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The Role of Tissue Culture and Novel Genetic Technologies in Crop Improvement

Proceedings of the Third Conference of the International Plant Biotechnology Network (IPBNet)

Nairobi, Kenya
January 8-12, 1989

Sponsored by the
Tissue Culture for Crops Project
Colorado State University
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(Editors)

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WELCOME ADDRESS

Steven Sinding

Thank you very much for that introduction. It is a great pleasure for USAID in general and for the AID Mission in Kenya in particular to welcome you to Nairobi and to have been selected along with KARI and the government of Kenya as the host of this very important conference. On behalf of AID, it gives me great pleasure to welcome all of you: leaders from the government, eminent scientists from around the world, and distinguished guests to the Third International Plant Biotechnology Network Conference.

This is a very important opportunity to share advances in the application of biotechnology to enhance crop productivity. World food production has to double within the next forty to fifty years if we assume a population growth of two percent over that period of time. For countries with even higher population growth rates like Kenya, the pressure to raise food production is even more severe. About three fourths of the necessary increase must come from intensified production on existing land and the rest into bringing new and marginal lands into production. Both paths will require new and appropriate technology. Biotechnology can contribute significantly to these requirements.

Specifically in Africa, major restraints to rapid increases in food production such as drought, salinity, diseases, and insect pests can potentially be overcome with the use of genetic engineering in adapting species to virtually all climatic and growing conditions. In addition, crop species could be developed that would produce greater yields that would have improved nitrogen fixation properties and that would be appropriate for smaller plots. Despite remarkable achievements to date and the tremendous potential for new technologies, scientists and development administrators have tremendous challenges ahead. I am certain that there will be lively discussions during this week about the appropriate mechanisms for the development and/or the transfer of these technologies into the developing world. Unlike the Green Revolution which emerged from international research centers and US land grant colleges, the Bio-Revolution is likely to be based largely in private industry and a few universities. To a layman, an obvious issue is how the new technology, which is being developed largely in European, Japanese, and American corporations and universities, will be transferred to the developing world. We will be very interested in hearing your suggestions as the role donor agencies like ourselves can play in this process.

As USAID's funding for this conference suggests, we as an agency are committed to supporting appropriate mechanisms for facilitating the application for biotechnology to advances in agricultural activity. Bilateral and multilateral efforts in this field have received significant levels of US support. In addition, our support to the International Agricultural Research Centers is firmly rooted in the belief that international efforts in commodity specific programs, using the latest technologies, has generated excellent returns in the past and will continue to do so in the future. In Kenya, USAID, as Bill Wapakala mentioned, is proudly providing substantial support to

the Kenyan Agricultural Research Institute (KARI) and the university development within Kenya. We are particularly interested in supporting closer linkages between KARI, the universities and the private research and development institutes within Kenya which will accelerate the development of new technologies. As with the case with the Green Revolution, we anticipate that those developing countries which have adequately-funded and well-managed research institutions will be the most successful in adapting biotechnology to their own agricultural requirements.

In closing, I want to extend my best wishes for a most successful conference and express our appreciation to KARI, the participating universities, ICIPE, and the government of Kenya for their excellent assistance in hosting this event. Let me say that we, at our office, stand ready to provide whatever assistance might be required of a logistical or administrative sort to solve any problems you might have.

I would like to introduce our final welcomer this evening, Dr. Murray Nabors, who is the Project Director of the Tissue Culture for Crops Project that has been funded by the Bureau of Science and Technology in AID/Washington since 1980. Dr. Nabors is a professor of botany at Colorado State University and I am sure all of you will see a lot of him in the course of the next five days. Thank you.

KEYNOTE ADDRESS

Murray W. Nabors

I would like to add my welcome to the welcomes that you have already been given to this Third IPBNet Conference on the application of plant biotechnology to agriculture. It is nice to see some familiar faces that have been associated with the Project for a number of years and were at the conferences we had in Fort Collins and Bangkok. It is also nice to see a lot of new faces here for the first time.

I would like to mention that one of the primary goals of this conference is to encourage interactions of the participants and, during the time we are here, to develop a sense of community among you. That is the reason for a couple of interesting things about this conference. One of them is that it is a very small conference; there are fewer than one hundred participants. Another is there are no concurrent sessions, so you do not have the joy of running from place to place and getting your aerobic exercise while attending the conference. We tried to put all of you on high floors of the hotel so you could run up and down the stairs, and I encourage you to do that whenever possible. The third characteristic of the conference is that there is a lot of free time. This is a delightful hotel and a delightful city so I encourage you to use that time to talk to each other and to develop official and unofficial collaborations between yourselves. Finally we have (and I will tell you some more about this later), built into this conference a number of round table discussions and on Wednesday morning we will have a very important session of audience participation relating to this.

I would like to thank William Wapakala and Steven Sinding for being here, taking their time to give us a greeting, and helping us with this conference. I would also like to especially thank Jim Gingerich, who is here in the background, and Cecil McFarland of the AID office staff for their consistent help over the past year and a half. I do not think this conference could have been put on without their help and we really appreciate it a lot.

The keynote speaker for this conference turned out to be me. It was originally going to be Dr. Don Plucknett of the CGAIR Secretariat in Washington, D.C., but he became ill and canceled all of his appointments in January. So I'm the replacement and you get to listen to me tonight. I would also like to thank some organizations that have supported speakers at the conference this year. They are Pioneer Hybrid, Pioneer Overseas, McCormick, Monsanto, Twyford Laboratories, CIP (International Potato Center) and ICRISAT.

I would like to talk a little bit about the realities of our world in terms of problems, and a little bit more about some of the possible solutions, because it is in the solutions to these problems that you come into play. Most of all, what I would like to do is stimulate your thinking about the solutions.

Among the realities of the world there are a lot of problems. Some of those problems relate to the physical make-up of things: problems related to human health, natural disasters, environmental concerns, and what not. We could make a long list. Other problems relate to the human nature of things: wars, over-population, child abuse, etc. I am going to focus on the limitation in food supplies, of which you are all familiar. This is a problem that has both physical and human constraints related to it. We know for sure that there is not enough food production in many areas. We also know that there are distribution problems even if there is enough food. The recent earthquake disaster in Armenia has brought to the forefront the fact that, even if there is relief available, it is sometimes impossible to get it all there in good time. There are also the political problems. As a result of these three sorts of things and others I have not mentioned, the sad fact is that 50% of the children in the world die before age one, and 50% of the remainder die by age 17, mostly of malnutrition and disease related to malnutrition. Those people who say that Darwinian natural selection does not apply to human populations are simply wrong; it does, these days.

Another fact is that food production problems are often related to environmental constraints. For example, about 25% of world agricultural land used now suffers from excess salts, chiefly sodium chloride. Another 25% suffers from excess acidity. Forty to sixty percent suffers from drought at some time during the growing season. One third or more of agricultural land has yields reduced due to disease. These are existing constraints that we have to deal with. Attempting to solve the problem, agriculturalists, research workers, economists, and others find themselves between a rock and a hard place. The rock is that there are many problems, the hard place is that resources are limited in terms of solving those problems. Another way to put that is that there are too many problems and too few solutions. A third way of looking at it is to say that each one of these problems limits agricultural production. Food production in our world is a black hole. It readily saps-up and utilizes any and all resources that are devoted to it, and still the problem is not completely solved. So the goal is to allocate resources in such a way as to maximize the human potential for solving problems in our world. In terms of food production, this means answering the question, "How can we best use the limited resources available to us to increase food production?"

What I am going to do tonight is suggest to you some solutions to the problems of increasing food production. These are going to be solutions with what I call "wrinkles." "Wrinkles," you know, are things that have to be ironed out before everything works perfectly. I have two young boys, and when they fight, which on some days is 100% of the time, I send them up to their beds for five minutes, and suggest that they can work the wrinkles out of this problem on how to get along together. I don't think I can send you up to your bed, although you are certainly welcome to do that if you want to, but I can suggest to you that, during the week here, you might have some time to think about <u>your</u> ideas from the perspective of being an active worker in this field, about the solutions that I have suggested, the wrinkles that are in them, and about other solutions that you could suggest as well. That is the kind of information that we will be interested in accumulating from you.

The best solution that I could suggest in increasing food production is an obvious one: develop appropriate new technologies which maximize the impact of the resources available to solve the problem. In this setting, I am

talking about plant biotechnology. Plant biotechnology has been around for a while, and in full-blown form for about five years; it has a pretty good head of steam. Initially, it was way over-promoted. It was going to solve all of the world's problems, certainly by next summer. This obviously didn't happen. Really, plant biotechnology is another tool for the plant breeder and agriculturalist to add to their arsenal. By now we are beginning to see some of the first successes of plant biotechnology. As we see the first successes, we also start to see some of the kinks that need to be worked out and some of the problems in the technology that need to be dealt with. So as I said in discussing these potential solutions to the problem of increasing food production, I am only going to suggest that the solutions might be in some of the problems associated with them, some of the so-called "wrinkles."

In developing any new technology, there is the question of basic research. Funding is extremely important for basic research. As many of you know, you spend probably more time searching around for funds in your research project than you do using them. Most of us are trained in scientific disciplines and probably we ought to have been trained as fundraisers. That certainly is, I think, my primary job. So that is a problem that needs to be looked at. The flow of information from basic to applied needs to be looked at. There is a tendency for basic researchers to want to stay in the lab and work on a problem without really thinking too much about possible applications down the road. I suggest to you that in a world of limited resources that is not a viable possibility.

Another problem with basic research is the fear that some people have of using the very newest technologies. For example, among tissue culture workers I find now-a-days a commonly held fear about getting into molecular biology, which is the latest kind of thing. "Oh gee, I don't know about this. I might have to go back and learn a few things." That kind of thing. We need to talk about that. We also need to talk about using collaboration because when resources are scarce, collaboration becomes very important. Also related to new technologies is the problem of the basic/applied interface. Historically in science this is a no-man's-land; nobody wants to deal with it. I remember very well when we were trying in the early 1970's to get our initial tissue culture project funded to look for salt tolerance, we were told by the practical agency, which was USDA at that point, that only the basic agency would fund that. Then when we went to the basic agency, which was at that point NSF, we were told, "Oh, the practical agency will fund that." This kind of thing still goes on to a degree. I will suggest to you, briefly, that solving the problem of creating a linkage or an interface between basic and applied sciences requires not only funding but also vision and collaboration.

Tissue culture has been doing this kind of thing for awhile; forging a link between the basic and applied sciences. And so tissue culture is now moving into a phase where we can really start to evaluate what works, what doesn't work, and how we can improve making that connection--getting the basic results out into the field so they can affect food production. Molecular biology is just starting this process of trying to move from a basic science with a lot of data into an applied science working on crop plants and on problems associated with crop plants. In molecular biology now there is extreme competition for funds. And while I think competition for funds is a healthy sort of thing, I think in molecular biology it is getting to the point where the competition is so intense that it is difficult for a lot of researchers in the area to get any research done.

On the issue of funding for new technologies, there are three things that I would say: 1) funding needs to be increased (remember I am not trying to provide any solutions, only outlining that they might exist), 2) funding needs to be streamlined so it doesn't take three to five years, in some cases, to get money from appropriations down to the researcher who is using it, and 3) funding needs not to be wasted. I think a tremendous amount of money is wasted in both basic and applied research to develop new technologies, partly because of duplication, partly because of lack of collaboration, and partly because of a lack of vision in planning in terms of research projects. I might just mention, for example, that there was a private company in the United States which, over a four-year period, put 85 million dollars down the drain in terms of biotechnology and had absolutely nothing to show for it. I suggest to you that if we divided 85 million dollars among the participants in this room and we played our cards right we might have a tremendous amount to show for it at the end of three or four years--at least I would hope so.

In terms of research problems in developing new technologies, we need to be very careful that we define those problems for a specific project and for a specific projected solution. There is a tendency for research proposals to look like a general plan for alleviating all of the ills of humankind. I think proposals and proposed projects need to have a projected solution, a strategy for obtaining that solution that includes a time table, activities, results, and means of verifying those results. I would also like to suggest that, in terms of developing new technologies, something like molecular biology needs to be planned in the laboratory or in national programs well in advance. We need to allow two or three years to make sure they have trained people, and money on hand to purchase the equipment, and to identify the problems, and probable and possible means of solving those problems.

The second general sort of solution that I would like to suggest to you, in terms of developing new technologies, is to encourage cooperation and networking to avoid duplication of effort and to facilitate information flow. I think the personal contact between researchers is just about as important as the research they are doing. This personal contact can be of two sorts. It can be horizontal; mainly contact between two researchers working on the same plant and the same problem. We have discovered in IPBNet that a lot of times there may be people in three or four different countries working on exactly the same problem, in exactly the same crop, and it is very useful to get those people together to chat. The personal contact can also be vertical. Two examples of that are 1) contact between basic workers and applied workers working on different ends of the same problem, and 2) contact between people in universities, people in government, and people in industries working on the same problem but from different sorts of approaches.

In terms of encouraging cooperation in networking, I think the computer-networks-information exchange, networks-fax, networks-whatever you want are extremely important. These are being developed now by FAO and by other agencies. I think information on these networks is extremely valuable to scientists and they are very, very important, if not more important, than the research being done. Also, I might add as an after-thought, we need to avoid what I would call "dueling networks." There is some of that going on in the world as well. I think that exchange of personnel, particularly on a short-term basis of six months or a year, is very useful between laboratories working on similar or identical projects. I think it is important to develop good working relationships with people in your field and I think that

conferences like this are one way of promoting that kind of thing. In terms of improving cooperation in networking, it is very vital to consider developing multi-national research teams to solve specific problems. I mention, as an example, Roger Beachy's work in the Cassava Network. Roger is here and hopefully he will say something about that. This is exactly the kind of thing we need to do to solve the problems and utilize the resources effectively.

We also need to consider how the small laboratories in the world utilize their expertise and their abilities as well as the resources of the bigger labs. To turn that around, we need to consider how the big labs can utilize the expertise and the knowledge of the small labs in many kinds of collaborative enterprises.

As a third possible solution to the problem of increasing food supply, I would like to suggest that we need to explore in detail the basic and applied interface. For example, I think a good deal of the germplasm produced in tissue culture experiments is simply wasted because its not brought into the field. We at the TCCP have learned that there are many valuable characteristics in this germplasm, and it really should not be wasted. Ronny Duncan at the University of Georgia and some of the people working with him have shown that our regenerated sorghum plants contain many kinds of useful mutations, some of them that we weren't even looking for (so-called somaclonal variants). Dr. Thavorn Vajrabhaya has a good track record in Thailand of getting his material out in the field, testing it, and seeing of what use it is for the plant breeder. This is an important reason why the basic/applied interface needs to be stressed, so that material that basic researchers are producing will be correctly utilized. I think it is quite okay, by the way, that some basic researchers are not particularly interested in the field studies; that's why collaborations exist. You do what you are interested in, and I'll do what I'm interested in.

In terms of bridging the basic/applied interface, we need to be training students in all three important areas relating to food production: 1) breeding, agronomy, and soil science; 2) cell biology, physiology, and tissue culture; and 3) molecular biology. We need students coming out of training programs, master's programs, and Ph.D. programs to be knowledgeable in all three areas at the same time. This would really help maximize the resources that we have available. We also need to focus more, I think, on establishing cooperative networks between these same three types of researchers. I eluded to this before and I probably will again because I think it is very important.

As fourth kind of solution to the general problem of increasing food production, I would like to suggest that training is very important, and particularly training of people to use the emerging technologies. The training needs to involve lots of different kinds of people: administrators—who I once thought were totally untrainable until I became one--students, scientists, and technicians. All of these people need to learn about new technologies and be able to apply them to their planning, scientific research, or the training that they themselves do. We also need to have training that varies in terms of the length of time. There is a crying need in many countries to have more people trained at the Ph.D. and master's level who have spent a lot of time investigating existing and new technologies and can apply them across the board. There is also a need for short-term training; workshops, conferences, or perhaps a visiting scientist who comes to learn

just one technique to apply to one problem. I also think that the training needs to be regional in nature. This is simply and plainly a way of saving money in terms of transportation and a way of making training available on a home-language basis. Dr. Victor Villalobos teaches a tissue culture training course in Spanish at CATIE. Dr. Mohammed Aaouine, IAV Hassan II, Morocco, is in the process of setting-up a similar training course to be taught in French. I think that these are the kinds of things that we really need to encourage.

A fifth way of implementing progress, of making sure that we have some solutions available that we are using to solve the problem of increasing food and food production, is to support national and international centers and programs. The International and regional centers are already well-organized and set-up. A number of national programs exist and more are forming all the time specifically relating, among other things, to how to apply biotechnology to solving particular problems. I think that these national and international centers need to be removed from politics and many of them are not. I think that they need to keep qualified people for a fairly long period of time to eliminate turnover. I think they need to facilitate matters, like attending conferences, getting visas and travel funds, and all that for individual scientists. These organizations, whether national or international, need to develop priorities; resources are scarce, no national program can solve all existing problems. We are involved in a kind of agricultural triage where you can decide what problems can be solved with existing resources, which can be solved in a short time and which will require a long-term investment of time and money. These organizations also need to put a priority on implementation. Resources need to be directed, I think, to local problems and this is where forming networks--consulting and cooperating with other people worldwide—can sometimes pool a lot of useful information. We find, for example, that there is quite a lot of useful information that can be transferred from the tissue culture of one cereal to the tissue culture of another, or even to the tissue culture of a legume. Planning and the implementing needs to be long-term so laboratories and the people manning them can be acquired and set-up well in advance of when the work needs to start. This is particularly important in molecular biology. It takes time to develop a cadre of trained people and to develop facilities to do molecular biological research.

Finally, in terms of the source of solutions that I think will contribute to solving the problem of increasing food production, I would like to suggest that we need to put more effort on answering these questions: "How is it working? Is what we are doing correct? How can we change what we are doing?" We need to get grass root views of the problems and the solutions, of how the implementation is going and how even little things could be improved to solve problems. I suggest that external reviews such as the one that the CGAIR centers have now are an important component and should be a part of all national and research programs. These results need to be published and made available so others can profit from their successes and also from their mistakes. This is where you play an important role. You are the grass roots. Most of you are scientists or administrators working with the problem of emerging technology, biotechnology, and plant biotechnology and trying to apply that to specific problems. I think you can help not only to find the problems but the solutions. So what is needed is your data, your ideas on the problems to be solved, your ideas on the "wrinkles" in terms of the solutions, and your feedback on how things are going, both in your work and in what you perceive in other sorts of programs and experimental entities.

The results of the round tables and the open discussions that we will have on Wednesday will be published in the proceedings and made available to AID, national programs, international programs, and also to individual scientists and policy-makers. I would really like to encourage you to think about the problems of increasing food production, the general solutions that I have suggested, and your own solutions, and then think about a lot of specifics. I think the best kind of specifics that could come from you would be, "How can I make my life easier? How can I accomplish more given the resources that I have? What could be done to facilitate the flow of more resources toward me?"

I think I tried to outline to you a little bit about the problem of increasing food production and my ideas of what the general types of solutions are. As I told you I would, I left lots of wrinkles. If I thought I could suggest how to solve all of those problems, I would have written a book long ago and become famous, rich, and all those kinds of things. I am glad that you are here, and I hope that you have a very enjoyable and profitable week. Thank you.

PLENARY PAPERS

MAIZE BREEDING FOR STRESS TOLERANCE

Stanley Barrance McCarter

ABSTRACT

The major constraints to maize production in Africa are defined in terms of inadequate water, poor fertility, and insect and disease damage. The success of conventional maize breeding procedures in combating these constraints is outlined. It is postulated that biotechnology could greatly accelerate progress in the following areas where conventional breeding for resistance has made only limited progress:

1. Stalk borers: Busseola fusca, Sesamia calamistis, and Chilo partellus.
2. Pests of stored grain: Sitophilus sp., Sitoroga cerealella, and Prostephanus truncatus.
3. Stalk and ear rots: Diplodia maydis, Gibberella zeae and Fusarium moniliforme.

Recent progress in the breeding of maize streak virus-resistant germplasm could be complemented through biotechnology by the incorporation of genes for resistance in susceptible hybrids extensively marketed in Africa.

INTRODUCTION

Maize is the single most important food crop in Africa, but harvested yields, in the region of one ton per hectare, are among the lowest in the world. Per capita food production in sub-Saharan Africa is often quoted as declining at a rate of approximately one percent per annum. There are many reasons for this unfortunate situation, some natural and some man made.

The purpose of this paper is to identify the major environmental and biological stress-inducing and yield-limiting constraints, to indicate the current status of breeding for tolerance to these stresses, and to give an indication where emerging biotechnologies could positively facilitate the breeding process.

YIELD LIMITING CONSTRAINTS

Water Stress

Periodic drought, caused by irregular and often inadequate rainfall, causes significant reductions in maize yields throughout Africa. This yield depletion is often accentuated by excessively high temperatures, and soils of low water-holding capacity and poor physical structure which limits rainfall infiltration. In addition, fear of substantial crop losses from drought also adversely influences the decision to use yield-enhancing inputs like improved seed and fertilizer.

Some of the damaging effects of short periods of water deficit and heat stress may be alleviated by adjusting the planting date, adopting water-conserving production techniques, or selecting cultivars of appropriate maturity. These are effective methods of reducing drought affects but they constitute drought avoidance rather than resistance per se.

A question that torments breeders, in the search for drought resistant genotypes, is whether selection for drought tolerance is more effective under stress or non-stress conditions. Lack of environmental control, high genotype x environmental interactions, and high error variances, experienced under water stress conditions, have generally encouraged breeders to select and initially test genotypes in non-limiting environments. The subsequent testing of relatively elite materials, in advanced trials over a number of locations and years, then attempts to quantify the stability of new cultivars in their target environment. Various forms of regression analyses may be used to assist in this selection process.

Breeders have taken some comfort from studies by Russell (1974) and Duvick (1977) which indicate that US Corn Belt hybrids of the 1970s are genetically superior to hybrids of past decades in their ability to withstand water stress. This improved performance may in part be a result of initial selection under high plant density and to the extensive testing of hybrids for yield stability. More recently Jensen and Cavalieri (1983) and Lafitte and Edmeads (1988) however, report that selection of drought-tolerant strains may be more efficient when practiced under stress and non-stress conditions in parallel. This procedure requires a uniform site and applied water controls to enable valid comparisons to be made. Lafitte and Edmeads (1988) demonstrated that selection for yield, with relatively low heritability under stress, may be facilitated by secondary trait measurements which have higher heritability and which are correlated with yield. Useful secondary traits which are easily measured and applied in practical breeding were identified as:

1. Relative leaf and stem extension rate, under stress and non-stress conditions measured over a one-week interval during rapid stem elongation;
2. Anthesis-silking interval; and
3. Leaf death score, assessed under severe water stress three to four weeks after flowering.

Since grain yield and correlated secondary traits are thought to be multigenically inherited, it should be possible to increase the frequency of these secondary traits leading to improved yield under drought conditions. Simultaneous yield evaluation of materials under both limiting and non-limiting water regimes should ensure that yield potential under near optimal conditions is not prejudiced.

Poor Fertility

Maize yields in most of Africa are also limited by poor fertility. In many countries there is little prospect of manufacturing, or importing and distributing, chemical fertilizer at prices that peasant farmers can afford. This implies the need to develop cultivars that are superior under conditions of limited soil fertility.

Preliminary studies by Lafitte and Edmeads (1988) showed significant genotype x environment interactions indicating that selection in nitrogen stress nurseries may be justified. As with selecting under moisture stress,

they found it to be beneficial to use the following secondary traits, which correlated with grain yield when measured in a nitrogen stress environment:

1. Leaf chlorophyll content, measured in vivo with a portable photometer;
2. Ear leaf area; and
3. Anthesis-silking interval.

