foundation in Philadelphia as a postdoc in the group of Prof. Walter D. Bonner Jr. (1914–1989). Bonner is known for his classic series of papers identifying the "alternative oxidase." In Philadelphia, Roland analyzed the activities of sucrose-purified mitochondria from mung bean hypocotyls and potato tubers. Among his major findings was the surprising observation of the inhibition of mitochondrial respiration by oxaloacetate which led him to develop a test proving the structural integrity of mitochondria (Douce et al. 1972).

After these 2 years in Pennsylvania, Roland Douce moved to La Jolla, California, to work on the chloroplast envelope membranes with Prof. Andrew A. Benson (1917–2015), the co-discoverer of the photosynthetic carbon reduction cycle, known today as Calvin–Benson cycle (Buchanan et al. 2007; Lichtenthaler et al. 2008, 2015). At that time, Benson was known for his research on plant glycerolipids and

✉ Jacques Joyard
jacques.joyard@wanadoo.fr

[1] Laboratoire de Physiologie cellulaire et végétale, Institut de Recherche Interdisciplinaire de Grenoble, Université Grenoble Alpes, CEA, CNRS, INRA, Grenoble, France

[2] Botany 2 (Molecular Biology and Biochemistry of Plants), Karlsruhe Institute of Technology, Kaiserstr. 12, 76133 Karlsruhe, Germany

Published online: 15 March 2019

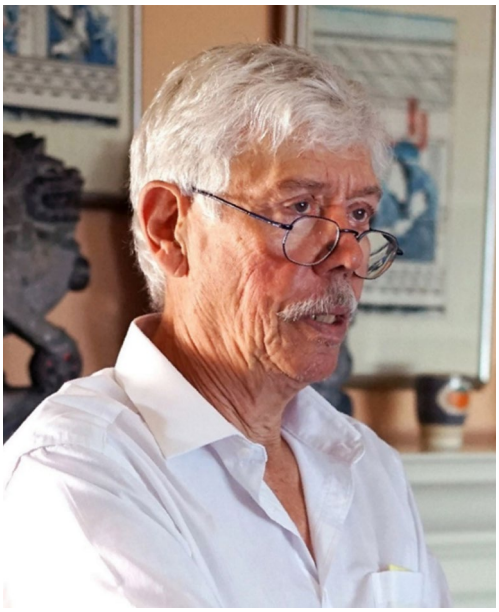

Fig. 1 Photograph of Roland Douce in 2014 in La Jolla, California. (Source: A. Nonomura)

had already detected the plant sulfolipid, which is unique for photosynthetic organisms. Starting with sucrose-purified spinach chloroplasts, Douce was the first to purify and properly characterize the chloroplast envelope, as a whole (i.e., a mixture of the outer and inner membranes) (Douce et al. 1973). This relied on the proper characterization of envelope marker enzymes, constituents (glycerolipids and carotenoids, etc.), and functions (galactolipid biosynthesis and carotenoid transformations) (Jeffrey et al. 1974; Douce 1974). The biochemical and functional characterization of the plastid envelope (inner and outer membrane) would determine the research of his group for almost the next 30 years. It brought him international recognition.

In 1974, Roland Douce was appointed Professor at the University of Grenoble, France, where he stayed after his retirement in 2004 as Professor Emeritus. During these years, he established an internationally known laboratory for plant cell physiology and biochemistry.

Other activities

Roland Douce was always open to scientific discussions and cooperation to better understand the dynamics of cell metabolism. In addition, he served as competent Adviser, Chairman, and Coordinator of various research programs and joined science activities.

Douce set up a research group at the University of Grenoble on the CEA (Commissariat à l'Énergie atomique) Campus. In 1979, he was appointed Scientific Adviser at CEA and his research group became associated with CNRS (Centre national de la Recherche scientifique). His project was titled, *Plastids-cytosol-mitochondria Interactions*, and the group later became the *Laboratoire de Physiologie cellulaire et végétale*, a research unit of Grenoble University, CNRS, and CEA. Douce was chairman of this laboratory until 1991. He remained a Scientific Adviser. The laboratory is still active at Grenoble Alpes University, CNRS, INRA, and CEA (Eric Maréchal, a former student of Douce, is Chairman).

Roland Douce found it essential to develop links between academic research and industry. In 1982, he became a Scientific Adviser to Rhône-Poulenc Agrochimie, and in 1986, he created the first CNRS laboratory associated with an industrial partner (initially Rhône-Poulenc Agrochimie, then Aventis Crop Science) in Lyon, France. The objective of this laboratory was to identify and describe the molecular level targets residing in chloroplasts or mitochondria for new herbicides, and it was dedicated to studies on the synthesis of amino acids and vitamins in higher plants.

In the 1980s, Roland Douce also played an important role in the resurgence of the Lautaret Alpine Garden, at 2100 m elevation in the Alps. This was followed by the creation of a high-altitude biological research station, the Joseph Fourier Station (https://www.jardinalpindulautaret.fr), in 1989. Its first two directors, Richard Bligny and Serge Aubert (1966–2015), were his former students. Research at the Station was open to scientists interested in understanding how light energy dissipates by investigating the specificities of photosynthesis and carbon metabolism in alpine plants.

From 1985 to 1990, Douce acted as Department Chairman at INRA. In 1992, he was appointed Member of the University Institute of France, Paris. From 1995 to 1998, he served as Director of Research at the École Normale Supérieure, Lyon, France. In 2001, he was elected as Senior Scientist at the University of Oxford, UK. From 2002 to 2004, he was the Director of the Institute for Structural Biology, in Grenoble (Fig. 2).

In addition, Douce was a regular participant and esteemed speaker at many international meetings, such as the International Congresses on Photosynthesis, the International Symposia on Plant Lipids, the ISPLs (Lichtenthaler 2004), see Fig. 3, as well as at various Gordon Conferences, and international meetings. On the 4th ISPL in 1980 held in Paris, he served as co-organizer.

