CONSULTATIVE GROUP ON INTERNATIONAL AGRICULTURAL RESEARCH

interim SCIENCE COUNCIL

Applications of Molecular Biology and Genomics to Genetic Enhancement of Crop Tolerance to Abiotic Stress

A Discussion Document

interim SCIENCE COUNCIL SECRETARIAT

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

This document comprises:

(a) Applications of Molecular Biology and Genomics to Genetic Enhancement of Crop Tolerance to Abiotic Stress - A Discussion Document

(b) Status of Breeding for Tolerance of Abiotic Stresses and Prospects for Use of Molecular Techniques (Annex I)

(c) Genetic Engineering for Abiotic Stress Tolerance in Plants (Annex II)

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PREFACE

TAC initiated its discussions on abiotic stress genomics at TAC 80 in March 2000 at ICARDA, Aleppo, Syria. Under the agenda item Trends in Science – Implications for CGIAR, TAC discussed the opportunities offered by the new sciences in improving the relevance, quality and impact of research in the CGIAR. In the area of biological sciences, TAC considered that the advances in molecular biology had important long-term implications for CGIAR's work on genetic enhancement and how that work could be organized in the future. As an input into the discussion at TAC 80 on the implications of the advances in molecular biology, the TAC Chair, Dr. Emil Javier, invited Dr. John Bennett of IRRI to submit a paper entitled *Status of Breeding for Tolerance of Abiotic Stresses and Prospects for Use of Molecular Techniques* (Annex I).

Dr. Bennett's paper pointed out that the CGIAR had yet to make a significant difference in difficult environments that were beset by abiotic stresses as a production constraint, namely drought, temperature extremes, soil toxicities, and soil nutrient deficiencies. The considerable genetic variation existing within several crop species opened up plant breeding as a feasible option for tolerance to abiotic stresses. Citing the example of drought resistance research at IRRI, the Bennett paper mentioned the use of a tool called Rice Gene Chips that contained half of the rice genes, allowing ready study of the drought stress-relevant genes. Rice's status as a model cereal had prompted IRRI to initiate a public platform on functional genomics, which was to involve other CGIAR Centres, NARS, ARIs and the private sector. He noted the CGIAR's strengths lay in its germplasm collections, its new capacity for genetic and molecular dissection of complex traits, its ability to conduct multidisciplinary plant improvement programs in target "stress" environments, and in its links with the national partners. The private sector was likely to provide genome information toward benefiting the poor.

Through separate presentations at TAC 80 by the Committee Members Drs. Usha Barwale Zehr and Hirofumi Uchimiya, TAC discussed emerging trends in molecular biology and associated sciences of biotechnology and genomics, and their implications for the CGIAR's strategic research on the genetic enhancement for generating improved germplasm. Dr. Barwale Zehr described genetic enhancement in the context of the activities chain from genetic resources management to product development, and the new research areas of genomics, bioinformatics and structural biology. Beneficial as they were, they created the need for monitoring and policy measures concerning biosafety, intellectual property and bioethics. Dr. Hirofumi Uchimiya discussed developments in genome science, including the publishing of the first draft of the human genome and the sequencing of the *Arabidopsis* and rice genomes. Micro-array technology linked to microchips allowed analysis of vastly greater amounts of gene information than previously possible, lending itself to researching, for instance, complex traits.

In offering potential applications in, among others, nutrition, pharmaceuticals, and NRM research, these novel biological approaches were giving the CGIAR new impetus to expanding its investment in this direction, guided by a dynamic strategy. To serve as a catalyst in applying genome research to developing countries' needs, the CGIAR had to be involved in collective genomics initiatives with adequate in-house competence and institutional linkages.

To carry forward its discussion, TAC requested its Standing Committee on Priorities and Strategies (SCOPAS) to prepare a discussion paper on crop abiotic stress genomics. At TAC 81 in September 2001 at CIFOR, Bogor, Indonesia, Dr. Hirofumi Uchimiya, SCOPAS member, presented a discussion paper prepared by him and entitled *Genetic Engineering for Abiotic Stress Tolerance in Plants* (SDR/TAC:IAR/01/27). The paper (Annex II) presented evidence suggesting that molecular understanding of the stress perception, signal transduction and transcriptional regulation of abiotic stress responsive genes may help engineer tolerance to multiple stresses through a "master gene" mechanism, with its associated economies of scale.

Based on the discussions at TAC 81, the Committee requested SCOPAS to commission a suitable consultant to prepare a fuller discussion paper on opportunities and priorities for abiotic stress genomics in the CGIAR, in consultation with CGIAR Centres, for consideration at the next meeting in April 2002. The paper should elaborate the following: relative importance of abiotic stresses; the CGIAR community's current work on the subject; assessment of NARS capacity; mode of coordinating the effort. The CBC and CDC voiced strong support, agreeing to facilitate the process and bringing all partners together.

At iSC/TAC 82 in April 2002 at CIP, Lima, Peru, Dr. Mike Gale delivered, by video-conference, a paper entitled *The potential application of molecular biology to genetically enhance crop tolerance to abiotic stress* (SDR/iSC:IAR/02/10), elaborating on the role of molecular biology and abiotic stress genomics in genetic enhancement and crop improvement.

Dr. Gale stated that crop genomics represented the state-of-the-art in plant genetics. Considerable work was already underway in the CGIAR aimed at stress tolerant crop improvement: the Centres, along with NARS and ARIs, were seeking an understanding of the genetic control of tolerance to the key stresses in their regions for their mandate crops, and applying the knowledge to breeding programmes.

The masses of DNA sequence and the associated novel and high throughput gene manipulation technologies are giving rise to a new science. The success so far registered in applying these to the abiotic stress tolerance problem has been limited to ARIs in developed countries and to 'model' species, namely A*rabidopsis* for broad-leaved crops and rice for cereals. However, it has been discovered that genome organization across species is more consistent than originally believed ("synteny"), and thus the knowledge of genetics underlying stress tolerance in the model species can likewise be transferred to the respective mandate crop species. A generic science was about to find application in all future crop improvement programmes in the developing world. However, cost-effectiveness and efficiency reasons would dictate: the sharing of rapidly improving technologies; greater outsourcing to providers of standard scientific services; and the assembly, in a "virtual" manner, of multi-disciplinary teams to share crop-specific information.

Dr. Gale concluded that it was an appropriate time to tackle abiotic stress head-on, given the motivation already in place, the experience of the ARIs in technology and model systems, the knowledge among the NARS plant breeders on stressed agricultural environments, and the CGIAR Centres' comparative advantage over mandate crops along with their links to the developing world as well as to industry.

Not only was there general agreement within iSC/TAC with what Dr. Gale had presented but support was expressed by donor representatives for a more concerted CGIAR

effort on abiotic stress genomics for crop improvement in drought prone areas. With the key question being how to organize the System's abiotic stress genomics research, the iSC requested Dr. Gale to finalize his paper by elaborating on a possible way forward, for consideration at iSC/TAC 83.

At iSC/TAC 83 in August 2002, at FAO/IPGRI Headquarters, Rome, Italy, Dr. Gale's revised discussion paper on abiotic stress genomics was introduced by Dr. Usha Barwale Zehr, SCOPAS member. Dr. Gale considered that a possible way forward could include the appointment of an independent CGIAR Genomics Facilitator with any Systemwide genomics service should be oversee by an International Stakeholder Steering Group. The iSC/TAC was pleased to note that a number of options proposed in the document were considered by the proponents of the Challenge Programme on Unlocking Genetic Diversity in Crops for the Resource Poor, that has a focus on abiotic stress.

The iSC/TAC accepted the revised Gale paper and shared it with the Group at AGM 2002 in Manila as part of the iSC Chair's report.

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by Mike Gale

interim Science Council SECRETARIAT

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

August 2002

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Summary

Only some 10% of the world's 13 billion ha is farmed, although one third of the total land area is considered as potentially suitable for arable agriculture to some degree. Even so, abiotic stress in one form or another, still limits production on most of the world's 1.4 billion farmed hectares. This is a problem that is not going to go away. For example, yield reductions due to drought stress are already serious, and they will increase. Irrigation will cease to be a practical solution as water becomes scarcer, and the irrigation already in place will continue to lead to yet more soil salinisation. High and low temperatures, acid soils and soils with high levels of metal ions continue to reduce productivity over vast tracts of land and will remain an agricultural challenge for the foreseeable future.

Solutions to the problem will be as diverse as the lands affected. However new, locally adapted and improved varieties will always be a central component in any package of engineering, agricultural management, sociological and political solutions. Moreover in these times of surpluses in developed countries, solutions to the problem of abiotic stress are laid firmly at the door of developing country agriculture. It is here that the most severe stresses are found and here the need for increased food production to feed an increasing population is greatest.

The significance of abiotic stress has not been lost on CGIAR plant breeders. There is considerable work aimed at stress tolerant crop improvement already going ahead. Together with NARS and ARIs, the Centers are working towards an understanding of the genetic and physiological control of tolerance to the key stresses in their regions for their mandate crops, and are beginning to apply the results in breeding programmes. Progress, albeit incremental, is real and demonstrates that the problem is tractable to a genetic approach. In short, breeding is a viable option.

However developments elsewhere tell us that the future will not be the same as the past. Our science is becoming more generic on one hand, and more expensive on the other. The pressure is building for more centralization. The science of abiotic stress resistance in the CGIAR could be the test-bed for a new way of working. Genetics itself is one such area, where a new science is emerging from the masses of DNA sequence and the associated novel and high throughput technologies. This new 'genomics' promises more rapid and more spectacular returns, but with expensive equipment, much of which has a short 'shelf life'. Some of these massively parallel genomics and gene manipulation technologies are already, and with some success, being turned on the abiotic stress tolerance problem in 'model' organisms by researchers at ARIs in developed countries. Some Centers are already tooling-up for plant genomics research. Another development is the discovery of 'synteny', where genome organization has been found to be much more conserved over species than was previously thought. Application of synteny will allow advances in our knowledge about stress tolerance and the underlying genetics to be transferred between crop species. Synteny will similarly allow CGIAR and NARS scientists to apply the array of genomics resources already available in the models arabidopsis and rice to their mandate crops.

However, in order to harness synteny and the new genomics in a cost-effective and efficient manner it will be necessary to develop new ways of doing science. These could involve: more rationalization and centralization and sharing of expensive and rapidly improving technologies; more outsourcing to providers of standard scientific

services and the sharing of skills by assembling multidisciplinary teams and networks in virtual centres that will work on a range of crop species. DNA science and the expensive equipment it begs, is identical for all organisms. Suddenly there is obvious potential for economies of scale in major collaborations.

The time could be right for a full-blooded assault on abiotic stress. Ongoing work shows that the motivation is already there. The question is not whether the work is needed, rather only when, how and what firepower should be brought to bear. A really effective collaboration will involve: the NARS with their germplasm collections, their knowledge of and, access to, stressed agricultural environments, and their plant breeders; ARIs with their experience of technology and model systems; the CGIAR Centers with their comparative advantage with the mandate crops, their collections and their networks to the developing world; and possibly industry as well.

Many of these ideas have already been incorporated in a Global Challenge project, ‘Unlocking genetic diversity in crops for the resource poor’, and, apart from a recommendation to compile lists of potential alternative crops for use in sub-optimal soils and climates, are not dealt with further at length.

Optimal organization of genomics science within the System is relevant and not dealt with elsewhere. It has become clear that the efficient application of genomics and the provision of genomics services within the CGIAR and for NARS partners will require a co-ordinated approach that is not in place today. This paper looks towards a time when basic genomics resources are available for all the mandated crops, and when all CGIAR and NARS researchers have access to sustainably state-of-the-art genomics platform technologies.

The conclusions are that an increasing amount of work will be outsourced to specialist companies, leading ARIs or other Centers. There will likely be strong financial and infrastructural reasons for centralizing other technologies, possibly micro-arrays today and soon the next generation of high throughput genotyping for marker-aided selection and germplasm characterization. There are also scientific reasons for sharing intellectual resources that are in short supply or unevenly spread around the System, such as bioinformaticists and physiologists. There is an opportunity to cost-effectively appoint a central Genomics Facilitator who will carry out market testing, organize key facilities and link groups of researchers around the System so that we can best exploit the new generic aspects of our science. The existing and active CGIAR Task Force on Genomics, with iSC oversight, will provide an ideal forum to discuss these developments.

The potential application of molecular biology to genetically enhance crop tolerance to abiotic stress – a discussion document.

Introduction

Only some 10% of the world's 13 billion ha is farmed. Apart from urban areas much of the remaining 11.5 billion ha are lands too hostile for any sort of agriculture [1]. Moreover almost all the land that is farmable is under conditions sub-optimal, often to a considerable degree, for plant growth. Alongside losses due to pests and diseases, a further 70% of yield potential has been calculated to be lost to unfavourable physiochemical environments, even in developed agricultures [2].

It is acknowledged that, in order to feed the eight billion mouths we expect by 2030, we will need to double world food production yet again. And we will. One component of that achievement will be the breeding of new varieties of food crops that will both improve yields on land presently being farmed on sub-optimal soils and extend our productive agriculture into lands which are currently barren.

Abiotic stress – extent of the problem

Drought. Unpredictable drought is the single most important factor affecting world food security and the catalyst of the great famines of the past. Moreover, because the world's water supply is fixed, increasing population pressures will ensure that the effects of successive droughts are more severe [3] because competition from industry will increasingly limit the water available for agriculture. Crops are voracious consumers, for example, for paddy rice 5000 l of water is needed to produce 1 kg of grain. At present an unsustainable 70% of the world's water is used for agriculture. By 2025 it is expected that most Asian countries will join those that already have water shortages. Uncertainties over global warming raise yet further concerns.

> Drought stress is a concern for most crops at most Centres for most regions. These include, IITA Cowpea in the Sahel, soybean and tropical maize in the Dry Savanna, ICRISAT Sorghum, pearl millet, chickpea, groundnut and pigeon pea, CIAT Bean in Mexico, C America and NE Brazil, IRRI Rice in Bangladesh, E India, Thailand and Indonesia. CIP, Potatoes in China, India, Southern Africa, Kazakhstan and Afghanistan. CIMMYT, Wheat in C and W Asia and N Africa and maize in sub-Saharan Africa. ICARDA All crops (except faba bean which is only grown under irrigation) in N Africa and Asia. ICRISAT all crops in India and the Sahel.

