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Symposium Summary and Future Perspectives

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Abstract

The Ninth International Symposium on Nitrogen Fixation with Non-Legumes, held in Leuven, Belgium in September 2002, clearly met the expectations of its organisers judging from the high quality of the review and the research papers presented. These displayed an advanced stage of maturity for this disciplinary area that now promises a productive future. The prospects for practical benefits from well established non-leguminous species, such as the casuarinas in symbiosis with *Frankia* or cyanobacteria with *Azolla* or *Gunnera*, as well as benefits from new inoculant biofertilizers for world agriculture incorporating diazotrophs that exert beneficial PGPR effects, such as *Azospirillum*, were made clear. With continuing basic research on these cooperative organisms now apparently assured, these prospects are excellent and the conditions allowing these economic and environmental benefits to be realised were examined in the symposium and are discussed below. Apart from more basic knowledge for natural ecosystems, these benefits will be both economic from increased crop yields but also environmental benefits, from N-sparing effects as a result of reduced losses of N from agricultural ecosystems by leaching to groundwater or by denitrification as the greenhouse gas, nitrous oxide. In the 21st century, such improvements in the efficient use of natural resources, while reducing impacts on the environment, are essential and the current stage of development of this research area is most opportune.

Keywords: Action matrix, biological nitrogen fixation, diazotrophs, genomics, non-legumes, PGPR

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1. Introduction

It is often the case that an area of basic research reaches a stage of maturity and its prospects for providing benefits to humanity appear much clearer. From the presentations and spirited discussions of more than 150 scientists at the 9th International Symposium on Nitrogen Fixation with Non Legumes, held at the Katholieke Universiteit, Leuven, Belgium from September 1–5, 2002, it was obvious that such progress has now been reached for this area of research, where appropriate operating principles are now available. Apart from the well-acknowledged role of microorganisms such as *Frankia* in the plant-microbial symbioses that were naturally selected long ago (e.g. the casuarinas and various cyanobacterial symbioses), there is now a golden opportunity for the range of plant rhizosphere organisms that have now been identified to contribute significantly to economic food production and to environmental health.

2. General Discussion

Biological nitrogen fixation versus plant growth promotion

This Symposium built on the many previous research advances made during the past three decades since the series began, inspired by the findings of the late Johanna Döbereiner of Brazil regarding potential benefits from *Azospirillum* growing in association with the Gramineae. In the 2002 Symposium, the role of other diazotrophs allowing sugarcane to be grown in Brazil with a smaller input of chemically-fixed nitrogen fertilisers, only half that used for most of the rest of the world's production, was described by Bob Boddey (Boddey et al., 2001) as one focus of the meeting. The Symposium program provided a guide to the understanding now reached into the mechanisms of such phenomena. In his opening address "Priority, please, for biological nitrogen fixation" Marc Van Montagu had presented a very strong case for the need to access the economic and environmental benefits from more strategically applied biological nitrogen fixation (BNF).

Successively, the following days of sessions focussed on the role of soil microbes, control of plant root development and interactive microbial quorum sensing, regulation of plant and microbial biochemistry and physiology, genomics and proteomics, microbial ecology and agronomy, providing the cross-disciplinary range needed to demonstrate the operating principles referred to above.

On the first day of the Symposium, in discussing an experimental framework for research in this area, Ken Giller (Giller and Merckx, 2003) issued a clear challenge regarding the lack of hard evidence for a major quantitative role of BNF by associative organisms, suggesting that this must be quite small when expressed as kilograms of N-input per hectare, compared to the total-N needed by crops. In fact, this likelihood has been acknowledged by most research workers in the area for some 20 years, since it was understood that diazotrophs associated with grasses do not need to contribute as much BNF as rhizobia need to with legumes taking the difference in N-content of grasses and legumes into account.

