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New Strategies, Methods, and Mechanisms for Agricultural Research

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New Strategies, Methods, and Mechanisms for Agricultural Research

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Model system thinking, methods, and approaches have dominated modern research, ranging from social sciences to laboratory-based biological and physical sciences and into agricultural research. Model systems are characterized by a minimization of variables, a reduction of "extraneous" influences, a choice of the simplest object of manipulation possible, and a requirement for incremental logic to be applicable continuously. These approaches have formed the bulwark of our current scientific knowledge and are still extraordinarily powerful *in certain realms.*

In agriculture, however, enormously diverse sets of organisms interact with a changing and highly heterogeneous environment within a fluid and complex social structure to produce a crop and an economic return, all of which then impact on and are impacted by both the environment and the society. It is slowly being appreciated that the complex nature of agriculture makes extrapolation from simple model systems largely irrelevant and in many cases counterproductive or destructive. However, our current modes of research are predicated on the intellectual and social framework developed through such model systems.

Modern industrial and "green revolution" modes of agriculture have dealt with this problem of complexity by making strident efforts to homogenize the environment, thereby making in effect a "model" system. Thus, application of soil fertility altering compounds such as nitrogenous fertilizers, potassium, and phosphate, rhizobial inocula, herbicides, pesticides, and growth regulators and intensive monoculturing are used to minimize environmental variables. Research, for instance plant breeding, can then be focused on this more tractable situation, where it is possible to evaluate reproducibly the performance of new genotypes or cropping systems.

It is increasingly clear, however, that from almost all viewpoints this mode of crop production is neither sustainable nor desirable and is highly disruptive both of environments, as often indicated by sharp declines in soil fertility, biodiversity, and productivity, and of social and economic structures. This is exacerbated further under the constraints characteristic of much of the economically disadvantaged world, the so-called less developed countries, which have limited and sometimes no access to the requisite inputs.

Thus, the problem can be distilled into one of policy imperatives. If centralized imposition of "solutions" on homogenized environments is counterproductive, then clearly approaches that encourage decentralized local solutions to be evolved to function sustainably under diverse environments will break the gridlock. It will be necessary to make bold steps to encourage the paradigm shifts necessary to achieve this, but it can be done through key interventions in methods.

Methodology for agricultural research must be developed *de novo* that will achieve three goals.

1. It must *decentralize* research to encourage local solutions to be developed at the level of individual farms, ecosystems, and regions.
2. It must *democratize* research to encourage broad-spectrum participation of farmers as active innovators with personal investments in the success of the innovations, and it must stimulate input and involvement from local research infrastructures, whatever their limitations.
3. It must *diversify* research to provide options for maximizing use of natural ecological balances and for incorporating an appreciation of the value of biodiversity in achieving the potential of agricultural systems, without compromising sustainability.

To develop such methods will not be trivial and will require a very substantial change in the mindset of the research community from a top-down approach to one that is much more responsive to farmers and those whose livelihoods and expertise lie with the land itself. It will also require a major shift from model systems to systems models. This change in mindset can only be achieved if the method itself not only addresses the needs and priorities of the agriculturalist, integrated over substantial time and space, but also can function under the constraints and priorities of the researcher.

The issue is not *technology transfer;* it is *problem transfer*. The issue is not the solving of problems for the Third World; it is making it more possible for local innovation and problem-solving to occur.

In this paper, I will outline first the **strategies** necessary to induce changes towards a more “systems’‘-oriented mindset that will productively deal with this conundrum, and the social, scientific, and infrastructural constraints that impede this change. Next I will give examples of novel **methods** that can be designed to implement these strategies and bridge this gap, methods that will function to establish a productive interaction between those whose lives largely revolve around model systems-the bulk of the research community-and those whose lives hinge on the performance of complex systems-the farming community. Finally I will discuss **mechanisms,** including a new institutional mechanism, CAMBIA, that will develop, disseminate, and support such methods so that they are broadly effective and generate innovation for social and environmental good.

STRATEGIES

Sustainability Requires Local Empowerment

Sustainable agricultural productivity and environmental and ecological preservation are of the utmost importance for the survival of humankind. Sustainability must be defined as much more than “environmental” or “agricultural,” because it is *people* who live in and shape an environment, and it is *people* who must develop, manage, and thrive on an agricultural system. Thus, human constraints and desires must be fully incorporated into any scheme for sustainability. However, as with environments and agricultures, human needs and social structures are highly context dependent and variable and do not lend themselves well to centralized problem-solving. The uncoupling of human requirements from our strategies makes agricultural research irrelevant and wasteful, whereas their integration can bring productivity and innovation.

Empowerment Requires New Approaches

The development of integrated approaches is difficult simply because the tools, both intellectual and technical, to address such complexity have not been adequately developed or appreciated. When developed, they must operate within the personal constraints of the tool user as well as function in the context of the problem. In fact, it is not so much an unwillingness to address this complexity that constrains progress as a desperate frustration and inability to do so. It is enabling people, farmers and researchers, to overcome this bottleneck that underlies the remit of CAMBIA, and this empowerment will be provided by new hierarchies of methods.

To shape new approaches, we must look critically and openly at both the strengths and the weaknesses of the existing systems and ways of defining and resolving problems.

Agriculture Cannot Be Separated from the Environment

Many regions of the developing world, which host the majority of the world's human population, have highly evolved traditional farming and marketing systems that have proven very robust and at their best have been sustaining soil fertility, environmental health, and economic production for millennia. These systems need not be romanticized to acknowledge the value of the empirical knowledge that they encompass. While it is certainly true that these systems cannot always make the necessary quantitative leaps required to feed an expanding urban population, many extremely important lessons can be learned from them, and they should be neither discounted nor necessarily replaced wholesale. It can be argued that research to enhance and empower these locally developed systems and their practitioners will be much more effective than will importation or imposition of new cropping and farming systems that may have been developed with different constraints and needs in mind.

In many cases it can be shown that the replacement of these agricultural systems has stimulated and heightened the problems that their replacement was designed to solve, for instance, the urbanization that can result from "efficient and labor-saving" agricultural systems.

The Model System in Science

The model system, in which variables are limited, the complexities reduced, and the components analyzed, has been and continues to be the dominant paradigm in experimental science. The approach of choosing a system that epitomizes the set of characters we wish to study in a manageable context has been of extraordinary value in increasing our understanding of the workings of organisms and of the world. The great biological model systems such as *Escherichia coli, Drosophila melanogaster, Caenorhabditis elegans, Arabidopsis thnliana,* and the mouse have all contributed through genetics, developmental and molecular biology to our understanding of the underlying mechanisms of organismal function. Thus far, however, our understanding of the interactions between organisms, the workings of complex communities of organisms, the balances and natures of ecologies, has not benefited from this approach, because the variables are so numerous and such an intrinsic part of interactive systems.