Roland Douce often presented his results in an unexpected manner with unusual comparisons. Hartmut Lichtenthaler recalls, that at the 5th International Photosynthesis Congress in Halkidiki, Greece, in September 1980, Roland started his lecture on the significance and function of the chloroplast envelope with a photo of a nice young girl, his daughter. Then he continued with: *One thing I know for sure, every single carbon atom in her body has twice passed*

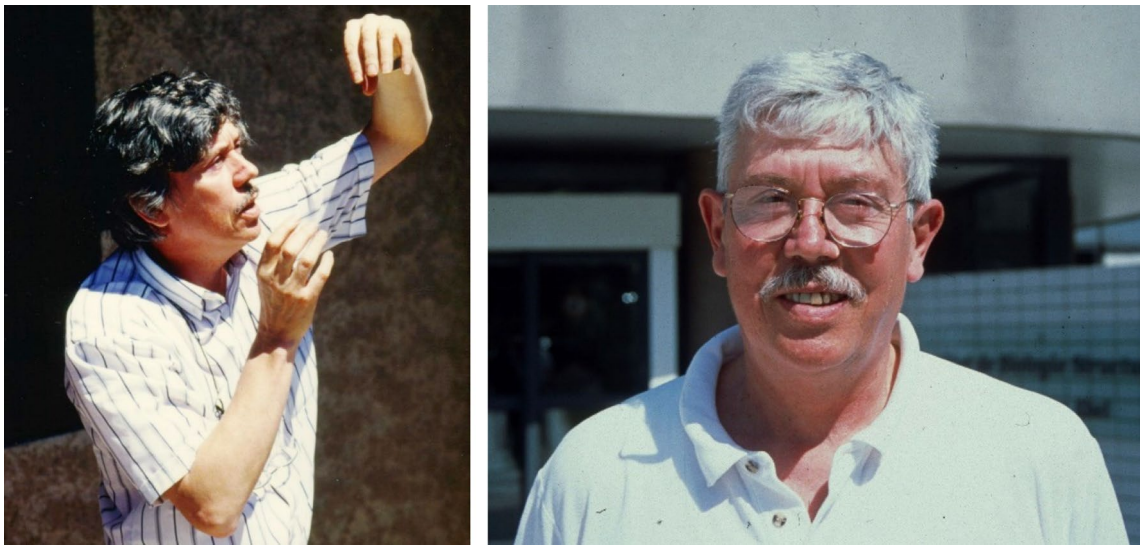

Fig. 2 Left: Roland Douce talking about plant metabolism during a lab meeting at the Lautaret Alpine Station (picture taken around 1980) and (right) in front of Institut de Biologie Structurale (Grenoble). Roland was the Director of this Institute from 2002 to 2004. (Source: LPCV, Grenoble and IBS, Grenoble)

Fig. 3 Roland Douce with colleagues in August 1986 on the 7th International Symposium of Plant Lipids (ISPL) in Sacramento, California. From left to right: Bernt Gerhardt (Münster, Germany), Roland Douce, Hartmut Lichtenthaler (Karlsruhe, Germany), and Norio Murata (Okazaki, Japan). (Source: H. Lichtenthaler)

the chloroplast envelope, the first time entering the chloroplast as CO_2 and the second time as part of a sugar molecule leaving the chloroplast. In this way, he also emphasized the essential role of the photosynthetic carbon fixation enzyme RuBisCO and the carbon reduction cycle in the chloroplast, known as Calvin–Benson cycle, for the nutrition of heterotrophic organisms including men.

Douce was very fond of Andrew Benson in La Jolla in whose lab he had spent 2 years from 1972 to 1974. In October 2007, Roland Douce, together with his friends Bob Buchanan (Berkeley) and Hartmut Lichtenthaler (Karlsruhe), all emeritus professors, organized a joint meeting with Andy Benson in Paris (Fig. 4). B. Buchanan and H. Lichtenthaler recall *At this meeting we were honoring our joint friend Andy Benson at his 90th birthday celebration at the historic Le Procope restaurant in Paris. Roland with his generous hospitality had organized a dinner at which we three presented Andy with the Special Issue of Photosynthesis Research, with papers dedicated to him* (Buchanan et al 2007). *This issue had been edited by us three. It was a wonderful celebration (*Lichtenthaler et al. 2008*). The presence of Andy's wife Dee added to memories of the meeting. Andy was one of Roland's heroes as he never failed to point this out when speaking in public. We know Andy was equally proud of Roland.*

Fig. 4 Roland Douce (right) with Andy Benson (middle) and Hartmut Lichtenthaler (left) in Paris, October 20, 2007 celebrating Andy Benson's 90th birthday. (Source: H. Lichtenthaler)

Searching for pure plant organelles and membranes: a global strategy for plant cell physiology

The strategy initiated by Roland Douce during his initial training and postdoctoral period relied on the development of new procedures to purify highly intact plant organelles–chloroplasts and mitochondria from various plant tissues as a first step toward their molecular and functional characterization. Once in Grenoble, this strategy was extended to all his projects on plant cell organelles involved in photosynthesis, respiration, and photorespiration.

One of the most conspicuous examples was the preparation and characterization of highly purified chloroplasts and their limiting envelope membranes from spinach leaves. The procedure developed in Benson's lab (Douce et al. 1973) was continuously improved to produce large amounts of highly purified envelope membranes (reviewed by Douce and Joyard 1979, 1990). Envelope fractions were almost totally devoid of contaminating membranes derived either from plastids (thylakoids) or from extraplastidial membrane systems (mitochondria, endoplasmic reticulum, etc.). In 1983, Block et al. described a method for the separation of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. An understanding as complete as possible of the biochemical nature of the two membranes of the chloroplast envelope was therefore made possible.

The purification method for mitochondria developed in Bonner's Lab (Douce et al. 1972) was markedly improved when Percoll was introduced (Neuburger et al. 1982). Reliable procedures were developed for preparing plant mitochondria in a high state of purity and morphological integrity from a wide variety of tissues (reviewed by Douce 1985; Douce and Neuburger 1989). Owing to continuous improvements and updates, these procedures (reviewed in Methods in Enzymology 148, Packer and Douce 1987) are still widely used by many groups around the world.

