External Evaluation

Indo-Swiss Collaboration in Biotechnology (ISCB)

to December 2011

Evaluators
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Caption cover:

Prof. B.K. Sarmah, Mr. Jean Noel Perrin, Prof. Udaya Kumar and Dr. S. Acharjee discussing ISCB insect resistant chickpea during a visit of the evaluators to the Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, India

Content

1. Executive Summary

The terms of reference set requested both a review and a forward look at institutional, programme and network levels of ISCB. The evaluators have taken an evidence-based approach to meet this remit and collected information using a variety of approaches.

A central point at the institutional and other levels was the very high regard that ISCB is held in by both scientists in India and its Department of Biotechnology. The particularly appreciated features are a long-term relationship that has allowed deep mutual partnerships to develop the quality of Swiss scientist contributions and the capacity building that this has underpinned. Also valued is ISCB's non-bureaucratic and efficient management approach ensures a dynamic ability to respond to change as needed. This has allowed good practices to be developed in the programme and then adapted in other DBT activities.

ISCB is well aligned to meet both DBT's remit and SDC priorities for research in the areas of food security and climate change resilient agriculture. Any change needed to ensure ISCB fits SDC global programmes in food security and climate change is minor and should be readily accommodated. There is value in ISCB remaining distinctive from the contributions of other donors and its responsiveness to DBT's priorities is important in this regard and should be maintained. Much of plant biotechnology is centred on molecular breeding or genetic manipulation which have long lead-in times. Such approaches must offer considerable long-term benefits not attainable by more rapid means to justify investment and research prioritisation. Partial mitigation of this issue relies on extracting any benefit identified mid-project and by including some projects in the portfolio that can mature within a decade.

There are recent successes from the programme in providing products to both public and private users of biotechnology. Opportunities have arisen to support Indian-based agribusiness. This should be encouraged when products emerge from their activities that support food security or climate resilient agriculture directly or indirectly for instance by enhancing rural livelihoods. It is important to ensure market needs are addressed both at project initiation and throughout its subsequent development. The value chain focus for all projects should be set and maintained by *Joint Apex Committee* (JAC) with an evidence base developed by *The Technology Advancement Unit* (TAU) founded in 2010 by ISCB. A fair and accurately applied exit strategy is needed when a project is not making appropriate progress along its value chain.

All networks can showcase successes but projects differ in their current position along the value chain. The Biofertilisers network is tightly integrated and has recently transferred products for evaluation by industry while continuing its public research activity. The two key issues for this network's products are the extent that biofertilisers can substitute for mineral fertilisers without risk of any loss to yield and the development of assured products with appropriate shelf life. The Pulse network has a range of projects at different stages in the value chain. The sucking insect resistance initially for chickpea is at the stage of public and private development but in common with all GM related approaches, regulatory issues and public acceptance rather than scientific issues may limit the rate of progress to the field. This network's other approaches for legumes have technical issues to resolve before a certain product is evident. The recent RNA interference work for cassava gemini virus control is based on strong science but is not developed enough yet to define the level of resistance and durability it can deliver. Innovation and new opportunities available from this network include modern sequencing approaches for plant virus diagnostics from the cassava project and possible enhancement of nodulation densities of legumes. The Wheat network has internationally significant basic research quality and the leads in terms of generating research plant material/identification of candidate genes will significantly contribute for the outputs to develop resilient crop varieties relevant to climate change effects in India. It has identified fungal resistance sources that have been provided to plant breeders.

The new ISCB programme has now been in place for three phases from 1999. The scientific collaboration has given much needed impetus and acted as a platform to undertake basic and translational research. The research plant materials developed so far and the genomics resource knowledge created in participating institutions has promise to make substantial impact on food insecurity or climate-change resilient agriculture in India. Moreover, the ISCB programme has also acted as catalyst to build linkages among the institution (DWR and PAU) to work in collaboration to utilise the plant resources and the collaborative linkage will go a long way and shows promise in joint leadership to develop crops resilient to climate change scenarios in India. Priority should be given within a balanced portfolio to the sub-set of activities that can achieve this soon and approaches adopted to promote and record the extent of uptake of products by Indian farmers.

New projects and possibly a further network should be considered. This would help maintain the engagement of Swiss research base as this understandably tends to decline as projects progress to an applied phase under the leadership of Indian partners. New networks require careful scoping to ensure a good fit with DBT priorities, the food security and climate resilient requirements of SDC and the availability of high quality Swiss and Indian scientists who are keen to form a partnership.

2. Recommendations

Recommendations are arranged at institutional (**I1-5**), programme (**P1-9**) network levels (**N1-9**) and possible new research areas (**R1-5**) with the section in the report underpinning each recommendation indicated (e.g. **S5.1**).

2.1. Institutional Level

I1 Both donors should maintain a continued commitment to joint leadership of the ISCB programme with the primary objective to achieve development impact ***(S5.1)***.

I2 ISCB should maintain the distinctive nature of its programme which is based on a long-term relationship with partners, focused objectives, a non-bureaucratic approach and strengthening of biotechnology in India through use of Swiss expertise and capacity building ***(S5.3).***

I3 In the context of ISCB, DBT should seek potential areas of collaboration with the Ministry of Agriculture, ICAR, and CGIAR institutes notably in publicly-led value chains where extension plays an important role ***(S5.3 and S5.4)***.

I4 For SDC, phase 4 provides an opportunity for the Swiss Global Programmes in Climate Change (GPCC) and food security (GPFS) to collaborate more systematically in India and to consider coordination with the Indo-Swiss Joint Research Programme ***(S5.2)***.

I5 The relationship between the Technology Advancement Unit of ISCB and other DBT platforms and incubators including the Platform for Translational Research on Transgenic Crops at ICRISAT should be appraised to stimulate synergies and avoid duplications ***(S5.3)***.

2.2. Programme Level

P1 ISCB should enhance its relevance to end-users by ensuring impact is the highest priority within project management with prioritisation of the development of climate resilient agricultural technologies over the secondary objective of capacity-building **(*S6.1*)**.

P2 ISCB should maintain a balanced portfolio with outputs or potential products likely to have impact in the short term (<5 years), medium term (5-10 years) and longer term (>10 years). The longer the development time the larger should be the benefits to be gained (***S6.1***).

P3 Only activities with a high probability of producing useful outputs or marketable products should advance into Phase 4. Resources should not necessarily correct constructs or products with inadequacies revealed in Phase 3 in preference to new projects drawing upon expertise in Switzerland and India. ISCB should assess its optimal amount of future networks/projects/research institutions and consider what new R&D areas should be added **(*S6.2*)**.

P4 Outputs of practical value should be developed as soon as they are evident through both public and other institutions (e.g. molecular markers for plant breeders and constructs and other resources to seed companies) **(*S6.2*)**.

P5 The ISCB structure should integrate specific capacities for systematic socio-economic context analysis and for the application of PCM methods at network level to reach development objectives **(*S6.4*)**.

P6 The value chain approach should be more fully embedded in the programme to support the overall aims of enhancing food security or climate change resilient agriculture. **(*S6.4*)**.

P7 TAU is encouraged to enhance its role by helping to inform partners what new stakeholders they should engage with as the project and its value chain develops. In this way they would have a foresight role as well as a responsive one. TAU needs to show its long-term value and develop products not just for their commercial potential but for any beneficial use. It needs to be a facilitator for all project outputs that have potential value **(*S6.4*)**.

P8 There need to be aims defined for each project and each network and then objectives that give a sense of direction. These objectives would be precise for the next work period and develop as the work progresses along the value chain. They would become more focused as the project develops. They should be time bound, have a geographical area or a target group and comply with a smart framework approach. This would correct an omission from Phase 3 **(*S6.5*)**.

P9 ISCB should consider founding a restricted access electronic site to which it can grant partners and others access. This could be managed by TAU and showcase products while raising awareness of ISCB related issues (***S6.5***).

2.3. Network Level

N1 All three networks evaluated should continue with varying amount of adjustment of current projects and new activities as indicated in recommendations below and later (***S7.1.1***, ***S7.2.1*** and ***S7.3.1***).

N2 Networks should take responsibility for ensuring their objectives are relevant to the value chain with support from TAU. With support from PMU and TAU they should ensure a more systematic collaboration and knowledge management of their work to enhance synergies and ensure a shared vision regarding objectives and outcomes for the network (***S6.3***).

N3 A project advisory committee of a few people knowledgeable in the field of effort and the market being addressed should be founded for each network. They should attend the annual meeting, review the value chain documentation and set next year's targets. They and TAU should ensure partners are aware of the potential contribution their network can make to rural livelihood. They should help realise this by interaction with stakeholders in the innovation system (e.g. industry, extension services and farmer associations) as project progresses **(*S6.5*)**.

N4 **Pulse, Cry:** this project needs to determine if the phenotypic abnormality and lack of stable expression can be resolved without long-term delay to product development. If the current difficulties in the cry gene approach are resolved the work could be extended from chickpea to pigeonpea. This would require the transformation efficacy for pigeonpea being in place from other sponsors **(*S7.1.2*)**.

N5 **Pulse, DREB1A:** The key issue for this project is how substantial and robust is the approach given it has worked in the glasshouse but not yet in field conditions? It is unlikely to be in demand by farmers if a robust and substantial effect is not provided **(*S7.1.3*)**.

N6 **Pulse, ASAL:** this project is progressing satisfactorily and ISCB should consider developing the effect of this lectin on nodulation if a) the evidence of efficacy of this serendipitous finding stands up to external assessment and b) a Swiss partner is identified to look at the fundamental aspect of how the lectin has this effect **(*S7.1.4*)**.

N7 **Pulse, introgression breeding:** It seems premature to begin such work until the efficacy, stability and lack of phenotypic abnormally is established for transgenic, parental lines (***S7.1.3 and S7.1.4***).

N8 **Cassava project:** this work is of high standard with a good interaction between Indian and Swiss scientists. It should identify future products and the time for their development by the end of Phase 3 (***S7.1.5***).

N9 **Biofertiliser:** This network provides an important outcome that can have significant impact within the next decade if its products are disseminated by public bodies such as agricultural universities and by private companies. The two key issues for the products is the extent that biofertilisers can replace mineral fertilisers without risk of any loss to yield and secondly the development of assured products with an appropriate shelf life. Success would justify further research to provide products for drier soils than at present (***S7.2.2, S7.2.3 and S7.2.4***).

N10 **Wheat:** This network produces high quality science within a field of high importance to India. It has strong international and national linkages that should be maintained. It needs to identify and emphasise its mid-project outputs to breeders and others to ensure it cannot be judged as over-emphasising basic science which is not the emphasis of ISCB. It is important to reassure its sponsors that development relevant benefits are provided for small farmers. In this regard, there is potential for increased sharing and coordination of results for synergies in India among the basic research (Delhi South Campus), pre-breeding (PAU) and application-oriented (DWR) partners. The resources generated in the network programme in terms of identification of candidate genes, stripe rust, leaf rust and powdery mildew resistant donor lines with resistant genes, RIL populations and the approach to phenotype for terminal heat stress under field condition is quite precise and innovative. These leads can now boost the product development in shorter period through coordinated efforts (***S7.3.2, S7.3.3 and S7.3.4***).

2.4. Possible New Research Areas

R1 Each area should fit DBT and SDC priorities and involve internationally leading Swiss scientist(s) to underpin project development and partner complementary Indian scientists. Research areas to be considered include those below ***(S7.4.1)***

R2 Plant biotechnology and crop responses to climate change ***(S7.4.2)***

R3 Post-harvest losses ***(S7.4.3)***

R4 Utilisation of bioproducts including biomass conversion ***(S7.4.4)***

R5 Biosafety of plant biotechnology ***(S7.4.5)***

R6 New tools for extension service processes ***(S7.4.6)***.

3. Summary of Terms of Reference

The main objective set in the terms of reference was to evaluate the current ISCB programme and to provide inputs and recommendations for developing future strategies and programme. A review was sought at the institutional, programme and network levels. The terms of reference are given in Annex 1.

4. Working Methods

The evaluators agreed in preliminary telephone and e-mail exchanges that their views would be evidence based. A range of ISCB documents and a list of scientific publications were provided by Dr. Doris Herrmann in autumn 2011. She supported the team very effectively in all administrative matters. The documents and a selection of the publications were read by individual evaluators. A questionnaire was circulated to partners and consultants and results analysed before meetings in India to help inform issues to consider. The itinerary for meetings in India from 6-13th December 2011 was determined by the evaluators and concentrated on the three networks, Pulse, Wheat and Biofertilisers on which >80% of ISCB funds are spent. A total of nine days were funded for meetings in India and the team was necessarily divided at times to maximise the number of meetings possible.

It met a range of partners in Pulse, Biofertiliser and Wheat networks, Dr. M.K. Bhan (Secretary to Govt of India, DBT and Chair of Joint Apex Committee (JAC) of ISCB), Dr. S.R. Rao (Advisor, DBT), Dr. Gerolf Weigel (Counsellor and Head Climate Change and Development Division (CCD), Embassy of Switzerland and Co-Chair of JAC), the staff of ISCB's Technology Advancement Unit (TAU), a seed company (Bioseed, Hyderabad) and Dr. D. Hoisington (Deputy Director General-Research of ICRISAT). An informal feedback session was held with DBT, CCD (SDC) and TAU staff at the end of the visit after prolonged internal discussion by the evaluators. The report was compiled via e-mail exchange by the evaluation team in early 2012 for submission by its agreed deadline of the end of February that year.

5. Evaluation at the Institutional Level

5.1. Context

ISCB is a long-lasting research and development partnership between India and Switzerland. The Department of Biotechnology (DBT) has maintained a strong interest in ISCB in recent past years, which supported continuity of the programme in a period of major institutional changes in the Swiss Agency for Development (SDC).The two funders have different mandates and roles within their administrations. DBT focuses on R&D in a variety of fields in Biotechnology. SDC, a part of the Federal Department of Foreign Affairs, is the main agency responsible for Switzerland's bilateral and multilateral development programmes and aid contributions. There is a common interest for the two countries in global concerns such as food insecurity and climate change consequences.

The Indo-Swiss Collaboration in Biotechnology programme started in 1974, with a wide range of biotechnology projects. It reoriented 1999 to become more focused thematically (on Agriculture and Environment) with a clearer aim to contribute to poverty reduction and food security. It is on these grounds that it was called the *new* ISCB, which is, at present, in its third phase (2007-2012). Under the current phase ISCB is co-funded by Switzerland and India with contributions of 4.8 Mio CHF and of 4 Mio. CHF (equivalent), respectively. DBT's relative share went up to 45% for the current phase, compared to 34% in the previous phase.