Modern Agriculture as a Model System

Agriculture in the North, and its myriad almost-identical export versions, has developed in perfect harmony with the model system approach, and its great "success" stories are built upon the same assumptions and are judged by the same criteria by the same people. We first homogenize the environment, adjusting with herbicides and pesticides the ecological competition, using nitrates, phosphates, and other fertilizers to "enhance" the soil fertility. Then, as with laboratory systems, but with more intent and design, we breed plants that cope well with this environment.

Laboratory bacteria such as *E.* coli, which normally grow in their natural environment of the vertebrate intestine with a doubling time of about 24 hours, can grow in a laboratory with a *20-minute* doubling time, when provided with glucose, oxygen, and numerous other limiting nutrients. Similarly, modern crops that have been subjected to rigorous plant breeding selections under just such controlled conditions perform "splendidly" in a homogeneous, simplified farming system.

This concept of the model system is so pervasive and, within its scope, so effective that few within the research community trouble to challenge it. It is extremely powerful, but like other powerful tools, it has a capacity for substantial abuse. The abuse of "model systems" approaches is the attempt to use the power, *as it stands,* for problems that are not in its domain or simply to choose to stay in the seductive realm of its effectiveness, a realm from which most real-world problems and conditions are excluded (TABLE 1).

In our current climate of rapid social and scientific change, technologies are evolving insufficiently coupled to problems. Increased specialization, which is a necessary outcome of the pace and breadth of new scientific progress, is encouraging this uncoupling. Thus, technology is transferred to those experiencing problems that, while powerful and exciting, may be completely inappropriate for solving their problems. This builds a nominal "problem-solving" ability that is often incapable of addressing *real* problems, and instead it develops a tendency to seek problems that fit the tools, rather than tools that fit the problems. As long as those who develop tools in the industrial world experience different problems and have different constraints, this will inevitably continue. If we are to have any enduring impacts on overcoming limitations in agricultural productivity without compromising sustainability and social justice, then *the problem must shape the technology* and *the problems must be solved by those who experience them.*

There is a tendency in science to ignore the development of methods, a scientist's toolkit, as being of secondary importance to the elite act of gathering knowledge and understanding ("basic" or "fundamental" scientific research). Furthermore, the application of these tools or the gathered knowledge to human problems is often regarded as being of tertiary importance, as manifested in the schism between physicists and engineers or between biologists and extension agents (or even farmers).

The problems in agriculture and the environment are infinitely more complex than are those of the molecular realm that is so successfully being mined in modern science, and their effects are correspondingly more devastating and urgent. Problems in agriculture and the environment are not solely or even largely "technical" but are more social. When the number of problem-experiencers is disproportionately large, as in problems of poverty, food self-sufficiency, and declining individual capacity in agriculture, then the difficulty is magnified.

To cope with this dilemma, the problem-experiencers must be encouraged to become tool-wielders, not to be passive appliers of external "wisdom." This must happen if not for reasons of social justice, then simply for logistical and pragmatic reasons as the number and diversity of the problems are so great. If this is to occur, however, there must be a dialogue and a coupling of tool developers with the problem-experiencers/solvers.

It is clear that here is a key point where our intervention can be effective at reorienting the *status* quo. CAMBIA is being developed as an example and as an enabling mechanism to provide the disenfranchised with some tools that they themselves help to design. These disenfranchised are first and foremost the farming communities of the world and also clearly comprise the scientific and agricultural research and development community whose job it is to support them.

TABLE 1. Comparison of the Characteristics of Model Systems, Traditional Farming Systems, and Industrial Farming

Model Systems	"Traditional" Farming Practice	Industrial Farming Practice
Homogeneous	Heterogeneous	Homogeneous monoculture
Site independent	Site dependent-context sensitive	Relatively site independent
Simple, lacking diversity	Complex and highly diverse	Simple, minimizing diversity
Eliminates variables	Variables are intrinsic	Minimizes variables through inputs and price supports
Reproducible	Irreproducible and risk-prone, risks buffered by diversity	Buffered by policies and subsidies, but highly risky over long times
Value reductionism	Values contextual intuitions	Places increasing value on reductionism

To illustrate this, I will describe a few of the methods whose generation will fall under CAMBIA's remit and then describe more about the mechanisms that will allow their interactive development, distribution, and support.

METHODS

Nondestructive Reporter Genes: The Fundamental Tools

One of the greatest paradigm shifts in experimental biology was the development of the concept of gene fusions and reporter genes. Pioneered in the 1960s by Ethan

Signer and Jon Beckwith, then at the Pasteur Institute, the idea has revolutionized our ability to analyze biological systems. The concept is outlined succinctly in FIGURE 1. A gene can be formally considered as consisting of two parts, one that "senses" the commands of the environment as transmitted by the cell (the *controller*) and directs the synthesis of a new product from the second component (the responder) (FIG. 1 a). The breakthrough of gene fusions was the idea that these formal concepts had physical reality and could be restructured to facilitate study.

To understand how a particular gene is controlled when measurement of the gene product is too cumbersome, gene fusion analysis can be performed. By leaving intact the controller sequences of the gene we wish to study, but replacing the original responder gene sequence with a new and easily studied gene, now called a *reporter gene*, *reporter* gene activity can then be measured and powerful inferences be made about the mechanisms of control of the target gene (FIG. 1b). Subsequent manipulations and changes of the controller will then cause corresponding changes in the activity of the reporter and illuminate elements of its biology. Moving the genes and DNA fragments around was originally extremely difficult and required extraordinary skills of bacterial genetics (the original papers are classics), but with the advent of recombinant DNA, the ability to manipulate genes *in vitro* and reintroduce them to the parent organism (DNA *transformation*), the possibilities expanded greatly.