Some traits, like the anthesis - silking interval, have been found to be extended by high plant density and by water and nitrogen deficiencies in a similar way. Genotypes selected for one stress type were observed to be more tolerant of other stresses. Russell (1984) and Lafitte and Edmeads (1988) concur that varying the plant density may be effective in creating various stress levels. The use of normal and high density plots, grown in adjacent nursery rows, may for many breeders serve as a potential substitute for specific stress nurseries.

Insects

Although the major insect pests are here identified as stalk borers and storage pests, it is conceded that important losses are also caused by a complex of other pests. The pests discussed below have been selected in recognition of their wide distribution, the economic losses they cause, and the relative lack of progress in selecting for resistance provided by current breeding methodologies.

Stalk Borers. There is a complex of stalk borers in Africa, but three are of particular importance:

1. Busseola fusca, the maize stalk borer, predominates in sub-Saharan Africa at altitudes above 500 m;
2. calamistis, the pink stalk borer, is important only in the coastal areas of East Africa;
3. Chilo partellus, the sorghum borer, is problematic in the low-altitude, sub-tropical areas of East and Southern Africa;

The biology of all three pests are similar, but they differ in geographical distribution, oviposition, migratory habits of young larvae, the over-wintering of pupae, and in generation interval.

Generally, moths emerge from pupae that over-winter in the stubble of the previous season's crop. Eggs are laid in batches and early instar larvae feed on the young leaves or sheath tissue. This feeding results in characteristic 'shot holes' in the leaves as they emerge from the whorl of the plant. Later, instar larvae may bore into stalks which disrupts the vascular system and frequently results in stalk lodging. In the post-flowering stage, larvae may enter the developing ears causing additional losses and creating infection sites for ear rot pathogens.

These borers are generally considered to be the most important pests of maize in Africa. Warui and Kuria (1983) reported average yield losses due to stalk borers in Kenya to be 18%. Control measures include the application of

appropriate cultural technologies or the use of insecticides. To date, little breeding work has focused on the development of resistant genotypes, although some recent work has been conducted by public agencies in Kenya and Nigeria.

Storage Pests. Several maize weevils (Sitophilus spp.), the grain moth (Sitotroga cerealella), and the larger grain borer (Prostephanus truncatus), cause serious grain losses in Africa. The maize weevil and the grain moth are universally important while the grain borer is mainly confined to Tanzania and Kenya.

These insects may infest the grain in the field and continue reproducing in storage until the grain is used. Control of silo temperatures and grain moisture content, good hygiene, and the use of suitable chemicals may virtually eliminate storage losses. In much of Africa, however, maize is stored in cribs, relatively exposed to the environment, providing good conditions for the insects and creating the possibility of substantial losses.

Many workers have reported less weevil damage in strains with longer and more prolific husk leaves. Breeders, however, need to take great care in adopting these as selection criteria as these husk characteristics have also been observed to increase susceptibility to ear rots where environments favor the causal pathogens.

Increasing kernel hardness has also been correlated with decreasing weevil damage. Serratos et al. (1987) report that phenols (principally ferulic and p-coumaric acids) located largely in the kernel pericarp, confer a direct antifeedant action. Furthermore, a good positive correlation was observed between phenolic content and grain hardness indicating that these phenols may affect grain hardness. Unfortunately, in developing hybrids with improved yields, breeders have a general tendency to select genotypes with softer dent kernels. There also would appear to be a general preference in Africa for softer grain for human consumption. Theses preferences should continue to lead to the selection of cultivars relatively susceptible to weevils.

Initial findings by Giga (pers comm) would indicate that the resistance mechanisms are different for each storage pest. The identification of factors conferring resistance to the grain moth and grain borer and their introduction into usable cultivars is a priority.

Diseases

Maize diseases limit yields by between 15-50% in Africa, as compared to a global mean of 9% (Fajemisin, 1986). Although over thirty diseases have been reported on maize in Africa, only those of major importance, wide geographical distribution, and the potential to significantly reduce yields will be discussed.

Maize Streak Virus. Maize streak virus (MSV) is considered one of the most important diseases in sub-Saharan Africa, Mauritius, Madagascar and Reunion, limiting yield from savanna to forest zones and from sea level to over 2000 m in altitude (Bjarnason, 1986).

The virus is transmitted by several leaf hopper species belonging to the genus, Cicadulina. Yield losses due to MSV are proportional to the number of plants infected and the severity of the infection. These losses are usually

greatest in later plantings, second season plantings, and around irrigation schemes, where conditions favor the build-up or carry-over of large hopper populations. Economic losses are not attributable to direct insect feeding, but to the transmission and expression of the virus.

Initial symptoms of MSV infection are white inter-veinal flecks which later develop into chlorotic streaks running parallel to the veins along the entire length of the leaf. As symptoms only appear on new leaf growth, it is possible to estimate the age of the plant when infection occurred. Early infection can cause severe stunting and failure of silk emergence, which may lead to total grain loss. Later infections, near flowering, may only slightly reduce ear size.

Improved cultural practices, for example early planting, the control of secondary hosts, and systemic insecticide spraying, may reduce infection. Such controls are difficult to apply in much of Africa because of lack of draft power for early land preparation and the prohibitive cost of chemicals. The development and distribution of MSV-tolerant cultivars to farmers is considered the only long-term solution.

Collaborative work between IITA and CIMMYT has resulted in the identification of resistance to MSV and its subsequent incorporation into a number of populations (Bjarnason, 1986). Kim et al. (1982) suggested that three genes, additive in nature but with modifiers, may be involved in conferring resistance.

Although a number of resistant varieties have been released through national programs, almost no resistant hybrid seed is presently marketed. Furthermore, the effective screening of segregating germplasm is best facilitated by inoculating young maize plants with viruliferous Cicadulina. This procedure necessitates the mass rearing of hoppers which requires resources beyond the means of most national and private breeding programs.

Leaf Spots. Helminthosporium turcicum, which causes leaf blight and Puccinia sorghi, which causes rust, occur generally in cooler environments above 1000 m, while H. maydis and P. polysora prefer warmer conditions prevalent below 1000 m. These pathogens favor warm humid conditions and initially affect the lower leaves. In susceptible genotypes the disease may progress up the plant until all the leaf area is lost. Generally, marketed maize cultivars have very good resistance to these pathogens a result of successful plant breeding.

Stalk and Ear Rots. Stalk and ear rots are among the most damaging diseases of maize in Africa. Yield reductions due to stalk rots may result either from poorly filled ears or from lodging. Ear rot pathogens not only reduce yield, but adversely affect grain quality and may produce undesirable mycotoxins. Diplodia maydis, Gibberella zeae, and Fusarium moniliforme are particularly important in Africa.

The development of stalk rots is encouraged by favorable early growing conditions, followed by post-flowering stress. This stress may be caused by drought, high plant density, insect and foliar disease damage or other factors which may reduce photosynthesis. Ear rot development is prolific when early growing conditions are unfavorable and the post-flowering period is accompanied by above normal rainfall.

Conventional breeding programs in Africa have made some progress against infection by the stalk rot phases of these pathogens by selecting for standability and 'stay-green' characteristics. The search for true resistance to the ear rot pathogens has largely been unsuccessful. Artificial inoculation techniques have not been as successful in Africa as in the United States. As a result, Breeders have tended to rely on the following indirect selection criteria:

1. elimination of very susceptible genotypes;
2. selection against stalk rots; and
3. selection against genotypes with erect ears, or too loose or tight husk leaf covering.

POSSIBLE APPLICATIONS FOR BIOTECHNOLOGY

Most of the major maize producing countries in Africa have or are in the process of developing improved cultivars largely by conventional breeding methodologies. While the technology exists to enable these new cultivars to more nearly express their genetic potential on farmers fields, there is an urgent need to encourage farmers to adopt these practices.

There is also, however, a need to develop cultivars with greater stability in the limiting environments in which they are grown by peasant farmers. Breeders could make a significant impact on African maize production by developing genotypes less sensitive drought stress and inadequate fertility. This may be accomplished by the modification of conventional breeding procedures to select in parallel under limiting and non-limiting conditions. Appropriate use of varying plant densities may be of considerable assistance.

Biotechnological methods should be applied to those constraints which are important to the African farmer, but have not successfully been combated by conventional breeding methodologies or which could be more rapidly combated using biotechnology. These include, in some order of priority, the following:

1. Stalk borer resistance. Such resistant cultivars may also limit the adverse affects of other maize pests;
2. Storage pest resistance. An additional benefit may be the protection afforded to maize seed;
3. Ear rot resistance;
4. Maize streak virus. The rapid incorporation of resistance into susceptible maize inbreds, already extensively marketed, may be an important supplement to current breeding progress.

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PROBLEMS AND PROSPECTS FOR GRAIN LEGUME BREEDING

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ABSTRACT

Grain legumes are an important source of nutrition, and contribute a substantial part of dietary protein in many parts of the world. Groundnut (*Arachis hypogaea* L.) is also an important source of edible oil.

There are large differences in yield per unit area, even ten-fold increases, between different locations, and between experimental plots and farmer's fields. Reduced yields are due to a range of factors, primarily climatic, edaphic and biotic. Among the major yield reducers are pests and diseases, drought, and adverse soil conditions.

In addition to breeding for yield *per se*, breeders have incorporated resistances to pests and diseases and other yield constraints. However, the primary gene pool that is easily accessible to the breeder lacks many of the characters needed.

A range of techniques need to be applied to grain legumes to overcome these constraints. Embryo rescue may be sufficient where the gene can be found in close relatives, but in many cases more sophisticated techniques are needed.

The priorities in breeding efforts for different crops will be discussed.

INTRODUCTION

Grain legumes are an important source of nutrition, and contribute a substantial part of dietary protein in many parts of the world. They are grown on a wide range of soil types and under varying conditions from cool temperate zones to humid tropics. Some are widely distributed; others, such as mungbean (*Vigna radiata* L. Wilczek) are more localized but still occupy an important position. Groundnut and soybean (*Glycine max* L. Merr) are also important sources of edible oil.

Grain legumes are important crops of developing countries (Table 1); in many cases they are essential to the nutrition of the population and the economy of the country.

Table 1. Areas (million ha) Under cultivation of selected important grain legumes, 1986.

	World	Dev'd Countries	Dev'g Countries	Africa	N\C America	South America	Asia
Beans, Dry[1]	26.2	2.1	24.1	2.6	3.1	6.2	12.9
Chickpeas[1]	10.5	0.1	10.3	0.4	0.2	<0.1	9.7
Pigeonpea[2]	4.1	0.1	4.0	0.5	<0.1	<0.1	3.5
Groundnuts[1]	19.7	0.9	18.8	5.4	0.8	0.4	13.1
Pulses, Total[1]	68.4	12.4	56.0	10.9	3.9	6.7	35.7

[1] From FAO Production Yearbook, 1986.
[2] From International Pigeonpea Newsletter 5:10-14.

Areas of production are defined by both edapho-climatic and socio-economic factors, and within these areas, production in each crop has been limited by a number of constraints. These can be classified broadly into biotic, such as pests and diseases, and abiotic, such as inclement soil and climatic conditions, and drought. In addition, there are constraints on availability of inputs (Table 2). Although there have been concerted international and national efforts to overcome these constraints, much remains to be done.

Table 2. National and International Activities in Grain Legume Improvement

- Constraints
  - Management
    - Pests, diseases, soil conditions, drought
    - Government policies/Infrastructure
    - Lack of inputs, marketing facilities
- Improvement Efforts
  - International Efforts
    - Consultative Group on International Agricultural Research
      - ICRISAT : Groundnut, Chickpea, Pigeonpea
      - CIAT : Dry beans (*Phaseolus*)
      - IITA : Cowpea, Soybean
      - ICARDA : Faba beans, Lentils, Chickpea
  - National Efforts
    - Developed countries
      - Rapid dissemination and use of new technologies
      - Improved management
    - Developing countries
      - Lack of inputs
      - Aware of new technology, but:
        - Extension services inadequate
        - Management often suboptimal

Recent advances in biotechnology have opened up new vistas for crop scientists and offer new hope to the farmer. This paper outlines the major constraints to production of some grain legumes, and suggests how these may be addressed. Groundnut, beans (mostly Phaseolus), chickpea (Cicer arietinum L.) and pigeonpea (Cajanus cajan L. Millsp.) are important in Asia, and groundnut, beans, and cowpeas (Vigna unguiculata L. Walp) are important in Africa (Table 1). Their importance has been recognized by the Consultative Group on International Agricultural Research (CGIAR) by their inclusion in the mandate of one or more international agricultural research institutes.

There are some excellent books on grain legumes and pests and diseases (Summerfield and Bunting 1980, Summerfield and Roberts 1985) and on tropical legume pathology (Allen 1983); it is not our intention to give a complete review of the subject, but to highlight some of the outstanding problems.

CONSTRAINTS TO PRODUCTION

Biotic Stresses

Grain legumes are attacked by a wide range of fungal, bacterial and viral diseases, and a host of insect and other pests. Some of these are distributed throughout the whole growing area of the crop, while others are restricted to certain continents (e.g. Groundnut Rosette Virus to Africa) or are only of local significance.

Fungal diseases. High levels of resistance to **ascochyta blight,** (Ascochyta rabiei Pass. Lab.), or **botrytis gray mold** (Botrytis cinerea Pers., ex Pers.) are not available in cultivated chickpeas. The levels of resistance available are about 5 on a 1-9 scale (1=resistant, 9=susceptible), and the recovery of such resistance in the progenies is low. A few related Cicer species have slightly higher levels of resistance (2 or 3 rating); however, these do not cross with chickpea, and embryo rescue may be needed to produce hybrids from these crosses.

Cowpea grown in the humid forest belt of West Africa is attacked by a wide range of diseases, including **cercospora leafspot** (Cercospora canescens), **powdery mildew** (Erysiphe polygoni), **fusarium wilt** (Fusarium oxysporum), **fusarium root rot** (Fusarium solani), **ascochyta blight** (Ascochyta phaseolorum), **phytophthora stem rot** (Phytophthora vignae and Phytophtora cactorum), and **verticillium wilt** (Verticillium albo-atrum), for which resistances are available. There is no reported resistance, however, to **anthracnose** (Colletotrichum lindemuthianum), **pythium stem rot** (Pythium aphanidermatum), **web blight** (Rhizoctonia solani), and **brown rust** (Uromyces appendiculatus), which are major causes of crop loss (Singh and Allen 1980).

Wild relatives of common bean (Phaseolus vulgaris) are good sources of resistance to fungal diseases. Resistance to **anthracnose** (Colletotrichum lindemuthianum (Sacc. and Magn., Bri. and Cav.), **rust** (Uromyces phaseoli (Pers. Wint.) and angular **leafspot** (Phaeoisariopsis griseola Sacc., Ferraris), has been identified in **scarlet runner** (P. coccineus), (CIAT 1987), but as numerous races of these pathogens exist, further work is needed to introduce stable resistance into P. vulgaris. Phaseolus coccineus is also resistant to **ascochyta blight** (Ascochyta phaseolorum Sacc.) and **beanfly** (Ophiomyia phaseoli Tryon.). Tepary bean (P. acutifolius) is resistant to **common bacterial blight** (Xanthomonas phaseoli), (CIAT 1988) and **leaf hoppers** (Empoasca kraemeri).

Early leaf spot, caused by Cercospora arachidicola and **late leaf spot** caused by Phaeoisariopsis personata, result in extensive defoliation in groundnuts and subsequent yield losses. Although there is some resistance in A. hypogaea, the best sources of resistance are the wild species. Arachis cardenasii is resistant to **late leaf spot,** and many lines have been produced incorporating this resistance. Resistance to **early leaf spot** has been identified in species of section Erectoides (ICRISAT 1987) which have not been crossed with A. hypogaea.

Resistance to **groundnut rust,** caused by Puccinia arachidis, has been identified in the cultivated germplasm. There are indications that a different gene is involved in the resistance that has also been reported in the wild species (Singh et al. 1984), and use of this could lead to improved stability of resistance. Germplasm with resistance from wild species have been produced at ICRISAT (Moss et al. 1988).

Bacterial diseases. Bacterial diseases have not been given high priority in many breeding programs, as bacterial infections do not frequently cause epidemics; however, yield losses can be as high as 40% (Allen 1983) and bacterial diseases are often difficult to control. Some resistance is available--such as to **bacterial leaf spot** and **stem canker** (Xanthomonas cajani)--in accessions of pigeonpea from Africa. Disease incidence often depends on presence of damage to the plant, either by insect or mechanical means, after which the bacteria invade the plant and multiply rapidly. Only a few genera of bacteria cause crop losses, and some species infect a wide range of legumes. Selection of resistance may be possible in cell cultures. A gene, with action similar to that of the viral coat protein gene, could be isolated and used, though there does not seem to be an urgent need at present.

Viral diseases. Viruses have a well-established reputation for devastating legume crops, as there is a wide range of virus diseases whose effects range from minimal yield loss to total destruction of the crop. The true picture is often confounded by difficulties of identification.

Peanut Stripe Virus has received much attention in the last few years. Previously of restricted distribution, it has spread to many countries, and is both seed-borne and aphid (Aphis craccivora Koch) transmitted. Extensive field screening of the germplasm has so far failed to find resistance in A. hypogaea, but resistance has been reported in A. diogoi, A. helodes and other wild species (Culver et al. 1987).

Virus diseases of cowpea are very important. Reported yield losses can be as high as 80% for cowpea severe mosaic. Sunn-hemp mosaic and cowpea banding mosaic can each result in losses of 40% or above (Singh and Allen, 1980). There are no reports of resistance to these viruses.

Pests. Moderate levels of resistance have been found in pigeonpea against **pod borer** (Helicoverpa armigera Hubner) and **pod fly** (Melanagromyza obstusa Malloch). These resistances are being incorporated into breeding lines. Moderate levels of resistance are available in chickpea for **pod borer** (Heliocoverpa); however, Heliocoverpa remains a major problem for both chickpea and pigeonpea, and a higher degree of resistance is needed to stabilize production.

The **pod borer** (Maruca testulalis), is the major world wide pest of cowpea. (Singh and Allen 1980, Steele et al. 1985). Some wild cowpeas and related species are resistant to **pod borer** Maruca (Singh, pers comm.).

Leaf hoppers (Empoasca spp.), **bruchids** (Zabrotes subfasciatus and Acanthoscelides obtectus), and **whitefly** (Bemisia tabaci) are pests of beans worldwide. **Whitefly** is more important as a vector of bean golden mosaic virus than in its role of yield reducer. **Bean pod weevil** (Apion godmani) is important in Latin America, and **bean fly** is the worst pest of beans in Africa. Resistance to **beanfly** has been identified in P. coccineus (CIAT 1987), and to **leaf hoppers** in P. acutifolius (CIAT 1988). Resistance to **bruchids** has been found in wild forms of P. vulgaris (CIAT 1988).

Abiotic Stresses

Many of the resource-poor farmers of the world who rely on grain legumes as their major source of protein grow them on soils which are low in nitrogen and phosphate, in areas which are subject to drought. Crops grown on acid soils are adversely affected by aluminum or manganese toxicity. This is particularly true for beans. Cowpea is grown under the same conditions as the major crops with which it is co-cultivated, which tend to be in the low input, rainfed systems of subsistence farming. Many of the cowpeas grown therefore suffer from low fertility levels and low moisture availability.

Soil salinity is increasingly important where chickpea and pigeonpea are grown. Cultivated germplasm does not have appreciable levels of resistance, but a moderate level of tolerance to salinity has been found in Atylosia albicans and A. platycarpa, and needs to be transferred to pigeonpea. Possibilities exist for single cell selection in suspension cultures.

Drought is especially limiting in groundnut production. The plants are susceptible to drought at flowering and at pod filling. The effect of end of season drought has been reduced by breeding short-duration varieties, but there is still a need to breed shorter duration varieties to fit crop rotations. A similar approach is applicable to chickpea which is grown on residual moisture.

Temperature extremes are another threat to which tolerance is needed. Cold tolerance would be valuable in chickpea when grown as a winter crop, and Cicer microphyllum has been identified as a source of resistance.

RECOMMENDATIONS

Wild species of many legumes are good sources of resistance. The first priority, therefore, is to screen the wild germplasm to identify desirable traits. Techniques of hormone treatment to prevent pod abortion and maintain ovule growth, and techniques of ovule and embryo rescue are widely applicable; however techniques, timings, concentrations, and media used are specific to each crop and need further attention.

Use of wide crosses in legumes is problematic as resulting hybrids have many undesirable features. Many generations of backcrossing are needed to regain the adaptability of the cultivated parent. The use of vectors containing resistance genes from wild species to transform adapted cultivars

overcomes these problems, and opens up the possibility of transferring these genes into completely unrelated genera.

Some solutions may have wide applicability in legumes, such as the use of the Bt and similar genes for insect resistance, and the use of gene coding for viral coat proteins to provide virus resistance. These genes have wide applicability, and should be transferred to legumes when the techniques for plant regeneration have been developed. Major thrusts are called for in these fields, both to transfer available genes to grain legumes, and to search for other genes.

Although biotechnology holds promise for the legume breeder, many of the grain legumes are not easy to regenerate from callus or single cells, and this aspect should receive attention. Much basic work is needed before the full potential of the germplasm, and the new techniques to utilize the available variation, can be realized.

Selection in cell suspension cultures shows promise for improving a number of traits. This technique could be applied for salinity tolerance and for disease resistance where no suitable source can be found in the existing germplasm.

There are a number of techniques which assist the legume breeder in crop improvement. The application of anther culture to generate haploids is one such technique. Isolation of the gene for resistance and subsequent development of probes for gene detection and vectors for asexual transfer would be beneficial in breeding efforts. Asexual transformation has the advantage of introducing a desirable gene without disturbing the adaptation of the recipient cultivar.

CONCLUSIONS

Biotechnology adds a new phase to the ongoing process of crop improvement in grain legumes. Some goals have been achieved by conventional means, and there are a number of adapted lines with resistance to certain pests and diseases which are, or shortly will be, contributing to increased yields. Much remains to be done, however—especially in the field of pest and disease resistance—to improve and stabilize yields of these important crops.

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SORGHUM BREEDING FOR STRESS TOLERANCE:

TISSUE CULTURE AND IMPLICATIONS FOR AFRICA

R. R. Duncan

ABSTRACT

Sorghum (Sorghum bicolor L. Moench) is extremely sensitive to acid soil stress conditions. A two-phase sequential breeding program under field stress conditions has resulted in development of sorghum genotypes tolerant to highly acidic soil conditions. The first phase of the breeding program (PHASE 1) selects plants for Mn toxicity tolerance at 20% soil cation exchange capacity (CEC) Al saturation. The second phase (PHASE 2) selects for Al toxicity tolerance (50% soil CEC Al saturation). Sorghum plants exhibit three very distinct growth responses (impact, pre-flowering, and post-flowering) during each phase. Utilization of in vitro-stressed tissue culture regenerants to bypass PHASE 1 development has provided limited success. Acid soil stress, herbicide, and drought tolerances are interrelated. Stress tolerance per se is important in stabilizing yield production. Biotechnological methods offer the potential for escalating stress tolerance breeding programs, but these methods should be exploited only as another "tool" in an overall breeding program. Tissue culture experts and traditional plant breeders must cooperate, to ensure that farmers in developed and in developing countries may realize the cumulative benefits.

INTRODUCTION

Developed and Developing Countries

Optimum environments for maximum plant growth and development rarely, if ever, occur in nature. Farmers, regardless of whether they are located in developed or in developing countries, must constantly deal with plant stress avoidance/tolerance for adequate yields. Plants are continuously bombarded with soil (acidity, salinity, alkalinity, compaction, water-logging) and environmental (rain, temperature) constraints that often uncontrollably cause yield instability. Soil and environmental stresses often trigger outbreaks of insects and/or diseases that can detrimentally influence crop yields. Too often, these unstable yields occur in the developing countries where food availability means life or death to millions of people.

New technology may bring larger harvests from a given area of farmland, but the foundation of this technological revolution is the breeding of crops adapted to the needs of low input, intensive and stabilized agriculture (Jennings 1976). This paper focuses on how a biotechnological technique such as tissue culture can be used as another "tool" to enhance a traditional plant breeding program.

