MODELS FOR INTEGRATING BIOTECH INTO CROP IMPROVEMENT PROGRAMS

Integrating new technologies into conventional plant-breeding programs warrants particular attention if the potential of these technologies to improve productivity and stability of crops is to be realized.[1] The sizeable investment required to launch biotechnology efforts will result in few practical benefits if they are divorced from the goals and needs of breeders. Although this understanding is widely recognized, little guidance exists for amalgamating the two approaches, with most examples arising from commercial research.[2] To be successful, biotechnology needs to be integrated at the research and organizational level—nationally and internationally.

Integration at the Research Level

Biotechnologies are being used to produce, select, or detect plant varieties not yet isolated by conventional breeding methods. Even so, the germplasm derived in the research laboratory and tested in the greenhouse still needs significant testing at the whole-plant level—plant breeding skills are necessary whether new variants arise from cellular, molecular, or sexual manipulations.[1]

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Programs that integrate research with the rapid agronomic testing of *in vitro*-derived materials usually consist of three phases (Figure 1). The first phase, setting project objectives, is a critical step and depends upon the working relationship between breeder and biotechnologist. To fulfill these objectives, decisions must be made on selecting germplasm, developing *in vitro* technologies, obtaining regenerated plants, increasing seed or vegetative materials, and finally, determining effective selection and testing procedures. Confusion occurs when objectives do not include provision for inserting *in vitro*-derived materials into testing and/or breeding programs.

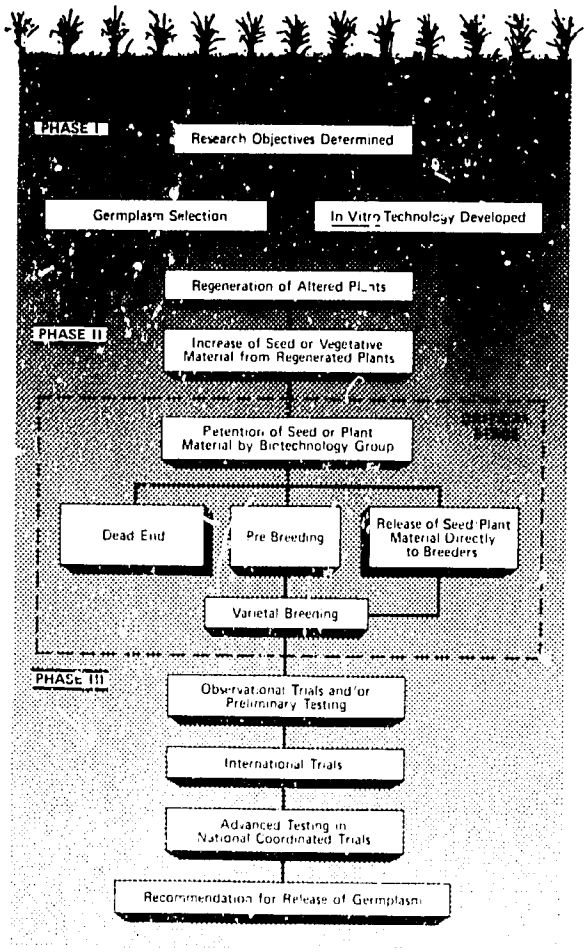

Figure 1. A model for integrating research between biotechnologists and crop improvement scientists. Integration is required to field-test *in vitro*-derived plants.

The second phase is the most critical. Here, decisions must be made as to how the seed or plant material produced by regenerated plants can be most effectively distributed, improved, and tested. Seed or materials ready for distribution face three possible fates: they may be evaluated and tested by the biotechnology department itself; they may be distributed to breeders for evaluation; or they may end up in a "dead end" if close working relationships between breeders and biotechnologists have not been fostered.

Pre-breeding can produce lines that stably express novel traits generated by biotechnological techniques. Breeders can select material for 1–3 generations after regeneration from an *in vitro*-derived source. Lines that exhibit both acceptable agronomic fitness and stable expression of selected traits are advanced to conventional plant breeders for varietal development (Figure 1). Pre-breeding assists both breeders and biotechnologists and functions similarly to its role in genetic resource programs, through which breeders accomplish germplasm enhancement and wide hybridization[5].