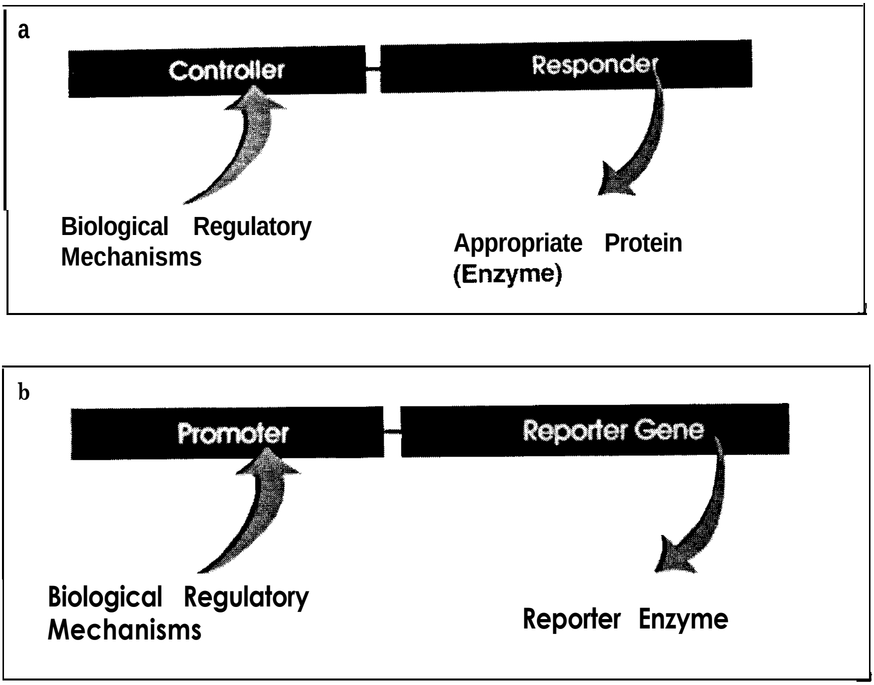

FIGURE 1. (a) Gene structure. (b) Gene fusions.

Choosing a reporter gene system that allows numerous simple assays can enormously increase the range and quality of measurements that are possible. The first of these, the LAC system, encoded a readily assayed enzyme, β-galactosidase, that to this day remains one of the premier tools of biological research, but it is hardly used in agriculture. It is not used widely in agricultural biology largely because the enzme is also a natural component of most agricultural systems, including plants. Thus, measurement of the enzyme is confounded by preexisting enzymes with the same specificity.

In plants and their associated microorganisms, the most widely used tool is the GUS gene fusion system, which encodes the enzyme β-glucuronidase (GUS). Like β-galactosidase, GUS is able to cleave a wide range of substrates producing colored or other compounds at the site of enzyme activity and in amounts proportional to enzyme activity. This enzyme is largely absent from the agricultural biota, and hence measurements and visualization of the enzyme can readily and sensitively be carried out, giving substantial information about the control of gene action in plants.

While the system has been widely used and has been essential for developing much of our understanding of plant gene function and in developing the tools of plant transformation, with thousands of citations in the literature, it has basically been used as an extension of the laboratory-based paradigm that started with LAC. There is a need to bring the enzyme (a large protein) and its substrates (small water-soluble compounds) together so that they can act to produce the color necessary to visualize activity. To do so typically requires that cells are broken or damaged to bring these components together; hence, assays of the enzyme are destructive and cannot readily be performed on living tissues or in the field. For instance, the blue- and magenta-colored plantlets in FIGURE 2 have been assayed for GUS using two different substrates (designed in collaboration with Biosynth AG of Switzerland) and have been killed in the process of analysis. With plants, which are intimately tied to their environments and which change with small fluctuations in the environment, this failing is enormous.

The next step in the evolution of gene fusions, one that will revolutionize agricultural research by bringing it back to the field, is to develop the tools for *in vivo* reporters for nondestructive analysis under field conditions. This subject is one of the principal foci of CAMBIA's research programs. The key criteria for such an *in vivo* reporter system are that it should:

- Be nondestructive
  Sampling should be avoided
- Be nondisruptive
  No alterations of physiology/performance of the crop
- Be generally useful
  Needs to function in most crops, including little-studied or orphan crops
- Be inexpensive
  Must be accessible to scientists in less developed countries
- Require little or no instrumentation
  Ideally chromogenic, producing easily recognized and measured colors
- Function under field conditions
  Questions of real value in agriculture require both field performance and availability to field scientists and farmers