Acid Soil Tolerance in Sorghum

Inter- and intra-species variability exists among various plants for tolerance to mineral stress (Foy 1983). Among the Gramineae species, rice

(Oryza sativa L.), oats (Avena sativa L.), and rye (Secale cereale L.) had high tolerance to excess concentrations of aluminum while millet (Pennisetum typhoides), wheat (Triticum aestivum L.), and maize (Zea mays L.) had intermediate tolerance. Sorghum (Sorghum bicolor L. Moench) and barley (Hordeum vulgare L.) were susceptible to high concentrations of Al in nutrient solutions (Tanaka and Hayakawa 1975).

Additional studies with sorghum have also shown sensitivity to various acid soil stress/infertility problems (Clark 1982, Clark et al. 1981). Sorghum genotypic variability has been demonstrated in nutrient solutions with low pH and Al toxicity (Bastos and Gourley 1982, Boye-Goni and Marcarian 1985, Furlani and Clark 1981, Furlani et al. 1983); in pot experiments with low soil pH (Brown and Jones 1977); in field experiments with variable acid soil stress levels (Duncan 1981abc and 1983a); and simultaneously in both nutrient solutions and in the field (Duncan et al. 1983b). Duncan (1982, 1983a,c) and Borgonovi et al. (1987) have devised field screening techniques to categorize specific genotypes for tolerance/susceptibility reactions to acid soil stress infertility problems. Sorghum germplasms have been released which will tolerate various acid soil stress levels (Borgonovi et al. 1982, Duncan 1981a and 1984).

Tissue Culture of Sorghum

Long-term, high-frequency plant regeneration has been obtained in sorghum (Nabors et al. 1983, MacKinnon 1986 and 1987a, b, Oberthur et al. 1983, Wernicke and Brettell 1960, TCCP Progress Report 1987a, Tomes 1985). However, once the generation process has been accomplished, in vitro selection and subsequent field evaluations must be conducted to ascertain 1) level of tolerance to environmental stress, 2) effect of in vitro selection on tolerance level, and 3) emergence of other useful variants from the regenerants. The Tissue Culture for Crops Project (TCCP) at Colorado State University has used NaCl, aluminum toxicity, and polyethylene glycol (PEG) as stressing agents in vitro. Additional information concerning NaCl tolerant sorghum callus (Braskaran et al. 1983) and Al toxicity selection in vitro (Smith et al. 1983) have been reported.

MATERIALS AND METHODS

Soils for Plant Stress Evaluation

The acid soil stress screening has been conducted on the Ultisols in the Appalachian (Blairsville), Limestone Valley (Rome), and Piedmont (Griffin) regions of the southeastern U.S.A. These soils are generally characterized by 1) coarse texture and poorly developed structure, 2) low organic matter (<1.5%), 3) moderate to strong acidity, 4) high leachability and low base status, 5) high weather-ability with predominately 1:1 non-expanding clays, 6) high Mn availability at moderate (pH 4.5-5.0) acidity levels, 7) high Al availability at soil pHs below 4.5, 8) low availability of P, high P fixing capacity, and high Fe- and Al-oxide contents, and 9) low water infiltration rates. Data in Table 1 provides additional specific characteristics of these six Ultisols. In general, the 50% Al saturation level has been targeted as the maximum acid soil stress level for effective development of tolerant sorghum genotypes (Borgonovi et al. 1987 and Duncan 1988).

Table 1. Specific soil characteristics of the Ultisols in the SE USA used for acid soil stress evaluation of sorghum genotypes

Location (Region)	Soil Type[f]	Soil pH		Ml Extr.[b]		KCl[c]	NH_4OAc[d]			Al[e]
		H_2O	KCl	Mn	P	Al	Ca	Mg	K	Sat.
				ug/g		$cmol_c\ kg^{-1}$				%
Blairsville (Appalachian)	Dyke cl	4.7	4.1	62[a]	19	0.80	2.23	0.32	0.61	20
		4.1	3.8	62	14	2.03	0.25	0.06	0.49	71
	Congaree sl	4.8	4.1	52[a]	9	1.57	1.41	0.18	0.52	42
		4.1	3.8	42	4	1.96	0.49	0.11	0.48	64
Rome (Limestone Valley)	Cedarbluff sl	4.8	4.0	36[a]	22	0.63	1.51	0.30	0.30	23
		4.3	3.7	37	26	1.16	0.73	0.17	0.32	48
Griffin (Piedmont)	Cecil scl	4.5	4.0	30[a]	6	0.59	1.24	0.26	0.21	25
		4.1	3.8	26	20	1.47	0.46	0.09	0.24	63
	Pacolet scl	4.6	3.8	6	3	1.67	1.05	0.50	0.27	48
		4.4	3.8	10	3	2.02	0.97	0.25	0.34	56
		4.1	3.7	6	10	1.74	0.15	0.05	0.15	83
	Appling csl	4.7	4.2	40[a]	8	0.38	1.24	0.17	0.13	19
		4.1	4.0	29	9	0.79	0.76	0.12	0.08	45

[a] Used for low level acid soil tolerance or suspected Mn toxicity screening (Phase 1)
[b] Extracted using Mehlich 1
[c] Extracted with 1 N KCl
[d] Exchangeable cations extracted with 1 M NH_4OAc, pH 7

[e] Percent Al saturation of the CEC = $\frac{Al\ (cmol_c\ kg^{-1})}{Al+Ca+Mg+K+Na\ (Cmol_c\ kg^{-1})} \times 100$

[f] Dyke = Typic Rhodudults; Congaree = Fluventic Dystrochrepts; Cedarbluff = Fragiaquic Paleudults; Cecil, Pacolet, Appling = Typic Hapludults

Sorghum Genotypes

Several genotypes have been identified as having some level of tolerance to acid soil field stress (Duncan 1981, 1982, 1983c, Duncan et al. 1986b). Data in Table 2 summarizes genotypes, pedigrees, country of origin, and relative stress response to field acidity problems and 50% Al saturation. Exotic accessions originating from Sudan, Tanzania, Uganda, Nigeria, and

Ethiopia have provided sources of acid soil tolerance. Additional accessions from Botswana, Kenya, Burundi, and Zambia are being targeted for possible sources of tolerance. Standard susceptible 'checks' currently being used in nutritional studies and in breeding programs include Tx430, Tx536, and TAM 428. The standard tolerant check is SC283 (IS7173c).

Table 2. Relative acid soil stress responses of selected sorghum genotypes[a]

Genotype	Additional Pedigree	Country of Original	Fertility Reaction[c]	Acid Soil Field Response[b]
SC283	IS7173	Tanzania	B	VT
SC694	IS7193	Nigeria	R	VT
SC991	IS12219	Uganda	B	VT
SC57	IS12569	Sudan	R	VT
SC689	IS2729	Uganda	R	T
SC574	IS83337	Pakistan	R	T
SC564	IS7142	Uganda	R	T
SC990	IS12211	Uganda	R	M
SC408	IS7542	Nigeria	R	M
SC279	IS7419	Nigeria	P	M
SC566	IS7254	Nigeria	B	M
SC175	IS12666	Ethiopia	R	M
SC112	IS12612	Ethiopia	R	M
SC326	IS3758	Ethiopia	R	M
GP140	(GP66XIS12564)	USA-Georgia	R	M
SC56	IS12568	Sudan	R	M
SC237	IS3071	Sudan	R	M
SC599	IS17459	Sudan	R	M
SC418	IS1335	Tanzania	R	M
SC322	IS1309	Tanzania	P	M
SC48	IS12564	Sudan	R	S
SC549	IS3625	Nigeria	R	S
TAM428	IS12610 derivative	USA-Texas	R	S
Tx2536	IS542 derivative	USA-Texas	R	S
Tx430	(Tx2536xSC170)	USA-Texas	R	SS

[a] Partially adapted from Duncan 1988
[b] VT = very tolerant; M = moderate/low level tolerance; S = susceptible; SS = supersensitive; T = tolerant
50% soil CEC Al saturation at pH 4.2-4.3
[c] Fertility reaction = crosses with Al cytoplasm. R = restorer (progeny all male fertile). P = partial restorer (progeny have 5-80% seed set). B = maintainer (progeny all male sterile).

Tissue Culture of Sorghum

The two TCCP reports (1987a, b) and the paper by G. E. Hanning contain details on the *in vitro* stress selection procedure and results.

RESULTS AND DISCUSSION

Acid Soil Tolerance Breeding Program

Field screening efforts to develop acid soil tolerance in sorghum have evolved into a dual-phase process (Figure 1). Phase 1 involves identification, evaluation, and screening in a soil pH range from 4.4-4.8 (suspected Mn toxicity and 20% soil CEC Al saturation) on six ultisols at three locations. Experimental hybrids are evaluated simultaneously at this level of stress and at pH >6.0. Subsequent generations are subjected to this level of stress at a minimum of two locations per year with surviving pedigrees from one location being grown at the other two locations the following year. Consequently, segregating survivors are subjected to acid soil stress conditions at all three locations and on all six soil types during PHASE 1 developmental (average 5-8 generations) stages. During F_2 to F_4 generations, family selection is exercised due to the large number of pedigrees in the program and also to possible "escapes" evolving from environmental conditions other than acid soil stress, i.e. evenly distributed rainfall during the season which enhances shallow rooting patterns. General adaptability, agronomic desirability, and disease resistance characteristics can be incorporated during PHASE 1 development. The germplasm base is generally broad enough to provide sufficient genotypes for proper segregation patterns. By-passing PHASE 1 or moving too rapidly during PHASE 1 development may eliminate potentially favorable genotypes or narrow the germplasm base to the point of detrimentally affecting the breeding program.

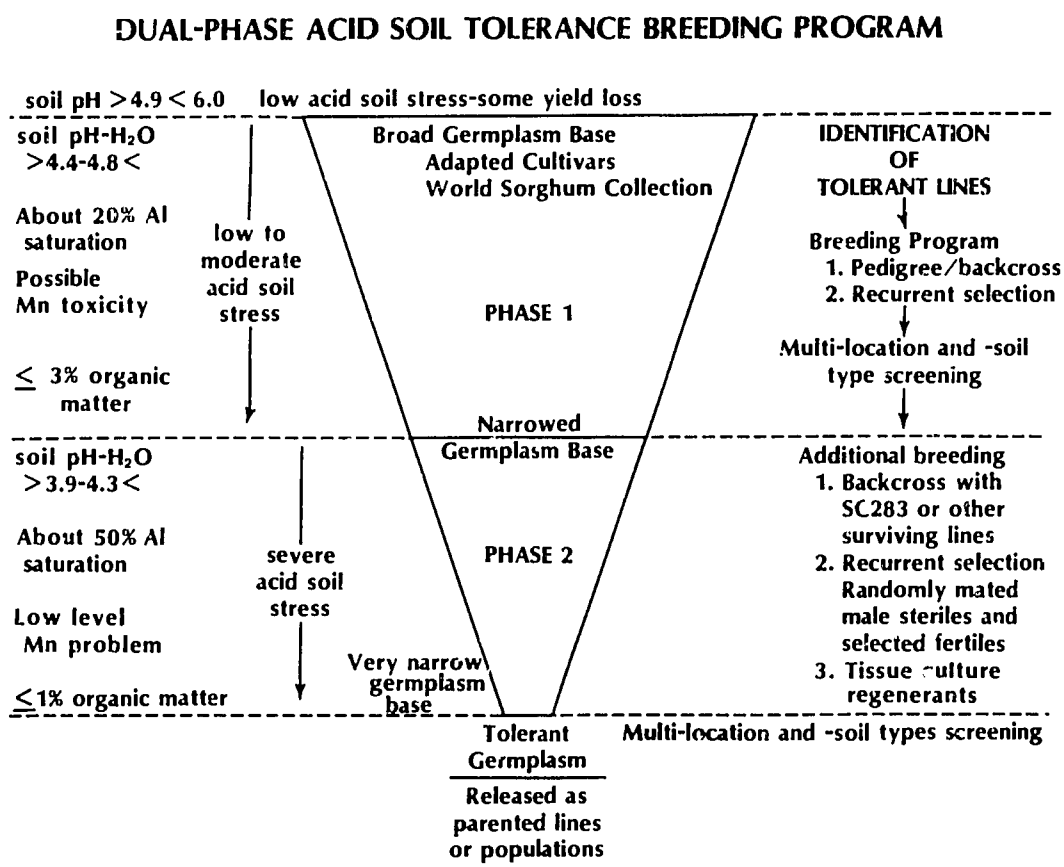

Fig. 1. Schematic of acid soil constraints, narrowing of germplasm base during development, and breeding approaches during the dual-phase program.

PHASE 2 selects for Al toxicity tolerance (50% soil CEC Al saturation) at a soil pH of 4.2-4.3 on the same soils as in PHASE 1. Only surviving pedigrees from PHASE 1 and selected genotypes (Table 2) which have good to excellent tolerance have been utilized in the pedigree breeding, reciprocal backcrossing, and recurrent selection-random mating population programs. Tissue culture-derived, $AlCl_3$ selected regenerants are being evaluated in PHASE 2 screening in an effort to bypass PHASE 1. Only three potentially acid soil field-tolerant regenerants have survived preliminary field stress testing.

Acid Soil-Tolerant Tissue Culture Regenerants

The initial evaluation of sorghum regenerants (Table 3) during 1986 in Georgia involved NaCl and PEG selected material (TCCP Progress Report 1987a). Lines planted in non-stress environments for seed increase were observed for somaclonal variation; however, little variation was observed within an R_1 line, except for variation in plant height, maturity, and insect resistance (9 lines with potential fall armyworm resistance). Among the 123 lines planted, 17 survived the acid soil stress (50% Al saturation) and simultaneous drought/heat stress conditions; only one line—an RTx430, salt-selected R_2 regenerant—produced seed. This was a significant finding since the original RTx430 is used as a susceptible check under acid soil field stress conditions (Duncan 1988). During 1987, 47 plots survived the acid soil field stress and 30 produced seed. Among the 30 lines which produced seed, 16 lines emerged from nonselected callus and 14 from NaCl-stressed callus culture. Overall, 53% of the 31 total lines derived from nonstressed cultures produced seed; 28% of the 50 lines from the NaCl-stressed cultures produced seed. Consequently, stress tolerance above that of the non-regenerated seed was found in regenerants from *in vitro* cultures (TCCP Progress Report 1987a). During 1988, three R_3 lines set seed under low pH (two Hegari lines and one line derived from RTx430). In R_1 evaluation, 6% of the lines derived from *in vitro* stressed cultures survived the severe low pH stress level, while 18% of the lines derived from $AlCl_3$ stressed cultures produced seed.

Table 3. Field evaluations in Georgia of tissue culture-regenerated sorghum for acid soil stress tolerance

Year	Generation(s)[b]	Number of R_1 Lines Represented	Number of Plots[a]	Approx. Number of plants	Number of Seed Per Plot	Number of Survivor Lines	Number of Plots Producing seed
1986	R_1	123	409	47,035	115	17	1
1987	R_1 & R_2	100	2700	310,500	115	47	30
1988[c]	R_1,R_3,R_4	69	2000	230,000	115	42	12

[a] Includes pH >6.0 seed increase block plus pH 4.2-4.3 acid soil stress block. Acid soil stress block is subjected to periodically severe drought and temperature stress.

[b] R_0 = regenerated plants, R_1 = first generation

[c] Includes sorghum and millet

Overall Acid Soil Field Tolerance (ASFT)

Breeding efforts to develop ASFT in sorghum involve the total impact of toxic and deficient elements in relation to plant growth and development. Many areas of the world have concentrated primarily on excess Al since that element is the predominant constraint. The field oriented program in Georgia not only must deal with toxic levels of Mn and Al and the deficiency problems (Ca, Mg, P) induced by these elements, but also with high temperature stress, low soil moisture and drought tolerance, and adequate root system development. Most sorghum genotypes are not well adapted to the humid, subtropical region of the southeastern U.S.A. and do not have sufficient insect and disease resistance to produce stable yields. Consequently, a dual-phase breeding program has been necessary to superimpose overall ASFT with yield capability and disease/insect resistance. Selection indices have been low (5-15% for PHASE 1, about 1% for PHASE 2 depending on pedigrees involved), but the index is gradually increasing with each generation and progress has been consistently steady.

Plant Response to Acid Soil Stress

The sorghum plant is thought to undergo three distinct response reactions to acid soil stress. These reactions are termed the impact, pre-flowering, and post-flowering responses (Duncan 1988). The impact response occurs from the onset of water imbibition by the seed following plant and encompasses the initial reaction of the radicle to the soil solution (and H^+ ion concentration), to the uptake of toxic elements and the deficiency of less available elements, and to subsequent physiological activity which triggers initial growth and development. Super susceptible genotypes simply do not emerge or they die within one to two weeks following emergence. Genotypes surviving this impact phase must contend with nutritional imbalances as well as drought stress caused by toxic-element induced root pruning. Tolerance to the grass-type herbicides Alachlor and Metolachlor is apparently associated with the ASFT impact response phase (Wilkinson et al. 1989) in sorghum; however, use of the herbicide is not essential for growth and development. The herbicides are simply used as another screening tool. Some genotypes produce sufficient vegetative matter and undergo panicle initiation (at about 40-45 days post-planting) in normal fashion; other genotypes are delayed or stopped completely in the transition to the reproductive stage. This incomplete pre-flowering response may be compatible with the drought tolerance mechanism. Enzymatic, hormonal, and physiological activities are operating during this response phase. Genotypes that successfully produce a panicle and grain in the panicle must successfully tolerate the post-flowering response to acid soil stress. Some genotypes are successful; others exhibit panicle or grain abortion and fail to develop and progress properly. Enzymatic, hormonal, and physiological activities are again operating during this response phase.

Genetics of ASFT

The overall acid soil tolerance reaction of sorghum genotypes to field stress is probably additive in inheritance, but individual elemental inheritance may involve varying degrees of dominance (Borgonovi et al. 1987, Boye-Goni and Marcarian 1985, Furlani et al. 1983).

CONCLUSIONS

1. Stress tolerance per se is important in stabilizing yield production. The higher the level of stress tolerance, the greater the probability of producing some yield regardless of environmental extremes.

2. Biotechnological processes such as tissue culture offer the potential for escalating stress tolerance breeding programs by concentrating on specific activities: proline production efficiency which is involved in adaptation to osmotic stress, nitrate reductase efficiency, Al Toxicity tolerance.

3. Tissue culture techniques should never dominate an overall plant stress development program. When properly applied with traditional breeding procedures, tissue culture can increase efficiency and cost effectiveness, thereby enhancing the breeding programs.

4. ASFT involves several complex mechanisms with must act simultaneously or sequentially to produce proper plant growth and development.

5. Step-wise in vitro selection techniques coupled with serial stress screening of callus variants will probably be needed before significant improvements in stress tolerance under field conditions can be accomplished. Non-regenerated plants must cope with multiple stresses under field conditions; regenerated plants must do the same.

6. Properly balanced biotechnological techniques and traditional breeding methods can ultimately benefit farmers in developed and in developing countries. If low input sorghum production is going to be improved and/or stabilized, improvements in nutrient use efficiency, drought tolerance, the plant root system per se, and tolerance to toxic elements must be realized.

7. Potential uses of tissue culture in developing countries might include:
   a) Improvement of nutrient uptake/use efficiency
   b) Improvement of drought tolerance
   c) Improvement in tolerance to toxic elements
   d) Transfer of disease resistance into locally adapted elite germplasm (such as virus resistance)
   e) Method of introducing quarantined germplasm without quarantine grow-out
   f) Using RFLPs, genetically fingerprint material in the Gene Bank and eliminate duplicates

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SESSION II: TISSUE CULTURE TECHNOLOGY

THE ROLE OF MICROPROPAGATION IN COMMERCIALIZATION OF PLANT BIOTECHNOLOGY

Irwin Y.E. Chu

ABSTRACT

In the last decade, plant tissue culture micropropagation has become one of the most important methods to reproduce crops that are difficult to propagate by conventional methods, e.g., by seed or cuttings. The speed of tissue culture technology development has been accelerated due to its practical commercialization in the marketplace. Tissue culture micropropagation offers many unique advantages compared to other propagation methods:

Rapid multiplication from single cloning materials
Product uniformity by totally controlled production system
High volume propagation in a short time
Heterozygous plants become marketable products
Delivery of genetically-engineered products into commercialization
Germplasm storage for production and gene banks
Improved phenotype by laboratory manipulation
Disease-free plants from meristem culture
Eased movement of product certification free of pathogens
Nonseasonal production by total control of production system.

For these reasons, tissue culture micropropagation will ultimately be the method of choice to successfully industrialize plant production.

INTRODUCTION

Within the last decade, tissue culture technology has rapidly been commercialized in the marketplace. Compared to other propagation methods, tissue culture micropropagation offers many unique advantages. Currently, the greatest application of tissue culture micropropagation is with ornamental tropicals; however, successful production systems for such crops as potato, asparagus and strawberry are beginning to replace traditional vegetative propagation methods. Overall, however, micropropagation is presently not cost-competitive as a method of propagation for many crops compared to conventional propagation methods (e.g. seeds, cuttings, division). The major limiting factor is the quantity of human labor currently required. Henceforth, to both maximize the cost competitiveness of micropropagation and expand its application to a wider range of plant species, production costs must be minimized through labor reduction and increased efficiency in output. The success and ultimate scope of the micropropagation industry are contingent upon successfully meeting these two challenges.

Advantages of Micropropagation

Advantages associated with the commercial production of plants by micropropagation may be categorized into three significant areas pertinent to the development and marketing of an improved commercial product:

A. Product Development 1) Fast growth 2) Product uniformity 3) Gross volume 4) Heterozygous products 5) Genetically-engineered products 6) Germplasm storage;
B. Product Enhancement 1) Improved phenotype 2) Disease-free plants;
C. Product Marketability 1) Product format 2) Eased movement of products 3) Nonseasonal production.

Production Cost Components

For a micropropagation business to become successful, it is essential that the company have an efficient, detailed production plan, maintain accurate production records, and continually evaluate its financial progress (Kyte 1983). The production plan should include both a thorough knowledge of the market for the plants produced, and the associated, calculated requirements for labor and space. Thus, it is essential to maintain accurate and current records from which production costs can be determined and product selling prices can be established (Donnan 1986; Strain 1980, 1981). Clear comprehension of tissue culture production costs is prerequisite to a meaningful comparison of potential micropropagation costs with conventional methods when evaluating new crops.

Determining Costs. There are several methods of determining micropropagation costs. Donnan (1986) suggested the following aspects for examination. First, the total production operation cost for a specific time period can be divided by the total number of units sold. Although this gives an overview of costs, this method does not enable a comparison of competitors' pricing. Second, the cost of operating a transfer hood position is calculated by dividing the total operating costs by the number of transfer hood positions, multiplying by the number of hours per position for the time in question, thus yielding the cost associated with operating a hood position for one hour. The crops and associated work rates must then be scrutinized to determine whether a profit is being made. In determining specific crop decisions, this method could be of great assistance. Third, the cost per square foot of shelf space can be determined (Strain 1980, 1981). An average cost of $1.75/square foot/week has been reported (Donnan 1986). Breaking this cost factor down demonstrates the significance of maintaining growth room shelves to capacity. Clearly, determining costs on a product-by-product basis is the most accurate method, whereby detailed records are maintained daily including transfer rate, cultures/vessel, multiplication rate, losses and space allocations. Greenhouse records (e.g., planting rates, finishing times, and losses) must be included for operations producing Stage IV products.

Cost Components. In order to ultimately monitor and reduce production costs, it is essential to examine specific cost components. Costs related to Stage IV (George and Sherrington 1984) liner production may be analyzed into various categories (Table 1): laboratory direct labor; utilities; depreciation; supervision (laboratory and greenhouse); planting direct labor and other production costs (e.g., repair, insurance, taxes and travel). An additional cost could include royalty payments for patented plant material.

