Nodular diagnosis for integrated improvement of symbiotic nitrogen fixation in cropping systems

Jean-Jacques Drevon, S. Gugliemni, G. Boyer, E. Lafosse-Bernard, Raphael Metral, Catherine Pernot, H. Vailhe

Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture
Current Plant Science and Biotechnology in Agriculture Volume 42, 2008, pp 51-55

Nodular Diagnosis for Integrated Improvement of Symbiotic Nitrogen Fixation in Cropping Systems

Nodular diagnosis consists of measuring the nodulation of a legume in an area of production, and relating it to the growth and subsequent yield of the legume (Drevon, 2001; Drevon et al., 2001; 2003). The objective of this procedure is to respond to the following questions: (i) does symbiotic nitrogen fixation (SNF) cover the N requirement of the legume in the cropping system? and (ii) what are the environmental factors limiting SNF in the area?

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NODULAR DIAGNOSIS FOR INTEGRATED IMPROVEMENT OF SYMBIOTIC NITROGEN FIXATION IN CROPPING SYSTEMS

J. J. Drevon[1], S. Gugliemni[2], G. Boyer[3], E. Lafosse-Bernard[4], R. Métral[2], C. Pernot[1] and H. Vailhe[1]

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The nodular diagnosis is based on sampling a site chosen from the fields of the bean farmers who agreed to participate in the agronomic survey on the nodulation of their plants. Each site was divided into two parts, one without N fertilization and the other receiving a non-limiting N fertilizer with the aim of establishing whether the N nutrition is indeed the major factor limiting the legume growth in that area. Once this was established, two practices (local practice versus an alternative) were assessed in an agronomic trial within the area. Thus, the sites of nodular diagnosis were multi-locational, so that tests could be shared with many extension agents. However, it was decided not to test more than one alternative bio-technique with farmers. A reliable number of sites for use was determined to be ten per area. At flowering, corresponding to the stage when the SNF potential starts to decline, 20 plants were dug out (20-cm depth) at four sampling points within a homogeneous site. Plants with roots and nodules were preserved in a cold room for subsequent measurement of their individual biomass of shoots and nodules.

Spatial Variation in Nodulation and Efficiency in Use of the Rhizobial Symbiosis

Figure 1 illustrates the variation in nodule number, from less than 5 up to more than 50, which was generally observed at each site with instances of the total absence of nodules on some or all plants surveyed in fields of common bean in rotation with wheat in Lauragais farming systems.

F. D. Dakora et al. (eds.), *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture*.

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A simple regression was obtained from plots of the nodulation parameter (number or biomass) and the growth of plants as shown in Figure 1. This allowed for calculation of the ratio of additional shoot growth for each additional nodule, i.e., the slope of the regression was considered as an assessment of the efficiency of use of the rhizobial symbiosis (EURS) at that site. This EURS varied significantly between sites.