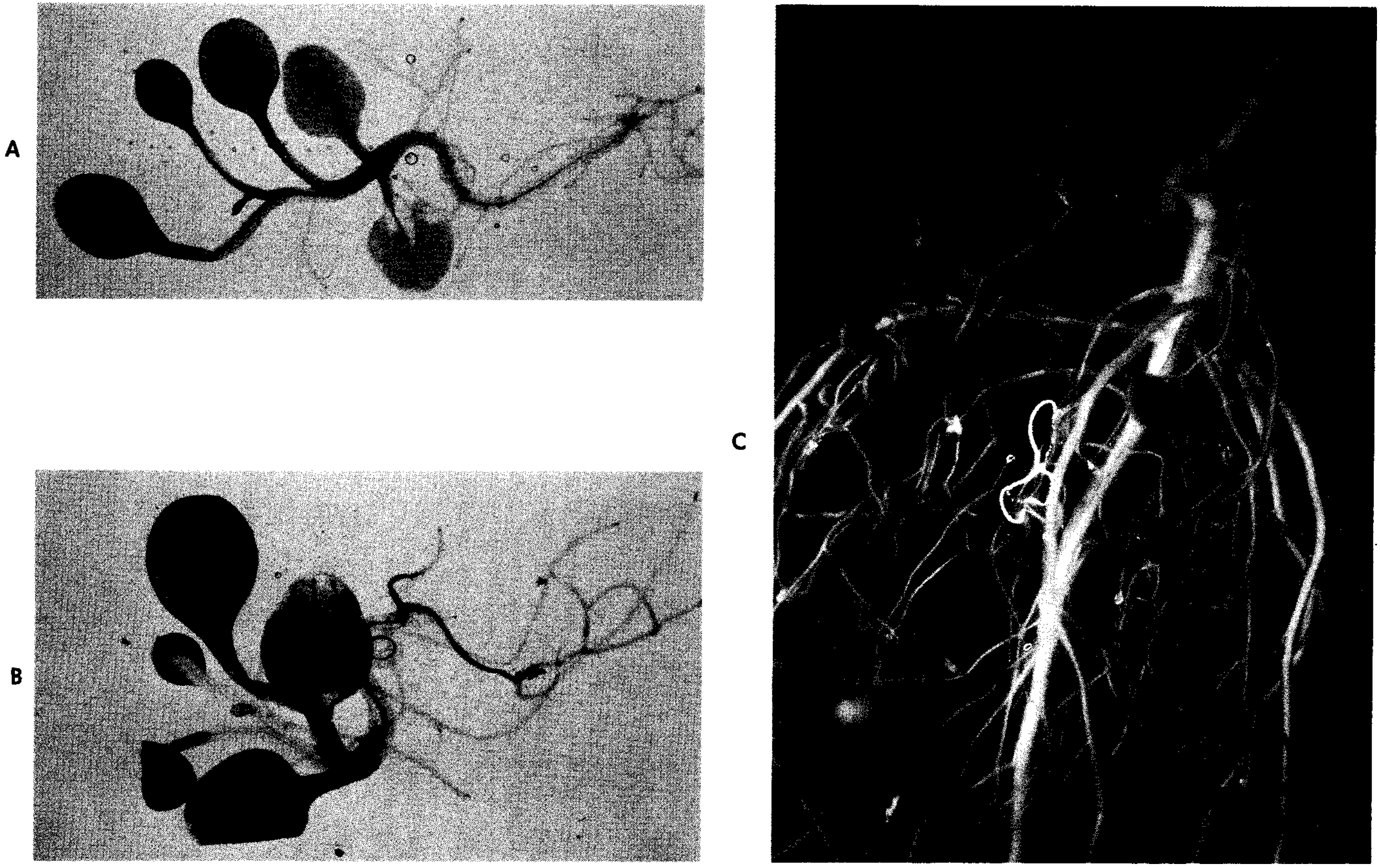

FIGURE 2. (A, B) Transgenic plants expressing the GUS reporter enzyme, assayed using GUS substrates which give rise to blue (**A**) or magenta (**B**) colored plants. **(C)** The GUS reporter system can be used to monitor the success of *Rhizobium* inoculation. These bacteria carry out symbiotic nitrogen fixation in nodules induced on the roots of legume plants. The efficiency with which a GUS-marked strain competes with indigenous strains for nodule occupancy can be assessed by the percentage of blue nodules following incubation in GUS substrate.

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Substantial progress is being made towards these goals in developing the next generation of markers. The basic concept being followed makes use of the structure of plant cells, which are surrounded by cell walls that, while permeable to sugar, water, and small molecules, retain proteins such as GUS and other enzymes. We are now working with and developing not only GUS, but also another marker with similar properties (our working name for it is Daughter Of GUS), so that both enzymes can be secreted from the cells across the cell membrane but remain trapped within the cell wall, rather like the leaves in a tea bag, where the simple water-soluble compounds can diffuse in to produce the color upon enzyme action.

Not only will this development revolutionize field analysis of gene action, but also it will open almost unlimited possibilities of transforming and analyzing difficult crops, such as most of those in the tropics. Many current methods of genetic transformation rely on tissue culture and genetic selection using antibiotics, which induce substantial stress on the plant and which are not suitable for perennial or woody crops or for most crops with little background of research. It is becoming increasingly clear that simple tools that will permit the scientist to easily visualize those cells and tissues that contain and express a new gene will allow novel developments that make these cumbersome selections unnecessary. Thus, crops such as cassava, yams, beans, chickpea and other grain legumes, agroforestry species, and so on, for which there is little hope of achieving the critical mass of research necessary to make even modest progress with current methods of molecular biology, will benefit greatly from tools that allow routine transgenesis with minimal infrastructures.

Sentinel Plants: Empowerment of Farmers and Crop Researchers

The concept underlying the development of ***sentinel plants*** or ***bioindicators*** is simple: the use of genetic engineering of plants to produce a suite of chromogenic enzymes that can be monitored cheaply and nondestructively in the farmers' fields, *whose levels are proportionate to any of a variety ofphysiological or environmental parameters that are of interest.* The basic concept is outlined in FIGURE 3, and builds on the nondestructive analysis tools just described. We do not need to think of gene fusions only as a tool for laboratory analysis or as simply a tool to study gene function. Gene fusions can be used to measure complex phenomena, even in the absence of mechanistic knowledge of how that phenomenon works.

The tools and methods to be developed are completely general in their application and could be used to cause a facultative plant color that is proportional to, for example, soil acidity (a biological pH meter), moisture, phosphate or other nutrients, or biotic or abiotic stresses. The ability to *measure or at least visualize* a phenomenon is a prerequisite to understanding and manipulating the character, even by empirical methods such as plant breeding or system manipulation. Thus, these tools should open limitless new possibilities for the agricultural and environmental sciences.

The empowerment feature is that the measurements will be done not only in the farmers' fields, but also by farmers and agronomists working in severe circumstances. It is these individuals who will be best placed to develop innovations in cropping systems or breeding that will make wise use of limiting resources.

One example is the need to measure nitrogen fixation efficiency in cropping systems. After water, nitrogen is the single most critical factor for agricultural productivity worldwide. Overuse of nitrogenous fertilizers causes extreme environmental problems, whereas inadequate nitrogen (as is the norm throughout most

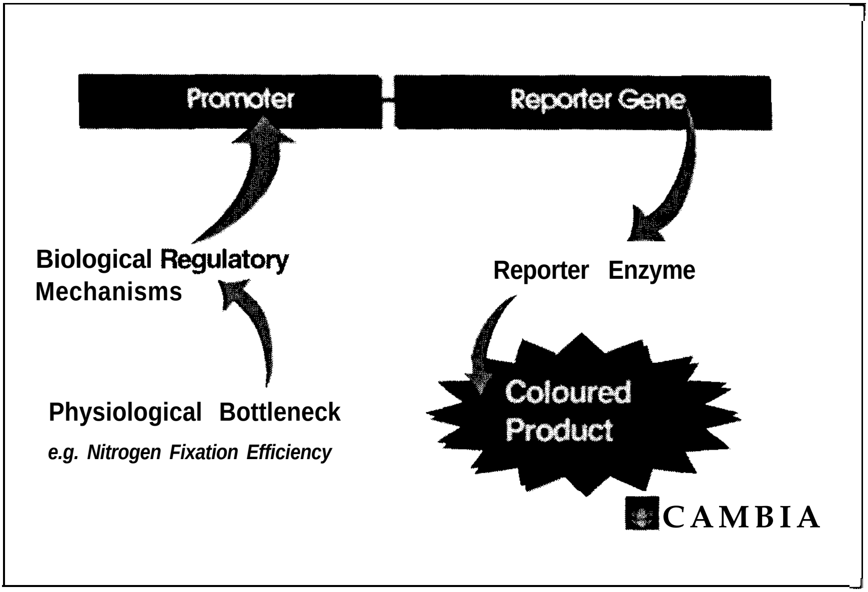

FIGURE 3. Gene fusions can report on complex physiological properties.

of the world's agricultural lands) can cause greatly reduced yields and declining soil fertility.