5.2. Switzerland / SDC

A timely shift in the nature of the international relationship with Switzerland occurred as India was increasingly perceived as an emerging economy with considerable capacities and means. As it was captured in the 1997 review of ISCB, the collaboration was bound to evolve from "aid administration" to a "true partnership" with mutual accountability. From a Swiss perspective, the overall engagement of SDC in India remained an open issue until 2008, at a time when the development agency was reducing the number of priority countries of its bilateral cooperation[1], and creating the new domain of Global Cooperation (SDC-GC). During that period, Switzerland/SDC strived to set up a new type of collaboration with India: *"The partnership programme involves a shift from traditional/classical development cooperation, towards a collaboration based on common interest, on joint ventures and shared investments, with the ultimate aim at reducing poverty. A key feature of this programme is the exchange of know-how and technologies between Switzerland and India and the promotion of south-south cooperation"*[2]

From 2008, SDC seeks to contribute through *Global Programmes* to the generation of experience and knowledge that are of global relevance, and to engage in institutional and policy processes on global issues, such as climate change or food security to reach policy outcomes at international and national levels. On this basis, and with the establishment of the Global Programme Climate Change (GPCC), SDCs strategic engagement in India shifted from a more traditional bilateral cooperation to cooperation on the climate change issue, seen as a global challenge to be tackled in India. The two main components of the SDC India programme focus

[1] Citation of Dr. KR Viswanathan, CCD India
[2] SDC in India 1963-2007, brochure, http://www.swiss-cooperation.admin.ch/india/en/Home/Publications

today on *energy* and *adaptation*. In the latter, the initiatives look at water resources, food security and agricultural biodiversity. The strategic decision to continue the international partnership under a new form suggests that the existing institutional links and partnerships are being valued by SDC, and that the years of collaboration and common work can be used as building blocks for this new partnership.

At SDC India – now represented in India by Climate Change & Development Division (CCD), Embassy of Switzerland - ISCB is considered to be at the interface between climate change and food security and is well integrated in the portfolio. The promotion of climate resilient crops in the current phase has gained in importance in Phase 3 of the programme and provides a real expression of both issues at an operational level with the choices made regarding research priorities. Generally speaking, it can thus be said that the ISCB strategy is in line with the new orientation of CCD and of the SDC-GC. However, at the head office in Berne, ISCB is the only programme to be managed from the Global Programme Food Security (GPFS). The GPFS manages a mixed portfolio of multilateral and bilateral contributions of a global character. Relevant to ISCB is notably its engagement in international agricultural research for development (it manages the Swiss contribution to the CGIAR) and agricultural extension/value chains. The resulting triangulation (GPCC-CCD-GPFS) is not perceived by CCD as an organisational complication but rather as an opportunity, since the Food Security dimension was retained as valuable for its programme.

ISCB was the first bilateral agreement in science and technology between the Swiss and Indian governments. A second cooperation in this field - the Indo-Swiss Joint Research Programme - was established between the Swiss Secretariat for Education and Research and the Indian Department of Science and Technology (DST) – DBTs parent department under the Ministry of Science and Technology. There are not apparent connections between the two programmes, beyond the hosting. From the Swiss side, both contributions/programmes are being managed by the Cooperation and Development Center of the Swiss Federal Institute of Technology in Lausanne. A third basis of collaboration is developing between SDC (GPCC and CCD) and the DST under the Indian Himalaya Climate Adaptation Programme-IHCAP. An overview would allow for a better comprehension of the boundaries and the overlaps, and the potential for institutional linkages and synergies between the different collaborations.

5.3. India / DBT

DBT is part of the Ministry of Science and Technology, in charge of biotechnology for all of India. Beyond coordinating basic and applied research and development activities of a decentralised set of research institutions in medical, animal, environmental and agricultural biotechnology, it fulfils mandates of promotional and of regulation science support nature (e.g. biosafety guidelines for GMOs). The DBT strives for India to "*achieve excellence in the field of Biotechnology for the benefit of the society, environment, entrepreneurs, trade and industry who are our partners in growth*"[3]. In this sense, it engages in promoting knowledge platforms (Biotechnology Information System Network) and in translational activities such as the setting up of industrial incubators aiming at technology transfer and product development. According to the Secretary of DBT, Dr. Bhan, an objective for the future is to "*balance science and translation*". As far as ISCB is concerned, it is in line with DBTs strategic orientation to foster agricultural development from a technical perspective. The new climate change focus has been welcomed by DBT.

[3] Vision of DBT, DBT website http://dbtindia.nic.in

DBT has a series of bilateral research programmes, notably with Germany, Sweden, UK, US, EU, Australia, Japan, Norway. It considers the collaboration with Switzerland to be of particular added value because of its long-term and low profile character, as it provides "*more quality than noise*"[4]. It is taken as an example for other bilateral collaborations according to Dr. Rao, the advisor to DBT in charge of ISCB. Furthermore, the quality of the technical and research capacity of the Swiss partners and the management of the programme in Switzerland are recognised. ISCB also benefits from technical advice based on DBT's knowledge of India and the commitment to it and the considerable experience of Dr Rao. A specific advantage of ISCB is the pragmatic and non-bureaucratic way the programme is being managed: This allows for changes, adjustments of the projects and, if required, also exits. This flexibility and the testing for new translational modalities – notably with the Technology Advancement Unit (TAU) - give ISCB an important pilot character according to DBT executives.

The most obvious link of DBT and ISCB to the consortium of centres of the CGIAR (Consultative Group on International Agricultural Research) is through the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) which is headquartered in Hyderabad. The scientific collaboration mostly concentrates on development of drought-tolerant chickpea and pigeonpea, two nutritious crops of importance for Indian farmers and for food security. Beyond that no systematic, institutionalised exchanges with the CGIAR system seem to exist. Some exchanges between individual institutions and researchers do occur but the lack of institutional links was noticed.

Agricultural biotechnology covers a substantial part of DBT's portfolio of projects and activities, and it is considered that the department contributes to agricultural development and food security coming from a technical side. India's Ministry of Agriculture is a close parent to DBT with its Department of Agricultural Research and Education, and in particular the Indian Council of Agricultural Research (ICAR) it hosts. The Council is the apex body for coordinating, guiding and managing research, education and extension in agriculture. With over 90 institutes and 45 agricultural universities it is spread across the country. ISCB does not have any direct institutional link to ICAR or to the Ministry of Agriculture in general but some of the partners (e.g. Directorate of Wheat Research, Indian Agricultural Research Institute) belong to institutions within the ambit of the ICAR and others (e.g. Punjab Agriculture University, Assam Agriculture University, Dharwad Agricultural University) are closely linked to it.

5.4. Observations Related to the Indian Context

Food security is a big issue in India, and climate change is perceived as a growing challenge for the future. Research in agricultural biotechnology (and to some extent genetic engineering) for an increased agricultural productivity is considered vital for India's future by many plant scientists and other stakeholders.

Central and state governments in India play important roles in dealing with food security both through policy-making and investment. Agricultural research and development is largely carried out by public institutions. In some sectors, such as in wheat, and in some states, government manages the food cycle from research to production to consumption, by buying the wheat at a set price (produced with seeds from the agricultural universities) from farmers to use it in its food security programmes in other parts of the country.

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[4] Citation of Dr. M.K. Bhan, Secretary, DBT, Chair of Joint Apex Committee of ISCB

Although there is an increasing appreciation of the need for establishing effective linkages along the value chain, India needs to develop and strengthen its public private partnerships through establishing new or restructuring existing organisational and institutional set-ups. Necessary regulatory, incentive and social responsibility mechanisms should be designed. The private sector should particularly be encouraged to invest in R&D. DBT has established a number of initiatives to support such partnerships[5].

Moving genetically manipulated research products down the value chain is a complicated procedure and no edible GM product has been commercialised so far in India. The only commercialised GM crop is Bt Cotton, which is being widely cultivated and about 90 percent of the total crop area for the country in 2011[6]. Although a non-food crop, a good proportion of the cotton seed enters the food and feed chains. The future pace of uptake of GM crops in India is currently difficult to determine. There are those in India who seek safe uptake without delay[7]. For instance, insect resistant crops are likely to eliminate considerable yield losses, reduce much pesticide use and lead to substantial welfare gains for consumers[8]. Other views emphasise perceived risks and ethical concerns that are important in defining public attitudes plus opposition to investment in India by foreign seed companies[9]. The issue of the biosafety of such crops has recently been reviewed by The National Academy of Agricultural Sciences, New Delhi, which should minimise the uncertainty created around GM crops[10].

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[5] **SBIRI** (Small Business Innovation Research Initiative, http://sbiri.nic.in/), **BIPP** (Biotech Industry Partnership Programme, http://dbtindia.nic.in/AboutBIPP.pdf) and **BIRAP** (Biotech Industry Research Assistance Programme, http://www.birapdbt.nic.in/)
[6] http://www.fibre2fashion.com/news/textile-news/newsdetails.aspx?news_id=106492
[7] http://sation.in/?page_id=737
[8] Krishna and Qaim, 2008, *Review of Agricultural Economics*, 30, 233–251
[9] http://www.greenpeace.org/india/en/What-We-Do/Sustainable-Agriculture/GE-campaign/
[10] http://naasindia.org/Policy%20Papers/policy%2052.pdf

6. Evaluation at the Programme Level

6.1. Mandate, Objectives and Impact

The ultimate goal to contribute to poverty reduction and sustainable agriculture/natural resource management by developing climate-resilient agricultural products is sound. But the two specific mandates of ISCB are weighed similarly: “climate resilience and food security”, which is in fact the main purpose, and “capacity building of Indian institutes”, that would rather constitute a result of ISCB and contribute to the achievement of the main objective.

The overall impression of the evaluation team regarding the R&D work done under ISCB is positive. Most of the projects are conceptually in line with the formulated mandate and with the climate resilience and food security dimensions, although it has not been yet demonstrated at impact level. Abiotic and biotic stresses have been properly addressed by the R&D projects and are likely to impact at large scale in the future[11].

Given the very upstream character of ISCB in the phase 3 (and previous phases), a major contribution can be observed in the capacity building in the research centres, from which a number of Indian scientists and students have benefitted. The cooperation with Swiss scientists provided a strong recognition of their competence and the high degree of interactions has been very positive for all ISCB networks. All partners did recognise the added value of the transnational collaboration.

Some of ISCB benefits to food security or climate change resilient agriculture may be long term (>10 years) whereas others may have the impact in the medium term (5-10 years) or even sooner (<5 years). ISCB should maintain a balanced portfolio that offers the prospect products with different development periods. It needs to ensure those with long development times offer the prospects of substantial benefits that justify their long term investment.

Important gaps between the upstream downstream levels of the value chain have been observed, except in a few cases such as licensing activity of the Biofertiliser network and the inclusion of both public and private licensees (e.g. Bioseed) in the Pulse network. The absence of methodological monitoring tools, such as objectively observable indicators[12] and clear development outcomes in defined timeframes reveals a weakness in the formulation of the overall programme.

The field of tension between an engagement in strategic basic research, which requires decades to come to intermediary research products and the need to contribute to agricultural products ready to be brought to the market or the end-user was identified in the nineties and remains a challenge today. This fact and the options regarding the nature and composition of the ISCB R&D portfolio need to be taken up in forthcoming strategic discussions regarding ISCB’s future. In this context, it can be observed that the number of networks has been shrinking: From originally 6 only 3 remain fully active, with one, pulse and cassava, being bigger and wider than the wheat and biofertiliser network.

[11] There are for instance about 90 millions of farmers involved in wheat, and the geographical impact will certainly involve a large number of states in India and possibly abroad.
[12] SMART indicators: Specific, Measurable, Achievable, Relevant and Time-bound

6.2. Intermediate Product Development

It is compelling that the ISCB review stated in 1997 that: "*major hurdles that are seen in the transfer of research results to industry and government are the "scale-up" aspects, the "downstream processing", the biochemical engineering processes, the economics of such processes and the overall attractiveness to industry*". The 2007 review, reiterated this observation by mentioning that "*there were deficits in bringing the products down the value chain*", and suggested that "*to bring one or more projects to the end of the value chain will be the primary goal of the next programme phase*"[13].

While considerable scientific progress has been made from basic research by the three ISCB networks that were reviewed, no product has yet progressed from these R&D partnerships for sale or dissemination by industry or state institutions. In this sense, development impact for the food insecure remains far off and ISCB needs to be aware of the concerns that this may raise for those who wish to see food security improvements in India soon. While most respondents understand the concept of the value chain and embed their research contribution in an overall development objective, currently there are few outreach activities specifically aimed at engaging in vertical exchange or integration activities. It seems the R&D partnerships remain largely disconnected, and the market, as the end-user, is often a minor issue when making strategic decisions. At the very least, ISCB needs to be aware and mitigate concerns of some stakeholders about the long lead in time before many biotechnology products have impact on food security or mitigate the effects of climate change. In this context it would be helpful for ISCB to show the overall balance of its portfolio by compiling the evidence base that substantiates the value chain progress of three networks (Biosensor, Biopesticide and Bioremediation). They were not considered in this evaluation because they have advanced beyond substantial ISCB funding. The Biopesticide work has been reviewed by an independent technical committee of DBT and funded by its Small Business Innovation Research Initiative[14].

The final aims of the research projects are generally relevant to the ISCB mandate but there is a risk that research processes end up in closed cycles. In such cases, the main direct outputs are post-graduate capacity-building, scientific publications and specific yet diffuse knowledge without any direct connection to any development relevant research product. This risk can be mitigated in part by ensuring all mid-project outputs of practical value are successfully transferred to end users.

6.3. Structure and Management – Creation of TAU

The role of the Swiss-led Programme Management Unit (PMU) is highly valued by Indian partners, and by DBT. The management done from Ecole Polytechnique Fédérale Lausanne (EPFL) is considered lean, efficient and flexible. Knowledge and experience of PMU lies primarily in biological science, a prerequisite for an interaction of content with research institutions and management skills. The main change in the structure of ISCB during Phase 3 was the creation of the Technology Advancement Unit (TAU) as an answer to the main recommendation of the last ISCB programme review (2007). TAU was strategically preferred to an Indian programme management unit, with a primary aim to concentrate on product development and technology transfer. This choice complements rather than duplicates the skills of PMU. TAU was set up as an independent project within ISCB. TAU had a late start in the

[13] Review of ISCB Programme Period 1999-2007, p.10

[14] Citation of Dr S.R. Rao, Advisor, DBT

phase (mid-2010) and its establishment was arduous. The initially difficult work environment and the change of hosting institution hampered the initial work of the small TAU team, which, at present, is still in a learning phase. Its first engagements aimed at tapping into the networks, understanding their challenges in terms of product development and identifying the related "gaps" in the value chain. TAU is the only management unit specifically created within DBT's international collaborations. Its pioneer role and the establishment of good practices by ISCB has a wider value for the Department's management of research and technology for instance within its new Biotechnology Industry Research Assistance Council (BIRAC)[15]. In addition to product development, ISCB has contributed to establishing good practices that have enhanced Indian industry-academy/research relationships. This favours investment of industry in R&D as the Indian economy matures. ISCB has an important future role in capacity building by demonstrating efficient pathways for dissemination of technology and products to both industry and public institutions. Given this importance, the roles of PMU and TAU should be verified as fully comprehensive and complementary for Phase 4 and their joint cost set at an appropriate proportion of the total budget.

TAU's mandate, as described in strategic documents, can be considered as broad: It is meant to function as a facilitator, advisor and supporter, provide access to expertise and skills to project partners, take some management responsibilities and contribute to the project cycle management (PCM) of the projects/networks. The TAU team sees its main contribution in the support of the networks to bring technology to maturity and to deliver quality products. A main activity consists of bringing different parties together and creating links to push for technology advancement and product development to occur. The main objective is to finalise licensing agreements with the private sector. Post-licensing activities of TAU would be limited to commercialisation matters, and no development outcome monitoring has been considered so far.