Structure and function of chloroplast envelope membranes

Extensive analysis of lipids within plant cell membranes was one of the first comprehensive observations by Douce and co-workers (reviewed by Douce and Joyard 1979, 1980, 1990). They demonstrated the unique chemical composition of chloroplasts: plastid envelope membranes, as well as thylakoids, containing galactolipids, sulfolipid, and phosphatidylglycerol. In contrast to extraplastidial membranes, chloroplast membranes are mostly devoid of phosphatidylcholine: only the outer surface of the outer envelope membrane contains this major cell phospholipid (Dorne et al. 1985). The major neutral glycolipids and phospholipids from envelopes of spinach chloroplasts were further analyzed with respect to proportions, positional distribution, and pairing of fatty acids, in collaboration with the group of Prof. E. Heinz (Germany) (Siebertz et al. 1979) (Fig. 5). In parallel, glycerolipids residing to the inner and outer mitochondrial membranes from various plant tissues were also analyzed in detail, thus providing one of the most comprehensive views of plant cell membrane lipids to date (Douce and Joyard 1980; Bligny and Douce 1980; Douce 1985).

Roland Douce was the first to demonstrate that envelope membranes from highly purified chloroplasts are yellow, because they contain carotenoids and lack chlorophyll. The

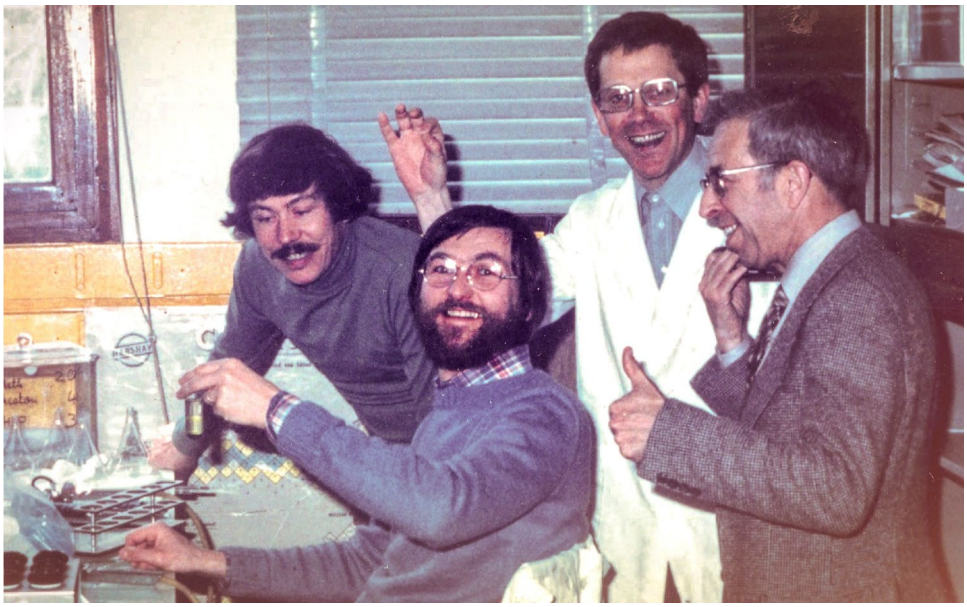

Fig. 5 Preparation of chloroplast envelope membranes (Grenoble, 1978). Roland Douce, Ernst Heinz (Hamburg, Germany), and JFGM Wintermans (Nijmegen, Netherlands)—two leading scientists on plant lipids—look at the centrifugation tube with the envelope preparation (held by Jacques Joyard). (Source: HP Siebertz)

individual types of carotenoids and the fact that the envelopes also contain several prenylquinones (plastoquinone-9 and tocopherols) were determined in a joint investigation of the labs of Hartmut Lichtenthaler with that of Roland Douce (Lichtenthaler et al. 1981). They also found that the relative prenyl lipid composition of the envelope is, however, quite different from that of photochemically active thylakoids. This aspect is also reviewed by Douce and Joyard 1979, 1980, 1990. The wide diversity of specific lipids, pigments, and prenylquinones in envelope and thylakoid membranes implies the existence of complex metabolic pathways closely associated with the chloroplast. As the envelope is the only permanent membrane structure of all types of plastids found in plant tissues, it was likely that it would play a central role in the biosynthesis of the specific chloroplast constituents. The extensive survey of envelope functions led to the characterization of all the enzymes for glycerolipid biosynthesis, centered on the Kornberg–Pricer pathway (Joyard and Douce 1977), that reflects the prokaryotic origin of the chloroplast. Further studies on the purification and molecular analyses of envelope enzymes involved in lipid metabolism, including MGDG synthase (Maréchal et al. 1994), were performed in collaboration with the group of Prof. Hiroyuki Ohta (Yokohama, Japan) (Miège et al. 1999).

Extensive studies of chloroplast envelope proteins (Joyard et al. 1982) were performed with the group of Prof. N.H. Chua (New York), using chloroform/methanol mixtures (for the most hydrophobic proteins), and proteolytic treatment of purified chloroplast to describe proteins residing on the outer surface of the outer envelope membrane. This paved the way for the characterization of marker proteins for each of the envelope membranes—an essential step prior to the preparation of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts (Block et al. 1983). This work allowed the determination of the respective role of each of the envelope membranes in the biogenesis of plastids (synthesis of polar lipids, pigments, prenylquinones, and chlorophyll precursors).

The picture emerging from the results obtained by Douce's group on chloroplast envelope membranes is that of a key player in the synthesis and turnover of all constituents of photosynthetic membranes during chloroplast development and photosynthesis (reviewed by Block et al. 2007). The envelope membranes are a flexible system that can divide, produce dynamic extensions, and interact with other cell constituents. They are also a major hub for integration of metabolic and ionic networks in cell metabolism (reviewed by Douce et al. 1984; Douce and Joyard 1990).

In this field, Douce's legacy is still alive in Grenoble. His former students are pursuing the study of glycerolipid metabolism and interactions between chloroplast, mitochondria, and other membrane systems. Several databases have been developed: (a) the AT_CHLORO database containing sub-plastidial and sub-thylakoidal localization of *Arabidopsis thaliana* chloroplast proteins obtained from MS-based quantitative proteomics experiments as well as curated function and localization of proteins (http://at-chloro.prabi.fr/at_chloro/) and (b) ChloroKB, a Web application (http://chlorokb.fr/) for visual exploration and analysis of the Arabidopsis metabolic networks in the chloroplast and related cellular pathways.

