

# Curriculum vitae

## 1. Personal:

**Name:** Dr. Luitjen Jakob De Kok

**Address:** Laboratory of Plant Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

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**Private address:** Schoolstraat 6, 9919 HX Loppersum, The Netherlands, Phone: +31 596 572691

**Date of birth:** May 12, 1952

**Place of birth:** Scheemda, The Netherlands

**Nationality:** Dutch

**Spouse:** Gertie A. De Kok-Van Dijk (date of birth: May 1, 1955)

**Children:** Daniël J.A. De Kok (date of birth: April 24, 1982)  
Gideon P. De Kok (date of birth: June 8, 1986)

**Education/Degrees:** College of Advanced Laboratory Education, Groningen (1973)  
Ph.D., Faculty of Mathematics and Physics, University of Groningen (1989)

**Academic employment:** Laboratory of Plant Physiology, University of Groningen, from September 1, 1972 - onwards

### Temporary positions abroad:

<i>Period:</i>	<i>Position:</i>	<i>University/Institute:</i>
March 1979 - August 1979	Visiting Research Fellow	Department of Plant Biochemistry, University of California, Riverside, USA
September 1979 - February 1980	Research Biochemist	Statewide Air Pollution Research Center, University of California, Riverside, USA
September 1986 - February 1987	Visiting Research Fellow	School of Agriculture and Forestry, University of Melbourne, Australia
May 1988 - August 1988	Visiting Researcher	Fraunhofer Institute for Atmospheric Environmental Research, Garmisch-Partenkirchen, Germany
September 2006 - onwards	Honorary Principal Fellow	School of Forest and Ecosystem Science University of Melbourne, Australia

**Current position:** Associate Professor

**Memberships:** Koninklijke Nederlandse Botanische Vereniging (KNBV)

## 2. Professional Service and Management:

### 2.1. Committees Groningen University:

- Faculteitsraad Wiskunde en Natuurwetenschappen (1980-1981).
- Subfaculteitsraad Biologie (1980-1981).
- Werkgroep CEES nieuwbouw Biologie (2001-2002).
- Werkgroep CEES nieuwbouw Biologie (2006-onwards).

### 2.2. Professional Service Elsewhere:

- Voorzitter schoolbestuur "CBS Roemte, Loppersum" (1993-1998).
- Koninklijke Nederlandse Botanische Vereniging (KNBV) - Dagelijks Bestuur Sectie Plantenfysiologie (1995-1996).
- Chairman of the Management Committee and Chairman of all Meetings of COST Action 829: "Fundamental, Agronomical and Environmental Aspects of Sulfur Nutrition and Assimilation in Plants" (1997-2003) (<http://www.cost829.org>).
- Chairman/Secretary of several symposia and workshops (see ad. 2.4).

### 2.3. Series Editor: Springer Plant Ecophysiology Series

**THE HANDBOOK SERIES OF PLANT ECOPHYSIOLOGY** ([www.springeronline.com](http://www.springeronline.com))

Series editors: Luit J. De Kok & Ineke Stulen (2001 - 2005)

Luit J. De Kok, Ineke Stulen & Malcolm J. Hawkesford (2006 - onwards)

The Springer handbook series of *Plant Ecophysiology* comprises a series of books that deals with the impact of biotic and abiotic factors on plant functioning and physiological adaptation to the environment. The aim of the *Plant Ecophysiology* series is to review and integrate the present knowledge on the impact of the environment on plant functioning and adaptation at various levels of integration: from the molecular, biochemical, physiological to a whole plant level. This Handbook series is of interest to scientists who like to be informed of new developments and insights in plant ecophysiology, and can be used as advanced textbooks for biology students.

**Molecular Analysis of Plant Adaptation to the Environment**

Series: Plant Ecophysiology, Vol. 1

Hawkesford, Malcolm J.; Buchner, Peter (Eds.)

2001, 292 p., Hardcover

ISBN: 1-4020-0016-2

**Significance of Glutathione to Plant Adaptation to the Environment**

Series: Plant Ecophysiology, Vol. 2

Grill, Dieter; Tausz, Michael; De Kok, Luit J. (Eds.)

2002, 280 p., Hardcover

ISBN: 1-4020-0178-9

**Nitrogen Acquisition and Assimilation in Higher Plants**

Series: Plant Ecophysiology, Vol. 3

Amâncio, Sara; Stulen, Ineke (Eds.)

2004, XIII, 299 p., Hardcover

ISBN: 1-4020-2727-3

**Root Physiology: from Gene to Function**

Series: Plant Ecophysiology, Vol. 4

Lambers, Hans; Colmer, Timothy D. (Eds.)