Salt. Some 380 million ha, almost a third of the area farmed, is affected by salt, and the associated water logging and alkalinity [4]. Sixty million ha are a direct result of over-irrigation, where a raised water table brings underground salt, particularly NaCl, to the surface. It is probable that this agricultural salinisation now degrades as much land as is put under new irrigation each year. Pressures on water use will ensure that the net productive irrigated land will go negative very soon and that secondary salinisation will become critical in Asia, Africa and S America [5].

> Salt stress is of particular significance for rice. IRRI Coastal salinity in Bangladesh, Orissa, Vietnam, Philippines and inland salinity in the Indogangetic plain and Thailand. ICARDA Secondary salinisation is a problem for all crops in C Asia.

Acid and degraded soils. Some 40% of the world's arable land is associated with acid soils, with pH less than 5, where growth is hindered by high aluminium or manganese content. This is particularly important in S America where some 380 million ha are affected, including almost the whole of the Amazon basin [6]. Other excess metal ion contents reduce the agricultural potential of other soils. For example iron toxicity is a major problem affecting rice production in W Africa.

> Acid soils are a widespread problem. IITA Cowpea and soybean in the humid rain forest. CIAT Bean in Africa and both bean and *Brachairia* in L America. IRRI Rice in Bangladesh, Indonesia and Philippines. CIMMYT maize in L America, SE Asia and Africa. Wheat in CWANA.
>
> Other metal toxicities and deficiencies. IITA Low P for soybean. CIAT Low P for bean and *Brachairia*. IRRI Zn, P deficiency in Bangladesh, Indonesia and the Philippines and Fe deficiency in Sri Lanka and the Philippines. WARDA Fe deficiency is widespread in Africa. CIP Low P in China, Africa and in the Andes.

Low and high temperatures. Temperature also limits the range and production potential of many of our crops, even at tropical latitudes [7]. Occasional and unpredictable periods of low temperature can be devastating to yields. For example, in the Andes 70% of land devoted to potato production is prone to cold stress [6].

> Cold stress is a rice problem for IRRI in Korea and Nepal. CIP, potatoes in the Andes. ICARDA Low temperature tolerance has become a problem associated with the shift from spring to autumn sowing for barley, lentils and chickpeas.
>
> Excessive heat is a problem for cowpea. IITA in the Sahel. CIP, for potatoes in S Asia.

In fact, abiotic stress tolerance, particularly drought, is the priority target trait for most of the CG Centers dealing with crop plants. In the present economic and agricultural climate, with food surpluses in developed countries, the focus of the private sector will continue to be protection from disease and improvements in aspects of quality. Even given the extent of the problem and although abiotic stress is a significant factor for production in developed countries, it is unlikely that genetic solutions will be actively sought by commercial breeding companies. If the problem is to be tackled at all, abiotic stress tolerance mechanisms and their genetic application in the crops of the developing world will have to be addressed by the public sector working in the developing world.

Gene mapping and marker development for genetic analysis and MAS in breeding

Genetic mapping as a prerequisite to genetic analysis is now part of standard plant breeding. Annex 1 shows clearly that base molecular maps are now available for most of the CGIAR's crops. Those that are the focus of international effort, e.g. rice, wheat, potatoes, can use the well-developed public maps. Base maps for many of the 'orphan crops', in which there is little international trade, have been made at Centers or in Center-ARI collaborations. Only a few very minor mandated species remain unmapped.

The mapping of quantitative traits where there is often little knowledge of the genetic control in advance of the analysis, such as is usually the case with stress tolerance, is usually carried out by 'QTL mapping'. This requires a scan of the genome, with markers every 10 cM or so to identify those regions where segregation of the trait is associated with segregation for the markers. The reason much denser base maps are needed is that only a subset of the available markers will segregate in any single population. These locations are the basis for establishing a marker aided selection (MAS) breeding programme for tolerance and for eventual map-based cloning of the genes underlying the QTLs. Annex 1 shows that key stresses in several crops are already being addressed in this way.

Breeders' markers that are closely linked to the target gene may be derived straight from the base molecular map. Today the ideal marker system will be micro satellites, also known as simple sequence repeats (SSRs), although over the next few years single nucleotide polymorphisms (SNPs), which are more amenable to high throughput methods, will take over as the ideal marker.

For rice, which will soon have the benefit of a full genome sequence, markers will never be a problem again. The sequence has been found to contain some 40,000 SSRs[8] and SNPs and, base pair deletions or insertions indels, are found in unique sequence at a rate of about 1% [9], which works out at about 24 in every gene! However, apart from in the major cereals, an adequate supply of good quality markers for all applications is still a problem for most CGIAR mandate crops. The status of the genetic maps and markers available for the CGIAR crops is outlined in Annex 1.

Genetic and physiological mechanisms that control stress tolerance

The physiological mechanisms underlying crop responses to stress and potential biochemical, physiological and architectural modifications that will allow crops to escape, avoid or tolerate stress are the subject of a vast literature. Two general approaches are taken in relation to varietal improvement, and both have their place. The 'empirical' approach proceeds from genotypic differences associated the best sources of tolerance in the cultivated crop or its wild relatives. Typically sources of tolerance are identified and then the underlying genetic control is investigated by QTL analysis in lines segregating for high and low tolerance. Although the identification of a predominant causal physiological mechanism is helpful, transfer of the improved trait to an already otherwise adapted variety can proceed simply for selecting for and accumulating 'beneficial' alleles. The second approach is often described as 'ideotype' breeding, in which specific morphologies or physiologies that might be expected to contribute to improved performance under stress are identified in diverse cultivated or wild germplasm and transferred to otherwise adapted varieties. The crossbreeding and, these days, marker-aided pyramiding of the underlying alleles is progressed in the same way in both approaches.

There is already considerable work underway at all CGIAR Centers to improve their mandate crops for stress tolerance. Almost all these breeding projects are being carried out in collaboration with NARS to address the major problems affecting their own agricultures. However many of these projects are crop- or geographical area-specific, even though the target tolerance and the technologies used to address the problem beg a collaborative, pan-stress, pan-crop, pan-Center approach.

For example drought, salt stress and cold temperature stress are all physiologically linked because all three stress environments result in limiting the crops' physiological access to water. Thus many of the strategies for improved tolerance are likely to be multiply applicable. These will include osmotic adjustment in roots and leaves to retain water, erecting hydrophobic barriers in roots and leaves to retain water, and improving aquaporin efficiency to speed water movement in the plant. Although tolerance mechanisms might be expected to overlap, escape or avoidance mechanisms are more likely to be stress specific. For example reducing time to flowering may escape late season drought but will not help in a chronic saline situation. Deeper roots may be able to reach the last of the water in a drought but would only aggravate salt stress where the salt is being brought to the surface by a rising water table.

With this background one would intuitively expect genetic control to be multigenic and complex, but to overlap somewhat in tolerance to the different stresses. This is exactly the situation found. Consider, for example, wheat and barley where the various reported genetic effects regulating responses to drought, salt and cold have been assembled on one comparative chromosome map, Fig 1 [10]. While ten or more QTL s are found for each trait, many overlap so that a few chromosomal regions are home to controlling factors for all three traits.

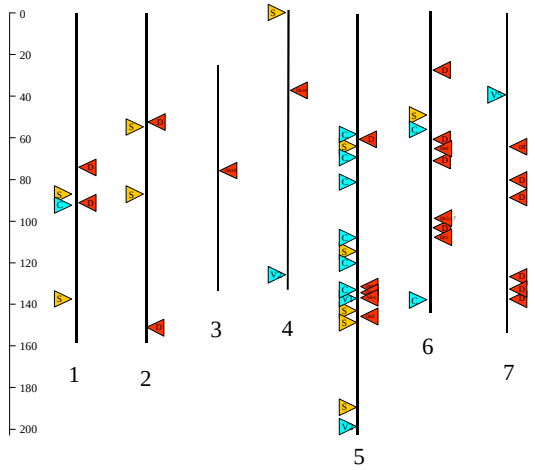


Fig. 1 Abiotic tolerance QTLs and major genes mapped on a composite Triticeae chromosome map. Salt tolerance in orange, cold tolerance in blue, drought tolerance in red.

The empirical approach to aluminium tolerance, where the selection screen is usually for improved growth of roots and shoots in Al supplemented nutrient solution at low pH, also reveals complex control. However, the network of genes is often dominated by one locus which accounts for a major proportion of the genetic variation e.g. [11,12]. These results identify MAS breeding priorities and also cry out to be followed by isolation of the key gene, either by cloning or the production of isogenic lines. Gene isolation and knowledge of the gene sequence can be critical steps in a project elucidate an understanding of the underlying mechanism.

A few stress tolerances do usually appear to be under the control of major genes, as revealed by genetic analysis. Submergence tolerance is a prime example, and it may be no coincidence that this trait lends itself to a straightforward and definitive selection screen. This is a key trait in SE Asia where some 25 million ha are prone to flash flooding which can completely submerge the rice crop for several days. Here a single locus, *Sub1*, has been shown to provide substantial tolerance [13,14].

Fig 2 Wheat, *Thinopyrum bessabaricum* (also known as *Agropyrum junceum*) and the man-made amphiploid, Tritipyrum grown in 250 mM NaC1. The amphiploid assumes some of the salt tolerance of the wild maritime grass parent. From Forster, B.P., Gorham, J.,& Miller, T.E. (1987). Plant Breeding 98:1, p.2, fig 1 "Plants of 'Chinese Spring' amplhiphloid & Ajunceum"

Mention must be made of the potential of wild relatives as potential donors. Wild species, where the *raison d'être* is survival rather than yield, are likely to retain useful variation that may have been bred out of the cultivated crop. There are many examples where genes for tolerance have been identified in wild relatives and have been used to transfer useful variation to cultivated crops. In some cases the wild species themselves have been used directly to create new crop species, e.g. Tritipyrum incorporating salt tolerant *Thinopyrum bessabaricum*, Fig 2[15]. Annex 1 shows that CGIAR breeding programmes are accessing this source of variation. The germplasm collections, which are mostly still relatively uncharacterized, will be central to any future stress tolerance initiative.

A possible alternative to varietal improvement is crop replacement. This is probably a viable socio-economic strategy only in extreme stress environments. Nevertheless NARS and their extension services should have reliable information about those crops which generally perform best in stress environments. These differences are exemplified by investigations into the responses of tropical grasses to aluminium stress. Signalgrass (*Brachairia decumbens*) was found to be far more tolerant than both close relatives and Al-resistant varieties of wheat, triticale and maize [16] Fig 3. It is probable that world-wide multi-centre, multi-crop studies, which are unlikely to be carried out by 'one-crop specialists', might reveal interesting alternatives in many stress situations, possibly even pushing back the borders of lands presently considered to marginal for agriculture at all. Overall the genetic dissection of stress tolerance for developing countries is receiving considerable attention, particularly in crops that already have advanced genetic maps. Initial understanding of the physiological and genetic controls most certainly informs breeding programmes. Marker-aided pyramiding of several genes is probably the only way forward for the transfer of improved phenotypes that have been shown clearly to be under control of multiple loci. However these methods have yet to impact stress tolerance breeding programmes. Although a few endogenous genes have been identified which are likely to have major beneficial effects when used as a transgene, these have not yet been applied to practical breeding.

Mapping by NARS and CGIAR Centres has identified a number of genes, usually as anonymous QTLs. Most of these genes have not

yet been associated with physiological mechanisms. Linkages between geneticist-breeders and physiologists could now pay dividends. The potential is there for application of these QTLs through MAS but this has not yet been generally successful. There is considerable scope for more collected wild and cultivated germplasm characterisation for stress tolerance. There is also a need to identify any potential alternative crops for severe or chronic stress environments. Any Systemwide initiative to quantify crop yield potential under stress should use common genotypes across stresses and regions.

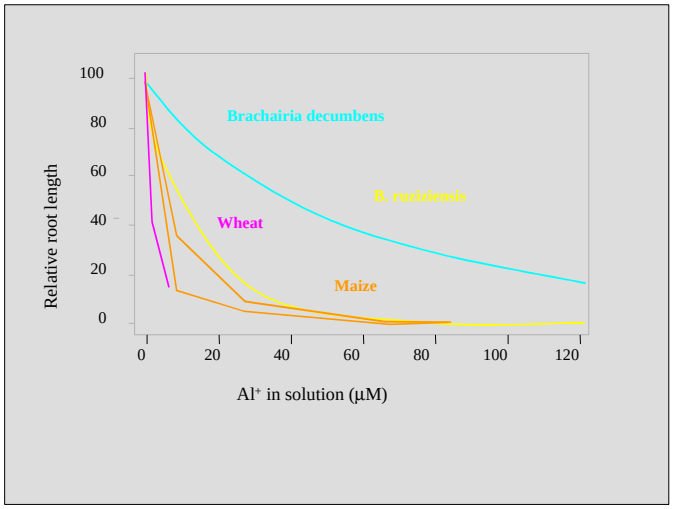


Fig 3 Signalgrass (*Brachairia decumbens*) is far more tolerant of high aluminium concentrations than other *Brachairia* species or 'tolerant' varieties of maize or wheat. Adapted from Wenzl et al (2001) Plant

Progress in breeding – slow but real

Some progress has been made in breeding for drought, salt and aluminium tolerance or avoidance. The CGIAR Center breeding programmes have played a major role in these advances, a small selection of which are listed below. In general these have involved incremental, rather than quantum jump, improvements and have been achieved by empirical selection and not, as yet, by MAS.