The engagement of TAU with the networks is very diverse and variable, as are the expectations of the different networks and research institutions. Depending on the advancement of the research of the networks and their stage in the value chain, TAU has been able (or not) to tap into the networks by addressing specific needs mostly regarding market information and licensing procedures. With some networks, there is hardly any exchange at all. TAU does not have one counterpart in the networks but interacts with each research institution, playing a (sometimes challenging) facilitator and brokerage role between the members of a network. The fact that TAU materialised much after the creation of the different networks makes its role rather reactive. While acknowledging the fact that TAU is still at an early stage of consolidation, there is a general opinion that the unit's role should be more than a "on demand" backstopper fulfilling specific activities. In the case of the Biosensor network, TAU seems to have played such a role by bringing "*the evidence to make a decision happen at JAC level*"[16] as they showed the limited market potential of the technology.

6.4. Monitoring of Development Outcomes

To make the Value Chain Approach (VCA) effective it is necessary to establish a common understanding among ISCB partners. The rationale of the VCA aims to increase the chances that end users will benefit from the new technology and ensure realistic innovations anticipate market constraints at a very early stage in product development. This includes the potential

[15] http://www.birapdbt.nic.in/uploads/birac_doc_new.pdf
[16] Citation of Dr. Pratibha Singh, TAU

needs for climate resilience and food security products and potential competitive advantages for targeted final users. This concept leads to a sound information mechanism including upstream knowledge and permanent progress and efficiency monitoring. Those principles are fully in line with those that have already been established during the Phase 3 of ISCB. However they have not been operational basically for two major reasons: a) because there are different understandings of the VCA vertical integration among partners but also horizontal interrelationships that may result in potential synergies within the network, b) the monitoring system has not been fully integrated into the overall institutional framework. The recommendation is to set up adequate tools that would contribute to improve the efficiency and effectiveness of the programme through a higher degree of interaction between all actors involved in the overall value chain.

The upstream knowledge of potential needs should be reflected through a market study that would delineate a) the product relevance with regards to climate resilience and weighted potential impact on food security, b) the potential advantages for final users, c) the potential breeding and delivery channels, d) the downstream post harvest and market constraints, e) a simulated cost structure analysis that is subject to continual updating. It is the JAC's responsibility to determine the value chain strategy and to select adequate indicators and milestones for each network weighting up this preliminary information with reference to the ISCB established goals.

The monitoring system has to be set up as an overall mechanism starting from the network level to reinforce the steering capacity of the JAC. The Project Cycle Management (PCM) approach, that involves identification, implementation and monitoring, would be the best way for drawing together and monitoring the information, because it ensures that each part of the project cycle is considered during the project's life, from research discovery to end users. Time frame, actors and cost analysis are the basic drivers that should be utilised in the VCA (Figure 1). Additionally, the VCA mapping should include research knowledge that may be disseminated horizontally within the networks, hence constitutes an extra output of ISCB.

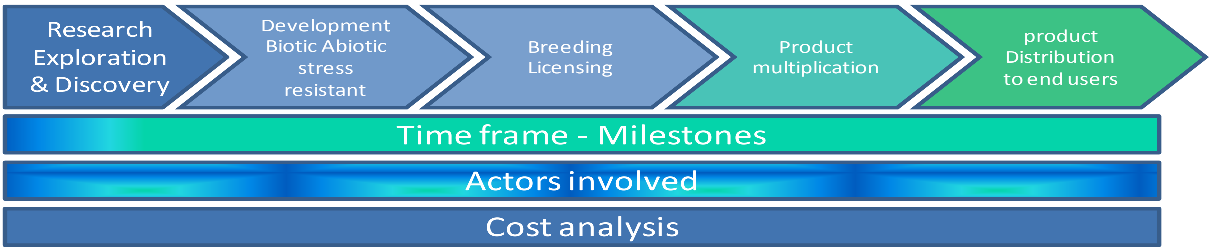

Figure 1: Generic mapping for ISCB projects along the value chain approach

In this context, the different research networks should be proactive partners to establish linkages at downstream levels at a very early stage provided that TAU is facilitating the information process. For instance, TAU should facilitate the identification of downstream outsourcing as licensors and assess the technical and socio economical constraints within the downstream chain. Additionally to this role of facilitator, TAU should bear the full responsibility of the ISCB-VCA monitoring, providing support to the different networks to monitor the VCA indicators and consolidate their annual reporting. Hence the TAU's mandate should focus on two main priorities: to facilitate upstream and downstream channels and monitor the overall VCA starting from network levels.

JAC's role in the strategic orientation of ISCB and project approvals should be assisted by this permanent monitoring mechanism in which the value chain analysis becomes a core element of the ISCB. First of all monitoring the VCA will provide a better traceability on product development and ensure adequate and realistic planning keeping within the ISCB main objective. It would also reinforce the JAC's capacity as mediator to resolve technical constraints related to product development. Finally, JAC may avoid failures in the value chain due to unanticipated drawback or even take timely arbitration to pursue or close a project, for instance when a risk of a nonperforming product has been identified or competitive advantages become critical.

The endpoint for each ISCB project and the expected time frame for its delivery need to be taken up in planning for Phase 4. Outputs need to be followed beyond licensing or other outcome to evaluate the impact on end users and determine the development benefit.

6.5. The Value of the Project Cycle Management (PCM) for ISCB

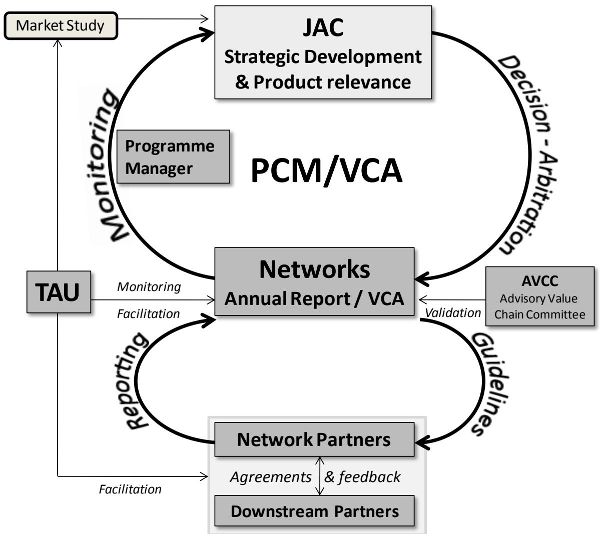

Figure 2: Organigram of Project cycle management for ISCB

Figure 2 describes how the PCM Value Chain Approach should operate at all levels. This mechanism confers a key role for JAC in establishing a strategic development, keeping a permanent control on product relevance. JAC's decision and arbitration process integrates the upstream and downstream levels, which means that the network partners are involved in monitoring the VCA. Information can be collected through the annual reports and TAU will facilitate the data collection as well as linkages and feedback from the downstream partners (licensing industries, private sector, NGOs, Farmer organisations, etc.). An Advisory Value Chain Committee (AVCC) could be established to validate data and planning during the Network Annual Meeting. The Programme Manager will also assess the progress of the VCA to evaluate the progress and efficiency of the ISCB and ensure the full commitment of the networks in the value chain approach.

At the very early stage, TAU will systematically initiate a market study for each network to define realistic product development in line with the ISCB objectives, and including the social and institutional environment, and the potential benefit for end users. This study will constitute a significant contribution for JAC to draw up the strategic development for each product, including the expected results and performance indicators that will be used by each network. The monitoring system will also contribute to provide clear indicators for external impact evaluations.

There would be value in adopting one or more of the standard approaches to manage projects. Examples include the GANTT chart approach proposed in the 1997 review which is now used internationally for many projects[17]. Another approach of relevance is the log frame approach which has been used by SDC and elsewhere in a development context[18]. The PRINCE2 system also has value[19] particularly for more complex projects particularly when the risk of delay of outputs from one researcher impacts on the progress possible for another.

There may also be merit in adopting an electronic system to monitor the progress of the networks, spread best practice and inform all of developments of interest. The site could have public and restricted access areas with parts of the latter available in controlled manner to other stakeholders or potential licensees.

6.6. Enhancing Institutional Links between ISCB and CGIAR Institutes

The only CGIAR partner in the networks is ICRISAT although the Wheat network has interactions with CYMMIT. However CGIAR has developed very efficient tools through one of its most important programmes, the GCP (Generation Challenge programme, based in Mexico) that focuses more and more on the value chain approach, so as to provide a positive answer to many donors who stressed the need for more efficient use of the research outputs, through improved trials and demonstration mechanism as well as a wider knowledge sharing. It could prove valuable for ISCB to establish linkages with the GCP, and either formally participates in the GCP dialogue or at least draw lessons from their experiences. The 2010 reform of CGIAR may make it difficult for new partners to be involved in this network. GCP acts as broker for partner institutions, bridging the gap between upstream and applied science and enhancing the NARS' research capacity to deliver product to resource-poor farmers in particular with its sub-programme 5 centred on capacity-building. There are two relevant instruments that are still operational under GCP to accelerate the technology dissemination process that could constitute a model for ISCB: a) the Integrated Breeding Platform (IBP) that provides developing countries access to modern breeding technologies and wide range of information previously unavailable that enhances the decision process and germplasm delivery, b) the second, more downstream instrument is the Community of Practice (CoP). It organises groups of scientists, breeders, and farmers to share advanced technologies and best practices and solve common problems. One of the major achievements of the CoP is to bridge the gap between the agricultural science, breeders and farmers.

Other lessons from the GCP/CGIAR is the importance given to the nature of linkages at all levels of the value chain, that was undertaken through a number of operational interactions with international experts making a substantial contribution to the capacity, recognition and self esteem of the local actors.

[17] http://www.ganttchart.com/
[18] http://www.swiss-cooperation.admin.ch/southernafrica/en/Home/Project_Cycle_Management/Monitoring/Framework_Description
[19] http://www.prince2.com/prince2-book-store.asp

7. Evaluation at the Network Level

7.1. The Pulse and Cassava Network

Coordinator: Prof. T. Hohn, *Institute of Botany, University of Basel, Basel, Switzerland*

7.1.1. Network as a whole

Overview of the network

India produces 66% of the world chickpea production (5.7 million tons in 2007) some of which is exported (mainly Kabuli types) while also importing (mainly Desi type) to meet domestic demand. This pulse provides a low cost protein source of particular nutritional value for vegetarians. Smallholder chickpea farmers require access to improved varieties for self consumption and to both maintain and increase their livelihoods and produce this affordable, high protein source for the poor. There is a difference between the varieties grown in northern and southern India but about 10 cultivars cover the majority of production in India. The three ISCB projects address the major biotic and abiotic stresses of the crop for India which are unlikely to be overcome by conventional plant breeding approaches.
This network has licensing partners. They are a) Mahyco (Maharashtra Hybrid Seeds co. Ltd), Mumbai and Aurangabad, b) Bioseed, Hyderabad, c) University of Agricultural Sciences, Dharwad and d) The Indian Institute of Pulses Research, Kanpur, from 2012.

Transnational collaboration

The network is an active international interaction with annual meeting (that in Jorhat in December 2011 was visited by three of the evaluation team). There have been examples of mutual benefit from the interaction. For instance the cassava project combined the expertise in plant virology and plant transformation with the RNAi research strength in Switzerland. There have been at least six visits of Indian academics up to 18 months to Swiss institutions and to CSIRO, Canberra, Australia linked to ISCB. This has enriched the academic experience of Indian staff, enhanced their technical ability, exposed them to new research avenues and raised their confidence to conduct research to international standards.

Capacity building

One consequence of enhancing of the Indian partners institution has been investment in their research infrastructure such as $8.7m to Assam Agricultural University from DBT who became aware of the quality of that institution’s work from ISCB. This has also led to at least one Indian scientist gaining additional international research funds for work on legumes. There has been training of post-graduates at PhD and MSc levels and enhancing of the expertise of research technicians.

Research publications

The network partners have published several papers in international journals such as *Transgenic Research PLoS ONE*, *Molecular Plant-Microbe Interactions* and *Nucleic Acids Research* in recent years establishing their scientific outputs are often of international standing.

Future of the network

The network is an effectively interacting grouping that has made progress and should continue. Individual projects within the network have different challenges to meet ensure they meet ISCB objectives for Phase 4 once these are defined partly in light of other recommendations in this evaluation. The principal challenges are considered below for each project (***S7.1.2**, **S7.1.3***, ***S7.1.4*** and ***S7.1.5***). New activities could be added or replace some of those currently in the network. Sources include innovations achieved in Phase 3 (e.g. see ***S7.1.4***) and new projects appropriate for this network such as those suggested in ***S7.4.2iii***, ***S7.4.3*** and ***S7.4.5*** some of which may need additional Swiss or Indian expertise.

7.1.2. Genetic enhancement of chickpeas using a chimeric *cry*2Aa gene to confer protection against pod borer

<u>Partners and institutions</u>

Prof. B.K. Sarmah (Coordinator), *Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, India*

Dr. K.K. Sharma, *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Genetic Transformation Laboratory, Patancheru, India*

Dr. H.C. Sharma, *International Crops Research. Institute for the Semi-Arid Tropics (ICRISAT), Genetic Transformation Laboratory, Patancheru, India*

<u>Aims and objectives</u>

Aim: Development of transgenic chickpeas expressing Bt-*cry2Aa* and Bt-*cry1Ac* genes to confer protection against pod borers.

Objectives: 1) Develop transformation system and reconstruct chimeric Bt genes for chickpea transformation 2) Introduce chimeric Bt genes into popular cultivar(s) through an *Agrobacterium*-mediated transformation system 3) Molecular analyses of transgenic events to confirm presence and expression of transgenes and identify homozygous lines 4) Insect bioassays to confirm level of resistance in selected transgenic lines against the target pest (*Helicoverpa armigera*) 5) Evaluation of the promising Bt lines for their non-target effects against arthropods under lab and/or greenhouse conditions 6) Incorporate insect resistant transgenic lines into an introgression breeding effort.

<u>Context</u>

The chickpea pod borer, *Helicoverpa armigera* (a lepidopteran insect) causes estimated global losses[20] of about $ 500 million/ year. No sources of pod borer resistance are available for chickpea plant breeders and so a transgenic approach has been taken as for the same insect when damaging cotton (cotton bollworm). Two cry genes (*cry1Ac* and *cry2Aa*) are being used for chickpea transformation. Cry2A proteins have a high-affinity binding site in the insect midgut that is different from that of *cry*1Ac[21]. This is important for pod-borer pest management as cross-resistance between these two types of cry proteins is rare so favouring their combined use in chickpea for durable resistance. Bt gene constructions were made at FMI Basel, Switzerland in Phase 1. The development of transgenic plants occurred in Phase 2 at CSIRO, Australia. This resulted in 54 lines expressing this *cry* gene at Jorhat of which 5 showed c100% mortality but all five imposed a reduction in chickpea plant size and seed yield in the glasshouse.

<u>Highlights</u>

Three of the evaluation team met with the Indian partners in this project at both Jorhat and at ICRISAT. The group at Jorhat showed a high commitment to the project. Their laboratories were well equipped for the molecular work and they had the containment glasshouses required for their contribution to the project. Dr HC Sharma is an internationally significant entomologist

[20] Estimated by AAU
[21] Hernández-Rodríguez *et al*.,2008, *Applied and Environmental Microbiology*, 74 (24), 7654-7659

whose expertise is of high value to the team. He showed the evaluators who visited ICRISAT the entomological facilities and the extensive containment glasshouse facility there needed for evaluation of many lines. ICRISAT also have the facilities required for contained field trials with chickpeas including natural infestations of pod borer. Dr KK Sharma has considerable experience in development of transgenic plants. Expertise and facilities at ICRISAT are important as Jorhat is far from areas of intensive chickpea production in India. Some of the results from Phases 1 and 2 of the project have been published[22].