The uniqueness of plant mitochondria

Roland Douce made a number of important contributions to mitochondrial research. Using purified mitochondria, he and his group characterized the features that set plant mitochondria apart from mammalian mitochondria, such as alternative NADH dehydrogenases, alternative oxidase, alternative pathways for malate oxidation, participation in photorespiration. In 1985, Douce summarized our knowledge about the structure, composition, and function of plant mitochondria transport in a landmark book (Douce 1985).

For instance, the question of the regulation of malate oxidation that was stimulated by the addition of NAD^+ was addressed and led to the demonstration that this stimulation was due to the existence of a specific transporter of NAD^+ in the inner mitochondrial membrane. Uptake and efflux operate to regulate the total matrix NAD pool size (Neuburger and Douce 1980). The existence of specific transporters for coenzyme A and thiamine pyrophosphate was also demonstrated (reviewed by Douce and Neuburger 1989) (Fig. 6).

In photorespiration, mitochondria take up glycine flooding out of the peroxisomes during photorespiration and convert it to serine by the glycine decarboxylase complex (GDC) and serine hydroxymethyltransferase (SHMT). Serine then leaves the mitochondria to return to the chloroplasts in the form of glycerate which re-enters the Benson–Calvin cycle. The necessity of a rapid destruction of glycine molecules explains why the mitochondrial matrix from photosynthetic tissues contains tremendously high concentrations of GDC, in contrast to mitochondria from non-green tissues. Our understanding of GDC complex structure, its components, and reaction mechanism is to a large extent due to work carried out by Roland Douce and his group (Douce and Neuburger 1999; Douce et al. 2001). Being fundamentally a physiologist, Douce soon understood the possible contribution of structural biology (crystallography and NMR spectroscopy) to the elucidation of enzymatic mechanisms. This pioneering work on GDC was done in partnership with scientists at the *Institut de Biologie Structurale* (Grenoble).

For the structural studies, components of the GDC complex were purified from pea leaf mitochondria. Emphasis was given to the lipoate-dependent H-protein that plays a pivotal role by acting as a mobile substrate between the other three proteins (Cohen-Addad et al. 1995). During this study, it was also shown that mitochondria possess all the necessary enzymatic equipment for *de novo* synthesis of tetrahydrofolate and lipoic acid, serving as cofactors for the functioning of glycine decarboxylase and SHMT (reviewed by Rébeillé and Douce 1999; Rébeillé et al. 2007; Ravanel et al. 2011).

Fig. 6 Roland Douce and Michel Neuburger hiking in the French Alps near Grenoble, May 1991. Alpine mountain hiking was one of Roland's favorite relaxation. Since 1974, Michel was the closest collaborator and friend of Roland Douce. Plant mitochondria were their common scientific interest. (Source: Ian Max Møller, Aarhus)

Deciphering enzymatic pathways for amino acids and vitamins

As the synthetic pathway of the essential branched amino acids valine and isoleucine is absent in animals, the enzymes involved—residing in the chloroplast—were expected to be good targets for a systematic search for herbicides. The huge body of molecular data obtained for this pathway developed by Douce Lyon group paved the way for the determination of the crystal structure of the acetohydroxy acid isomeroreductase, the enzyme which catalyzes the conversion of acetohydroxy acids to dihydroxy valerates (Biou et al. 1997). This study, done in collaboration with the *Institut de Biologie Structurale* (Grenoble), is the archetype of what physics can bring to biology, both in terms of fine resolution of protein structures and modeling of active sites and catalysis.

The group also determined the functional and structural characteristics of chloroplast enzymes involved in the biosynthetic pathways for methionine and cysteine. They studied, e.g., mechanisms that are responsible for homeostatic regulation of methionine and of its immediate precursor, S-adenosylmethionine (AdoMet)—the knowledge of which has practical implications, e.g., in herbicide design (Ravanel et al. 1998). Cysteine synthesis is catalyzed by serine acetyltransferase (SAT) and o-acetylserine (thiol) lyase (OAS-TL) in the cytosol, plastids, and mitochondria of plants. Moreover, Roland Douce addressed the metabolic pathways for coenzymes,

including vitamins. Indeed, the Lyon laboratory elucidated the mechanisms of biotin biosynthesis and protein biotinylation in higher plants (Alban et al. 2000). By combining the efforts of his two groups in Lyon and Grenoble, Douce demonstrated the central role of mitochondria in catalyzing one or several steps of the synthesis of two vitamins (folate and biotin) and one non-vitamin coenzyme (lipoate) (reviewed by Rébeillé and Douce 1999; Rébeillé et al. 2007; Ravanel et al. 2011).

Using metabolic NMR to describe the plasticity of plant cell metabolism

In the early eighties, Roland Douce and his group developed Nuclear Magnetic Resonance (NMR) as a new technology for in vivo and in vitro analysis of plant physiology and biochemistry (Martin et al. 1982) especially in the study of plant autophagy processes (Aubert et al. 1996). A limited CO_2 supply (e.g., stomata closure) causes a cascade of reactions: consumption of vacuolar sucrose (Rébeillé et al. 1985); a decrease of phosphorylated sugars, starch breakdown as well a decline in cell respiration due to the decrease of mitochondria per cell (Journet et al. 1986). During this process, proteins and polar membrane lipids, including phospholipids and galactolipids are degraded, whereas phosphorylcholine and amino acids like asparagine steadily accumulate. These essential metabolic changes deliver early endogenous signals that contribute to trigger rescue metabolism (Gout et al. 2011).

Roland Douce promoted the improvement of the high-resolution NMR spectroscopy technique for its utilization in plant studies. Basically, NMR can be used to measure different nuclei in cell extracts, including H, N, C, and P in vitro as well as in vivo (C and P). With improved NMR, it was possible to simultaneously measure the concentration of a great number of metabolites and also the pH of different cell compartments (Gout et al. 2014). In addition, perfusion systems designed for cell and solid tissue were created (Roby et al. 1987) that allowed for the first time the analyses of perfused plant materials. Undoubtedly, NMR methods will continue to permit the elucidation of metabolic networks under changing physiological conditions and stress constraints (reviewed by Bligny and Douce 2001).

The remarkable wide range of Roland Douce's scientific achievements is documented in about 500 original publications, review articles, proceedings, and books, some of which are listed below under References.

