भाकृअनुप
ICAR

वार्षिक प्रतिवेदन

ANNUAL REPORT

2011-12

राष्ट्रीय पादप जैव प्रौद्योगिकी अनुसंधान केन्द्र
NRCPB

राष्ट्रीय पादप जैव प्रौद्योगिकी अनुसंधान केन्द्र

NATIONAL RESEARCH CENTRE ON PLANT BIOTECHNOLOGY

(Indian Council of Agricultural Research)

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Genomes of Pigeonpea, Tomato and Linseed have been sequenced

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Preface

With the ever increasing population and decrease in arable land, there is only option of vertical growth, which can be accomplished by application of biotechnology. Possible threat of climate change has increased the load of biotic as well as abiotic stresses on agriculture and complicated the issue. Application of genetic engineering has already proved its potential and turned out to be a spectacular success in twenty-nine countries, a majority of them in developing countries. Biotechnology has the potential to take care of almost every aspect of agriculture, be it crop improvement, crop management, crop protection or post harvest management. Education and public awareness on the pros and cons of the application of biotechnology in agriculture is extremely important for the implementation of the technologies for the benefit of the masses.

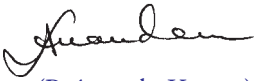
NRCPB is the only ICAR institute which is exclusively involved in Plant and Agricultural Biotechnology research and related academic activities in the country. It has contributed significantly on human research development; training manpower in the field of plant biotechnology and making substantial progress through biotechnological approaches since the inception of this Centre. It is a great sense of satisfaction for all of us that during the reporting year the contribution of this centre has been recognized at the National level. Sardar Patel Outstanding ICAR Institution Award was conferred to recognize the outstanding performance made by NRCPB during the year 2010-11 where as Mahindra Samriddhi Krishi Sansthan Samman is the recognition for Public Sector organizations committed to a broad policy on agriculture that has become instrumental in changing the lives of hundreds of farmers. I take this opportunity to congratulate and thank all the staff of NRCPB for these achievements. I would also like to assure that our efforts will be continued with great vigour and enthusiasm towards the improvement of Indian Agriculture.

On the research front, five ongoing projects were formally concluded during the reporting year. Subsequently, after reorganizing the research activities, new Research Projects would be prepared from the year 2012-13 as per the suggestions of the Research Advisory Committee (RAC) of NRCPB. One of the significant achievements during this year was pigeonpea (Arhar) genome sequencing. This is the first plant genome sequenced entirely through a network of Indian institutions and it will provide a highly valuable resource for pigeonpea variety improvement.

NRCPB has continued its momentum on development of products/ processes and transferring them to end users for further use. During the reporting year three patents were filed and nine MOUs were signed with different agencies including private companies and academic institutions.

NRCPB has co-hosted 'International Conference on Plant Biotechnology for Food Security: New Frontiers' along with the Society for Plant Biochemistry and Biotechnology in which more than 750 participants from all parts of the world including the most eminent scientists in the area of Plant Biotechnology presented their research work.

This Annual Report elaborated the different activities of this Centre covering research achievements, human recourse development and other institutional activities. I thank Dr. P. K. Mandal, Dr. Prasanta Dash, Dr. Rhitu Rai, Dr. S.K. Sinha and Dr. Rampal Niranjan for their help in preparation of the Annual Report.



New Delhi
Date: 12 July, 2012

(P. Ananda Kumar)
Project Director

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विशिष्ट सारांश

वर्ष 1993 में स्थापित, राष्ट्रीय पादप जैव प्रौद्योगिकी अनुसंधान केन्द्र (एन.आर.सी.पी.बी.), पादप आण्विक जीवविज्ञान और जैव प्रौद्योगिकी के क्षेत्र में अनुसंधान, शिक्षण और कार्मिकों के प्रशिक्षण में भारतीय कृषि अनुसंधान परिषद् के अग्रणी अनुसंधान संस्थानों में से एक हैं। केन्द्र ने फसल सुधार में प्रासंगिक मूल और प्रयोगिक अनुसंधान में निरन्तर अपनी गति बनाई रखी है जिसके परिणामस्वरूप 2011–12 के दौरान बड़ी संख्या में प्रकाशन पेटेण्ट और खोजें जारी किए। इसमें बाह्य निवेशों की दृष्टि से आश्चर्यजनक वृद्धि दर्ज की गयी है जिसके परिणामस्वरूप न केवल प्रायोगिक अनुसंधान के लिए बेहतर उपकरणों पर आधारित ज्ञान को बल्कि समूचे देश के विकास में उन्नत अनुसंधान को बढ़ावा देने के लिए अत्यधिक प्रशिक्षित मानवीय शक्ति को भी बढ़ावा मिलेगा। वर्ष 2011–12 के दौरान दो राष्ट्रीय पुरस्कारों, सरदार पटेल आउटस्टैंडिंग आई.सी.ए.आर. अवार्ड 2010 और महिन्द्रा कृषि संस्थान सम्मान–2012 को प्राप्त करने से इस संस्थान की प्रगति स्पष्ट है।

वर्ष 2011–12 के दौरान इस संस्थान की अनुसंधान उपलब्धियां पांच मुख्य शीर्षों के अंतर्गत प्रस्तुत हैं: पादप जीन और प्रवर्तकों का पृथ्थकरण, जैविक प्रतिबल सहिष्णुता के लिए पराजीनी, जैव प्रौद्योगिकी और जलवायु परिवर्तन, उत्पादकता संवर्धन हेतु जैवप्रौद्योगिकीय पद्धति और जीनोमिक्स और आण्विक संकेतक। 2011–12 के दौरान संस्थान की अनुसंधान, शिक्षण और प्रशिक्षण की उपलब्धियों को नीचे संक्षेप में प्रस्तुत किया जाता है।

पादप जीन और प्रोमोटर

इस वर्ष एक सूत्रकृमि के प्रति अनुक्रियात्म जड़ विशिष्ट प्रवर्तक को अलग किया गया और उसके लक्षणों का निर्धारण किया गया जो विशेष रूप से खरोंच/गाँठ उत्तकों, जो सूत्रकृमि संक्रमण (*Meloidogyne incognita*) की अनुक्रिया में तैयार होते हैं, में अभिव्यक्त होता है। तदनन्तर इनका उपयोग RNAi का उपयोग करते हुए आक्रमण करने वाले सूत्रकृमियों के जीनों को लक्ष्य करने हेतु किया जाएगा जिससे सूत्रकृमि संक्रमण के विरूद्ध एक प्रभावशाली नियंत्रण उपलब्ध होगा।

भारतीय सरसों (*Brassica juncea*) से एक पैथोजीन इंडियूसिवल प्रोमोटर अलग किया गया है और उसके लक्षणों का इसके विभिन्न cis–तत्वों जो *in-silico* विश्लेषण के द्वारा SA, JA, इथिलीन, जल बाद और जैविक प्रतिबल के लिए अनुक्रियाशील हैं, निर्धारण किया गया है।

कपास के बीजकोषों उत्तकों में प्रमुखता से योगदान करने वाले एक तथाकल्पित जीर्णता प्रोटीन के एक प्रवर्तक की पहचान की गयी है और आदर्श पादप तंत्र में उसे विधिमान्य बनाया गया है। यह प्रवर्तक *एराबिडोप्सिस*, तम्बाकू और कपास बीजकोषों में क्षणिकता में सुसंगत और उच्च अभिव्यक्ति देने में समर्थ है। इस प्रवर्तक का उपयोग कपास में कीट प्रतिरोध के लिए जीन अभियांत्रिकी हेतु *cry2Aa* जीन को हटाने के लिए किया जा रहा है।

जैविक प्रतिबल

NPRI, जो एक फफूंद विरोधी जीन है, कार्यात्मक दृष्टिकोण से प्रतिरक्षा संकेतन में शामिल ट्रांसक्रिप्सन कारक का सक्रियकारक है, को पूर्व में पृथक किया गया था। भारतीय सरसों (ब्रासिका जंसिया) को एल्टेरनेरिया बैसिकी जो ब्लैक लीफ स्पॉट बिमारी का कारण है, के विरोध में प्रतिरोधक के विकास के लिए NPRI जीन से स्थानांतरित किया गया। गैर पाराजीनी वंशक्रमों की अपेक्षा पाराजीनी पौधे पच्चास प्रतिशत अधिक प्रतिरोधकता (लीजन के आधार पर परिकलित) दर्शाये। इस लीफ स्पॉट प्रतिरोधक सरसों के विकास के उद्देश्य के अन्तर्गत, सरसों के वाइल्ड संबंधियों जैसे कैमेलिया सेटाइवा एवं सीनापिस अल्बा के तुलनात्मक अध्ययन प्रतिरोध के स्तर के लिए किया जा रहा है। यह देखा गया है कि एल्टरनेरिया प्रतिरोध के लिए वाइल्ड संबंधी ज्यादा सहिष्णु है इसलिए इस पारस्परिक क्रिया में शामिल जीनों को समझने का प्रयास किया जा रहा है।

एक पूरे लंबाई के लेक्टिन जीन चना से पृथक किया गया और rolc प्रोमोटर के नियंत्रण में एक युग्मक वेक्टर में प्रतिरूपित किया गया। इस रचना (construct) का उपयोग बैसिका जंसिया

CV पूसा जयकिशन को रूपांतरित करने में किया गया। इस तरह विकसित किये गये पाराजीनी का मुल्यांकण एफिड बायोऐसे के द्वारा किया गया।

चना के प्रोटीयज बाधक जीन का पीसीआर परिवर्धन एवं प्रतिरूपण PCAMBIA1300+NOST में किया गया। प्रमाणित प्रतिरूप से, PCAMBIA1300+NOST युग्मक वेक्टर के प्लासमिड डी एन ए पृथक किये गये। PCAMBIA1300 जिसमें पहले से ही NOT+CPPI उपस्थित थे, उनमें rolc प्रोमोटर सम्मिलित किया गया। पूर्ण रूप से तैयार कन्सट्रक्ट को EcoRI एवं SalI से रेसट्रिक्सन के बाद सुनिश्चित किया गया जो 1.8 केबी के डी एन ए टुकड़े जिसमें rolc + CPPI + NOST एवं युग्मक वेक्टर की मुख्य भाग शामिल है, को निकलता है।

2,4-DAPG जो विस्तृत प्रभावशाली प्रतिजैविक है, के उत्पादन के नियंत्रक का पता लगाने के लिए सीडोमोनास फलोरीसेंस (पीजीपीआर) स्ट्रेन S218, Tn5 उत्परिवर्ती लाइब्रेरी बनाया गया, और इसे टोमैटो बैक्टेरियल विल्ट रोगाणु, रोल्सटानिया सोलानासियम, के विरूद्ध व अत्यधिक प्रभावशाली पाया गया। एक कार्य प्राप्ति उत्परिवर्ती की पहचान हुई जिसमें अधिकतम प्रतिजीविता पाया गया एवं आणविक विश्लेषण से पता चला कि इस उत्परवर्ती का सुगर परिवाहक जीन ट्रान्सपोजोन के द्वारा विध्न हुआ है।

एन–टर्मीनल ट्रांसिट पेप्टाइड अनुक्रम का उपयोग करते हुए Bt प्रोटीन (Cry1Ac) को क्लोरोप्लास्ट में लक्षित किया गया एवं सायटोसोलिक एवं एन्डोप्लासमिक जनिका लक्षित Bt प्रोटीन के अभिव्यक्ति, स्थिरता एवं क्षमता की तुलना की गयी। Cry1Ac का स्तर ER लक्षित वंशक्रमों में अत्यधिक था परन्तु सायटोसोलिक वंशक्रमों में कम जबकि Cry1Ac का स्तर क्लोरोप्लास्ट लक्षित वंशक्रमों में दोनों स्तरों के बीच था। कोशिकांगों में Cry1Ac प्रोटीन के स्तर की मात्रा अधिक होने के कारण, इनका न्यूनतम विकास एवं अधिकतम स्थायित्व था। इसलिए Bt प्रोटीन की अभिव्यक्ति को किसी एक कोशिकांग में लक्षित कर काफी हद तक बढ़ाया जा सकता है।

इन सिलिको तकनीक का प्रयोग करते हुए, क्रॉसा चावल जीनोम में एक जिंक फिंगर न्यूकसियेज की रचना एवं संस्लेषण किया गया। कुल नौ स्वतंत्र वंशक्रमों का विकास क्रॉसा किस्म में किया गया जिनमें कोई भी लक्षित पौधें में नहीं पाया गया।

कपास संबंधित जीन, AKS-1, जो पत्तियों में सबसे ज्यादा अभिव्यक्ति दर्शाता है, को प्रोमोटर के अध्ययन के लिए चुना गया। यह प्रोमोटर कपास में Bt-ICP जीन की अधिक अभिव्यक्ति के प्रयोग के लिए किया जा सकता है।

बैसिलस थूरिनजियेंसिस के द्वारा पैदा किया AHL लेक्टोनेज, जो कोरम को नष्ट करता है, जीवाण्विक बीमारियों को वश में करने का एक दूसरा तरीका है। भारत के विभिन्न प्राकृतिक वास से पृथक किये हुए प्राकृत Bt आइसोलेट एवं Bt टाइप स्ट्रेन की जांच aiiA की उपस्थिति के लिए की गई। AHL लेक्टोनेज ने कुछ Bt स्ट्रेन को सुनिश्चित किया जिसकी प्रतिरूपण का कार्य पूरा कर लिया गया है। अनुक्रम निर्धारण एवं विश्लेषण कार्य प्रगति पर है।

IR–64, एक लोकप्रिय भारतीय चावल की किस्म का ग्लाइफोसेट सहिष्णु पाराजीनी, कोडोन अनुकूल कृत्रिम CP4-EPSPS जीन, जिसकी सजातीयता ग्लाइफोसेट के लिए कम है, से रूपांतरित किया गया है। पाराजीनी वंशक्रमों में गुणात्मक प्रोटीन अभिव्यक्ति की जांच की गई और छः पाराजीनी वंशक्रमें सुनिश्चित पायी गयी। खरपतवार नियंत्रक के वायोएसे जांच से सिद्ध हुआ कि पाराजीनी चावल 1 प्रतिशत तक व्यावसायिक राउंडअप को सहन कर सकता है जबकि यह मात्रा चावल के खरपतवार को खेतों में मार देता है।