Progress

A total of 68 new events are positive by PCR for *cry2Aa*. So far protein expression has been confirmed for four events one of which expressed at a high level. T_1 progeny from different events are being grown for segregation analysis to confirm single or low copy number insertions. In addition, a *cry1Ac* truncated gene has been used and 13 lines with a single copy of the transgene insert obtained. Resistance has been achieved but only two lines (BS100B and BS100E) showed a near normal phenotype. The work is now generating transgenic lines using new versions of chimeric *cry1Ac* / *cry2Ab* gene with a full length *cry1Ac* or using a targeting peptide to export the Cry protein to the chloroplast in attempt to reduce phytotoxicity. No useful chimeric *cry1Ac* gene lines have been generated so far. Three lines with detectable level expression of *cry2Ab* gene are being analysed.

Outputs and products

Collaborations with other partners have provided an encouraging development of a value chain approach of this ISCB project. Assam Agricultural University has already agreed non-exclusive licenses with Mahyco and two public sector partners. The lines transferred are 2 high and 1 medium expresser. They have also transferred one *cry1Ac* line.

Impact

Success with the project would result in eliminating the severe loses that Lepidopteran pests impose on chickpea. The transfer of technology to private seed companies provided the prospect of parallel effort on certain crops beyond public breeding effort. The project has also had considerable benefit in building research capacity at Assam Agricultural University.

Challenges and a forward look

There is some evidence that Cry proteins can cause abnormal phenotypes in plants (see Acharjee S. *et al.*, 2010 *ibid*). Therefore, a key issue for *cry2a* in this work is can a level of expression be achieved that is sufficient to control stem borer but less than needed to cause phytotoxicity? This may not be a robust approach. Possibly this risk can be avoided if the *cry2b* gene now being used does not show a phytotoxic effect. A second approach is targeting the cry protein to the chloroplast using a transit peptide in the expectation that this will avoids the toxicity at high expression levels as achieved in earlier work by others[23]. A further issue is the level of expression of *cry1a* is typical of that recorded in some earlier work but very low compared to other work[24] whose construct has been mimicked in this project. One possible approach may be to match the transgene sequence to the codon usage of chickpea in the hope that increases expression levels.

An ability to advance any Cry protein providing resistance to pod borer would be valuable progress. A benefit is that cry proteins have been well characterised internationally for their biosafety and so should pass scrutiny by Indian regulatory authorities. The need is to establish

[22] e.g. Acharjee S. *et al.*, 2010, *Plant Science*, 178, 333-339
[23] Wong et al,. 1992, *Plant Molecular Biology*, 20, 81-93
[24] Miklos, J.A. *et al*., 2007, *Crop Science,* 47,148-157

each transgene as a stable homozygous line(s) with pod borer resistance but without an abnormal plant phenotype. There seem little value in assessing non-target effects or initiating an introgression programme with additional cultivars until that position is reached. Once chickpea lines expressing two distinct Cry proteins have passed all regulatory hurdles they can be crossed to achieve pyramided resistance.

It is recommended that progress is considered carefully at the end of phase 3 to assess whether or not a product can be advanced rapidly on the basis of lines with stable expression, pod borer resistance and without an abnormal plant phenotype. If not, a decision needs to be taken about the chance of current problems being overcome soon using approaches such as those listed above. This could be a role for Swiss scientists in this ISCB project.

7.1.3. Characterisation and evaluation of transgenic events of chickpea containing the *DREB1A* gene for tolerance to drought stress under contained greenhouse and field conditions

Partner and institution

Dr. K.K. Sharma, *Genetic Transformation Laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India*

Aims and objectives

Aim: To improve the response of chickpea to terminal water stress by their transgenic expression of a transcription factor, dehydration-responsive element-binding protein 1A (DREB1A).

Objectives: to evaluate the response of already generated transgenic events (T6 generation) and parent controls to terminal water stress in both the glasshouse and then the field by assessing a range of parameters including a) the pattern of water uptake b) transpiration efficiency c) the pattern of flowering d) yield and e) harvest index.

Context

Drought stress is a major constraint for chickpea in India so this project is highly relevant to impacts of climate change particularly as production is increasing in dry areas within Southern India. Chickpea is normally sown into moist soils but water and temperature stress intensifies as it grows which reduces flowering, pod number and yield. These effects can be reduced by a) early maturation (for which conventional breeding has made progress), b) better water use by the plant and c) prolonged tolerance of dry conditions which is the focus of the project.

Phase 1 of the ISCB project developed an efficient protocol for chickpea transformation and developed lines over-expressing proline which is known to have a positive value in mitigating the consequences of plant drought stress. These did not provide an expected benefit under dry conditions. In Phase 2 of the ISCB project, the promoter of the water stress induced gene studied in Phase 1 has been used to enhance expression of a dehydration responsive element binding (DREB1A, a transcription factor). Over 18 transgenic events with this constructs were advanced to T6 generation and characterised by detection and integration of the gene using PCR, RT PCR and Southern analysis. Several *rd29A:DREB1A* events had relatively higher transformation efficiency and 4 transgenic events (RD2, RD7, RD9 and RD10) were selected for further study in Phase 3.

Highlights

The ability to transform chickpea with sufficient efficiency to generate groups of 50 independent transformation events per constructs is a highly valuable general contribution to chickpea improvement. The collaboration between ICRISAT molecular biologists and plant physiologists is a distinctive characteristic of the project that provides a highly thorough characterisation of the additional attributes of transgenic lines.

Progress

Phase 3 has evaluated lines RD2, RD7, RD9 and RD10 for a range of parameters of relevance to withstanding drought stress in the glasshouse. It has studied patterns of water uptake and flowering, transformation efficiency, yield and harvest index. Significant differences from an untransformed control were found for abortion of flowers (RD7, 9 and 10) pod weight (RD7, RD10 in 2009 and RD2 plus RD7 in 2010) and shoot weight in 2010 (RD10).

The work then advanced to field evaluation at ICRISAT where no rainfall was allowed after planting into well watered soil contained in deeper cylinders than roots will penetrate (lysimeters). This allowed water use and the other parameters already assessed in the glasshouse to be measured. Unfortunately no significant differences were established in the field although there was some indication of benefit particularly for RD2 and RD7 based on pod and total weight. This trial is to be repeated in 2012 but the likely cause of the lack of effect is not certain with only temperature differences between the field and glasshouses suggested as a possible basis for the lack of benefit under field conditions.

Outputs and products

Some transgenic lines have value according to a contained glasshouse-based assessment of the consequences of over expressing DREBA1 on other gene expression. In addition thorough approaches for assessing drought tolerance of chickpea lines and varieties have been demonstrated.

Impact

Drought tolerant chickpea would provide considerable benefit for small farmers in India in terms of food security and livelihoods. Success in this project could be of value for developing similar traits in other crops

Challenges and a forward look

Over-expressing DREB proteins has resulted in transgenic plants that are more tolerant to drought, salt, heat, and freezing stresses[25]. However it is uncertain which if any single gene can confer appreciable drought stress on chickpea and more generally whether or not any single gene approach can provide a level of drought tolerance that famers will value in chickpea. Currently the benefits of over-expressing DREB1A during drought stress in chickpea have not yet provided a robust approach and a certain benefit.

The researchers seek to characterise the differentially expressed genes in roots/shoots of *DREB1A* transgenic lines under drought stress in the contained greenhouse. Such an approach may provide insights to the difference glasshouse and field trials to-date but does not results directly in a product for field use.

It is recommended that progress is considered carefully at the end of Phase 3 to assess whether or not a product can be advanced rapidly based on any currently available DREB1A lines. ICRISAT estimates that a further two years or more is needed before the potential increase yield can be estimated. The key issue relates to uncertainty that a robust benefit is available of increased drought tolerance with sufficient value to farmers to be worth development as a product. If not, then more lines are required and one or more additional gene (s) needs to be over-expressed. In both cases a product is in unlikely to be delivered soon as expected at the onset of Phase 3.

[25] Lata and Prasad, 2011, *Journal of Experimental Botany*, 62, 4731–4748

A suggested product development time-line provided by ICRISAT of 12 years to release the final product assumes no regulatory delays and is conjecture given the lack of successful field evaluation to date. This provides an example of the need for JAC to determine if any project should be continued if only moderate progress on the overall value chain is achieved. It is certainly premature to begin an introgression phase before a parental line with substantial improved drought tolerance is achieved or to initiate a biosafety package for a line not yet available.

7.1.4. Development of sucking pest resistant chickpea cultivar using biologically safe *Allium sativum* leaf agglutinin (ASAL)

Partner and institution

Prof. S. Das, *Plant Molecular and Cellular Genetics, Bose Institute, Kolkata, India*

Aims and objectives

Aim: To demonstrate that ASAL provides resistance to the aphid *Aphis craccivora* when expressed transgenically in chickpea.

Objectives: 1) analyses of T_1 plants generated in project Phase 3; 2) assess the biosafety of ASAL protein and 3) advance progenies of selected lines.

Context

Damage by insects that suck phloem sap is important in at least 50% of the chickpea areas of India particularly in the northern regions of the crop area. This work is deploying a lectin of garlic (*Allium sativum*) from leaves of the plant (ASAL). It is a mannose binding lectin with an LD_{50} of 3 to 20 µg per ml for different aphids on artificial diets. The level of expression in transgenic chickpeas is up to 4ng/ µg total protein. In a bioassay 80% of the aphids survived on wild type plants and only 20% on transgenic lines. It is uncertain how ASAL functions. It possibly binds to the same receptor in the aphid mid-gut as do virus particles. Alternatively the lectin may bind to a chaperone protein.

Chickpea was transformed and ASAL expressed under control of the CaMV35S constitutive promoter in previous ISCB phases. That work established these plants provided resistance to the aphid, *Aphis craccivora*.

Highlights

This work has progressed at the rate expected and its researchers are well organised and competent. Evidence for this is that they have published several papers in the last five years of the project in international journals. They have developed products of value for sucking insect control that can be developed both by private companies and public breeding effort. They also have recent, unexpected results suggest that transgenic expression of ASAL enhances nodulation by legumes.

Progress

They have multiplied already existing transgenic chickpea lines developed with chimeric ASAL: gene constructs under CaMV35S promoter and generated lines expressing this lectin under control of a phloem tissue specific rolC promoter. Both promoters provided sufficient expression of ASAL to cause 80-90% mortality for lines feeding on the T3 generation of the transgenic lines. Food safety analysis of ASAL established a *prima facie* case for food safety. ASAL has no significant matches to allergenic proteins in standard data bases and is rapidly digested by simulated gastric fluid containing pepsin which digests polypeptide proteins in ingested food within the mammalian stomach. Sera from humans known to be hypersensitive to food ingredients showed no response to the lectin. Mice showed no clinical changes when dosed with ASAL which seemed not to bind to tissues sections. ASAL is stable below 55ºC and so would be present in food that included uncooked chickpeas.

Outputs and products

This project has demonstrated the potential of ASAL to control *A. craccivora* on chickpea and may have potential to control other sucking insects. The technology has been licensed to one or more Indian seed companies but can also be advanced by public research from crops such as chickpea that are unlikely to be prioritised by private companies. ASAL may also have potential to enhance nodulation formation in chickpea and possibly other legumes.

Impact

This work has the potential to have a considerable impact on food security particularly if ASAL controls other sucking insects in addition to *A. craccivora* and in particular if it controls virus vector pests such as whitefly.

The transgenic chickpea is likely to reach growers through public breeding effort which is a main aim for ISCB. This project demonstrates a further route to generating impact for ISCB products. The ASAL technology has been transferred to Mahyco and Bioseed. Bioseed are concentrating on hybrid crops such as rice and vegetable (tomato, lady's finger, hot pepper), and cotton for which monetary value can be recovered for added traits. Bioseed can address the needs of the full range of growers from large scale to smallholders. They seek to target 20% of the regions that cover 80% of the production as already for Bt cotton. They use a combination of field trials and on farm demonstrations within an extensive network to validate the value of the added trait and make the case for uptake of improved seed by small farmers. The incremental benefits would be similar for small and large farms and not less than 4x the incremental cost of the new seed which also provide other characteristics of agronomic value. The benefit of interest to ISCB is that livelihood income of small farmers can be enhanced if the quality and quantity of the marketable produce is enhanced. The timeline to marketing is estimated as 8-9 years and milestones have been clearly defined to include biosafety and regulatory issues from proof of concept through field and large scale trials to commercialisation.

Involvement of industry also provides sound feedback on plant biotechnology issues through existing forums such as seed congresses in India which some 200 companies attend. Bioseed would welcome and willingly contribute to the implementation of a web information exchange site for instance to inform and improve the regulatory process at both institutional biosafety committee and national genetic engineering approval committee levels.

Challenges and a forward look

The results to-date provides a careful and progressive demonstration that ASAL does offer resistance to the cowpea aphid. There is value in generating more lines to obtain a highly effective line for either development for use or to act as a parent in an introgression breeding programme.

The value of ISCB funding such effort would be enhanced if ASAL controls major pests of key Indian crops in addition to chickpea. The investigators should be encouraged to make the case that ASAL has the potential to control other phloem feeding insects. Chickpea is a host for some other hemipteran insects including *Bemisia tabaci* (a whitefly). There is a need to minimise the direct damage they cause and also for vector control of the plant viruses they transmit. A key priority could be begomoviruses (Gemini viruses) such as Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV). They are both transmitted by *B. tabaci* and their control would add durability to the cassava project for RNAi-mediated virus control which is another ISCB project. Control of virus transmission by *B. tabaci* is a major international challenge for crop protection.

ASAL needs to be progressed carefully to be certain there are no biosafety issues. A *prima facie* case is made that it is a safe ingredient in food but more work is needed. The no observable adverse effect level (NOAEL) has yet to be determined and so the margin of safety for consumers of raw and cooked chickpeas is not known. Caution is needed to ensure accidental or deliberate misinformation does not arise that confuses ASAL from leaves with another lectin from garlic bulbs which is an allergen[26]. Possibly commercial companies developing regulatory packages for ASAL may make available information of value to the public research effort with chickpea. Alternatively, ISCB should consider funding such work in Phase 4 by a specialist food toxicological laboratory or company in Switzerland or achieving this via the contacts of the platform for translational research on transgenic crops set up by DBT at ICRISAT.
A recent, serendipitous finding is that ASAL apparently increases nodulation of transgenic chickpea significantly with an attendant increase in both nitrogen and phosphate content particularly when the lectin is under control of the RolC promoter. The effect is reported as large enough to be of agricultural significance and so it is justified to determine if this effect occurs in a contained field trial as well as under glasshouse conditions as a preliminary to more work on this trait.