- Many releases of drought and acid soils tolerant tropical maize varieties released worldwide, e.g. the acid soils tolerant CORPOICA H-108 and H-111 for Colombia, the Pool 25 and population 28 lines for acid soils used the Brazilian programme, ZM421, 521 and 621 recently released in Southern Africa which are both tolerant of low nitrogen and mid-season drought. (CIMMYT)
- Rice releases listed as salt tolerant for Bangladesh, e.g. PSBRc 84, 86 and 88. PSBRc 88 has good eating quality and is planted even in non-saline areas (1999, IRRI)
- Drought tolerant banana variety, FHIA01, bred and released in Honduras, now released in Tanzania and in trial in 50 other countries (INIBAP)
- Heat tolerant potato variety, Unica, released in Peru (1997, CIP)

- Series of durum wheat and barley varieties that have extended the range of these crops in Syria. Chickpea varieties which have facilitated the switch from spring to autumn sown crops (ICARDA)
- Release of drought tolerant Nerica lines, first in Cote d'Ivoire and now particularly in Guinea (1998, WARDA)
- Release of Mulato, a *Brachairia* Al tolerant variety for Mexico and C America (2001, CIAT)

Progress has however been hampered by the perception that, in some situations, stress tolerance and high yields are incompatible. The view that higher yields under stress conditions are incompatible with higher yields under good conditions [17,18,19] invokes the need for independent targeted breeding programmes of specialized varieties. In particular it has been argued that, as drought 'stress intensifies, high yield potential and drought resistance become mutually exclusive'[17]. Counter to this is the conclusion that the improved yield of hybrid maize in the US, where there have been steady improvements since the 1930s, is mainly all the result of selection for response to tolerance to stress [20]and potential yields have not changed over this period, Fig 4.

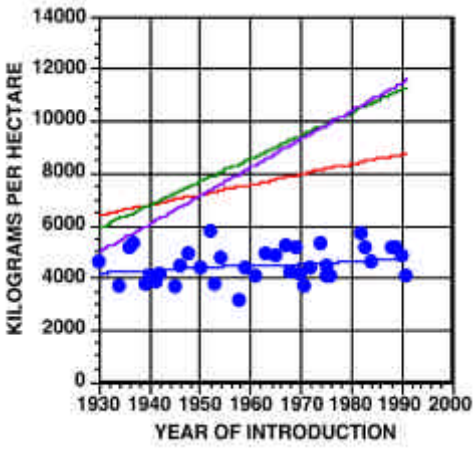


Fig. 4 Grain yield of maize hybrids regressed onto year of introduction at four planting densities. 10,000 plants ha (i.e. at 1 m spacing) in blue, 30,000 in red, 54.000 in green and 79,000 in blue. Maximum yield potential per plant has not altered over the past 70 years. Increased yielding ability is due to improved tolerance to abiotic stress. From Duvick (1997) in 'Developing drought and low N-tolerant maize', CIMMYT

Yet others [21] believe that decentralized participatory breeding with local partners provides the most viable means of breeding locally adapted lines, and at the same time provides an acceptable compromise accommodating the two opposing views. It may well be that there are good physiological reasons as to why the former view may be true for some crops for some stresses at some levels of intensity.

> Breeding for stress tolerance will proceed more efficiently once it is clear whether, for individual crops and specific stresses, yield potential under stress is controlled by the same genes as yield under optimal conditions. The conclusion will dictate breeding strategy.

Appropriate screens

Selection screens appropriate to the field conditions that new varieties might experience are often problematic. Field trial climatic factors such as drought and temperature are often unpredictable, while uniform stress conditions are difficult to achieve in trials for edaphic stresses. Also different stresses are often found together, for example salinity problems are rarely all due only to common salt, NaCl. In fact stress the field is rarely due to a

single factor. For example multiple metal toxicities and deficiencies are often found simultaneously, and these are farmers' field conditions that are very difficult to match in the laboratory or glasshouse.

Of course, in the later stages of a breeding programme, empirical selection under field stress conditions is still probably the available approach for most breeders working in developing countries. Usually breeders will simply select those lines that remain the greenest after a period of stress, even though it is well known that plants retain chlorophyll even after all growth has ceased Fig 5 [22], i.e. that survival is not the same as productivity. The recent developments in thermal imaging and chlorophyll fluorescence imaging [23,24] may provide rapid, economic, non-invasive selection criteria applications over a range of crops and stress programmes.

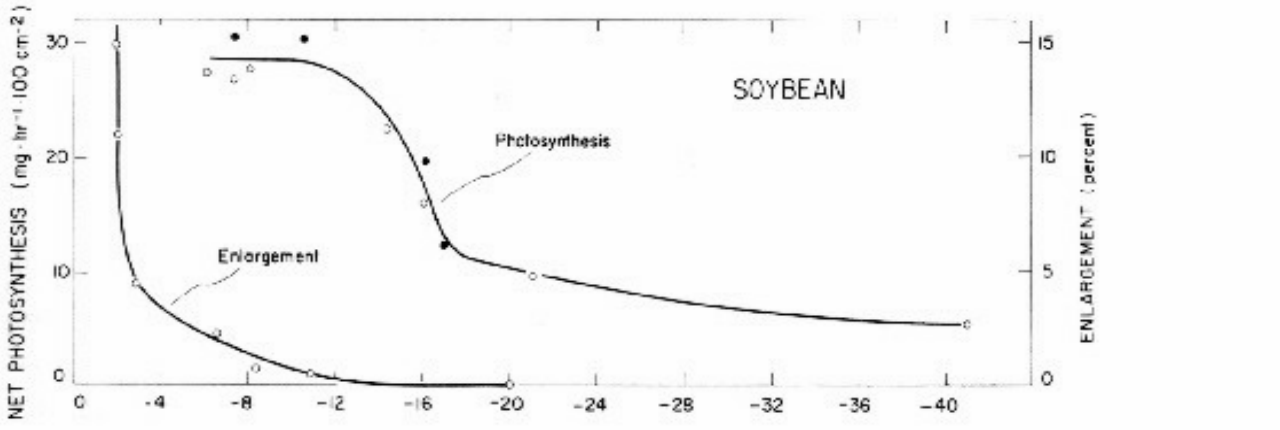


Fig 5 'Green' is not the same as 'growing'. As water potential is lowered (as in increasing drought or salt stress) chlorophyll retention persists after all growth has ceased.
From Boyer, J.S. (1970) Plant Physiol 46, p234 Fig 1 "Rates of leaf enlargement and net photosynthesis in corn, soybean & sunflower plants at various leaf water potentials".

Good uniform trial sites for stress tolerance selection are not common and where possible should be shared over breeding programmes. The involvement of physiologists with experience of appropriate imaging technologies could benefit a range of CGIAR stress tolerance programmes.

Genomics – the new genetics

Developments over the past decade, arising particularly from the human genome programme, have led to a new phase of plant genetics. 'Plant genomics' is the application of the newly available vast amounts of genomic DNA sequence, using a range of novel high-throughput, parallel and other technologies. In plants a 'whole genome' DNA sequence is available as yet only for arabidopsis, which was 'finished' in 2000. A 'draft' raw almost complete sequence of indica rice has been deposited in the public databases by the Beijing group[9] and a similarly complete sequence of Japonica is available within a private company [8].

The fully annotated public DNA sequence of rice, 88% complete at the moment, will be finished later this year. Undoubtedly more species will follow. Possibly maize will be the next major crop plant to be sequenced, at least for 'gene-rich' regions of the genome. Technologies which are included under the umbrella of 'genomics' are: automatic DNA sequencing, where one machine can read two million base-pair a day; microarrays and DNA chips where tens of thousands of genes can be scanned for activity levels at the same time; automated genotyping machines that can assay tens of thousands of DNA diagnostic points a day. In fact it will soon be possible to monitor whole genomes for genetic markers or gene expression on single chips. Transformation technologies that allow the facile and efficient genetic modification of almost all crop plants can also be considered genomics technologies.

Genomics is still in its infancy. Genomics technologies, beyond the now conventional molecular biology technologies, are being taken up by CGIAR Centers and by NARS. High throughput capillary DNA sequencing machines and micro-arrayers are in place in the Centers, transformation as a research tool is available for most mandated species (see Annex 1).

> The CGIAR Task Force for Genomics met in April 2002 to consider the Systemwide accountability and organisation needed for flexible, efficient, sustainable, cost effective genomics for the mandate crops. Some consensus was achieved and more can follow.

Synteny and comparative genomics

A second development, which has also emerged over the last decade, is the discovery that gene content and gene order is much more conserved over even quite distantly related species that was previously envisaged. This is known as 'synteny' [25].

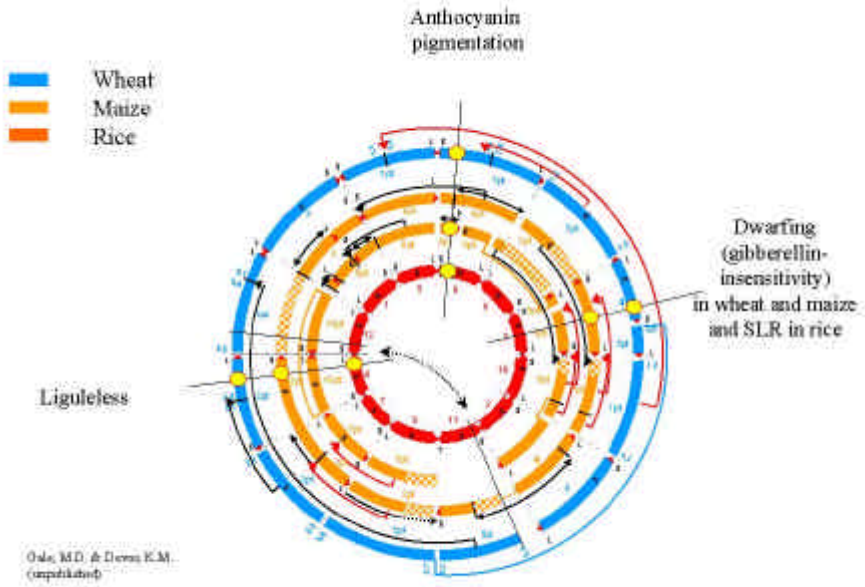


Fig 6 Crop circles. The genomes of the three major cereals aligned syntenously so that homoeologous genes lie on radii. Three homoeoallelic series are shown, including the *Rht* (wheat), *D8* & *D9* (maize) and *SLR* (rice) dwarfing genes. M.D. Gale and K.M. Devos, unpublished

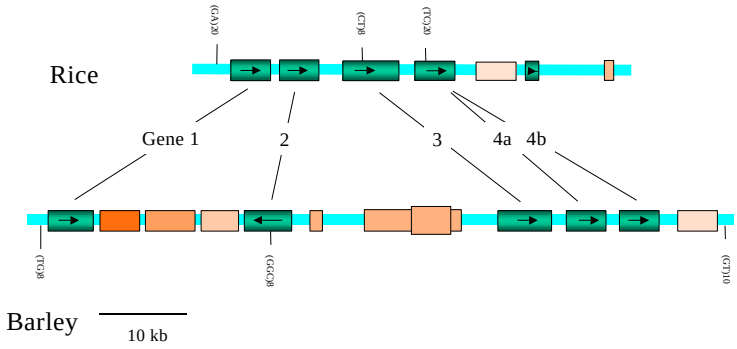


Fig 7 Gene content and gene order is remarkably conserved between rice and barley. As is commonly found, one gene is duplicated in one of the genomes. Also the genes in barley, which has the larger genome, are interspersed with large repeated elements. Adapted from Dubcovsky et al (2001) Plant Physiol 125:1342

Synteny over the grasses, where the phenomenon is most clearly documented, has recently been tested in shotgun rice genomic sequence produced by a private company [8]. Almost all the cereal genes, of known and unknown function, and all of the proteins they code for are found to have corresponding genes in the rice sequence. The maps of the genomes of the grasses, which include all the cereal crop species that have evolved over the past 60 million years [26], can be aligned so that the location of a gene known in any one gene can be predicted in all the others [25], Fig 6. A corollary of synteny is, of course, that gene function and role in control of agronomic traits can also be predicted across all the cereals. We should note that the similarity between genomes is restricted to the genes themselves and that intergenic regions differ greatly between even quite closely related species, e.g. as shown in Fig 7, and give rise to the large variation in genome size found in the grasses [27].

The key issue here for the application of genomics tools in the CGIAR crops is that all of the rice resources can be applied directly to the genetic analysis of wheat, maize, barley, pearl millet, finger millet and sorghum. First generation comparative maps have been published for rice and all these genomes.

It turns out that the 240 million years that separate the grasses from the broad leafed plants, the eudicots, has degraded precise map correspondence to the point where the retained synteny does not have predictive utility[8,28,29]. However the arabidopsis sequence and the arabidopsis genomic resources are available and applicable to broad-leafed crops, e.g. tomato [30]. The genome relationships are close within the Crucifereae, which includes arabidopsis and the brassica crops. The Solanaceae and the Crucifereae are more distant from one another at an estimated 150 million years. Nevertheless the arabidopsis gene organization can still be used to aid genetic analysis in tomato [31]. The syntenic relationships between arabidopsis and the majority of the broad-leafed CGIAR crops, particularly the legumes, are not well established (Annex 1).

However other models are emerging which will aid genetic analyses in these crops. The DNA sequence of a legume model, probably *Medicago* (alfalfa), will be available in the foreseeable future. Also work is progressing rapidly with the tomato genome to bring this species up to model status for all Solanaceous crops.

Breeders and geneticists of cereal crops should become familiar with the relationship of the rice genome organisation and that of their own crop.

Breeders of broad-leafed crops where the genome relationships with arabidopsis are not known should have access to the arabidopsis sequence, and should be beginning to establish the syntenic relationships. Novel bioinformatics applications will be are necessary for full application of synteny to crop improvement.

Genomics applications in CGIAR mandate crops in relation to abiotic stress tolerance

In order to employ genomics to address the problems of abiotic stress in mandate crops and to be able to exploit synteny, rather than simply rely on solutions formulated in the models, a basic genomics infrastructure in the crop itself is required. The bare minimum is probably a molecular framework map of the chromosomes, a large DNA insert library, and a facile (and reasonably efficient) transformation system capable of delivering relatively large numbers of engineered plants. The map will have markers every 2 or 3 cM and will have a number of anchor loci, RFLPs or ESTs marked with SSRs or SNPs, that will allow comparisons with the appropriate models. The library will probably be a better than 2-times coverage BAC library with insert sizes in excess of 100 kb.