2005, XVI, 272 p. 14 in colour., Hardcover

ISBN: 1-4020-4098-9

## 2.4. Meetings Organized:

Has directly been (and is) involved in the organization of several symposia, workshops (listed). In addition, has been invited to chair sessions of several other symposia, workshops, which have not been listed.

- "5th International Symposium on Biochemistry and Metabolism of Plant Lipids", Groningen, The Netherlands, June 7-10, 1982.
- "Symposium on Nitrogen Metabolism in Higher Plants", Haren, The Netherlands, April 9-12, 1985.
- "Workshop on Sulfur Nutrition and Assimilation in Higher Plants; Fundamental, Environmental and Agricultural Aspects", Haren, The Netherlands, March 28-31, 1989 (Secretary).
- European Science Foundation Research Conference on "Effects of Elevated CO<sub>2</sub> levels, Air Pollutants and Climate Change on Natural Plant Ecosystems - Impact on Tree Physiology". Aghia Pelaghia, Crete, Greece, April 3-7, 1992 (Chairman).
- "2<sup>nd</sup> International Workshop on "Sulfur Nutrition and Assimilation in Higher Plants; Physiological Functions and Environmental Significance", Garmisch-Partenkirchen, F.R.G., April 21-25, 1992 (Vice-Chairman).
- "3<sup>rd</sup> International Workshop on Sulfur Nutrition and Assimilation in Higher Plants", Newcastle, UK, April, 9-13, 1996.
- "4<sup>th</sup> International Symposium on Responses of Plants to Air Pollution", Egmond aan Zee, The Netherlands, April 1-5, 1997 (Chairman).
- "1<sup>st</sup> Joint Meetings of the Working Groups of COST Action 829" on "Regulatory Aspects of Uptake and Reduction of Sulfate in Plants in Relation to the Metabolic Need for Growth", Goslar, Germany. January 31 - February 2. 1998.
- Meeting of Working Group IV of COST Action 829" on "Environmental Aspects of Sulfur Nutrition in Plants", Pulawy, Poland, November 6-7, 1998.
- 30<sup>th</sup> Air Pollution Workshop, April 20-23, 1998, Appalachian State University, Boone, North Carolina, USA.
- "Meetings of Working Groups I and III of COST Action 829" on "Sulfur and Crop Quality: Molecular and Agronomical Strategies for Crop Improvement", Naples, Italy, January 8-10, 1999.
- "4<sup>th</sup> International Workshop on Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Molecular, Biochemical and Physiological Aspects", Wengen, Switzerland, April 6-10, 1999.
- "31<sup>st</sup> Air Pollution Workshop", April 25-30, 1999, U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, USA.
- "Meetings of Working Groups II and IV of COST Action 829" on "The Role of Sulfur Compounds in Plant Stress Adaptation", Lisbon, Portugal, January 7-9, 2000.
- "Joint Meeting of Working Groups I and III of COST Action 829" on "Plant Sulfur Metabolism and Plant Adaptation to Pests", Graz, Austria, September 1-3, 2000.
- "Combined Meeting of Working Groups I and II of COST Action 829" on Plant Sulfur Metabolism at the Beginning of the 21<sup>st</sup> Century", Seville, Spain, February 2-3, 2001.
- "12<sup>th</sup> World Fertilizer Congress", August 3-9, 2001, Beijing, China.
- "Combined Meeting of Working Groups III and IV of COST Action 829" on "Sulfur-Nitrogen Interactions in Plants", Oulu, Finland, September 7-8, 2001.
- "5<sup>th</sup> International Symposium on Responses of Plant Metabolism to Air Pollution and Global Change", Pulawy, Poland, November 1-4, 2001 (Chairman).
- "5<sup>th</sup> International Sulfur Workshop on Sulfur Nutrition and Assimilation in Higher Plants; Regulation, Interaction, Signaling", Montpellier, France, April 11-14, 2002.
- "Combined Meeting of Working Group I & III of COST Action 829" on "Improving Sulfur Nutrition in Plants - Molecular and Agronomic Approaches", Rothamsted, U.K., September 13-14, 2002.
- "Combined Meeting of Working Groups I-IV of COST Action 829", on "Progress in Plant Sulfur Research 1997-2003", Goslar, Germany, May 15-18, 2003 (Chairman).
- "7<sup>th</sup> International Symposium on Inorganic Nitrogen Assimilation in Plants", Wageningen, The Netherlands, June 23-27, 2004.