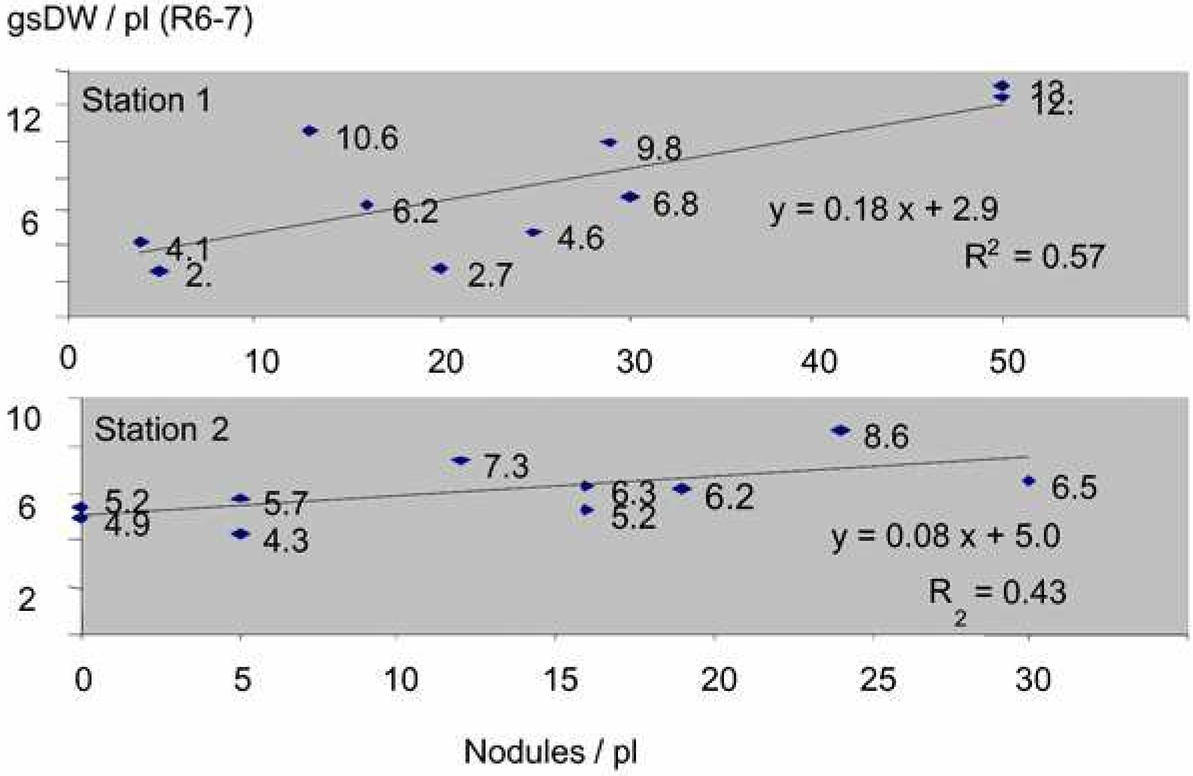

Figure 1. Efficiency in utilisation of the rhizobial symbiosis varies among fields in Lauragais.

The comparison of growth and yield of common bean with or without an optimal N fertilization within each site (Figure 2) made it possible to assess whether the nodulation or the shoot is the determining variable of the regression. As observed in site 5 and 6, the higher growth with N fertilization established that the low nodulation at those sites was the major factor that limited bean yield. These were fields where improving SNF could directly contribute to yield improvement. This may be obtained by inoculation with a native rhizobia isolated from sites with high EURS. This was not the case with sites 1 and 2 where it was found that SNF could complement soil N efficiently to support such a high bean grain yield of 3 t ha^{-1}. However, exceptionally higher grain yield than 4 t ha^{-1} were found at site 3, although the EURS was relatively high in the non-fertilized part of the site. This result suggested that the plant genotype did not have an effective capacity for SNF. This prompted the farmers to request genetic improvement of the lingot type beans, including (among other selection parameters) the height of the pods in order to decrease the losses at harvest.

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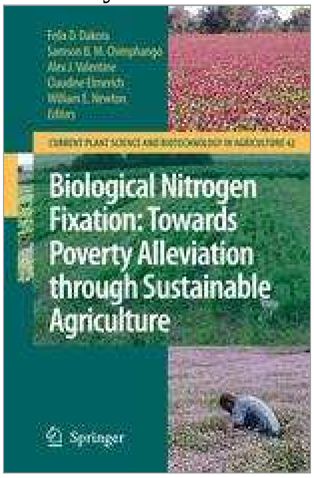

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: Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture

Book Subtitle
: Proceedings of the 15th International Nitrogen Fixation Congress and the 12th International Conference of the African Association for Biological Nitrogen Fixation

Book Part
: I

Pages
: pp 51-55

Copyright
: 2008

DOI
: 10.1007/978-1-4020-8252-8_15

Print ISBN
: 978-1-4020-8251-1

Online ISBN
: 978-1-4020-8252-8

Series Title
: Current Plant Science and Biotechnology in Agriculture

Series Volume
: 42

Series ISSN
: 0924-1949

Publisher
: Springer Netherlands

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Proceedings of the 15th International Nitrogen Fixation Congress and the 12th International Conference of the African Association for Biological Nitrogen Fixation

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ISBN: 978-1-4020-8251-1 e-ISBN: 978-1-4020-8252-8

Library of Congress Control Number: 2008930755

Cover Illustration: A field trial with lentil showing the response to inoculation, and fields of clover in bloom. Photographs courtesy of John Howieson, Murdoch University, Western Australia and reproduced with permission.