Instead, the beneficial role of organisms such as *Azospirillum* has been considered for more than a decade now as much more to do with their promotion of better crop plant growth and higher yields of grain, rather than with the amount of BNF expressed as kg ha^{-1}. Furthermore, Barbara Reinhold-Hureck rightly questioned speculative calculations presented to suggest that only limited photosynthate would be available to diazotrophs growing in the rhizosphere. This viewpoint ignores the thermodynamic power of sink strength to dictate that more carbon will flow into a region where it is being consumed, particularly for endophytes growing in high numbers of the order of 10^7–10^9 cells per g fresh weight between rice plant cells, where they are known to express their *nif* genes (Roncato-Maccari et al., 2003). Moreover, the various cooperative phytohormonal and nutritive effects of these organisms are possibly more important than BNF in improving the yields of their associated crop plants.

Indeed, a case could even be made that globally, more N_2 is currently being fixed than is needed to supply the world's crop plants, when the total of industrially fixed N_2 and BNF is considered. The inefficient use of chemical N-fertilisers clearly generates serious environmental impacts, when the pollution of ground-water by nitrate and the emission of greenhouse gases such as nitrous oxide are considered. The hypothesis that the role of associative diazotrophs can be valued as allowing more efficient utilisation of all plant nutrients including N, while providing an opportunity for some highly targeted BNF when conditions are appropriate, should be tested in future research. Considering the role of agronomy as simply a process of supplying sufficient inorganic chemical nutrients for growth has now been thoroughly discredited by numerous failures in field trials to obtain yield responses to applications of urea or superphosphate. It will be an ongoing thesis of this Symposium that a more biological or organic approach to crop growth and farming is justified, fostering the role of these cooperative PGPR microbes wherever possible.

It is surprising how often that PGPR are found to be diazotrophs. Beneficial PGPR effects by diazotrophs involve an interactive matrix of factors contributing to crop yields, including phytohormonal effects controlling plant morphology, nutrient N and P mobilisation, biocontrol of pathogens and others (Dobbelaere et al., 2003). Is this association statistically significant or a result of selective bias in sampling? This possible involvement needs to be critically tested. The diazotrophic PGPR strains are certainly able to conduct BNF for their own benefit while helping crop plants such as rice, sugarcane, maize and wheat to better obtain their supplies of inorganic nitrogen and phosphorus from the plant-organic cycle.

The evidence presented for this at this Symposium allows the conclusion that a small investment in BNF by these organisms may allow much greater efficiency in the cycling of plant nutrients, effectively reducing the need for inputs of chemically-fixed N and providing a significant N-sparing effect. This means that N is kept in the cycle of plant growth-ammonification-nitrification-plant growth, rather than leached as nitrate or denitrified as N_2O, requiring more fertilizer-N or BNF to replace these losses. If the plant cycle can be sustained to prevent such losses, only the nitrogen actually removed in produce needs to be replaced in farming systems and the overall need for BNF would be markedly reduced.

Thus, a quantitative assessment of their cooperative benefits as diazotrophs when growing in association with crops and pasture plants must include these N-sparing effects. If so, the contribution of these diazotrophs to agricultural ecosystems would be comparable to that of rhizobia for legumes grown in rotation or ley farming systems. A hypothesis could be tested that free-living diazotrophs have a special role in natural ecosystems to exert this N-sparing effect, while they carry out small amounts of strategically important BNF at critical stages of the plant cycle of plant growth and decomposition, allowing ammonia or nitrate to be re-absorbed by plant roots.

New research findings

A large amount of basic research into the relevant plant-microbial interactions has now been described, as illustrated in the outputs of this Symposium. In Jan Kijne's session on specificity between microbes and root development Ulrike Mathesius recalled Phillip Nutman's focus hypothesis for lateral roots of fifty years ago, amidst reports of recent research on the quiescent centre, stem cells, on polar auxin transport. There is ample scope for constructive interaction by PGPR organisms with plant roots (e.g. Dazzo et al., 2003; Perlova et al., 2003). Christina Kennedy related how the development of a robust model for studying the interaction of sugarcane and *Gluconacetobacter* showed that possession of the *nif* gene allowing BNF is needed to achieve the full beneficial effect of this obligate endophyte on the growth of sugarcane plants. We were reminded (Meeks, 2003) of the complexity of cyanobacterial symbiotic interactions with hornworts (*Anthoceros* spp.) and the puzzle of why a self-sufficient autotroph should become a microsymbiont. Depending on the cultivar, rice roots respond differently to inoculation with *Azoarcus*, as demonstrated by proteomics of the interaction described by Barbara Reinhold-Hureck. The central role of the P_{II} protein and its paralogues in the regulation of nitrogen metabolism of such diazotrophs was emphasised in Mike Merrick's physiology session (Forchhammer, 2003; Zhang et al., 2003).