- "6<sup>th</sup> International Symposium on Plant Responses to Air Pollution and Global Change: from Molecular Biology to Plant Production and Ecosystem", Tsukuba International Congress Center, Tsukuba, Japan, October 19-22, 2004 (Vice-Chairman).
- "6<sup>th</sup> International Sulfur Workshop on Sulfur Nutrition and Assimilation in Higher Plants", Kazusa Akademia Center, Kisarazu, Chiba, Japan, May 17-21, 2005.
- Cross-talk between Metabolic Pathways involving Sulfur in Plants, FAL Braunschweig, October 21-22, 2005.
- "7<sup>th</sup> International Symposium on Responses of Plant Metabolism to Air Pollution and Global Change", Edinburgh, Scotland, September 2 - 7, 2007.
- "7<sup>th</sup> International Sulfur Workshop on Sulfur Nutrition and Assimilation in Higher Plants", Warsaw, Poland, May 13 - 18, 2008.

## **2.5. Invited lectures and seminars:**

Has presented many lectures, seminars and oral presentations at society meetings, conferences, workshops and during visits to universities/institutes in the Netherlands and abroad for instance in Austria, China, Belgium, Denmark, France, Germany, Greece, Poland, Portugal, Ukraine, Sweden, United Kingdom, USA on various topics in plant physiological research viz. lipid metabolism, adaptation of plant to chilling temperatures and frost, the significance of glutathione in the plants, the impact of elevated CO<sub>2</sub> on plants, regulatory aspects of plant sulfur metabolism, metabolism and/or toxicity of air pollutants. Several of these presentations were given upon invitation and/or during visits abroad in order to discuss cooperative research programs, grant proposals etc.

Hereby, a selection of invited lectures/seminars from 1997 - onwards:

- 4th International Symposium on Responses of Plants to Air Pollution, Egmond aan Zee, The Netherlands, April 1-5, 1997 - "Impact of H<sub>2</sub>S on plants".
- 30<sup>th</sup> Air Pollution Workshop, Appalachian State University, Boone, North Carolina, USA, April 20-23, 1998 - "Integration of Mechanistic Responses to Pollutants: Genetic, Molecular and Biochemical Basis of Response of Plants to Air Pollutants"
- 31<sup>st</sup> Annual Air Pollution Workshop, Oregon State University, Corvallis, USA, April 25-30, 1999 - "Atmospheric H<sub>2</sub>S as Sulfur Source for Plants: Consequences for Sulfate Uptake and Assimilation".
- 1st Joint Meetings of the Working Groups of COST Action 829 on Regulatory Aspects of Uptake and Reduction of Sulfate in Plants in Relation to the Metabolic Need for Growth, Goslar, Germany. January 31 - February 2, 1998 - "Impact of sulfurous air pollutants on uptake and reduction of sulfur in plants".
- 4th International Workshop on Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Molecular, Biochemical and Physiological Aspects, Wengen, Switzerland, April 6-10, 1999 - "Sulfur stress in plants: deficiency and excess".
- Max Planck Institute of Molecular Plant Physiology, Golm, Germany, May 31, 2000 - "Plant response to a changing atmosphere: impact on carbon, nitrogen and sulfur utilization".
- Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany, June 29, 2000 - "Interaction between atmospheric and pedospheric sulfur nutrition in plants".
- Joint Meeting of Working Groups I and III of COST Action 829 on Plant Sulfur Metabolism and Plant Adaptation to Pests, Graz, Austria, September 1-3, 2000 - "Expression of sulfate transporters and regulation of sulfate uptake by curly kale as affected by sulfur nutrition and atmospheric H<sub>2</sub>S exposure".
- International Symposium on Elemental Sulfur for Agronomic Applications & Desert Greening (ESAA-DG), Abu Dhabi, United Arab Emirates, February 24-25, 2001 - "Sulfur Nutrition and Regulation of Sulfur Fluxes in Plants".
- Sino-German Workshop on N<sub>2</sub>O Emission from Agroecosystems, Shenyang, China, October 8-11, 2001 - "The plant foliage as source and sink of nitrogenous and sulfurous gases".
- 5<sup>th</sup> International Symposium on Responses of Plant Metabolism to Air Pollution and Global Change, Pulawy, Poland, November 1-4, 2001 - "Sulfurous air pollutants: toxins or nutrients for plants".