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

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15th INTERNATIONAL NITROGEN FIXATION CONGRESS

was supported by

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(Technical Centre for Agricultural and Rural Cooperation,
operating within the Cotonou Agreement between the
African, Caribbean and Pacific Group and the European Union)

National Research Foundation, Pretoria

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Edgar DaSilva

(1941–2007)

This volume is dedicated to the memory of Edgar DaSilva, former Director of the Division of Life Sciences at UNESCO, in recognition of his long-term interest in biological nitrogen fixation research. Edgar joined UNESCO in 1974 and was responsible for the life-sciences programs. His name is associated with the UNESCO Microbiological Resource Centers (MIRCEN) program, which was initiated in 1975 in partnership with the United Nations Environment Program (UNEP), and the United Nations Development Program (UNDP). He was involved in the implementation of five Biotechnology Education and Training Centres (BETCEN), one on each continent. The MIRCENs and BETCENs proved to be very successful and offered training to young scientists from developing countries as well as opportunities for international co-operation. He developed fellowship programs for research and training both within UNESCO's biotechnology program and also with partners like the American Society for Microbiology (ASM) and the International Union of Microbiological Societies (IUMS). One of his ambitions was that research carried out in developing countries should be recognized and given the necessary support. To this end, in 1985, he was instrumental in the launch of a journal, which is now published by Springer as "World Journal of Microbiology and Biotechnology". Edgar wrote numerous articles and reviews for many journals, especially those in the fields of microbiology and biotechnology. After his retirement in 2001, he continued his visits to many countries and also served as co-editor of the "Biotechnology" theme for the UNESCO-sponsored "Encyclopaedia for Life Support Systems". We thank Lucy Hoareau, Division of Basic and Engineering Sciences at UNESCO, for supplying this dedication.

Preface

Poverty is a severe problem in Africa, Asia, South America and even in pockets of the developed world. Addressing poverty alleviation via the expanded use of biological nitrogen fixation in agriculture was the theme of the 15th International Congress on Nitrogen Fixation. Because nitrogen-fixation research is multidisciplinary, exploiting its benefits for agriculture and environmental protection has continued to attract research by diverse groups of scientists, including chemists, biochemists, plant physiologists, evolutionary biologists, ecologists, agricultural scientists, extension agents, and inoculant producers.

The 15th International Congress on Nitrogen Fixation was held jointly with the 12th International Conference of the African Association for Biological Nitrogen Fixation. This joint Congress was hosted in South Africa at the Cape Town International Convention Centre, 21–26 January 2007, and was attended by about 200 registered participants from 41 countries world-wide. During the Congress, some 100 oral and approximately 80 poster papers were presented. The wide range of topics covered and the theme of the Congress justifies this book's title, *Nitrogen Fixation: Applications to Poverty Alleviation*.

Crop yields depend on many factors, but primarily on three major inputs; the capture of the Sun's light energy as chemical energy through photosynthesis, a source of water, and on the availability of a fixed-nitrogen (either mineral or organic) source. This Congress dealt with the last of these three major inputs. An enormous reservoir of nitrogen resides in the atmosphere as nitrogen (N_2) gas, however, this atmospheric nitrogen is not directly usable. It only becomes available to the biosphere through biological nitrogen fixation (BNF), a process that only the simplest microorganisms have developed. Through associations with these nitrogen-fixing microorganisms, plants can, in turn, derive a significant proportion of their fixed-nitrogen requirement for growth from BNF. The most agriculturally important associations are those of legume crops (for example, soybeans, peas, and feed legumes, like alfalfa and clover) with *Rhizobium* bacteria, where a tight symbiotic relationship occurs within a specially developed organ, the nodule, usually on the roots of the plants. An ecologically significant association involves *Frankia* microbes with trees and shrubs, which help reclaim devastated soils, and through inter-cropping can enhance the growth of valuable lumber-producing trees. Other associations include cyanobacteria with plants and trees as well as the more informal associative and endophytic associations of microorganisms with grasses, most particularly with sugarcane, where possibly all of its fixed-nitrogen requirement can be supplied by BNF. These examples clearly illustrate why BNF is a key metabolic process for food production and the maintenance of life on Earth.