रेशों की विकास के आणविक प्रक्रिया को समझने के लिए जीनोम वृहत् ट्रांसक्रिपटोमिक्स की तुलनात्मक विश्लेषण गौसिपियम हिरसुटम सीवी MCU5 उत्परिवर्ती एवं रेशा सहित बीजांड के बीच किया गया। विभिन्न अवस्था में ऊर्ध्व संचालित ट्रांसक्रिप्टो की संख्या की तुलना में निम्न संचालित ट्रांसक्रिप्टो की संख्या अधिक पायी गयी जो मुख्य रूप से रेशों के प्रारंभ एवं दीर्घीकरण अवस्था में अधिक थी। यह विस्तृत ट्रांसक्रिप्टोम डाटा रेशों की कोशिका के विकास में शामिल जीनो के बारे में बहुमुल्य जानकारी प्रदान करती है।

आखिरी वर्ष एराब्डॉपसिस के FPS2 जीन का उपयोग पाराजीनी उत्पन्न करने में हुआ था। एफिड को अच्छी तरह वश में करने के लिए FPS2 जीन को CaMV 35S प्रोमोटर के साथ

प्रतिरूपित किया गया जिससे की फारनेसायल डाइफॉस्फेट का स्तर बढ़ सके। एराब्डॉपसिस पाराजनी जो इस कन्सट्रक्ट के इस्तेमाल से उत्पन्न हुआ पाराजनी की अभिव्यक्ति की पुष्टि करता है। और इसी क्रम में कीड़ों की बायोअसे के लिए गुणात्मक जांच की पुष्टि की गयी। एफपीएस–पाराजीनी से निष्कासित वाष्पशील को GC-MS फ्रोफाईल से भी सत्यापित किया गया।

अजैविक प्रतिबल

जीवाणु से प्राप्त CSP जीन, पौधों में विभिन्न स्ट्रेस (तनाव) को सहन करने की क्षमता को बेहतर किया है। मिट्टी से पृथक किये हुए विभिन्न जीवाणुओं में, कम तापक्रम को सहने करने की क्षमता वाले जीवाणुओं को जांचा गया। उनमें से पांच आइसोलेटस, *Pseudomonas sp* एवं *Providencia* sp के रूप में पहचाने गये। इन सभी जीवाणुओं से पूर्ण लंबाई के CSP जीन परिवर्धित एवं अनुक्रमित किये गये। इसी तरह उच्च तापक्रम को सहन करने के लिए उत्तरदायी जीवाण्विक जीन की bioprospecting का भी प्रयास किया गया।

विभिन्न गर्म जल वाले झरनों से 45–72 डिग्री सें. तापक्रम पर उपलब्ध मिट्टी एवं जल के नमुने लिऐ गये। जो अइसोलेटस 55 डिग्री सें. से उच्च तापक्रम पर जीवित पाये गये उन्हें आगे के विश्लेषण एवं उच्च तापक्रम के लिए उत्तरदायी जीन के पहचान के लिए चुना गया।

वर्तमान में बहुत सारे हेलोफाइटस के अध्ययन पौधों में लवण प्रतिबल से संबंधित शोध के लिए किया जा रहा है। करीब 800 bp लंबे SOS1 जीन के प्रतिप्रवाह अनुक्रम (Upstream seq) *Salicornia brachiata* (लवण मृदोदभिद पौधा) से प्रतिरूपित (clone) एवं अनुक्रमित किया गया।

Hydrilla verticillata (l.f.) पौधा में प्रकाश संश्लेषण से संबंधित प्रक्रिया में एक अद्‌भुत क्षमता है, जो अपने पत्तियों में क्रान्ज एनाटोमी के अनुपस्थित होने बावजूद उच्च तापक्रप पर C3 से C4 प्रक्रिया में परिवर्तित हो जाता है। C4 एवं C3 प्रक्रिया को प्रेरित करने के लिए इन पौधों को दस दिनों के लिए 34 डिग्री सें. एवं 22 डिग्री सें. पर रखा गया। इन पौधों के पत्तियों से पांच अग्र व्यवकलित लाइब्रेरी बनाये गये एवं पाये गये समृद्ध डीएनए को प्रतिरूपित किया गया। करीब एक हजार प्रतिरूपों को चूना एवं अनुक्रमित किया गया। अभी वायोइनफॉरमेटीक्स से संबधित विश्लेषण किये जा रहे हैं।

ऐनेकिसन घुलनशील, बहुक्रियात्मक, वसा बंधनकारी प्रोटीन है जो कि बड़े बहुपित्रेक कुल से कोडीत होते हैं एवं तमाम प्रोकेरियोटीक एवं यूकैरियोटीक क्षेत्रों में फैले हुए हैं। ऐसे प्रोटीन्स में Ca^{2+} एवं वसा संबंधी संकेतों को जोड़ने की क्षमता होती है जो विभिन्न जैविक एवं अजैविक पर्यावरण को संयोजित करते हैं। चावल की एक किस्म नगीना 22, जो अनावृष्टि सहिष्णु प्रजाति है, से Annexin 6 gene (Ann0sI6) को, 587 bp प्रतिप्रवाह खंड से एवं 210 bp कोडीत खंड से, परिवर्धित किया गया है। इसी क्रम में गस जीन को pBI121 दुविआधारी वेक्टर के साथ जोड़ कर अनेक विलोपित कनस्ट्रक्ट (P1-5AnnOsI6) भी बनाये गये हैं। इस तरह बनाये गये कन्स्ट्रक्ट के डीएनए टुकड़ों में बहुत सारे सीस एलिमेंट उपस्थित है जो हार्मोन की उपस्थिति में अपनी प्रतिक्रिया प्रस्तुत करते है एवं प्रतिबल प्रेरित जीन अभिव्यक्ति को नियंत्रित करते है। दीर्घकालीन विश्लेषण के लिए पराजनी तंबाकु P4AnnOsI6 कन्स्ट्रक्ट का उपयोग करके, तैयार किये गये हैं। pBI121 के साथ रूपांतरित पौधों में मूलभूत अभिव्यक्ति को देखा गया है। पौधों के विभिन्न अंगों में गस के अंतरीय अभिव्यक्ति को विभिन्न प्रतिबल की उपस्थिति में देखा गया है। यह अध्ययन अजैविक प्रतिबल की दशा में, एक क्रियात्मक, कार्यशील प्रोमोटर की आशाजनक नतीजा प्रस्तुत करता है जिसे आगे अध्ययन के लिए जांचा जा रहा है।

बाजरा *(Penniseteum glaucum)* विभिन्न पौधों की जातियों में, एक विशिष्ट जाति है जिसमें विभिन्न पर्यावरणीय प्रतिबल को सहन करने की असाधारण क्षमता हैं। इस जानकारी को ध्यान में रखते हुए बाजरा में सूखे से संबधीत सुदृढ युग्मविकल्पी (Allele) ढुंढने के लिए प्राइमर अभिकल्पित किये गये। इन प्राइमरों के अनुक्रम, डेटाबेस में उपलब्ध विभिन्न नमी प्रतिबल से संबंधित जीन अनुक्रमों के आधार पर किया गया एवं तदोपरांत अभ्यर्थी जीन को बाजरा से परिवर्धित किया गया। विभिन्न जीनोटाइप/प्रजनन लाइन/ स्पेशिज से P5C5 जीन परिवर्धन का सफल प्रयास किया गया। एवं संपूर्ण दूरी के P5C5 जीन को प्राप्त करने के लिए रेश (RACE) तकनीक का प्रयोग किया गया जिसके लिए 5'–3' प्राइमर अभिकल्पित किये गये एवं फलस्वरूप संपूर्ण दूरी के P5C5 जीन प्राप्त कर लिए गये। 1276 bp न्यूक्लियोटाइडों के P5C5 जीन अंततः परिवर्धित एवं

अनुक्रमित किया गया। इसके आगे, इस फसल के एक महीने पुराने बीचड़ों में शुष्क प्रतिबल प्रेरित किया गया और निषेधात्मक (suppresive) DNA लाइब्रेरी तैयार किया गया। अनुक्रमित डेटा की टिप्पण से जीनों की समजातता, जैविक एवं अजैविक प्रतिबल से पायी गयी।

अपने निरंतर प्रयास को जारी रखते हुए, चना में शुष्क प्रतिबल से संबंधित जीनों की पहचान क्रियात्मक जीनोमिक्स का प्रयोग करते हुए, इस वर्ष इएसटी (ESTs) जो AP2/ERF प्रोटीन कोडीत करते है, को पृथक किया गया। ऐसा पाया गया कि ये इएसटी शुष्क प्रतिबल के फलस्वरूप अपनी अंतरीय अभिव्यक्ति प्रस्तुत करते हैं। इ आर एफ (ERF) सब फैमिली के जीन जिसमें 58–60 अमीनो अम्ल के AP2 प्रक्षेत्र (Domain) पाये जाते हैं, AGCCGCC सीस–एक्टीग एलिमेंट को पहचानते हैं, जो GCC बॉक्स जाने जाते हैं, मुख्य रूप से जैविक एवं अजैविक प्रतिबल प्रतिक्रिया में शामिल होते हैं। चना के इ आर एफ फैमिली के कुछ सदस्यों की अभिव्यक्ति का अध्ययन आर टी क्यू पी सी आर (RT-qPCR) से किया गया। इस अध्ययन में इन जीनों के अभिव्यक्ति का स्वरूप पौधे के अंगों में बहुव्यापी एवं कुछ में ऊतक विशिष्ट अभिव्यक्ति पाया गया।

ICC4358 (शुष्क सहिष्णु) एवं ICC1882 (शुष्क संवेदनशील) के संकरण से उत्पन्न पुनर्योगज अन्तः प्रजात वंशक्रम में नायलोन माइक्रोअरे द्वारा पूर्व से अभिज्ञात अंतरीय अभिव्यक्त यूनीजीनों को जांचा गया। MYB ट्रांसक्रीपसन कारक, सल्फेट परिवहाहक, बीटेन एल्डीहाइड डीहाड्रोजीनेज, ट्रासक्रीपसन नियामक प्रोटीन एवं ट्रांसक्रीपसन दीर्घीकरण कारक जो यूनीजीनों से कोडीत होते हैं, की अभिव्यक्ति उच्च जड़ जैव भार रील (RIL) में टर्मीनल शुष्क प्रतिबल की दशा में बढ़ जाता है। अन्य वर्गों के जीन जैसे ATP बंधनकारी प्रोटीन, कायनेज संबंद्धी प्रोटीन, पीआरपी, कैल्शियम बंधनकारी प्रोटीन, WD40 प्रक्षेत्र प्रोटीन आदि जिनकी अभिव्यक्ति ICC4958 में बढ़ जाता है, रील के उच्च जड़ जैवभार शुष्क की दशा में कम हो जाता है।

उत्पाकदता वृद्धि

सरसों में संकर ओज प्रजनन कार्यक्रम सतत रूप से जारी हैं। मोरीकेन्डीया अरवेंसीस *(Moricandia arvensis)* CMS पद्धति पर आधारित बहुत सारे संकरों का मूल्यांकन किया गया, जिनमें से दो संकरों, संकर 23 एवं संकर 2007ए, ने बीज पैदावार में 18–30 प्रतिशत श्रेष्ठता अपने शह किस्म वरूणा पर सिद्ध किया है। ये परिणाम यह सिद्ध करते है कि पर्यावरण का असर संकर के पैदावार पर होता है अतः श्रेष्ठ संकरों की पहचान के लिए विभिन्न वर्षा में बहुस्थानाक–खोज की आवश्यकता है। ब्रासिका में, विभिन्न कार्यिकी अध्ययनों ने साबित किया है कि बीजों में उपस्थित रक्षित पदार्थ को बनाने में सिलिक भित्ती के प्रकाश संश्लेषण मुख्य रूप से जिम्मवार होते हैं। इसलिए अभी भी बीज पैदावार में पत्तियों के प्रकाश संश्लेषण का महत्व एक रहस्य बना हुआ है। *B. rapa* के हरे सीलिक की अपेक्षा में एक श्वेत सीलिक उत्परिवर्ती में पर्णरहित की बहुत ही कम मात्रा अंकित किया गया है। उत्परिवर्तन के आनुवंशिक लक्षण को समझने के लिए श्वेत एवं वाइल्ड प्रकार के बीच के प्रजनक बनाये गये, जिसमें यह पाया गया कि हरे सीलिक की अपेक्षा श्वेत प्रकार प्रभावशाली है एवं एकल अनानुवंशीक लक्षण की तरह पृथक होते हैं। परन्तु बीज के रंग या कोष्ठक की संख्या एवं इस लक्षण के बीज कोई सहलग्नता नहीं पायी गयी और ये परिणाम, वर्तमान में व्याप्त धारणा जो बताती है कि सीलिक प्रकाश संश्लेषण का योगदान बीज पैदावार में है, से भिन्न है। कोशिका विभाजन के दौरान गुणसुत्र के पृथकरण में गुणसुत्र बिन्दू हिस्टोन प्रोटीन महत्वपूर्ण भूमिका निभाता है और CENH3 के आनुवंशिक अभियांत्रिकी ने यह सिद्ध किया है कि यह ऐरॉब्ड़पसीस में अगुणित विप्रेरण किस्म बनाने में उपयोगी है। चूकि ऐसे अगुणित विप्रेरण किस्में प्रजनन प्रक्रिया में तीव्रता ला सकते हैं, ब्रैसिका जून्सिया में अग्रणित विप्रेरण किस्म के विकास के लिए अनुसंधान के प्रयास शुरू किये गये हैं। यह प्रयास मूल CENH3 प्रोटीन का अभियांत्रिक CENH3 टेलस्वाप प्रोटीन के स्थानापन्न से होना है।