- Sulphur Information Day on “Everything you ever wanted to know about sulphur”, Institute of Plant Nutrition and Soil Science, Braunschweig, Germany, November 27, 2001 - “Why do plants need sulphur”.
- 5<sup>th</sup> International Sulfur Workshop on Sulfur Nutrition and Assimilation in Higher Plants; Regulation, Interaction, Signaling, Montpellier, France, April 11-14, 2002 - “Impact of atmospheric H<sub>2</sub>S deposition on growth and sulfur metabolism of *Allium cepa* L”.
- Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, May 15, 2002. - "Interaction between atmospheric and pedospheric sulfur nutrition".
- Combined Meeting of Working Groups I & III of COST Action 829 on Improving Sulfur Nutrition in Plants - Molecular and Agronomic Approaches, Rothamsted, U.K., September 13-14, 2002 - “Influence of atmospheric H<sub>2</sub>S nutrition on the expression of different sulphate transporter genes in curly kale”.
- 9<sup>th</sup> International Conference of The International Fertiliser Society on Sulfur Fertilisers: Demand, Production and Use, Robinson College, Cambridge, U.K., December 16-17, 2002 - “Sulphur in Plant Physiology”.
- 35<sup>th</sup> Air Pollution Workshop, Delta Vancouver Airport Hotel, Vancouver, BC, Canada, April 9-10, 2003 - "Plant physiological responses to pollutants: CO<sub>2</sub>, S, N, O<sub>3</sub>, heavy metals an introduction".
- Annual Main Meeting of the Society for Experimental Biology, Heriot-Watt University, Edingburgh, U.K., March 30 - April 2, 2004 -"Whole plant regulation of sulphur uptake and assimilation in relation to both the need for growth a the potential sink capacity for secondary sulphur compounds".
- Sino-German Workshop on Aspects of Sulphur Nutrition of Plants, May 24-29, 2004, Shenyang, China. "Biochemical pathways of sulphur in plants".
- 7th International Symposium on Inorganic Nitrogen Assimilation in Plants: From the Genome to the Agro-Ecosystem, Wageningen, The Netherlands, June 23-27, 2004. "Is there a direct interaction between the uptake and assimilation of nitrogen and sulphur?"
- 6<sup>th</sup> International Symposium on Plant Responses to Air Pollution and Global Change: from Molecular Biology to Plant Production and Ecosystem, Tsukuba International Congress Center, Tsukuba, Japan, October 19-22, 2004. - "Uptake and metabolism of atmospheric sulfur gases: interaction with pedospheric sulfate utilization".
- 37<sup>th</sup> Air Pollution Workshop and International Symposium, Banff, Alberta, Canada, April 25-28, 2005 - "Negative versus positive functional plant responses to air pollution: A study with SO<sub>2</sub> and H<sub>2</sub>S in establishing cause-effect relationships".
- 6<sup>th</sup> International Sulfur Workshop on Sulfur Nutrition and Assimilation in Higher Plants, Kazusa Akademia Center, Kisarazu, Chiba, Japan, May 17-21, 2005 - "Expression of sulfate transporters in *Brassica oleracea* L. as affected by sulfur nutrition".
- '40 Jahre Schwefelforschung - Aus Anlass der Pensionierung von Prof. Dr. Ahlert Schmidt', University of Hanover, Germany, June 17-18, 2005 - "Sulfur metabolism and plant stress adaptation".
- Cross-talk between Metabolic Pathways involving Sulfur in Plants, FAL Braunschweig, October 21-22, 2005 - "Shoot to root interaction between uptake and assimilation of sulfur in plants".
- Symposium on "Sulfur-Containing Defence Compounds: Pivotal Players in Plant Stress Tolerance" October 4-7, 2006 - "The physiological significance of plant sulfur nutrition in sulfur induced resistance"

## **2.6. Reviewer for Grant Proposals:**

Has been invited to review grant proposals from several American, Austrian, Dutch and English funding agencies and organizations.

## **2.7. Reviewer for the Journals:**

- Acta Pharmaceutica
- Annals of Botany
- Botanical Bulletin of Academia Sinica
- Encyclopedia of Life Sciences
- Environmental and Experimental Botany
- Journal of Cereal Science
- Journal of Experimental Botany
- Journal of Plant Nutrition
- Journal of Plant Nutrition and Soil Science
- Journal of Sea Research
- Journal of the Science of Food and Agriculture
- New Phytologist
- Physiologia Plantarum
- Phytton
- Plant and Soil
- Plant Biology
- Plant Physiology
- Tree Physiology

## **3. Teaching:**

### **3.1. Undergraduate and graduate courses:**

Has more than twenty-five years experience in teaching, organization and coordination of undergraduate and graduate courses on plant physiology, ecotoxicology and has supervised more than fifty undergraduate research studies/projects and colloquia.