Additional resources that will be of value include a collection of ESTs (transcribed gene sequences), a comparative map and some ‘knockout populations. The ESTs will often be comparative collections from stressed and non-stressed plant tissues. The comparative maps will align syntenous chromosome regions of the crop with the model. The knockouts will probably be mutation or deletion libraries in which genes have been disabled at random, although T-DNA tagged or transposon-tagged population, as are available or under development in arabidopsis and rice, are also possible when a good transformation system is available.

Extensive public sector genomics resources are available for maize, wheat and rice. Genomics resources for other CGIAR crops, such as pearl millet, sorghum and *Musa*, are being created in collaborations with ARIs. Other crops such as bean, cassava and *Brachairia* are being worked up within the CGIAR System and yet others, such as forage legumes, beans and chickpeas, have yet to be started (see Annex 1).

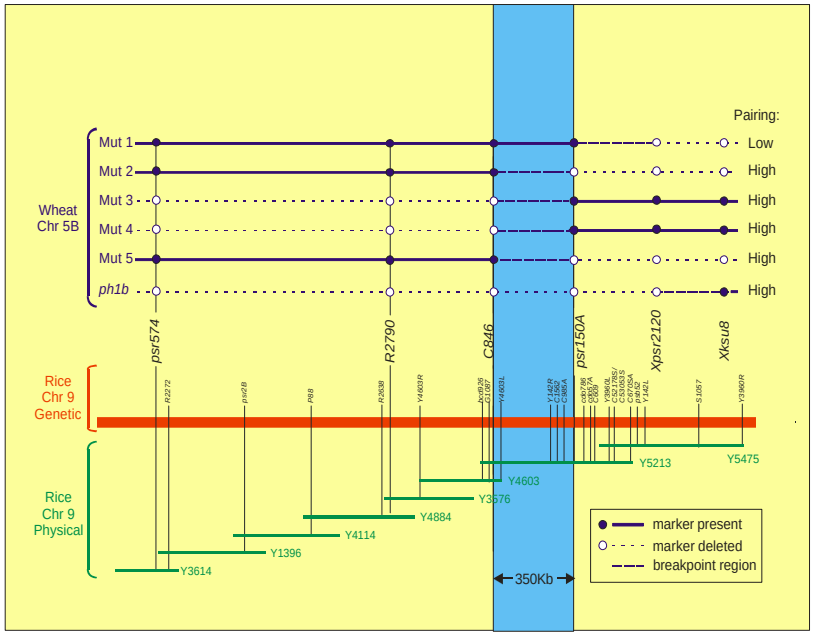
At the very least base genetic maps should be available for all CGIAR crops (a microsatellite-based map would today be the work of a 3-year post-graduate student). This will open up all species to MAS breeding. Further resources may easily be added at a later date. Links with ARIs or direct outsourcing should be explored for EST and BAC library production.

Gene cloning

There are many ways to clone genes, however if all that is available is a map location of a stress tolerance gene and there is no indication of its precise function, then the process will probably be either to identify possible candidate genes by their map position or to find genes whose expression is associated with the trait. Certainly in order to find out unequivocally what any gene actually does it will generally be necessary to clone it first.

Map-based cloning

Fig 8 Deletion tiling. In an experiment to isolate a wheat gene, *Ph1*, which controls chromosome pairing, the critical wheat chromosome was aligned with the syntenous chromosome in rice. Then a series of 'knock-out' deletions in wheat, identified by their *Ph1* phenotype, were produced. The individual wheat deletions are Mb long. However the minimum overlap region of these deletions (shown in blue) defines a mere 350 kb region of rice genome in which to search for candidate genes. Adapted from Roberts et al (1999) Genetics 153:1909



When the alignment of the crop and model maps is available candidate genes mapping in the region of the QTL in the crop can be identified directly from the model DNA sequence. With around 30 genes per map unit, as in rice, it will usually be necessary refine the map location of a QTL, which has been reduced to an identifiable major gene by this time (a process sometimes referred to as 'Mendelisation'), in large segregating populations. BAC contigs, overlapping linear series of large insert clones, can also be made in the crop itself using DNA landmarks gleaned from both the crop molecular map and the model sequence.

A novel method of map-based cloning, known as 'deletion tiling', involving the generation of a number of deletions that include the target gene in the crop, and using the minimum overlap to identify candidates in the model, is being pioneered in wheat, Fig 8 [32]. This method has the advantage that variation is not required for either the target gene or the flanking DNA regions, and it could find many applications in CGIAR crops.

Microarrays

Candidate genes will also emerge from microarray analyses. Genes that are induced by stress are ideal for comparative microarray analysis. A typical experiment will be to challenge an array of ESTs with RNA extracted from stressed and unstressed tissues. A comparison of the two will identify genes that are up-regulated in the stressed tissues and those which are down-regulated or switched off, Figs 9 and 10. Whether these represent genetic cause or effect is another challenge. Ideally one would like these experiments to scan entire genomes because, until all genes are available, any microarray experiment will always be incomplete. This will be possible in the near future for the 25,000 arabidopsis genes and the 50,000 rice genes, but not for other crops for some time. Mini-array's can however be built from collections of ESTs assembled from random cDNA libraries, or from more targeted collections made from cDNAs collected from stressed tissues. Even more targeted will be the special 'stress arrays' made up of all the expressed genes for which there is any evidence of implication. Stress arrays are being contemplated in several CGIAR Centers. Other

technologies such as cDNA-AFLP and differential display can also identify critical gene sequences.

A major NSF grant [33] has recently been completed which has investigated the use of arrays to investigate salt tolerance. This project has made a commendable start to cataloguing stress inducible genes in halotolerant and salt-sensitive plants. The results are generally relatively complex For example, in rice 10% of the genes were significantly up- or down-regulated after 1 h of salt stress [34]. An added complication is that almost half of the genes available as ESTs or as hypothetical genes in genomic DNA sequence have, as yet, completely unknown functions.

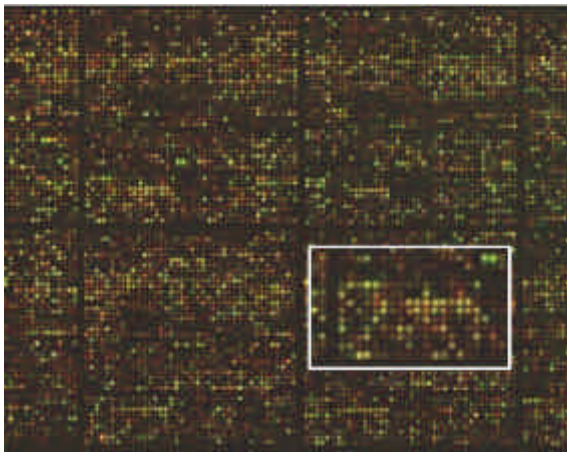


Fig 9 Micro-arrays can carry 20,000 genes on a 2 cm^2 plate. When arrays are probed with RNAs from, say, stressed and unstressed plants computer enhanced imaging identifies genes that are under-expressed (in red) and over-expressed (in green).

Plainly microarray analysis has a role to play and the CGIAR groups should be gaining experience of the technology. However it is equally clear that microarrays alone will provide complex correlative data which will require considerable further refinement using, for example, knockout phenotypes and QTL location-related map data in order to tease out the causal genetic elements. Candidates from the arrays that coincide with the candidates from map-based approach will be of significant interest.

Comparisons of the DNA sequence or the hypothetical protein product sequence with other isolated genes of known function can provide a lot of information. However while some half of the genes revealed by whole plant genome sequencing remain of unknown function, it will often be necessary to attempt to elucidate function by various reverse and forward genetics methods including transformation and knockout analysis, in both the crop and the

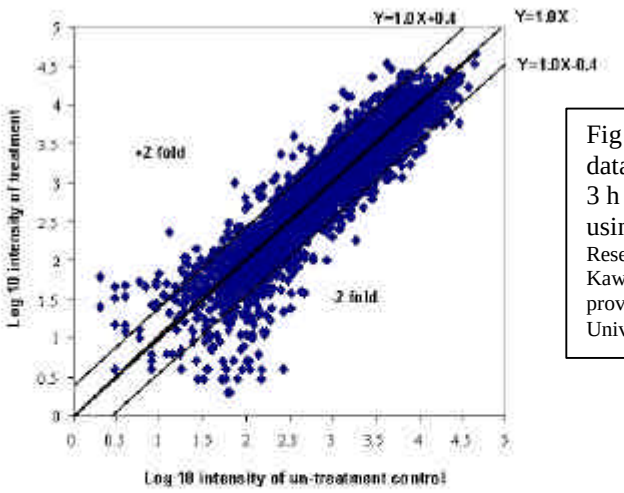


Fig 10. Microarray data. Rice response to 3 h drought stress, using 6,400 ESTs. Research by Shinji Kawasaki. Figure kindly provided by Hans Bohnert, University of Illinois.

model. The definitive experiment will usually involve the production of transgenics with an expected phenotype.

Knockout populations

Various T-DNA or transposon tagged populations are available in rice and arabidopsis. These reverse genetics 'gene machines' allow the identification of lines in which any gene of interest is disabled. These lines can then be investigated to identify a phenotype that may give clues as to the genes function. A recent development, TILLING (from 'targeted induced lesions in genomes') [35,36] allows production of targeted knockouts and also the creation of allelic series in any gene. TILLING populations are available for arabidopsis and are under investigation for rice.

Chemical or irradiation mutant populations in the crop itself will allow a forward genetics approach. Stress tolerant lines can be identified by the simple expedient of subjecting populations to drought, high salt, cold temperatures, submergence etc. and selecting vigorous survivors. The challenge then is to link the phenotype with a deleted gene for which the sequences of candidates will be a good starting point.

> With the notable exception of rice, CGIAR germplasm curators have not entered the field of knock-out populations in mandate crops. CGIAR Centers and NARS have significant comparative advantage in having the facilities to grow and maintain large populations, and they have the expertise in the crop to recognize and screen for key knockout phenotypes. These populations will be central to functional genomics efforts and, especially for the minor CGIAR crops, their availability should encourage collaborations with ARI researchers working in model species. The opportunity costs of not taking an international lead of this sort should at least be evaluated for all of the mandate crops.

Transformation in the crop and the model

Genetic transformation is now possible for most crop species. However 'possible' is not the same as 'efficient'. CGIAR Centers need to be able to produce at least tens, and ideally hundreds, of low copy insert transgenics for any construct (the need for many lines is demonstrated by the range of phenotypes produced in any single transformation experiment, see Fig 14). Good systems exist for many broad-leafed crops and for most cereals. Legumes are probably the most recalcitrant group of crop plants. The status of CGIAR Center transformation capability is shown in Annex 1. Transformation is often seen only as means of making transgenic crops. Indeed, the ideological debate surrounding transgenics notwithstanding, it is inconceivable that we will penetrate far into the 21st Century and its looming food shortages without needing to use all the technology that we have available. However for the time being, transformation has another use as the ultimate test of function of any candidate gene, either in the model or the crop.

Initial attempts will usually employ constructs of the beneficial allele of the gene linked to a constitutive promoter, such as CMV35S, in over-expression experiments. Early transgenic trials will also usually involve antisense constructs that will provide information by negating the effects of the gene. Later trials in the crop itself will probably employ specific

alleles of the gene in constructs with promoters that target the effects to specific tissues, such as roots or developing seeds, at particular developmental stages. Transgenics in the crop itself will likely be in an already otherwise adapted genetic background, and these may serve as breeders' lines for eventual introduction into the main stream breeding programmes.

Leads from model species

Research into the molecular basis of abiotic stress tolerance is being carried out mainly in model species, particularly arabidopsis. Although this area of our science is still in its infancy there are some 200 references and claims in the reputable scientific press. Genetic transformation experiments to improve stress tolerance are beginning to yield some promising results.

A particularly encouraging approach is the use of transcription factors, regulatory elements that control batches of genes, including those which are induced by stress. One such is *CBF1* in arabidopsis, which is the likely regulator of the cold acclimation response. Over-expression of *CBF1* enhances the levels of a swathe of cold-regulated genes to mimic the effect of cold acclimation that provides subsequent resistance to freezing, and provides protection against cold temperature damage, Fig 11 [37]. Transcription factors act as 'master switches' and provide one means of rationalizing and exploiting the information obtained from gene expression microarray analyses.

DREB1A is another transcription factor that regulates expression of a further range of stress tolerance genes. Over-expression of *DREB1A*, again in arabidopsis, activates expression of a range of genes and results in improved drought, salt and freezing tolerance [38].

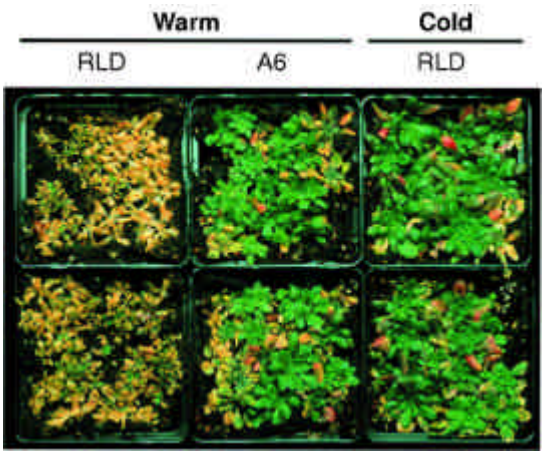


Fig 11 Effect of *CBF1* over-expression in arabidopsis, *Left*: Non- acclimated controls after freezing for 5 days; *middle*: Non-acclimated transgenics after freezing, *right*: Acclimated controls after freezing. Reprinted with permission from Science 280, p 105, fig 3 "Freezing survival of RLD and A6 Arabidopsis plants", Jaglo-Ottosen et al.

Interestingly it was noted that, when *DREB1A* was driven by CaMV35S, a strong constitutive promoter, normal growth of the plants in an unstressed environment was severely retarded. However the simple expedient of driving *DREB1A* with a stress inducible promoter reduced adverse side effects and further improved tolerance. Negative pleiotropic effects on fruit yield were also seen in tomato with CaMV35S driven yeast *HAL1* gene, which enhances K^+/Na^+ selectivity and maintenance of water status [39].