Nitrogen can be obtained "free" from atmospheric sources by fixation into useful forms through some symbioses between legumes and bacteria. This renewable nitrogen source is within the economic means of poor farmers who cannot afford chemical inputs. However, the proportion of nitrogen that a legume crop derives from fixation can vary from 0–100%, and to breed for maximum efficiency of nitrogen fixation or to develop cropping systems that wisely use it, we must be able to measure this proportion. Unfortunately, this symbiotic fixation is virtually impossible to routinely measure under field conditions, especially in the developing world, and hence to adjust and improve. Thus, we are largely unable to make intelligent use of this remarkable natural source of the greatest limiting nutrient in agriculture.

Recently, through the work of two Australians, David Herridge and Mark Peoples, it has been discovered that in many of these symbioses, including those in soybean, pigeonpea, common beans, and many tropical legumes, the proportion of nitrogen derived from fixation can be measured in the laboratory through quantitating ratios of "signature" compounds-the ureides, allantoin and allantoic acid--to other nitrogen-containing compounds, such as nitrate or amino acids.

The existing technologies to measure these compounds, and hence to make inferences about nitrogen use efficiency, require substantial laboratory infrastructures, are relatively expensive, and yield a low throughput. To empower the farming community and the agricultural research community, we need to develop methods that will allow fast, routine, and inexpensive analysis in the field.

One avenue of our research is geared towards coupling this discovery to the development of *bioindicator plants* or ***sentinels*** that will instantly reflect levels of the different signature compounds in plants growing in the field. In this example, DNA controller sequences that sensed changes in the levels of the key signature compounds would be identified and would be engineered so that the activity of reporter genes, the genes coding for GUS and Daughter Of GUS for example, would reflect this. Thus, a simple color assay carried out in the field which indicated the relative activities of GUS and DOG would immediately inform the farmer about nitrogen fixation efficiency (FIG. 4).

This then would open up limitless possibilities for evaluating the genetic or the environmental contributions to nitrogen fixation efficiency and allow altogether new management and breeding options to be developed based on the actual field performance.

Another area of importance in nitrogen-fixation research is the population dynamics of the nitrogen-fixing *Rhizobium* bacteria. An example of the use of the GUS reporter system to monitor the efficiency of inoculation of legumes with *Rhizobium* is given in FIGURE 2C.

Apomixis: The Ultimate Tool for Agriculture and the Environment?

The two most important scientific and social innovations for the future of humankind apply to sexuality. The first relates to humans and is effective birth control or *sex without seeds*. The second relates to plants, seeds without sex-apomixis.

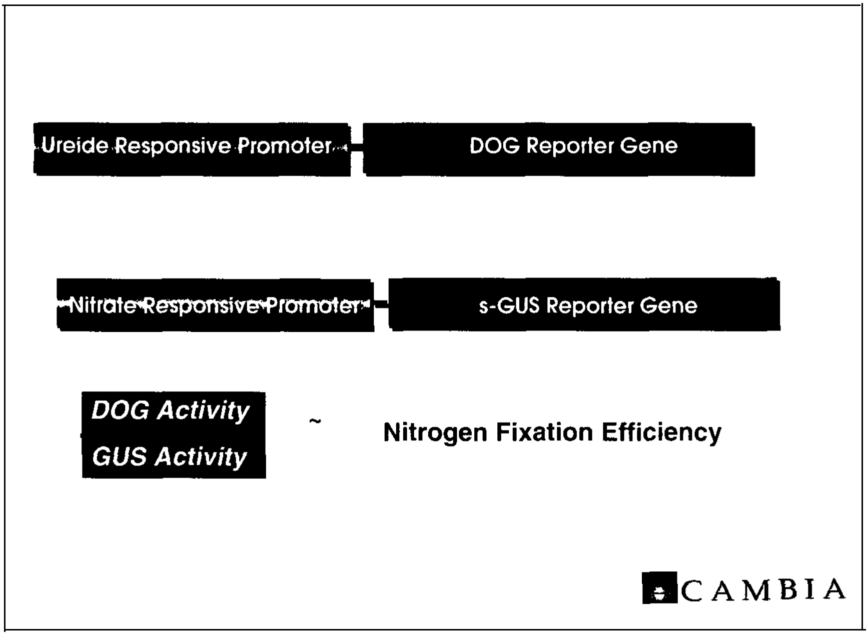

FIGURE 4. The ratio of expression of two different gene fusions can indicate efficiency of nutrient use.

Apomixis is a phenomenon by which seed is formed without fertilization of the gamete by pollen (it's a form ofparthenogenesis), and it results in the propagation of the maternal genotype without segregation or reassortment of characters. Apomixis occurs naturally in numerous species distributed among a large number of plant families and by several diverse mechanisms. However, it occurs in very few crop plants, and when it does, it occurs by mechanisms that are not readily usable.

The introduction of apomixis *(production of true-breeding seed without fertilization)* as a controllable and efficient trait in many diverse crop plants would easily herald the single most profound change in agriculture since the dawn of cultivation. A few of the changes that could ensue are:

- preparation of almost limitless numbers of hybrid cultivars from almost every crop species into which the trait will be introduced, greatly expanding the diversity of utilized genetic resources and providing the benefits of heterosis through hybrids to the small landholder and for the numerous crops that have never had hybrid technology available;
- immediate fixation of heterozygous genotypes, including those made through wide crosses, opening up almost unlimited possibilities for new breeding strategies and methods in sexual and vegetatively propagated crops;
- plant breeding could become extremely quick and responsive to microenvironments, cropping conditions, pathogen populations, and markets and market opportunities, stimulating extraordinarily diverse strategies for agroecosystem management and optimization. This would fundamentally reverse the tendency to homogenize cropping systems to match breeding and management possibilities and would encourage "boutique breeding" and context-dependent enhancements;
- propagation of hybrid seed directly by the farmer without the need for inbreds or male steriles and without recourse to frequent seed purchases;
- true-seed propagation of traditionally vegetatively-propagated crops, including many crops of particular importance to the small farmers of the tropics and subtropics, such as cassava, potato, sweet potato, and yams, with concomitant elimination or reduction of disease, substantial increases in germplasm flows, and enormous expansion of growing regions;
- elimination of anthesis and fertilization-related crop losses (e.g., anthesis drought, heat stress, pollinator failures, submergence, or pathogenesis), a major cause of devastating reductions in crop yield and reliability;
- substantial increases in yield in some crops due to additional photosynthate availability through elimination of male flowers/flower parts;
- breeding specifically for endosperm performance in locally adapted varieties without compromising agronomic traits in non-autonomous apomicts.