As exhibited in Table 1, labor requirements currently total 47% of the production cost. Although labor costs can and do reach higher levels depending on the particular crop, Table 1 is representative of the prevalent situation. For example, if increased subculture frequency is required to maintain acceptable multiplication rates, a crop then requires greater labor. Other workers have reported labor components in the range of 40% (Sluis and Walker 1985), 60% (Donnan 1986), to more than 70% (Anderson and Meagher 1977). Analysis of the entire production process reveals Stage I (George and Sherrington 1984), or the initiation stage, as the most costly. Since relative output is low, losses may be exorbitant due to contamination and specialized labor requirements. As soon as a culture is passed on to Stage II and multiplication rates increase, the associated production cost on a per unit basis declines and achieves a relatively stable level (Henny et al. 1981). It should also be noted that the laboratory start-up phase of a new operation can be quite expensive, due to considerable capital investment in facility and equipment with initial low product output. The higher the percent usage of the facility, the lower the related production costs on a unit basis (Strode and Abner 1986). Thus, once a laboratory decides to substantially increase its production capacity, more equipment, growth rooms and multiple shifts will be needed. Initially, the costs on a per unit basis will again be high, until the expanded facility can be operated at maximum capacity and efficiency.

Table 1. Relative Cost Components Associated With The Production Of A Typical Crop. (Twyford Plant Laboratories, Inc. study)

Laboratory Direct Labor	15%
Utilities	9%
Depreciation	7%
Supervision (laboratory and greenhouse)	23%
Planting Direct Labor	9%
Other Production Costs	37%
Total	100%

Current World Activity And Market

Existing micropropagation companies. There has been a progressive expansion of commercial micropropagation laboratories since the 1970's. Presently, there are over 250 such companies in the United States (Jones 1986). The larger labs have over 100 employees and produce high-volume crops, specifically foliage plants and ornamental species. Existing laboratories can be categorized on the basis of product-type offered, ranging from Stage II or III cultures, Stage III bare-root plantlets, to finished Stage IV liners. The product type is usually representative of whether the company is equipped with acclimation and greenhouse facilities.

The worldwide micropropagation industry has been especially active in the past five to ten years, with commercial laboratories located primarily in Australia, Belgium, Canada, France, Israel, Italy, Japan, the Netherlands, the United Kingdom, and the United States. The global expansion of the micropropagation industry has increased potential markets and assisted in creating new product opportunities, specifically for tropical crops (e.g., banana and oil palm).

Current Products and Markets

The most widely propagated crop in the U.S., and the world, is orchids, followed by foliage and flowers, predicated on information by George and Sherrington in 1984, as exhibited in Table 2.

Table 2. The Percentage Of World And USA Commercial Micropropagation Labs Producing Specific Crops (Chu 1986).

Crops	90 USA Labs	115 World Labs excluding USA	205 World Labs including USA
Orchids	60%	56%	58%
Ferns	15%	21%	19%
Foliage & Flowers	28%	37%	33%
Woody Crops	15%	17%	16%
Tropical Plantation Crops	3%	9%	6%
Vegetable Crops	5%	13%	10%
Fruit Crops	4%	23%	15%

A 1985 survey of actual units produced/year in diverse crop categories by U.S. laboratories records the following (Jones 1986): 32.0 mill units of foliage plants; 7.0 mill units of flowering plants; 20.5 mill to 25.5 mill units of woody ornamentals with the total U.S. production at 60.7 mill to 65.7 mill units. Twyford Plant Laboratories, Inc. has conducted their own marketing research for the major tissue culture products in North America (Table 3), Europe (Table 4) and Asia (Table 5). In North America, Syngonium is the most produced crop at 16.0 mill units/yr., with ferns ranking second at 12.0 mill units/yr., and Spathiphyllum third at 10.0 mill units/yr. In Europe, however, the number one product is gerbera, being produced at 18.0 mill units/yr., while ferns rank second at 12.0 mill units/yr. In Asia (Japan, Taiwan, Korea, Malaysia, Thailand and China), foliage plants rank number one, being produced at 12.0 mill units/yr., while orchids rank second at 11.0 mill units/yr., and sugar cane stands third at 8.0 mill units/yr. It is noteworthy to point out the total number of units produced per year for each area: North America at 84.7 mill units/yr.; Asia at 55.7 mill units/yr.; Europe at 66.5 mill units/yr. This information indicates that the globalization of the micropropagation industry has been quite successful.

Table 3. Major Tissue Culture Products in North America.

Crop Category	Products	Units (Millions)
Bulbs	Lily 1.5	
Cut Flowers	Gypsophila	0.2
	Gerbera	1.5
Foliage Plants	Spathiphyllum	10.0
	Syngonium	16.0
	Ferns	12.0
	Ficus	1.5
	Dieffenbachia	3.0
Fruits	Strawberry	3.0
	Raspberry	1.0
Landscape Plants	Rhododendron/ Azalea	8.0
	Nandina	2.0
	Day Lily	2.5
Plantation Crops	Sugar Cane	0.2
Pot Flowers	African Violet	3.0
	Orchids	5.0
	Gerbera	2.0
Rootstocks	Apple	0.1
Seed Production	Brassica	3.0
	Ornamentals	1.5
Trees	Birch	0.2
Vegetables	Asparagus	0.5
	Potato	2.0
Other		5.0
TOTAL		84.7

Table 4. Major Tissue Culture Products in Europe.

Products	Units (Millions)
African Violet	6.0
Ferns	12.0
Ficus	4.0
Gerbera	18.0
Landscape Plants	3.0
Lily	8.0
Musa	1.0
Philodendron	2.5
Roses	2.0
Spathiphyllum	1.0
Syngonium	4.0
Other	5.0
TOTAL	66.5

Table 5. Major Tissue Culture Products in Asia.

Products	Units (Millions)
Carnation (Mother Stocks)	2.0
Foliage Plants	12.0
Gerbera	2.0
Gypsophila (Mother Stocks)	0.5
Limonium	1.5
Musa	3.5
Ornamentals (Misc.)	5.0
Orchids	11.0
Potato	1.0
Strawberry	1.0
Sugar Cane	8.0
Rootstocks	0.2
Other	8.0
TOTAL	55.7

Improved Production Systems for the Future

The ultimate extension and application of micropropagation technology to other crops, such as plantation, forest and vegetable crops, depends on a reduction in their current production cost and ultimate selling price (Rowe 1986). To enter markets currently propagated by seed, such as vegetable

crops, the cost of micropropagated material must be able to compete favorably with seed-derived transplants. The two major approaches to reduce production costs include: 1) optimization of production systems by defining conditions to efficiently produce even larger numbers of plants; and 2) reduction in the human labor requirement as much as possible through the application of mechanization throughout the production process.

Optimization of Production Systems. Measures should be taken to both optimize current production protocols to obtain high rates of plant regeneration without sacrificing product quality, and to design new production systems for the future. The laboratories which have the vision of the future micropropagation business, will be the ones which will be able to meet the challenge of increased efficiency and productivity. To optimize current production systems, several factors are worth consideration: 1) in vitro culture conditions, including culture media and subculture schedule; 2) minimization of mutant or off-types; 3) production of phenolic compounds in vitro; 4) vitrification of cultured material; 5) minimization of contamination; 6) successful acclimation and establishment of Stage II or III plant material; and 7) successful cold storage of in vitro material (Hussey 1983; Hartman 1985).

Mechanization of Micropropagation. At present, commercial micropropagation laboratories utilize production systems based on standard microbiological and horticultural practices which have been developed over the past ten to twenty years. These methods have been successful until recently, while production quantities remained reasonably low. However, the current objective of increasing production and reducing costs to capture even greater market shares cannot be met with current production technology (Sluis and Walker 1985). Although there have been numerous improvements over the years in the development of current production protocols and systems development for an increasing number of species, the advancement which is now required to permit further expansion of markets is that of mechanization. Mechanization could be applied in all phases of plant production by a commercial plant micropropagation company in the form of automation, computerization, and even robotics. The key target areas include culture media preparation, subculture and division of multiplying cultures, harvest of shoots or micro-cuttings for rooting, and planting of shoots or plantlets. In addition, materials handling systems in the greenhouse as well as packing and shipping areas contribute to overall productivity and efficiency.

Recently, an automated micropropagation system based on liquid culture has been developed in Israel by Plant Biotech Industries Ltd. (Levin et al. 1988). Their 'Vitromatic System' integrates a bioreactor or fermentor with a bioprocessor in a closed system. Either shoot organogenesis or somatic embryogenesis is utilized as the chosen regeneration routes, depending on the species. In addition, they developed an automated transplanting machine which transfers plantlets to soil mix in cell trays to be finished in the greenhouse. Plant Biotech Industries has applied this technology to several species, including ornamentals, vegetables and trees, and is now conducting grow-out trials to determine the degree of any possible off-typing associated with their system. If successful, this development could revolutionize commercial micropropagation.

The use of robots, especially during the labor-intensive stages of the subculture process (harvesting of shoots to be rooted and even planting), has

potential for improving efficiency. The major obstacle to overcome when using robots is the complex growth habit of the cultures, usually clumps of shoots and nodal explants which could be difficult to separate by mechanical dissection (Rowe 1986; Walker 1986). The use of a robotic system to locate plantlets on the culture medium, pick them up and place them into containers has been evaluated (Deleplanque et al. 1985; Miwa 1987). Miwa (1987) has developed a culture transplant robot with a shape memory alloy actuator and a "plant antenna" sensor to locate the plantlets based upon their emission of a weak electrical charge into the culture medium. Row et al. (1987) have also developed a robot and the necessary computer software to interface with a vision control system to both hold and excise nodal potato microcuttings.

A major challenge in the utilization of such robotic systems is that their working speed is still slow when compared to human operators. In addition, the high investment cost associated with the development and commercial application of such robotic systems still limits their immediate usefulness in the reduction of micropropagation production costs.

CONCLUSIONS

Tissue culture micropropagation will emerge as a major means to propagate many important crops in the agricultural and horticultural industries. Through the combined efforts of 1) reduction of production costs 2) increased of efficiency, and 3) production of plants with "added value," micropropagation will achieve a greater importance in the marketplace as a superior method of plant propagation.

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IN VITRO SELECTION IN CEREAL AND LEGUME TISSUE CULTURE AND FIELD EVALUATIONS OF REGENERATED PROGENY

G. E. Hanning, A. S. Kumar and M. W. Nabors

ABSTRACT

Plant regeneration is the first step in utilization of a tissue culture system for *in vitro* selection. Regeneration for the cereals—rice, wheat, millet and sorghum—has been previously reported by the TCCP. Plant regeneration has been accomplished for pigeon pea, moth bean, and tepary bean in the past year. *In vitro* selection for cells tolerant to NaCl, $AlCl_3$, and polyethylene glycol (PEG) has been studied for all of the cereals and some of the legumes. *In vitro* selection levels of the stress agents are chosen by measuring the reduction in embryogenic callus fresh weights and plant regeneration rates. Three regenerated lines of sorghum have been identified in two seasons of field evaluations as having fall armyworm resistance, acid soil tolerance, or drought/salt tolerance.

INTRODUCTION

The Tissue Culture for Crops Project was established to expand and improve tissue culture techniques of the major cereal and grain legume crops. A second area of tissue culture was to investigate and determine the value of using *in vitro* selection as a technique to produce germplasm with enhanced field performance on saline soils, acid soils with high exchangeable aluminum levels, and in drought conditions.

Plant regeneration procedures developed at the TCCP have been reported for rice (Raghava Ram and Nabors 1984; Heyser *et al*. 1983; Siriwardana and Nabors 1983), wheat (Heyser *et al*. 1985; MacKinnon *et al*. 1987a), sorghum (MacKinnon *et al*. 1987a,b) and moth bean (*Phaseolus acutifolius*) (Kumar *et al*. 1988a,b). *In vitro* selection has been conducted with NaCl, $AlCl_3$, and PEG as selection agents. This paper will describe the grain legume tissue culture, improvements in sorghum tissue culture, *in vitro* selection for $AlCl_3$ and PEG in sorghum, and field evaluations for several regenerated sorghum lines.

RESULTS AND DISCUSSION

Grain Legume Plant Regeneration

Tissue culture of grain legumes has been very difficult. At the TCCP, Dr. Suresh Kumar has developed plant regeneration techniques for moth bean (*Vigna aconitifolia*), tepary bean (*Phaseolus acutifolius*) and pigeon pea (*Cajanus cajan*).

Mothbean and tepary bean. Callus was initiated from young leaf tissue on MS medium with 1 mg/l 2,4-D. Three-week-old callus was then transferred to liquid MS medium. The proportion of densely cytoplasmic cells increased when the cells were cultured in L-6 medium with 10 mg/l 2,4-D. Filtration of the cells at each of five consecutive subcultures resulted in the isolation of

plant regenerating cell lines (Kumar et al. 1988a,b). The cell lines were maintained in L-6 medium with 1 mg/l 2,4-D and 0.5 mg/l zeatin. Partially differentiated green cell aggregates were obtained when the suspensions were transferred into liquid L-6 medium with benzylaminopurine (BA) or zeatin. These structures were transferred to L-6 agar medium with BA for further development. Complete plantlets were obtained on the same medium with naphthaleneacetic acid (NAA) and kinetin.

Pigeonpea. Cell suspensions were initiated by transferring the leaves of seven-day-old seedlings to liquid, modified L-6 medium with 5 mg/l 2,4-D and 1 mg/l BA. After two weeks the cells below 500 um were transferred to L-6 media containing 10 mg/l 2,4-D and 10% PEG (MW=4000). After three subcultures with filtrations at every step, a green cell suspension with isodiametric and densely cytoplasmic cells was obtained. The cell line was maintained in L-6 medium with 5 mg/l 2,4-D and 5.0% PEG. Somatic embryos were developed when small cell aggregates were transferred from maintenance to L-6 medium with kinetin, gibberellic acid (GA_3), and indolebutyric acid (IBA). These structures developed into plantlets when plated on L-6 medium with BA and 2% sorbitol.

Sorghum Plant Regeneration

Effect of Genotype. The minimum requirement for a tissue culture system is plant regeneration. To have an efficient system for in vitro selection, embryogenic callus must be identified and maintained for the duration of the selection and plants recovered after a lengthy period in culture. The initial problem is the identification of embryogenic (E) callus. Over the past two years, a large number of embryos from four cultivars have been plated. The cultivars Sc283 and M35 produced the most E callus, followed by Hegari, and RTx430 which produced the least E callus (Table 1).

Table 1. Effect of genotype on the percent of embryos planted which produced E callus.

Cultivar	N	Percent*
RTx430	320	1.30 ± 0.83
Hegari	776	5.49 ± 1.48
Sc283	860	20.66 ± 5.50
M-35-1	282	32.30 ± 12.15

* Mean ± standard error

Establishment of R_0 plants. The R_0 plants are often less vigorous and require greater care in the greenhouse than plants from seed. From an in vitro aluminum selection experiment, the fate of the plants were monitored. As total passage time increased, percent of sterile plants and premature deaths increased. Within a passage time, higher stress levels increased the percentage of sterile plants and premature deaths.

Sorghum Selection In vitro

Aluminum Selection Levels. The genotype Sc283 exhibits acid soil tolerance in the field. Our objective was to correlate and evaluate its tolerance to $AlCl_3$ *in vitro*. Stress levels of 150 ppm $AlCl_3$ and above caused callus death. At the 75 ppm $AlCl_3$ level, E callus weight declined and later increased suggesting adaptation to the stress (Figure 1). Plants have been recovered from all treatments except the 300 ppm level.

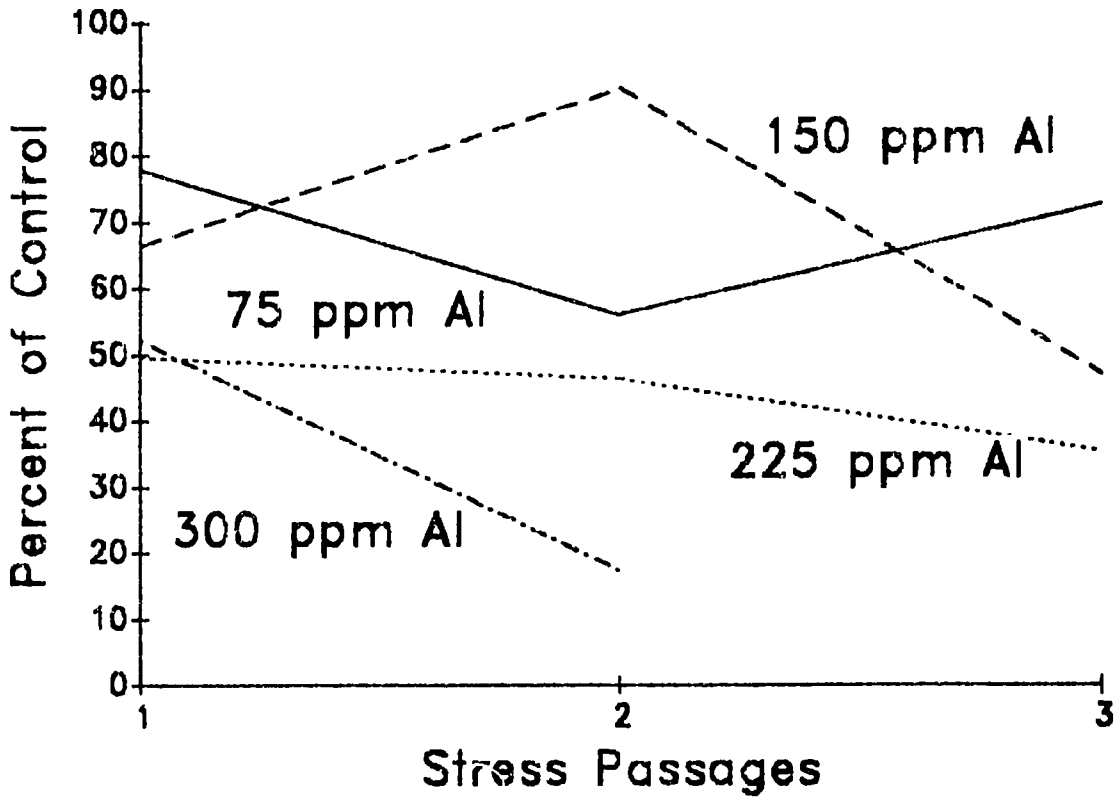

Figure 1. Effect of $AlCl_3$ on E callus growth as percent of control for the sorghum genotype Sc283.

Testing R_3 Seed. Seed (R_3) harvested from an R_2 plant (2024-7-19) growing on acid soils in Georgia was compared to non-regenerated Hegari on $AlCl_3$-containing media. The E callus weight of the R_3 line (identified as 2024) was similar to the Hegari. The R_3 seed was able to adapt to 200 ppm $AlCl_3$ added to the media while the Hegari could not (Figure 2). Shoots have been obtained from 2024 selected on 200 ppm Al but not from Hegari on 200 ppm Al.

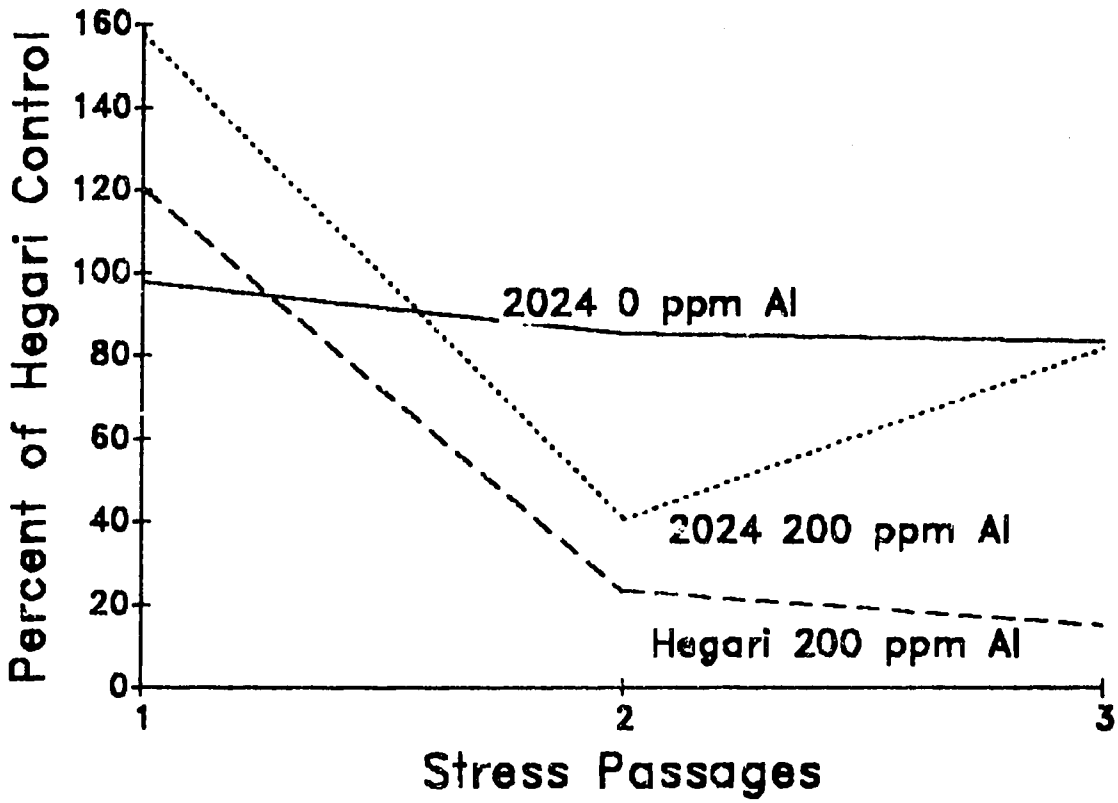

Figure 2. Effect of $AlCl_3$ on Hegari and on R_3 seed of an Hegari line collected from acid soil.

PEG Selection Levels. A derivative of M-35-1 from ICRISAT, genotype 4101 (M-35), developed by Dr. Charles Sullivan at University of Nebraska, has shown good drought tolerance. We have obtained the line to evaluate its in vitro tolerance to PEG.

The levels of 12 and 15% PEG reduced E callus growth from the initial passage on stress (Figure 3). The 9% PEG treatment enhances callus growth for three passages before callus growth declined. By the sixth passage, callus growth on all levels was less than 20% of the control. Plants have been obtained from the third passage on stress from all treatments.

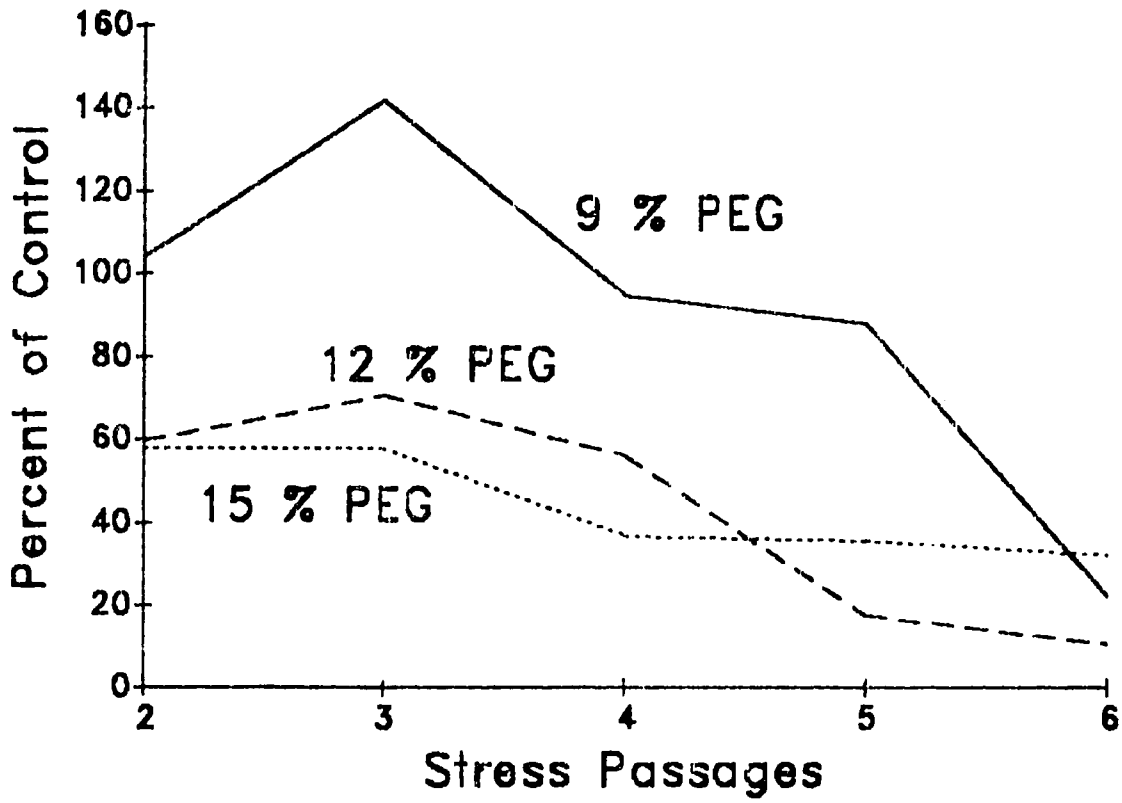

Figure 3. Effect of PEG on E callus growth of the sorghum genotype M-35 as percent of control.