The third and final phase includes steps required for evaluating and advancing the new germplasm. Initially, special field tests may be needed to screen for *in vitro*-derived traits. Beyond these initial screening tests, coordinated national and international testing programs are the same for conventionally and *in vitro*-derived germplasm. Testing that combines these two sources of germplasm requires that managers and breeders recognize new sources of genetic variation as well as heed recommendations from biotechnologists. This arrangement ensures that *in vitro*-derived lines enter field tests, quarantine grow-outs, disease nurseries, germplasm exchange, or international trials in a timely way.

Working Relationships

Restrictions on testing *in vitro*-derived lines become obvious when field arrangements are finalized. Conventional breeding programs cannot easily accommodate unexpected development material without at least a season's notice. Testing pipelines are committed to lines generated the previous season and superior lines cannot be held back from advancement (Figure 1). Mutually understood responsibilities for advancing and improving lines, as well as recommendations for releasing germplasm, can alleviate these constraints while ensuring the stable expression of traits in agronomically useful germplasm. Working relationships between biotechnologists and plant breeders fall into three broad categories: collaborative, opportunistic, and independent.

1. Collaborative interaction. In this model, which provides the framework for interdisciplinary scientific teams, conventional plant breeders are involved at the project's inception. A research agenda is charted with mutually defined objectives. The program necessitates the breeder's involvement in all three phases, while the biotechnologist may only be involved in Phase I and part of Phase II. Responsibility for breeding, evaluation, and recommendation for release, as well as provision of data to biotechnologists, belongs to the breeder.

2. Opportunistic interaction. This model includes programs in which initial collaboration is absent and direct relationships between plant breeders and biotechnologists do not exist. Many conventional plant breeders have adopted a "wait and see" attitude concerning biotech-derived material. This perspective is based on time and resource constraints and the need for genetically stable germplasm. Breeders request seed from biotechnology units based upon observations taken from screening trials or special tests. Breeders are then involved, independent of the biotechnology unit, half-way through Phase II and in all of Phase III. Biotechnology units, of course, can simultaneously develop and test lines from the same material.

3. Independent development. This model encompasses biotechnology programs that develop material through Phases I and II independently of plant breeders. Even in the final phase, the biotechnology staff makes recommendations for line advancement and varietal release without consulting with breeders. This operational style has the greatest propensity for generating confusion and conflict, especially with regard to responsibilities for varietal release. Also, independent operations necessitate biotechnologists stepping into applied plant breeding and field evaluation, areas in which they have little or no experience.

Organizational Integration

Organizational models must be taken into account if biotechnology is to have a positive impact on crop improvement. Successful cultivar development combining *in vitro* and conventional breeding technologies is achieved by expanding research from either initial focus—biotechnology or plant breeding (Table 1). Organizational integration coordinates this expansion at the level of the individual scientist, department, or organization.

Optimally efficient research integration is determined, in part, by the organizational setting involved and the technologies employed. If re-

search and product development capabilities are not contained within the same organization, they must be established through licensing arrangements, collaborative research, or research networks. In fact, this sort of integration has been encouraged through increased support for biotechnology by developmental agencies, private firms, and the Rockefeller Foundation [6-8].

Biotechnology research at universities, international agricultural research centers, and in the private sector, tends to be organized in units or institutes (scientific/industrial parks), reflecting the variety of goals and technologies employed[9]. In contrast, plant breeding is seldom set apart as a special unit or institute. Most plant breeders are members of agronomy or horticulture departments, and many work in commodity-oriented private companies. When plant breeders form close associations with growers, scientists of other disciplines, and with those producing and marketing varieties, it assures that product development proceeds in a timely manner.

Integrating Biotech Applications—International Efforts

Research administrators are keenly interested in identifying realistic applications of biotechnology that enhance crop improvement. This is especially true for developing nations, which are interested in developing or transferring those technologies that allow them to keep pace. Optimal integration of these applications is achieved through multi-disciplinary teams that determine objectives directly supporting and in agreement with the goals of conventional plant breeders[10]. Collaborative research necessitates dedicated support from breeders for evaluation and testing. Biotechnology research efforts are ready for integration when routine regeneration of *in vitro*-derived plants is attained.