This list of possible impacts is so striking and so comprehensive that it is well worth considering apomixis, if developed to achieve the goals on the list for a broad and diverse group of users, as being the most important target for international agricultural research. And yet it is almost always viewed as comprising a "fringe" element within agricultural research priorities and the community, and it has never been the subject of a concerted effort.

Traditional approaches to developing apomictic crops involving the introgression of apomictic traits or "tendencies" from wild or related material into crop species have generally been extremely disappointing and limited in their potential. However, it is now becoming clear that *de nouo* generation of the trait or "synthesis" of a suitable controllable apomixis through molecular biology will be a viable and productive avenue. Advances in modern research biology, molecular biology, and genetic engineering now make it likely that within the next 10 years this can be achieved.

In just the last few years, for example, conditional nuclear male sterility has been genetically engineered into numerous crops by transforming plants with chimeric genes that direct and limit the expression of toxic gene products (an RNA degrading enzyme derived from bacteria in the best known case) into the male flower parts. This gives rise to a dominant nuclear male sterile phenotype with efficiencies approaching 100%. When combined with a genetically engineered ***restorer*** line that encodes a specific inactivator of the toxin, a universal and completely reversible two-line male sterility system is achieved. There is every reason to believe that this mechanism, with few variations or exceptions, will be applicable to most crop species; thus far, it is effective in both monocots and dicots, both autogamous and allogamous. This has been carried out largely by teams in the private sector with the goal of developing commercial and proprietary hybrid production for many crops. However, while elegant, it is only the beginning of what can be done by adjusting the sexual systems of plants through molecular intervention.

Apomixis, the combination of the failure to reduce the female gamete through meiosis with a concomitant ability to initiate embryogenesis and endosperm formation without pollen-mediated fertilization, is dramatically more powerful and versatile than a male sterility approach, if designed correctly, but for many reasons it is less likely to receive substantial private sector investment. The principal reason is the difficulty of ownership. Basically, apomixis more than any other technology could be a democratizing and decentralizing agent in agriculture, an empowerment of the communities that actually live on and work the land to dictate the improvements and verify the sustainability and performance of those improvements.

Development of Apomixis through a Transgenic Mechanism

To develop an apomictic trait that functions in many or most crop plants will require several parallel avenues of research. These should be dictated by a strict assessment of the ultimate desired properties of the trait. Although some criteria can vary depending on the implementation of the apomictic trait, there are nonetheless clear, non-negotiable criteria that must be adhered to in the design of the trait. Specifically, the innovation should be designed explicitly to result in a socially and economically equitable and environmentally and ecologically sound practice.

Criteria for a Successful Apomictic Mechanism

Envision a scenario in which an apomictic "cassette" that produces several linked phenotypes, can be generated through molecular biology. This cassette would function as a dominant trait in virtually all crops to which it is introduced to:

- block entry of the embryo mother cell into meiosis and thus produce a fully functional unreduced female gamete,
- block the development of male sexual structures,

- allow fully autonomous development of the embryo and endosperm without pollination,
- have this condition be fully penetrant, and further
- have the condition be dominant but conditional, where the default state is apomictic, but upon application of a nonproprietary, inexpensive natural compound. the trait is fully suppressed so that crosses can be performed in either direction.

This scenario may be the best-case situation and should be borne in mind for the research and development of apomixis. Although many scenarios for the development of apomixis as a useful tool can be generated, virtually all of them require certain "technical" constraints that exist in natural apomicts to be overcome to make the trait truly useful to agriculture and to a broad community. Key among these is the requirement that the apomixis be conditional and suppressible by the farmer/breeder.

CAMBIA will be focusing substantial attention and research towards apomixis as well as coordinating international efforts to achieve a socially and environmentally sound apomixis methodology. The research necessary to achieve this will take several parallel approaches, including:

- detailed molecular and cellular analysis of model apomicts,
- genetic screens for apomictic mutants in *Arabidopsis thaliana*,
- analysis of plant genes by complementation of meiotic mutants in yeasts,
- development of tools to delimit and control gene expression within target plants, and
- development of tools for farmer/breeder control of transgene expression.

Responsible development of apomixis *must* involve generation of mechanisms to ensure both environmental and ecological restriction of the trait. This could probably be achieved through a concomitant male sterility engineered as early in the development of the male floral organs as possible. This trait could best be envisioned as being facultative, so that on demand, the trait could be passed to sexually related species through conventional breeding methods. However, the penetrance of the sterility in the absence of its suppression must be high.

It is equally important to ensure that the apomictic trait is expressed as the default condition, but suppressible at the will of the farmer or breeder. Molecular mechanisms need to be developed that will allow breeders to switch the trait off to perform a sexual cross and then release the switch to allow reversion of the F1 to apomictic mode. Thus, systems comprising compounds that can be applied to induce or repress defined corresponding promoters need to be developed. These in turn must be developed with a view to ensuring that the small farmer, breeder, and agronomist in the less developed world has free and easy access to the methods. Although some such systems have already been described by laboratories in the private sector using proprietary agrochemicals, the proprietary nature of the tools and the lack of availability of both genes and chemicals make these unacceptable candidates. Rather, these compounds must be chosen to be available to even the most resource-limited of the plant improvement community. Interesting candidates could be compounds such as β-glucuronides that are present at high levels in animal urine, but not present in plants, and that have a well-characterized effect of inducing bacterial genes (the *gus* repressor/operator). Engineering this system into transgenic plants could then allow even farm-level control of the trait.

Biodiversity Indexing Technologies

Much attention is currently being focused on biodiversity issues, with the decline in genetic diversity of cropping and forestry systems being of particular urgency. However, all policy as well as research and development strategies are constrained by the inability to rapidly, inexpensively, and unambiguously characterize or "index" this genetic diversity. The development of technologies that would do so in an automated, low cost, and high-throughput manner and that could function under the limiting infrastructures of much of the less developed world would revolutionize our ability to sensibly and sustainably manage and conserve our natural resources and to make substantial improvements in agriculture without decreasing our genetic base.

One of CAMBIA's technology development goals is precisely this, that is, to make use of the breakthroughs in molecular biology, automation and fluids engineering, computational hardware, and informatics that have been stimulated by the international Human Genome Initiative and to adapt and modify these to produce robust technologies that can be used to develop DNA sequence-based diagnostics or "fingerprints" from biological materials.