Current teaching obligations:

- 1<sup>st</sup> years' course "Physiology of Plants" ('Basisopleiding van Bachelorsopleiding Biologie') - Lecturer, practical course leader, course and semester coordinator.
- 2<sup>nd</sup>/3<sup>rd</sup> years' course "Ecophysiology of Plants ('Specialisatiefase van de Bachelorsopleiding Biologie')" - Lecturer, practical course leader, course coordinator.

In general the different courses have received a positive evaluation and the students appreciate their content and quality.

### **3.2. Ph.D. Supervisor and Referent/Co-Promotor:**

- Frank M. Maas: "Responses of Plants to Sulfur Containing Air Pollutants (H<sub>2</sub>S and SO<sub>2</sub>)", University of Groningen (Ph.D. project funded by STW); Thesis defense: October 9, 1987 (referent).
- Fokke Buwalda: "Effects of Atmospheric Hydrogen Sulfide on Thiol Metabolism in Higher Plants", University of Groningen; Thesis defense: November 26, 1993 (referent).
- Tom A.W. van der Kooij: "Response of *Arabidopsis thaliana* to Elevated CO<sub>2</sub> and SO<sub>2</sub>", ISBN 90-367-1193-2, University of Groningen (Ph.D. project funded by Shell, Nederland B.V.); Thesis defense: March 24, 2000 (referent).
- Sue Westerman: "Response of Sulfur Assimilation in Curly Kale to Atmospheric Hydrogen Sulfide" - ISBN 90-367-1330-7, University of Groningen; Thesis defense: November 20, 2000 (co-promoter).
- Mark Durenkamp: "Metabolism of Atmospheric Hydrogen Sulfide in Onion", ISBN 90-367-2403-1, University of Groningen; Thesis defense: October 28, 2005 (co-promoter).
- Liping Yang: "SO<sub>2</sub> Nutrient or Toxin for Chinese Cabbage", ISBN 90-367-2391-4, University of Groningen (Ph.D. project funded by NOW-WOTRO) - Thesis defense: December 5, 2005 (co-promoter).

- Anna Castro: "Atmospheric NH<sub>3</sub> Deposition, S and N Metabolism in Curly Kale", ISBN 80-367-2513-5, University of Groningen; Thesis defense: March 10, 2006 (co-promotor).

## 4. Research:

### 4.1. General Statement of Major Research Interests:

#### 4.1.1. Introduction:

During the last decades my research has generally been focused on investigating the physiological processes underlying the mechanism of adaptation of plants to the environment. The significance of membrane lipids and antioxidants (e.g. glutathione) in the adaptation of plants to various stress factors e.g. chilling, frost, salinity and air pollution has been studied in detail. In addition the consequences of global change, in particular the effect of elevated CO<sub>2</sub> and NH<sub>3</sub> levels on plants was investigated. During the last decades studies on the physiological significance of sulfur metabolism in plants have become one of my major research topics. The regulatory aspects of sulfur uptake and assimilation in plants in relation to the metabolic requirement for growth and the signal transduction pathway therein involved are currently investigated.

#### 4.1.2. Overview of major research achievements during the last decade:

The research during this period has been focused on the interaction between atmospheric and pedospheric sulfur nutrition in plants as tool to study the regulatory aspects of sulfur metabolism as related to the requirement for growth.

It has been established that plant shoots form a sink for sulfurous air pollutants viz. SO<sub>2</sub> and H<sub>2</sub>S. The impact of these sulfurous pollutants on plants appears to be paradoxical; they may be phytotoxic at relatively low atmospheric levels ( $\geq 0.03 \mu\text{l l}^{-1}$ ; De Kok and Stulen 1998). From our current studies it is evident that chronic atmospheric SO<sub>2</sub> and H<sub>2</sub>S levels of  $0.05 \mu\text{l l}^{-1}$  and higher (levels which may arise in polluted areas) may also contribute substantially to the plant's sulfur nutrition. Absorption of sulfur gases by the shoots may even be beneficial when the sulfur supply to the roots is limited (De Kok et al. 1998, 2000, 2002; De Kok and Tausz 2001). There was a direct interaction between the regulation of uptake and assimilation of atmospheric sulfur gases by the shoots and the uptake of sulfate by the roots and its reduction in the shoot.