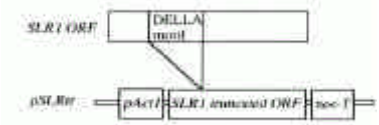
Fig 12 Over-expression of the antiport gene, *AtNHX1*, provides tolerance to salt in arabidopsis at levels up to 200 mM. Reprinted with permission from Science 285, p1258, fig 3 "Salt treatment of wild-type plants and plants overexpressing at At NHX1", Apse, M.D. et al. Copyright 1999 American Association for the Advancement of Science

Specific 'ideotype' approaches have also been tried. For example, it has been argued that plants should be able to exploit ions to achieve osmotic adjustment and internally distribute these ions to keep sodium away from the sites of metabolism. To achieve just this a vacuolar Na^+/H^+ antiport, *AtNHX1*, was over-expressed to provide protection up to about half seawater salt levels, Fig 12 [40]. Similar effects have been demonstrated with *AtNHX1* over-expression in oil seed rape, *Brassica napus* [41].

There are many opportunities using the transgenic approach, including, eventually, to produce lines that can be entered into mainstream breeding programmes. Novel genes can be expressed with increasing precision, as more tissue and developmental time specific promoters become available. Endogenous genes can be over-expressed, or negated by the use of antisense constructs.

Interestingly, synteny can also be exploited to good effect. Once the molecular basis of beneficial alleles in any species, has been discovered, including in models like arabidopsis, it is possible to engineer the equivalent homoeologous genes in the target crop with the same alterations in DNA sequence. An excellent example of this approach is the recent targeted engineering of rice to produce a GA-insensitive dwarf phenotype. In 1997 the GAI gene, which produced a gibberellin insensitive dwarf phenotype was isolated from arabidopsis[42].

Fig 13 *SLR*, at the rice homoeologue of arabidopsis *GAI* and the wheat 'Green Revolution' *Rht* genes can be engineered to produce the equivalent dwarf phenotype for rice. From Ikeda, A. Ueguschi-Tanaka, M. Sonoda, H. et al (2001), The Plant Cell 13, p 1006 fig 8B "Truncation of the DELLA motif in SLR 1 leads to a dwarf phenotype". Copyrighted by the American Association of Plant Biologists and reprinted with permission.



Soon it was it was possible to demonstrate that rice homoeologues (found in rice ESTs) of the arabidopsis gene mapped to locations in cereal genomes that coincided with the location of the 'Green Revolution' wheat semi-dwarfing genes. Moreover the allelic difference between tall and dwarf phenotypes of both rice and wheat were based on the same 51 base-pair (17 amino acid) deletion in homoeologous genes[43]. Just last year, a Japanese group[44] added the last step when they were able to demonstrate that they could engineer the equivalent rice gene (*SLR*), for which no dwarf mutant had ever been found, with the same 51 bp deletion and produce GA-insensitive dwarf transgenic plants (Fig 13), and thereby avail rice of a completely new and potentially very valuable form of short straw. The same paradigm can, and undoubtedly will, be used to transfer alleles between quite distantly related plants and will no doubt be of value for transferring stress tolerance genes between crops and between wild species and crops.

Finally we should note that the process of transformation is not yet an exact science, as demonstrated by the range of reduced height phenotypes produced by transforming rice with *pSLRtr* [44] (Fig 14). An efficient transformation system will produce a range of 'alleles' from which to choose the 'ideal' phenotype.

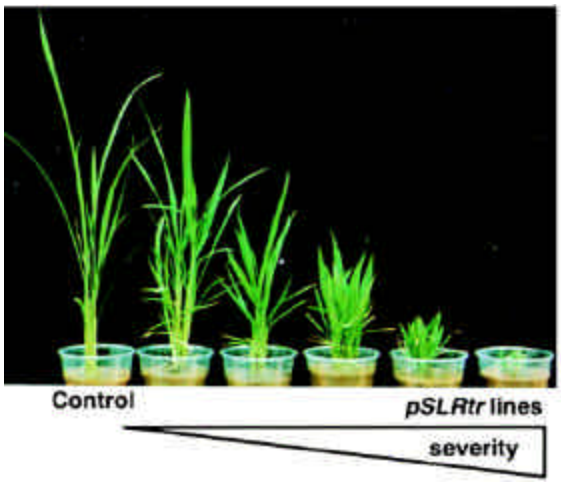


Fig 14. The same *pSLRtr* construct produces a range of transgenic 'alleles'. From Ikeda *et al* (2001) Plant Cell 13:999

There appears to a tremendous potential in these results on stress genetics, either in the direct application of model plant gene constructs in crops or in the modification of endogenous crop genes to emulate the effective model plant alleles. The arabidopsis *DREB* genes have already been transferred to wheat with most encouraging effects on salt tolerance[45]. Most CGIAR Centers have effective transformation systems for their own mandate crops and could enter into collaborations to obtain the necessary genes, at least for research purposes.

Nevertheless we should not forget Abraham Blum's warning that '...any claim for a genetic modification of stress resistance that is presumed to impact crop performance in agriculture will remain on paper unless proven …. under field conditions'[45]

Germplasm collections – allele mining and association genetics

The collections held in trust by the CGIAR Centers and by many NARS have major roles to play in both candidate gene discovery and, once genes have been identified as being important in the control of a trait such as stress resistance, identifying the range of alleles available at that locus. It is very clear that these collections will increase in importance. Everything that can be done should be done to provide added value over the next few years.

'Allele mining' will involve PCR-extraction and sequencing of the different versions of genes found in varieties, land races and wild relatives. Variation in gene sequence may then be correlated with the stress tolerance performance of the accession, and may well identify the best alleles for future transgenic experiments.

'***Association genetics'*** is a collection-related development that the CGIAR Centers are particularly well placed to exploit. This new area of science derives from human genetics where analysis of large segregating populations is not possible. Genes associated with any trait are identified by correlation of phenotype with specific alleles at linked markers. In plants this involves scanning collection accessions for variation at marker loci dispersed over the genome (genotyping) and then correlating, for example, performance under stress of the genotypes with allele dis-equilibrium around the genome. This a young science in plant biology, however the potential is great for discovering novel genes of adaptive significance and for providing added value to the collections. It will not go unnoticed that, once the collection has been 'genotyped', the same data is applicable to any trait of interest.

> CGIAR Centers are advantageously positioned to develop plant association genetics. The biodiversity discussion has increased public awareness of the value of *ex situ* collections and any genotyping and further characterization will increase their value still further. Again the technology and the analyses will be generic so there is much to be gained by close Systemwide collaboration

Conclusions

Abiotic stress is a major constraint to food production and one that will grow in significance as we approach the increasing world food shortages in the developing world that will characterize the first half of the 21st Century. Aid and technology may be available from the North but the problem is one for the developing world alone. New crop varieties that will produce more in increasingly marginal agricultural environments will be desperately needed. These varieties will be bred only by the CGIAR System and their national agricultural programme partners.

Considerable collaborative work, usually with NARS and often with ARIs, is already underway for most mandate crops from most Centers. Levels of expertise and motivation are high. However the various projects are crop and region specific and are being carried out in relative isolation.

The new science of plant molecular biology is beginning to impact all of our work, including stress tolerance research and breeding. Comparative genomics in particular promises new opportunities and is developing fast. Researchers in ARIs are already making discoveries that will impact stress tolerance breeding, and they are highly motivated to work

with developing world problems. CGIAR scientists have been quick to appreciate this and are collaborating with key academics and are beginning to import and install the technology. However the notion that any single Center can keep at the cutting edge of this fast moving and expensive field must be unsustainable. The science is generic and the need for centralization, rationalization and outsourcing will become obvious to everybody soon. The centralization, at least to virtual centres, could extend to personnel with key skills as well as technologies.

The sooner we start the better prepared we will be for the future. A pan-crop, pan-Center global collaboration involving NARS, key ARIs and even industry, to approach the problem of abiotic stress tolerance, building on what has been achieved already, could be the vehicle around which to begin to build this new way of doing science. The notes below will provide a basis upon which to open the discussion.

Options for the way forward

In the CGIAR there is already a wide understanding of the importance of stress tolerance. There is tremendous motivation, and considerable ongoing work, to breed new varieties of CGIAR crops with improved tolerance to stresses. Most Centers have incorporated molecular biology into their science and their breeding. Genomics technologies are beginning to be absorbed by the leading Centers. Links are already being made with the ARI scientists working in the area of abiotic stress tolerance. Technology transfer is continuing through the established CGIAR Center-NARS networks.

Even since the first draft of this discussion document in April 2002, progress has been made. The ‘fast-tracked’ Global Challenge project ‘Unlocking genetic diversity in crops for the resource poor’, already submitted by CIMMYT, IRRI and IPGRI, has incorporated the potential of molecular genetics and genomics to mine the CGIAR germplasm resources for novel genes and alleles that can be employed to improve the characteristics of varieties in developing countries, particularly for abiotic stress resistance. In fact an approach to drought tolerance is included as an example for ‘proof of concept’. So with regard to abiotic stress comments will be restricted to a list of goals for a successful co-ordinated approach and a few suggestions for further work that have not been incorporated into the GCP. More attention will be devoted to ways forward to build a pan-System corporate knowledge of genomics technologies and to provide a genomics infrastructure that will make the CGIAR System the preferred partner for national programmes and provide the System itself with state-of-the–art technology for the foreseeable future.

Abiotic stress research in the CGIAR Centers. The arguments developed above indicate that there is a need for a highly co-ordinated pan-crop approach that seeks out and exploits the comparative advantages of all the partners. The approach will also exploit the generic aspects the new genetics and comparative genomics.

The overriding goal must be to produce varieties that will extend the range of arable agriculture and yield more under stressed conditions to provide improved food security for the poor of the world.

Sub-goals will include:

- Implementation of an initiative which will underpin stress breeding programmes by providing good science and the best tools to address different problems, without duplication, including:

    - novel genes, and improved versions of genes, to address abiotic stress tolerance
    - improved breeding tools for more efficient incorporation of these genes in new varieties, e.g. better selection screens, better molecular markers, more efficient transformation protocols
    - information on the most appropriate breeding strategies, e.g. whether, for specific crops and specific stresses, breeding can be integral to the core programme, should comprise a separate specialized programme or would be better outsourced to participatory programmes
    - improved knowledge of the physiology and biochemistry underlying stress tolerance.

- Provision of a framework to ensure a continual flow of information between keys ARIs, Centers and NARS. Also the provisions of a forum where ARI scientists can be exposed to the problems encountered in developing countries, and at the same time allow Centers and NARS access to ARI stress science and generic technology.

- Exploitation of the generic experience in molecular genetics and genomics technology, and exploitation of the new opportunities arising from the discovery of synteny between crop genomes, and between crops and models.

- Bringing key skills together to link with breeders to address the problems, e.g. molecular physiology, bioinformatics, genomics technology, IP management etc., particularly where all these skills are not present in a single Center

A successful initiative will exploit the comparative advantages of all potential partners:

- NARS – national breeding programmes will have access to the most relevant stress trial sites, increasingly NARS will have molecular biology and genomics expertise, together with skills which are becoming uncommon in the CGIAR, such as physiology and biochemistry. NARS will know local market drivers and will be able to interact will local relevant industry, such as plant breeding and seed companies.

- ARIs – Advanced Research organizations will provide access to state-of-the–art genomics, and, notably, a few genomics laboratories specializing in abiotic stress. ARI's will provide access to arabidopsis genomics, including the first whole genome arrays, bioinformatics, and biochemical pathway research.

- CGIAR Centers have a specialized knowledge of the mandate crops, including genetic transformation, availability of field and glasshouse space for trials, and a growing bioinformatics, molecular genetics and genomics capacity. CGIAR Centers hold the key germplasm collections. The Centers have special relationships with NARS and networks in place to transfer technologies

- Industry – The multi-national agbiotech industry does undertake some fundamental and strategic and controls some results relevant to Centers' needs. The first whole genome rice arrays are likely to be available from industry. Smaller specialized, often local, companies can provide market-tested genomics service providers, e.g. BAC libraries, DNA sequencing, which will provide benchmarks for Centers planning their in-house/outsourcing research strategy.

The goals and objectives above are embodied in the 'Unlocking genetic diversity in crops for the resource poor' Global Challenge programme. One outstanding recommendation concerning crop replacement should be considered. Although crop improvement to tolerate local will probably be preferred it will be very valuable for agronomists to have available lists of alternative crops that are inherently more tolerant of particular sub-optimal soil types or particular adverse climatic conditions. The compilation of such information for global application would be very valuable indeed.

Genomics and genomics resources in the CGIAR. It is very clear that genomics will play an increasingly important role in CGIAR science and crop improvement. It is equally clear that genomics platform technologies are the most expensive, and probably the most rapidly advancing, that the System has ever had to accommodate. Therefore an early single pan-System policy for the acquisition and deployment of these technologies is imperative.

The goal should be to provide Centers and NARS partner's access to the appropriate genomics resources for all the mandated crops and sustainable access to state-of-the-art platform technologies and plant genomics capacity.

An initiative to achieve this would have a number of sub-goals:

- Should be cost-effective and achieve economies of scale while still being flexible and responsive to new developments.
- Providing the CGIAR Centers and NARS access to the rapidly changing state-of-the-art genomics technology.
- Allowing smaller Centers working with wider portfolios of marginal crops to learn from the experiences of the Centers concentrating on the major staples.
- Providing a framework to ensure a continual flow of information between keys ARIs, Centers, NARS and commercial technology suppliers.
- Providing Centers and NARS access to model plant, e.g. rice, *Arabidopsis*, *Medicago*, tomato etc., genomics resources.
- Developing ways of outsourcing *between* Centers
- Providing access to common negotiated sources for standard within-crop genomics services.
- Achieve a minimum basic 'in house' genomics infrastructure at the Centers for each crop, e.g. genetic maps, comparative maps with models, BAC libraries, EST collections, efficient transformation methods, genotyped QTL mapping populations (possibly also 'knock-out' libraries), to be shared across the System and with NARS and as vehicles for collaborations with ARIs.