Sorghum Field Evaluations

A total of 206 R_1 lines were grown as a seed increase in Georgia in 1986. Ninety of those lines were also grown on low pH soil (Figure 4). Only one line, 2075, derived from RTx430, set seed in that evaluation.

In 1986, there was a heavy, natural infestation of fall armyworm (FAW). Dr. David Isenhour from Tifton, Georgia evaluated the field and selected nine lines which had less feeding damage. In 1987 and 1988, these nine lines were evaluated under artificial infestation. One line (2442) has shown tolerance in both years to whorl feeding of the fall armyworm.

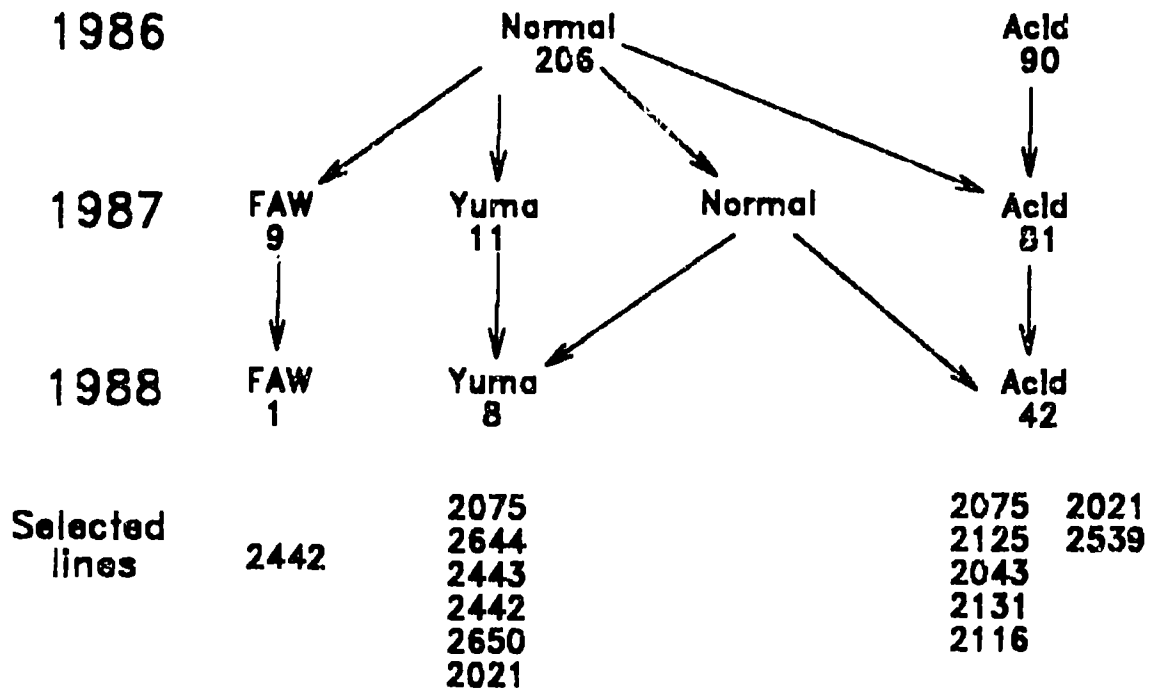

Figure 4. Flow diagram describing field sites and number of lines evaluated and a list of selected lines. FAW is fall armyworm evaluated at Tifton, GA. Normal is seed increase field at Griffin, GA and acid is a low pH field also at Griffin, GA. Yuma is a field site at the Yuma Experiment Station.

In 1987, 81 R_2 lines were evaluated on acid soils. Of the 81 lines, 13 had been tested as R_1 in 1986; 23 out of the 81 lines set seed. In 1988, 31 lines were evaluated on acid soil. They were selected based on performance in 1986 and 1987. All 31 lines were planted using seed from the 1987 acid plot. Two lines from the seed derived on acid in 1987 set seed; one line set seed in all replications. None of the 13 Hegari non-regenerated check plots survived in this area of the field.

In 1987, a site in Yuma, Arizona originally selected through Pioneer Hybrid Seed Company was planted as a salt evaluation. Eleven lines were evaluated and eight lines were re-evaluated in 1988. Five lines showed superior tolerance to the heat, salt, and drought conditions as compared to their non-regenerated checks.

In summary, of the 206 lines grown in 1986, 159 lines were evaluated for acid, 11 lines for drought, and 9 lines for FAW. One line in both the acid and salt environments and one FAW line have been identified as very promising. The acid-tolerant line and the drought-tolerant line were both from non-stressed cultures. The FAW lines were in culture 11 total passages with 5 passages on 9 g/l NaCl. These three lines—2021 (acid), 2075 (drought) and

2442 (FAW)--also showed tolerance to a second stress. Both 2442 and 2021 are among the top lines in Yuma and 2075, an RTx430 line, set seed in 1986 and 1988 on acid soils.

ACKNOWLEDGMENTS

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SESSION IV: NOVEL BIOTECHNOLOGIES

BIOTECHNOLOGY APPLIED TO POTATO IMPROVEMENT

John H. Dodds

ABSTRACT

The potato is an important basic food crop in many developing countries. It is also a crop plant which is highly amenable to the new techniques of biotechnology. It is susceptible to infection by Agrobacterium, and is easy to regenerate from somatic tissues thus making genetic engineering a feasible approach for genetic improvement. Experiments are described showing the use of transformation to insert genes for protein improvement, and pest and disease resistance. Other biotechnological techniques such as embryo culture, protoplast culture and anther culture are routinely used on potato. In more applied research potato tubers can be induced under in vitro conditions, this allows in vitro selection and improved methods for potato conservation and distribution.

INTRODUCTION

The application of tissue culture and rapid propagation methods for potato production continues to become more widely used in both developed and developing countries. While rapid propagation consists of a number of methods for rapid increase in the number of propagules, tissue culture techniques can be applied, not only to increase propagation rates, but also to modify the germplasm itself.

It is important to see tissue culture not as a scientific discipline but rather as a range of techniques. These techniques are of differing degrees of complexity forming a complete spectrum of technologies. The most important feature for agricultural application is the integration of these techniques to improve potato production in its widest possible sense. In this presentation, individual technologies of differing degrees of sophistication are analyzed indicating the existing and potential impact of the said technique on potato production.

For many years tissue culture has been applied to improve potato production by means of micropropagation, pathogen elimination, and germplasm conservation (Roca et al. 1979; Roca et al. 1978; Slack 1987); however, some of these techniques are still being refined and improved (Espinoza et al. 1987; Dodds et al. 1986). Intermediate level technologies, such as in vitro tuberization (Toval et al. 1985) and embryo and anther culture, are having some direct application on germplasm distribution and germplasm improvement (Sonnino 1984). The most sophisticated technologies, such as genetic engineering and protoplast fusion, have potential to improve potato production but care must be exercised in the translation of that 'potential' into reality. This article will analyze this technology spectrum in relation to its impact on potato improvement.

In vitro Germplasm Conservation

A number of tissue culture methods have been applied for conservation of potato germplasm in vitro, these include the use of growth-retarding compounds (Westcott 1981), reduction in incubation temperature (Schilde-Rentschler 1979), and less commonly, freeze preservation by cryopreservation (Grout and Henshaw 1978; Towill 1981). Most potato programs apply tissue culture germplasm conservation to some extent, as in the maintenance of a few genotypes used in a seed program, or they may have a major germplasm collection such as that at CIP with over 3000 clonal accessions of potato.

A number of advantages exist for in vitro germplasm collections over that of field maintained collections. The material is available year-round, it is protected from environmental and pathogen risks, and it is relatively simple to produce multiple copies of the collection to maintain duplicates in different geographical locations.

Use of Growth Retardants. A wide range of chemical growth retardants have been tested on in vitro potato plantlets. The objective in using these compounds is to lower the growth rate of the in vitro plantlets in order to lengthen the time between sub-cultures.

Maleic hydrazide (MN), an active compound in a number of commercially available growth retardants, has been shown to promote tuberization in cultured stem sections of S. tuberosum cv. British Queen (Harney et al. 1966). Diamonozide (B995) is normally used extensively as a foliar spray on ornamental plants such as chrysanthemum and azalea; however, Humphries and Dyson (1967) reported growth retardation and a 10% increase in tuberization after spraying it on plants of S. tuberosum cv. Majestic. Phenolic compounds such as trans-cinnamic acid (TCA) have been shown to stimulate tuberization from stem cuttings in vitro. Abscisic acid (ABA) is present in potato tubers and it is involved in the control of dormancy where it has been used to act as a natural growth retardant.

An alternative approach to use of growth retardants has been increasing the osmotic pressure of the medium, by adding sucrose or the metabolically inactive sugar alcohols mannitol and sorbitol, in order to reduce the water available to growing cultures (Cram 1984).

Growth Regulation by Reduced Temperature Incubation. Plants, like most organisms, live within a fairly restricted temperature range. Plant growth processes are under the control of a large number of regulatory enzymes. Biochemically, each of these enzymes has a well-defined temperature optimum. It follows, therefore, that if the in vitro plants are maintained at temperatures either significantly above or below this optimum, their growth will be restricted, and care must of course be taken not to over-stress the plant. If temperatures fall much below 320° C in potato, then cold damage will kill the plants; likewise temperatures much above 28° C will cause excessive heat stress. Within this range of temperatures, plants will survive and grow; however, the time period between sub-cultures changes markedly on either side of the 6° C mark in potato. To date, 6° C seems to be the optimal growth inhibition temperature for potato plantlets while maintaining high viability (Henshaw et al. 1980).

Although some experiments were performed to study survival of material maintained between 0° C and -12° C, the success of these experiments was limited. Some success has been achieved by dropping to an extremely low temperature (-196° C) using techniques of cryopreservation (Withers 1985; Bajaj 1979; Kartha 1985; Henshaw et al. 1985; Grout and Henshaw 1980).

Cryopreservation of Germplasm. A comprehensive review by Withers (1980) (and references cited therein) has discussed fully all aspects of the cryopreservation of plant cell, tissue and organ cultures. Only the basic principles and methodology of freeze-preservation will be discussed in the following sections, since any attempt to briefly survey and discuss the literature already available would go beyond the scope of this paper.

The successful cryopreservation of a particular plant tissue requires that damage caused by ice crystal formation within the individual cells of the tissue is either prevented or minimized. This has led to the adoption of two basic approaches to cryopreservation: either ultra rapid freezing or slow/stepwise freezing. The process of rapid freezing results in the formation of ice crystals within the cells which are of microscopic size, and which do not disrupt the internal organelles and membranes of the cells; however, thawing must be carried out rapidly enough to prevent recrystallization. Henshaw (1975) has reported the use of this method for successfully freeze-preserving potato shoot-tips.

Pathogen Elimination

The use of meristem culture coupled with thermotherapy or chemotherapy has for many years been an important component of pathogen elimination in potato seed programs. The techniques cannot only increase marketable yield by producing pathogen-tested plants, but also facilitate germplasm exchange by removing virus infections which allow compliance with quarantine regulation (Hewitt and Chiarappa 1977). The establishment of pathogen elimination programs has greatly aided the international distribution of potato germplasm and, indirectly, potato production. It is worth noting, however, that experiments are in progress in several institutions to streamline pathogen (virus) elimination procedures. The use of a wide range of antiviral chemicals included in the culture medium and *in vitro* heat treatment of *in vitro* plantlets should lead to more efficient general pathogen elimination procedures (Slack 1987).

In vitro Micropropagation

Many potato seed programs used *in vitro* pathogen-tested plantlets as starting material. The initial stages of the seed program can make use of varying amounts of *in vitro* micropropagation depending on the size and location of the program. The basic methods used, however, are very similar in most institutions and are based on the rapid growth on solid or liquid culture media of single node cuttings or stem explants. The basic micropropagation methods used at CIP and many other institutions are as described next:

Single Node Cutting Procedure. Single nodes with leaves are excised from small *in vitro* plantlets; in some genotypes the large leaves are carefully removed. The removal of the leaves ensures uniform growth of the newly developing shoots. If large leaves are left on the single node pieces it is believed that hormones from the senescing leaf can inhibit growth of the newly

developing shoot. Each node is then inoculated onto the surface of agar-solidified medium (Espinoza et al. 1984). The axillary bud quickly grows out (Figure 1), and in three to four weeks a plantlet with six or seven more nodes becomes available for sub-culture.

Liquid Shaken Cultures. This type of culture is performed to rapidly produce large numbers of nodes for subsequent nodal cutting preparation. Stem cuttings, each with three to four nodes, are taken from the plantlets; the large leaves are removed. Each stem piece is placed in 15 ml of liquid medium (Espinoza et al. 1984) and the flasks are shaken at 80 rpm. After two to three weeks of rapid growth, each flask contains 60 to 70 nodes.

Once a suitable number of small plantlets have been produced, single node cuttings are prepared and grown in vitro until they can be transferred to beds or pots for tuber production (See Figure 1).

Rooting and Transfer to Non-sterile Conditions. After removal of the large leaves, between 16 and 25 excised, single nodes of in vitro plantlets are placed on agar-solidified medium in a suitable container. When the plantlets are 3-5 cm high and have developed a good root system (Figure 1), they are ready for transplanting into pots or beds containing a suitable, highly organic mixture (Figure 1).

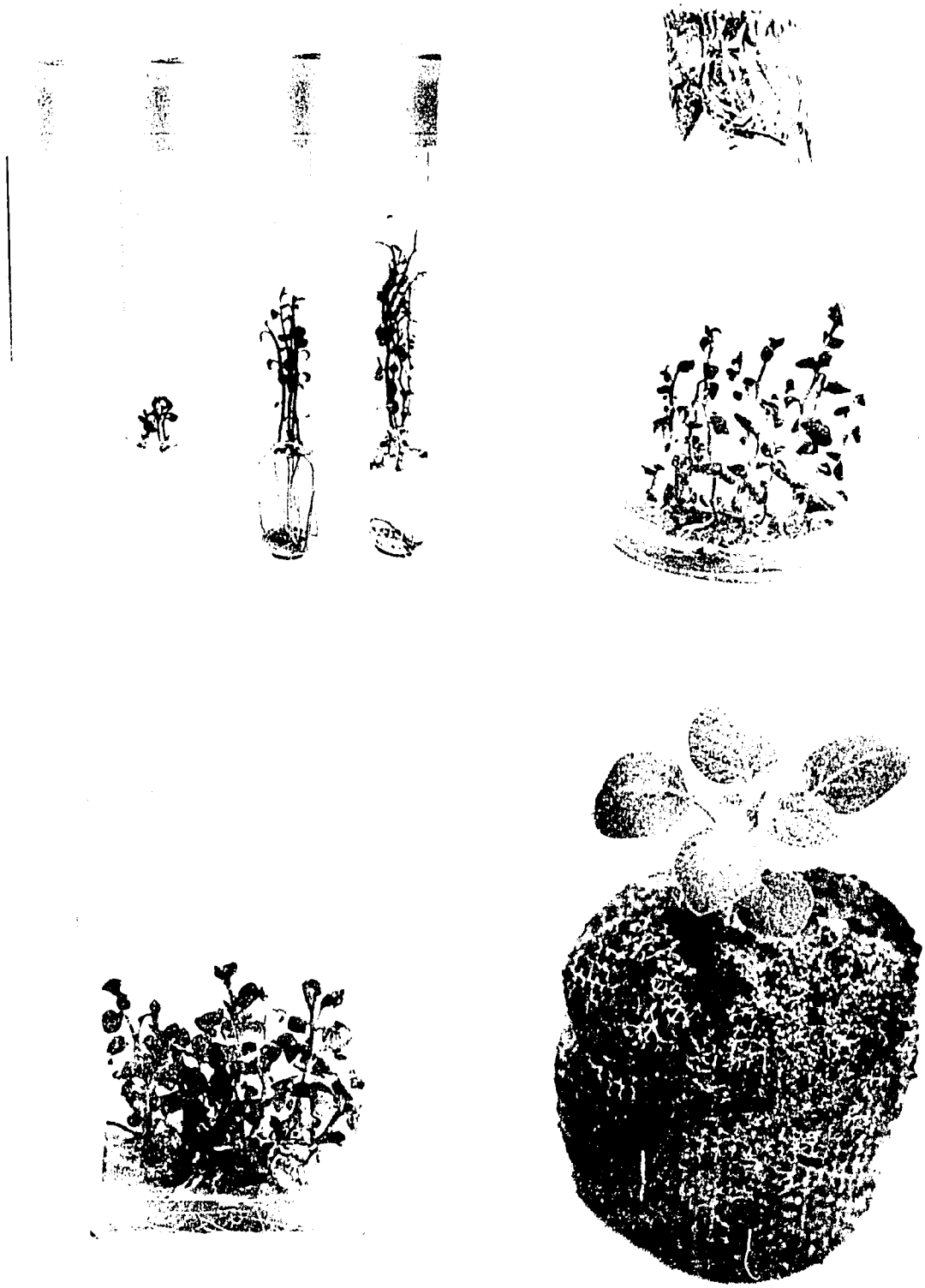

Figure 1. (a) Growth of single mode cuttings on solid media. (b) Shaken cultures with stem segments in liquid medium containing GA_3. (c) Plastic box with individual rooted plantlets ready for transfer to pots or seed beds. (d) <u>In vitro</u> plantlets a few days after transplant to jiffy pot.

These basic technologies have enhanced dramatically the amount of high quality seeds produced in many programs. In recent years, a number of institutions have been using in vitro potato plantlets transplanted at high densities (i.e. $100/m^2$) to produce small, high quality tubers for subsequent field multiplication.

In vitro Tuberization

In recent years, interest has developed in many countries on the induction of potato tubers under in vitro conditions. Several different methods are available to bring about the induction process (Towill 1981; Estrada et al. 1986). At CIP, we have developed a rapid, cost effective method that involves the addition of Chlorocholine Chloride (CCC) and sucrose to the liquid medium used for propagation (Tovar et al. 1985). We have shown that this technique is not only rapid and efficient but is applicable to a wide range of potato genotypes.

Figure 2a shows diagramatically the basic methods used for the induction process, while Figure 2b shows tubers induced under these conditions. Institutions in many developed and developing countries have started in vitro tuberization programs. The tubers are normally utilized in two different ways: 1) for distribution of germplasm either nationally or internationally or 2) as an additional component to the standard methods of rapid propagation used in a seed tuber production program.

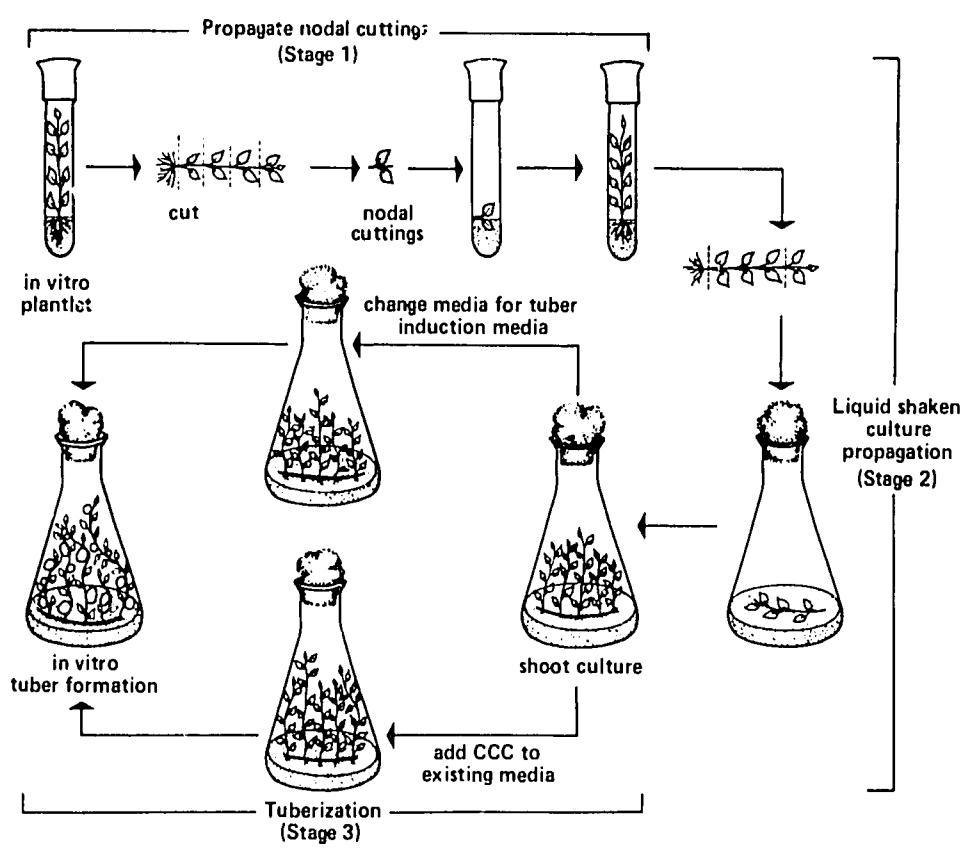

Figure 2a. Schematic representation of the basic methodology used for in vitro tuber induction at CIP.

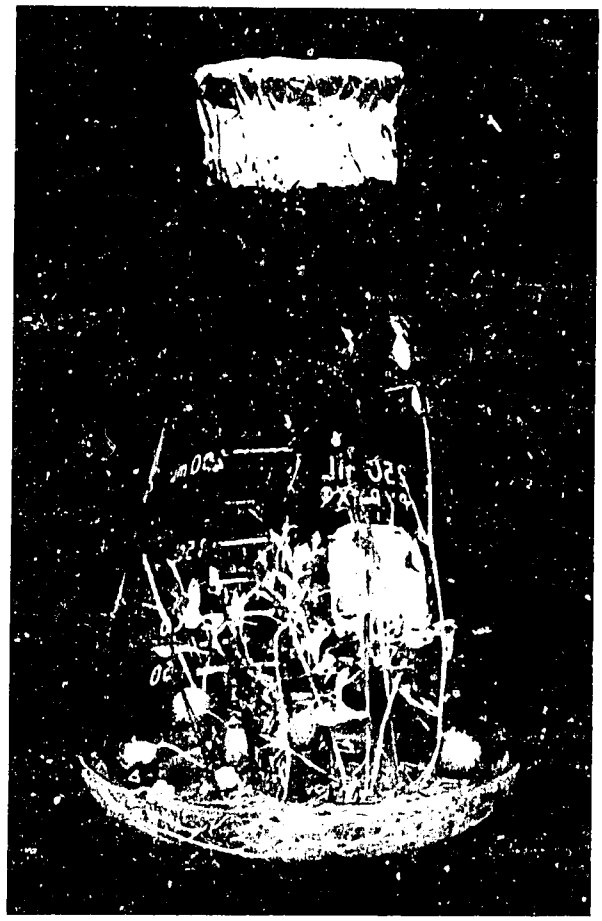

Figure 2b. A typical flask containing in vitro tubers after one month on induction medium.