नींबू में बीजाण्डकलज बहुभ्रूगता संचालित जीनों के विलगन का अनुसंधान कार्य जारी है, एवं नींबू के पूर्व बीज का ट्रांसक्रिपटोम विश्लेषण का कार्य सूक्ष्म व्यूह द्वारा प्रतिवेदित समय के दौरान किया गया। बहु एवं एकल भ्रूणिय कल्टीवारो के पूर्व प्रफुल्लन चरण के जीन अभिव्यक्ति में महत्वपूर्ण भिन्नता पायी गयी है। बहुभ्रूणीय लक्षण से संबंधित ट्रांसक्रीप्ट को पहचानने का प्रयास किया गया है। इन ट्रांसक्रीप्टो के कार्यात्मक टिप्पण और नियत अंकन से यह पाया गया है कि अधिकांश (20 प्रतिशत) ट्रांसक्रीप्ट एच एस पी से सम्बद्ध है जो पूर्व में SSH अध्ययन से हमलोगों द्वारा पहचाने गये ट्रांसक्रीप्ट भी है।

सरसों में आनुवंशिक अभियांत्रिकी के द्वारा पैदावार बढ़ाने का प्रयास पादप हार्मोनों की सान्द्रता का माडुलन के द्वारा किया गया है। पौधों के सायटोकायनीन समस्थापन संचालित जीनो जैसे सायटोकायनीन ऑक्सीडेज/डीहाइड्रोजिनेज का विलगन सरसों मे पीसी आर आधरित प्राइमरों जो एरॉब्डॉप्सीस जीन समजातता का प्रयोग कर, किया गया है। प्रतिरूपित जीन CKX5 है, चूकि इसकी 99 प्रतिशत समानता एराब्डोपसीस थैलियाना के CKX5 से है। जड़ों में CKX जीन के प्रभावशाली अभिव्यक्ति के लिए, जड़ विशिष्ट प्रोमोटर एराब्डॉपसीस से किये गये हैं। वर्तमान में CKX5 जीन का पृधक्कृत प्रतिरूपण जड़ विशिष्ट प्रोमोटर के साथ का कार्य प्रगति में है।

फास्फोरस का उपापचयी क्रियाओं जैसे प्रकाश संसलेशन, श्वसन, ग्लाइकालिसिस, रेडॅक्स संतुलन एवं ऊर्जा संस्लेषण में केन्द्रीय भूमिका अदा करता है। और इस तरह यह न्यूक्लिक अम्लों एवं फास्फोलिपिड के निर्माण में महत्वपूर्ण होते हैं। फास्फोरस समस्थापन के माडुलन के फलस्वरूप पौधों के विभिन्न अंगों में फास्फोरस उद्रग्रहण संतुलन, गतिशीलन, एवं विभाजन, करना आवश्यक है। वर्तमान में ट्रांसक्रिप्सन कारक जो फास्फोरस समस्थापन के लिए जिम्मेवार है के पहचान का कार्य जारी है।

जीनोमिक्स एवं मार्कर

अरहर भारतीय उप महाद्वीप, दक्षिण पूर्व एशिया एवं पूर्व अफ्रिका का एक महत्वपूर्ण दलहन फसल है। अरहर की प्रचलित किस्म 'आशा' का जीनोम अनुक्रमन सफलता पूर्वक प्रतिवेदित वर्ष में पूरा कर लिया गया। विस्तृत अनुक्रम समीप जो अरहर के अनुमानित 858 Mb आकार के जीनोम के 60 प्रतिशत हिस्से को दर्शाता है, का उपयोग जीन विश्लेषण, दुहराव की मात्रा एवं एस एस आर लोसाई को पता करने में हुआ। कुल 59,515 पूर्वानुमानित जीन में 47004 प्रोटीन कोडीत जीन एवं 12511 जीन टीई से संबंधित थे। सभी प्रोटीन कोडीत जीन को फिर से अनेक कार्यात्मक श्रेणी में वर्गीकृत किया गया। इसके आगे 366 जैनिक SNP मार्करों पर आधारित अरहर का अति घनत्व अंतःनस्ल संदर्भ आनुवंशिक मानचित्र विकसित किया गया। अरहर के अति परिवर्तनीय HASSR मार्करों के विकास एवं प्रमाणीकरण से स्पष्ट हुआ कि पूर्व में प्रतिवेदीत BAC end व्युत्पन्न जीनोमिक एसएसआर मार्कर के अपेक्षा में HASSR मार्कर में बहुरूपता ज्यादा थी। यह नतीजा HASSR मार्करों का अरहर के आणविक प्रजनन में अति सम्भावित उपयोग को दर्शाता है।

अरहर एवं सोयाबीन फैबेसी कुल के एक ही क्लेड से संबंधित है। इसी क्रम में अरहर एवं सोयाबीन का तुलनात्मक विश्लेषण किया गया। अरहर एवं सायेाबीन के बीच निम्न स्तर के समन्वय, संकेत करता है कि ये दानों में संभवतः केवल एक ही जीनोम आम है और दोनों ही प्राचीन एम्फिप्लॉड है। भारतीय कृषि अनुसंधान परिषद के नेतृत्व में विभिन्न भारतीय संस्थानों के समूह के द्वारा अरहर जीनोम का अनुकमण पूरी तरह पूरा किया गया है जो कि अरहर के किस्मों के विकास के लिए एक बहुमूल्य संसाधन है।

करीब 238 GB गेहूं के जीनोम का अनुक्रमण डेटा TGAC, BBSRC, नौरविच, यूके से प्राप्त किया गया एवं जिसका फिर विश्लेषण किया गया। इसी तरह चेक गणराज्य से 56832 BAC प्रतिरूप भी प्राप्त किया गया जिसका SNAP shot तकनीक द्वारा फिन्गर प्रिटिंग किया जा रहा हैं। गेहूं में शुष्क सहिष्णुता एवं संबंधित लक्षण के लिए QTL मानचित्रण का कार्य प्रगति में है। 206 पुनयोगज अंतःप्रजात वंशक्रमों वाली मानचित्रण जनसंख्या का विकास, WL 711 सूखा संवेदनशील किस्म एवं C306 सूखा सहिष्णु किस्म के संकरण द्वारा किया गया। गेहूं में कुल 37 QTL पहचाने गये जो दस सूखा सहिष्णु लक्षण से संबंधित थे। सूक्ष्म ब्यूह अभिव्यक्ति खाका एवं QTL मानचित्रण के उपयोग से ढेर सारे सूखा सहिष्णु अभ्यर्थी जीन निर्धारित किये गये। पेरेन्टस एवं दो RIL बल्क में अंतरीय अभिव्यक्त हुए संभवत वे सुखा सहिष्णु के अभ्यर्थी जीन हो सकते हैं। चावल, ज्वार एवं मक्का के समन्वय जीनोमिक भाग भी निर्धारित किए गए जो सुखा सहिष्णु जीनों को आश्रित करते हैं।

गेहूं में बीज भंडारण प्रोटीन के लिए NILs के चरित्र–चित्रण का कार्य प्रतिवेदित समय में सतत रूप से चल रहा है। गेहूं में रोटी बनाने की गुणवत्ता के लिए, HMW, LMW, ग्लायडीन, एल्बयूमीन एवं ट्रीटीशिन के लिए विभिन्न युग्मविकल्पी (Allele) का मूल्यांकन किया गया। ग्लूटेनीन युग्म विकल्पी GluDtd, GluB1b, GluA3d एवं ट्रीटीशिन युग्मविकल्पी TriD1a, TriA1a ने एक सा स्पष्ट प्रभाव दिखाया। जबकि ग्लायडीन युग्म विकल्पी का कोई सार्थक प्रभाव नहीं दिखाई पड़ा। इन

युग्मविकल्पी का प्रजनन कार्यक्रमों में रोटी बनाने वाले गुणवत्ता के विकास में संभावित भूमिका है।

बासमती चावल में सुगंध एक बहुत महत्वपूर्ण मात्रात्मक लक्षण है जिसके जटील वंशानुगतता की वजह से इस लक्षण के लिए जिम्मेदार जीन की पहचान किसी भी एक विश्लेषण से कर पाना कठीन है। इस संदर्भ में छः अतिसुगंधित और छः बिना सुंगधित RIL के दो बल्क समुह बनाये गये। ऐसा करते हुए 160 अंतरीय अभिव्यक्ति जीन का विश्लेषण किया गया, जिनमें 16 दो बल्कों के बीच में QTL भाग के aro4.1 हिस्से में जबकि दो QTL भाग के aro3.1 हिस्से पाये गये ।

चावल के दो किस्म नगीना 22 जो सुखा एवं ताप सहिष्णु एवं IR64 जो सुखा एवं ताप संवेदनशील है, का उपयोग कर SNP आधारित आण्विक मानचित्रण पुनयोगज अंतःप्रजात वंशक्रमों से विकास किया गया। यह मानचित्रण एक उचित फिनोटाइप डाटा के उपलब्ध होने के बाद ताप एवं सुखा सहिष्णु QTL की पहचान में बहुत उपयोगी साबित होगा।

एक दलीय एवं द्विदलीय स्पेशिज के माइक्रोसेटेलाइट के वितरण का अध्ययन किया गया। कुल 797, 863 एस एस आर, छः जीनोमों में निर्धारित किये गये जिसमें तीन एक दलीय समुह (ब्रैकीपोडीयम, ज्वार, चावल) तीन द्विदलीय समूह (ऐरॉब्डॉपसिस, मेडीकागो, पॉपलस) पौधे है।

गेहूं में स्पॉट ब्लॉच की बिमारी जो *B. sorokiniane* से होती है, मुख्य रूप से भारत के गर्म एवं नमी वाले हिस्सों में पायी जाती परन्तु आजकल ठंडे इलाकों में भी फैल रहा है। स्पॉट ब्लॉच के लिए प्रतिरोधक लोसाई की पहचान के लिए अध्ययन जारी हैं और इस संदर्भ में HD2932 संवेदनशील किस्म एवं SW89-5422 और Chirya1 सहिष्णु दाता के बीच संकरण कराया गया एवं F1 को अग्रवर्ती किया गया। वर्तमान में दो F2 जनसंख्या का मूल्यांकन का कार्य किया जा रहा है। इस बिमारी से संबंधित ज्ञात एसएसआर मार्कर्रों का उपयोग करके पेंरेन्टस की जांच की जा रही है।

पूसा सुंगध 2 के दो स्वतंत्र समयुग्मजी पराजीनी वंशक्रमों, जो AtDREB1A जीन अपने साथ वहन किये हुए, की पहचान एवं जाँच सुखा एवं ठंडे के प्रतिक्रिया को देखने के लिए किया गया। T4 पौधों पर ठंडे प्रतिबल के प्रयोग को दुहराया गया एवं DREB जीन की बढ़े हुए अभिव्यक्ति को पाया गया।

ब्रासिका में अल्टेरनेरिया ब्लाइट प्रतिरोध के क्रायात्मक जीनोमिक्स का अध्ययन वरूणा किस्म के SSH लाइब्रेरी से प्राप्त 456 प्रतिरूप के अनुक्रमण से किया गया। जब प्रतिरूप के अनुक्रमों को एराब्डॉपसिस के EST डेटाबेसों से ब्लास्ट किया गया तब 248 अंतरीय अभिव्यक्त जीन भिन्न क्रायात्मक श्रेणी से संबंधित पाये गये। 29 दिनों के प्रतिबलित *S. alba* के पौधों से भी SSH लाइब्रेरी बनाया गया। कुल 750 पुनर्योगज प्रतिरूप जिनमें अंतरिय अभिव्यक्त ट्रांसक्रिप्ट थे, पाये गये। 144 अभियर्थी जीन जिन्हें लाइब्रेरी से अनुक्रमित किया गया वे ट्रांसक्रिप्सन कारक एवं अन्य उपापचयी क्रियायें के समजातक पाये गये।

''चावल में नमी की कमी एवं कम तापक्रम के फिनामिक्स'' विषय पर एक सामुहिक परियाजना, राष्ट्रीय निधि, रा.कृ.अ.प. के धन से प्रतिवेदित वर्ष में शुरू किया गया। चावल के विभिन्न विकासात्मक चरणों से प्राप्त ट्रांसक्रिप्टों, जो सार्वजनिक रूप से उपलब्ध विभिन्न प्रयोग से प्राप्त है, का विश्लेषण सूखे से प्रतिक्रयाशील जीनों को पहचानने के लिए किया गया। सुखे की दशा में IR64 (संवेदनशील) के तुलना में Nagina 22 (सहिष्णु) में छः जीनों की अभिव्यक्ति उच्च स्तर की पायी गयी जिनमें पाँच जीनों की अभिव्यक्ति (नगीना 22 के बीचड़ों में) दो गुणा से ज्यादा पाई गई। यह अध्ययन पूर्व में सूखे की स्थिति में उत्पन्न सूक्ष्मब्यूह डेटा से की गई। इसके उपरांत जीनों को परिवर्धित एवं pGEM-T वेक्टर में प्रतिरूपति किया गया। इन जीनों का अनुक्रमण एवं पादप अभिव्यक्ति वेक्टर में शुष्क प्रेरित AtRD29A प्रोमोटर एवं मूलभूत ZmUbq1 प्रोमोटर में क्रार्यात्मक वैधीकरण के लिए किया गया।

चावल के किस्म पूसा सुगंध 2 की आनुवंशिक रूपांतरण cry1Aabc से किया गया। इस प्रयोग के उपरांत 23 कल्पित रूपांतरण को पाया गया जिनका विश्लेषण Cry1Aabc विशिष्ट प्राइमरों से पी सी आर तकनीक से किया गया। इन कल्पित रूपांतरणों में छः में पाराजीन के पी.सी.आर. परिवर्धन के द्वारा वास्तविक पाया गया और फिर पौधों को संवर्धन कक्ष स्थिति में स्वॉलराइट में सख्त किया जा रहा है।