There are eight new technologies relevant to conventional crop improvement programs. Four of these—clonal propagation, *in vitro* conservation, pathogen-free plant production, and diagnostics—produce or screen clonal material derived through micropropagation. These technologies are most often used with finished lines developed by breeders or materials collected for storage in gene banks. Genotypes are subsequently tested, either at the cell or whole plant level, for genetic integrity, indexed for presence of disease, processed through quarantine, and distributed as germplasm to plant breeding programs.

Germplasm derived from these technologies is integrated with research programs in Phase I (see Figures 1 and 2). The collaborative model, when used here, develops interdisciplinary scientific teams that coordinate research involving production of variants, pathogen diagnosis, and germplasm distribution. Organizational expansion to develop these technologies begins with initiatives based in biotechnology units or genetic resource programs (Table 1). Further expansion, to include breeding programs, is not warranted.

1. Rapid clonal propagation. In vitro clonal propagation can multiply plant material much faster than traditional vegetative propagation[11]. Successfully combining explant sources, media, and culture conditions has enabled micropropagation of a number of plant species, particularly root and tuber crops. Micropropagation seems to be especially successful for tropical crops such as banana, plantain, cassava, potato, sweet potato, yam, and oil palm. It also shows promise in fruit crops, medicinal plants, ornamentals, and forest trees.

Major advantages of *in vitro* systems—regardless of whether plants arise from meristem/shoot-tip culture, somatic embryogenesis, or organogenesis—are: rapid and reproducible multiplication rates; pathogen-free conditions; the ability to propagate species that are difficult to propagate vegetatively; and, production on a year round basis[12].

2. In vitro conservation. The potential to conserve crop genetic resources with *in vitro* technologies has added a new dimension to germplasm conservation. New tissue culture methods have provided innovative ways to conserve clonally propagated materials and recalcitrant seeds[13]. *In vitro* conservation, which began with shoot cultures, now embraces shoot-tip, cell, callus, and embryo cultures[14]. These culture techniques have been used extensively with cassava, sweet potato, potato, and banana at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, the Centro Internacional de la Papa (CIP) in Lima, Peru, and the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria[15,16].

Tissue culture is now part of many germplasm conservation programs. India's National Bureau for Plant Genetic Resources, for example, has established a National Facility for Plant Tissue Culture Repository which is responsible for research on *in vitro* conservation including cryopreservation of seeds, anthers, pollen grains, calli, and organs[17].

While *in vitro* technologies offer new avenues in preserving crop germplasm that cannot be stored as seed, it is not yet possible to ensure the genetic stability of cultures. Genetic variability generated during tis-

TABLE 1. Organizational options for combining biotechnology with conventional crop improvement programs

	I. Initiatives beginning with conventional plant breeding		
	INDIVIDUAL SCIENTIST	DEPARTMENT	SEED COMPANY OR NATIONAL PROGRAM FOR VARIETAL RELEASE
Description:	Plant breeder expands own research to include biotechnology or obtains external support	Plant breeding department adds personnel to develop task-oriented biotechnology expertise	Institution with plant breeding department in place adds autonomous biotechnology unit
Setting:	Universities, national agricultural research systems	Private sector, universities, international agricultural centers, national agricultural research systems	Private sector, universities, international and national agricultural research centers
	II. Initiatives beginning with biotechnology		
	INDIVIDUAL SCIENTIST	UNIT CORPORATION	AUTONOMOUS INSTITUTE
Description:	Biotechnology research complements or seeks out independent breeding program	Biotechnology research effort acquires varietal breeding and development expertise	Autonomous biotechnology center established without formal ties to plant breeders
Setting:	Universities, international agricultural research centers, Rockefeller Foundation	Venture capital companies, agrochemical companies, university biotechnology units	National and university institutes, UNIDO center, TCCP

sue culture, referred to as somaclonal variation, is ubiquitous[18]. It must be evaluated on a crop-by-crop basis and balanced against other sources of instability in the field. Field gene banks and *in vitro* conservation will each assume roles in complementary conservation strategies. Restriction fragment length polymorphism may become useful in determining genetic stability; scientists at CIP are testing this on micropropagated potatoes[19].