It has become evident that the foliar uptake of the sulfur gases predominantly proceeds via the stomates and both stomatal conductance and internal (mesophyll) resistance determine the rate of uptake. However, the factors determining the internal resistance towards SO<sub>2</sub> and H<sub>2</sub>S are quite different. The stomatal conductance is generally the predominant determining factor for foliar uptake of SO<sub>2</sub> and the internal resistance towards SO<sub>2</sub> is very low, since SO<sub>2</sub> is highly soluble in the cell sap and is rapidly hydrated and part of it either enzymatically or non-enzymatically oxidized to sulfate (Fig. 1). There was a nearly linear relation between the uptake and the atmospheric level of SO<sub>2</sub> in *Arabidopsis thaliana* and *Picea abies* (De Kok and Tausz 2001). SO<sub>2</sub> exposure resulted in a strongly increased sulfur content of the shoot, which in the greater part could be ascribed to an accumulation of sulfate. It has become evident that this accumulation was due to the absence of a direct regulatory control of the rate of uptake of SO<sub>2</sub> versus its metabolism (De Kok and Tausz 2001; Fig. 1).

In contrast to SO<sub>2</sub>, the internal resistance primarily determines the rate of H<sub>2</sub>S uptake, viz. by the rate of metabolism of H<sub>2</sub>S. The atmospheric H<sub>2</sub>S is directly metabolized into cysteine with high affinity and subsequently into other organic sulfur metabolites, including glutathione and proteins. In contrast to SO<sub>2</sub>, the uptake rates of H<sub>2</sub>S showed saturation curves in all plant species tested, indicating that the rate-limiting step for H<sub>2</sub>S uptake is a metabolic process, the incorporation of sulfide into cysteine. The uptake kinetics are determined by the *in situ* activity of *O*-acetylserine (thiol)lyase (catalyzes the synthesis of cysteine), the degree of association of *O*-acetylserine (thiol)lyase with serine acetyltransferase (the latter catalyzes the synthesis of *O*-acetylserine (OAS)) in the cysteine synthase

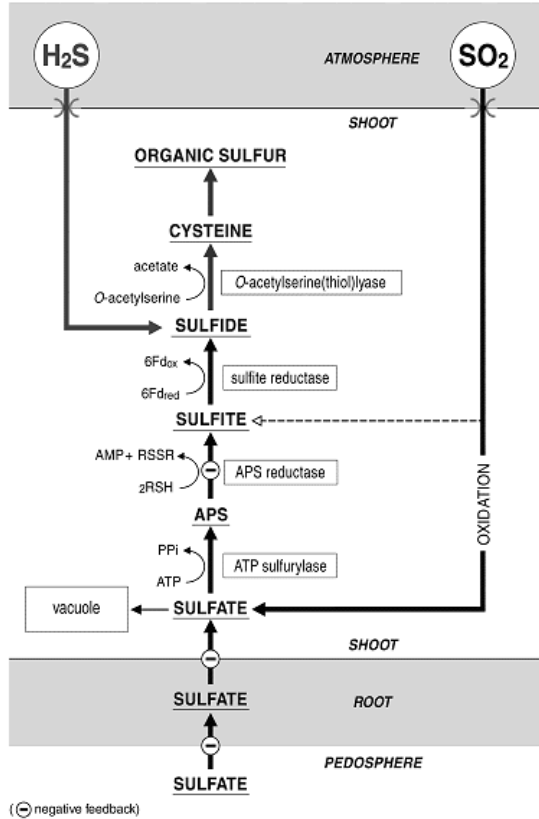
complex, the affinity of *O*-acetylserine (thiol)lyase for sulfide and the *in situ* availability of OAS. The use of transgenic plants overexpressing serine acetyltransferase and *O*-acetylserine (thiol)lyase and the measurement of the *in situ* levels of OAS will be used in order to assess the factors determining the uptake of H<sub>2</sub>S by plants (De Kok et al. 1998, 2000, 2002; De Kok and Tausz 2001; Fig. 1).

