Plant Sciences Research Programme

Annual Programme Report 1995

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ODA

NATURAL RESOURCES RESEARCH DEPARTMENT

P*lant Sciences* P*rogramme*

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Semi-arid Production System.
Purpose 1. Production of target crops on impoverished soils in semi-arid conditions increased by the selection and genetic enhancement of cultivars.

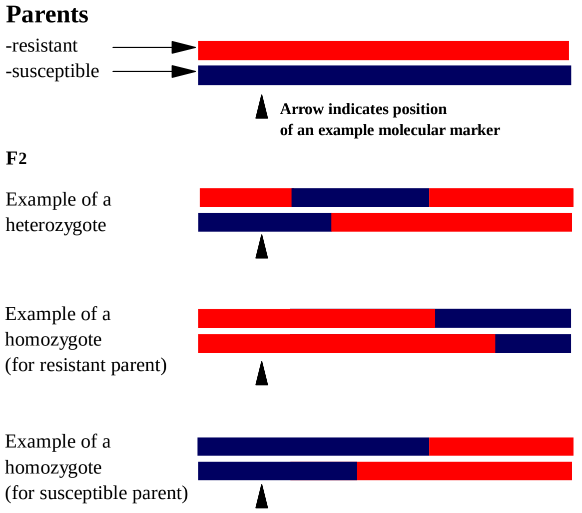
R6041. *QTL mapping of downy mildew resistance and marker-assisted selection in pearl millet.*

Pearl millet downy mildew is the most important biotic constraint on the crop in India and in many African countries and the disease is accorded the highest-ranked priority in pearl millet, after drought, in the ICRISAT medium term plan. In order to produce this rating, ICRISAT has calculated the total value of benefits, benefit cost ratio, and indices for poverty focus, gender focus and sustainability. Successful breeding of resistance to downy mildew would have a total pay off of US $75 m per year, and would benefit 115 million poor people.

The breeding of downy mildew resistance into susceptible male-sterile lines using backcross breeding has been a long term objective of the ICRISAT breeding programme and for many years considerable resources have been spent on backcross breeding programmes for disease resistance. For various reasons, (see box on next page), this programme has been unsuccessful.

Project **R6041** is using marker-assisted selection to breed for downy mildew resistance in male-sterile lines that are of great importance to ICRISAT, Indian seed producers, and farmers.

How molecular markers are used to find where genes having effects controlling a trait such as downy mildew resistance are located



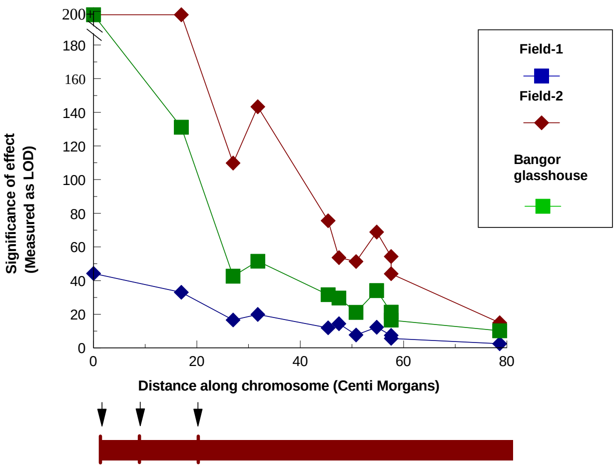
The inheritance of the chromosomes in the F2 is determined by molecular markers that identify the DNA of each parent.

All of the F2 plants (or their progeny) are then screened for disease.
At each point on the chromosome the plants are grouped into
-homozygous susceptible
-heterozygotes
-homozygous resistant

If there are differences between the classes for disease resistance it is likely that there is a gene controlling resistance at this point on the chromosome.

This gene is called a quantitative trait locus (QTL). By testing many points along the chromosome a map of the effects can be produced (see next page). The area of the map with significant effect is where the genes controlling the trait are likely to be.

A map of linkage group 1 showing the importance of different parts of the chromosome in imparting resistance to downy mildew disease.



The beginning of the chromosome is important and contains the resistance gene.
The three example markers (indicated by arrows) can be used for marker assisted selection.

All that is now required is to select for the trait using marker-assisted selection.

A conventional backcross breeding programme is used but instead of selecting for the target gene by phenotype i.e. by selecting plants having disease resistance in our case, the molecular marker of the resistant parent is selected for in the region of the chromosome where the gene has been located.

This procedure is simple, and requires very few plants to be screened. The genotypes of the plants in the backcross breeding programme can be determined with great precision. Examples from the programme, currently underway, to transfer resistance from ICMP 85410 into 843B can be seen on the following page.