The first case being developed is the pressing issue of tree biodiversity, thereby empowering local management, breeding, and conservation strategies. Through collaborations with The Institute for Genomic Research (TIGR), one of the leading institutions in the Human Genome project, and the International Board for Plant Genetic Resources (IBPGR), the leading international center mandated to deal with genetic diversity, several major scientific instrument companies, and the CSIRO Division of Forestry, CAMBIA plans to streamline and adapt the use of PCR-mediated gene amplification (polymerase chain reaction, a key new technology), with automated DNA sequencing to generate a simple technology whereby the forestry community will have rapid and inexpensive methods to unequivocally identify and characterize the genetic diversity of both natural and managed tree provenances. The utility of these technologies for conservation strategies for rainforest and other threatened species will also be immense.

Interestingly, the technologies that CAMBIA will be developing will be equally applicable to virtually all crop germplasm characterization. Internationally sanctioned and standardized molecular indexing technologies could provide the teeth necessary to facilitate scientific, legal, and political accountability for the flow of genetic resources and biodiversity from the less developed nations of the tropics and subtropics to the industrialized developed economies.

MECHANISMS

By what mechanism can we then begin to integrate these disparate ideas, innovations, and priorities? Clearly the development of new methods to operate under field conditions by those who experience the problems will be effective, but how will these methods come to be? And how can they be distributed and supported in an interactive fashion if the scientific community has other constraints and priorities? The extraordinary short-term demands on the attention and resources of public sector research organizations, such as universities or national programs, and their necessarily local or regional foci make it extremely difficult for these organizations to function in a service role, while maintaining a strategic research and innovation capability. The private sector is perhaps even more constrained to use methods and tools "off the shelf' to generate products that can yield short- to medium-term profits.

CAMBIA (Center for the Application of Molecular Biology to International Agriculture) is a new international research and education initiative with the specific mandate to develop and support novel tools and methods for agricultural innovation. These approaches will be based on molecular biology, as in the examples of transgenic bioindicators for measuring nitrogen fixation efficiency just discussed, but they will not generally require molecular biology for their use. Their primary goal will be to stimulate local problem-solving and thus to greatly enhance the power of scientists and farmers to identify and solve their own agricultural problems in a socially equitable and environmentally sound manner.

This problem of inadequate analytical tools for use in the field confronts the agricultural research community worldwide, be it public or private sector. Through development of such tools, CAMBIA will benefit research in the developed world and the developing world alike. There are, however, a number of constraints that particularly hinder such research in developing countries. CAMBIA will have teams dedicated to addressing these constraints:

CAMBIA's Research Divisions:

Molecular Genetics

e.g., Nondestructive Reporters

Field and Environment Analysis

e.g., Population Dynamics, Sentinel Plants, Biodiversity Indexing Technologies

Adaptation and Implementation

e.g., Cassaua *Postharvest Deterioration, Transgenesis* of *Orphan Crops*

CAMBIA's Support Divisions:

Education

Skills and Understanding

Communication and Information Services

Information, Computers and Motivational Support

Infrastructure Development and Support

Research Capacity

Molecular Genetic Resource Service

Materials and Critical Services

Examples of the work of the first two divisions, Molecular Genetics and Field and Environment Analysis, have been given earlier.

The third research division, Adaptation and Implementation (AI), will concentrate on adapting and implementing tools developed at CAMBIA and elsewhere for scientific problems of particular importance in developing countries.

The Adaptation and Implementation Division

Inadequate Attention to Research on Orphan or Tropical Crops

The major crops grown and consumed in less developed countries and the cropping systems and farming systems that produce them are often very different from those of the economically advantaged temperate countries. Many of these crops have been neglected even in conventional agricultural research and are often referred to as "orphan crops." A major disincentive to research on hitherto neglected crops is the extensive background work that must be carried out. To encourage "bootstrapping," by both advanced laboratories and laboratories from less developed countries, into research problems on these crops and systems, CAMBIA will provide the key components necessary to initiate and sustain productive projects on these crops.

One area in which the Adaptation and Implementation division will play a prominent role is in the establishment of a transformation facility to develop routine and transferable protocols for the DNA transformation of such crops. Such a facility could be of great service both to national agricultural programs, universities, and research institutes and to the International Agricultural Research Centres. The MGRS, to be described, will also play a key role in facilitating research on orphan crops.

Inadequate Awareness of the Potential for New Approaches

Often a problem is given very low priority for research not because of its low importance, but because of a perception of a low probability of success. This perception is rarely balanced with an awareness of the rapidly expanding possibilities through modern research biology. Another role of scientists within the Adaptation and Implementation division is therefore to help adapt tools developed at CAMBIA and elsewhere to local crops, resource constraints, or situations and to demonstrate their effectiveness in overcoming a few carefully chosen agricultural problems. For instance, CAMBIA, together with Food and Agriculture Organization of the United Nations (FAO), Centro Internacional de Agriculture Tropical (CIAT), International Institute for Tropical Agriculture (IITA), and the Natural Resources Institute of the United Kingdom, is currently working towards a proposal to use state-of-the-art molecular methods to attack the fundamental problem of postharvest deterioration of cassava, a principal food for up to 800 million people worldwide.

In addition to the three research divisions, CAMBIA will have four divisions dedicated to support services. One support division, the Molecular Genetic Resource Service (MGRS), will provide key biological materials to the research community,

The Molecular Genetic Resource Service

Insufficient Access to Genetic Materials and Key Technologies

A major problem confronting researchers trying to establish molecular biology-based research programs is the difficulty in obtaining the necessary genetic materials. The MGRS division will address this problem by providing a service that proactively acquires, assesses, and distributes public domain DNAs perceived to be of benefit to the international agricultural community. It will also provide specific key technical services to laboratories in developing countries, such as synthesis of short DNA primers or protein microsequencing, to help them overcome certain technical bottlenecks that might otherwise substantially impede their research. MGRS will provide these services to less developed countries at subsidized rates and in local currencies.

Absence of Molecular-Genetic Materials for Little-Studied Crops

Additionally the MGRS will assist with the bootstrapping ofresearch on orphan and little-studied crops by developing and providing genomic and cDNA libraries from crops and their associated biota, such as insects. These libraries will be extensively characterized and mapped. Also, the CAMBIA MGRS will develop extensive molecular genetic maps through automated technologies and make these readily available to the International Agricultural Research Centre (IARC), to the National Agricultural Research Service (NARS), and to universities to stimulate breeding programs. Technologies will be developed to stimulate and facilitate DNA-based population studies of pests, pathogens, and other associated biota, including CAMBIA BIT, the Biodiversity Indexing Technologies group.

A second CAMBIA support division, the Infrastructure Development and Support (IDS) division, will seek to facilitate the actual practice of innovative and locally relevant science in countries with constraints imposed by limited resources and infrastructure.