The policy should enable the build-up of pan-System corporate knowledge and capacity of technologies, including:

- ESTs, cDNAs and BAC library production
- marker development – SNPs and, possibly still, SSRs
- high throughput (HTP) genotyping – both for SSRs and SNPs
- association genetics as applied to in-house germplasm collections
- map and comparative map construction and application in mandated crops
- fully genotyped segregating populations for QTL applications
- comparative genomics and bioinformatics, particularly between mandated crops and models
- insertion/mutation populations, TILLING
- handling and storing genomics resources, using laboratory information management systems
- microarrays – both built in-house and 'bought in' Affymetrix-type arrays
- proteomics
- high throughput DNA sequencing
- transformation protocols

All of the above technologies are, or will soon, be required by all Centers. The issue is whether any particular technology is best centralized within Centers, centralized somewhere within the System or outsourced altogether. Centralization and outsourcing between Centers will certainly become necessary as technology, and associated costs, evolve to soon become beyond the scope of any one Center. Costs are, of course, not the only determining factor. Convenience, service level, relationships between customers (particularly between Centers and NARS) and training considerations will all play their part.

Precisely these issues were considered two years ago and reported in the TAC 'Systemwide review of plant breeding methodologies in the CGIAR' [46]. The review emphasized that outsourcing and, especially, outsourcing *between* Centers should become common for some technologies. The report also noted that centralization should be considered for technologies that had 'broad utility for all Centers', were 'so expensive that individual Centers cannot afford it', and where' information transfer was synergistic'. In the intervening two years at least two of the CGIAR's genomics technologies have moved into this group.

Below most of the technologies are briefly considered from this point of view.

ESTs, cDNAs and BAC library production should probably all be contracted out, and there are excellent suppliers out there. These are resources that will be revisited over and over, for which quality is paramount. Quality large insert BAC libraries in particular are very reliant on experience and the availability of the appropriate colony picking robots, which will not be present in any Center.

Molecular marker development. This is still a critical activity for many crops (see Annex 1). SSRs are generally identified in various enriched libraries or directly from EST sequences. Libraries will probably be made in collaboration with expert ARI labs and the sequencing contracted out directly. SNP detection in the non-staples (where e-detection is not possible) will again probably be by sequencing PCR copies of cDNA sequences from different varieties. A job for outsourcing.

Of course the marker only acquire real value once they have been located in a map framework. This mapping will probably be carried out within Centers (see **genotyping** below)

Genotyping – High throughput genotyping should by now be part of most CGIAR breeding programmes. Using SSRs, although there is trend among industrial groups for global centralization, I would still recommend development of relatively HTP systems within Centers. These should extend to liquid handling robots for mass PCR and automatic reading of fluorescent labeled products. Proximity to the users – breeders, germplasm curators and geneticists – and the availability of a local system for training purposes argue for in-Center systems. These facilities could also be a focus for specific crop NARS breeders' use and training.

All indications are that MAS and germplasm collection characterization will soon move to HTP SNP genotyping. At present the favoured SNP format has not yet emerged, however when it does it will probably be beyond the financial reach of individual Centers. There are other factors mitigating for centralization of such a facility. These include large economies of scale and value in having collections of primers at a single site. Also Centers should be incentivised to use a centralized site which represents even a small part of their budget.

A watching brief should be kept on developments, particularly at international breeding companies with interests in multiple crops.

Map construction and development of genotyped QTL mapping populations will be carried out within Centers, although the development of crop-model comparative maps will likely be carried out in collaboration with ARIs with expertise in the models.

Mutation and deletion populations, other forms of 'knock-out' lines, possibly 'targeted induced lesions in genomes' (TILLING) populations have not, other than in rice at IRRI, been considered at the Centers. Such genetic stocks play a key role in functional genetics, i.e. assigning function to anonymous gene sequences. The Centers, with their specialized knowledge of the crops and, generally, the space and the manpower to grow large populations under good agronomic conditions, have a comparative advantage in the production of such stocks for the mandated crops. The leveraging power of such resources in the promotion of interactions with ARIs and even companies is obvious. The Centers should carefully consider the opportunity costs of not producing such resources.

Microarrays are of two main types. The first are the high quality, high-density GeneChips produced commercially by a commercial company, Affymetrix, using short 16-32-mer gene-specific oligonucleotides built up on the chip. These include the recently produced 24,000 gene (400,000 spot) whole arabidopsis genome arrays (and, in the near future, barley and rice whole genome arrays, and, in the foreseeable future, wheat, maize etc). The only option at the moment is to buy such chips in (about $700 each or $4,800 including sample processing for a minimal experiment) and process them on an Affymetrix Genechip system costing around $150,000. The second type usually use 'spotted' cDNAs or larger 50-70-mer gene specific oligos and can be made within academic labs. Modern arrayers can work up to 20,000 spots, and cost around $50-100,000. This sort of facility will usually be associated with significant liquid handling capacity to enable the large numbers of PCR reactions necessary. Also, significant -80°C freezer space will be needed to accommodate the

growing amplified cDNA resource. Finally, in a perfect world, one will validate all amplification products by resequencing, so a HTP sequencer may also be required.

It is generally acknowledged that the value of special arrays, such as the rice stress arrays being developed at IRRI, is very dependent on their quality, and this means dedicated expert technical staff associated with a facility. Chip production could be outsourced or centralized within the System, with obvious advantages and disadvantages, but clearly the development of multiple facilities around the Centers is not the best option. Among the advantages the development of quality controlled libraries of cDNAs all in the public sector or for which IP issues are known to have been centrally negotiated. Since it will, for quality control purposes, probably also be necessary to run the hybridizations at the same site, gene expression databases will be developed which are available to all users. A Systemwide facility will be useful for training of other Center and NARS staff. Return on capital outlay is also a factor with an in-house facility. The thinking is that a top-flight arrayer purchased today would remain 'current' for three to four years. Bioinformatics support, both for the direct analysis of results and comparative analyses of the rapidly growing array result databases, is vital and must be factored in to an in-System facility.

Proteomics. Protein analysis, such as performed 'time-of-flight' mass spectrometers is currently outsourced. The cost of the equipment and the rapidly changing state of the art will probably ensure that outsourcing is favoured for the time being.

DNA sequencing. For relatively large-scale sequencing, e.g. several BACs, ESTs in the 1,000s, outsourcing will be the preferred route. This is a very competitive commercial market. Local sequencing capacity within Centers may still be justified for small jobs. Nevertheless, other issues, like the present Indian policy of not allowing DNA to leave the country, may also convince Centers to retain some sequencing capacity.

Laboratory management systems. It will soon become clear that laboratory information management systems (LIMS) are vital for tracking samples and maintain quality control in the laboratory. At present there are none in use around the System (although CIAT are exploring options). The eventual benefits will be large if all Centers were to use the same, or compatible, systems.

Genetic transformation is most definitely required in-house and for all crops, although development of the technology may be best carried out in collaboration with ARIs.

The way forward. For cost effective pan-System provision of state-of-the-art genomics infrastructure and technologies iSC might consider the appointment of an independent **'CGIAR Genomics Facilitator**'. The initial JD might include:

- a constantly updated trans-national review of outsourced providers and costs for DNA sequencing, DNA library production, proteomics analyses, micro-array facilities and high-throughput genotyping, i.e. constant market testing
- a constant review of outsourcing possibilities between Centers
- act as a clearing house for CGIAR and NARS genomics related queries
- a review of LIMS available, with a view towards harmonization across the System.
- undertake a special study of the advantages and costs associated with CGIAR centralized micro-array and HTP SNP genotyping services.

and, if such facilities move ahead

- the collection of international genomics resources under appropriate MTAs for use with all CGIAR Centers and their stakeholders
- interaction with local managers to establish service level agreements and appropriate financial structures
- commission the development of a web-based tracking system whereby CGIAR and NARS customers can follow the progress of their samples in real time and automatically receive results. This is a key component of any effective and competitive service.

I believe that any Systemwide genomics service should also be overseen by an **International Stakeholder Steering Group**. This group will include technology experts (probably managers of service laboratories in developed countries), CGIAR representatives (probably at the DG or DDG level) and NARS representatives. The role of the group will be ensure that the service(s) are state-of-the-art, competitive and appropriate for the major CGIAR and NARS customers.

Line management and financial structures will of course need considerable discussion. Operational models like that of Central Advisory Service (CAS) for intellectual property matters at ISNAR should be explored. Also it is possible that the independent facilitator could be closely aligned with, or even be part of, the ISNAR Biotechnology Service (IBS). Plainly a Genomics Facilitator will regularly report and interact on activities through the existing CGIAR Genomics Task Force and the post could report to the System through the chair of that group.

Further activities. Yet another role of a Genomics Facilitator might also assemble and work with multidisciplinary teams, again in actual or virtual centres, across and over crops to address specific issues. The CGIAR Genomics Task-force is an excellent vehicle through which such groupings could meet:

- Crop type groups. Cereals, legumes and roots and tuber groups have already been initiated. Certainly the development of stress arrays and anchor markers over related genomes will be crop group activities. These groups will have also specific comparative bioinformatics needs

- Bioinformatics. CGIAR over crops bioinformaticists are already linked through Systemwide projects to ARIs in the US and the EU and have skills appropriate to all crops.

International meetings, such as Plant and Animal Genome that is held at San Diego every January, provide excellent opportunities for CGIAR scientists to interact with international academics in:

- Crop groups. These already exist and many individual crops are already the subject of international meetings and annual meetings at PAG. A global *Musa* genomics consortium has also recently been formed [47]

Annual meetings organized by CAS at ISNAR or elsewhere for:

- Intellectual property managers. Already in place with CAS at the hub, common systems and corporate knowledge, particularly in dealings with industry, are vital. Similarly common IP arrangements should be anticipated across the System for collaborative grants with ARIs, particularly IP for humanitarian use. The Systemwide IP group should also be the preferred partner for NARS.

So finally, rapidly moving research cusps, increasingly expensive technologies, more obvious links between traits and over crops, and increasing technological capacity in NARS are all indicative of more rationalization, more centralization, more outsourcing, and more virtual groupings over institutions. The time when we adopt new ways of working cannot be put off much longer.

An appropriate first step forward would be to convene a meeting of the key CGIAR stakeholders to formulate ways in which the new science can be brought to bear in the most efficient manner to deliver the new crops that developing country agricultures need. Such a workshop would be organized by the CGIAR Task Force on Genomics.

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ANNEX 1

Original

Center	Crop	*Genetic maps*	*Comparative maps*	*Mapped SSRs*	*QTL mapping*	*BACs*	*ESTs*	*Micro-arrays*	*DArT*	*Transformation*	*Insertion/mutation libraries*	*Alien introgression from*
IPGRI	Musa	***	.							**		
(INIBAP)	*M. acuminata*		.			*** & **	***					
	M. balbisiana		.			**	0					
CIAT	Cassava	****	.	***	***	***	[851] (starch)	**	*	***	.	
	Bean	****	.	***	P def, drought	(***)	***leaf & root	** P def	**	.		
	Brachiaria	***	.	***	* Al toxicity	.	**root	*Al	*	**	.	
	Rice	[4000]	Cereals	[400 + 000s ex sequence]	***	[Several libraries]	[105,000]			****	Ac/Ds (USDA)	****O. rufipogon* *O. glaberrima* *O. barthii*
WARDA	Rice	.	Cereals	see above		see above	see above					***
IRRI	Rice	.	Cereals	see above	drought salinity Zn, Fe, P def Fe toxicity	see above	[see above] Panicle (root, shoot)	drought salinity	**	****	Mutation/(Activation mutation)/TILLING	**** many sources
CIP	Potato	[900]	Tomato	[100]	carbohydrate metabolism/glycoalkaloid s/cold stress	At least 5 available. More underway	[94,000] & 5core tissues/late blight resistance	***	**			
	Sweet potato	***	.	**	**	***	(***)			**		
	ARTCs	.	.	.	.	.	.	.	.	.	.	.
	(Quinoa)	.		.	.	.	.	.	.	.	.	.
IITA	Musa	.	.	.	.	.	.	.	.	***	.	.
	Cassava	(****)	.	***	***	.	*	.	.	*	.	.
	Yam	***	.	11	**	.	0	.	.	.	.	.
	Cowpea	**	.	***	***	(*)	[45]	.	.	*.	.	** *V. vexillata*
	Cocoa	[372]	.	[200] and more available	.	10x under production at CIRAD	.	.	.	.	.	.
	Tropical maize	.	Rice	.	.	.	[see below]	.	.	.	.	.
CIMMYT	Maize	[5-7,000]	Rice	[1,800]	drought acid soils cold stress	(****)	[167000]	Collab with Pioneer		***	**T-DNA	
	Bread wheat	[1,500]	Rice	[600]	drought	Several A, D, AB, and soon ABD genome libraries]	[190,000]			****		
ICARDA	Durum wheat	[1000]	Rice	[400]	drought	(****)	[2,000 + most of the 6x ESTs]		.	.	.	
	Barley	(****)	Rice	[600]	drought	Several	[247,000]	**	.	****	.	
	Lentils	60	Medicago	?	drought cold stress	(****)	.	.	.	***	.	
	Faba bean		Medicago	.		.	.	.	.	.	.	
	Kabuli chickpea		Medicago	.		**	.	.	.	.	.	***C. reticulatum*
	Forage legumes		Medicago	.		.	.	.	.	.	.	
ICRISAT	Pearl millet	250	Rice	73 [+more unmapped)	seedling heat, terminal drought, insoluble P aquisition	3x Tift23DB leaf	0 (but salinity stress ESTs under invetsigation			**	**mutation	
	Peanut	[117] +[375 in wide cross]	Medicago	0 (but 310 unmapped)	Drought, foliar disease resitance	Non e	0	.	.	***	.	several
	Chickpea	76	Medicago	76	drought	8x	**drought (roots)	.	.	**	.	*C. reticulatum*
	Pigeon pea	*	Soybean	** (but 10 unmapped)	.	.	.	.	.	**	.	*P. sericeus*
	Sorghum	[3000]	Rice and other major cereals, bufflegrass, bermuda grass	300 [+more unmapped] & 220 pending from ICRISAT	Staygreen, Al toxicity	[3 libraries - 2 BTx623, *S. propinquum*]	[85,000]	.	.	**		*S. propinquum* and others