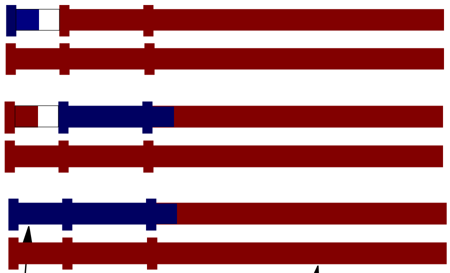
Some Advantages of Marker-Assisted Selection in Backcross Breeding

The programme has revealed why many conventional backcross programmes fail. Despite carefully made backcrosses to the recurrent parent, progeny derived exclusively from self pollination, because the crossing has failed, have been found in one of the backcross programmes. Other workers in other crops are also finding the same result. Hence, in conventional breeding, breeders are often not working on the genetic material that they assume they are, because crossing fails more often than expected.

Of course, the other reason why backcrossing fails is the misclassification of a plant for the presence of the donor gene (disease escape instead of disease resistance) and its use as a parent in further backcrossing. All of these problems, and others such as the need to make time and resource-consuming selfed generations to identify a recessively controlled character, are avoided in marker-assisted selection.

Marker-assisted selection for downy mildew resistance in pearl millet. The results so far.

Examples of useful genotypes found at the BC2 stage



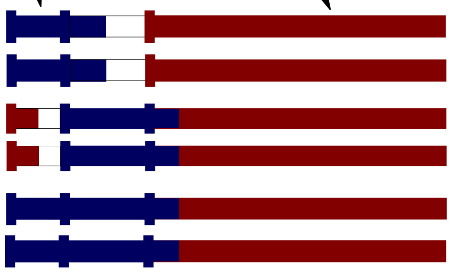
Solid colour indicates very high probability that genotype is of that parent. (Extremely rare double recombination events could mean that this is not correct. It has no practical implications for the success of the programme.)

Shaded colours indicate the greatest probability of the genotype. Between a resistant parent marker and a susceptible parent marker there is a crossover between the two genotypes, the exact location of which is not known unless more markers are evaluated.

Donor parent genotype

Recurrent parent genotype

Target genotypes



The final genotypes that can be produced after further backcrossing and one generation of self pollination.

The resistant parent genes are present in three differing lengths at the part of the chromosome shown to be involved in disease resistance.

After disease screening, the genotype with the best combination of high disease resistance and greatest genetic contribution from the recurrent parent genotype will be used.

Semi-arid Production System.
Purpose 1. Production of target crops on impoverished soils in semi-arid conditions increased by the selection and genetic enhancement of cultivars.

R5487 *Seedling thermotolerance in pearl millet.*

In India, pearl millet is the principal grain and fodder crop in arid and semi-arid areas bordering on the Thar desert in the states of Rajasthan, Haryana, and Gujarat, with Rajasthan accounting for 45% of the total area under pearl millet in India. Although pearl millet shows considerable environmental adaptation to these marginal areas, its yield there is not only low but also highly variable. Seed and biomass yields are severely constrained by extremes of temperature and by unreliable and irregular rainfall. Poor seedling establishment, and consequent crop failure, result from the extreme high temperature prevalent at the start of the growing season. Improvement of the adaptation of seedlings to high temperatures has been given by ICRISAT an estimated annual value of success of over $44 million.

Collaborative research on seedling thermotolerance conducted at IGER and ICRISAT has resulted in many successes including:

- the development of screening systems for seedling thermotolerance and their use in a selection programme over two cycles of bidirectional selection
- the production of lines and experimental varieties of differential thermotolerance providing sources of tolerance to breeders
- the construction of skeleton RFLP-based maps and production of lines for QTL analysis
- the identification of linked molecular markers not only for seedling thermotolerance but also for other traits such as downy mildew reaction, grain mass, and flowering time.

Rapid screening techniques for seedling thermotolerance, along with molecular markers for this trait, greatly improves the efficiency with which breeders can select for thermotolerance. Material developed in this programme is appropriate for use directly in ICRISAT's pearl millet breeding programme, both for population improvement and in the development of elite hybrid parents with improved tolerance expressed in hybrids.

The products of this research contribute directly to the stability of pearl millet grain and fodder production in the harshest production environments by reducing losses of seedlings during early establishment due to high soil temperatures. This will in turn result in smaller areas needing to be resown and fewer problems in terminal drought stress at the end of the season due to delayed effective sowing date. With heat tolerant cultivars and improved crop management practices, farmers will obtain optimum plant stands more regularly. This will improve yield and yield stability for both grain and stover.
The use of molecular marker techniques for physiological traits is discussed in the box below.

QTL Mapping of Secondary Traits for Indirect Selection

Research on trait identification is intended to enable breeders to identify easily measured traits that can be selected to give an indirect response in the target trait such as seedling thermotolerance. This can be indirectly selected for by using a more easily screened secondary trait such as low electrolyte leakage from heat stressed seedlings under controlled conditions. Using conventional techniques, proof that this indirect selection could work required either difficult quantitative genetical analyses, that are subject to large errors, or experimental proof from expensive selection experiments.