ब्लास्ट प्रतिरोधक पराजीनी वंशक्रमों में Pik^h(Pi54) जीन की अभिव्यक्ति में प्रतिप्रवाह एलिमेंट की सहभागिता एवं जीन अभिव्यक्ति के प्रकृति के अध्ययन के लिए, Pi54 एक्सान विशिष्ट प्राइमरों के उपयोग से qRT-PCR किया गया। Pi54 जीन की

अभिव्यक्ति की तुलना TP-*Pi54*-2, TP-*Pi54*-15 एवं ताइपाई 309 के 0–96 घंटे के पोस्ट इनाकुलेशन (hpi) पर की गई। इन जीन की अभिव्यक्ति TP-*Pi54*-2, TP-*Pi54*-15 में क्रमशः 2.31 एवं 1.5 गुणा अधिक 72 hpi पर पायी गई। इसके विपरीत संवदेनशील युग्मविकल्पी की अभिव्यक्ति ताइपाई 309 में कम पायी गई। *M. oryzae* के 72 hpi संक्रमण प्रतिक्रिया से प्रतिरोधक TP-*Pi54* एवं संवदेनशील ताईपाई 309 सूक्ष्म ब्यूह आधारित ट्रांसक्रिप्टोम विश्लेषण में जीन अभिव्यक्ति में सार्वभौमिक परिवर्तन पाया गया। पाराजीनी चावल वंशक्रमों, जिनमें *Pi54* जीन स्थित है, का संक्रमण *M. oryzae* स्ट्रेन PLP-1 72hpi से कराने के उपरांत संपूर्ण जीनोम में जीनों की सह अभिव्यक्ति को समझने के लिए सूक्ष्म व्यूह विश्लेषण किया गया। पराजीनी वंशक्रमों में विभिन्न प्रतिरक्षक प्रतिक्रियाशील जीन की अभिव्यक्ति अधिक पायी गयी।

पूरे विश्व में चावल की उत्पादन, विनाशकारी बिमारी राइस ब्लास्ट की वजह से प्रभावित होता है, जो *Magnaporthe oryzae* के कारण होता है। Pita एक प्रभावशाली ब्लास्ट प्रतिरोधक जीन है जो भारतीय परिस्थिति में असरकारी है। देश के विभिन्न हिस्सों से एकत्र की हुई की चावल के लेंडरेश में इस की युग्म विकल्प की माइनिंग की गई। Pi-ta ऑर्थलाग अनुक्रमों को 220 चावल के एक्सेंसन के डेटाबेसों से प्राप्त किया गया एवं विश्लेषण में शामिल किया गया। पांच अद्वितीय एवं नया Pi-ta प्रोटीन परिवर्ती भारतीय लैंड रेश में पहचाने गये।

''अजैविक प्रतिबल सहिष्णु के लिए जीनों की बायो प्रोस्पेकटींग एवं युग्मविकल्पी माइनिंग'' सामुहिक परियोजना जो एनएआईपी, भा.कृ.अ.प. से निधिवद्ध है, के अंतर्गत पिछले दो वर्षों में 7000 जीनोटाइप जिनमें मूलभूत वर्ग संस्थापित है, का दविगुणन एवं फिनोटाइपिंग रंग से लेबेल्ड माइक्रोसेटेलाइड मार्कर लोसाई जो एक समान रूप से चावल के बारह गुणसुत्रों पर वितरीत है, स्वचालित खंड विश्लेषण पद्धति से चालू वर्ष में किया गया। SSR जीनोटाइपिंग के द्वारा, 440 युग्मविक्लपी औसतन 12.57 युग्मविकल्पी प्रति लोकस के साथ पाया गया। जीनोटीपिक एवं फिनोटीपीक डाटा का उपयोग कर एक मीनीकोर, जिनमें 228 जीनोटाइप शामिल है, की पहचान की गयी। अभिलक्षित वर्ग के एस एन पी (5246) जो अजैविक प्रतिक्रियाशील जीन में शामिल है, के लिए ज्ञात मीनीकोर वर्ग का पुनः जीनोटाइपिंग किया जा रहा है। जीन अभिव्यक्ति प्रोफालिंग से 1977 एवं 2930 जीनो क्रमशः ऊर्ध्व नियंत्रण एवं निम्न नियंत्रित, कम से कम दो गुणा परिवर्तन पर पाया गया।

507 ज्ञात उत्परिवर्ती वंशक्रमों को खरीफ 2011 में सींचित परिस्थिति में पैदा किया गया जिनमें 382 का चरित्र चित्रण फिनोटीपिकली DUS विवर्णक के अनुसार किया गया। 382 उत्परिवर्ती एवं नगीना 22 का डी एन ए फिंगरप्रिंटिंग किया गया एवं सात उत्परिवर्ती के डेटा से पता चला कि सभी में मोनोजेनिक वंशानुगतता है।

पांच सौ उत्परिवर्तीयों का 25 प्रतिशत PEG6000 में अनुवीक्षण करने पर नमी प्रतिबल सहिष्णु के लिए कार्य प्राप्त उत्परिवर्ती (Gain of fumction mutant) पहचाना गया, जो आकृति एवं आनुवांशिकी दृष्टिकोण से नगीना 22 के समान थे।

उच्च भूमि चावल की किस्म नगीना–22 में EMS प्रेरित उत्परिवर्ती सर्वत्र केन्द्रों में उत्पन्न किया गया जिसे परियोजन सहयोगियों में वितरीत किया गया। इन वंशक्रमों के सबसेटो का विभिन्न महत्वपूर्ण लक्षणें के लिए फिनोटाइपिंग किया गया। इस प्रक्रिया में शुष्क प्रतिबल सहिष्णु, लवण सहिष्णु, कुशल फास्फोरस उपयोग, और जैविक प्रतिबल जैसे वैक्टेरीयल ब्लाइट एवं ब्लास्ट प्रतिरोधक के लिए तथाकल्पित उत्परिवर्ती पहचाने गये। इसके अतिरिक्त पच्चीस (25) उत्परिवर्तीयों में वंशानुगतता का अध्ययन भी किया गया जो एक जीन से नियन्त्रित पाये गये। ये सभी डेटा को चावल उत्परिवर्ती डेटाबेस में जोड़ा गया।

एक स्थानीय डेटाबेस फसल पौधों के जीनोमिक संसाधन के लिए केन्द्र पर पौधा जीनोम डाटाबेस के नाम से विकसित किया गया। अभी तक इस डेटाबेस में 79 पौधा स्पेशिज के बारे में जानकारी उपलब्ध है जिनमें धान्य, सब्जी, फल, तिलहन, दलहन, रेशा एवं अन्य फसल शामिल हैं। इस डेटाबेस में कुल 1,62,76,956 अनुक्रम प्रविष्टीयाँ संग्रहित कर ली गई हैं, जिन्हें NCBI (www.ncbi.nlm.nih.gov) के वेबसाइट से डाउनलोड किया गया है। इन अनुक्रमों को www.nrcpb.org के वेबसाइट से एक्सेस किया जा सकता है।

अतिसूक्ष्म प्रौद्योगिकी

AuNPS की हरीत संश्लेषण एक पर्यावरण अनुकूल एवं व्यवहार्य विकल्प है। जड़ प्रणाली के वृद्धि प्रतिक्रिया पर $KAuCl_9$

के विभिन्न सान्द्रता का प्रभाव निर्धारित करने के लिए वाइल्ड प्रकार के एराब्डॉपसिस के बिचड़ी को जल संवर्धन के द्वारा उपजाया गया। इसके बाद बिचड़ो को वैसे पोषक तत्व माध्यम में स्थानांतरित किया गया जिसमें विभिन्न सान्द्रता के $KAuCl_4$ मौजूद थे। यह अध्ययन AuNPs के हरित संश्लेषण के व्यवहार्यता को दर्शाता है।

मानव संसाधन

केन्द्र आरंभ से ही सक्रिय रूप से मानव संसाधन के विकास के लिए काम करता आ रहा है। इस केन्द्र पर वर्तमान में 46 छात्र/छात्राएं अपने स्नातकोत्तर एवं पी.एच.डी. कार्यक्रम को जारी रखे हुए हैं। इसके अतिरिक्त विभिन्न विश्वविद्यालय एवं संस्थानों के पच्चास से भी ज्यादा छात्र/छात्राएं पादप प्रौद्योगिकी के विभिन्न क्षेत्रों में प्रशिक्षण प्राप्त कर चुके हैं।

Executive Summary

National Research Centre on Plant Biotechnology (NRCPB), established in the year 1993, is one of the premiere research institutes of ICAR, engaged in research, teaching and training of personnel in the area of Plant Molecular Biology and Biotechnology. The centre has continued its momentum in conducting basic and applied research relevant to crop improvement resulting in many publications, patents and discoveries during 2011-2012. It has witnessed stupendous growth in terms of external funding to carry out research which would result not only in increased knowledge based on better tools for applied research but also highly trained manpower to undertake advanced research and development for the country as a whole. Progress of this Institute is evident from the two national level awards received during the year 2011-12; **Sardar Patel Outstanding ICAR Institution Award 2010** and **Mahindra Samriddhi Krishi Sansthan Samman – 2012.**

Research achievement of the institute during 2011-12 is presented under five major heads: Isolation of Plant Genes and Promoters, Transgenic for biotic stress tolerance, Biotechnology and climate change, Biotechnological approaches for increasing productivity and Genomics and molecular markers. Sailent research, teaching and training achievements of the centre during 2011-2012 are presented below in brief.

Plant Genes and promoters

A novel nematode-responsive root-specific promoter has been isolated and characterized this year which specifically expresses in gall/knot tissues which are formed in response to root knot nematode (*Meloidogyne incognita*) infection. This will be further utilized to target genes of invading nematodes using RNAi and thereby provide very effective control against nematode infection.

A pathogen inducible promoter has been isolated from Indian mustard (*Brassica juncea*) and characterized for its different cis-elements responsive to SA, JA, Ethylene, water stress and biotic stress by *in-silico* analysis.

A promoter of putative senescence protein that confers high expression in boll tissues of cotton has been identified and validated in model plant systems. The promoter was able to confer consistent and high *GFP* expression in Arabidopsis, tobacco and transiently in cotton bolls. This promoter is now being used to drive the expression of *cry2Aa* gene for genetic engineering of cotton for insect resistance.

Biotic Stress

NPR1, an antifungal gene, functionally an activator of transcription factors involved in defense signaling was previously isolated and used to transform Indian mustard (*Brassica juncea*) to develop resistance against *Alternaria brassicae* which causes black leaf spot. Transgenic plants showed 50% more resistance (as calculated by size of the lesions) compared to the non-transgenic lines.

Under the same objective of developing leaf spot resistant mustard, the wild relatives of mustard like *Camelina sativa* and *Sinapis alba* are being compared for level of resistance. It was observed that wild relatives are more tolerant to the *Alternaria* infection and hence efforts are on to understand the genes involved in this interaction.

A full length lectin gene has been isolated from chickpea and cloned under control of *rol*C promoter

in a binary vector. This construct was used for transformation of *B. juncea* cv Pusa Jaikisan. Transgenics so developed have been evaluated for function by aphid bioassay.

PCR amplification and cloning of Chickpea protease inhibitor gene in *pCAMBIA1300+NosT was carried out*. Plasmid DNA of pCAMBIA1300 with NosT (pCAM1300+NosT) binary vector was isolated from the clone confirmed above. *rolC* promoter was incorporated in pCAMBIA1300 already having NosT+CPPI binary vector. *W*hole construct prepared was confirmed by restricting with *Eco*RI and *Sal*I which gave fall out of 1.8kb fragment which included *rolC*+CPPI+NosT and backbone of binary vector pCAMBIA1300.

With an aim to tap the regulators of 2, 4 DAPG production, which is a broad spectrum antibiotic, a Tn5 mutant library of a PGPR, *Pseudomonas fluorescens* strain s218 was constructed and found to be highly efficient against tomato bacterial wilt pathogen, *Ralstonia solanacearum*. A gain of function mutant was identified with enhanced antibiosis and the molecular analysis of mutant revealed that the gene disrupted by transposon is a sugar transporter.

Bt protein (Cry1Ac) was targeted to chloroplast using the N-terminal transit peptide sequence and compared with cytosolic and endoplasmic reticulum -targeted proteins for analysis of Bt protein expression, stability and efficacy. Cry1Ac level was the least in cytoplasmic lines and the highest in ER targeted lines, while Cry1Ac level was in between in chloroplast targeted lines. Higher Cry1Ac protein level in organelle-targeted lines is due to less degradation and more stability. Therefore, Bt protein expression can be substantially increased by targeting it to any one of the organelle.

Gene targeting using *in silico* approach, a zinc finger nuclease (ZFN) pair that recognizes and cleaves a specific nucleotide sequence in *UGT* (UDP-glucosyl transferase) gene in Crossa rice genome was designed and synthesized. A total of nine independent lines were developed in Crossa variety and none of them were found in the targeted plants. The inability of the ZFNs when tested *in vitro* was attributed to ZFNs failing to recognize the DNA with the target sequence. Hence, ZFN proteins were redesigned for a single target site. Constructs for stable transformation have been made and rice transformation is in progress.

To facilitate high expression of Bt-ICP genes in cotton, a cuticle related gene AKS-1 having highest expression in leaves was selected for promoter characterization. Promoter sequences obtained of around 800 bp downstream to ATG. Second round of genome walking is under process. This promoter will be characterized by expressing GUS and comparing it with 35S CaMV and Rubisco small subunit (RbcS) promoters.

To understand the molecular mechanisms implicated in fibre development, a lintless-fuzzless mutant of *Gossypium hirsutum* cv. MCU5 was compared with fibre bearing ovules of wild-type by genome wide transcriptome analysis. Analysis of transcriptome data revealed that number of down-regulated transcripts was more as compared to up-regulated transcripts at various stages analyzed and the percentage of down-regulated transcripts was found to be high at fibre initiation and elongation stages. These down-regulated genes may be having crucial role in fibre cell differentiation and early elongation. This comprehensive transcriptome data provides valuable information on genes implicated in fibre cell development.