7.1.5. Genetic diversity and RNAi-based control of cassava mosaic viruses in India

Partners and institutions
Prof. T. Hohn (Coordinator), *Institute of Botany University of Basel, Basel, Switzerland*
Prof. K. Veluthambi, *School of Biotechnology, Madurai Kamaraj University, Madurai, India*

Aims and objectives
Aim: Cassava with resistance to two distinct forms of mosaic viruses of this crop in India
Objectives: 1) Cloning of Sri Lankan cassava mosaic virus isolates, 2) demonstration of their infectiousness in *Nicotiana benthamiana* and 3) Construction of Rep hairpin RNA genes for RNA silencing

Context
This project was initiated in Phase 3 of ISCB. Cassava is a highly productive starch rich food that costs little to cultivate and has resilience to several biotic and abiotic stresses. It offers flexibility to Indian resource-poor farmers as either a subsistence or a cash crop. India has the highest productivity for this crop in the world (around 28 t/ha for about 250, 000 ha). It is an important human food security crop in Kerala and an important commercial crop for starch in Tamil Nadu and Andhra Pradesh where 90% of the cassava starch produced in India is harvested with a consequent high importance for the agricultural economy of these two states. The Madurai Kamaraj University has established linkages with the industry and a Farmer Cooperative Society (Sagoserve) to ensure the relevance of the project based on the co-operative's role in helping >50% cassava farmers to market their produce. This reduces the risk of market dominance by middlemen and traders who seek to influence prices for raw cassava tubers and products derived from them. The projected cassava tubers demand-supply gap in the industrial sector was estimated to be 1.5million tons and rising by Madurai Kamaraj University at the start of the project. This university estimates marketable products of cassava from the ISCB project are likely by 2017 and it has established informal linkages with the downstream members of the product chain to support commercialisation.

[26] Clement, and Venkatesh, 2010, *International Immunopharmacology,* 10, 1161-1169

A severe and widespread disease of cassava is caused by forms of whitefly transmitted bergomoviruses (Gemini viruses) of which two distinct forms occur in India. Both these cassava mosaic viruses (CMV) are being studied in this work (Indian, ICMV and Sri Lankan, SLCMV). Gemini viruses gain their name from having genomes consisting of two circular DNA components (A and B genomes). A characteristic of these viruses is the frequency of re-arrangements of their genomic DNA so generating differences between isolates that have consequences for transgenic approaches for viral resistant plants.

The approach to transgenic resistance favoured in this work depends on RNA interference (RNAi). The RNAi mechanism has many roles in plants but an important one is defending cells from plant viruses. The plant cell recognises unexpected RNA such as that which may originate from a virus. Two types of small RNA molecules are central to RNAi. They are microRNA and small interfering RNAs. They bind to other messenger RNA (mRNA) molecules and so can prevent the new protein being specified from being generated. This work seeks to manipulate that natural mechanism to generate transgenic cassava that are resistant to both ICMV and SLCMV.

A widely used approach to generate RNAi effects in plants involves constructs with DNA sequences that specify both the sense and antisense of a targeted gene (hairpin constructs) with a high levels of sequence similarity. The presence of the DNA template *in planta* results in generation of RNA molecules which in the current work are some of those expressed by CMV. This initiates the RNAi mechanism. There are precedents for success of an RNAi based resistance to a begomovirus transmitted by whitefly. In that work the viral gene targeted encodes the only protein essential for viral genome replication[27] preventing its expression. The current work targets different CMV sequences.

<u>Highlights</u>

The work has provided preliminary evidence that silencing by the approach they have adopted has the potential to control SLCMV. They have made strong progress in defining variation in SLCMV isolates infecting cassava in India and propose a rapid sequencing/bioinformatics approach to identify both known and newly detected viruses in Indian crop plants. Clearly there are prospects for significant, future impact for cassava farmers from the outputs of this project.

<u>Progress</u>

A collection was made of virus infected plants from seven different locations in South India to determine the extent of variation in the genomes of SLCMV. The A genome sequences were all very similar with 97-99 % nucleotide identity to each other and high similarity to reference sequences. Sequence comparisons allowed highly conserved sequences to be identified. Genomic regions with little variation and few point mutations among isolates in this region reduce the risk of new isolates overcoming the resistance. This informed the design of constructs that were targeted at conserved sequences of the viral genome. They have generated conventional hairpin constructs and regions of slight mismatch (bulging construct) along the sequence to tolerate any variation among different viral isolates. The symptoms of virus infection can suppressed for two isolates of SLCMV by bombarding the model plant *Nicotiana benthamiana* (a close relative of tobacco) with the bulged RNAi construct. The next major step is to transform stably both *N. benthamiana* and cassava to demonstrate the transgenic resistance to SLCMV is effective.

They seek to improve on the less than certain current diagnostic approaches to plant virus detection. The approach they favour is based on analysing populations of 21-24 nucleotides of short interfering RNAs generated by the plant's natural plant RNAi mechanism in response to viral infection. It is now possible to sequence large populations of such molecules (deep

[27] Aragão and Faria, 2009, *Nature Biotechnology,* 27, 1086-1088

sequencing) and use bioinformatics to reconstruct the whole genome from the data obtained so unequivocally identifying the presence of either a known or newly detected virus. This would have an initial benefit in identification of viruses present in Indian cassava crops.

Outputs and products

Innovation comes from deploying bulging hairpin constructs to avoid mismatches in sequence that may occur among isolates of CMV which would reduce the effectiveness of RNAi. There is value in extending in the deep sequencing approach which has already been applied to diagnoses of plant viruses[28] and advanced for its high potential[29].

Impact

The impact of this technology can be judged when the approach demonstrates significant levels of disease caused by CMV isolates in cassava are prevented. They are in discussion with an Indian seed company about wider utilisation of their technology and perhaps also the diagnostic sequencing for viral identity.

Challenges and a forward look

By August 2012, researchers expect to have defined which viral genes to target by RNAi and show efficacy in *N. benthamiana*. If successful, the key issue is the availability of an efficient cassava transformation from the effort of others in Switzerland. This is essential if progress towards ICMV and SLCMV resistant cassava is to be achieved. However this work has only been funded through one phase of the ISCB programme so with the above caveats overcome there is a clear value in exploring the potential of delivering a product in the next phase of ISCB.
The expectation is that RNAi plants will pass Indian regulatory scrutiny as no novel protein is generated in contrast to most other approaches based on expression of a novel protein. A precedent is available from Brazil. Its National Technical Commission on Biosafety has approved an RNAi mediated resistance to a bean golden mosaic virus (see context) which was developed by public research[30].

[28] Kreuse *et al*., 2009, *Virology*, 388, 1-7
[29] Wu *et al*., 2010, PNAS, 107, 1606-1611
[30] http://www.biofortified.org/2011/10/brazilian-virus-resistant-beans/

7.2. The Biofertilizer Network

Coordinators: Dr. A. Adholeya (India), *The Energy and Resource Institute, New Delhi, India* and Dr. P. Mäder (Switzerland)*, Research Institute of Organic Agriculture (FiBL), Soil Sciences Division, Frick, Switzerland*

7.2.1. Network as a whole

Overview of the network

There are wide variations in the extent of fertiliser consumption patterns across India and among its states. The profitability of fertiliser use often relates to the portion of the yield that matches the cost of the fertiliser. Phosphate (P) and potassium (K) are more expensive than inexpensive sources of nitrogen (N, e.g. urea) and so provide a lower return ensuring the resource-poor farmers are particularly unlikely to afford optimal applications of such fertilisers. The Government of India promotes an integrated nutrient supply system which involves combined use of mineral fertilisers, organic manures and biofertilisers. This requires greater use of biofertilisers which are often criticised for providing poor and inconsistent crop responses[31]. Biofertiliser efficacy is likely to be influenced by soil factors and they may less readily provide the appropriate balance of NPK than mineral fertilisers.

This project seeks to provide a reliable basis for increased biofertiliser use in India. This network is appropriately centred on rice and wheat which receive 31.8% and 21% respectively of the total fertiliser consumption in India.

Transnational collaboration

The Indian partners met the evaluation team during its visit to India. In addition, there are three Swiss based projects. They are: 1) *Short and mid-term effects of biofertilisers on wheat yield and quality in different environments* (Dr. P. Mäder Research Institute of Organic Agriculture (FiBL), Soil Sciences Division, Frick, Switzerland 2) *managing arbuscular mycorrhizal (AM) fungi for sustainable agriculture: Molecular tools for strain-specific tracing and quantification of AMF applied as biofertilisers (*Prof. A. Wiemken, University of Basel, Institute of Botany, Basel, Switzerland) and 3) *Application and transfer to India of molecular tools for monitoring of bacterial PGPR bioinoculants, particularly Pseudomonas spp., in wheat crops in tropical soils* (Prof. T. Boller, Institute of Botany, University of Basel, Basel, Switzerland).

The evaluators only met with those members of the Biofertilizer network with projects based in India (IIT, TERI and GBPUAT) who have established a strong collaboration. There are biannual meetings of the principal investigators (most recent in Basel in September 2011) and other meetings among Indian partners with information exchanges and material transfers. IIT has also established a close collaboration with GBPUAT regarding the flocculation process for the bacterial component of the biofertiliser. There has also been an exchange of material with Basel to support development of molecular probes for the project within Switzerland.

Capacity building

The level of international exchanges between India and Switzerland occur does not seem to be as intense with annual reports for the last three years logging only few short term visits to Switzerland. This probably reflects the strategic/applied phase of this phase of the project but raises an issue of the importance of the Swiss research contribution in the future.

[31] ftp://ftp.fao.org/agl/agll/docs/fertuseindia.pdf

Research publications

The network publishes in national journals and book chapters with some outputs in specialised international standard journals in their discipline such as *Soil Biology* and *Biochemistry and Applied Soil Ecology*. This mix is perhaps as expected given they carry out strategic work well focused on national needs but the network does generate some outputs of international interest.

Future of the network

The network has made the most progress of the three evaluated towards products that may reach growers within the time span of Phase 4. An appropriate role in that future phase would be to enhance the prospect of ensuring reliable and economic products are available that are valued and used by Indian farmers. A second challenge is to extend the range of products to a wider range of agroecologies and soils. The principal challenges within these contexts are considered below for each project (***S7.2.2***, ***S7.2.3*** and ***S7.2.4***). New activities appropriate for this network are suggested within ***S7.4.2iii*** and ***S7.4.4*** but in the latter case that project may require additional Indian or Swiss expertise.

7.2.2. Mass scale multiplication of Plant Growth Promoting Bacteria and development of consortium formulations with suitable carriers

Partner and institution

Prof. V. Bisaria, *Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi (IIT), New Delhi, India*

Aims and objectives

Aim: to provide a cost effective basis for production of plant growth promoting bacteria for use by Indian farmers so reducing their application rates of costly inorganic fertilisers

Objectives: 1) mass scale multiplication of plant growth promoting bacterial (PGPR) strains R62 and R81 and 2) development of carrier based formulations of PGPR.

Context

The work at IIT centres on fluorescent pseudomonad bacteria that promote crop growth in various possible ways including directly by biofertilisation and phytostimulation and indirectly by producing compounds that suppress some soil borne plant pathogens and by inducing the plant's systemic resistance to pathogens. The need is to provide large quantities of these plant growth promoting bacteria. They can be produced in mass cultures using a batch system, a batch-fed system or continuous culture. The current project favours the fed-batch system. It supports the production of more bacterial colony forming units (cfu) in controlled conditions than a batch process and underpins repeatable growing conditions for the bacteria. This supports quality control which in the current context is the production of more viable bacteria with a predictable shelf life. Adoption of a fed-batch production system requires initial study of the conditions required and operator skill in developing the process for routine use.

Highlights

Sufficient product has been developed from a fed-batch bioreactor of 14L over 35h to coat sufficient seeds to sow about 476 ha. The product can be stored for short periods and is now ready for transfer to commercial and other partners for mass production and distribution to growers.

Progress

A basic medium that supports bacterial multiplication has been enhanced to achieve higher cell mass, production of a bacterial iron chelator (siderophore) and a phenol (2,4-diacetylphloroglucinol, DAPG) produced by the bacterial strains under study that contribute to their biocontrol and anti-plant pathogen attributes. Their addition at the start of the fermentation process reduces risk of contamination with other microorganisms. Initial success was based on a 14 liter batch system fed with glycerol. This established the upper asymptote for cell dry weight and siderophore was reached after about 35 hours incubation. Improvements were made by using a system in which the culture is fed either exponentially as the cell density increased or with a feedback to maintain constant growing conditions (fed-batch systems) The bioreactor was obtained by a company with its headquarters in Switzerland (Infors HT) but software from another (Labview 2009, a USA-based international company) best suited the current application. The exponential feeding system proved unsuitable but a feedback loop that maintained a constant pH as the cell number increases provided excellent results. The results can be summarised as the colony forming units (cfu) of 4.7×10^9 cfu after 13.8h obtained when a batch system would be terminated is raised to 2.8×10^{11} cfu with a fed-batch approach at constant pH over 35 hours. This is a harvest of about a 60x increase over the batch process.

Outputs and products

A fed-batch bioreactor based process has been developed that enhances the production of plant growth promoting bacteria in 35h. The fed-batch system provides a level of control over the bioreactor process that supports a repeatable production which is essential for successful market uptake. The product after formulation is stable when stored in a poly-house for short periods allowing product to be accumulated for a limited time before direct or indirect distribution to growers. IIT favour inclusion of public bodies in product uptake to monitoring uptake and help ensure small farmers benefit. Otherwise they perceive the risk that a market orientated approach may favour only better resourced growers.

Impact

The product has been licensed to Indian companies but the rate of growth of uptake will not be apparent for several years. If the products provide a fertiliser that reduces the cost of fertiliser used by poor farmers there could be widespread benefits to the rural economy.

Challenges and a forward look

There is a need to support transfer of the technology to industry to ensure production maintains a high and reliable standard of product for seed coating. There would value in a rapid assay to confirm stored formulated product or that applied to seed has retained full activity before use so underpinning a critical need for maintenance of product quality. The quantity of seed that can be coated for each run of the fed-batch 14 litre bioreactor has been estimated. Thought needs be given to the scale-up process. Prolonged storage without quality loss is important to meet flexible demand because it is difficult to anticipate grower demand before a planting season starts. There are limits to the quantity of product that can be generated and disseminated in response to demand which often occurs just before planting.

There is a need to determine the economics of production and the incremental additional cost to marketed seed relative to the cost of proportion of commercial fertiliser that is replaced. A cost: benefit analysis should be provided for farmers using just PGPR bacteria and also when mixed with the mycorrhizal fungi being developed by TERI. Such analysis would determine the potential for widespread use rather than niche markets (e.g. organic production) which is important to maximise the impact on resource poor farmers.

There is value in selecting initial areas for commercial use where there is high level of farmer innovation to ensure the product is appropriately used and reliable feedback is provided. This provides a role for state extension workers.
Any new organisms of value are needed for drier soils and other agroecologies than the current bacteria. There is a role for IIT in optimising the production of these organisms for seed coating.

7.2.3. Development of technology for *in vitro* mass production of selected AMFs and field validation demonstration of finished product on wheat in India

Partner and institution

Dr. A. Adholeya, *The Energy and Resource Institute (TERI), New Delhi, India*

Aims and objectives

Aim: to deliver commercial combined bioinoculant product for sustainable and low-input agriculture based on arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) using strains identified to be of value in earlier phases of ISCB.
Objectives: 1) develop processes for mass production of the bioinoculants, 2) explore formulations and storability of the product, 3) develop application technologies for the product, 4) validate new formulations and application rate of the combined biofertiliser in small scale and field trials and 5) develop appropriate technology for safe and efficient PGPR product delivery to farmers.