Honors

For innovative research, Roland Douce received various honors:

1982: Silver Medal of the Centre National de la Recherche Scientifique (CNRS)
1990: Corresponding Member of the Académie des Sciences, Paris
1996: Member of the Académie des Sciences, Paris, Section Integrative Biology
1995: Corresponding Member Award of the American Society of Plant Biologists (ASPB)
1997: Member of the National Academy of Sciences (USA)
2003: Officier de l'Ordre National du Mérite, France
2009: Officier de la Légion d'Honneur, France
2009: Fellow of the American Society of Plant Biologists (ASPB)
2013: Lifetime Achievement Award for Photosynthesis of the Rebeiz Foundation, USA (Fig. 7)
2015: Commandeur de l'Ordre National du Mérite, France (Fig. 8)

Personal Memories of Roland's colleagues and friends

Thomas Bach, Professor Emeritus, University of Strasbourg, France, remembers: *In a plenary lecture at the 10th International Symposium on Plant Lipids in Paris in 1994, Roland Douce reported on the biochemical characterization of functional plant mitochondria. It was possibly the presentation with the most "French-sounding" English the audience might have ever been listening to throughout their lives, but the talk was presented in such an enthusiastic manner that everyone was just fascinated.*

Christoph Benning, MSU Foundation Professor at East Lansing, USA, writes: *I met Roland over 30 years ago when I visited him as a young grad student in Grenoble. He was generous and inspirational to me, when I was stuck with my experiments on sulfolipid biosynthesis in the chloroplast, and I have admired him ever since.*

Bob Buchanan, Professor Emeritus, University of California, Berkeley, recalls: *The late night swim we enjoyed in an idyllic New Hampshire lake during the 1973 Gordon Research Conference on Photosynthesis. After an evening session, Roland and I swam to the middle of the lake and then treaded water while we had a great discussion of the recent work on photosynthesis stemming from the conference. Roland had presented his exciting experiments in which he succeeded in separating the outer and inner chloroplast envelopes. As always, his presentation was punctuated with his memorable French pronunciation of "membrane."*

Nam Hai Chua, Professor Emeritus, former head of a lab at Rockefeller University, New-York, USA, recalls: *Roland was a good friend and has made important contributions to*

Fig. 7 Roland Douce, Constantin Rebeiz, and Robert E. Blankenship at Champaign, Illinois (USA). RD and REB received the 2013 Lifetime Achievement Award for Photosynthesis of the Rebeiz Foundation, USA. (Source: LPCV)

Fig. 8 Roland Douce and Pierre Joliot (Paris) in Meylan (France), when RD was raised as Commandeur de l'Ordre national du Mérite. (Source: Marie-France Joyard)

our knowledge and understanding of plant lipids and indeed plant biochemistry. We remembered the many good times we shared when he stayed with us at Scarsdale and when we visited him in Grenoble. His French accented English reminded me of Maurice Chevalier!!!

David A. Day, Professor at Flinders University, Adelaïde, Australia, remembers: *I met Roland Douce at a plant mitochondria meeting in Marseilles in 1978, where a young and very flamboyant Roland took centre stage, greatly impressing the international audience. I later had the privilege of working with Roland and his amazing research group at the CENG for a year in 1983, in which I learnt more than at any other time in my career. Roland had a touch of the genius about him and was an inspirational leader, as well as a supportive and at times hilariously humorous friend. Roland made many seminal contributions to the field of plant bioenergetics, both with plastids and mitochondria, and will be remembered as one of the greats of plant science.*

Michel Delseny, Emeritus Director of research at CNRS, University of Perpignan, recalls: *Roland was a fascinating and passionate teacher and trained many young scientists in France and abroad in plant physiology and biochemistry including me. In addition to excellently leading his lab at the university, he played an essential role in reviving the Alpine Botanical Garden at Col du Lautaret at about 2100 m altitude, encouraging several of his co-workers to invest their time and knowledge in this activity* (Fig. 9). *Roland was passionately in love with his beautiful alpine mountains near Grenoble and, on several occasions, he enjoyed inviting his colleagues there for hiking and scientific meetings. In our mind we will keep the memory and image of an outstanding, brilliant scientist, of a generous and cheerful colleague and friend, always curious and open to novelty and always ready to share his passion for the secrets of plant biology and nature.*

Emmanuelle Douce, daughter of Roland Douce, writes: *My father nourished an intimate feeling of deep friendship for his scientific colleagues and in particular a loving respect for his mentor Prof. Andy Benson in the USA. Although my father was already very ill, he made in 2014 again a trip to USA (Illinois and California*, Fig. 10*) in a way as if a bird was flying back to his homeland. Yes, his stay in the US was not only work, it was friendship under the sign of happiness and freedom. At the very last stages of his life, he was speaking to me in a very poetic and beautiful style. This surprised me but thinking a while I figured out that one cannot be a good scientist without being a poet in the sense of creativity, the love for humanity and the observation of the beauty of nature. This makes a whole scientific community*

Fig. 9 Roland Douce (left) with Richard Bligny and Jacques Bourguignon in September 2015 at Lautaret Alpine garden during the visit of the French Academy of Sciences. (Source: Jacques Joyard)

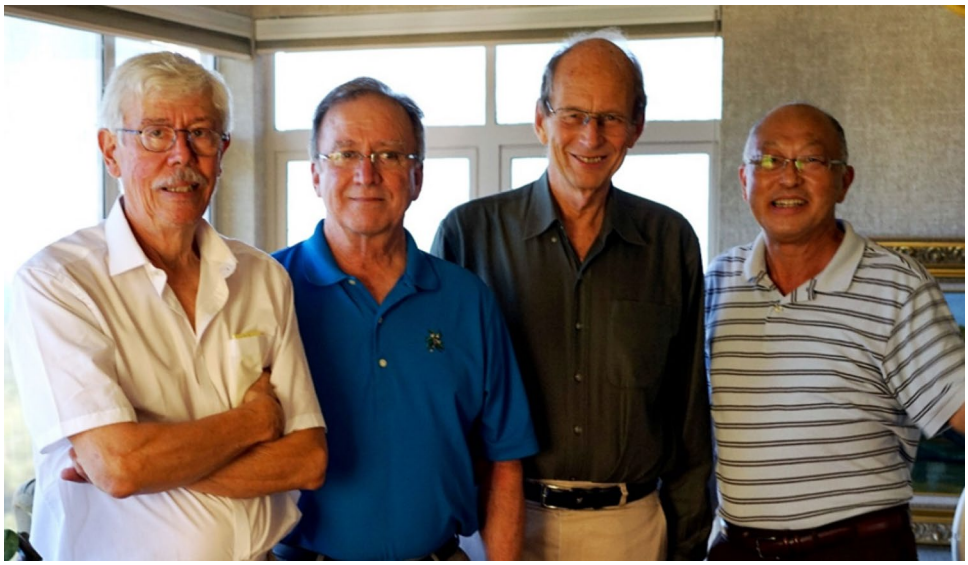

Fig. 10 Roland Douce together with Barry Holtz (Austin), Bob Buchanan (Berkeley), Arthur Nonomura (Powel, Ohio) at the 97th birthday of Andy Benson on September 24, 2014 in La Jolla, California. (Source: Photosynth. Research 2015)

a sensitive one despite a very rational appearance. These qualities are absolutely necessary in our agitated world today.