Transgenic rice tolerant to glyphosate has been developed by transforming popular Indian rice cultivar, IR-64 with codon optimized synthetic *CP4-EPSPS* gene encoding an EPSPS enzyme having less affinity towards glyphosate. The qualitative protein expression in the transgenic lines has been tested and six transgenic lines were found to be positive. Herbicide bioassay test confirmed that the transgenic rice can tolerate up to 1% commercial Roundup, the dose used to kill rice weeds in field conditions.

Quorum quenching by AHL lactonases produced by *Bacillus thuringiensis* is another approach being pursued for control of bacterial diseases. Screening of native Bt isolates recovered from diverse habitats in India as well as Bt type strains for the presence of *aiiA (autoinducer inactivation),* AHL-lactonase genes revealed few Bt strains to be positive from which cloning has been done. Sequence determination and analysis is in progress.

To achieve a better control of aphids, the Arabidopsis FPS2 gene utilised last year to generate transgenics, was cloned under CaMV 35S promoter to have enhanced level of farnesyl diphosphate (FDP). The transgenic Arabidopsis lines generated using this construct has been confirmed for expression levels of the transgene and also qualitatively tested for functionality by insect bioassays. The increase in the volatiles extracted from FPS-transgenics has also been verified by GC-MS profiles.

Abiotic Stress

The bacterial csp genes have been shown to improve the tolerance to various stresses in plants. Microbes isolated from soil were screened for tolerance to cold temperature (4^0C). Five of the isolates were identified as *Pseudomonas* sp. and *Providencia* sp. Full length *Csp*A gene was amplified from these microbes and sequenced. Similarly, bioprospecting of bacterial genes responsible for tolerance to high temperature was attempted. Soil and water samples were collected from different hot water springs with temperatures ranging from 45°C-72°C respectively. Isolates growing above the temperature of 55°C were selected for further analysis and identification of genes responsible for tolerance to high temperature. In relation to salinity stress in plants, increasing number of halophytes are being studied. Nearly 800 bp long upstream sequence of *SOS 1* gene from halophytic plant *Salicornia brachiata* has been cloned and sequenced.

Hydrilla verticillata (L. f.) has distinction with respect to photosynthesis in higher plants, as it switches to a C4 cycle from C3-cycle state at high temperature, and also lacks specialized Kranz type leaf anatomy that is found in terrestrial C4 species. *H. verticillata plants* were incubated at 34°C for 10 days to induce C4 photosynthesis, and 22°C to maintain the C3 state. Five forward subtracted libraries were generated from leaves of plants and enriched DNA fragments were cloned. Nearly one thousand clones have been picked and sequenced. Presently, Bioinformatic analysis is underway.

Annexins are soluble, multifunctional, lipid binding proteins encoded by large multigene family spread throughout the eukaryotic and prokaryotic domains. They are capable of linking Ca2+, redox and lipid signaling to coordinate development with response to the biotic and abiotic environment. During the reporting period, a 587 bp upstream region from the initiation codon and a 210bp of the coding region of Annexin6 gene *(AnnOsI6)* from a rice drought tolerant variety Nagina22 were amplified and deletion constructs made (P1-5$_{AnnOsI6}$) by fusing with reporter gene GUS in binary vector pBI121. The fragment harbored several known cis-elements that respond to hormone treatment and regulate stress induced gene expression.

For long term analysis transgenic tobacco were developed with the P4$_{AnnOsI6}$ construct. Constitutive expression was observed in control plants transformed with pBI121. Differential expression of GUS was observed in various plant organs under different stress. This study provide promising results for an active functional promoter under abiotic stress which is being further investigated.

Bajra (*Penniseteum glaucum*) is one of the few plant species which has remarkable ability to tolerant different environmental stresses. To look for robust allele of the drought responsive genes primers were designed based on heterologous sequence information available in the database for the candidate genes for moisture stress tolerance and amplification of the candidate genes were carried out in *Penniseteum glaucum.* Amplification of P5CS gene in other cultivars/

genotypes/species/ breeding lines is being attempted and successful amplification obtained. RACE primers were designed and 5'-3' RACE carried out to amplify the full length P5CS gene in *Penniseteum glaucum*. A 1276bp nucleotides of P5CSwas amplified and sequenced. Further in this crop, drought Stress was induced in one month old seedlings and suppressive cDNA library was constructed. Annotation of sequencing data reveals homology with genes related to biotic and abiotic stresses.

In continuation of our efforts to identify genes involved in drought tolerance in chickpea using functional genomics, this year, ESTs coding for AP2/ ERF protein were isolated as differentially expressed in response to terminal drought stress, from chickpea (*Cicer arietinum* L.). The ERF subfamily genes, having AP2 domain of 58-60 amino acid, are mainly involved in response to biotic and abiotic stresses by recognizing the *cis*-acting element AGCCGCC, known as the GCC box. An expression study of few members of chickpea ERF family using RT-qPCR showed ubiquitous expression pattern in all the organs while some exhibit tissue specific expression. Further, the expression pattern of previously identified differentially expressing unigenes was investigated in RILs generated from cross between ICC4958 (drought tolerant) and ICC1882 (susceptible) using a nylon macroarray . Unigenes encoding MYB transcription factor, Sulfate transporter, Betaine aldehyde dehydrogenase, transcription regulator protein and transcription elongation factors etc. were upregulated in High Root Biomass (HRB) RILs (similar to the ICC4958 response) under terminal drought stress condition. Interestingly, another set of genes including ATP binding protein, kinase associate protein, PRP, Calcium ion binding proteins, WD40 domain protein etc. which showed up regulation in ICC4958 (HRB line) were down- regulated in RILs of HRB under drought condition.

Productivity Enhancement

Heterosis breeding in mustard programme was continued and several hybrids based on *Moricandia arvensis* CMS system were evaluated of which two hybrids namely, Hybrid 23 and Hybrid 2007A showed 18-30% superiority for seed yield over the check variety Varuna. These results show significant influence of environment on yield of hybrids and need multi-location trials over the years to identify superior hybrids.

In Brassica, physiological studies have shown that silique wall photosynthesis makes major contribution to the seed reserves. Hence, the significance of leaf photosynthesis to seed yield is remains a mystery. A significant lower amount of chlorophyll content was recorded in an albino silique mutant in *B. rapa* than that of green silique. To understand the genetic nature of the mutation, crosses were made between the albino mutant and wild type, and albino silique was found to be dominant over green silique and segregated as a monogenic trait. But no linkage was detected between this triat and seed colour or locule number and seed set albino esilique plants were normal. These results are at variance with the current view regarding the contribution of silique photosynthesis to seed yield.

Centromere histone proteins play pivotal role in chromosome segregation during cell division and genetic engineering of CENH3 has been shown to be useful in creating haploid-inducer lines in Arabidopsis. Since such haploid-inducer lines could accelerate breeding, work has been initiated to develop haploid-inducer lines in *B. juncea* by substituting native CENH3 proteins with engineered CenH3 tailswap protein.

Research work isolation of genes governing nucellar polyembryony in citrus was continued, and a microarray analysis of citrus ovule transcriptome was carried out during the reporting period. A strong difference in gene expression was found between the pre-anthesis stages of poly- and mono-embryonic cultivars. Attems were made to identify transcripts related to polyembryony. Functional annotation and target description of these transcripts indicates that

majority (20%) of these transcripts belong to HSPs and also includes the transcripts we identified in previous SSH study.

Modulation of phytohormones concentration in mustard by genetic engineering to increase yield was attempted. Genes governing cytokinin homeostasis in plants such as cytokinin oxidase/dehydrogenase (CKX) was isolated from mustard by designing PCR based primers using *Arabidodpsis* gene homology. The gene cloned is *ckx5* as it showed 99% similarity to the *ckx5* of *Arabidopsis thaliana*. For efficient expression of *ckx* gene exclusively in roots, a root specific promoter was isolated from *Arabidopsis*. Currently, cloning of *ckx5* under root specific promoter is under progress.

Phosphorus (P) plays a central role in metabolic processes like photosynthesis, respiration, glycolysis, maintenance of redox balance, and energy synthesis and is an indispensable building block for the biosynthesis of nucleic acids and phospholipids. To modulate P homeostasis, plants must balance P uptake, mobilization, and partitioning to various organs. Now efforts are underway to identify transcription factors (TFs) regulating Pi homeostasis.

Genomics and Markers

Pigeonpea (***Cajanus cajan***) is an important grain legume of the Indian subcontinent, South-East Asia and East Africa. Genome sequencing of popular pigeonpea variety "ASHA" was completed during the reporting period successfully. The large sequence contigs, representing about 60% of the estimated 858 Mb size of the pigeonpea genome were used for the analysis of genes and repeat contents of the genome and mining of SSR loci. Total 59,515 genes were predicted out of which 47004 were protein coding genes and 12511 genes were TE related. All protein coding genes were further classified into several functional categories. Further, high density intra-species reference genetic map of pigeonpea based on 366 genic-SNP markers were developed. Development and validation of hyper variable hassr markers for pigeonpea reveraed that the HASSR polymorphism was much higher than the earlier reported for bac-end sequence derived genomic SSR markers. This underlines the high potential utility of the hassr markers in pigeonpea molecular breeding.

Pigeonpea and soybean belong to the same clade Millettieae of the plant family Fabaceae, a comparative analysis of pigeonpea and soybean has been carried out. Low level of synteny between pigeonpea and soybean suggests that they might have only one genome in common and both are ancient amphiploids. This is the first plant genome sequence completed entirely through a network of Indian institutions led by the Indian Council of Agricultural Research and provides a valuable resource for the pigeonpea variety improvement.

During the reporting period, approximately 238 GB wheat genome sequencing data was received, from TGAC, BBSRC, Norwich, UK which were further analysed. Similarly a total no. of 56832 BAC clones were obtained from Czech Republicand they are being fingerprinted using SNaPShot Technology.

Mapping QTLs for drought tolerance and associated traits in wheat is in progress. A mapping population of 206 RILs derived from across between drought sensitive high yielding wheat variety WL711 and drought tolerant traditional wheat variety C306 was used for this purpose. Total thirty seven QTLs were identified for ten drought related traits in wheat. Further a number of candidate genes for drought tolerance using a combination of QTL mapping and microarray expression profiling approaches has been identified. Transcriptome profiling of the parents and Recombinant Inbred Lines (RILs) bulks with extreme phenotypes revealed five genes underlying this QTL that were differentially expressed between the parents as well as the two RIL bulks, suggesting that they are likely candidates for drought tolerance. Syntenic genomic regions of rice, sorghum and maize were identified that also harbour genes for drought tolerance.

Characterization of Near isogenic Lines (NILs) for seed storage proteins in wheat continued during the reporting period. Thirty one NILs comprising different alleles of HMW, LMW, gliadins, albumins and triticin were evaluated for bread making quality. Glutenin alleles *GluD1d, GluB1b, GluA3d* and triticin alleles Tri*D1a,* Tri*A1a* shows consistent positive effect on dough stability which was determined by farinograph. Gliadin alleles have no significant effect on bread making quality except *GliB1c*, GliA1g,* and *GliA1h* which shows negative effect on SDS sedimentation volume and loaf volume. *GluD1d, TriD1a, TriA1a* alleles shows consistent positive effect on farinograph dough stability, SDS sedimentation and loaf volume. NILs of *GluD1d, GluD4a, GluB1b, TriD1a* and *TriA1*a shows consistent positive and *GluA3c, GliB1c*, GliA1g,* and *GliA1h* shows consistent negative effect on bread making quality when compared with HD2329. These alleles are of potential value in breeding programmes designed to improve bread-making quality.

Aroma, a quantitative trait, is one of the most important quality attributes of basmati rice. The complex inheritance of this trait makes it difficult for the identification of genes responsible for aroma using any one single analytical approach. Two extreme bulk groups were made comprising of six highly aromatic and six non-aromatic RILs. RNA of each segregating line were pooled together and used for making cDNA copies through in vitro hybridization. The differential expression of genes was analyzed and the number of differentially expressed genes to about 160. 16 between the RIL bulks are found to be co-located in the QTL region aro4.1, whereas 2 genes have been identified to be differentially expressed in the QTL region aro3.1. Proteomics approach is now being employed to further validate these results/genes.

A molecular map is developed in rice solely based on SNPs from a biparental recombinant inbred population developed from the parents Nagina22 and IR64, which are drought and heat tolerant and susceptible cultivars respectively. This map will be very helpful in identifying genomic regions (quantitative trait loci-QTLs) associated with heat and drought tolerance once appropriate phenotypic data sets are available.

Microsatellite distribution in Monocot and dicot species were studied during the reporting year. A total of 797,863 SSRs were identified for the six genomes including three of each monocot *(Brachypodium, Sorghum and Rice);* and dicot *(Arabidopsis, Medicago* and *Populus)* plant species.

Spot blotch of wheat caused by *B.sorokiniana* is a destructive disease of wheat particularly in the warm and humid parts of India and is slowly spreading into cooler traditional areas of wheat also. Studies are continuing to identify the loci underlying spot blotch resistance so as to incorporate durable resistance into the high yielding popular varieties of India. Fresh crosses were effected between the susceptible parent HD2932 and the resistant donors SW89-5422 and Chirya1 in Rabi 2010-2011 and the F1s were advanced in summer 2011. Currently the two F2 mapping populations are being evaluated. Screening of the parents using mostly SSRs including known linked markers to spot blotch resistance is in progress.

Earlier two independent homozygous transgenic lines of Pusa Sugandh2 carrying *AtDREB1A* gene were identified and screened for their response to drought and cold. Experiment on cold stress was repeated for T_{4} plants. Up-regulated expression of the *DREB* gene was observed in stressed transgenic plants over control.