3. Pathogen-free plant production. Virus-free tissue derived from meristem culture has been obtained from over 50 species and used to produce virus-free plants[20]. Combining methods such as chemotherapy, thermotherapy, and antibiotic treatment can effectively eliminate viral pathogens during growth in culture[21]. Pathogen-free tissue culture technologies facilitate germplasm exchange, have the potential to upgrade quarantine services, and are increasingly used to distribute disease-indexed plants to plant breeders worldwide[22]. National quarantine programs must keep pace with *in vitro* technologies by providing for the expeditious examination, certification, and release of imported *in vitro*-derived clonal materials.

4. Molecular diagnostics. While containing plant material *in vitro* is a barrier against infection, it is important to verify that such cultures are pathogen-free. Rapid diagnosis of pathogens is an important, immediate application of biotechnology. Immunoassays or nucleic acid hybridization present significant advances in time and sensitivity over both serological and traditional virus detection methods[23]. Such advances offer powerful new tools for quarantine and germplasm exchange programs (Figure 2). Both technologies are already being used to detect viruses such as tungro, grassy stunt, and ragged stunt on rice in Asia and barley yellow dwarf on small grains in Africa and Latin America[24]. A nucleic acid spot hybridization kit can detect potato spindle tuber viroid in the field. Regional scientists collect samples, place them on nitrocellulose membranes, and return them to CIP for hybridization and autoradiography[25].

The other relevant technologies—embryo rescue, somaclonal variation, anther culture, and non-sexual gene transfer—produce or facilitate production of *in-vitro*-derived germplasm. This germplasm must be evaluated within conventional breeding programs and represents an output of Phase I (Figure 1), as opposed to being an input derived from the former technologies. This difference affects the working models adopted between breeders and biotechnologists. Variation produced through biotechnology, when in concurrence with breeding objectives, will more likely be accepted for selection. The biotechnologist has the challenge of providing effective *in vitro* selection schemes to the breeders. This can be established early using the collaborative model, or left to the judgement of the breeder as in the opportunistic model. Setting objectives should clarify responsibilities as to evaluation and development of variation. The optimal model, both at the research and organizational levels, is the one that allows for rapid examination of and cultivar development from this variation.

Expanding research to include these technologies can begin with conventional breeding programs or with biotechnology research (Table 1). Organizational integration in private companies has placed *in vitro* objectives within biotechnology units and utilized breeders for finalizing products. This is a costly option, but it is often the most rapid. Organizations lacking this coordinated approach will require extensive outside arrangements to develop or screen *in vitro*-derived variation.

5. Embryo rescue. Wide hybridization has been a long-standing interest of breeders, who look beyond the primary gene pool to improve crops[26]. Following sexually-induced hybridization, tissue culture techniques nurture the otherwise abortion-prone embryo through cell divisions until it eventually regenerates an F1 plant. Renewed interest in interspecific and intergeneric hybridization, coupled with new hormonal and pollination techniques developed for *in vitro* systems, have accelerated the uses of embryo rescue in crop improvement.

The cytogenetics unit in the legume program at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is using ovule and embryo rescue, together with hormone treatment during pollination and ploidy manipulations, to overcome fertility barriers in crossing peanut with over a dozen of its wild relatives. A number of interspecific derivatives showing resistance to late leaf spot and/or rust have been included in the All India Coordinated Research Project on Oilseeds[27]. And scientists at the International Rice Research Institute (IRRI) have used embryo rescue in conjunction with wide hybridization between domesticated rice (*Oryza sativa*) and wild rice, (*O. officinalis* and *O. australiensis*). Using these wide crosses, the scientists have transferred broad-spectrum resistance to brown planthopper, the vector for grassy stunt virus in tropical Asia, to domesticated rice[28].