Christine Foyer, Professor at the University of Leeds, UK, writes: *I remain indebted to Roland for introducing me to INRA and providing his unstinting support to me during my transition to a permanent post. I first met Roland when I worked in the lab of David Walker in Sheffield, where Roland visited from time to time. David and Roland shared a passion and understanding of the "intact" chloroplast, an organelle that they proved was best analysed when both envelope membranes were intact. I will never forget how Roland inspired me by his dynamic enthusiasm for the envelope membranes and their functions. He always referred to the chloroplast stroma as being so rich in proteins that it was like "glue". Even now I think of the stromal glue separating the thylakoid and envelope membranes. Roland was one of my heroes. A light has gone out of the world with his passing.*

John L. Harwood, Professor, Cardiff University, Wales, UK, recalls: *Roland was a truly outstanding scientist - one of the very best that France has produced since WW2. He was great company. I remember when he came to stay with us for three weeks in my lab. As you can imagine, my wife Sue was nervous about cooking for someone from a land of culinary excellence! I told her just to do something routine and Roland had two extra helpings of what is called 'shepherd's pie'! He was also popular with students - his strong French*

accent and unique phraseology lending sparkle to his talks. When discussing the separation of plant membrane–'look it is not a dirty brown but a BEAUTIFUL orange' or 'you add the inhibitor; see what happens–puff, ABSOLUTELY DEAD' I had never seen the students so attentive. They loved him!

Ernst Heinz, Professor Emeritus, Hamburg, Germany, recalls: *I met Roland Douce for the first time in 1976 during the international plant lipid meeting in Karlsruhe. Just two years earlier he had published his paper in Science that the chloroplast envelope is the site of galactolipid biosynthesis. We arranged an informal visit to his laboratory to address some questions of galactolipid metabolism. During two short visits and a series of experiments with Jacques Joyard we could publish a paper that can be considered as an early precursor of the lipidomics analyses of a purified membrane system prevailing in many recent analyses (*Siebertz et al. 1979*). Our success and the intensive interaction with Roland and his group resulted in close personal contacts lasting for more than 40 years. We will remember Roland as a most inspiring and wonderful colleague and an extraordinarily successful scientist.*

Barry Holtz (President of iBio, Inc., USA) remembers *when Roland Douce walked into the Benson lab in 1972 and occupied the bench space next to mine. He came with an enthusiasm and confidence that lifted the room immediately into a new level of intensity. We were friends for over 40 years. Each year brought new data, new ideas and new enthusiasm into our knowledge of plant biochemistry. I would often travel to France to see my friend, but it was also a renewal for me to focus on good science and meeting Roland's challenge to think about science that really matters. He was the glue that brought together a great team of scientists that have wonderfully enriched our field of endeavor. He will be sorely missed, but those of us fortunate enough to be in his circle are forever blessed. Godspeed Roland Douce.*

Pierre Joliot, Emeritus Director of research at CNRS, IBPC, Paris, recalls: *Roland Douce demonstrated as a researcher an enthusiasm that he knew how to communicate around him. In the long relationship of friendship that linked us, one of my best memories was a collaboration during which Roland had come to my laboratory to prepare intact chloroplasts from spinach leaves. I was able to measure how much Roland manifested the same skill and passion for practice or the more conceptual aspects of our work. The whole laboratory was able to share the joy of Roland, who loudly expressed his enthusiasm for the perfection of his preparation by shouting: It's dynamite!.*

Hartmut Lichtenthaler, Emeritus professor at the Karlsruhe Institute of Technology, Germany: *Roland and I were both coined by our two years stay in California, he with Andy Benson in La Jolla and me with Melvin Calvin in Berkeley, and we both had a favor for an open discussion which we had positively experienced in the States. Personally, we met first on the 2nd International Symposium on Plant Lipids (2nd ISPL) organized by me in July 1976 in Karlsruhe, and in openly discussing plant metabolism and new research approaches, we quickly became friends. This was a lifelong friendship.*

Eric Maréchal (former student, present head of the laboratory set up by Roland Douce in 1974) writes: *Roland founded our lab and directed it from 1979 to 1991 providing guidance to many of us. We keep the memory of an immense scientist, understanding like no other the metabolism and physiology of plants. It will take time to appreciate his scientific legacy on the knowledge of carbon and nutrients' metabolism, the biosynthesis and roles of vitamins, the biology of mitochondria and chloroplasts. In Benson's lab, he began the study of plastidic lipids (with a paper in Science on galactolipid synthesis in the chloroplast envelope) that we are still pursuing here. He was a precursor of the functional study of plant protein complexes by structural approaches, and he also led another study unit, the Institute of Structural Biology. Roland was one of the great professors of our university, making the most complex processes crystal clear. His bioenergetics course was fabulous (30 years ago already)! He always took the time to explain to the students with passion. Many of us have grown as scientists following his criticism and advice. A page is turned for our lab. We lost one of its most passionate explorers.*

Norio Murata, Professor Emeritus, National Institute for Basic Biology, Okazaki, Japan, writes: *It is unbelievable that Roland is no more in our society of plant science. He tremendously contributed to research in plant science in all of his life with strong passion. His influence is invaluable.*