Functional genomics studies on *Alternaria* blight resistance in Brassica carried out with sequencing of 456 clones from the SSH library of cv. *Varuna*. Sequences of the clones were subjected to BLAST analysis with EST database of *Arabidopsis*, which revealed 248 differentially expressed genes belonging to different functional categories. Besides 176 ESTs did not have any match in the *Arabidopsis* sequence databases. A SSH library was also constructed using

29 day stressed plants of *S. alba*. A total of 750 recombinant clones containing the differentially regulated transcripts were obtained and sequenced from this library which revealed 144 candidate genes, which are homologous to transcription factors and genes belonging to other metabolic pathway.

A network project on "Phenomics of moisture deficit and low temperature tolerance in rice" has been initiated during reporting year with funding from National fund, ICAR. Publically available transcriptome data generated from various experiments at different developmental stages of rice were analyzed to identify drought responsive genes. Six genes showed high levels of expression in Nagina22 (tolerant) as compared to IR-64 (susceptible) under drought conditions in the microarray data generated from previous study, of which five had more than 2 fold increase (in expression in Nagina22 seedlings. The genes were amplified and cloned in cloning vector pGEMT. Sequencing of these genes was performed and subcloning was done in plant expression vector under drought inducible *AtRD29A* promoter and constitutive *ZmUbq1* promoter for functional validation.

Genetic transformation of rice using cry1Aabc was carried out in Pusa Sugandh 2. A total of 23 putative transformants were obtained, which were analysed for the presence of transgene through PCR using *cry*1Aabc gene specific primers and six putative transformants were confirmed positive for the PCR amplification of transgene. Presently, plants are hardened in soilrite at culture room condition.

In order to study the nature of gene expression and confirm the involvement of upstream elements in regulating the expression of *Pi-k*^{h} (*Pi 54*) gene in blast resistant transgenic lines, quantitative Real Time RT-PCR (qRT-PCR) was performed using *Pi54* exon specific primers. Expression of *Pi54* gene was compared after 0 to 96 h post inoculation (hpi) of TP-*Pi54*-2, TP-*Pi54*-15, and Taipei 309. *Pi54* gene was found to be expressed 2.31 fold higher in TP-*Pi54*-2, 1.5 fold higher in TP- *Pi54*- 15 after 72 hpi. In contrast, the susceptible allele was found to express lower in Taipei 309. Global gene expression changes happening in response to the infection by *M. oryzae* at 72 hpi, in resistant TP-*Pi54* and susceptible wild Taipei 309 (TP309) were investigated and compared using microarray based transcriptome analysis. In order to understand the genome wide co-expression of genes in the transgenic rice line containing *Pi54* gene, microarray analysis was performed at 72 h post inoculation of *M.* oryzae strain PLP-1. Various defense response genes were found to be up regulated in transgenic lines.

Rice blast caused by *Magnaporthe oryzae* is the most destructive disease affecting rice production worldwide. *Pita* is one of the dominant blast resistance genes which is effective under Indian conditions. Alleles of this genes has been mined in land races of rice collected from different parts of the country. Besides, *Pi-ta* orthologue sequences of 220 rice accessions were retrieved from the database and included in the analysis. *Pi-ta* orthologues of Indian land races were found scattered in most of the major haplotypes indicating its heterogenous nature Five unique and novel Pi-ta protein variants were identified from the Indian land races.

Under the ongoing network project on "Bioprospecting of genes and allele mining for abiotic stress tolerance" funded by NAIP of ICAR, to identify a minicore reference set in rice, 7000 genotypes constituting the core set were collected, multiplied and phenotyped in the last two years. These accessions were genotyped at 36 fluorescent dye labelled microsatellite marker loci, uniformly distributed across all the 12 rice chromosomes, using an automated fragment analysis system in the current year. SSR Genotyping yielded a total of 440 alleles with an average of 12.57 alleles per locus. Using both genotypic and phenotypic data sets, a minicore constituting a total of 228 genotypes was identified. The identified minicore set is further being genotyped for a selected set of SNPs (5246) present in abiotic

responsive genes. Gene expression profiling revealed 1977 up regulated and 2930 down regulated genes at a minimum of two fold change.

During the reporting period, a total of 507 selected mutant lines were grown in *Kharif* 2011 under irrigated conditions of which 382 were phenotypically characterized according to DUS descriptors. DNA Fingerprinting of Nagina22 and these 382 Mutants was carried and analysis of the seven mutants data two years, revealed monogenic inheritance for all of them. A gain of function mutant for moisture stress tolerance was identified from screening of 500 mutants in 25% PEG6000, which was found morphologically and genetically similar to the wild type, Nagina22.

EMS induced mutants of upland rice variety Nagina-22 generated (> 20000) across centres have been redistributed among project partners and sub-sets of these lines have been phenotyped for various important traits. This has led to identification of putative mutants for moisture stress tolerance, salt tolerance, efficient phosphate utilization and biotic stresses such as bacterial blight resistance and blast resistance. A set of 382 mutants from the mutant garden was characterized at appropriate growth stages according to DUS descriptors. In addition to this, the study of inheritance was done for more than 25 mutants, most of which were found to be governed by single genes. All these data have been added to the Rice Mutant Database (RMD).

A local database of genomic resources of crop plants were developed at this centre and named it as Plant Genome database. Sofar this database consisted of information for 79 plant species, which include cereals, vegetables, fruits, oilseeds, legumes, fibers and others. A total of 1,62,76,956 sequence entries are stored, which were downloaded from NCBI (www.ncbi.nlm.nih.gov) web site. These sequences can be accessed from www.nrcpb.org web site.

Nanobiotechnology

Green synthesis of AuNPS is an environment-friendly viable alternative. To determine the effects of varying concentrations of $KAuCl_4$ on the growth response of the root system, wild-type Arabidopsis seedlings were grown hydroponically. Subsequently, the seedlings were transferred to the nutrient medium supplemented with different concentrations of $KAuCl_4$. The study clearly demonstrated the feasibility of green synthesis of AuNPs.

HRD

Since its inception, the centre has been actively engaged in human resource development. At present 46 students are pursuing their Ph.D and M.Sc. degree programme from our centre. In addition more than 50 students from different universities and institutions were trained in various areas of plant biotechnology.

About the Centre

- NRCPB and the Mandate
- Staff Position
- Financial Statement
- Resource Generation


लाल बहादुर शास्त्री केन्द्र
LAL BAHADUR SHASTRI CENTRE

NRCPB

National Research Centre on Plant Biotechnology (NRCPB) is the premiere research institution of the Indian Council of Agricultural research (ICAR), engaged in molecular biology and biotechnology research. The Biotechnology Centre, established in 1985, as part of the Indian Agricultural Research Institute (IARI), was upgraded to a National Research Centre on Plant Biotechnology in the year 1993, with a vision to impart the biotechnology advantage to the National Agricultural Research System (NARS). The NRCPB has acquired, in the past, an excellent infrastructure in terms of equipment and other physical facilities and also a high degree of scientific competence. Development of transgenic crops for biotic and abiotic stress management, exploitation of heterosis through marker and genomic approaches, marker assisted selection and molecular breeding of major crops for productivity and quality enhancement, search for novel genes and promoters for efficient native and transgene expression are the major activities taken up by the centre. There is now considerable emphasis on structural and functional genomics of crop species such as rice, pigeonpea, chickpea, cotton, tomato and wheat in the centre. In addition to research, the centre is contributing significantly to competent human resource development by way of offering regular M.Sc and Ph.D programmes by partnering with PG School, IARI.

In order to develop strong inter-and intra-institutional linkages for promoting and strengthening plant biotechnology research in the ICAR system, the Centre has been identified as the lead centre. It has established strong linkages with various research institutes in the country including ICAR, CSIR, State Agricultural Universities and CGIAR institutes like ICRISAT. The center is now placing considerable emphasis on the development of products, processes, patents and research publications in journals with high impact factor. It also encourages and practises Public-Private Partnership (PPP) mode for commercializing the products of genetic engineering.

Mandate

- To undertake basic plant molecular biology research for understanding molecular mechanisms underlying basic biological processes;
- To develop capabilities of devising tools and techniques of biotechnology and genetic engineering for crop improvement;
- To use the knowledge gained and technologies developed for advancing agricultural development;
- To serve as a national lead center for plant molecular biology and biotechnology research and create trained manpower in the area of plant biotechnology.

Staff Strength of the Centre

Staff	Sanctioned Strength	Filled	Vacant
Scientific	34+1 (RMP-PS)	28+1	06
Technical	16	13	03
Administrative	18	07	11
Skilled Supporting Staff	01	01	-
Total	70	53	16

Financial Statement 2011-12

(Rs. in Lakh)

	Plan		Non-plan	
	Allocation	Utilization	Allocation	Utilization
Capital	257.00	256.48	10.00	7.16
Revenue				
Establishment Expenses	0.00	0.00	442.00	441.40
Traveling Allowances	0.21	0.21	2.00	1.93
Research and Operational Expenses	151.00	150.81	40.00	39.77
Administrative Expenses	189.57	190.22	73.00	74.35
Miscellaneous Expenses	2.22	2.26	0.00	0.83
Total	**600.00**	**599.98**	**567.00**	

Resource Generation

Sale of Farms Produce	0.00
License Fee	0.00
Leave Salary and Pension Contribution	0.00
Interest Earned on Short Term Deposits	39.31
Income Generated from Internal Resources	85.35
Miscellaneous Receipts	5.01
Total	**129.67**

Funds Received from Externally Funded Projects

Externally Funded Projects	415.97
Consultancy Projects	9.94
Total	**425.91**

Research Achievements

1. Isolation of Plant Genes and Promoters
2. Development of Transgenics for Biotic Stress Management
3. Biotechnology and Climate Change
4. Biotechnological Approaches for Increasing Productivity
5. Genomics and Molecular Markers
6. Honorary Scientists' Projects

1. Isolation of Plant Genes and Promoters

Isolation and characterization of nematode-responsive root-specific promoter

Last year we reported isolation of an Arabidopsis line exhibiting nematode-responsive and root-specific GUS expression. This year we isolated another nematode-responsive root-specific promoter and this is all the more specific because expression is restricted only to gall/knot tissues which are formed in response to root knot nematode (*Meloidogyne incognita*) infection. Using *in silico* approach, meta-profile analysis of microarray datasets for root specificity and response to nematodes led to the identification of specific genes. This gene showed consistent up regulation at 7-day post infection (using root knot nematode) in the gall tissues (Fig. 1.1). Upstream region (1525 kb) of this gene was cloned and fused with *GUS* reporter gene. GUS assay in transgenic plants confirmed that the upstream sequence directs the expression of the *GUS* reporter gene specifically in galls in response to nematode infection. These regulatory sequences would be immensely helpful to target gene of invading nematodes (by eliciting RNAi) and thereby provide very effective control against nematode infection.

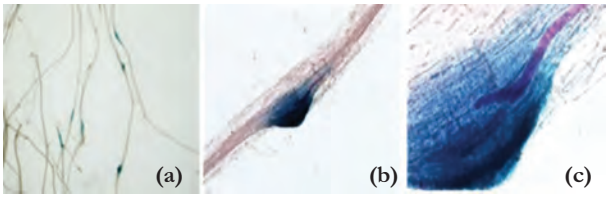


Fig. 1.1: In response to root-knot nematode infection, transgenic *Arabidopsis* plants harbouring the identified promoter along with reporter *GUS* gene shows expression of GUS in response to nematode infection in galls present in roots (a), a magnified view of root section with gall shows GUS expression (blue colour) and J2 worm (red colour) (b) and still higher magnification clearly shows GUS expression and nematode (c).

Functional genomics of drought tolerance in chickpea

Identification and characterization of chickpea ERF transcription factor

As a crop of arid and semi-arid regions, chickpea often suffers from the terminal drought stress which limits the productivity. Identification of candidate genes involved in drought tolerance is one of the important steps towards conferring drought tolerance in chickpea. Several candidate genes associated with drought tolerance in chickpea were identified in our previous studies. Recently, ESTs coding for AP2/ERF protein were isolated as differentially expressed in response to terminal drought stress, from chickpea (*Cicerarietinum* L.) using cDNA SSH libraries and computational biology approach. The ERF family contains a single AP2/ERF domain, and are sometimes further divided into two major subfamilies, CBF/DREB subfamily and the ERF subfamily. The ERF subfamily genes, having AP2 domain of 58-60 amino acid, are mainly involved in response to biotic and abiotic stresses by recognizing the *cis*-acting element AGCCGCC, known as the GCC box. An expression study of few members of chickpea ERF family using RT-qPCR was performed. Interestingly, some of the ERF transcripts showed ubiquitous expression pattern in all the organs while some exhibit tissue specific expression (Fig. 1.2). Several members of AP2/ERF transcription factors have been shown as regulators of drought stress responses in diverse plant species. Additionally, the expression pattern of