Key - resources

* Planned
**In progress
***Basic
****Advanced

Key - transformation

*Possible
**Routine
***Efficient
****Very efficient

() developed elsewhere
? Some doubt about availability

Legume crops
Cereals
Roots & tubers

Notes

Maps, approx. numbers of RFLPs, SSRs, morphological etc points only. AFLP, ISSR, DAF and RAPD markers are excluded

SSRs, nos of publicly available mapped markers

Annex I

CONSULTATIVE GROUP ON INTERNATIONAL AGRICULTURAL RESEARCH

TECHNICAL ADVISORY COMMITTEE

Status of Breeding for Tolerance of Abiotic Stresses and Prospects for Use of Molecular Techniques

TAC SECRETARIAT

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

March 2001

Status of Breeding for Tolerance of Abiotic Stresses and Prospects for Use of Molecular Techniques

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Executive Summary

Drought, salinity and phosphorus deficiency illustrate the range of abiotic stresses that are faced by farmers in developing countries. Most crop species show considerable genetic variation in tolerance to the major climatic and chemical stresses. Plant breeding is therefore a viable option for improving productivity, reducing farmers' risks and bringing marginal land into use. The CGIAR Centers have a comparative advantage in many aspects of abiotic stress research because of their germplasm collections, their new capacity for genetic and molecular dissection of complex traits, and their ability to conduct multidisciplinary plant improvement programs in target environments. The combined resources of the CGIAR for this work are immense but are underutilized. Investment by the CGIAR in the new tools for gene discovery will produce breakthroughs in our understanding of abiotic stress tolerance that will benefit all the mandated crops. The advances that the Centers are poised to make will find application also in developed countries which are increasingly concerned with the same problems. Improvements in drought tolerance have been responsible for much of the recent increase in yield in maize and have great potential for water saving and risk reduction for rice farmers. Genes responsible for tolerance of salinity, aluminum toxicity and phosphorus deficiency are now close to hand. The impact of these discoveries will be deep and enduring in the communities currently affected by abiotic stress.

Importance of abiotic stresses for the CGIAR mandate

Abiotic stresses arise from extremes of climate such as drought, flood and cold, from soil toxicities of such elements as Na, Al and Fe, and from soil deficiencies of elements like P and Zn. In places where climatic extremes are of regular occurrence and predictable, agricultural activity is usually very limited and dependent populations are small, but in many other places where these stresses occur in an unpredictable manner the agricultural activity may be intense and the dependent populations large. It is in these latter regions that abiotic stresses are major contributors to food insecurity and poverty for hundreds of millions of the rural poor. Drought alone affects more than 70 million hectares of rice-growing land world-wide. Soil toxicities and deficiencies on the other hand render more than one hundred million hectares of agricultural land marginal for agriculture, again limiting production and creating poverty for millions. Farmers in these environments adopt a risk-aversion strategy of low inputs, resulting in low outputs, poor human nutrition and reduced educational and employment opportunities, especially for girls. The rural poor are particularly badly affected because of lack of access to alternative sources of employment or food.

Plant breeding for abiotic stress tolerance – a viable option

Most crop species show considerable genetic variation in tolerance to the major climatic and chemical stresses. Plant breeding is therefore a viable option for improving productivity, reducing farmers' risks and cultivating marginal land. The CGIAR Centers have a comparative advantage in these breeding activities because of their germplasm collections, their new capacity for genetic dissection of complex traits, and their ability to conduct multidisciplinary plant improvement programs in target environments. The Centers have released several high-yielding cultivars with enhanced tolerance of abiotic stresses, including Al-tolerant rice from CIAT, cold-, salt- and submergence-tolerant rice from IRRI, and drought-tolerant maize from CIMMYT. However, these new cultivars represent incremental gains that could certainly be exceeded if more investment were made in the needed multidisciplinary research (Table1).

Partnerships for multidisciplinary research

It is now a particularly auspicious time for the CGIAR to dedicate itself to a concerted effort to improve tolerance of abiotic stresses in the mandated crops. Advanced research organizations have developed the necessary analytical tools for understanding the mechanisms of stress tolerance. Most of this work is conducted in the public sector, often in basic studies on *Arabidopsis thaliana*. The leading laboratories are eager to be partners in the enterprise. The private sector, with its traditional focus on protecting plants from biotic stresses, at present have little interest in abiotic stresses and may share their new genomic resources with the CGIAR Centers for the benefit of the poor. The linkages between the Centers and their NARES partners are particularly important in defining the research agenda, conducting the breeding programs in realistic environments and ensuring impact.

Table 1 summarizes the steps in enhancing abiotic stress tolerance in CGIAR mandated crops. Also listed are the principal disciplines and partners required at each step. An important early step is identification of target environments and their associated stresses. This information will be used in designing the selection screens for conserved germplasm and

breeding materials and planning the evaluation trials. The early identification of recipient cultivars can greatly accelerate the breeding program and define the baseline performance against which genetic improvement will be judged. The identification of beneficiaries helps in determining the relative contributions of genetic enhancement and crop management to overcoming abiotic stress. Poor farmers will rely more on genetics than management, but expectations should be realistic: it is likely that innovative research on crop and natural resource management in relation to abiotic stresses will reveal cost-effective ways in which poor farmers can increase their productivity and income. Just as the Green Revolution varieties of wheat and rice encouraged massive government infrastructure schemes for irrigated environments, we can expect that new germplasm for the fragile rainfed environments will encourage local innovation in management by and for poor farmers.

Table 1: Partners in developing cultivars with tolerance of multiple abiotic stresses

Stages in development of stress tolerant cultivars	**Principal disciplines**	**Principal partners of CGIAR Centers**
Identify beneficiaries and define target stresses and environments	Economics, agronomy, soil chemistry	NARES, farmers' organizations
Identify elite cultivars to be recipients of stress tolerance	Economics, breeding, physiology	NARES, farmers' organizations
Decide balance between genetics and crop management	Economics, breeding, physiology	NARES, farmers' organizations
Devise appropriate screens for stress tolerance	Physiology, biochemistry, molecular biology	AROs*
Screen germplasm for donors of stress tolerance	Physiology, biochemistry, molecular biology	NARES
Identify mechanisms of tolerance in donors	Physiology, biochemistry, molecular biology	AROs*
Identify genes conferring tolerance	Biochemistry, molecular biology, genomics	AROs*, private sector
Pyramid different mechanisms in elite genetic backgrounds	Breeding, molecular biology	NARES
Combine multiple tolerances in elite backgrounds	Breeding, molecular biology, physiology	NARES
Evaluate breeding lines in target environments	Agronomy, physiology	NARES
Disseminate improved cultivars and evaluate impact	Anthropology, economics	NARES, farmers' organizations

*Includes linkage to public research on abiotic stresses by the Arabidopsis community

Understanding the mechanisms of abiotic stress tolerance

The screening of germplasm collections for different mechanisms of tolerance to a particular stress must be based on sound physiological principles and take into account the target environment and the timing of stress relative to the growth cycle. Yield under stress is often used as a preliminary criterion that can be applied to thousands of accessions, with more discriminating tests being applied subsequently to identify accessions with different mechanisms of tolerance. If the CGIAR Centers do not invest adequately in the

characterization of the germplasm that they hold in trust, it is unlikely that anyone else will do so and a valuable resource will remain unexploited.

Germplasm accessions with high tolerance of a particular abiotic stress are usually not directly useful for agriculture. The genes conferring stress tolerance must be introgressed into improved backgrounds, a task often rendered difficult by the genetic complexity of the trait and our poor understanding of it at the molecular level. Another limitation is the difficulty of applying a uniform level of stress over a field. Great skill and considerable expense are involved in exposing a population of a thousand breeding lines (or a thousand genebank accessions) to uniform stress from drought, salinity, iron toxicity or zinc deficiency. And lack of control of soil type and texture and general climatic conditions can lead to *genotype* x *environment* interactions that confound even
the most carefully planned experiments. Finally, the target environment is unlikely to feature a single abiotic stress: submergence, drought, iron toxicity and Zn deficiency may be encountered in a single season at a single location. New cultivars with multiple tolerances of abiotic stress are essential.

CGIAR Centers have responded to these challenges by developing interdisciplinary teams focused on specific, high-priority stresses, such as drought, salinity and aluminum toxicity. The powerful new tools of biotechnology have been allied with skills in physiology to design informative experiments. Some blind alleys have been entered, but progress overall has been encouraging. Breakthroughs in one crop are frequently relevant to other crops because of the common background of plant development and metabolism. Studies on one stress may help studies on other stresses because of common principles operating in diverse stress-response pathways.

A major theme in stress biology is the relationship between the evolutionary history of a crop and its whole-plant response to stress. Wild relatives of crop plants generally adopt a fail-safe strategy that, in times of stress, allocates scarce resources to just a few seeds to ensure their vigor as seedlings in the next generation rather than attempting to fill all seeds. In adapting a crop to productive agriculture, it may be necessary to supply just enough crop management or genetic improvement to prevent plants from responding to stress by unnecessarily adopting the same fail-safe strategy. Common principles that apply over species and over stresses are emerging from current studies, raising the possibility of multiple payoffs within the CGIAR system from investments made in any particular crop or stress.

Molecular tools

Completion of the Arabidopsis and rice genome sequences was announced in 2000 and 2001, respectively. The rate of development of new molecular tools will increase dramatically. For example:

- Microsatellite-based simple sequence repeats (SSR), widely used for marker-aided selection, were until recently quite laborious to find and map. Now they will be available in vast numbers, and few genes of interest will be far from a marker.

- IRRI's activities in proteomic analysis of drought and salt responsiveness in rice led frequently to the detection and partial sequencing of interesting proteins not present in any sequence database. Now the genes corresponding to these proteins reside in databases and wait to be mined.

- Until recently we were unaware of the full complement of plant genes and were ignorant of the genes responsive to any given stress. Now Rice GeneChips carry 24,000 genes and can be regarded as a "mother array" from which trait- or stress-specific "baby arrays" can be readily derived. The "baby arrays" will be cheaper to make and use and easier to interpret.

Other useful molecular tools are also becoming available, such as insertional mutants in rice and maize, and deletional mutants in rice. These resources will allow a direct connection to be made between a gene and a phenotype. IRRI is developing a public platform in functional genomics to which many different institutes will contribute (IRRI itsef, its NARES partners, other CGIAR centers, AROs from developed and developing countries, and the private sector. The position of rice as the model cereal means that this public platform will be useful for the functional genomics activities of CIAT, CIMMYT, ICARDA, ICRISAT and WARDA as well as IRRI.

Table 2 summarizes the current prospects for isolation of stress tolerance genes from CGIAR mandated crop. The analysis is based on information about mapping of major genes and QTLs for abiotic stress tolerance in rice and other crops and about the Arabidopsis genomic initiatives. It assumes also that the public platform for rice genomics will begin operating as planned in the next 12 months. The probability of success is given a higher rating if major genes or QTLs for tolerance of the indicated stresses have been closely mapped in a cereal or if homologous Arabidopsis genes have been identified, sequenced and annotated. The difficulty in applying each stress uniformly over a large mapping population was also taken in account in judging whether QTLs might be isolated; traits that are difficult to screen for, and hence to map accurately, were given a low rating.

Table 2: Probability that four molecular approaches will lead to the discovery of genes able to enhance abiotic stress tolerance in the field

Stress	Probability of success			
	Isolation of major gene from mapping population	Isolation of QTL from mapping population	Homologues of Arabidopsis genes	Candidate genes from functional genomics
Drought	poor	fair	good	good
Salinity	good	fair	high	high
Cold	poor	poor	good	fair
Aluminum toxicity	good	poor	high	good
Fe toxicity	fair	poor	high	good
Zn deficiency	fair	poor	good	fair
P deficiency	good	poor	good	good

Drought research – a case study

Drought is the most important and most intractable of the abiotic stresses. As the water crisis deepens, the emphasis is on water saving through irrigation systems that are water-efficient. This means developing plants that are high-yielding even when grown under recurrent mild water deficit. At IRRI we use the term "aerobic rice" to refer to both water-efficient irrigated rice and rainfed rice made much more productive through limited irrigation. It is likely that research on water-efficient irrigation will benefit from studies on drought tolerance under rainfed conditions. Table 3 summarizes these and other opportunities to understand drought tolerance and apply the knowledge in breeding programs.

Table 3. Opportunities for enhancing drought tolerance

Strategy	Examples
Genetics – drought escape	Short duration plus seedling vigor to reduce yield penalty
Genetics – drought avoidance	Deep roots with root tips able to penetrate hard pan (rice)
Genetics – drought tolerance	1) Enhanced expression of transcription factors that are master switches of several drought tolerance pathways. 2) Osmotic adjustment in roots and leaves to retain water. 3) Hydrophobic barriers in roots and leaves to retain water. 4) Aquaporins (water channels) to speed water movement. 5) Altered hormonal signaling among roots, leaves and seeds
Genetics and water management	Aerobic rice for water saving in irrigated environments and high yields in upland environments

One traditional approach to increasing yield under drought is to avoid the stress through cultivation of short-duration varieties. This approach is most effective in areas with a likelihood of drought early or late in the season, but it is necessary in a good season to accept the yield penalty implicit in a shorter growth cycle. A modern improvement on this approach is to reduce the yield penalty by enhancing early seedling vigor, so that the crop gets off to a faster start; genes for this trait have been identified and can be used in traditional breeding or in genetic engineering. A second approach is to grow normal duration varieties with increased root density at depth, to facilitate extraction of water from a greater soil volume. Quantitative trait loci (QTLs) for root density at depth have been detected and efforts to identify the corresponding genes and exploit them for breeding are under way. This research is greatly assisted by the development of physical maps of rice and other crops, as well as the completely sequencing of the rice and Arabidopsis genomes. However, deep roots are almost invariably associated with poor tillering and low yield

A keenly awaited development is the isolation of genes controlling root elongation under drought. Some of these genes cause the drought-affected roots to enter a quiescent state in which they are less vulnerable, while other genes may stimulate growth of unaffected roots in the same plant. Another group of genes under intense study are those that help rice roots to push through the hard pan ~15 cm below the soil surface to reach moist soil underneath.