Infrastructure ***Development and Support Division***

Inadequate Infrastructure for Local Innovation and Research

Most tools used for molecular biological research are not developed with the practical constraints that face researchers in developing countries in mind. For example, while it is possible to buy a few enzymes as dry powders, a form in which they can be readily and stably transported prior to subsequent reconstitution, almost all are supplied as the fresh enzyme. This requires costly transport on wet or dry ice within a very short period of time, something that is almost impossible to achieve in tropical countries. Similarly, equipment is rarely designed to withstand conditions of extreme temperature, humidity, dust, or frequent power outages and variations, and a breakdown in a country where it can take at least 6 months before a service can be carried out is disastrous.

The CAMBIA Infrastructure Development and Support division will focus on these logistical and material problems. It will, for instance, help to develop mechanisms to efficiently obtain or locally produce key unstable or expensive reagents such as enzymes, and it will provide advice to international and national agencies. Through its innovative CAMBIA QUERIES service (Quarterly Evaluation of Research Instrumentation, Equipment and Services), it will test, evaluate, and recommend equipment and services found to be particularly robust, cost effective, and functional and will provide advice and services for optimizing laboratory and equipment design and maintenance. Indeed, even in the industrialized world, there is no authoritative unbiased organization that provides this service. As such CAMBIA QUERIES will fill a crucial niche in the marketplace to stimulate responsive and responsible manufacturers and providers of services to the research community worldwide.

As well as suffering from practical difficulties in research, such as a lack of biological materials and physical infrastructure, researchers in such environments frequently suffer from a chronic and demoralizing lack of current information and exclusion from the interactive processes that comprise creative scientific research. This constraint will be addressed by the CAMBIA Communication and Information Services (CIS) division.

Communication and Information Services

Inadequate Information Quantity, Quality, and Timeliness

Even more than in other fields, the amount of information a biological researcher must have at her or his fingertips is increasing precipitiously. This information is scattered throughout scientific journals, books, and manuals, and much of it may not exist in published print at all, residing instead in the informal network that is created by contacts between laboratories. Laboratories in developing countries can rarely afford many journals, and those they do receive arrive months after publication and often years after the information has become available on the informal network.

Professionally critical filtering of the information glut is naturally achieved in the industrial world through this informal network of scientists. These filters are essential, but not available to most scientists in the less advantaged countries. The CAMBIA Communications and Information Services division will develop innovative, critical, and comprehensive interactive communication methods together with the Infrastructure Development and Support division and its partners and collaborators. This division will also focus on acquisition and development of robust and intelligent hardware/software combinations for use in modeling and analysis of field and laboratory data.

Isolated and Ineffective Researchers

It is now becoming appreciated that productivity and effectiveness in research are heavily influenced by the enthusiasm, persistence, and motivation of the scientists and students. This in turn can only come from a combination of local appreciation for one’s work with a satisfying personal integration into, and support from, the wider international science community. Such motivational support is best provided through interactive communication mechanisms that comprise as much listening as speaking. The CIS will develop interactive bidirectional communications that encourage awareness not only of the latest scientific advances, but also a converse awareness and appreciation of the problems being experienced by scientists working under adverse conditions.

Finally, one of the most critical areas for any researcher is the need for access to educational opportunities for the acquisition of new skills or to learn about new scientific developments that could be of importance to them. Inasmuch as the goal of CAMBIA is to develop new tools and to provide them to the research and, ultimately, the farming communities, education in its many forms will be a key focus of CAMBIA and will be the remit of the Education Division.

The Education Division

Educational and Training Constraints

The technical training of scientists throughout the world is highly variable. Different cultures and countries have placed widely differing emphases on components of the scientific paradigm, sometimes to the complete exclusion of others. And yet it is the integration of successful problem identification with flexible approaches to problem-solving that characterizes the best scientific method. The CAMBIA Education Division will be dedicated to ensuring that collaborating students, scientists, educators, and countries obtain not just the tools developed at CAMBIA, but also the skills, flexibility, and understanding required to implement and troubleshoot them. This division will function not only through scheduled training courses at CAMBIA and abroad, but also interactively with the substantial flux of visitors to CAMBIA and through working visits by CAMBIA staff to collaborating laboratories and field stations in the developing world, so that the counterpart scientists can directly communicate the particular challenges and limitations in their own situation to CAMBIA's staff. In addition, education specialists will assist in the formulation of local curricula, for instance in local universities in the less developed countries, that stimulate integrated problem-solving skills and flexible strategic thinking.

Effective Transfer of Practical Skills and Understanding

As well as having a good theoretical background, scientists and technicians must have considerable practical skills and experience to utilize the available tools. A key component underpinning the research and education capabilities of CAMBIA, therefore, will be the CAMBIA Post-Doctoral Program. Internationally recruited and highly skilled post-dots, while based at CAMBIA, will spend variable periods, for instance 6-8 weeks, each year in laboratories with compatible interests in developing countries. These will be young and active bench scientists who will be able to transfer the latest skills and approaches directly to counterpart research staff. The post-dots will also facilitate a true, bidirectional communication of problems, bringing back to CAMBIA a real understanding of the problems and challenges faced by counterpart researchers in developing countries. This will aid in the development of new, creative solutions.

Brain Drain

A frequent problem encountered when young scientists from less developed countries do gain the opportunity to carry out postgraduate study in laboratories in other countries is that they become disconnected from their own culture and from the research needs and priorities of their own countries. Thus, they frequently remain working in advanced institutions overseas, and the goal of enhancing the research capability of their country of origin is not met. CAMBIA will therefore have an innovative postgraduate program, whereby students can register for a degree at CAMBIA, in collaboration with the Australian National University. They will spend up to a few months each year working in their home country and the rest of the time at CAMBIA working on development of methodology of particular relevance to their home country needs. This will also facilitate bidirectional transfer of information and skills and will ensure that the students retain strong links-cultural, personal, linguistic, and scientific-to their original countries and thus are much more likely to benefit their home country through returning and carrying out research there.

CONCLUSIONS

We have made great strides with the model systems approach in science, but this is not yet sufficient to address the complexities of agricultural systems. Thus, new tools that can be wielded by farmers and local agricultural and environmental researchers to assist them in understanding the complexities of their own local agricultural systems are needed. Until recently, there was no organization with the specific remit to develop such tools, and it falls well outside the financial and other constraints of previously existing private and public organizations. CAMBIA will fulfill this role, and by working in partnership at all levels to develop such tools, it will benefit the agricultural community and those who depend upon it throughout the world.

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