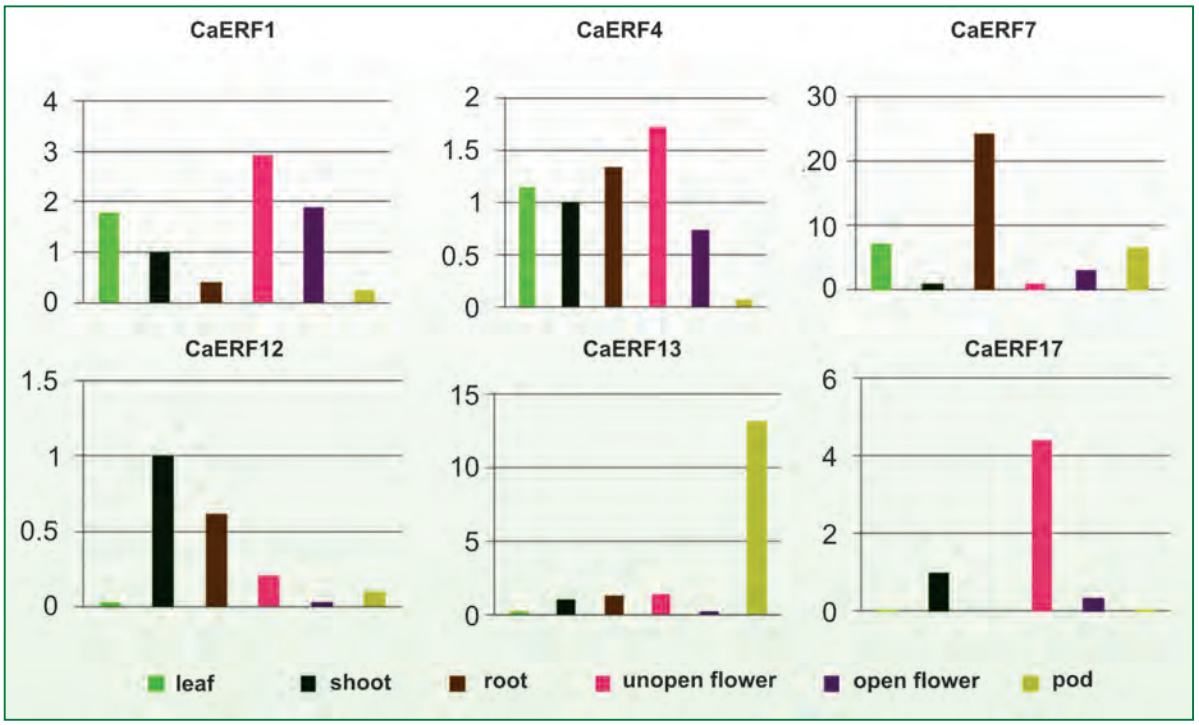


Fig. 1.2: Expression profile of CarERF genes. Relative fold change of six CarERF genes analyzed using QPCR under different tissues *viz.* leaf, shoot, root, unopened flower, opened flower and pod

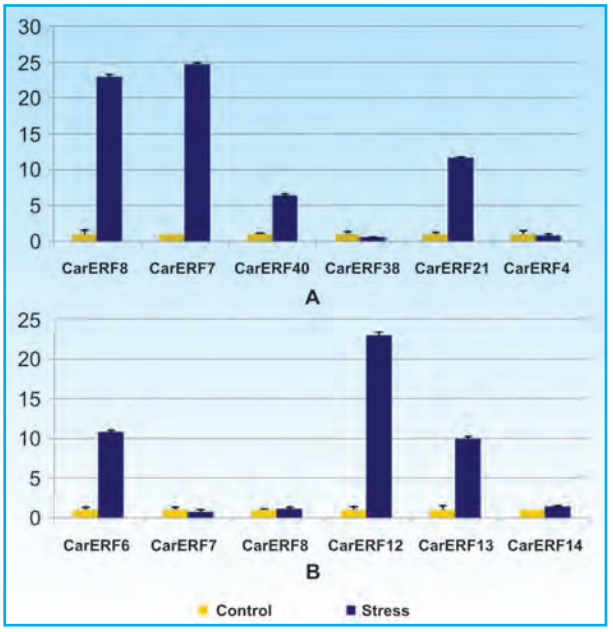


Fig. 1.3: Expression profile of CarERF genes. Relative fold change under drought stress (A) and wilt stress treatment (B) using QPCR. Y-axis represents relative expression value and X-axis represents control and stress samples. Error bars show standard error between three biological replicates

some genes under drought and wilt stress conditions was studied by RT-qPCR (Fig. 1.3). CarERF8 and CarERF7 showed higher up regulation under drought stress while, CarERF12 showing twenty folds up regulation under wilt stress. Similar study is going on other members of ERF family identified from chickpea cDNA SSH library.

Expression analysis of differentially expressed genes in selected RILs under terminal drought stress

We analyzed genotype specific response of 830 unigenes and identified 152 differentially expressed unigenes (136 up-regulated and 16 down-regulated) between drought tolerant (ICC 4958) and susceptible (ICC 1882) lines in response to terminal drought stress. The results obtained from macroarray analysis were validated using qRT-PCR analysis. qRT-PCR confirmed the differential expression of these genes under terminal drought stress conditions.

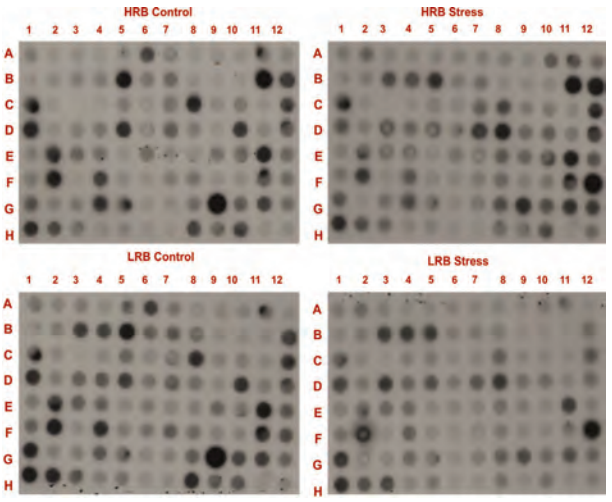


Fig. 1.4: Representative images of macroarray. 152 differentially expressed genes between ICC4958 and ICC1882 were screened on selected RILs. HRB: High Root Biomass lines, LRB: Low Root Biomass lines

Further, in order to investigate the expression pattern of these genes in RILs generated from cross between ICC4958 and ICC1882, a nylon macroarray with the selected differentially expressed genes were constructed (Fig. 1.4). Ten RILs each, of extreme HRB (High Root Biomass) and LRB (Low Root Biomass), were selected for this analysis. Bulks of these lines (10 each) were constructed by pooling equal amount of total RNA from each RIL. RNA samples were labeled during first-strand cDNA synthesis in the presence of α-^{32}P dCTP and used as probes. Well watered (control) and water stressed samples of both HRB and LRB were used for macroarray analysis.

The analysis of the macroarray revealed that 21.32% of total analyzed genes (Cluster II, in heat map, Fig. 1.5) showed up regulation in HRB RILs (similar to the ICC4958 response) under terminal drought stress condition. The Cluster II contains unigenes encoding MYB transcription factor, Sulfate transporter, Betaine aldehyde dehydrogenase, transcription regulator protein and transcription elongation factors etc. Role of these genes in various stress condition has been well documented in several plant species.

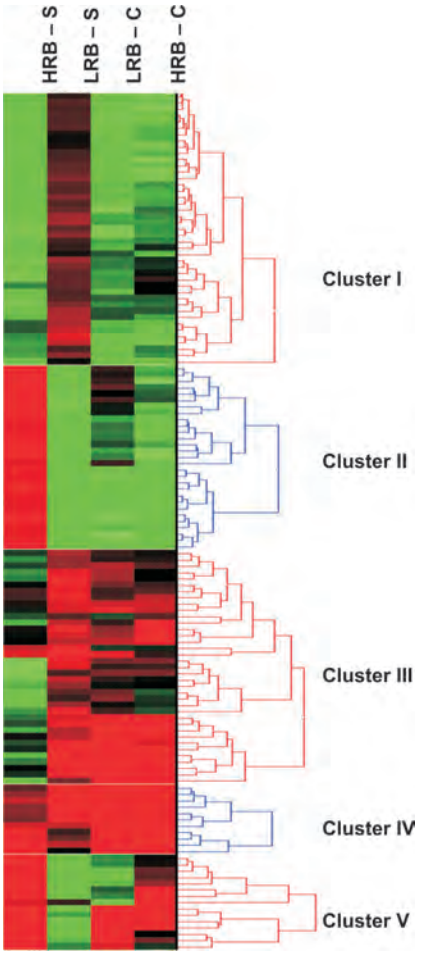


Fig. 1.5: Hierarchical clustering (average linkage and Euclidean distance matrix with the minimum similarity of 0.5) of 136 unigenes was performed using HCE version 3.5 beta web tool. Clustering of unigenes based on normalized signal intensity into five clusters (I, II, III, IV and V). HRB-C: High Root Biomass Control, HRB-S: High Root Biomass Stress, LRB-C: Low Root Biomass Control, LRB-S: Low Root Biomass Stress. Colour scale (from green to red)represents the range of expression level.In the heatmap red represents normalized expression values greater than themean, green colour represents expression less than the mean andcolour intensities in between the two represent the magnitude ofthe deviation from the mean

Interestingly, some of genes showing up regulation in ICC4958 (HRB line) were down- regulated in RILs of HRB under drought condition (cluster I in Heat map). This set of genes includes ATP binding protein, kinase associate protein, PRP, Calcium ion binding proteins, WD40 domain protein etc.

Genetic engineering for *Alternaria* resistance in Indian mustard

Alternaria brassicae which causes black leaf spot in *Brassica juncea* can lead to great yield loss particularly in favourable conditions. Full length cDNAs of many antifungal genes from SA-and JA- induced *Brassica juncea* libraries were isolated. Among many, NPR1 is one such antifungal gene isolated in the lab. NPR1 is an activator of transcription factors and is involved in defense signaling. Four transgenic lines of *Brassica juncea* having NPR1 gene were subjected to *in vivo Alternaria brassicae* infection.Transgenic plants showed 50% more resistance (as calculated by size of the lesions) compared to non-transgenic plants (Fig. 1.6). This work has to be repeated to confirm the results. Screening of other transgenic lines has to be done.

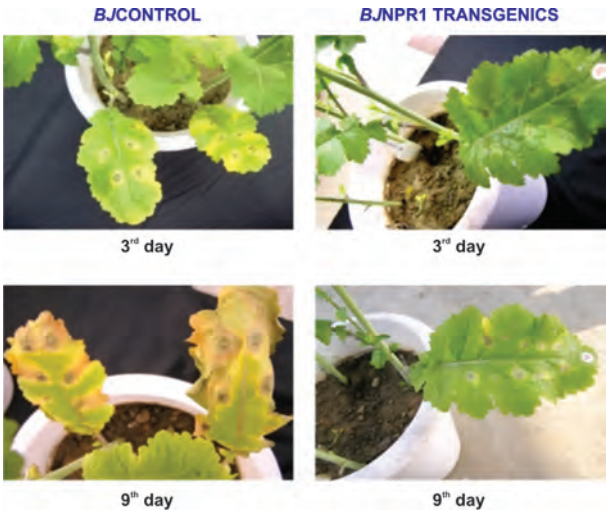


Fig. 1.6: *Brassica juncea* non- transgenic (control) and transgenic (with NPR1) plants after infection with *Alternaria brassicae* infection

Wild relatives of *B. juncea* (*Camelina sativa* and *Sinapis alba*) are much more resistant to Alternaria as compared to Brassica. Therefore, it is important to understand how the wild relatives behave in the presence of Alternaria. Infection patterns of *Alternaria brassicae* on *Brassica spp* and its wild relatives (*Camelina sativa* and *Sinapis alba*) were studied. On *B.juncea*, symptoms appeared after 2 days. Within 5days lesions as big as 10mm were seen. On *Sinapis alba* symptoms appeared after 2 days. On 5th day lesions were seen. But lesions were only of 5mm size. *Camelina* showed no symptoms and was highly resistant (Fig. 1.7). Understanding mechanism of resistance in wild relatives will help in developing Alternaria- resistant Brassica plants.

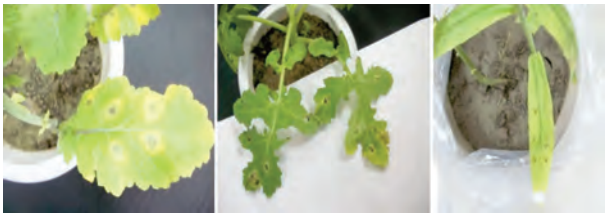


Fig. 1.7: Response of *B.juncea* and its wild relatives (*S. alba and C.sativa*) *to A. brassicae* infection. Symptom development in *Brassica juncea* (a), *Sinapis alba* (b) and *Camelina sativa* (c) after five days of infection

Isolation of pathogen-inducible promoter

Presently most of the transgenics being developed all over the world are with constitutive promoters.

Fig. 1.8: *B. juncea* chitinase gene promoter sequence (about 2 Kb) depicting basic elements like TATA and CAT boxes, and elements responsive to various stresses and defense signaling hormones

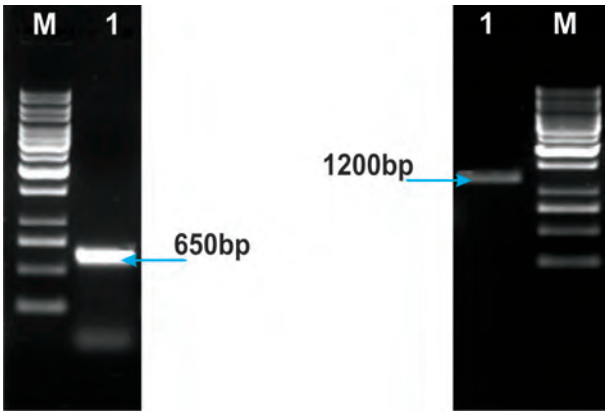
This constitutive expression of the foreign gene taxes unnecessarily the energy demand on the plant. Therefore it is important to isolate specific promoters which are tissue- specific, development specific, and in the present study the pathogen-inducible promoter. A 2.5kb promoter of chitinase gene (induced by pathojen) was isolated in one go from *B.juncea* and sequenced. Different cis-elements responsive to SA, JA, Ethylene, water stress and biotic stress were identified by *in-silico* analysis (Fig. 1.8).

Isolation, cloning and validation of novel genes and promoters for developing insect resistant crop plants

The world population is increasing at an alarming rate and is expected to be doubled in the next 40 years. There is a need for food production increase by 250 percent. This includes the exploration of dwindling resources for the achievement. So clearly, biotechnology with its ability to improve yield, quality and nutritional value will help in feeding today's and tomorrow's population. The application of plant transformation to the control of plant pests has become one of the major practical success stories of plant biotechnology in the past decade.