6. Somaclonal variation. The increased variability observed in plants regenerated from tissue culture has spawned interest in exploiting somaclonal variation for crop improvement. Novel traits from somaclones of potential value to breeders are being explored in tropical and temperate crops. Somaclonal variation is most effective with cereals and may prove especially useful for perennial crops that must be grown out for many years before they reproduce. Scientists are screening somaclones for variants tolerant to salt and other environmental stresses[29]. However, significant variation exists within gene pools available to most breeders, so generating variation in the laboratory alone is only a partial accomplishment. The stability and ultimate value of *in vitro*-induced variation under field conditions needs to be confirmed.

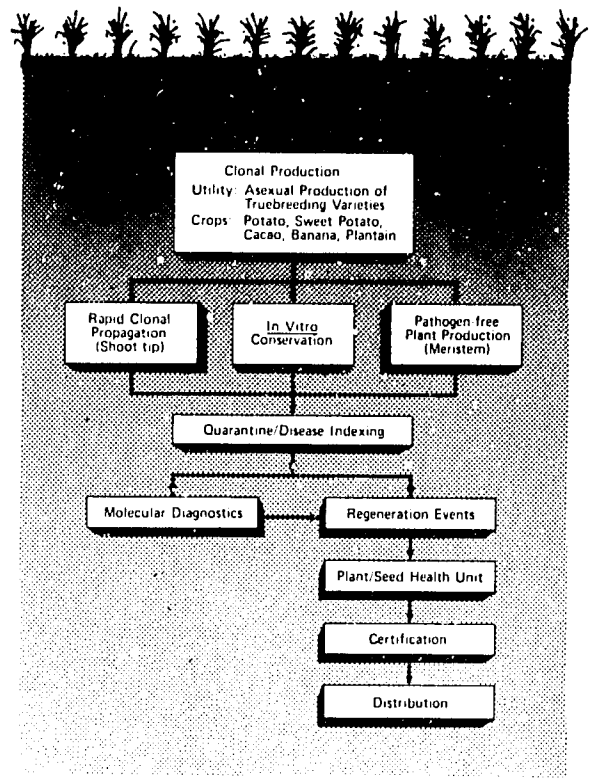

Figure 2. Distribution of clonally propagated germplasm produced by *in vitro* technologies.

7. Anther culture. Culturing pollen mother cells, once they are isolated from anthers, produces haploid plants that can be screened rapidly for desirable traits. Anther culture has the potential to compress breeding cycles, increase selection efficiency, provide for early expression of recessive genes, and expose gameto-

clonal variants. Currently, about 19 months elapse between initial anther culture and the first replicated yield trials. IRRI has established specific programs using anther culture to support conventional plant improvement programs. The most important of these are: production of homozygous lines from F1 crosses for salinity, cold and drought tolerance, and blast resistance; improvement of callus production and plant regeneration efficiencies; and, performance of observational and yield trials in regenerates from selected crosses[30].

8. Non-traditional methods of gene transfer. Cellular and molecular approaches identifying, accessing, and transferring useful genes include protoplast fusion, gene transfer through the use of bacterial or viral vectors, and direct gene transfer through microinjection or electroporation[31]. Although gene transfer has been successful in model systems, problems remain with each step necessary for gene manipulations in crop plants. Identifying important genes is hindered by limited knowledge of plant genomes, transferring genes is limited by vectors and methods that are often plant-specific, and regeneration of the altered plant cell to an entire plant has been difficult to achieve in a number of crops.

Recent advances combining gene transfer, strong promotors, and protoplast technologies, however, have provided encouraging results in both monocots and dicots[32]. As transformation efficiency increases, breeders and biotechnologists will rely on gene bank accessions as sources for genes to be isolated, cloned, and transferred. This enhanced demand for germplasm will require greater collaboration between biotechnologists and genetic resource components of crop improvement, including evaluation[33].

Biotechnology can facilitate plant breeding by identifying useful genes for nonsexual transfer, providing better understanding of genetic and physiological processes in plants, improving the ability to diagnose plant diseases, improving the nutritional quality of crops, and providing new methods for identifying resistance to such stresses as salinity, pests, diseases, and herbicides[34]. Purposeful integration of biotechnology with conventional crop improvement programs, as opposed to this integration occuring on its own—or not occuring at all—increases opportunities to maximize the efficiency of funding, personnel, and facilities while accelerating the delivery of cultivars to the end-user—the farmer.

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