The Congress theme, the application of BNF to sustainable agriculture, poverty alleviation, and environmental concerns, was well covered and included the introduction of nitrogen-fixing legumes into local small holdings, appropriate use of both soil and water, the use of indigenous soil microbes to provide N and P, and good agricultural practices generally. In addition, the basic sciences that underpin these more applied aspects were also well represented through presentations and progress reports, among others, on the fundamentals of nitrogen fixation (including a nitrogenase – the enzyme responsible for the chemical conversion of N_2 gas to ammonia – that can fix N_2 at 92°C!), plant breeding, plant and microbial molecular biology, legume-*Rhizobium* genetics, genomics, gene expression, evolution of symbioses, and nodulation physiology, stress responses, bioremediation, and forestry.

Sustainable agriculture not only depends on appropriate agricultural practices but, to maintain high yields, it requires the use of plant cultivars that respond to environmental constraints. In addition to classical plant-breeding technology, the modern-day engineering of high-performance crops and symbiotic associations can now access and use the incredible insight acquired through plant and bacterial genomics. Both topics were well represented in the Congress. Indeed, refined gene-sequence maps of the model legumes, *Lotus japonicus* and *Medicago truncatula*, have led to exciting genomic work in other legumes. On the microbial side, research on *Sinorhizobium meliloti* and *Mesorhizobium loti* as well as the broad host range *Rhizobium* NGR234 has reached the post-genomics era. The recent and rapid progress made in sequencing the genomes of *Azotobacter vinelandii*, *Azospirillum brasilense*, *Herbaspirillum seropedicae*, *Azorhizobium caulinodans*, *Gluconactetobacter diazotrophicus* and *Frankia* was also reported.

Moreover, there were reports of photosynthetic bradyrhizobia that lack the *nodAC* genes, which are necessary for Nod-factor biosynthesis and, therefore, for initiation of symbiosis. This discovery opens new opportunities for research in the area of plant-microbe signaling during the early stages of symbiotic establishment. New exciting data showed that members of the ß-proteobacteria (only recently recognized as microsymbionts), isolated from Mimosoideae can infect and fix N_2 in Papillionoideae. All this plus a cyanobacterium that anticipates sunrise each morning!

Taken together, the excellent technical presentations and the lively discussions that ensued, both during and after the oral and poster sessions, are clear indicators of the success of the Congress. We hope that this volume will serve as a living reminder of those sweet moments in Cape Town. On a sad note, during the preparation of these Proceedings, we learned of the untimely death of Edgar DaSilva, who was an avid supporter of biological nitrogen-fixation research. We feel it only appropriate to dedicate these Proceedings to his memory.

Finally, we wish to thank all the individuals who helped to organize this Congress and all those who gave their support, including Ms. Helen Zille, the Mayor of Cape Town, Mr. Ben Durham, who represented the RSA Department of Science and

Technology, Professor Wieland Gevers, the CEO of the Academy of Sciences of South Africa, and Dr. Ibrahima Ndoye of the AABNF. We also thank the sponsoring organizations, listed elsewhere, without whose financial support the Congress would not have been possible. We especially thank Elizabeth Danckwerts (and her Mum) for their commitment and tireless effort in organizing the Congress. We sincerely appreciate Vicki Newton for her hard work, dedication, and guidance towards getting these Proceedings published. Lastly, we thank Edwina Felix for her voluntary service to the Congress and all the postgraduate students who assisted during the Congress. All protocol observed!

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