Genome sequences of diazotrophs now become available. Christina Kennedy described how the complete genomic sequence for the chromosome of *Azetobacter vinelandii* had become available recently as a result of another special project. The major work now in progress to map the genomes of *Gluconacetobacter diazotrophicus* (Ferreira et al., 2003) and that of *Herbaspirillum seropedicae* was described in the session chaired by Fabio Pedrosa; this is very timely and a highly appropriate project for the country that discovered these diazotrophs; hopefully, these DNA sequences will be made available to researchers in this area as soon as possible just as the *A. vinelandii* genome now is.

Potential benefits on farms

So we can conclude that new possibilities for genetic modification may soon be available for all these key diazotrophs. The agronomy session led by Yaacov Okon showed just how much evidence (Dobbelaere et al., 2003) there is now regarding beneficial PGPR effects for field crops such as maize, even without genetic modification. However, as the discussion revealed, this is an area still needing much more applied research. We still do not know if the positive effects of inoculation with organisms like *Azospirillum* can carry over from one crop to the next, as is often the case with rhizobia for nodulation of legumes.

It is now time to consider how these benefits from such basic research can be made available to poorer farmers, because their needs are urgent. Genomics and proteomics may allow discovery of plant genes promoting PGPR effects in crop plants (Vargas et al., 2003; Perlova et al., 2003) and also for a more beneficial role for BNF in these systems. For example, there was suggestive evidence presented from a major program of inoculation of 1.5 million ha in Mexico with *Azospirillum* indicating large increases in grain yield (Dobbelaere et al., 2001) that more primitive cultivars of maize may contain genes more favourable for positive responses to inoculation. Generating transgenic plants favouring endophytic colonisation by diazotrophs, improving their access to carbon substrates and modifying diazotrophs to favour ammonia transport to plant tissues are now all possibilities for generating true symbiotic character in these associations.

3. Conclusions

Farmers with limited resources and needs everywhere (Reeves et al., 2002) could benefit from biofertilizer technology, but only if due attention is paid to the following essential factors:

- The development of critical quality control and quality assurance regarding the effectiveness of inoculant biofertilizer products, guaranteeing that such products can function in the field,
- Clear field evidence regarding those conditions under which responses to biofertilizer applications will be substantial enough for farmers to use them. Such products should only be employed where there is a good probability that their application will improve yields, with a significant benefit-cost ratio.

The conditions for the realisation of such benefits, and the basic research still needed, were well discussed at this Symposium (e.g. Goddard et al., 2003). Here, we advance the idea that an action matrix of mutually cooperative factors needs to be considered in any attempt to optimise the benefits possible in such systems. Because of the multi-factorial operation of such action matrices in the real world (Kennedy, 2001), non-linear responses can be expected when varying input factors, often with surprising results. For example, the use of multiple bacterial strains (Nguyen et al., 2003) raises many possibilities for obtaining balanced responses, depending on the crop cultivar and particular environmental circumstances, that needs further investigation. However, the very complexity of such responses demands a systematic, quantitative approach to their assessment to avoid confusion in the analysis of these cooperative effects.

Many of the key papers presented at the Symposium are being published in this Special Issue of *Symbiosis*, a most appropriate choice of journal considering its goals, and the reader is referred to these papers. No inference should be taken from the fact that not all the contributions to the symposium are mentioned in this article, since major advances can emerge from any of the studies.

Together with similar publications from other recent symposia (Kennedy, 2001; Kennedy and Choudhury, 2002) these will provide the scientific basis by which the agronomic and environmental benefits referred to above can soon be achieved. It is anticipated that the extent of these advances will be reviewed at the 10th International Symposium to be held in Mexico in 2005, where its native maize or Indian corn is expected to be a focus for attention.

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