Genetic (QTL) mapping not only shows in a much clearer fashion how traits are genetically correlated, but where they are correlated on the chromosome, making it much easier to say with certainty whether indirect selection will give responses in the target trait. It is also possible to say whether combined selection for more than one trait will be effective, because genes are selected at different locations on the chromosomes. QTL mapping still allows marker-assisted selection to be done if it is more economic than selecting for secondary traits.

Two secondary traits of seedling thermotolerance have been mapped (Figure 1). It can be seen that the sand bed thermotolerance test identifies more genes than the electrolyte leakage test. Nonetheless, the analysis shows that selection for electrolyte leakage will result in a response for increased thermotolerance in the sand bed. Even though selection for electrolyte leakage will produce a lower response it may still be economically worthwhile, since it is an easier trait to evaluate. The most crucial information as to where the genes are for the primary trait are not shown on the map. The inbred lines cannot be screened in the field for seedling thermotolerance because they fail to survive at all due to inbreeding depression. Attempts will be made to identify the QTL for field thermotolerance using less inbred material.

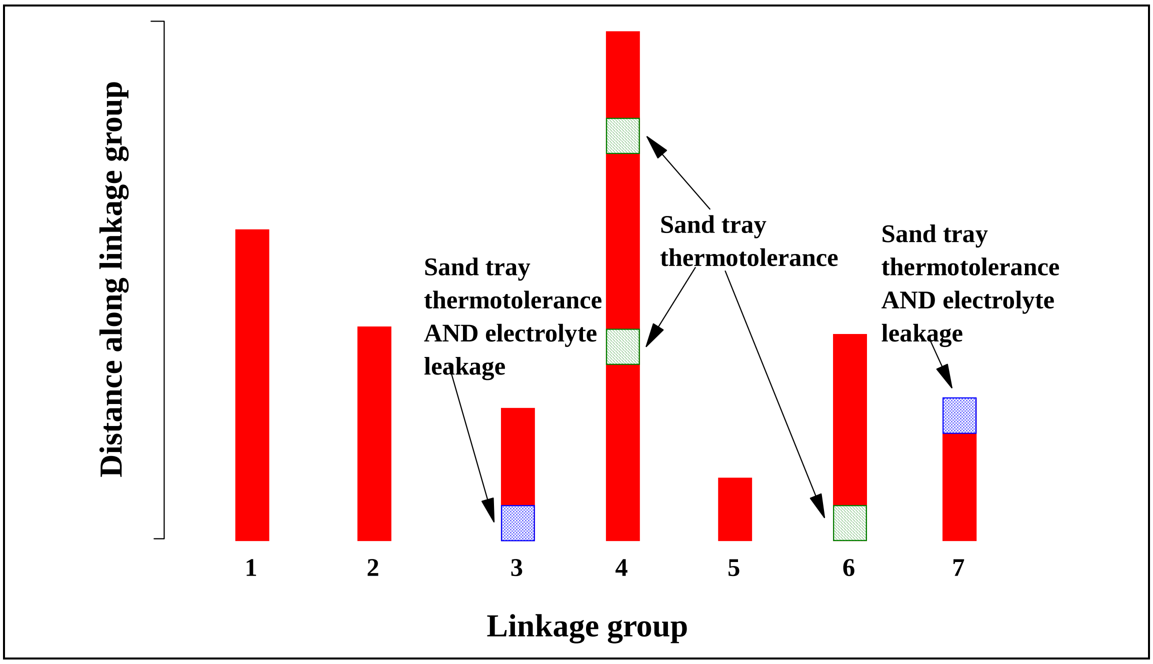


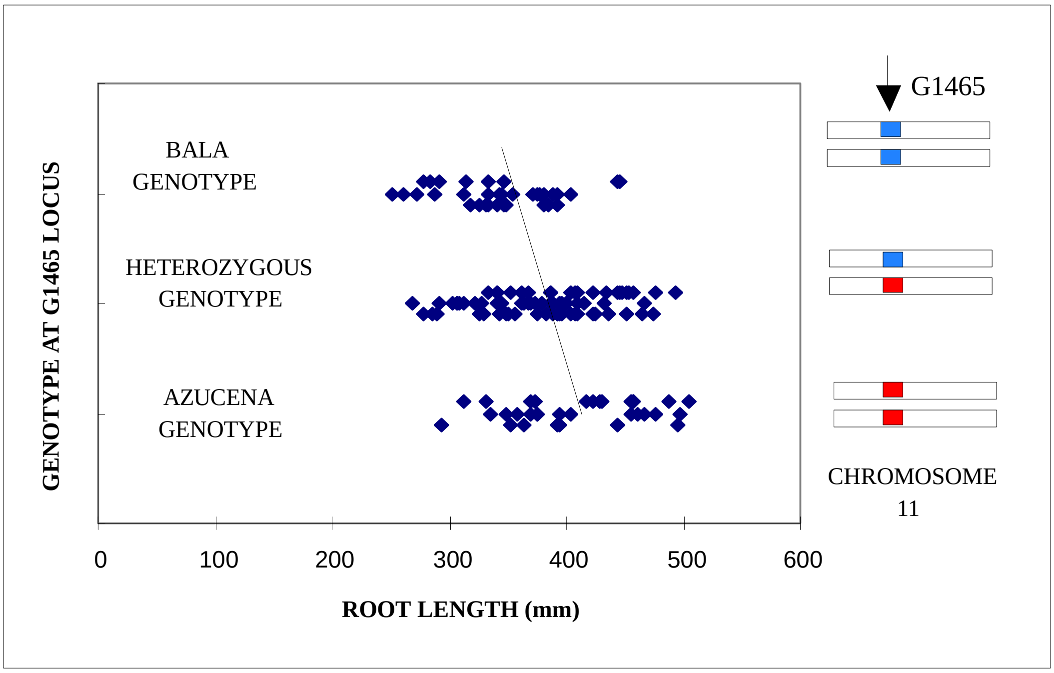
Fig. 1. QTL map of two traits, thermotolerance assessed in a sand tray in controlled environments, and electrolyte leakage from heat-stressed seedlings

Semi-arid Production System.
Purpose 1. Production of target crops on impoverished soils in semi-arid conditions increased by the selection and genetic enhancement of cultivars.

R4631. *Drought resistance in rice; plant physiology, molecular genetics and field experiments*

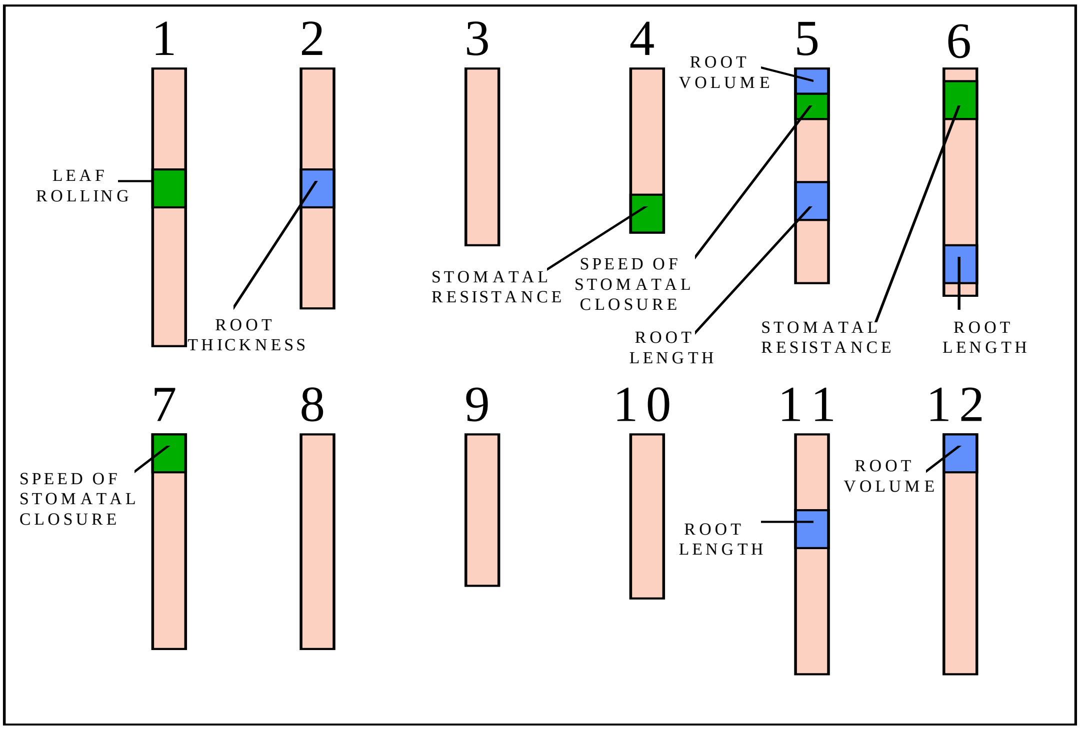
Average yields of upland rice (14% of the global area under rice cultivation) are in the order of 1 tonne hectare^{-1} season^{-1} which compares unfavourably with 3.5 tonne hectare^{-1} for irrigated rice. Drought is the major abiotic yield-limiting stress in upland rice, so improvement of drought resistance will have a significant impact on world rice yield, especially in poorer countries.

Several research institutes are examining the possibility of manipulating root characteristics in breeding programmes to improve drought tolerance. Since it is too resource-consuming (and too slow) to measure root traits routinely, quantitative trait loci need to be identified, so that marker-assisted selection can be employed as a more efficient method of producing cultivars with greater drought tolerance.