Context

The project is developing a biofertiliser product for commercialisation that is a mix of an arbuscular mycorrhizal fungus (AMF) and plant growth promoting rhizobacteria (PGPR) using isolates selected to be of value in earlier phases of ISCB.

Highlights

The ability of the biofertiliser to reduce conventional fertiliser applications from recommended levels by 25% was consistently achieved in a wide range of locations often on farmer fields with a 50% reduction sometimes obtained without reducing yields. Consistency is important as the cost saving from reduced fertiliser use would be readily exceeded if even a small yield penalty was involved.

Progress

The products have been trialled most recently in both bean (*Vigna*) and wheat at sites in two states. The product provided 4-8% and 9-12% increase in wheat grain yield relative to control plots not receiving the biofertiliser in Punjab and Hisar respectively. Ten field demonstration trials were also conducted in farmers' fields in Haryana, Punjab, Rajasthan and Madhya Pradesh. This established that the biofertiliser provided the largest increase yields when used with a recommended fertiliser application rate but it could also compensate for either a 25% or a 50% reduction in added fertiliser.
The data to-date does not readily define the added benefits of combining PGPR and AMF preparations or the advantage of the synthetic AMF of this project and either natural or other commercial preparations. It is therefore uncertain how distinctive are the ISCB products from those developed by others.
Extending the shelf-life of the product has been a priority for research. Lyophilisation of the bacterial cultures was carried out and three different cryoprotectants used to determine if they extended shelf life. No treatment prevented a decline in bacterial colony forming units (cfu) to less than 10% of the original levels after 60 days.

Outputs and products

A consistent but not large benefit of the selected strains of AMF and PGPR used in the biofertiliser product has been well established by TERI, its collaborators and by the G.B. Pant University report. The benefits apply to a wide range of soils for wheat and bean crops (plus rice, see G.B. Pant University report). The main benefit is to reduce the recommended fertiliser rate by 25% with probable cost saving to farmers. There is limited evidence that the ISCB products provide a greater benefit than other sources of AMF. This weakens the case for commercial advantage beyond know-how achieved by this network.

Impact

This product has been licensed through DBT in December 2011 to several Indian companies on non-exclusive licence basis. This provides a multiple opportunity for markets to be developed.

Challenges and a forward look

The partners deserve credit for advancing the product to its current status. There is a need to improve storage of the products. The claim is made that a useful inoculum persists for 6 months of storage. However less than 1% of initial cfu are present at that time. Therefore, there is a need to apply 100x more cfu than needed at planting to provide an adequate shelf life. This adds considerably to the bioreactor production costs of PGPR at levels needed for a marketed product.

The current product would benefit from molecular diagnostics to demonstrate any benefits to growers can be correlated with the presence of the introduced organisms. Ideally this should be a quantitative or semi-quantitative measure rather than just detection of presence. Such a resource would benefit commercial partners. There is also a need to develop the range of products to suit a wide range of soils in targeted Indian states.

The economic case for biofertilisers needs to be made. It is uncertain that the benefit to growers of reducing fertiliser demand is substantial enough to ensure market uptake. An alternative marketing strategy would be use in supplementing recommend fertiliser applications rates to raise yields. This needs to be compared with the yield benefit when additional fertiliser to the same additional cost is applied. It is also uncertain if the benefits are for all growers including those with limited resources. Possibly organic production could use the biofertiliser as a supplement to organic fertilisers but any benefits to this market have yet to be demonstrated.

7.2.4. Effects of bio-fertilisers on yield and quality of wheat based cropping system under different experimental trials and developing the formulation of PGPR

Partner and institution

Dr. A. Sharma, *Department of Biological Sciences, GB Pant University of Agriculture and Technology (GBPUAT), Pantnagar, India*

Aims and objectives

Aim: (inferred) to develop a knowledge basis that supports uptake of ISCB biofertiliser products in wheat-rice cropping systems.

Objectives: 1) to assess agronomic performance, mycorrhizal colonisation, soil and crop quality of the natural AMF inocula and the PGPR in a wheat-rice rotation; 2) as above for using a synthetic mycorrhizal consortium; 3) to test the new formulations and develop application protocols; 4) to define the minimum level of mineral fertilisers for the optimum yield with biofertilisers; 5) to monitor persistence of PGPRs under wheat/rice; 6) to validate the results in field trials with independent partners; 7) to establish demonstration field trials and 8) to assess the impact on climate based on the input data (theoretical analysis).

Context

One aim of the Indian government is to increase biofertilisers use but this requires the extent of a reduction in mineral fertilisers to be determined that can be achieved without a yield loss. In addition the past criticism of poor and inconsistent crop responses[32] to biofertilisers needs to be overcome. This work addresses these needs and seeks to ensure reliable application of biofertilisers by a wide range of farmers in many locations.

Highlights

The biofertilisers were proven to enhance yields of rice and wheat. Their use offsets a need for a minor part of the recommended fertiliser dose. This may be of economic value to resource poor-farmers but the situation is made more complex when poor growers are unable to afford the recommended fertiliser application rates. Progress has been made on using flocculation to enhance shelf-life of the PGPR but that problem has not yet been fully overcome.

Progress

Rice yields were enhanced by 15-22% relative to unfertilised plots when PGPR was applied with a natural AMF inoculation or a mix generated by TERI. However this increase was much less than achieved by applying the recommended fertiliser level. A similar effect was obtained in two wheat trials with about a 15% and 26% gain using the two biofertiliser preparations relative to the unfertilised crop but a greater increase of 54% and 63% when the recommended fertiliser level were used instead. The benefit of the bioinoculants of 8-16% was recorded for 9 wheat cultivars. Therefore, a key issue is can the biofertiliser application offset some of the inorganic fertiliser use? The loss of production is c 8% and 16% by using biofertiliser at only 75% and 50% respectively of the recommended application rates. Clearly such losses do not support an increase in the Indian wheat harvest but they may benefit a resource poor farmer if the cost of the biofertiliser-dressed seed is less than purchasing uncoated seed and sufficient fertiliser for the recommended application rate.

A second key factor is the shelf life of the biofertiliser preparation. This seems to be a major issue in that cfu declined to 10% of the initial level in <60 to 105 days with some evidence that low temperatures retarded a presumed exponential decline in viability. Evidence was provided that a flocculated culture at 4°C or 25°C retained viability for 1 month but this trial has not been extended for several months.

A key commercial need is the identification of the microbial isolates provided in the seed dressing relative to other similar organisms already present in the soil. Progress has been made with identification of PGPR isolate R62 (marker developed by University of Neuchatel, further developed to a quantitative tool by University of Basel and applied by GBPUAT) although surprisingly the sequence from the specific primers used was only 97-98% of that expected. The corresponding primers for the second isolate R81 are in progress (collaboration of University of Basel and GBPUAT). The recently completed sequencing of the two bacteria may open large future potential. There seems as yet no method to quantify densities after their addition to correlate abundance with the benefits obtained.

Outputs and products

Biofertilisers have been field trialled and shown that they can have a beneficial effect on crop yields.

[32] ftp://ftp.fao.org/agl/agll/docs/fertuseindia.pdf

Impact

The reduced application rates of mineral fertiliser could be appreciable if the biofertilisers products are taken up widely by wheat and rice farmers. This would support the Indian government's aim to reduce national fertiliser demand as these crops receive about 50% of all fertilisers applied in the country.

Challenges and a forward look

GBPUAT has identified the need for vertical connections with industry and final users. This may help them extend the current emphasis of their technology on hydrated soils to drier soils. Such a development also has relevance to assuring climate change resilience of agriculture in such soils.

A key question for shelf life is what loss of cfu is permissible for the preparation to be essentially the same quality as on its first production? This needs to be defined. For instance monthly decline rates of 32%, 10% or 1% are needed for the product to be 10%, 50% or 90% of initial cfu at production if applied 6 months later. More work is required to achieve such levels of persistence. The alternatives are a production system that can deliver many more cfu per seed at coating than required with immediate planting or a reliable product that can be distributed and used within one month of production.

Replacement of all fertiliser use by the various biofertilisers involves a yield penalty. The addition of biofertilisers to recommended fertiliser levels provided an additional benefit. Replacement of 25% of the recommend fertiliser use by biofertilisers prevents a yield loss. However the economics of the procedures are not provided. A first attempt for improvement by the partners follows: as an approximation the cost of fertiliser is c Rs8,000/tonne[33] and usage varies but is often about 0.15 tonnes/ha[34] so the saved fertiliser is approximately 8000 x 0.15 x 0.25 = Rs 300/ha minus the additional cost of biofertiliser coated seed This suggests the saving to the farmer is small. In contrast the average yield of wheat and rice in India has a value of Rs 37,000/ha and Rs 62,000/ha[35] suggesting improving yields by supplementing full fertiliser use with biofertilisers which stimulates outputs by c 8% is the economically superior approach. It returns about Rs 3,000/ha and Rs 5,000/ha to the grower for wheat and rice respectively. If this analysis is correct critical questions are a) what would be the benefit of additional mineral fertiliser to the same cost of biofertiliser and b) does the approach have a higher potential for raising yield when supplementing recommended mineral fertiliser levels rather than as a strategy to reduce fertiliser demand in India?

[33] http://www.fao.org/docrep/009/a0257e/A0257E06.htm
[34] ftp://ftp.fao.org/agl/agll/docs/fertuseindia.pdf
[35] http://faostat.fao.org/site/567/default.aspx#ancor (2009 data)

7.3. The Wheat Network

Coordinator: Prof. B. Keller, *University of Zurich, Institute of Plant Biology, Zurich, Switzerland*

7.3.1. Network as a whole

Overview of the network

This network underpins the development and utilisation of technology for wheat breeding to develop new varieties with: i) improved endogenous resistance to fungal diseases and heat tolerance through better understanding of wheat genomics and ii) design and implementation of marker assisted breeding approaches to address consequences of climate change.

Transnational collaboration

The quality of the science and the importance of the research field provide opportunity for internationally significant collaborations which are in place. This raises the profile, competence, confidence and quality of Indian partners and their wheat research to an internationally significant standard. The ongoing activities are on relevant basic research at the genomic level will substantially contribute to the focused translational research.

The Indian partners met the evaluation team during its visit in India. In addition, there is one Swiss partner: *Genomics for improvement of fungal disease resistance in wheat*, Prof. B. Keller, Institute of Plant Biology, University of Zurich.

Capacity building

There is clear evidence of the value of international collaboration with a record of short and long-term visits by Indian scientists to Switzerland listed in annual reports. In addition, one of fourteen Beachel Borlaug international Fellowships in 2010 was awarded to a young Indian scientist (Ms. Amandeep Sandhu) to work within the network in Switzerland[36]. The expertise created and linkages developed with Swiss partner will help build a state-of-the-art plant molecular breeding laboratory at Punjab Agricultural University.

Research publications

There have been several publications in internationally significant journals including one in the pre-eminent scientific journal *Science* in 2009[37].

Future of the network

This network should continue. It has provided internationally significant outputs and is active in an area of critical concern for India, its wheat harvest. Its research attracts high international interest and it is well placed to enhance ISCB's interaction with CGIAR institutes (e.g. CYMMIT) if this is favoured by the sponsors. The network's principal challenges are considered below for each project (***S7.3.2**, **S7.3.3*** and ***S7.3.4***). It needs to ensure its progress along the value chain is continually recorded and any mid-project opportunities for utility are exploited. The work programme of Phase 4 is defined by the status of progress at the end of Phase 3 as the biotic and abiotic stresses it addresses require continual effort to counter their detrimental effect on wheat yields. It is well placed to be involved in new project areas such as ***S7.4.2*** and in particular ***S7.4.2ii***.

[36] http://www.monsanto.com/ourcommitments/Pages/mbbisp-past-awardees.aspx#2010awardees

[37] Krattinger *et al*., 2009, *Science,* 323, 1360-1363

7.3.2. Genomics for improvement of fungal disease resistance and terminal heat tolerance in wheat

Partner and institute
Dr. K. Singh, *Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India*

Aim and objectives

Aim: Development of wheat genotypes tolerant to both biotic (powdery mildew, stripe and leaf rust fungi) and abiotic (heat) stresses.

Objectives: 1) identify closely linked markers for powdery mildew resistance gene transferred from *T. monococcum/T. boeoeticum;* 2) identify closely linked markers suitable for marker assisted selection (MAS) for stripe rust resistance gene transferred from *Triticum monococcum/T. boeoeticum*; 3) identify closely linked markers suitable for MAS for leaf rust resistance gene transferred from *T. monococcum/T. boeoeticum*; 4) agronomic evaluation of introgression lines generated from the previous phases of the project; 5) transfer stripe and leaf rust resistance genes to a set of elite lines by integrating MAS with conventional breeding and 6) map components of heat tolerance in the wheat cultivar Halna (K-7903) using the recombinant inbred line (RIL) population developed at The Directorate of Wheat Research (DWR) of India.

Context

Wheat is subject to both major abiotic (higher temperature regimes) and biotic (rusts and stripe disease) stresses in the North-West plains of India especially in the states of Punjab, Haryana, and parts of Rajasthan and Utterpradesh. These stresses cause annual, global yield loss of wheat that are estimated to be 25% with increases likely due to future impacts of climate change. For instance, in Punjab, yield fluctuations of 4.69 T/ha in year 2000 to 4.2 T/ha in 2002, 2004 and 2006 may be due to high variability in ambient temperatures. It is projected that Indian wheat yields need to increase to 100 million tonnes to meet national demand. Thus it is critical to focus the wheat improvement programme on stabilising the crop's yields through in-built tolerance to important stresses. These stresses impact highly on resource-poor farmers who have a substantial share in Indian wheat production.

There is limited information on genes that impart resistance to those pathogenic fungal races that cause rust and stripe diseases. Moreover, the transfer of genes from wild relatives to superior genetic background of wheat has met with mixed results due to a linkage drag that limits the commercial utility of the generated wheat lines. In addition virulence of pathogens is enhanced by climate change with new strains of fungi causing rust and rust disease emerging that increase wheat susceptibility to higher temperature regimes.

The transfer of rust and stripe resistance gene with minimum linkage drag forms the basis for development of RILs using the diploid A genome species, *Triticum monococcum* and *T. boeoeticum* plus the closely related A genome of hexaploid wheat. The high yielding lines developed from these materials will be useful in Indian wheat breeding programmes.

Highlights

The team has developed substantial insights into biotic stress especially those related to rust and stripe diseases of wheat. The visits of project personnel to the Swiss partner lab. has helped to build expertise both in basic and applied aspects of molecular breeding and this has contributed immensely to the project's progress. The expertise created and linkages developed with the Swiss partner are supporting the development of a state-of-the-art plant molecular breeding laboratory at Punjab Agricultural University. This helps to attract future funding for the wheat development programme and has built the competency of team to participate in the international wheat genome sequencing project. The concept and approach to identify and transfer resistant genes from *Triticum monococcum* and *T. boeoeticum* is quite innovative and may be rewarding. The team has published outputs in international journals of repute.

Progress

A high density linkage map that includes molecular markers based on simple sequence repeats (SSR), restriction fragment length polymorphisms (RFLP) and diversity array technology (DArT) has been developed for the wheat A genome using a recombinant inbred line (RIL) population derived from the cross *T. monococcum/T. boeoeticum*. This map can be used to generate a physical map for the chromosomes of the A genome of bread wheat.