At CIP, the use of in vitro tubers offers some advantages for distribution of germplasm. The tubers are produced under in vitro conditions from pathogen-tested plantlets and thus comply easily with international quarantine requirements. Unlike in vitro plantlets, however, if the package is delayed in transit there are fewer problems of losing the material. Figure 3a shows freshly packed, in vitro tubers for export while Figure 3b shows the same tubers after eight weeks at 22° C packed in the same way as distributed tubers; clearly even after this period of time material can still be utilized.

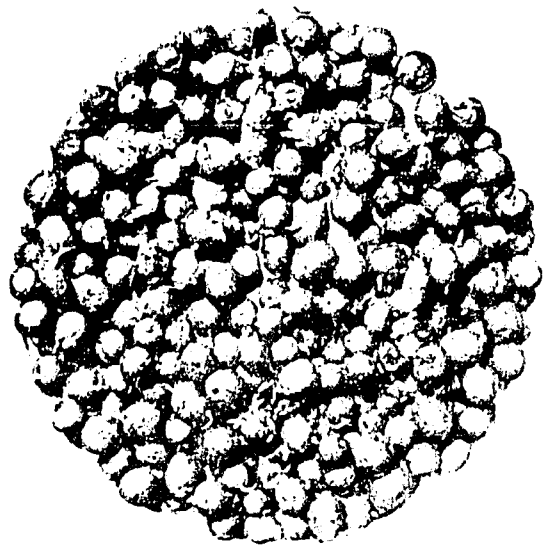

Figure 3a. Sterile petri dishes containing in vitro tubers, these dishes are packed with cotton wool or vermiculite and shipped air freight to their destination.

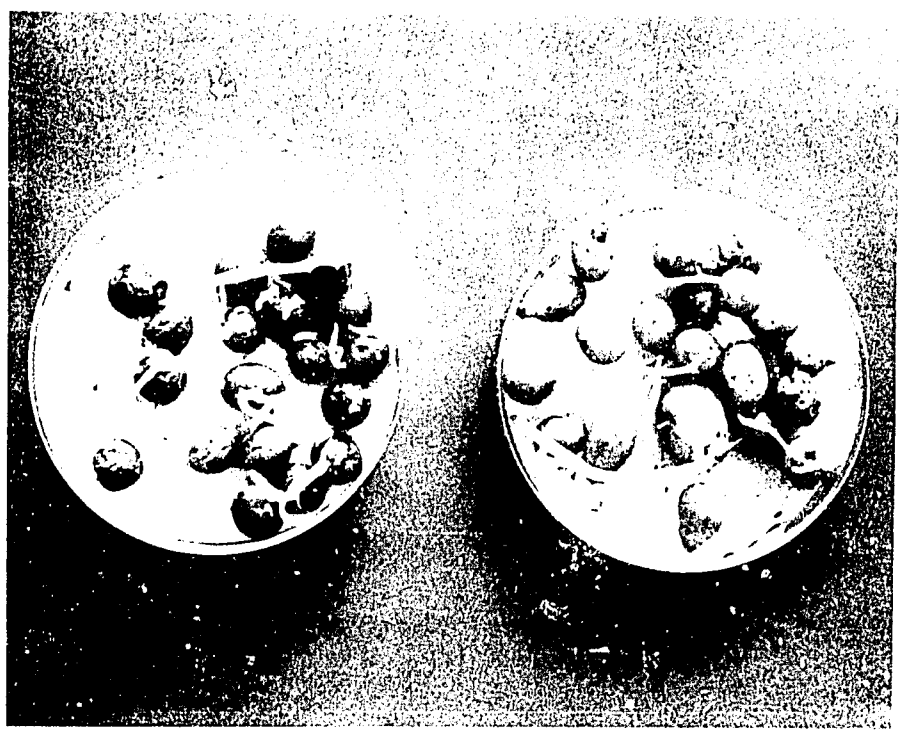

Figure 3b. A similar group of in vitro tubers after four months of storage at room temperature. Although sprouts are long, plantlets can be rescued from this material.

Molecular Biology and Cell Genetic Manipulation

In collaboration with the Biochemistry Department at Louisiana State University (LSU), CIP has developed a project to insert genes into potato plants by cell genetic manipulation. In this project, *Agrobacterium* plasmid vectors (Jaynes *et al*. 1986; Dodds 1987) are being used in a system of gene transfer similar to those being employed by several laboratories worldwide, including laboratories in Europe, the United States, Brazil, Mexico, and India. The primary objective of the project is to enhance the nutritional value of the potato by obtaining the supplementary production of a synthetic protein rich in essential amino acids. The synthetic protein is produced from a gene that has been synthesized by machine in the LSU laboratory (Figure 4).

Through this research collaboration, inserting the synthetic gene into potato and sweet potato plants has been successful. In the case of potato, evidence has also been obtained that the gene is transcribed and produces a corresponding messenger RNA (mRNA), which is then translated in the plant to produce the synthetic protein (Espinoza *et al*. 1987). Figure 4 shows these translational steps. This gene synthesis, cloning and transfer project has shown that the technology now exists to introduce new and valuable traits into the potato. However, much more research, as well as possible regulatory measures by the respective countries will be needed before these transgenic plants can be released and used in national programs.

On the basis of the success with the synthetic protein gene, CIP is making plans to use the gene cloning and transfer technologies to introduce resistance to insects and diseases into potato (Jaynes *et al*. 1987). Through collaborative links, CIP hopes to use the technologies to generate potato germplasm with resistance to viroid and virus infections by inserting gene sequences that will interfere with virus and viroid replication. This approach would allow potato plants to be grown for more generations in the field before degeneration of seed due to increased infestation by these pathogens.

Collaborative research is also being developed to obtain genes for resistance to diseases caused by bacteria such as *Pseudomonas* and *Erwinia*. For these experiments, we are using genes that have been purified and that code for proteins with known, potent antibacterial activity. Such genetic engineering approaches to bacterial disease resistance in potato may allow us to develop clones with resistance to both *Erwinia* and *Pseudomonas*. Hopefully these procedures will not adversely affect the existing, agronomically important characters of the plants.

Gene Fragment 1

READING DIRECTION OF TOP STRAND (A)->->->

AATTCGGGGATCGTAAGAAATGGATGGATCGTCATCCATTTCTTCATCCATTTCTTAC
GCCCCTAGCATTCTTTACCTACCTAGCAGTAGGTAAAGAAGTAGGTAAAGAATG

GATCCATCCATTTCTTAAGAAATGGATGAAGAAATGGATGACGATCCATCCATTTCTT
CTAGGTAGGTAAAGAATTCTTTACCTACTTCTTTACCTACTGCTAGGTAGGTAAAGAA

CATCCATTTCTTCATCCATTTCTTACGATCAAGAAATGGATGAAGAAATGGATGAAGA
GTAGGTAAAGAAGTAGGTAAAGAATGCTAGTTCTTTACCTACTTCTTTACCTACTTCT

AATGGATGAAGAAATGGATGCATCCATTTCTTAAGAAATGGATGAAGAAATGGATGAA
TTACCTACTTCTTTACCTACGTAGGTAAAGAATTCTTTACCTACTTCTTTACCTACTT

GAAATGGATGACGATCGATCGTAAGAAATGGATGACGATCCATCCATTTCTTACGATC
CTTTACCTACTGCTAGCTAGCATTCTTTACCTACTGCTAGGTAGGTAAAGAATGCTAG

CCCG
GGGCTTAA

<-<-<-READING DIRECTION OF BOTTOM STRAND(B)

SEQUENCE OF PROTEIN A->->->

GlyAspArgLysLysTrpMetAspArgHisProPheLeuHisProPheLeuThrIleHisProPheLeu->
->LysLysTrpMetLysLysTrpMetThrIleHisProPheLeuHisProPheLeuHisProPheLeuThr->
->IleLysLysTrpMetLysLysTrpMetLysLysTrpMetLysLysTrpMetHisProPheLeuLysLys->
->TrpMetLysLysTrpMetLysLysTrpMetThrIleAspArgLysLysTrpMetThrIleHisProPhe->
->LeuThrIlePro

SEQUENCE OF PROTEIN B->->->

GlyAspArgLysLysTrpMetAspArgHisProPheLeuThrIleAspArgHisProPheLeuHisPro->
->PheLeuHisProPheLeuLysLysTrpMetHisProPheLeuHisProPheLeuHisProPheLeuHis->
->ProPheLeuAspArgLysLysTrpMetLysLysTrpMetLysLysTrpMetAspArgHisProPheLeu->
->HisProPheLeuLysLysTrpMetAspArgLysLysTrpMetLysLysTrpMetThrIleHisProPhe->
->LeuThrIlePro

Figure 4. (a) A synthetic DNA sequence coding for a protein rich in essential amino acids (SP47). This gene can be translated in either direction A or B to produce two synthetic proteins (1) and (2) whose amino acid composition is shown in (b).

CONCLUSIONS

The range of sophisticated techniques available in tissue culture is clearly a broad one. Some of these techniques are already having a direct and significant impact on potato production and their use is widespread. Other techniques, which offer great potential for direct or indirect improvement of potato production, are still at a developmental stage and time is needed before the possible benefits of these types of research are transferred to farmer fields.

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GENETIC APPROACHES TO STRESS TOLERANCE IN PLANTS

Oluf L. Gamborg, J. T. Colbert, I. John, Y. Zafar, A. S. Kumar, M. W. Nabors

ABSTRACT

Several genetic approaches are being investigated in attempts to transfer stress tolerance to crop species. The principal ones are intergeneric crosses and gene cloning and transfer technologies. The source materials are highly tolerant, but distantly related species. A species known as kallar grass, (Leptochloa fusca) which is extremely salt-tolerant and also has associative nitrogen fixation and C4 photosynthesis, is the source material. The kallar grass is being used in the production of hybrids with rice (O. sativa cv. Basmati-370) by protoplast fusion as a means of transferring salt tolerance to rice. Plant regeneration through tissue culture has been achieved in both species. The necessary protoplast technology is being developed.

The objective of the molecular biology program is to obtain genes which can be used to confer salt tolerance to crop plants. The cell materials being used are suspension cultures of kallar grass. The cells are salt stressed at 20 g NaCl per liter of medium. Subtraction (cascade) hybridization has been used to obtain an enrichment of cDNA corresponding to mRNA species that are induced by salt stress. A cDNA library has been prepared using poly(A)RNA isolated from salt-stressed cells.

INTRODUCTION

Salinity remains one of the oldest and most serious environmental problems. The total area affected by mineral toxicity is about 25% of the world's potentially arable land (Raghava Ram and Nabors 1985). Saline soils are found in North America as well as in many developing countries. In South and South East Asia alone an area of fifty million hectares is adversely affected by salinity (Boje-Klein 1986). The areas affected by salinity are increasing. Nearly one third of the 230 million hectares irrigated worldwide has become saline (McWilliams 1986). In Pakistan alone, 40,000 hectares are lost annually due to salinization (Jones and Gorham 1986).

A large number of plant species are productive under a wide variety of environmental stresses. Tolerance to stress conditions varies between species due to differences in heritable traits. Plants have been observed to grow in extremes of salinity combined with alkalinity or acidity with aluminum toxicity or drought. Such highly tolerant species have potential as germplasm for introducing tolerance into crop plants.

Halophytic species grow throughout their life cycle in salt marshes, estuaries or saline deserts, which demonstrates that plant production is feasible under saline conditions (Shay 1988). Such highly tolerant species are potential sources of genes for the salt tolerance traits. The halophytes have the genetic, biochemical and structural features for coping with high salinity and sometimes in combination with alkalinity (Malik et al. 1986). Any information on the genetics of the salt tolerance traits, particularly at the molecular level, would be valuable and facilitate a systematic transfer of

salt tolerance across incompatibility barriers in plants. The information gained through molecular biology also would be an avenue towards an understanding of salt tolerance genes and their regulation.

Standard plant breeding through sexual crossing is being employed on a modest scale to develop salt-tolerant cultivars and progress has been made in certain crops (Akbar 1986; Srivastava and Jana 1986). Such transfer of salt tolerance from alien, tolerant species to crop plants is being attempted in wheat (Dvorak and Ross 1986; Mujeeb-Kazi and Bernard 1985) and in rice (Akbar 1986). Information on the genetics of salt tolerance is very limited (Shannon 1984); however the trait is probably polygenic (Tal 1984). Although considerable information is available on the physiology of how plants cope with salinity, the identity and regulation of salt tolerance genes are still to be determined (Tal 1984; Lauchli 1984). The technologies of molecular biology, genetic manipulation, and tissue culture (biotechnology) have advanced to permit their application in the search for genes and gene products related to salt tolerance and the transfer from alien species to crop plants (Fillatti et al. 1987; Beachy et al. 1987). There is evidence for salt tolerance response proteins in crop species, but no report at this time that they are directly related to stress tolerance.

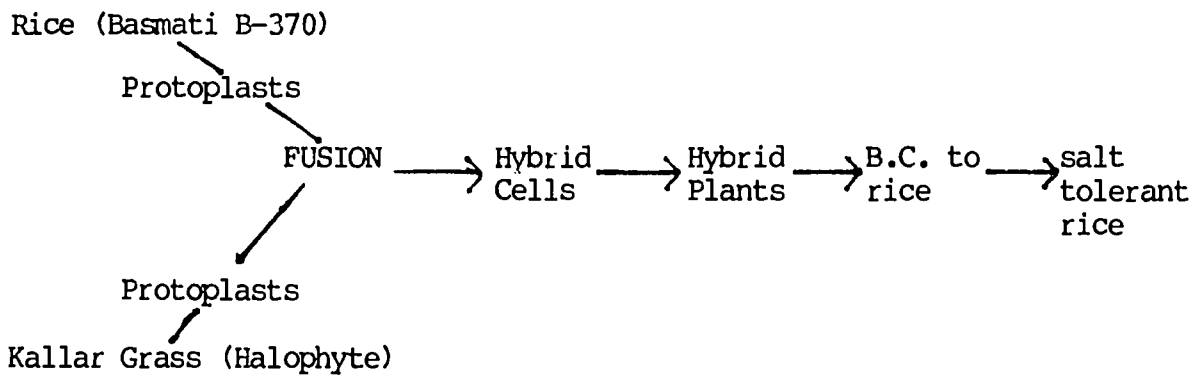
The Tissue Culture for Crops Project and its collaborators are investigating the use of protoplast fusion hybridization and molecular biology in attempts to transfer salt tolerance from wild species to crop plants. One of the procedures consist of producing somatic hybrids between a wild species and rice by protoplast fusion. The somatic hybrids will then be backcrossed to rice to transfer the trait. Molecular biology is applied to obtain genes which could be used to confer salt tolerance to crop plants. These studies include a halophytic grass species and a cereal.

Salt Tolerance Through Protoplast Fusion

The technologies of protoplasts, including the production of protoplast fusion hybrids, has been available for some time (Gamborg et al. 1974; Kao et al. 1974). The original research indicated the biological and technical feasibility of the procedure. The approach has been used in combination with breeding programs in several crop plants to produce hybrids of species and genera (Austin et al. 1985; Pelletier 1984; Toriyama et al. 1987).

Protoplast fusion hybridization is being implemented at TCCP to produce hybrids as a means of transferring salt tolerance and other traits from a halophytic grass species to rice. The project is a collaboration between TCCP and the Nuclear Institute for Agriculture and Biology (NIAB), Pakistan. The rice (*Oryza sativa*) is the fine grained, uniquely flavored, indica type Basmati (B-370), which has very low tolerance to salinity. The Basmati is the principal export rice for Pakistan. The grass species is *Leptochloa fusca* (kallar grass) which is halophytic. Kallar grass is a primary colonizer in salt affected soils of Pakistan (Malik et al. 1986). The plants are also photosynthetically efficient (C4) and have associative nitrogen fixation (Malik and Zafar 1986). Thus there are prospects for transferring several traits to rice through the hybridization process. The basic procedures for accomplishing somatic hybridization of the two species are not available and thus must be developed.

The following is an outline of the strategy for the transfer of salt tolerance from kallar grass to rice by protoplast fusion hybridization:



B.C. = backcross

It is necessary to develop the plant regeneration procedures and the essential protoplast technologies for each of the species before proceeding to the fusion hybridization (Gamborg 1986).

Plant regeneration. Methods have been developed for callus induction and plant regeneration from mature embryos of the Basmati rice (cv. 370). In the MS or N6 media (Gamborg 1985), the callus consisted of embryogenic (E) and non-embryogenic (NE) sections when cultured in the presence of 2,4-D and kinetin or benzyladenine (BA) (Zafar et al. 1989). When the E callus was transferred and cultured on MS medium with 3 uM IAA and 2 uM BA, plant regeneration was achieved. The cultures were maintained on the same medium for more than eight months following which it was also possible to regenerate plants. Embryogenic calli were also produced from kallar grass tissues and plants were regenerated. Both callus initiation and growth were extremely slow. The medium which supported callus growth was the AA composition by Toriyama et al. (1986). It was observed that the efficiency of callus formation and plant regeneration was reduced with increasing age of the seeds.

The strategy for producing somatic hybrids will require the use of protoplasts and the regeneration of plants from single cells. Suspension cultures are a useful source of protoplasts. It has been possible to establish rapidly growing cell suspension cultures of basmati rice and kallar grass (Zafar et al. in preparation). The development of a protocol for plant regeneration from the suspension cultures has not been feasible. Work is in progress to develop the necessary protoplast technology, including plant regeneration, of the two species.

Molecular Biology of Stress Tolerance

The modification of gene expression is a common occurrence in plants exposed to environmental stress (Sachs and Ho 1986). When plants are exposed to a stress they frequently produce stress-induced proteins. Heat shock proteins have been observed in several plant species (Key et al. 1985;

Mansfield and Key 1987). Specific proteins have also been detected in plants exposed to drought stress (Vartanian et al. 1987).

Exposure of plants and cultured cells to salinity also has been observed to result in gene activation and production of novel proteins. Barley plants exposed to salt stress produced 12 mRNA's in roots and 9 mRNA's in shoots which coded for polypeptides of 18 to 50.5 KD (Ramagopal 1987). Polypeptides were also induced in tobacco cells exposed to NaCl concentrations of up to 25 g per liter. A 26 KD protein was studied in salt-adapted and unadapted cells (Singh et al. 1985). King et al. (1988) isolated a cDNA coding for the 24 KD polypeptide from salt-stressed tomato roots. Results of studies on wheat and wheat-Elytrigia hybrid plants have shown the occurrence of up to 18 polypeptides, which were either repressed or up-regulated during salt stress (Gulick and Dvorak 1987).

Molecular Genetics Program at TCCP

The goals of the molecular biology program are to obtain the genes which could be used to confer salt tolerance to crop plants. The rationale is to investigate species which tolerate and complete their life cycle growing in high levels of salt and attempt to isolate the genes responsible for the salt tolerance trait. Since one of the principal objectives is to transfer salt tolerance to cereals, such as rice and wheat, the salt-tolerant species being used is the halophyte kallar grass (Leptochloa fusca) (Malik et al. 1986). The experimental materials being used are suspension cultures. Results have shown that, when the cells are cultured in media containing 20 g per liter of NaCl, the growth is reduced to about 50%. Such cells are considered to be stressed.

Initial experimentation has been performed to determine the presence of stress-induced proteins in cells of the kallar grass. That was investigated by isolation of total RNA from which the Poly(A)RNA was obtained. After in vitro translation of the poly(A)RNA, the polypeptides were analyzed by SDS polyacrylamide gel electrophoresis. There were no detectable stress-induced polypeptides observed from cultures stressed with up to 3% NaCl for up to 96 hours; however, this analysis would only detect changes in relatively abundant poly(A)RNA species. Dramatic changes in the levels of relatively rare poly(A)RNA species would be undetectable.

The research is a collaborative program with Dr. J. Colbert, formerly of CSU's Department of Biology and now of the Iowa State University Botany Department.

Cascade (Subtraction) Hybridization

The method of subtraction hybridization (Sargent 1987) has been employed as an approach to detect subtle changes in gene expression. The method makes it possible to obtain an enrichment of low level mRNA's as the complementary DNA clones, which can be used as probes. In the procedure poly(A)RNA from cells, which had not been salt stressed, were hybridized with single-stranded cDNA derived from salt-stressed cells. The hybridization mixture was then fractionated on a hydroxyapatite column. The cDNA species corresponding to mRNA species present in the non-stressed cells form RNA-DNA duplexes. The resulting double-stranded molecules bind to the column under the conditions used. The cDNA species that corresponded to the mRNA species, which were

present at the elevated levels in the poly(A)RNA isolated from salt-stressed cells, remained single-stranded and did not bind to the hydroxyapatite. The results of initial experiments indicated that approximately 17% of the radioactivity in the cDNA population from salt-stressed cells was increased in abundance relative to the mRNA population in the non-stressed cells. These results show that it may be possible to enrich the population of cDNA's from salt-stressed cells of kallar grass for messages which are induced by salt. The procedure is being used to prepare salt stress-enriched cDNA probes for use in the screening of the cDNA library. Such a library has been produced from poly(A)RNA isolated from salt-stressed cells of kallar grass. The library consists of about one half million clones with an average size of 1000 BP.

Salt Stress Regulated mRNA Induction in Rice

Most crop species are sensitive to salt stress to varying degrees (Maas 1986). During salt stress, the plants of a particular species may respond by producing specific proteins. The research rationale in the TCCP program is to investigate plants of particular crops in which cells or regenerated plants have been selected for increased salt tolerance by *in vitro* screening. The investigation to date has focused on studies on rice cells to determine their response to salt stress. The response was measured by the production of polypeptides by *in vitro* translation using poly(A)RNA from salt-stressed and non-stressed cells.

Experimental Procedures. A cell suspension culture (RB 387) of Basmati rice (B-370) was used for the experiments. The cells were cultured in L6 medium (Kumar *et al*. 1988) and were used six days after subculture as inoculum. In the initial experiments, the cells were tested at 0 to 30 g per liter NaCl for time periods of up to 48 hours. After treatment, the cells were harvested, frozen in liquid nitrogen, and stored until RNA isolation. Cells of the same age were also used in experiments to test the effect of other salts ($NaSO_4$, KCl, $CaCl_2$ and $AlCl_3$, $MgCl_2$) at similar concentrations. Tests with PEG at 300 mg/l and ABA at 10 mg/l were also included. In these experiments the cells were harvested after 24 hours. The effect of salt stress was studied with seedlings grown in hydroponics in nutrient solutions which contained up to 30 gm/l NaCl. Total RNA was isolated from each sample. The poly(A)RNA was then isolated by affinity chromatography on a poly(U) Sephadex column (Colbert *et al*. 1985). The *in vitro* translation was performed with the poly(A)RNA in a nuclease-treated, rabbit reticulocyte lysate. The polypeptides were analyzed by electrophoresis using 18% SDS polyacrylamide gel. The gels were stained with Coomassie blue, dried and exposed to Kodak X-ARS film. The results to date have shown that several polypeptides are produced in response to stress by NaCl, $AlCl_3$, PEG as well as ABA (Table 1).

Table 1. Stress Induced Proteins Produced by Rice Cell Cultures and Seedling Tissues in Response to Stressing Agents.

Stressing agent	Proteins (KD)
CELL CULTURES:	
Sodium chloride	16.3, 20, 24
Aluminum chloride	26, 39.8
Abscisic acid	20, 21.4, 24, 30, 39.8
SEEDLING/SHOOTS:	
Sodium chloride	17.6, 20, 40, 47, 51.5
SEEDLING/ROOTS:	
Sodium chloride	31.6, 51.5, 53.4

In rice shoots five different mRNA's were induced that encoded proteins of 17.6 to 51.5 kDa within twelve hours of salt stress, while the synthesis of three other proteins of 53.4, 51.5, and 31.6 kDA were enhanced in seedling roots in response to stress. The mRNA which encoded 51.5 kDa protein was induced both in shoots and roots exposed to NaCl for twelve hours or more.