Identification of a QTL for hydroponic root growth on Rice Chromosome 11 by regression of root length with the genotype of RFLP probe G1465 in population of 178 F_2 progeny from a cross between two drought resistant rice varieties, Azucena and Bala.

Molecular markers such as RFLPs (Restriction Fragment Length Polymorphisms) can be used to identify the location of genes controlling physiological traits. The figure on the left shows how the RFLP probe G1465 identifies such a QTL (Quantitative Trait Locus) in a cross between two drought resistant rice varieties, *Azucena* and *Bala*. Those individuals which have inherited the middle of chromosome 11 from the *Azucena* parent have longer roots in hydroponics than those that inherited that genetic material from the *Bala* parent.



Location on the 12 rice chromosomes of QTLs for physiological traits theoretically related to drought resistance in an F_2 cross of 2 drought resistant rice varieties, Azucena and Bala.

The figure above shows the location of genes controlling many physiological traits related to drought resistance in the *Azucena x Bala* population which is being studied in Bangor.

Field analysis of drought resistance in this population will begin in the Philippines in January 1996. Those parts of the genome which confer drought resistance will be identified. It will then be possible to identify those traits which actually confer drought resistance in the field. For example, if the *Azucena* genotype for probe G1465 correlates with drought resistance, powerful evidence that long roots confer drought resistance is obtained. In addition, this work will provide breeders with molecular markers suitable for breeding for drought resistant rice.

High Potential Production System.
Purpose 1. Production of system commodities increased through appropriate selection and genetic enhancement of cultivars.

R5061 and R6121 *Transgenic rice for nematode resistance in West Africa*

P. Vain, B. Worland, A. Kholi, M. Leech, M. C. Clarke, H. Atkinson, J. Snape and P. Christou

Rice is the largest cereal crop in the developing world where half the population depends on it as the major source of nutritional calories. The costs of agrochemical use and crop losses due to pests and diseases total billions of dollars annually. *Meloidogyne* spp. (root knot nematodes) damage upland rice crops in both Asia and Africa. Their wide host range threatens other crops in rotation with upland rice and intermittently flooded, lowland rice. *Hirschmanniella* spp. (rice root knot nematodes) damage lowland rice, but only when cropping is intensive. *Pratylenchus* spp (root lesion nematodes) occur in virtually all Asian and African upland rice crops and can cause up to 50% loss of yield.

The thrust of these projects at the JIC (**R6121** *A general system for rice transformation using biolistic bombardment*) and the University of Leeds (**R5061** *A novel basis for crop resistance to root knot nematodes*), is to provide a basis for control of all nematode pests of rice.

The project is demonstrating that genetic engineering can improve the currently inadequate control of plant parasitic nematodes in subsistence agriculture. A safe and effective technology is being adapted for developing world use. Rice has been chosen for the initial work because it is host to a range of nematode species. Upland rice is damaged by several nematodes including Root-knot (*Meloidogyne* spp) and Lesion nematodes (*Pratylenchus* spp) which together occur in 50% of all fields causing up to 70% loss of yield. Losses occur in lowland rice to a range of nematodes including rice root nematodes (*Hirschmanniella* spp) and *M. graminicola*.

The Centre for Plant Biochemistry and Biotechnology, University of Leeds has developed plants that are highly resistant to nematodes. Resistance is conferred by transgenic expression of a modified rice seed protein. Both root-knot and cyst-nematodes (*Heterodera* and *Globodera* spp.) are unable to grow effectively on roots expressing this modified cysteine proteinase inhibitor (Oc-IAD86)[2]. They fail to lay the eggs they require to achieve damaging population levels. The modified cystatin may prove to be effective against all nematode pests of rice and other major crops.

We have utilised a variety-independent gene delivery system. This involves particle bombardment using an electric discharge device to deliver DNA-coated gold particles into immature rice embryos[1]. Efficient DNA delivery, selection and transgenic plantlet recovery procedures have been put in place and are now in routine use. Transgenic plants from important West African varieties (ITA 212, IDSA 6, LAC 23, WAB56-104) and an elite *indica* cultivar adapted to SE Asian conditions (IR64) have been recovered and characterised molecularly using PCR, Southern and Western blots.

Transgenic plantlets are recovered routinely 8-10 weeks following gene delivery. Following molecular characterisation to confirm presence of the gene of interest, the plant material is analysed using Western blots to determine levels of expression of the transgenic protein. Such plants as well as additional lines are currently undergoing bioassays to evaluate whether transgenic plants expressing the gene are resistant to nematodes, in growth chamber tests. We expect that second generation constructs driven by tissue specific promoters will have distinct advantages over constructs being expressed constitutively in the rice plants. Targeted gene expression will be the focus of the next stage of our programme, which should produce material suitable for evaluation in the greenhouse and in the field.