The water-soluble non-protein thiol content constitutes only a small proportion of the organic reduced sulfur in plants (about 2%; De Kok and Stulen 1993). It has been shown that upon H<sub>2</sub>S exposure, part of the atmospheric H<sub>2</sub>S metabolized in the shoots can be revealed in the water-soluble non-protein thiols, which content was rapidly increased upon exposure in all tested plant species (De Kok et al. 1998; De Kok and Tausz 2001). Glutathione ( $\gamma$ -glu-cys-gly) is generally the most abundant thiol compound present in plant tissue, and its content comprises 90% or more of the water-soluble nonprotein thiol fraction. However, upon H<sub>2</sub>S exposure the increase in thiol content was not solely due to an enhanced glutathione level, but it was accompanied by a relatively higher enhanced level of cysteine and of  $\gamma$ -glutamyl-cysteine, the latter compound predominantly in the dark. The nature of the change in size and composition of the thiol pool in plant tissue upon H<sub>2</sub>S exposure requires further to be examined. It is still questionable to what extent the increase in thiol levels upon H<sub>2</sub>S exposure represents either a surplus of reduced organic sulfur temporarily stored in a specific subcellular compartment of cells or an altered but dynamic equilibrium in the size of the thiol pool (De Kok et al. 1998, 2000, 2002; De Kok and Tausz 2001). The chloroplast is likely to be the predominant site of sulfate reduction, and of cysteine and glutathione synthesis under normal conditions. However, upon H<sub>2</sub>S exposure part of the cysteine may not be synthesized in the chloroplast but in the cytosol (see above), and may be beyond the existing systems of feedback control of assimilation. Furthermore, little is known about differences in participation between the different leaf cells in sulfate reduction and assimilation. Part of the absorbed atmospheric H<sub>2</sub>S may be metabolized in cells, which normally do not fulfill a role in sulfur assimilation but still have the ability to synthesize cysteine. Transgenic plants overexpressing or depressing *O*-acetylserine (thiol)lyase in cytosol or chloroplast have been used in order to study the nature of the change in thiol composition upon H<sub>2</sub>S exposure.

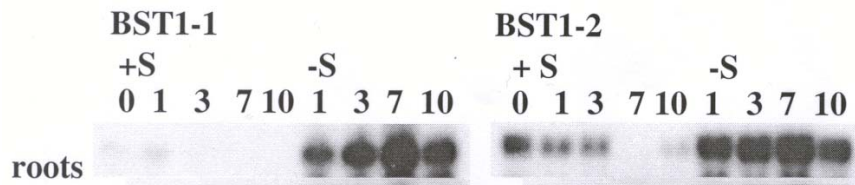
In *Brassica oleracea* the uptake and metabolism of both H<sub>2</sub>S by the shoot and sulfate by the roots appeared to be in tune and adjusted to the actual sulfur requirement for growth. The total sulfur content and the N/S ratio of both shoot and roots of *Brassica* were hardly affected upon prolonged H<sub>2</sub>S exposure. Even not at atmospheric levels higher than 0.2  $\mu\text{l l}^{-1}$ , which have been established to exceed the sulfur requirement of this species (De Kok et al. 2000, 2002). However, H<sub>2</sub>S exposure of *Allium* resulted in a substantial increase in the total sulfur content in the shoots (Durenkamp and De Kok 2004). *Allium* species (e.g. onion, garlic, leek) contain alliin, secondary sulfur compounds, viz. stereo-isomers of *S*-alk(en)ylcysteine sulfoxides, which are synthesized with cysteine as precursor. The alliin and their precursors appear to be a sink for reduced sulfur and its content is related to level of sulfur nutrition. Likewise, the H<sub>2</sub>S-induced increase in the sulfur content in the shoots of *Allium*, could indeed for the greater part be ascribed to an accumulation of alliin and their precursors (Durenkamp and De Kok 2002). H<sub>2</sub>S exposure had a negative feedback on regulation of the sulfate uptake and its transport to the shoot in *Brassica oleracea* (De Kok et al. 2000, 2002; Fig. 1). H<sub>2</sub>S exposure also affected the assimilatory sulfate reduction and resulted in a substantial decrease in the activity of adenosine phosphosulfate (APS) reductase in shoots of *Brassica*. This enzyme is considered to be one of the rate-limiting enzymes in the sulfate reduction pathway (De Kok et al. 2000, 2002; Fig. 1).

In order to investigate the regulatory mechanisms, pedospheric sulfate and atmospheric H<sub>2</sub>S supply were manipulated in *Brassica*. Sulfate deprivation of *B. oleracea* seedlings induced a rapid increase of the sulfate uptake capacity by the roots, accompanied by an increased expression of genes encoding specific sulfate transporters in roots and other plant parts (Buchner et al. 2004, Fig. 2). More prolonged sulfate deprivation resulted in an altered shoot/root partitioning of biomass in favor of the root. *B. oleracea* was able to utilize atmospheric H<sub>2</sub>S as S-source, however, root proliferation and increased sulfate transporter expression occurred as in S-deficient plants. It was evident that in *B. oleracea* there was a poor shoot to root signaling for the regulation of sulfate uptake and expression of the sulfate transporters. cDNAs corresponding to 12 different sulfate transporter genes representing the complete gene family were isolated from *Brassica* species (Buchner et al. 2004). The sequence analysis classified the *Brassica* sulfate transporter genes into 4 different groups. The expression of the different sulfate transporters showed a complex pattern of tissue specificity and regulation by sulfur nutritional status (Fig 2). The sulfate transporter genes of Groups 1, 2 and 4 were induced or up-regulated under sulfate deprivation, although the expression of Group 3 sulfate transporters was not affected by the sulfate

status. The significance of sulfate, thiols and *O*-acetylserine as possible signal compounds in the regulation of the sulfate uptake and expression of the transporter genes is currently under investigation.