In cell cultures exposed to NaCl, the proteins of low molecular weight, ranging from 16.3 to 24 kDa, were overproduced. Most of the mRNA's encoding these proteins were induced within twelve hours in cells exposed to $\geq$ 0.75% NaCl. These proteins were also accumulated in response to $CaCl_2$, Na_2SO_4 and KCl, but not with $AlCl_3$ or $MgCl_2$. The cells exposed to ABA for 24 hours triggered the transcription of mRNA's found in both salinity and non-osmotic metallic ion-exposed cells. These findings suggest that tissue specific transcriptional mechanisms regulate gene expression in rice when exposed to stress factors.

OPPORTUNITIES AND ADVANTAGES OF BIOTECHNOLOGY

The overriding advantage of using tissue culture and associated biotechnologies is improved efficiency. The data in Table 2 makes a comparison between using standard breeding procedures alone and in combination with tissue culture and biotechnologies. Generally, the time required for the development of new varieties could be reduced by as much as one-half by incorporating a biotechnology procedure. These are estimates and are based on crops in which the technologies are available for application.

Table 2. Variety development for yield and quality traits* by genetic improvement.

Traits	Comparative Time Requirements (years)	
	Standard Breeding	Biotechnology and Standard Breeding
Stress tolerance	6-8	4-6
Disease resistance**	8-10	4-6
Insect resistance**	8-10	4-5
Weeds/herbicide tolerance	7-8	5-6
High solid		5-6
Increased protein quality		feasible

* Multigenic traits
** These are ongoing efforts because genetic changes occur in the pathogen and pest

The genetic engineering technologies may have particular potential for significant economic pay-offs and crop security because of "added value" of improved nutrition, yield enhancement, and more permanent resistance traits. It has been estimated that by 1991 most of the major annual crop plants will have been subjected to genetic manipulation and field testing will be in progress or completed (Ratafia and Purinton 1988). The development of the newer technologies and the basic information has been confined largely to countries which are relatively more advanced industrially. The countries which at this time are less developed and have relatively less economic resources and skilled expertise could now begin to take advantage of these technologies and information.

It is important that collaboration be encouraged and established between centers and scientists in developed and developing countries. The initial needs in developing countries are usually a shortage of trained personnel and leaders, and limitations in facilities. It is imperative that any consideration to implement biotechnology be preceded by the preparation of a plan with short-term and long-term objectives and goals. The plan should include provisions for training and facility development.

The countries which are less developed agronomically might have the most to gain from applications of biotechnology. The reasons for that are the greater efficiency and effectiveness of the procedures which could result in more rapid progress in advances in production practices and improvement strategies.

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ROLE OF MOLECULAR TRANSFORMATION IN CROP IMPROVEMENT FOR DEVELOPING COUNTRIES

Roger N. Beachy

ABSTRACT

Several years ago we reported that introducing a gene encoding the coat protein (CP) of tobacco mosaic virus (TMV) by transformation into tobacco and tomato plants conferred resistance to infection by tobacco mosaic virus. It was later demonstrated that these "transgenic" tomato plants had a high level of resistance under field situations.

Subsequent to the report of engineered coat protein-mediated protection against tobacco mosaic virus, other research groups reported that a similar approach was used to protect plants against infection by alfalfa mosaic virus, potato virus X, cucumber mosaic virus, and tobacco rattle virus. Each of these examples lead us to conclude that resistance against a variety of different viruses can be achieved in transgenic plants by introducing a gene that encodes a coat protein identified to that of the virus against which resistance is desired.

To apply this type of engineered coat protein protection to a targeted plant, it is essential to have 1) a basic understanding of the pathogen, 2) a cloned DNA representing the viral coat protein, 3) the necessary DNA fragments (transcriptional promoter and polyadenylation signal) to produce a functioning chimeric plant gene encoding the coat protein, 4) an appropriate gene delivery system, and 5) a tissue culture system to regenerate plants from cells. Although well-equipped research labs in many locations may be able to carry out the entire process, other lab groups will need to collaborate to achieve their goals. Nevertheless, such collaborations may be useful and essential to achieve resistance against virus diseases for which other more classical forms of resistance are lacking.

INTRODUCTION

The development of techniques for genetic transformation of plants has lead to the production of plants with novel characteristics (Weising et al. 1988). Plants resistant to a variety of herbicides, specific insects and virus diseases have been the outcome of the introduction and expression of single genes in transgenic plants. It is anticipated that seeds carrying these novel gene traits will be available for distribution and sale by the mid-to-late 1990's.

While the techniques that are used to create genetically superior plant varieties have been developed primarily in U.S. and European laboratories, the applications will have great impact on agricultural problems in developing countries. This is due in large part to the fact that gene transfer technologies will bring new solutions to agricultural problems that previously had no solution or which required expensive control measures. However, because of the relative sophistication and need for specialized techniques

that are involved in genetic modification, including gene isolation and characterization, gene transfer and selection and regeneration of plants from modified cells, it is essential that research groups in less developed countries establish collaboration with advanced laboratories in order to take advantage of these technical developments in the near future.

As an example of the types of techniques that are involved in plant genetic transformation, the development of virus resistant plants will be related. We first described the production of virus resistance in 1986 (Powell Abel et al. 1986) after which other research groups in the U.S. and Europe confirmed the general methodology. Although the methods for producing virus resistance are known, considerable effort remains to be expended to confer virus resistance to each new plant species or cultivar.

METHODS AND MATERIALS

Construction of a Gene for Virus Resistance

The initial report of genetic transformation to confer virus resistance involved construction of a gene that encoded the coat protein (CP) of TMV (Powell Abel et al. 1986); other subsequent reports to confer resistance to alfalfa mosaic virus (Tumer et al. 1987; Loesch-Fries et al. 1987; van Dun et al. 1987), cucumber mosaic virus (Cuozzo et al. 1988), potato virus X, (Hemenway et al. 1988), tobacco rattle virus (van Dun et al. 1987), potato virus Y, and tobacco etch virus (D. M. Stark and R. N. Beachy, manuscript submitted for publication), each involved construction of a CP gene. Each of these examples describes a pathogen that belongs to a different group of plant viruses, with considerable variation in virion structure, genome organization and replication strategy. Basic understanding about the targeted virus contributes greatly to development of the experimental protocol for isolation of the CP gene (Beachy et al. 1987). When the sequence of the viral genome (in the majority of cases the genome is single-stranded RNA) is known, it is relatively straight forward to isolate a DNA copy of the CP coding sequence; in other situations the the protocols are less precise, but equally successful. To isolate the TMV CP, we used standard techniques to synthesize a DNA copy of the viral RNA using reverse transcriptase and an appropriate DNA obigonucleotide primer complementary to the 3' end sequences of TMV-RNA (Goelet et al. 1982). DNA polymerase I was used to synthesize the second strand of DNA, and double-stranded DNA copies (cDNAs) of TMV-RNA were replicated after ligation to a plasmid and transformation of the modified plasmid to E. coli. The resulting bacterial colonies that contained CP sequences were identified by molecular hybridization reactions. It is also possible to isolate CP sequences by screening bacterial colonies with antibody raised against the virus (see Beachy et al. 1987 for review). After identifying colonies that contain CP sequences, the cloned DNAs were characterized by DNA sequencing and the derived amino acid sequence compared with that of the viral CP.

After isolating a complete cloned cDNA of the TMV (or other virus) CP gene, the DNA is ligated to DNA sequences that control the expression of the gene when introduced into a plant chromosome. Such sequences are required to ensure transcription of the gene, efficient translational initiation of the transcript, and to ensure polyadenylation of the transcript (Rogers et al. 1986). The resultant chimeric gene is then introduced into leaf disc (or other) cells utilizing an intermediate plasmid (in our case the plasmid was

made available from collaborators at Monsanto Company, St. Louis, Missouri) and a disarmed strain of Agrobacterium tumefaciens. Whole plants were regenerated from the cells into which the gene was introduced by virtue of their capacity to grow on media containing the antibiotic kanamycim (Horsch et al. 1985). Primary transformants were analyzed to determine levels of gene expression and CP accumulation and seeds were collected from plants that were of interest. Seedlings produced from these plants were used in experiments to determine disease resistance.

RESULTS

Coat Protein Protection

Tobacco and tomato seedlings that accumulated TMV coat protein (CP+) and those that did not (CP-) were inoculated with increasing concentrations of TMV, then observed for infection and disease development. Plants that were CP+ either escaped infection, became infected on inoculated leaves but did not develop a systemic infection, or developed systemic infections with attenuated symptoms significantly later than did CP- plants. In these experiments each of the CP- tobacco plants became systematically infected within five days when inoculated with TMV at 0.01 ug/ml, while 100 ug TMV/ml was needed to cause each of the CP+ plants to develop symptoms in five days. On the basis of these results we refer to the degree of resistance to TMV in CP+ plants as 10^4 greater than CP- plants.

Similar results were obtained with tomato plants that expressed the TMV coat protein gene. In fact, the degree of resistance was somewhat better than in tobacco. In tomato, resistance was generally reflected in infection being confined to inoculated leaves (Nelson et al. 1988).

In both tomato and tobacco plants it was often observed that, even when plants developed symptoms, they were more mild than in CP- plants. In these cases the degree of visual chlorosis, mottling, and leaf distortion (if any) was attenuated in degree when compared with CP- plants. Thus, there are three manifestations of disease resistance in the transgenic plants that express the TMV CP gene. First, fewer sites of infection were established upon inoculation with virus; second, the rate of spread of virus from inoculated leaves to other leaves was much reduced in CP+ compared with CP- plants; third, if systemic infection occurs, disease symptoms were noticeably more mild in CP+ than CP- plants. Taken together it is apparent that expression of a TMV CP gene can protect plants against virus infection and disease development.

Is Coat Protein Protection Effective Under Field Situations?

To date a single paper has described the results of a limited field trial designed to test CP protection under field situations (Nelson et al. 1988). In this experiment tomato plants that contain TMV CP were planted in a glasshouse then transplanted to a field. After two weeks, plants were mechanically inoculated with TMV (10 ug/ml or 40 ug/ml) and observed over the succeeding three months for virus infection, spread, and disease development. The CP+ plants displayed greater resistance in the field than did CP- plants: 1) less than 10 percent of the plants developed infection; and 2) symptoms were substantially attenuated on CP+ plants that developed infection compared to CP- plants. Equally as important, the CP+ plants suffered no loss of yield

while CP- plants suffered a 30-35 percent yield loss due to virus infection. Furthermore, the Agronomic characteristics (i.e., number of flowers, number of fruit set, the rate of fruit ripening, etc.) of the CP+ plants were identical to non-inoculated CP- plants. The results of these experiments indicate that CP-mediated protection will be effective to protect against, or reduce, the incidence and extent of diseases caused by plant viruses.

Other Examples of Coat Protein Protection

As indicated earlier, workers in a number of different laboratories around the world have successfully demonstrated that CP protection is effective to control a variety of different viruses. Although work published to date describes protection to members of the solanaceae (tobacco, tomato, and potato), it is expected that CP protection will be effective to control a variety of plant viruses in many different plants. In each of the examples reported, a chimeric CP gene from virus in question was expressed in transgenic plants. By this approach resistance against viruses in six different groups have been described, including **ilarviruses, potexviruses, tobraviruses, tobamoviruses, cucumoviruses,** and **potyviruses.** In the latter example (D. M. Stark and R. N. Beachy, in preparation), resistance against tobacco etch virus and potato virus Y was generated in tobacco through the expression of a CP gene from another **potyvirus**, soybean mosaic virus, a non-pathogen on tobacco. These results and others involving several different **tobamoviruses** allow us to speculate that the concept of "multivalent resistance" may occur in CP-mediated protection. In other words, a single CP gene will, in addition to protecting the plant against other viruses in the same taxonomic virus group. In practical terms this means that it will not be necessary to isolate a CP gene from each different virus (more than 100 diseases have been ascribed to different **potyviruses**); rather, several representative genes may provide protection against a variety of different diseases.

Can Coat Protein-mediated Protection be Implemented in Developing Countries?

It is recognized that there are a great number of virus diseases in tropical and sub-tropical regions of the world that are not serious problems in temperate regions. This is largely due to weather patters, to abundant alternate hosts, and large populations of insect vectors, each of which contribute to high inoculum potential. There are few, if any, natural sources of resistance to most of these diseases, and plant breeders and farmers have little recourse but to suffer the yield losses caused by the pathogen. Thus, whether on small, subsistence farms or large commercial farms, it is common to see considerable damage to all or most crops, including tomato, cucumber, sweet potato, yams, papaya, cassava, and peanut, to name a few.

Many national or regional laboratories have established research programs to eliminate viral pathogens through tissue culture techniques; however, when virus-free materials are planted in the field, they are again subjected to high inoculum pressures and are generally re-infected within several years. The process then is repeated. It could be very useful to have any significant degree of virus resistance available to reduce the frequency of costly tissue culture manipulations, and to maintain yield potentials of the crop. The approach is, of course, applicable to both the vegetatively propagated crops, where the pathogen is often spread through cuttings or root tuber pieces, and to seed propagated plants, through which viruses are generally eliminated.

Because of the conditions described above, it is highly likely that crops of the tropics and sub-tropics, including those of many less developed countries, can benefit enormously from the techniques of genetic engineering and coat protein protection. However, implementing the technology requires a degree of experimental sophistication that may be absent in all but a limited number of laboratories in less developed countries. Although it is important to strive for the establishment of national or regional research centers to carry out experiments to transform plants, it is likely that such facilities will be available in the near future in only a few locations.

Until such time as facilities become widely available, problems can be addressed and solved through establishing cooperative research arrangements with other laboratory groups. Such arrangements will make it possible to produce new or improved plant varieties with virus resistance in relatively short periods of time without the large financial investments that accompany establishing the capability of doing this type of research. The potential rewards of this research are enormous, and time is short in which to meet the food needs of the rapidly increasing population in many less developed countries. It is essential that effort be taken to initiate the process to meet these needs.

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ROUND TABLE DISCUSSIONS

ROUND TABLE NO. 1

NATIONAL POLICIES AND STRATEGIES FOR BIOTECHNOLOGY

Dr. A. I. Robertson, Convenor
Dr. M. W. Nabors, Recorder

Dr. E. T. Acquah, Contributor
Dr. A. Mujeeb-Kazi, Contributor
Mrs. F. M. Wambugu, Contributor

A. What should be the strategic goals of a national program in biotech?

1. There should be plant biotechnology programs established by national governments. Included in the programs should be provisions for setting priorities. Problems should be identified which are solvable, and which are important to the agriculture of a particular country.

2. Scientists and administrators should consider the need for "avenues" to communicate and convince national governments and people in authority of the status and applicability of biotechnology.

B. How should these goals be implemented?

1. There needs to be links between organizations dealing with plant biotechnology within each country. These links will facilitate communication and eliminate duplication. They will ensure that research data and germplasm are utilized.

2. Strategies for directions and goals for the research are needed. The strategies should incorporate plant biotechnology with conventional research in basic and applied programs.

3. There is a need for advisors from overseas to help formulate and establish national programs.

4. Access to training and graduate education is critical, because skilled personnel at all levels are needed.

5. Collaboration between national academic institutions and research laboratories, as well as with foreign, advanced research institutes is essential for efficient use of available methodology and avoidance of duplication.

C. How can USAID best help implement these goals?

1. The USAID can assist by providing monetary support, as well as by putting national laboratories in communication with researchers in developed countries which may be able to assist. See No. 5, above.

2. The International Plant Biotechnology Network (IPBNet), and other information and research networks, can play important roles in facilitating access to training and in establishing collaborations. The IPBNet Directory

is an example of a vital source of information on research being conducted in other countries, as well as monetary resources available to researchers worldwide.

ROUND TABLE NO. 2

DEVELOPMENT AND APPLICATIONS OF BIOTECHNOLOGY

Dr. Y. L. Nene, Convenor
Dr. O. L. Gamborg, Recorder

Dr. M. W. Oggema, Contributor
Dr. O. U. Onokpise, Contributor
Dr. Victor Villalobos, Contributor

A. What are the current needs for new technologies in developing countries?

The panel recognizes that it cannot generalize about developing countries in regard to their capabilities and potentials in biotechnology. Some countries officially designated as developing have facilities and capabilities, including trained scientists, on a level with developed countries, but some countries have not yet developed this capability. In general, however, developing countries lack many resources including financial. Moreover, factors such as irregular power and water supplies adversely affect the efficiency of a laboratory.

Within this framework it was difficult to generalize about which technologies should be adapted or developed. The establishment of a tissue culture laboratory was proposed as a first step, and has indeed been done by many countries. The availability of such facilities has many potential applications including micropropagation, production, maintenance and distribution of disease-free stock (either as germplasm or for eventual use in food production) and screening for stress tolerance.

Screening of germplasm was recognized as an essential component of any national program. Where hybrids could be established by conventional means, use of embryo culture is recommended. The panel was of the opinion that the achievements in protoplast technology development to date indicated that it should only be used in special cases.

It was recommended that very careful consideration be given before developing countries use the molecular biology of gene cloning and recombinant DNA technologies for gene isolation. Molecular biology facilities are more expensive than those used for tissue culture; however, they may be justified, and may be an excellent economic investment when the procedures are employed to alleviate serious losses and there are no other sources of resistance. Developing countries usually do not have facilities for chemical control of vector insects nor do they have a winter season which reduces vector populations naturally. The losses due to viruses and the returns from introducing viral coat protein genes are examples of where developing countries could gain considerably from the application of molecular biology. Several developing countries could take advantage of this technology when genes become available in suitable vectors since tissue culture can be used for gene transfer to plants.

While the panel discussed genetic improvement of crops, the need for accurate disease screening was considered. Recent developments in enzyme linked immuno absorbent assay (ELISA) have brought this technique to the stage where it could be readily adopted in screening.

B. Should new technologies be adapted or developed by the developing countries?

While recognizing the initiative and inventiveness of developing country scientists and their enthusiasm in developing new technologies, the panel recommended that, in the interest of short-term and long-term gains, they should make use of techniques which have already been developed and are ready for application. Most of these techniques need to be adapted to desired varieties or species and the technical reproducibility verified. The adaptation of regeneration techniques to different species or varieties was cited because the conditions can vary between species and even varieties; however, countries that have the capabilities and resources could justifiably develop technologies according to identified needs with species of national importance.

C. How will new technologies be implemented within existing national organizations?

The panel stressed the need for thorough planning within national government departments or organizations prior to establishing national programs for biotechnology application. It would be advisable to involve input from interdisciplinary groups, which would include not only personnel with experience in in vitro technologies but also those with responsibilities in the areas of potential applications, such as plant breeders, pathologists, and other specialists.

National government organizations should make use of consultants as needed in all phases of planning and reviews. It was considered essential to develop long-term (5-10 years) and short-term plans with program objectives and goals. The panel also recommended the involvement of universities and national research institutes to set-up collaborative projects which could involve graduate students and sharing of major high-cost facilities and equipment.

The successful application of any of the biotechnology procedures requires a team approach involving breeders, pathologists and other specialists in addition to those who have the expertise and experience in tissue culture. The applications also require access to the necessary facilities including greenhouse and field testing sites, and depending upon the nature of the programs, genetics, biochemistry and other basic science laboratories.

There is a need for setting-up regular reporting and discussion sessions between research leaders and scientists within the regional centers. The panel also proposed and agreed that establishing collaboration with research scientists and laboratories abroad would be highly desirable assuming there are mutual interests and benefits.

The role of international centers and institutes was discussed. The national programs should take full advantage of new technologies, new germplasm and information available from the centers. They have made valuable contributions in the past and continue to do so. Collaborations with the centers should be set-up where possible and advantageous.

The point was made by the panel that many crops of importance to developing countries are not mandated or food crops. They may nevertheless contribute to the national economy of a country. Application of new technologies to such crops would be fully justified. Examples are vegetable oil and plantation crops.

A program in research and application of biotechnology will require continuous access to training. The research workers and technical staff need access to new information and technologies, and this may be achieved through short-term technical training as well as through graduate studies and post doctoral research abroad. Research and program leaders need to have support for study visits to progressive research laboratories to ensure they keep abreast of new advances in information and technologies and avoid duplications. Workshops or topical conferences also serve important functions in bringing together scientists from different disciplines. Networks are another valuable means of communication and exchange of information among scientists.

ROUND TABLE NO. 3

NETWORKING: EXISTING COLLABORATIONS AND FUTURE GOALS

Dr. R. N. Beachy, Convenor
Ms. K. L. Wright, Recorder

Dr. J. H. Dodds, Contributor
Dr. F. J. Novak, Contributor
Dr. S. K. Hahn, Contributor

A. What are the existing technical and information networks and their respective foci?

Upon reviewing the various research and information networks existing for crop improvement programs, the panel identified three primary areas of networking efforts. These are: 1) research networks with specific crop foci and germplasm exchange; 2) technical assistance networks represented by scientific exchanges and training programs; and 3) information dissemination networks which provide regular research publications, database systems, and annual workshops and/or conferences. The panel recognized that their findings were not exhaustive but assisted in uncovering information of interest to Conference participants, and warranted post Conference follow-up. (Please see TCCP Newsletter #10 for an overview of plant biotechnology/crop improvement networks and their coordinators.)

Research networks were identified as those which focus on specific crop improvement programs. The International Agricultural Research Centers (IARC's) have established several programs linked to specific crop mandates and regional crop improvement issues. These networks work to make elite breeding lines, cultivars, and hybrids available to scientists, as in the case of the Cooperative Cereals Research Network (CCRN), coordinated from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Other programs work to combine collaborative efforts from the IARC's, national programs in developing countries, and institutions from Europe or the United States to identify a common research goal, such as the Cassava-Trans Project at Washington University in St. Louis, Missouri. This program, supported by the French Research Organization "ORSTOM" and the Rockefeller Foundation, works to demonstrate viable means of using biotechnology to provide new sources of virus resistance in cassava for application in tropical countries.

The technical assistance component, as seen in scientific personnel exchanges and short-term and long-term training programs, is often incorporated into existing research networks. Programs such as those sponsored by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency (FAO/IAEA) have focused on inter-regional efforts to further training in new technologies in agriculture. One program, organized to address problems through <u>in vitro</u> resistance systems in plants using mutation induction, is composed of scientists from developing and developed countries who meet every two years to gather updated research information. Other short-term tissue culture training courses have existed for several years, organized through the International Cell Research Organization (ICRO) of the United Nations, through UNESCO. The program in plant biotechnology works with national research institutions and individual scientists to organize and implement various training courses.

Information networks exist in lesser numbers, but serve as important linkages for the international scientific community. The International Information System for the Agricultural Sciences and Technology (AGRIS) and the Current Agricultural Research Information System (CARIS) of the FAO are examples of two international cooperative information systems. The AGRIS focuses on the world agricultural literature such as periodicals, reports, books, conference proceedings, etc. The CARIS documents research institutions, research programs and projects in developing countries. The International Board for Plant Genetic Resources (IBPGR) has compiled an extensive In Vitro Conservation Database which functions as an information service to the scientific community. This computerized database is maintained and continually updated to provide information related to aspects of in vitro conservation and associated areas of research (i.e. propagation and disease indexing).