The above approaches involve drought escape or drought avoidance. Similar progress is being made in relation to drought tolerance. Research on Arabidopsis has identified a master switch that controls genes involved in tolerance of drought, salt and cold – all stresses

that cause a water deficit in cells. When the sensitivity of this switch to stress is increased, the ability of the plants to tolerate all three stresses is greatly enhanced. A rice homologue of this gene has been isolated and similarly modified in the expectation of achieving drought tolerance in rice. Genes for osmotic adjustment to water loss have also been mapped in several cereals. These genes control the cellular accumulation of amino acids, sugars or ions such as potassium; high concentrations of these small, osmotically active chemicals enable cells to retain water and hence their normal structure. Genes encoding aquaporins, water-channel proteins, may help plants to acquire and distribute available water faster, while genes controlling the deposition of hydrophobic barriers between cells and on the surface of leaves help create barriers to the loss of water to soil and atmosphere, respectively.

The list of exciting avenues for drought research includes the study of the genes controlling the long-range signaling between roots and leaves (to close stomata and reduce water-loss) and between roots and developing grain (to ensure that available resources are allocated to a few seeds rather than spread thinly over many).

Should all of these possibilities be pursued? Until recently, each stress biologist tended to focus on one or two avenues that seemed promising. The efforts were fragmented, uncoordinated and impossible to evaluate within a broad perspective. Now, with the advent of genome-wide tools such as microarrays, gene chips and proteomics, these disparate approaches can be integrated into a single approach, with the hope of identifying the key events and intervention points. The fact that the Rice GeneChips produced by Affymetrix contain 24,000 out of the 40-50,000 genes of rice means that the behavior of most of the relevant genes under drought stress can be studied unbiased by personal preferences or blinkered by ignorance of whole pathways. However, the interpretation of this vast outpouring of data will require access to special genetic resources and special knowledge of traits and environments. Here lies the comparative advantage of the CGIAR Centers and their NARES collaborators.

Priorities for the CGIAR

If poor farmers had access to cultivars with enhanced tolerance of abiotic stresses, they would reduce their economic risks, improve the livelihood and nutrition of their families, put their marginal land to work, and protect the environment by providing an alternative to slash-and-burn activities. Many farmers in developed countries would also wish to see such advances made by the CGIAR Centers applied to their own crops that increasingly face similar problems. To achieve these advances, CGIAR and NARES scientists must work together and with others to take advantage of the germplasm resources held in trust by the Centers' gene banks. Links with scientists in advanced laboratories, including the Arabidopsis community, will be essential to achieve rapid progress. CGIAR Centers can work together on environmental characterization, germplasm evaluation protocols, genomics analysis, molecular breeding strategies, crop management strategies and research on participatory plant breeding. The CGIAR Centers should remain major supporters of public sector genomics initiatives for mandated crops; it is not clear how willing and able agricultural biotechnology companies will be to share their genomics information.

An initial five-point program for CGIAR research on abiotic stresses is summarized below:

- Apply new screening protocols to the CGIAR GeneBanks to discover germplasm with novel stress-tolerance mechanisms;

- Combine diverse mechanisms to enhance yield under stress without jeopardizing yield in the absence of stress;

- Use functional genomics for gene discovery to improve molecular breeding strategies for stress tolerances;

- Use farmer participatory evaluation of new stress tolerance cultivars under diverse conditions;

- Develop CG-wide working groups on drought and salinity to share advances, especially in functional genomics.

Annex II

SDR/TAC:IAR/01/27

CONSULTATIVE GROUP ON INTERNATIONAL AGRICULTURAL RESEARCH

TECHNICAL ADVISORY COMMITTEE

Genetic Engineering for Abiotic Stress Tolerance in Plants

TAC SECRETARIAT

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

September 2001

Genetic Engineering for Abiotic Stress Tolerance in Plants[1]

1. Introduction

A number of abnormal environment parameters such as drought, salinity, cold, freezing, high temperature, anoxia, high light intensity and nutrient imbalances etc. are collectively termed as abiotic stresses.

Abiotic stresses lead to dehydration or osmotic stress through reduced availability of water for vital cellular functions and maintenance of turgor pressure. Stomata closure, reduced supply of CO2 and slower rate of biochemical reactions during prolonged periods of dehydration, high light intensity, high and low temperatures lead to high production of Reactive Oxygen Intermediates (ROI) in the chloroplasts causing irreversible cellular damage and photo inhibition.

In response to dehydration or osmotic stress a series of compatible osmolytes are accumulated for osmotic adjustment, water retention and free radical scavenging. Similarly, overexpression of certain enzymes such as superoxide dismutase, ascorbate peroxidase and glutathione reductase has been implicated in free radical detoxification and scavenging of free radicals under oxidative stress.

2. Complexed stresses by osmoticum, dehydration and salinity

Proline has been recognized as a potent and compatible osmoprotectant which is accumulated in high concentrations in glycophytes and halophytes in response to osmotic stress such as drought and high salinity. Two important enzymes for the biosynthesis of proline i.e. ? 1 –pyrroline-5-carboxylate synthetase (P5CS) and ? 1 –pyrroline-5-carboxylate reductase (P5CR) have been cloned from several plants and their expression studied under various abiotic stresses and ABA application.

Some transgenic plants expressing a high level of P5CS mRNA also accumulated high level of P5CS protein. The transgenic plants produced 10 to 18-fold more proline than the control plants. Under drought stress the proline content increased from about 80µg/g fresh leaf (before stress) to about 3000µg/g (after stress) in control and from 1000µg/g to an average of 6500µg/g in transgenic lines. Wilting in the transgenic plants was less severe and delayed by 2-3 days in transgenic plants as compared with the wild type (WT) control plants. Their results demonstrated that proline acts as an osmotic protectant and its increased production in the transgenic plants increased tolerance to drought and salt stress.

Glycinebetaine, a quaternary amine, is another important compatible solute, which is widely distributed among plants and protects plants on exposure to salt and cold stress. In plants like spinach and barley betaine is synthesized from choline by oxidation of choline to betaine aldehyde and then to betaine. The first step is catalyzed by choline mono-oxygenase while the second by nuclear coded gene for betaine aldehyde dehydrogenase. The transformed plants grew slowly at 200 mM NaCl whereas none of the WT plants grew. The accumulation of glycine betaine through genetic engineering in Arabidopsis enhanced its ability to tolerate salt and cold stress.

[1] Prepared by Hirofumi Uchimiya for SCOPAS

3. Anaerobiosis / anoxia

Most plants are highly sensitive to anoxia during submergence. An important aspect of the adaptation to oxygen limitation include metabolic changes such as avoidance of self poisoning and cytoplasmic acidosis, maintenance of adequate supplies of energy and sugar. During anoxia, ATP and NAD+ are generated not in the Krebs cycle and the respiratory chain but via glycolysis and fermentation. A number of enzymes of the anaerobic pathways such as alcohol dehydrogenase and pyruvate decarboxylase induced during anoxia have been cloned and characterized.

4. Heavy metal

Optimum growth and productivity and even cultivation of most of the plants is severely restricted in soils with elevated levels of one or more inorganic ions such as sodium in saline soils; Al, and Mn in acidic soils and heavy metals Cu, Zn Pb, Ni, Cd etc. due to mining, industrial affluents and other human activities.

In plants with genetic resistance to Al toxicity, the Al exclusion and uptake from root tips have been found to be correlated to their increased capacity to release organic acids such as citric acid which chelates Al3+ outside the plasma membrane. Transgenic tobacco and papaya that overexpressed a citrate synthase gene (CSb) from Pseudomonas aeruginosa in their cytoplasm. Tobacco lines expressing CSb had up to 10-fold higher level of citrate in their root tissues and one of the lines released 4-fold citrate extracellularly whereas in papaya there was only 2 to 3-fold increase of citric acid production. Increased production of citric acid was shown to result in Al tolerance in both the species.

5. Heat and Cold

Temperate and subtropical plants are highly susceptible to high temperature during early tillering, flower initiation, anthesis and grain filling stages leading to substantial reduction in their productivity. In response to high temperature all organisms, including plants, synthesize a set of proteins called as heat shock proteins (HSPs) which have been classified into several families according to their molecular masses. The induction of HSPs at permissive temperatures have been associated with the acquisition of thermotolerance to withstand short periods of an otherwise lethal temperature.

The chilling sensitivity of plants is closely correlated with the degree of unsaturation of fatty acids in the phosphatidylglycerol of chloroplast membranes. Plants with a high proportion of cis-unsaturated fatty acids, such as spinach and Arabidopsis, are resistant to chilling, whereas species like squash with only a small proportion are not. The chloroplast enzyme glycerol-3-phosphate acyltransferase seems to be important for determining the level of phosphatidylglycerol fatty acid unsaturation. Thus they demonstrated for the first time that the level of fatty acid unsaturation of phosphatidylglycerol and the degree of chilling sensitivity of tobacco can be manipulated by transformation with cDNAs for glycerol-3-phosphate acyltransferases from squash and Arabidopsis.

6. Shading

Optimum supply of nutrients and efficient photosynthesis are conducive to biomass production but the allocation of assimilates within the developing plant determines the harvest index and economic yield. In pure stand canopy as well as in mixed cropping, competition for

light energy invokes shade avoidance syndrome manifested by rapid growth and extension of stem and petiole at the expense of leaves, storage and reproductive organs thus predisposing plants to lodging, susceptibility to diseases and insect pests and a lower harvest index. Although the development of semi-dwarf varieties of wheat and rice in the 60s has led to their higher harvest index and grain yield by overcoming some of the defects of the tall genotypes yet the competition among plants for light energy continues to operate in canopies under intensive cultivation practices. The photosynthetic pigments in plants absorb the visible radiation (400-700 nm) and reflect and transmit far red (FR) radiation beyond 700 nm. The FR wave band between 700-800 nm predominating in the dense plant stands have been implicated in proximity perception for initiating shade avoidance syndrome. The FR reflection signals are perceived by the photoreceptors called phytochromes which possess distinct photo sensory functions. Phytochrome (phyA) mediating the inhibition of stem growth on etiolated plants in response to FR wave length 710-720 nm is rapidly degraded and down regulated in light grown plants. Transgenic tobacco lines expressing a high level of heterologous oat phyA apoprotein have been produced.

The level of growth inhibition of transgenic plants correlated with the level of phyA production. Under field trials at various planting densities from 20 to 100 cm, the transgenic plants were indistinguishable from the WT plants at the lowest plant density but became progressively shorter as the plant density increased. This phenomenon termed as “proximity conditional dwarfing” led to a 15 to 20% increase in harvest index (expressed as leaf biomass as a proportion of total biomass) in transgenic plants under high plant density thus demonstrating the suppression of shade avoidance response under high level of phyA expression. Further understanding of the molecular basis of interaction of various phytochromes among themselves and with R : FR rations in natural light environment may help to change crop plant architecture to avoid shade stress and obtain maximum production under high plant density, mixed cropping and agroforestry.

7. UV – B

The high influxes and absorption of UV-B radiation affects terrestrial plants through damage to DNA directly or indirectly through formation of free radicals, membranes by peroxidation of unsaturated fatty acids, photosystemII, phytohormones and even symbiotic relationship of plants with micro-organisms.

A number of secondary metabolites such as flavonoids, tannins and lignins are increased at elevated levels of UV-B radiation which screen UV-B and protect the cellular components against the UV-B damage.

8. Oxidative stress

A number of abiotic stresses such as extreme temperatures, high light intensity, osmotic stresses, heavy metals and a number of herbicides and toxins lead to over production of reactive oxygen intermediates (ROI) including H2O2 causing extensive cellular damage and inhibition of photosynthesis.

9. Perspectives and strategies for improving tolerance

The work on genetic engineering of tolerance to abiotic stresses began piece meal within a decade of the molecular understanding of pathways induced in response to one or more of the abiotic stresses. In most of the cases the transgenes expressed faithfully but only a

limited level of tolerance was provided under stress conditions as compared to the non-transformed wild type plants. In many cases the transgenic plants had morphological abnormalities and slower growth under nonstressed environment. The level of many compatible osmolytes responsible for osmotic adjustment was too low to be effective per se in providing the required water retention and osmotic adjustment.

The use of multiple tolerance mechanisms for one or more of the abiotic stresses through stepwise or co-transformation may help to achieve high levels of tolerance for commercial exploitation. The QTL mapping of stress tolerance in certain species, comparative mapping and map based cloning in plants may be used to screen genes which function under stress as well as those induced and expressed in response to stress.

Molecular understanding of the stress perception, signal transduction and transcriptional regulation of abiotic stress responsive genes may help to engineer tolerance for multiple stresses.

Understanding the molecular mechanism for providing protection against biotic and abiotic stresses may lead to a generalized master mechanism for stress tolerance. Optimum homeostasis is always a key to living organisms for adjusted environments. Thus, abiotic stress accompanying a number of biological phenomena must be precisely investigated by consideration of plant homeostasis.

Table 1. Genetic engineering of plants for tolerance to abiotic stresses

Stress	Gene/Enzyme	Source
Osmotic	Delta-pyrroling-5-carboxylate synthetase (P5CS)	Mothbean (*V. aconitifolia*
Drought and Salinity	Mannitol-1-phosphate dehydrogenase (*mt1D*)	*E. coli*
Cold and Salt	Choline oxidase (*cod* A)	*Arthrobactor globiformis*
Salt	Choline dehydrogenase (*bet* A)	*E. coli*
Cold	Omega-3-fatty acid desaturase (*fad* 7)	*Arabidopsis*
Drought	Trehalose-6-phosphate synthase	Yeast
Drought	Levan sucrase (Sac B)	*Bacillus subtilis*

Table 2. Genetic engineering of plants for tolerance to heavy metal

Stress	Gene/Enzyme	Source
Cadmium	Metallothionein-I (MT-I)	Mouse
Copper	Metallothionein-like (PsMTA)	Pea
Aluminium	Citrate synthase (CSb)	P. *aeruginosa*