**Fig. 1.** Assimilation of sulfurous air pollutants in the shoot and sites of feedback regulation of the uptake and the assimilation of pedospheric sulfate (derived from De Kok et al. 2002). Regulation of sulfate uptake and transport may occur via allosteric inhibition of the sulfate transporter proteins and/or repression/depression of the sulfate transporter genes by sulfate, reduced sulfur compounds (cysteine/glutathione) or *O*-acetylserine as possible regulating candidates. Regulation of APS reductase is likely also mediated by reduced sulfur compounds. APS, adenosine 5'-phosphosulfate; Fd<sub>red</sub>, Fd<sub>ox</sub>, reduced and oxidized ferredoxin; RSH, RSSR, thiol compound (reduced and oxidized, possibly glutathione).



**Fig. 2.** Expression of Group 1 sulfate transporters in roots of *Brassica oleracea* upon sulfate deprivation. Plants were grown on 25 % Hoagland nutrient solution with (+S) or without sulfate (-S) for up to 10 days (derived from Buchner et al. 2004).

#### 4.1.3. Outline of current and future research:

The molecular/biochemical, physiological and agronomic and environmental aspects of sulfur in plants are further integrated in close co-operation with other European research groups. Studies are carried out on the understanding of the regulatory aspects of sulfur uptake and assimilation in plants in relation to the metabolic requirement for growth and the signal transduction pathway therein involved. The relations between expression of the various genes involved in sulfur metabolism, the level of transporters and enzymes involved in sulfur metabolism and the actual fluxes of the sulfur metabolites under various physiological conditions is investigated. Small scale  $^{35}\text{S}$ -H<sub>2</sub>S and  $^{35}\text{SO}_4^{2-}$  exposure experiments are carried out to get more insight into the regulatory signals involved in the shoot/root interactions and potential and effectiveness of the channeling of reduced sulfur into sulfur metabolites. Levels of crucial substrates and sulfur metabolites in both roots and shoot that are altered in their fluxes, either by exposure to H<sub>2</sub>S and/or by molecular engineering of key enzymes are correlated with

expression levels of sulfate transporters and the enzymes of sulfur assimilation. This will contribute to a better understanding of the 'whole plant' regulation of the uptake and assimilation of sulfur in relation to the metabolic need for growth and of the rate limiting steps and regulatory signals involved. The cross-talk between sulfur metabolism and other nutrients (*viz.* nitrogen, selenium) is further evaluated. Studies are carried out with a variety of *Brassica* cultivars differing in sulfur demand for growth and possibly with mutants and transgenic lines of *Arabidopsis* with modified and enhanced sulfur assimilation/sulfur management, and with *Allium* species, which have a high sink capacity for sulfur though their potential to synthesize secondary sulfur compounds, *viz.* alliins. The objectives of the further studies are (i) to assess tools to sensing of plant sulfur status, (ii) to identify important metabolic/environmental regulating signals, (iii) to resolve the molecular components of the regulatory mechanisms and (iv) to determine the impact of sensing/regulation on source-sink relationships of sulfur (resource allocation) and the cross-talk with other nutrients. In addition, the relation between plant sulfur status and its resistance to environmental stress and pests will be evaluated, since based on profound understanding of the influence of the sulfur supply on plant resistance, environmentally friendly fertilizing strategies can be developed which help in keeping agro-ecosystems healthy with minimal or no use of pesticides. The current research is carried out in close cooperation with other research groups providing additional expertise in molecular (transcriptomic and metabolomic), environmental and agronomical approaches:

- Dr. Malcolm J. Hawkesford and Dr. Peter Buchner, Rothamsted Research, Harpenden, U.K.
- Dr. Holger Hesse and Dr. Rainer Höfgen, Max Planck Institute of Molecular Plant Physiology, Golm, Germany.
- Prof. Dr. Ewald Schnug and Prof. Dr. Silvia Haneklaus, Federal Agricultural Research Center, Braunschweig, German .
- Dr. Ineke Stulen, University of Groningen, Haren, The Netherlands.
- Dr. Michael Tausz, University of Melbourne, Creswick, Australia

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