Rice cystatins are widely consumed as part of the human diet and they do not inhibit our digestive enzymes. Safety will be enhanced further by restricting expression of Oc-IAD86 to roots rather than the consumed parts of resistant plants. We seek to provide resistant plants that can be used even when extension advice is not available and the grower is unaware of any nematode problem. We expect to develop durable resistance suitable for intensive use. ODA already has the right to distribute this technology freely within the developing world for a range of crops. The resistance will be transferred to other subsistence crops once effective resistance has been demonstrated for rice.

We are hopeful that transgenic rice plants expressing resistance to nematodes will make a significant contribution to assuring food security in Africa and Asia. An important advantage of our programme is its linkage to other programmes supported by the ODA and the Rockefeller Foundation. These programmes are designed to generate elite rice germplasm resistance to major virus and insect pests for immediate deployment in the field in rice growing countries. The nature of our rice programme research is very applied and the major deliverable will be field trials of transgenic plants in West Africa and Asia.

References

1. Christou, P., Ford, T.L., Kofron, M. 1991, Bio/Technology 9:957-962
2. Urwin, P.E., Atkinson, H.J., Waller, D.A., MacPherson, M.J. 1995. Plant J. 8:121-131.

High Potential Production System.
Purpose 1. Production of system commodities increased through appropriate selection and genetic enhancement of cultivars.

R5473cb. *Salt tolerance in wheat - from lab to land.*

Demand

The associated problems of waterlogging and salinity are major constraints to increasing agricultural productivity in both India and Pakistan. About 7 M ha of land in the arid and semi-arid regions of India have gone out of production or give very low yields due to soil salinity or alkalinity. A large additional area is less severely affected by salinity, although it still causes considerable yield reductions.

Yield losses of wheat in moderately salt-affected areas of Pakistan average about 64% and it can be assumed that the loss in India is similar. Agricultural output may be increased by increasing productivity on areas unaffected by salinity, but this requires the use of additional inputs. Increase in agricultural output would be aided by using marginal lands and water to increase agricultural production, and for this the development of more salt-tolerant wheat is required.

Research outputs

Techniques for screening wheat varieties for salinity tolerance have been developed in previous ODA-funded projects (**R3788**, **R4463**) in Bangor and at the John Innes Centre (JIC), Norwich, and personnel from research institutes in Pakistan trained in their use.

Uptake

Wheat lines with enhanced tolerance have been selected using these techniques at the Universities of Agriculture in Faisalabad and Peshawar, Pakistan. SARC 1, an improved selection from the salt-tolerant Pakistani wheat LU26S, and a mutant from the Indian salt-tolerant landrace Kharchia local, have been multiplied and distributed to farmers in the Punjab and NWFP, with the work in NWFP being partly funded from the current ODA project **R5473cb**. Approximately 1700 kg of each genotype were supplied in 1995, an average of 20 kg per farmer, and both have shown enhanced production over locally-recommended material in saline areas.

Farmers in both provinces are keen to sow these lines, but the Universities have been unable to meet demand due to a lack of dedicated facilities for seed production. As a result, farmers are being encouraged to multiply and distribute the seed themselves. Further assistance towards the costs of seed multiplication and distribution is being provided by the Australian government under an ACIAR programme. The distribution of the seed, and information about the new material, is also being aided by the formation of associations of salt-land users by both Universities.

Project **R5473cb** is co-ordinating a number of on-farm trials in different types of saline environments throughout the two provinces, using these two farmer-preferred lines and other material. As the project develops over the next three years it will be possible to test many more lines. Facilities for seed multiplication in the northern areas of NWFP over the summer will be provided, allowing the raising of two generations a year and increasing the availability of seed.

Promoting the research outputs

In a traditional breeding programme it can take nine generations from the initial crossing to a variety's entry into National List trials. The use of doubled haploid breeding allows the production of genetically-uniform material in early generations, and so speeds-up the breeding process by several years. This technique has not yet been widely adopted in developing countries. The production of the initial cross is simple, but the procedure requires the use of carefully controlled conditions, and is quite a specialised task. To package and promote these research outputs the senior wheat breeder from CSSRI in Karnal, India, was attached to the JIC for 4 months in 1995 to work on doubled haploid breeding. He has reported enthusiastically upon his visit, and is keen to use the technique under the next phase of the project, which will provide the necessary equipment to establish an effective programme at that Institute. The next phase will also provide further training for breeders.

Further screening of germplasm is being carried out in Bangor on material that includes landraces from saline regions of Pakistan and advanced breeding lines from India. The selected lines have been distributed to and are being crossed by plant breeders in both countries. Selections for improved agronomic characters have also been made from the landraces.