With an emphasis on biotic stress tolerance, the team has mapped and transferred genes for resistance to rust and stripe diseases from diploid A genome species *T. monococcum* and *T. boeoeticum* into hexaploid wheat. The stripe-rust resistance gene (APR genes) was validated as effective against virulent races of rust and stripe pathogens and so supersedes the widely used Yr27 gene. The leaf rust resistance gene is effective against the prevalent leaf rust fungal races in India.

Two stripe rust and two leaf rust resistant genes have been transferred into hexaploid wheat using *T. durum* as bridging species. The genes have been mapped to chromosome region but the closely linked markers have not yet been developed.

A novel powdery mildew resistance gene has been identified from *T. boeoeticum.*

The major limitation that remains is the development of diverse maps with more SSR and single nucleotide polymorphism (SNP) markers. A more focused effort in future may provide a more useful outcome than achieved to-date.

Outputs and products

Two leaf rust and two stripe resistance genes have been transferred into the hexaploid wheat background. DNA markers linked to these genes have been identified. Stripe rust, leaf rust and powdery mildew resistant stocks with genes transferred from *T. monococcum/T. boeoeticum* have been made available to wheat breeders. Several genes of evolutionary importance for wheat resistance have also been mapped.

Impact

The outstanding work conducted by a PhD student has been recognised and awarded Monsanto Beachel Borlaug Fellowship. The team has published project data in several international journals of repute.

Stripe rust, leaf rust and powdery mildew resistant donor lines with resistant genes from *T. monococcum* and *T. boeoeticum* will improve substantially the likelihood of developing resistant cultivars by conventional and marker assisted selection.

Challenges and a forward look

Promising progress has been made towards development of wheat resistance to both leaf rust and stripe rust fungi. However, a major hurdle remains of identifying markers closely linked to resistance to stripe and leaf rust for gene pyramiding through molecular breeding.

Rusts will always remain a major challenge for management to sustain and improve wheat productivity. This requires more effort to identify, map and transfer new stripe rust and leaf rust resistance genes to wheat cultivars from different wild species.

Aegilops speltoides (the probable B genome donor of wheat) is resistant to all foliar diseases including rusts and blights. The group has made an initial observation that this species is also tolerant to heat, drought and submergence. However, the critical physiological responses to these stresses need to be defined. The progress made in a DBT funded parallel and a complementary project has generated backcross progeny with *Ae. speltoides* accession PAU

3809. This work is encouraging the identification of large number markers and effort on gene mining to advance wheat improvement and build resistance to abiotic and biotic stress.

7.3.3. Molecular characterisation and genetic enhancement of fungal disease resistance and terminal heat tolerance in wheat

<u>Partner and institute</u>
Dr. R. Tiwari, *Directorate of Wheat Research, Karnal, India*

<u>Aim and objectives</u>
Aim: Genetic enhancement of wheat cultivars for fungal disease resistance and thermal heat stress.
Objectives: 1) molecular marker enrichment of genic region for identification of highly diagnostic marker of adult plant leaf rust resistance, *Lr34;* 2) powdery mildew resistance characterisation in wheat through molecular markers for utilisation in breeding programme and 3) firming up of mapping population for terminal heat tolerance and molecular mapping of associated traits.

<u>Context</u>
Increased fluctuations in ambient temperatures may have contributed substantially to a past, recent decline in productivity of wheat in India. Stripe and rusts are biotic stresses that have been identified as a potential threat to wheat productivity in combination with climate change. Wheat is prone to rusts and powdery mildew which are widespread in all wheat growing area of India. The leaf rust pathogen, *Puccinia triticina* is highly variable with changes in its virulence rendering new cultivars grown in India susceptible to it. A recent upsurge in stripe and rust in Northern India during the wheat cropping season has been linked to fluctuating temperatures. The focus of this research project is to maintain the productivity of Indian wheat crops by development of genotypes that are tolerant to heat induced fungal (stripe and rust) induced biotic stress.
Genotypes are also required that possess resistance to a second biotic stress, powdery mildew. In addition terminal heat stress is emerging as another direct abiotic stress that affects wheat productivity in most of Northern Peninsular India. Improving thermal heat stress tolerance of wheat is an important national priority.

<u>Highlights</u>
A nucleus molecular breeding programme was initiated at The Directorate of Wheat Research (DWR) Karnal in its Biotechnology division with collaboration and inputs from the Swiss partner, Prof. Keller.
The strong wheat breeding programme at DWR made an exceptional contribution to an important output from the second phase of the ISCB programme. They identified Lr34 gene in several donor lines that provides some resistance to all races of *P. triticina*. The gene was utilised in a breeding programme.
Some of the developed lines of stripe/rust resistance proved quite promising in preliminary yield trials.
The major highlight of the programme has been an approach to characterise the population of plants for heat stress tolerance. Innovative methods have been designed to phenotype in a small growing area with more precision than before.

<u>Progress</u>
Research has mainly addressed i) stripe rust and ii) thermal heat stress tolerance.

Emphasis has been on characterising a RIL developed in the background of Cappelle Desprez and its substitutive lines with cultivar PBW 343 against prominent stripe rust pathogen 75584. Other genes were involved in addition to Yr1b on chromosome 2D. A three population approach was adapted to identify markers/genes other than Yr16. This population was also evaluated for powdery mildew resistance.

The programme on thermal heat stress tolerance is still at an exploratory stage. The RIL population derived with Halna and RS4014 parents is the major output but the population has yet to be characterised at the molecular level. However, fairly innovative and focused phenotyping approaches are being implemented by the physiology group which may provide useful leads. Emphasis is on starch biosynthesis genes during grain filling and exposure to high temperatures but progress is at a preliminary stage.

The number of markers utilised to-date is far too few. One proposal is to outsource for DArT based genotyping. Development of a diverse map is a priority for the research.

Collaboration of South Campus, New Delhi with DWR should be added to the network to examine the value of utilising the temperature-specific transcriptome. The need to validate these stress genes is a limitation to progress.

Outputs and products

There is a clear continuity of progress from Phase 2. The leads have been utilised for genetic enhancement of rust resistance in Phase 3 of the ISCB programme.

The RIL population generated is of value. Some genotypes of promise that regulate the rust resistance and tolerance have been identified that need validation. Progress has been made with co-dominant markers Lr34/Yr18 that have the potential both to identify resistant breeding material and assist the understanding of the genetic mechanism of heat tolerance in the Halna cultivar.

The approach to phenotype for terminal heat stress under field condition is quite precise and innovative.

Impact

Impact on the wheat improvement programme is provided by identification of some good recombinants for heat tolerance plus elite lines with stripe rust resistance that have potential for varietal trials and for identifying good donor lines.

Challenges and a forward look

A genetic enhancement programme should be initiated to identify quantitative trait loci (QTL) donor lines for stripe, rust and powdery mildew. The challenge is to saturate the map to support QTL discovery. A more focused programme in addition to attempts to use DArT markers would enhance progress.

Horizontal resistance must be improved by introgressing (pyramiding) two or three relevant genes for geographical locations where stripe and powdery mildew isolates are variable and in epidemic proportions. Transcript profiling of *Puccinia* spp. to characterise its genes that are related to pathogenesis is also an area that would benefit from attention.

A particular challenge is combining tolerance to heat stress to that for stripe/rust resistance. In this context, a highly focused emphasis is crucial that is centred on precise phenotyping of the population (Halna K7903 and Raj 4014). The innovative phenotyping approaches that have been initiated need further refinement and their ability to identify the relevant traits established. Association with other groups is recommended to accelerate the phenotyping of RIL populations.

7.3.4. Functional genomics of thermal tolerance in wheat

Partner and institute

Prof. P. Khurana, *Delhi University, South Campus, New Delhi, India*

Aim and objectives

Aim: Screening to identify heat tolerant genotypes and characterise functional genomics of heat tolerance in wheat

Objectives: 1) to undertake comparative physiological and molecular analysis of *Triticum aestivum* and *Aegilops* sp. with respect to thermal stress tolerance and 2) functional and comparative genomics of genes associated with thermal tolerance in wheat and *Aegilops* sp.

Context

Wheat is a temperate, annual crop not optimally suited to cropping where temperatures during grain filling may reach 40^{o}C. High temperature stress affects almost every stage of wheat development from germination to grain filling. Furthermore, a likely consequence of climate change is variable ambient temperatures that affect the wheat responses and its production. A critical understanding of transcriptomes helps understand the responses of wheat to different temperature regimes. Moreover those wild relatives of wheat that have an enhanced ability to withstand heat stress have value for a comparative molecular analysis. Therefore, mapping and characterising expressed sequence tags (ESTs) offers a manageable approach to the functioning of the complex wheat transcriptome. A significant increase in our understanding of molecular basis of plant stress tolerance will allow breeders to address the problem of built-in resistance against high temperature stress.

Highlights

The institution has a good infrastructure for molecular research and the team has expertise in the genomics and transformation of wheat with its knowledge of functional genomics enhanced from association with rice and *Solanum* genome projects. Several differentially up-regulated genes have been classified. Ongoing project activities centre on relevant basic research to improve understanding of the molecular mechanism of heat tolerance at a genomics level. Temperature tolerant cultivars are being identified by physiological screening. The suppression-subtraction approach is being adopted to identify differentially expressed genes and to clone temperature responsive genes from genotypes with differential stress responses. There is a strong sustained interaction with the Swiss partner and several articles have been published in international journals of repute.

Progress

Indian wheat genotypes have been screened using various developmental strategies. Both stress regulated gene transcriptome and suppression-subtraction hybridisation has been utilised to identify differentially expressed genes and ESTs generated from unfertilised flowers. The data generated has been submitted to an international data base (GENBANK). Genes of potential relevance have been identified based on their putative function and their relevance validated by cDNA microarray and RT-PCR analysis. Full length clones of a sub-set of genes of interest have been characterised using functional genomics. Comparative genomics is being deployed to identify their homologues in wild relatives (*Aegilops* sp.). However, a more relevant approach to functional validation would be to study these genes by differential expression in relevant cultivars/wild relatives.

Output and products

The work has characterised 4-5 genes associated with thermal stress tolerance in wheat. It has progressed molecular and functional characterisation of the selected/novel ESTs, which is useful for introgression in the existing marker-based selection. It has developed sequencing of a subtractive library for unfertilised flower following heat stress. A comparative physiological/biochemical analysis has been carried out of the growth adaptive features of bread wheat (*T. aestivum* heat tolerant variety Halna and Raj4014).

Impact

Once characterised, the promising candidate genes associated with heat tolerance in wheat are exploitable in wheat improvement programmes to develop heat tolerant varieties by deploying either marker assisted selection or technologies for genetic modification. The genes can also be used to screen the germplasm based on DNA-based sequence specific markers. The elucidation of the signalling pathway involved in heat stress responses underpins future better control over the consequences of changing climatic factors. The outputs have the potential for immense impact on the wheat improvement programme while also contributing to the knowledge base at genomics level.

Challenges and a forward look

State-of-the-art genomic technologies have considerable relevance to an enhanced understanding of the mechanisms of thermal tolerance in wheat and other crops. The screening methodologies used for selection of heat stress tolerant genotypes can be extended to *T. durum, T. speltoides, T. dicoccum* and *Aegilops* spp. and to promising parental lines for breeding. The ESTs generated would be analysed for use as gene/allele specific markers in breeding programmes. Promising genes can be studied in wild relatives and such comparative genomics has relevance to both product development and improved understanding of heat tolerance in wheat.

7.4. Possible Future Projects or Networks for ISCB

7.4.1. Forward planning

ISCB held a workshop of 23 participants including partners and external experts in New Delhi on 23 October 2010 to assist in the design and planning of a fourth phase of the programme. Several potential research projects that build on existing networks or initiate a further one were considered. Areas of biotechnology discussed in some detail were in the fields of: 1) the consequences of climate change for Indian agricultural production and 2) post-harvest crop loss or wastage. In addition summary consideration was given to a) biofuels, b) the nutritional value and improvement of crops in relation to organic farming, c) use of bi-products such as rice and wheat straw, d) micronutrients, e) the specific post-harvest problems of fruit and vegetable production, f) soil nutritional management and g) plant nutrient uptake. There is a need to define projects that fit with funding agencies priorities and identify both Indian and Swiss partners capable of completing high quality research and practical outputs. Some of the areas of the workshop are considered below by this evaluation.

7.4.2. Plant biotechnology and crop responses to climate change

Models that relate climate to changes in crop yield have been based on several parameters including the importance of a crop to improve food security in a region and the median predicted impact of climate change. S. Asia was identified as a region without sufficient adaptation measures for millet, groundnut and rapeseed and to a lesser extent wheat[38].
ISCB programmes can contribute to the development of climate resilient crops for India but this requires informed debate to prioritise effort amongst existing options. ISCB could consider focusing on three diverse but relevant problems that have profound relevance in food security. They are considered below (i-iii).

i) *Climate resilient crops*: ISCB could facilitate identification of crop lines with intrinsically high water use efficiency (WUE) and appropriate temperature optima and characterise them using molecular tools at the genomics level. Research interventions should aim to improve grain quality and productivity in the target environments while avoiding any anti-nutritional qualities. Such an approach will contribute to food security by sustaining or enhancing productivity and by improving the nutritional quality of the produce from these crops.

ii) *Wheat:* the productivity of this crop and rice pose critical challenge under climate change consequences for India. The elevated temperature regimes/fluctuations may have detrimental effect on crop growth and performance. The demonstrated decline in wheat in the specific northern plateaus of India to terminal heat. It is this warming trend and the increased fraction of rainfall falling as heavy precipitation while this crop is growing that harms yields[39]. It has also been suggested recently that warming in India presents an even greater challenge to its wheat crop than implied by previous modelling studies with the crop's sensitivity to very hot days being a key issue[40]. These concerns necessitate a comprehensive strategy to ensure the future wheat harvest of India. This requires the basic issues of adaptations to climate change effects to be addressed which include a) improving water use and water use efficiency; b) phenology-

[38] Lobell *et al.*, 2008, *Science*, 319, 607-610
[39] Lobell *et al.*, 2011, *Science*, 333, 616-620
[40] Lobell *et al*., 2012, *Nature Climate change,* published online 29th January 2012, DOI: 10.1038/ncclimate1356

duration of the reproductive and grain filling periods; and c) strategic understanding of high temperature induced acquired tolerance mechanisms.
The existing variability for diverse traits across the genotypes and related species should be exploited but the 'transgenic' option is also of value for developing climate resilient cultivars of wheat within the context of new discoveries in functional genomics.

iii) *Groundnut/brassicas and pulses:* pulses in Indian context are highly relevant for food and nutritional security in India. They are often grown under rainfed conditions and frequently experience profound flower/fruit drop in response to drought and heat stress. Strategic basic research and developing genomic resources provides the platform to initiate the pre-breeding options. The pulse network could consider high temperature, water use efficiency stress induced acclimation response during reproductive growth as main constraints on this crop in addition to pod borer and drought tolerance already considered. There is scope to exploit the trait and the genetic diversity in the existing germplasm and a pre-breeding QTL discovery programme for both biotic and abiotic traits has relevance. The output from phase 3 has demonstrated that transgenics have a role to play in pulse improvement and provide options to transfer traits from other genomes. Pyramiding of relevant traits is now emerging as an option by transgenics.