Ian Max Møller, Professor Emeritus, Aarhus University, Denmark, who spent April–August 1991 in the laboratory of Roland Douce, recalls: *The lab in Grenoble was run in a very professional way. Roland had the ability to find, and retain, superb senior collaborators. It is clear that such a lab is very likely to flourish when the head of the lab is full of excellent ideas. Roland was charismatic, and perhaps even flamboyant. The lectures he gave in his very characteristic 'Frenglish' were always entertaining and scientifically excellent.*

Mikio Nishimura, Professor Emeritus, National Institute for Basic Biology, Okazaki, Japan, remembers *many things when Roland visited Nagoya University and the National Institute for Basic Biology and when my spouse, Ikuko and I collaborated in Grenoble. He was a big plant organelle and lipid biologist and had great impacts on Japanese scientists. We were very impressed by the fact that Roland carried out mitochondrial characterization using the oxygen electrode by himself besides all his other work. Roland was a really great experimental biologist for his entire lifetime.*

John B. Ohlrogge, Professor Emeritus, Michigan State University, East Lansing, USA, recalls: *Roland Douce was*

one of the greatest plant biochemists of our generation. I was a postdoc in the late 1970's and I remember how impressed I was after reading and learning from his landmark papers on the chloroplast and mitochondria. I later heard his seminars that were filled with energy and his unique brand of enthusiasm. His legacy will continue through the inspiration he provided to so many other scientists.

Michel Rohmer, Professor Emeritus, University of Strasbourg, recalls: *I often met Roland Douce in Paris at the Academy of Sciences on the occasion of sessions, seminars and prize committees. It was a pleasure to talk to him and we often had lunch together. This was the occasion to discuss not only science, but also culture, hiking, botany, photos, traveling. Roland was for me a very friendly and cultivated man. He organized a session for the life sciences section of the Academy in the Alps close to the botanical garden of the Col du Lautaret. There we spent two days in a convivial atmosphere, with a botanical excursion in the alpine meadows on the last morning. Bringing people together for scientific and general discussions was a major concern for Roland*.

Naoki Sato, Professor at the University of Tokyo, Japan, remembers: *When I worked in Grenoble as a visiting scientist in 1989, Roland was always active in seriously and passionately discussing with any topic including my strange experiments leading to the discovery of the PEND protein (*Sato et al 1993*), which he strongly supported to publish later*.

Jürgen Soll, Professor at Ludwig-Maximilians Universität, Planegg-Martinsried, Germany, recalls: *I spent a couple of month in the laboratory of Roland Douce, once in 1979 and again in 1984. Roland impressed me by his deep knowledge in plant metabolism but more so by his enthusiasm for science and passion for his co-workers. He became one of the few role models for me in academic science*.

Final remarks

Roland Douce had a fulfilled life as a dedicated scientist and he acquired great international recognition as an outstanding scientist, understanding like no other one the dynamics of plant cell metabolism and physiology. Passion is the word that characterizes him the most. His leadership in Science was coupled with a very strong commitment to teaching: Roland Douce was an inspiring and enthusiastic academic teacher, an excellent instructor for his graduate students. Many members of the French scientific community owe him their vocation for plants. Roland Douce is survived by his wife Danielle, his daughter Emmanuelle, and his grandson Cosme.

The international plant science community, his former graduate students, his colleagues, and friends mourn for a passionate scientist, a great personality, and an esteemed friend. For one of us writing this Tribute (JJ), it was an honor to have worked with Roland for almost 40 years. For the other of us (HKL) writing this Tribute, it was a great pleasure and honor to have cooperated with Roland as colleague and friend for more than 40 years. The sophisticated science approaches, the hospitality, the fine humor, and kind spirit of this distinguished scientist will be deeply missed.

Acknowledgements We deeply thank all friends and colleagues who expressed their wishes to pay tribute to Roland Douce. We also thank Marie-France Joyard, Ian Max Møller, Arthur Nonomura, and HP Siebertz for providing photos. Our special thanks go to Michel Neuburger and Richard Bligny for their invaluable contribution to this article. They were the first members (together with JJ) of the group set up in 1974 by Roland Douce in Grenoble.

References

Major review articles and books

Alban C, Job D, Douce R (2000) Biotin metabolism in plants. Ann Rev Plant Phys Plant Mol Biol 51:17–47

Bligny R, Douce R (2001) NMR and plant metabolism. Curr Opin Plant Biol 4:191–196

Block MA, Douce R, Joyard J, Rolland N (2007) Chloroplast envelope membranes: a dynamic interface between plastids and the cytosol. Photosynth Res 92:225–244

Douce R (1985) Mitochondria in higher plants: structure, function, and biogenesis. Academic Press, Orlando, p. 327

Douce R, Joyard J (1979) Structure and function of the plastid envelope. Adv Bot Res 7:1–116

Douce R, Joyard J (1980) Plant Galactolipids. In: Stumpf PK (ed) Biochemistry of plants, a comprehensive treatise, vol 4. Academic Press, Davis, pp. 321–362

Douce R, Joyard J (1990) Biochemistry and function of the plastid envelope. Ann Rev Cell Biol 6:173–216

Douce R, Neuburger M (1999) Biochemical dissection of photorespiration. Curr Opin Plant Biol 2:214–222

Douce R, Bourguignon J, Neuburger M, Rébeillé F (2001) The glycine decarboxylase system: a fascinating complex. Trends Plant Sci 6(4):167–176

Douce R, Neuburger M (1989) The uniqueness of plant mitochondria. Ann Rev Plant Phys Plant Mol Biol 40:371–414

Douce R, Block MA, Dorne AJ, Joyard J (1984) The plastid envelope membranes: Their structure, composition, and role in chloroplast biogenesis. Subcell Biochem 10:1–84

Packer L, Douce R (eds) (1987) Plant cell membranes. In: Methods in enzymology, vol 148. Academic Press, New York, p. 762

Ravanel S, Gakière B, Job D, Douce R (1998) The specific features of methionine biosynthesis and metabolism in plants. Proc Natl Acad Sci USA 95(13):7805–7812

Ravanel S, Douce R, Rebeillé F (2011) Metabolism of folates in plants. Adv Bot Res 59:67–106

Rébeillé F, Douce R (1999) Folate synthesis and compartmentation in higher plants. In: Kruger NJ, Hill SA, Ratcliffe RG (eds) Regulation of primary metabolic pathways in plants. Proceedings of the phytochemical society of Europe, vol 42. Springer, Dordrecht