Future directions

A highly salt-tolerant line, KTDH19, produced by doubled haploid breeding from a cross between the salt-tolerant Indian wheat Kharchia 65 and a sodium-excluding UK breeding line, TW161 was also tested by project **R5473cb**. This cross showed great potential in field trials in Spain, demonstrating that sodium exclusion had been transferred into the Kharchia background, and showing enhanced grain yield over Kharchia (Figure 2), a variety that, although showing a poor performance in good environments, generally maintains its yield in saline conditions.

However, in highly saline environments in India and Pakistan the results were not so clear, and yields, although better than previously-selected material, were lower than Kharchia (Figure 3). One of the main reasons for this was late maturity, as grain-filling was adversely affected by the high temperatures in April and May. Mutation breeding is being used in Bangor to attempt to induce earliness to this line, and the M1 generation will be sown shortly.

The parents of KTDH19 were selected solely on physiological traits, without consideration of their potential performance in the field. Further doubled haploids are being produced from backcrosses between KTDH19 and agronomically well-adapted Indian and Pakistani wheats: F_5 material from the first of these is being tested in Spain this season and, if successful, will be sown in India and Pakistan in autumn 1996. A major part of the next phase of the project will be to develop a cultivar database, to enable the parents to be selected with regard to the intended environments of their progeny.

The project has shown that sodium exclusion and enhanced yield potential can be transferred into an existing salt-tolerant wheat variety. The priority now is to transfer these characters into a genetic background better adapted to the high-input target environments of the irrigated wheat zone in India and Pakistan.

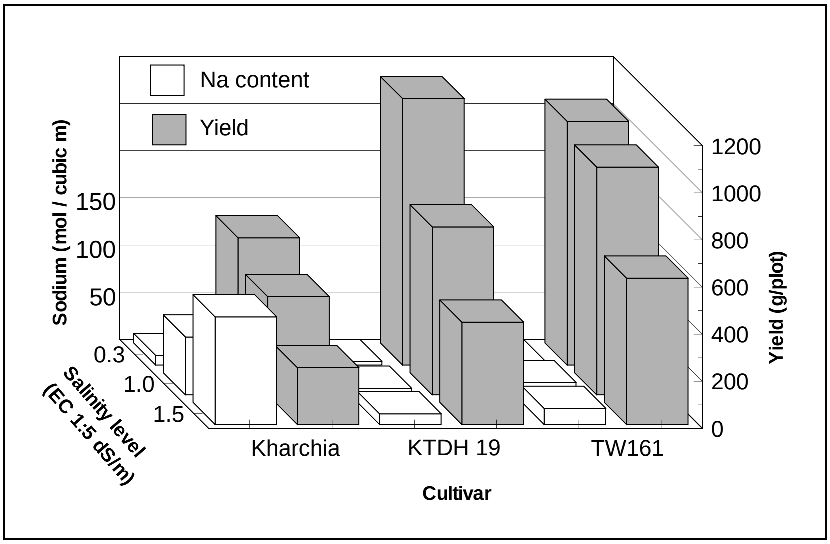


Figure 2. Effect of salinity on grain yield and sodium content of wheat, Zaragoza 1994/95

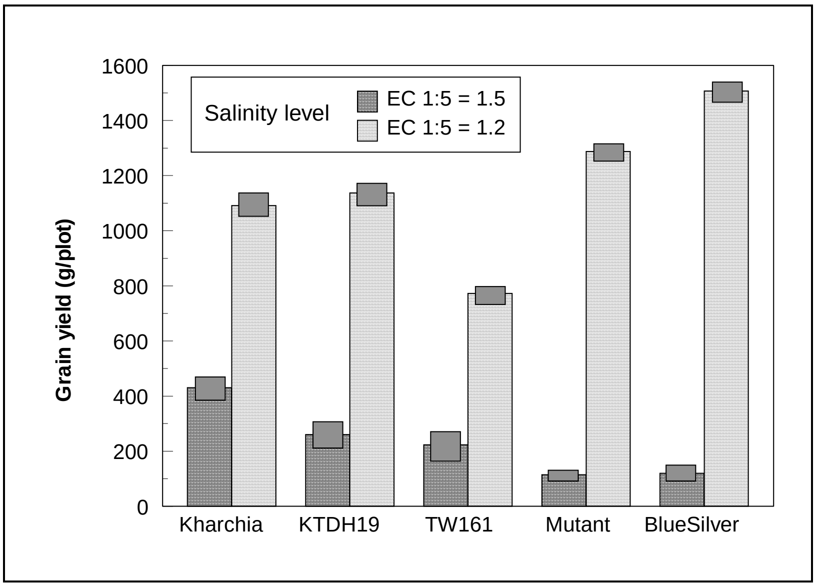


Figure 3. Effect of salinity on grain yield of wheat, Peshawar 1994/95. Standard errors are indicated by the boxes at the top of each bar.

Semi-arid Production System.
Purpose 2. Production of target crops on impoverished soils in semi-arid conditions increased by physiologically appropriate agronomic practices.