A project within the biofertiliser network could concentrate on additional products for drier soils than required by current products. Both networks could also be extended to include plant uptake nutrient uptake and soil nutritional management.

7.4.3. Post-harvest losses

The loss figures estimates for cassava, pulses and wheat and in India are of the order of 10-12%[41], 9.5%[42] and 9.3%[43] respectively of which insect damage is responsible for up to half this value for pulses and wheat. This may not be sufficient to prioritise biotechnology for insect control over other appropriate approaches of value for low income countries farmers for which biotechnology is not relevant such as improving harvest techniques, farmer education and storage facilities[44]. ISCB did consider *Callosobruchus* (a beetle) storage pests in Phase 1. As this work ended it is assumed that a project would only be of interest if new anti-insect proteins are available that controls this insect effectively such as cry3 and vip3 proteins.

7.4.4. Utilisation of wheat and rice bi-products

The ISCB workshop of October 2010 identified issues relating to waste, residue management and straw burning to enhance organic material into the soil as researchable problems. One relevant approach is the production of biochar. The scale on which this is done varies with Anila type cooking stove being one basis for local char production[45]. The stove was developed by India's National Institute of Engineering and there are international biochar associations in both India and Switzerland with activity in universities in both countries. A pilot-scale study in Tamil Nadu established that the type of plant material used to generate the biochar influences its macro- and micro- nutrient content. An application rate of 6.6 metric tons / hectare was needed

[41] http://www.fao.org/docrep/V4510E/V4510E08.htm
[42] Reddy, 2009, *Economic & Political Weekly*, 52, 73-80
[43] http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_WHEAT.pdf
[44] http://www.fao.org/fileadmin/user_upload/ags/publications/GFL_web.pdf
[45] http://www.biochar-international.org/technology/stoves

to initiate carbon accumulation in laterite soils, encourage soil particle aggregation and increase available phosphate and other soil nutrients[46].

A second approach is to use crop residues as biomass feedstock for one or a more of a wide range of conversion processes resulting in chemicals for industry or fuels (e.g. ethanol), and energy for electricity or heat. Engineering is important to improving the technology but there are also challenges for biotechnology. A key limitation is that lignocelluloses are resistant to anaerobic digestion but they can be degraded by pre-treatment with microbes or biochemicals. For instance, a set of complex microbial agents increased yields of biogas by 33% from rice straw[47]. The technical challenge is to generate economically competitive products from rice or wheat straw.

The main issue for ISCB is how to use biotechnology to improve biochar or straw use to benefit small-scale Indian farmers. A call for proposals could determine if appropriate innovation is offered. If it involved microbial research it would provide an addition to the current interests of the biofertiliser network.

7.4.5. Biosafety of plant biotechnology

India has a well-developed science base in this area, comprehensive guidelines for researchers[48] and practical experience from growing one product, Bt cotton. DBT may favour projects that support its mandate and the widening of the range of biosafe and acceptable transgenic plants grown in India. Switzerland currently has a moratorium on cultivation of genetically modified crops but has recently completed a comprehensive research programme to underpin development of its future policy in this area[49]. That programme has projects that have relevance to Indian priorities including co-existence of transgenic and conventional crops, several aspects that relate to the cultivation of transgenic wheat, environmental concerns and societal issues. A synergy is likely between Swiss science expertise in this area and that in India that could underpin the DBT mandate.

An ISCB project on biosafety could centre on its products likely to be field trialled and later commercialised so advancing them along their value chain. It would be important not to duplicate work to be carried out by private seed companies holding licences to ISCB technology but to complement such effort for other, poverty-focused applications. Alternatively, the ISCB project could centre on general issues such as novel approaches that relate to biosafety of transgenic crops of value to develop Indian agriculture. An important, distinctive and high quality contribution for ISCB would be a priority given the current, considerable activity in India in this research field. This may require India, Swiss or other international experts to evaluate concept notes submitted by prospective partners. Chosen projects should go beyond describing areas of difficulty and be enabling in character to support the DBT mandate.

7.4.6. New tools for extension service processes

This project encompasses a range of very different project areas that could be scoped. For instance development of lab or in-field diagnostics based on both immunological and molecular approaches such as PCR based techniques and next generation sequencing. There are many approaches[50] that could be developed to enhance plant pathology diagnostics in India. As a

[46] Mankasingh *et al*., 2011, *Applied Geochemistry,* 26, S218-S221
[47] Zhong *et al*., 2011, *Bioresource technology*, 102, 11177-11182
[48] http://dbtbiosafety.nic.in/
[49] http://www.nfp59.ch/e_index.cfm
[50] Burns R. ed., 2009, *Methods in Molecular Biology,* Volume 508, Humana Press, ISBN 978-1-58829-799-0

specific example, the cassava project's use of a deep sequencing approach could be extended to diagnose further plant viruses (see S9.5-9.6).

There would also be value in research to enhance the uptake of technical innovation emerging from the ISCB programme. The outputs would be to ensure extension workers can identify when an ISCB product has value for an individual farmer's circumstances, to optimising use and identify market opportunities offered by higher quality or increased outputs[51].

[51] http://www.fao.org/sd/2003/KN0603a_en.htm

8. Annex

8.1. Annex 1: Terms of Reference

8.1.1. Background and introduction

The Indo-Swiss Collaboration in Biotechnology (ISCB) is a bilateral research and development programme, jointly funded by SDC (Swiss Agency for Development and Cooperation, Switzerland) and DBT (Dept. of Biotechnology, Ministry of Science and Technology, India). It was initiated in 1974. For the first 25 years, a wide range of projects in biotechnology were supported. Since 1999, after a comprehensive landmark external evaluation, a new ISCB programme (with a specific set of guiding principles and strategic orientation including the concept of Innovative Value Chain) was initiated. It is supporting several joint research and development projects (networks with at least one Swiss and one Indian project partner), which focus explicitly on agriculture and environment. The first mandate of the programme is to develop climate resilient products and processes which have an impact on poverty reduction, food security and sustainable management of natural resources in India. The second mandate is the capacity building of individual researchers and Indian institutes as well as to promote R&D partnerships between Swiss and Indian institutes and private companies with strong economic, social and ecological relevance.
The new ISCB is in its third phase 2007-2012. For the current phase, 4 million CHF and 4.8 million CHF have been provided from DBT and SDC respectively. The programme includes about sixty scientific staff of 20 different research institutes (14 India). The focus of the third programme phase is to steer projects towards product development.

After a good decade working on the specific subjects, projects and often with project partners on a consistent basis, scientific results of a high level were achieved, also with publications of a number of articles in international journals of high repute. In addition, several projects are collaborating with the private sector, taking an important step concretely towards product development. It is felt that an external evaluation of the ISCB, at the programme level together with a review of guiding principles and strategic orientations, will be appropriate. Especially, the evaluation should aim to shape the future phase of the programme.

In addition, this evaluation will be an opportunity to review the programme and its objectives within its evolving institutional context: India's DBT is heading towards its 12th five year plan (2012 to 2017). At SDC head office, ISCB is now part of the portfolio of the Global Programme Food Security (GPFS), which elaborated its five year strategic framework (2010-2015) recently. In India, SDC is since 2011 represented by the Climate Change and Development Division (CCD) of the Embassy of Switzerland. The programme shall also be reviewed within the broader international Agricultural Research for Development (ARD) System, itself facing profound changes.

The planned evaluation is thus meant to assess ISCB's networks, projects and initiatives at the programme level, including the results, outputs and outcomes together with lessons and experiences, regarding research products, processes and partnerships, and their impact on poverty reduction, food security and climate resilience in India. At institutional level the programme should asses in terms of its being embedded in the international ARD architecture, and in SDC's and DBT's strategic frameworks and objectives. The extended recommendation part of the evaluation will be the basis for the Joint Apex Committee (steering committee of ISCB) to elaborate the ISCB's future strategic orientation (phase IV).

8.1.2. Evaluation team

The evaluation team consists of five members. The expertise of the team members covers all key elements of the proposed evaluation and the team is built up of half Indian and half Swiss/International members. Two members agreed to take over the role of the Chair respectively Co-Chair of the team.

<u>Indian:</u>

- Prof. RB Singh (President National Academy of Agricultural Sciences, Delhi): Chair
- Prof. Udaya Kumar (UAS Bangalore)

<u>Swiss/International:</u>

- Prof. Howard Atkinson (University of Leeds, UK): Co-Chair
- Mr. Jean Noel Perrin (Free Lance Consultant, France)
- Dr. Pierre-Andre Cordey (GPFS, SDC Berne)

8.1.3. Objectives and tasks

The main objective of the evaluation will be to review the ongoing ISCB programme and to provide inputs and recommendations for developing future strategies and programme. What is the relevance, appropriateness, effectiveness and efficiency of the programme, especially in the context of the guiding principles and strategic orientations in which ISCB is rooted. The review as well as the recommendations should cover programme, network and institutional aspects.
In the following some bullet points to may be included in the report. However, these points should only be taken as very broad frame and starting point for discussions. Other aspects considered to be important by the evaluation team should be added.

<u>Review:</u>

- Institutional level
    - Position of ISCB strategy in India first of all in view of DBT and national research strategies
    - Position in Switzerland and strategy of SDC first of all GPFS
    - Position in the context of Climate Change and Development Division of the Embassy in Delhi (representing SDC) implementing SDC Global Programme on Climate Change
    - Position towards the international ARD system first of all of CGIAR's
    - Contribution of ISCB on institutional orientation and of state structures (e.g. DBT or ISCB partner institutes)
- Programme level
    - Is the scientific focus (biotechnology of environment and agriculture) still adequate?
    - Is the defined mandate still adequate?
    - Relevance of activities concerning mandate capacity building and product/technology development.
    - Are the innovation pathways defined the right ones to make valuable products available to the end-user?

  - Relevance of the activities and products regarding food security and adaptation to climate change for the improvement of smallholder livelihoods and climate resilience in agriculture (-> Contribution of ISCB to poverty reduction in rural India – results chain)
  - Review of current management structure (one common steering committee of both funding agencies, two management units with different tasks) and project collaboration (organisation in networks)
- Network level
  - Short review of achievements of individual networks and joint projects in view of mandate (and research quality)
  - Are the ISCB research networks adequately connected to the right private sector partners (in terms of successful product development and market uptake)?
  - (Scientific) focus of individual networks still adequate?
  - Other (scientific) focuses to be relevant for India

<u>Recommendation:</u>

- Institutional level
  - Suggestions to improve position of ISCB with Swiss (e.g. SDC/ CCD of the Embassy), Indian (e.g. DBT) and international (e.g. CGIAR) strategies
  - Possibilities to improve contribution of ISCB on institutional orientation and state structure
- Programme level
  a) Focus and mandate
     - What should the programme focus on in the next phase, what should its mandate be?
     - Mandate still capacity building and product development (e.g. focus on basic research could be alternative or should we extend value chain with mandate to link products to end-user through incentives)?
     - Focus still on climate resilient agriculture and environment with impact on food security, climate change and poverty reduction?
  b) Structure and organisation
     - Keep principle of networks?
     - Other changes in management structure or network organisation?
- Network level
  - Recommendation for each present network and project* about continuation, reorientation or logical conclusion.
    *Pulse Network, Wheat Network, Biofertilizer Network, Cassava Joint Project, Biopesticide Project (present project already financed by other source for product development), Bioremediation Joint Project (present project already defined to be stopped), Biosensor Project (future currently under discussion)
  - Recommendations for possible new focuses of networks.

8.1.4. Organisation

- One common report should by prepared by the evaluation team.
- Prof. RB Singh was designated as Chair and Prof. Howard Atkinson as Co-Chair of the evaluation team. Chair and Co-Chair will upon discussions with the other members decide about procedure, divide tasks among the team members and will lead meetings and calls. The Co-Chair will additionally coordinate with Doris Herrmann (Programme Management Unit (PMU), Lausanne) visits and will consolidate the different contributions of the team members to one report.

- D Herrmann will help in the coordination by e.g. make travel arrangements or organise meetings with project partners or other involved parties.
- One basis of the report will be documents about ISCB provided by D Herrmann. Upon request D Herrmann could brief the evaluation team members about ISCB in a call or meeting.
- The other basis will be meetings during a visit of the evaluation team in India. Meetings will be arranged among team members and between team members and project partners/funding agencies/programme management. During this visit at least a draft of the report should be established.

8.1.5. Deliverables

- Report which includes the aspects mentioned in the objectives and tasks (see above).
- Optional, presentation of the report at a JAC (Joint Apex Committee of ISCB) meeting by one of the team members.

8.1.6. Time schedule

Time schedule depends on the availability of the evaluation team members and will be finalised among the team members.
Suggestion:

- July to September 2011: Finalise contracts with team members, definition of timelines of first of all visit in India.
- October 2011 to December 2011: reading of documents by the evaluation team members, finalising of Indian visit programme by the members (with help of D Herrmann if necessary). Organisation of India visit by D. Herrmann (confirm visits, organise travels).
- First half December 2011: Visit in India of the evaluation team, first draft report.
- February 2012: Final report available.

8.1.7. Number of working days of evaluation team members

- One working day for preparation of visit in India / reading of documents
- Two days for international/local travel for international team members, one day local travel for Indian team members
- Up to eight working days for visit in India
- Three days for preparation and revision of report
- For the Co-Chair two additional working days for the coordination and consolidation of the report

8.1.8. Information and documents provided

<u>Briefing in telephone call or meeting with Doris Herrmann (PMU, Lausanne)</u>

- Background, history and principles of ISCB
- Overview individual projects with focus on present stage concerning product development
- Explaining tasks
- Comments to provided documents

Meetings (or calls) with DBT, SDC, TAU, PMU and selected project partners

The evaluation team should define a meeting plan for a visit in India. D Herrmann will provide detailed contact details and will help with the organisation of the visits. TAU (Technology Advancement Unit, Delhi) and Climate Change and Development Division of the Embassy Delhi may support D Herrmann in the organisation.

Documents

Documents will be provided as electronic softcopies (hardcopies can be provided upon request). In **bold**, documents with short background information about programme (history, objectives and organisation) and projects (documents 1 and 12) as well as information collected for planning phase IV (12 and 13). *In italic*, documents with more detailed information about programme and projects in general (2) and present phase (9-11). The rest of the documents gives information about phase I (4 and 5), phase II (7) and planning phase III (8) as well as two example of external reviews of ISCB (3 and 6).

1. **Blue brochure of ISCB (2011)**
2. *ISCB Book – a decade of experience (2011)*
3. ISCB review – base and principles for new ISCB programme (1997)
4. Internal review ISCB phase I (2003)
5. Final report phase I (2005)
6. External review phase I and II, i.e. 1999 to 2007 (2007)
7. Final report phase II (2008)
8. Report of planning workshop phase III (2007)
9. *Annual report to SDC 07/08 (2008)*
10. *Annual report to SDC 08/09 (2009)*
11. *Annual report to SDC 09/10 (2010)*
12. **Background paper for planning workshop October 2010 (2010)**
13. **Proceedings of planning workshop October 2010 (2010)**

Documents upon request

- Proposals individual projects phase III
- Annual reports of individual projects phase III

Please note that an abstract of each project proposal is available on the ISCB website (http://iscb.epfl.ch/) and abstracts of the annual reports of each project are available in the Annual Reports to SDC.