B. How do international networks affect exchanges between national and international programs? How can this be improved?

The panel discussed the effectiveness of international networks based on their ability to incorporate existing research programs and assist in further technology transfer among participating scientists. Due to the large area of research topics and crop material included in the existing networks, it is important to evaluate successful linkages based on issues of priorities and goals of individual programs. Therefore, broad statements cannot be made concerning evaluation of specific networks' effectiveness. Several questions were raised however, which addressed the transfer of technologies and information dissemination as a global priority. Participants requested that more information be released concerning membership in professional societies, such as the International Association of Plant Tissue Culture (see Newsletter #10). Feelings were also expressed by Conference participants which reflected a sense of professional isolationism in remote areas. The problem of duplicated programs and research efforts was also mentioned as an area in which international networks can assist by releasing information not readily available. Another suggestion for improving networks and participation was to call upon increased assistance from private industry to participate in national and international collaborative research programs. Regional training programs, conferences, and workshops are a visible means of increased research exchange. The suggestion was presented to the panel that post-conference workshops be established after international meetings to assist scientists in the use of specific techniques and the application of various methodologies or research findings addressed during the previous conference.

C. How can IPBNet contribute to and interface with other existing networks?

The current and future roles of the International Plant Biotechnology Network (IPBNet) was discussed by panel members and Conference participants. Those participating in the discussions saw IPBNet as a connecting link to various plant biotechnology programs (including those of the TCCP) involving research, training and information exchange. It was suggested that IPBNet remain term "crop neutral," since several of the existing networks are crop specific. With a more general orientation, IPBNet would continue to collect information on a wide variety of research topics and programs and distribute it to interested parties. Participants would like to see the information

services current provided by IPBNet (database and literature system) expanded to include other areas such as molecular biology and plant breeding.

Another means of interfacing more effectively with crop programs is for IPBNet to assist in identifying and placing scientists into existing training programs (both long- and short-term). Regional training programs in tissue culture are seen as a beneficial way of addressing an area's basic research needs, using plant material of regional importance. Specialized training from advanced laboratories, involving technologies not readily available in developing regions, is still needed. Often times scientists, as well as donor agencies, need assistance in identifying and matching the most applicable program for the individual or the institution he or she represents. The IPBNet could assist in this service.

The need for IPBNet Conferences was discussed and seen as an excellent opportunity for continued collaborations and a free flow exchange of current and future research direction. Cooperation from the USAID Missions and national research programs is essential for the success of all regional programs, particularly conferences. The Nairobi Conference was heavily supported by the Nairobi AID program, which enabled strong pre- and post-conference organization. The ultimate growth of networks such as IPBNet will continue to rely on the participation from individual scientists and research organizations to successfully introduce and maintain future plant biotechnology programs.

ROUND TABLE NO. 4

FINANCIAL RESOURCES: NEEDS AND OPPORTUNITIES

Dr. A. W. Von der Pahlen, Convenor
Ms. J. L. F. Ketchum, Recorder
Dr. I. Y. E. Chu, Contributor
Dr. E. C. Quisumbing, Contributor

A. What are the obstacles to obtaining available resources?

1. Lack of trained manpower in the biotechnology field resulting in lack of awareness of the availability of biotechnology as a new tool for agricultural research and development.

2. Lack of appropriate skills or expertise in the preparation of an acceptable project proposal.

3. Inadequate or insufficient information on biotechnology research being undertaken in other research institutes within the country or outside. A research proposal for an activity which had already been undertaken or is currently being undertaken elsewhere will not get a positive response from donors.

4. Lack of consultation and rationalized programming at the national and regional or international levels resulting in intense competition for limited resources.

5. Presence of more pressing problems which require priority attention.

6. Availability of conventional "tools" or approaches which are less expensive or sophisticated than biotechnology.

7. Lack of a clear understanding of the agency's part or role in biotechnology. (This may be related to #1 above.)

B. What are the requisites for successful (biotechnology) projects or proposals?

1. Adequate support from policy (decision) makers at all levels in the government to make possible items #2 - 6 below.

2. Assurance and availability of continued funding from the government and/or donors.

3. Availability of a "critical mass" of trained manpower with sufficient experience in conventional laboratory techniques in plant breeding, plant pathology, microbiology, and plant physiology.

4. Availability of suitably equipped laboratories with reliable power and water supply.

5. Involvement in biotechnology research and development projects which are internationally coordinated by IARC's or renown research institutes in developed countries.

6. Participation and collaboration in international research networks in biotechnology research and development.

7. Proper integration of laboratory trials and results with field trials under the applicable environments.

8. Clear presentation in the project proposal of planned activities indicating: 1) the problem which is being addressed, 2) the economic, social and political implications of the problem, 3) the probability of success, and 4) a cost/benefit analysis of the biotechnology if successfully developed.

C. How can the obstacles to obtaining resources be overcome?

1. Make decision makers at various levels of government aware of the potential contribution of biotechnology to the solution of pressing national problems.

2. Initiate training programs to develop the necessary trained manpower for biotechnology research and development. This could start with the sending of a few selected staff to non-degree research internships in IARC's or research institutions with strong programs in biotechnology and by sponsoring scholarships in biotechnology at the MS or PhD degree levels.

3. Prepare well-conceptualized research proposals preferably those which focus on the solution of highly visible problems. The strategy is to generate a series of success stories which would highlight the value of biotechnology as a valid and useful tool for agricultural development. This approach is related to #1 above and would condition or convince decision makers to allocate adequate resources to ensure that the potentials of biotechnology could be properly exploited. (Please also see Item B.8 above.)

4. Seek out and participate in biotechnology research networks such as the IPBNet and those of IARC's like CIP, IITA, and others. This will provide national scientists the opportunity to exchange information and materials, and to develop collaborative strategies for solving common problems, e.g. exchange of disease-free germplasm and modern varieties, rapid propagation of perennial crop hybrids, etc.

5. Understand the intended donor's mandates and interests, funding priorities, project proposal format and technical and financial information required. Some donors follow different project proposal formats and evaluation/processing procedures depending on the magnitude of the funding being requested.

6. If convinced that you have a worthwhile proposal, it will pay to be persistent in looking for or approaching donors. Biotechnology is a solution looking for a problem and persistence will eventually lead you to a donor with the "right" problem.

7. Look toward the commercialization of the biotechnology. The developer of the technology can generate income through royalty arrangements or profit sharing which can be used for funding new biotechnology research and development projects.

8. Provide services like training, technical assistance (consultancy) and provision of planting materials to government agencies and the private sector for which fees can be charged. Such fees can be utilized to support the continued operations of the organization.

ABSTRACTS

The following abstracts did not appear in the "Abstracts of the Third International Conference of the International Plant Biotechnology Network," or have been revised since its printing.

EFFECT OF MUTAGENIC TREATMENTS ON SOMACLONAL VARIATIONS IN WHEAT

X.Y. Cheng, M.W. Gao, Z.Q. Liang and K.Z. Liu. Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University, Hangzhou, China

A number of wheat genotypes were treated with gamma-rays, sodium azide and EMS before tissue culture and immature embryos from M_1 plants or plants shortly after gamma-irradiation were used to initiate callus cultures. Thousands of plants have been regenerated and were used to investigate the effect of mutagenic treatment on the regenerated plants and somaclonal variation in M_3R_2 and M_2R_2 generation. The results indicated that mutagen-induced damages in terms of reduction in plant height, fertility, tillering ability and spike length were not remarkable in the regenerated plants as compared with untreated control. In M_3R_2 generation, only sodium azide treatment had significantly higher frequencies of somaclonal variations than control. Increase in the variation frequencies were observed when explant embryos were irradiated with 250 and 500 gamma-rays and the highest frequency appeared when embryos were exposed to 500 R gamma-rays on the 5th day after anthesis. Enlarged variation spectra were also resulted from mutagenic treatments and most of the recovered variants were undesired for plant improvement.

ESTABLISHMENT OF MORPHOGENIC SUSPENSION CULTURES IN RICE (*Oryza sativa* L.)

X.Y. Cheng, M.W. Gao and Z.Q. Liang. Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University, Hangzhou, China.

Calli were induced from the seeds of 107 rices, including 90 indica rices and 17 japonica rices on N_6 medium supplemented with 0.5 mg 2,4-D, 4 mg NAA and 0.5 mg 6-BA (per liter), and response of the calli to liquid culture was investigated in an attempt to screen genotypes suitable for suspension culture. Five out of the 90 indica rices and 12 out of 17 japonica rices were found to be capable of proliferating well in liquid N_6 medium when their calli were transferred into the liquid medium within a suitable period of callus ages, which differed in different genotypes. After 4 to 6 month's culture in the same N_6 medium, well dispersed cell suspension cultures were obtained in 5 japonica and 2 indica rices. These suspension cultures consisted of fine clusters of round, cytoplasmic and fast growing cells, which had doubling times in total cell volume of 7 to 12 days. Plant regeneration occurred in all of the suspension cultures when they were transferred to 2,4-D-free differentiation media, even when the cultures were 10 to 12 months-old, although the regeneration abilities were lower and varied with the cultures.

PRIORITIES IN THE USE OF BIOTECHNOLOGY FOR FOOD - CROP BREEDING IN INDONESIA

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Biotechnology has offered a wide variety of techniques to enhance crop breeding. However, not all of the available techniques are applicable in Indonesia and other developing countries where research resources are limited. Priorities should be determined on the basis of the importance of the crops, the significance of the breakthroughs offered, and the probability of success of the research work. The importance of the crops is determined in the light of economical, social, and political considerations. The significance of any particular technique depends on the magnitude of the problems it may solve or the new opportunities it may open. The probability of success of any particular biotechnology research work is implied from past experiences in this area. Several possible techniques for different crops were discussed.

THE ECONOMIC IMPLICATIONS OF BIOTECHNOLOGY: A BRIEF REVIEW OF THE CASE OF POTATOES IN KENYA.

Leopold P. Mureithi and Boniface F. Makau. Department of Economics, University of Nairobi, P.O. Box 30197, Nairobi, and, Ministry of Planning and National Development, P.O. Box 30005, Nairobi, respectively.

This study set out to assess the level and rate of adoption of certified seed potatoes which are developed through biotechnological processes. The study went on to determine the incomes and employment generated in the use of certified seed potatoes in Kenya. The findings were that certified seed potatoes were grown in roughly twenty per cent of the land area under potatoes and that additional profits due to the use of certified seed potatoes were in the region of fifty per cent. Both direct and indirect employment was found to be higher for certified than for non-certified seed potatoes. There is, however, need to address the issue of cash flow problems associated with the higher costs of growing certified seed potatoes so that farmers can adopt this worthwhile technology. This would include, among other things, facilitating credit availability and the streamlining of marketing outlets.

EFFECTS OF ALUMINUM ON THE GROWTH OF PEANUT CELL SUSPENSION CULTURES

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Peanut (Arachis hypogea line 7920B-3B) cell suspension cultures derived from leaf callus were grown in reduced strength of L-6 liquid maintenance medium containing 4.00 uM 2,4-D + 2.50 uM kinetin, pH 4.0. The cell suspensions treated with different levels of aluminum; 0, 50, 100, 200, 300, 400 uM, supplied as $Al_2(SO_4)_3.18H_2O$, were cultured in 50 ml liquid media in 250 Erlenmeyer flasks. Cell growth was determined after two weeks of culture by measuring the Packed Cell Volume (PCV). The results obtained showed that the PCV was significantly reduced in aluminum-treated cultures. The cell suspensions with added aluminum appeared brown and the degree of browning increased in intensity with the increase of aluminum concentrations. When observed under the microscope the aluminum-treated cell suspensions contained numerous broken cells and the cell clumps were smaller in size and number. The degree of these stress responses due to aluminum toxicity varies with the amount of aluminum applied.

CLONING OF COFFEE IN ZIMBABWE

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SUMMARY

There is a need in Zimbabwe for a system for the in vitro propagation of Coffee arabica. This will facilitate the movement of germplasm around the country as well as the rapid propagation of commercial hybrids. Somatic embryo induction in cultures of Coffee arabica leaf explants without subculture is reported. Induction was possible with both material from the field as well as that from in vitro plantlets. Somatic embryos were then grown into plantlets in light. The medium for the induction of somatic embryogenesis consisted of half strength Murashige and Skoog inorganic salts supplemented with 10 mg/ml thiamine, 50 mg/ml cysteine, 100 mg/ml caseine hydrolysate, 30 g sucrose, 100 mg/ml myo-inositol, 1 mg/ml 2-ip, and 5 mg/ml IBA. The explants were in the form of leaf discs from mature leaves. After two months of culture in the dark at 28°C somatic embryos in the form of globular organoids began to appear at the cut edges of explants taken from F_2 hybrids growing in the field. This was also true for the in vitro plantlets. A reproducible protocol for the genesis of at least 30 somatic embryos per leaf disc is available.

RESEARCH PROGRAM ON BIOTECHNOLOGY BOGOR RESEARCH INSTITUTE FOR FOOD CROPS (BORIF), BOGOR, INDONESIA.

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To support the agricultural research on food crops, pioneering research may be needed to obtain information useful and needed for more efficient applied research. In the next five years development planning, the Central Research Institute for Food Crops (CRIFC) through Bogor Research Institute for Food Crops (BORIF) tries to develop the program for tissue culture laboratory in order to catch up the development of biotechnology in the modern countries. For this purpose, biotechnology research of BORIF try to use the principles of genetic engineering to grow and manipulate cells in the test tubes in order to improve methods of germplasm collection and storage, disease control, and conventional method of plant breeding. In the beginning, the research will focus on plant tissue culture for mass propagation of agricultural crops. In the more advance, anther culture and protoplast culture are included in the five years planning research of the Institute. These procedures are expected to reduce the time and cost required to breed some food crop lines by conventional method resulting in considerable saving in developing new varieties. Possible steps needed are: mobilization of the existing CRIFC capabilities in biotechnology, promotion of the utilization of existing biotechnological process as well as research and development of new processes, and manpower development through trainings.

BIOTECH TARGETS FOR AFRICA

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SUMMARY

Can any African country afford not to have:

(1) A quarantine tissue culture lab for import and export of in vitro material
(2) A commercial lab for the production of clean cassava, sweet potato, Irish potato, etc.
(3) A biotech lab capable of embryogenesis from leaf discs, from callus, from protoplasts?

Problems in getting started, cost effectiveness, critical mass and basic kits will be discussed.

TISSUE CULTURE FOR VIRUS-FREE POTATO PROPAGATION PROJECT IN INDONESIA

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The main constraint in the effort of increasing potato production in Indonesia is the availability of good quality seed. Two techniques which are used in the production of good quality seed (disease-free potato seed) are meristem culture and rapid multiplication techniques and they have been adopted by several countries. In Indonesia those two techniques were used to produce disease-free potato since 1984 supported by USAID to construct the facilities, to purchase the equipment and to finance the training and the travels. One permanent screen house of 200 sqm was established at Lembang Horticulture Research Institute (LEHRI) in Bandung. Two rooms were modified to be used as incubation and preparation rooms. Net houses were also built to produce tuberlets. Training, workshop and comparative study to other countries have been done not only by LEHRI staffs but by extension workers and the farmers as well. In the country trainings were held at Lembang, West Java (two times), Sidomulyo, East Java (once) and Malang also East Java (once) and attended by 105 participants from extension workers, seed farms and farmers. Some studies on meristem culture media mass propagation, stem cutting production and tuberlets production were carried out and the results were put forward in the report. The project was successful, however, it must be followed up by other projects which will involve the private sector to engage in seed business.

MERISTEM CULTURES OF LOCALLY POPULAR VARIETIES OF SWEET POTATO ON DIFFERENT MEDIA

Nguyen T. Thanh-Tuyen and Flora Mia Y. Duatin. Tissue Culture Laboratory, Visayas State College of Agriculture, ViSCA, Leyte 6521-A, Philippines

Apical and axillary meristems (0.6-0.8 mm) of fifteen locally popular varieties including the native and newly developed hybrids of sweet potato (*Ipomoea batatas*) in the Philippines were plated on five different culture media and evaluated for their *in vitro* performance. The latter was recorded as frequencies of shoot emergence and plantlet regeneration, average number of plantlets regenerated per meristem and presence of callus prior to plant regeneration.

Different levels of response of the plated explants were observed in the various genotypes. However, the effects of culture media appeared more prominent than that of genotypes. ViSCA 1 medium and those adopted from IITA and IBPGR publications consistently produced callus on cultured meristems of all varieties included in the investigation. Culture growth and plant regeneration were slow on ViSCA 2 and CIP medium but no callus formation was noted in all genotypes. Regeneration of multiple shoots was occasionally observed on the two media. The absence of callus in the process of plant regeneration from meristem culture of sweet potato assures the true-to-typeness of the propagated plant materials.

IMPROVE POTATO MICROTUBER PRODUCTION BY MANIPULATING SUCROSE AND BENZYLADENINE

Gustaaf A. Wattimena, Agus Purwito, D. M. Puspitaningtyas. Department of Agronomy, Bogor Agricultural University (IPB), Bogor, Indonesia

The numbers and earliness of *in vitro* microtubers produced as well as their fresh and dry weight were analyzed for the effect of sucrose and benzyladenine. The experiments were factorial consisting of 4 levels of sucrose and 3 levels of benzyl adenine. The shoot liquid medium was MS (salt and organics), 3% sucrose and 0.5 ml/l A-Rest. The liquid tuberization medium was the same as shoot medium, but A-Rest was substituted by 400 mg/l Cycocel.

The earliness of tuberization was affected by sucrose and benzyladenine. Sucrose without benzyladenine was able to induce 100% of tuberization one week after adding of tuberization medium. Increasing sucrose concentration from 3 to 9% increased the number of microtubers produced per bottle and average microtuber size. Further increase of sucrose to 12% decreased both the numbers of microtubers produced as well as microtuber size. Average microtuber fresh weight and dry weight were affected by the interaction of sucrose and benzyladenine. The maximum average fresh weight and dry weight were produced by the treatment combination of 12% sucrose and 2 mg/l benzyladenine. 9% sucrose produced the higher average microtuber fresh weight while 12% sucrose produced the higher microtuber dry weight.

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Orals

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Tissue Culture Investigations of Graft Incompatibility Among *Persea* Species

Robert P. Adams
DNA Bank-Net: A Network of DNA Banks for the Conservation and Utilization of DNA

Abdel-Fattah H. Belal, Mohamed S. Radwan, Alaa Bondok, and Mona Karora
Growth of Cultured Alfalfa (*Medicago sativa* L.) Cells in Response to Sodium Chloride

Tishu Cai and Larry G. Butler
Somaclonal Variation in High Tannin Sorghums

Xiongying Cheng
Effect of Mutagenic Treatments on Somaclonal Variations in Wheat

Pablo S. Jourdan
Somatic Hybridization for the Manipulation of Cytoplasmic and Nuclear Traits in *Brassica*

N. Kumaravadivel and S. R. Sree Rangasamy
Anther Culture and Somatic Embryogenesis in *Sorghum bicolor* (L.) Moench

M. Mahadevappa and K.P. Viswanath
Simpler Technique of Developing Male-sterile Lines in Indica Rice

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Cytological Studies of Anther Derived Plants in Rice

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Abdul Mujeeb-Kazi
Practical Applications of Conventional and Novel Genetic Manipulation Procedures in the Triticeae

S. Y. C. Ng
Application of Tissue Culture in Cassava and Yams

Frantisek J. Novak
Banana and Plantain (*Musa* spp.) Mutation Breeding: Results and Perspectives with Tissue Culture Technologies

D. M. Okioga, L.M. Muriithi, S.P. Gichuru, and W.G.M. Ottaro
Rapid Propagation of Pyrethrum (*Chrysanthemum cinerariifolium* Vis.) by Tissue Culture Techniques

D. Padmanaban and C. Sudhersan
In vitro Cultivation of a Medicinal Plant *Enicostemma axillare* (Lam.) Raynal (Gentianaceae)

Cai Qihua, Gao Mingwei, and Liang Zhuqing
Studies on *In vitro* Mutation Techniques in Hybrid Rice Improvement

A. Ian Robertson
Biotech Targets for Africa

Nguyen T. Thanh-Tuyen and Bernardita C. Paduano
Somatic Embryogenesis and Plant Regeneration in *Zea mays*

Dirk Vuylsteke, Rony Swennen, and Sang KiHahn
Practical Applications of Tissue Culture Technologies for the Improvement of Plantain and Cooking Banana: The Need for Resistance to Black Sigatoka Disease in Africa

Gustaaf A. Wattimena, Agus Purwito, and D.M. Puspitaningtyas
Improve Potato Microtuber Production by Manipulating Sucrose and Benzyladenine

Joseph G. Wutoh
Biotechnology and Associated Constraints in the Development of Rapid Seedstock Multiplication System in the Tropical Roots and Tuber Crops in Africa

Abdelouahhab Zaid and Harrison Hughes
In vitro Acclimatization of Date Palm (*Phoenix dactylifera* L.) Plantlets: I. Effect of Polyethylene Glycol on Water Loss

Posters

Emmanuel Turkson Acquah and Nyochembeng Leopold Mbehuchon
Economics of Rapid Seed Multiplication of Xanthosoma Sagittifolium (Cocoyam - Macabo) Through Tissue Culture

A. H. Belal, M.N. Loo, and D.W. Rains
Study of the Effect of Sodium Ions on Growth of Cultured Alfalfa (*Medicago sativa* L.) Cells

Xiongying Cheng, Mingwei Gao, and Zhuqing Liang
Establishment of Morphogenic Suspension Cultures in Rice (*Oryza sativa* L.)

Ahmad Dimyati
Priorities in the Use of Biotechnology for Food - Crop Breeding in Indonesia

Tigst Demeke and Harrison Hughes
Rapid Aseptic Micropropagation of *Phytolacca dodecandra* Through Shoot Tip Culture

N. Kumaravadivel and S. R. Sree Rangasamy
Direct Somatic Embryogenesis from *Sorghum bicolor* (L.) Moench Leaves

M. Mahadevappa, K. Govindaraju, and R.A. Krishnamurthy
Performance of Locally Developed and Exotic Hybrids in Indica Rice

M. Marziah
Effects of Aluminum on the Growth of Peanut Cell Suspension Cultures

M. Marziah
The Importance of pH in Aluminum Toxicity Studies in Plant Cell Suspension Cultures

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Cloning of Coffee in Zimbabwe

Leopold P. Mureithi and Boniface F. Makau
The Economic Implications of Biotechnology: A Brief Review of the Case of Potatoes in Kenya

J. Ndeberi
In vitro Cultivation of Cereals: Nutrients Requirement Approach

Stanley Ndunqu and Michael J.S. Magambo
Callus Induction and Regeneration from Maize (*Zea mays*) Embryo Cultures

C. E. A. Okezie
Hormonal Requirements for *In vitro* Development of Mature and Immature White Yam (*Dioscorea rotundata* Poir, (*Dioscoreaceae*) Embryos

Thipmani Paratasilpin, Apichart Kaosa-ard, and Pimchai Apavatjrut
Anther Culture of Teak (*Tectona grandis*)

Mukunda Ranjit and Nalini S. Upadhyaya
In vitro Propagation of Apple Rootstocks (*Malus prunifolia* L. and *Malus domestica* Borkh. Var. MM 106) and CV. 'Anna' (*Malus domestica* Borkh.)

Sudjoko Sahat, Azis A. Asandhi, Asih K. Karyadi, and Iteu M. Hidayat
Tissue Culture for Virus-free Potato Propagation Project in Indonesia

A. H. Tarmizi, K. Paranjothy, and A.H. Halim
Low Temperature Storage and Possible Role of Exogenous Abscisic acid (ABA) in Inducing Cold Tolerance in Oil Palm Polyembryogenic Cultures

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Potato Micropropagation and Seed Improvement in Rwanda

Nguyen T. Thanh-Tuyen and Dolores I. Apurillo
Histological Evidence of Somatic Embryogenesis in Coconut (*Cocos nucifers* L.)

Nguyen T. Thanh-Tuyen and Flora Mia Y. Duatin
Meristem Cultures of Locally Popular Varieties of Sweet Potato on Different Media

Montakan Vajrabhaya and Thavorn Vajrabhaya
Development of Salt Tolerant Rice Varieties Using Tissue Culture Technique

Joseph G. Wutoh and Oghenekome U. Onokpise
Application of Biotechnology in Breeding for Root Rot Disease Resistance in Cocoyam (*Xanthosoma sagittifolium* (L.) Schott. L.)

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