R6395. *Seed priming to improve crop establishment in Zimbabwe and India. The establishment of research linkages.*

Good crop stand establishment is a pre-requisite for the efficient use of resources such as water and light and plant stand is a major determinant of yield. This is particularly true in the semi-arid tropics where there is a delicate balance between supply of, and demand for, water. High and rapid germination and emergence determine good stand establishment, and the related vigorous early growth of seedlings often produced higher yields. Observations in many semi-arid areas suggest that stand establishment, particularly of cereals such as sorghum (the fifth most important cereal in the world) and millet (a crop of immense importance to the world's poor people), is often extremely poor. Patchy stands and the need to replant commonly occur for many reasons, both physical and socio-economic. However, even when good quality seed is sown properly into soil at the optimum moisture content, stands may still fail to establish properly if hot, dry weather follows sowing. In such cases the surface layers of the soil dry quickly, and soil temperature rises rapidly. Under certain circumstances, surface crusts and hard layers form impenetrable barriers to shoot emergence and root penetration.

Only seeds which germinate rapidly and emerge before soil surface conditions deteriorate too far will be able to emerge and form viable adventitious root systems with access to moisture deeper in the soil. Intra-specific variation in germination and emergence rates exists in many crops but previous work has suggested that priming seeds i.e. soaking them in water before sowing can speed up emergence and improve early growth of sorghum. This project seeks to extend the promising work on sorghum to other varieties and crops in an attempt to address serious and widespread establishment problems reported from Zimbabwe and India.

On-farm and on-station field trials are not yet complete but laboratory studies in the UK have confirmed the potential of seed-priming (Figure 4). Soaking seed for 8 hours in water reduced germination time by 38% and 33% in millet and sorghum, respectively, while emergence of sorghum from soil was advanced by 12 hours (16%). In areas where evaporation rates may reach 10-12 mm per day, a saving of 12 hours could make all the difference between establishment success and failure.

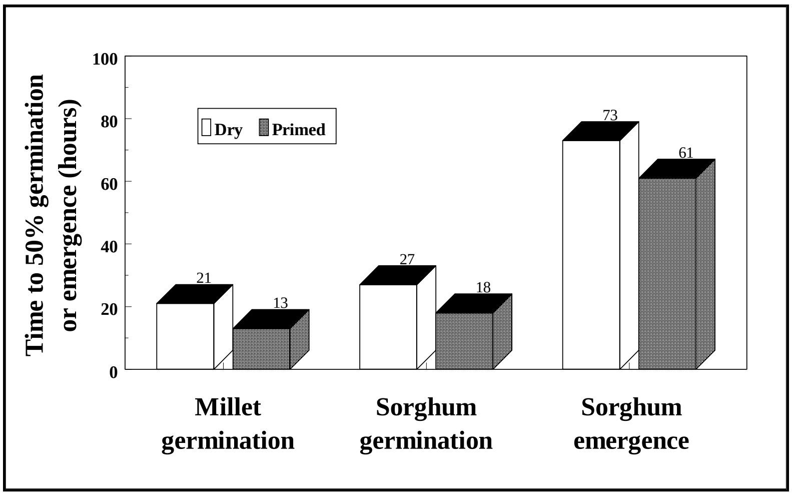


Figure 4. Sorghum and pearl millet response to seed priming

There appears to be some variation amongst genotypes in the detailed response to priming and this has important implications in minimising the risk of using the technique. Table a. shows the range of this variation amongst some maize genotypes tested so far. This sort of information is essential for the development of robust, farmer-friendly seed priming techniques.

Table a. Safe limits for seed-priming in maize.

Cultivar	Country	*Estimated* safe limit for priming (hours)
R 201 (hybrid)	Zimbabwe	16
Desi 2 (local Dudly *dent*)	India	24
Desi 1 (local Sameri *flint*)	India	36
Shweta	India	36

Seed priming is not unknown to farmers in semi-arid Zimbabwe, who sometimes soak maize seeds if planting has been delayed and the soil has dried out likewise farmers in the KRIBP area of Gujarat, Madhya Pradesh and Rajasthan will soak their chickpea seeds in similar circumstances. Neither group has considered seed-soaking and planting under optimal conditions or extending the technique to other crops (e.g. maize in India, sorghum and millet in Zimbabwe). Early results, at least from controlled environment experiments, suggest that benefits are likely from both approaches.

Initial collaboration has been with KRIBP, a bilaterally-funded NGO with strong extension links in India and with the Department of Research and Specialist Services, the research arm of the Ministry of Agriculture in Zimbabwe. On the strength of early results, an invitation was received from ICRISAT to screen millet and sorghum germplasm for its reaction to seed priming. The material provided is to be tested under hot, dry, conditions in Rajasthan as part of work relating to seedling thermotolerance in pearl millet (**R5487**).