Rébeillé F, Alban C, Bourguignon J, Ravanel S, Douce R (2007) The role of plant mitochondria in the biosynthesis of coenzymes. Photosynth Res 92(2):149–162

Original research papers

Aubert S, Gout E, Bligny R, Marty-Mazars D, Barrieu F, Alabouvette J, Marty F, Douce R (1996) Ultrastructural and biochemical characterization of autophagy in higher plant cells subjected to carbon deprivation: control by the supply of mitochondria with respiratory substrates. J Cell Biol 133:1251–1263

Biou V, Dumas R, Cohen-Addad C, Douce R, Job D, Pebay-Peyroula E (1997) The crystal structure of plant acetohydroxy acid isomeroreductase complexed with NADPH, two magnesium ions and a herbicidal transition state analog determined at 1.65 Å resolution. EMBO J 16:3405–3415

Bligny R, Douce R (1980) A precise localization of cardiolipin in plant cells. Biochim Biophys Acta 617(2):254–263

Block MA, Dorne AJ, Joyard J, Douce R (1983) Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. I. Electrophoretic and immunochemical analyses and II. Biochemical characterization. J Biol Chem 258(21):258(21):13273–13280

Buchanan BB, Douce R, Lichtenthaler HK (2007) Andrew A. Benson. Photosynth Res 92:143–144

Cohen-Addad C, Pares S, Sieker L, Neuburger M, Douce R (1995) The lipoamide arm in the glycine decarboxylase complex is not freely swinging. Nat Struct Mol Biol 2(1):63–68

Coulon-Morelec MJ, Douce R (1968) Diphosphatidylglycerol of higher plants. I. The structure of plant diphosphatidylglycerol. Bull Soc Chim Biol (Paris) 50(9):1547–1559

Dorne AJ, Joyard J, Block MA, Douce R (1985) Localization of phosphatidylcholine in outer envelope membrane of spinach chloroplasts. J Cell Biol 100(5):1690–1697

Douce R, Christensen EL, Bonner WD Jr (1972) Preparation of intact plant mitochondria. Biochim et Biophys Acta 275:148–160

Douce R, Holtz RB, Benson AA (1973) Isolation and properties of the envelope of spinach chloroplasts. J Biol Chem 248(20):7215–7222

Douce R (1974) Site of biosynthesis of galactolipids in spinach chloroplasts. Science 183(4127):852–853

Gout E, Rébeillé F, Douce R, Bligny R (2014) Regulation of cell respiration by ADP and Mg^{2+}. Proc Natl Acad Sci USA 111(43):E4560–E4567

Gout E, Bligny R, Douce R, Boisson A, Rivasseau C (2011) Early response of plant cell to carbon deprivation: in vivo ^{31}P-NMR spectroscopy shows a quasi-instantaneous disruption on cytosolic sugars, phosphorylated intermediates of energy metabolism, phosphate partitioning, and intracellular pHs. New Phytol 189:135–147

Jeffrey SW, Douce R, Benson AA (1974) Carotenoid transformations in the chloroplast envelope. Proc Natl Acad Sci USA 71(3):807–810

Journet EP, Bligny R, Douce R (1986) Biochemical changes during sucrose deprivation in higher plant cells. J Biol Chem 261:3193–3199

Joyard J, Douce R (1977) Site of synthesis of phospatidic acid and diacylglycerol in spinach chloroplasts. Biochim Biophys Acta 486:273–285

Joyard J, Grossman A, Bartlett SG, Douce R, Chua NH (1982) Characterization of envelope membrane polypeptides from spinach chloroplasts. J Biol Chem 257(2):1095–1101

Lichtenthaler HK (2004) Thirty years of international symposia on plant lipids. In: Biacs P and Gerely P (eds) Proceedings of the 16th plant lipid symposium. Mete Publisher, Budapest, pp. I–XXXI

Lichtenthaler HK, Buchanan BB, Douce R (2008) Honoring Andrew Benson in Paris: a tribute on his 90th birthday. Photosynth Res 96:181–183

Lichtenthaler HK, Buchanan BB, Douce R, Govindjee (2015) Andrew A. Benson, 1917–2015. Photosynth Res 124:131–135

Lichtenthaler HK, Prenzel U, Douce R, Joyard J (1981) Localization of prenylquinones in the envelope of spinach chloroplasts. Biochim Biophys Acta 641:99–105

Maréchal E, Block MA, Joyard J, Douce R (1994) Kinetic properties of monogalactosyldiacylglycerol synthase from spinach chloroplast envelope membranes. J Biol Chem 269:5788–5798

Martin JB, Bligny R, Rébeillé F, Douce R, Leguay JJ, Mathieu Y, Guern J (1982) A ^{31}P nuclear magnetic resonance study of intracellular pH of plant cells cultivated in liquid medium. Plant Physiol 70:1156–1161

Miège C, Maréchal E, Shimojima M, Awai K, Block MA, Ohta H, Takamiya K, Douce R, Joyard J (1999) Biochemical and topological properties of type A MGDG synthase, a spinach chloroplast envelope enzyme catalyzing the synthesis of both prokaryotic and eukaryotic MGDG. Eur J Biochem 265:990–1001

Neuburger M, Douce R (1980) Slow passive diffusion of NAD + between intact isolated plant mitochondria and suspending medium. Biochem J 216:443–450

Neuburger M, Journet EP, Bligny R, Carde JP, Douce R (1982) Purification of plant mitochondria by isopycnic centrifugation in density gradients of Percoll. Arch Biochem Biophys 217:312–323

Rébeillé F, Bligny R, Martin JB, Douce R (1985) Effect of sucrose starvation on sycamore (*Acer pseudoplatanus*) cell carbohydrate and Pi status. Biochem l 226:679–684

Roby C, Martin JB, Bligny R, Douce R (1987) Biochemical changes during sucrose deprivation in higher plant cells. Phosphorus-31 nuclear magnetic resonance studies. J Biol Chem 262:5000–5007

Sato N, Albrieux C, Joyard J, Douce R, Kuroiwa T (1993) Detection and characterization of plastid envelope DNA-binding protein that may anchors plastid nucleoids. EMBO J 12:555–561

Siebertz HP, Heinz E, Joyard J, Douce R (1979) Characterization of lipids from chloroplast envelopes. Eur J Biochem 101:429–438