Completion of partial lectin gene from Chickpea to full length gene

Total RNA from developing seeds of chickpea was isolated using LiCl method and mRNA was isolated using Oligotex mRNA isolation Kit (Qiagen). From the partial sequence of lectin gene the nested set of complementary primer was designed. 5'RACE was carried out using Ambion's RLM RACE Kit. The 650 bp amplicons (Fig. 1.9A) was eluted from the gel and cloned in pGEMT Easy vector and sequenced. Forward and reverse primer from the two amplicons was designed and a full length amplicons of size 1200bp was amplified (Fig.1.9B), cloned, sequenced and yielded a full-length sequence with an ORF of 834 bp.



(A) (B)

Fig. 1.9: (A) 5' RACE of partial cDNA of Chickpea lectin gene (B) Full length amplicon of Chickpea lectin gene

Construction of plant transformation vector using chickpea lectin gene

The rolC promoter was amplified from the plasmid with specific primer having *Xba*I/ *Eco*RI restriction site and cloned in pUC 29 vector. Similarly, chickpea lectin cDNA was amplified using primer having *Eco*RI/*Not*I and cloned in pUC vector. Both rolC promoter and lectin gene were subcloned in binary vector pORE4 (Fig. 1.10). This gene construct was mobilized into *A. tumefaciens* GV3101 by direct freeze thaw method for transformation of *Brassica juncea*.

Fig. 1.10: Diagrammatic representation of Chickpea lectin gene and rolC promoter in a binary vector pORE4

Transformation of *Brassica juncea* using developed construct

The hypocotyls and stem segments from *in vitro* grown *Brassica juncea* cv Pusa jaikisan plants were cut and infected with *Agrobacterium* harboring chickpea

lectin gene construct for 30min, blotted dry and sub cultured on MS media supplemented with only plant growth regulators. After 2 days they were transferred to regeneration medium having cefotaxime and after 10 days transferred to selection medium having kanamycin as a selection agent till the green shoots appeared (Table 1.1 & 1.2).

Table 1.1: Transformation of *Brassica juncea* cv. Pusa Jaikisan (hypocotyls)

No. of explants co-cultivated	No. of explants transferred in selection medium	No. of explants showing green shoot formation	Total No.of shoots developed
300	145	22	35
250	105	19	34
408	187	32	44
327	132	24	32
256	65	12	21

Table 1.2: Transformation of *Brassica juncea* cv. Pusa Jaikisan (stem segments)

No. of explants co-cultivated	No. of explants transferred in selection medium	No. of explants showing green shoot formation	Total No.of shoots developed
48	15	14	30
52	10	8	16
59	19	11	24
56	21	12	28
78	34	15	33

Segregation of Transgenic Mustard harboring pigeon pea lectin gene

The T1 seeds of transgenic mustard were selected on selection medium (1/2 MS media supplemented with 100mg/l kanamycin) (Fig 1.11) and after hardening seedlings were subsequently transferred to net house and Phytotron for further growth. T-2 progeny was also raised to generate the material for aphid bioassay experiments.

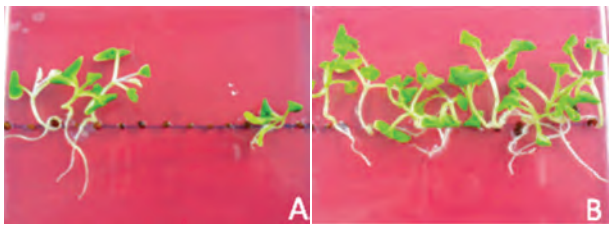


Fig. 1.11: T-1 seeds germination on Kanamycin selection medium

Molecular analysis of transgenics

RT PCR of T-1 generation Transformants harboring pigeon pea lectin gene:

Total RNA from control and transgenic lines was isolated and analyzed by RT-PCR to detect the level of expression of transgenes. RT-PCR analysis with *npt* II primers showed the ~750bp amplification corresponding to gene transcript and no amplification

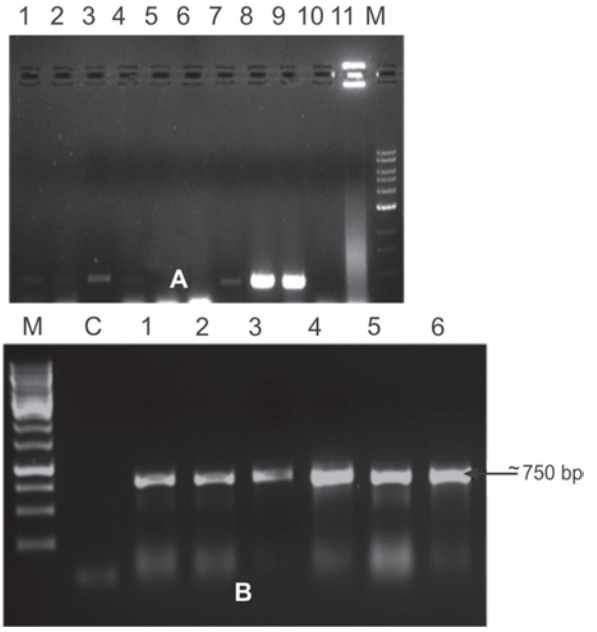


Fig. 1.12: RT-PCR analysis of T1 Plants of *B. juncea* (A) with gene specific primers (B) with *npt II* primers

was detected when RNA from control plants was used (Fig. 1.12 A). Similarly, RT-PCR of total RNA with gene using specific primers showed the amplicons at 832 bp (Fig. 1.12B).

Evaluation of transgenics for the targeted trait expression:

Aphid bioassay:

T-1 plants of *Brassica juncea* were used for aphid bioassay. The aphids (*Lipaphis erysimi*) were collected from the mustard fields of IARI Campus, New Delhi. Leaves were collected from transgenic as well as control plants and placed onto petri plates with their cut ends dipped in agar medium. 15 aphids were placed over each leaf kept in three replicas for each individual transgenic line as well as control untransformed plants. Experiment was carried out in the germinator maintained at 25±1 °C, 75% relative humidity and 16:8 light: dark photoperiod. The effect of lectin gene on survivability of aphids was assessed by the total number of insects survived on individual transgenic plants at the end of 5 days (Table 1.3).

Table 1.3: Mortality percentage of aphids on *Brassica juncea* cv. Pusa Jaikisan

Transgenic lines	No. of dead insects	Mortality %
Control	2	3.34
PT5	5	11.11
PT7	3	6.67
PT13	5	11.1
PT23	7	15.55
PT48	8	17.77

Partial cloning of sugar transporter gene, involved in regulation of antibiotic, 2,4DAPG production

The Plant Growth Promoting Rhizobacteria (PGPR) suppress plant diseases by production of antibiotics, which has proven to be a promising and sustainable alternative for biological control of the soil borne plant pathogens. Among the antibiotics, 2, 4-diacetylphloroglucinol (2,4-DAPG or PHL), a polyketide compound, has broad-spectrum activities like antiviral, antifungal, antibacterial, antitumor and phytotoxic properties. Hence, with an aim to tap the regulators of 2, 4 DAPG production, a Tn5 mutant library of a PGPR, *Pseudomonas fluorescens* strain s218 was constructed. This strain has been tested to be highly efficient against tomato bacterial wilt pathogen, *Ralstonia solanacearum*. The mutant library with 4500 transconjugants was further screened for gain of function, only one mutant designated as Mu65 showed more antagonism against *R solanacearum* as evidenced by its bigger inhibition zone (Fig.1.13). Southern hybridization was carried out using mob region of Tn5 as probe, which confirmed a single site of Tn5 insertion in the chromosomal DNA of s218 (Fig. 1.14).

The flanking region of Tn5 in Mu65 has been cloned by genome walking and sequenced. The cloned product shows homology to sugar transporter family.

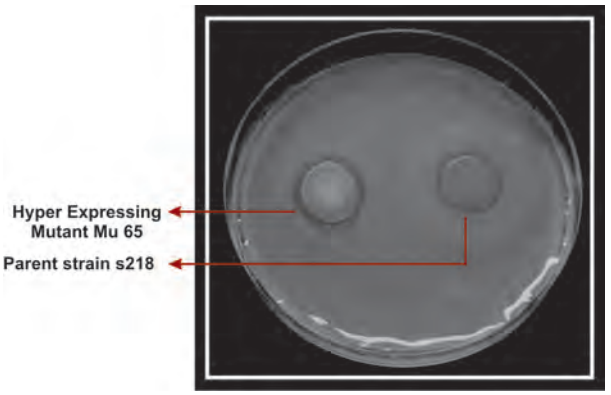


Fig. 1.13: Dual inoculation agar disc assay showing bigger inhibition zone in mutant 65 compared to parent s218

Fig. 1.14: Southern analysis of mutant Mu65

The full length gene will be cloned and functionally validated by complementation of the mutant.

B. Cloning and expression of 2, 4 DAPG biosynthetic genes from PGPR s218

The biosynthetic genes *phl* A, *phl* B, *phl* C, *phl* D for the antibiotic 2,4 DAPG are sufficient to confer the ability to produce 2,4DAPG on non 2,4 DAPG producing recipient strains. Hence, we isolated and cloned all the four biosynthetic genes from the *Pseudomonas fluorescens* strain s218 by parallel gene approach. The PCR amplified products (Fig. 1.15) for all the four genes have been sequenced and confirmed after cloning. Further, protein expression of these genes is being ascertained by cloning in expression vector pET29A in *E. coli* strain BL21 as host. Fig.1.16 shows the SDS-PAGE profile of PhlB protein of 16kD which corresponds to the relative molecular mass of PhlB.

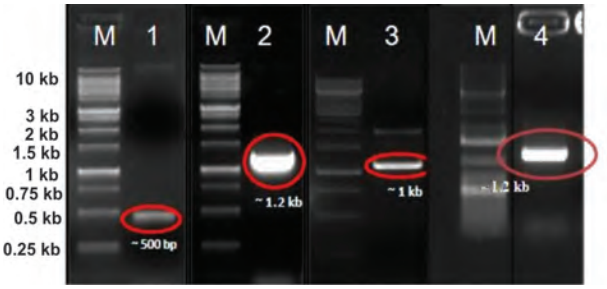


Fig. 1.15: PCR amplification and cloning of 2,4DAPG biosynthetic genes. M: 1kb ladder, 1: phl B, 2: phl C, 3: phl D and 4: phl A

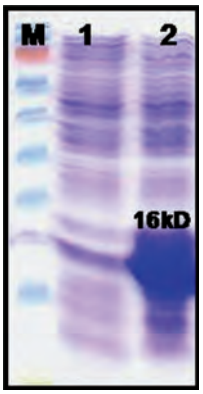


Fig. 1.16: SDS-PAGE analysis of PhlB protein

2. Development of Transgenics for Biotic Stress Management

Organelle targeting of *Bt* Cry1Ac

Bt protein (Cry1Ac) was targeted to chloroplast and compared with cytosolic and endoplasmic reticulum -targeted proteins for analysis of Bt protein expression, stability and efficacy. Chloroplast targeted transit peptide sequence was amplified from *Nicotiana tabacum* and fused to 52 end of *cry1Ac* (Fig. 2.1). Genetic transformation of tobacco was done for validation and analysis of efficiency of the construct. Chloroplast targeted plants were analyzed by PCR analysis. Both chloroplast targeted (CAc) and ER targeted (EAc) plants were compared with cytoplasmic Cry1Ac (Ac) tobacco plants. Comparative expression analysis of Cry1AC protein were done by quantitative ELISA, Western and quantitative real-time PCR (q-PCR). Although steady-state level of *cry1Ac* RNA was the highest in Ac lines, comparative study of protein expression by quantitative ELISA and Western Blotting analysis showed higher level of Cry1Ac protein in organelle-targeted lines (Fig. 2.2). Cry1Ac level was the least in Ac lines and the highest in EAc lines, while Cry1Ac level was in between in CAc lines.

Higher Cry1Ac protein level in organelle-targeted lines is due to less degradation and more stability. Therefore, Bt protein expression can be substantially increased by targeting it to any one of the organelle.

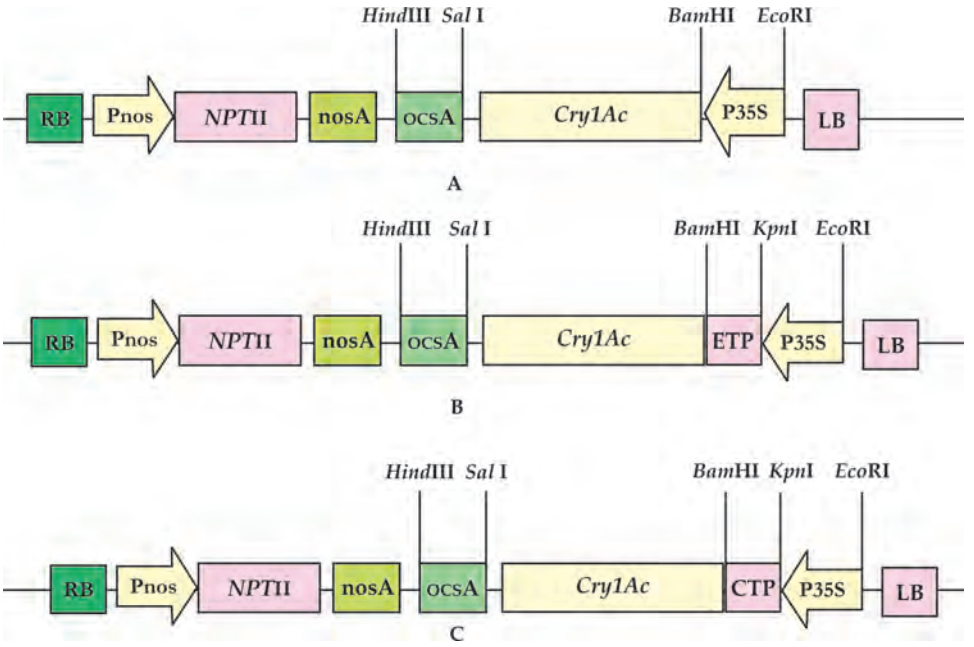


Fig. 2.1: *Cry1Ac* constructs used for organelle targeting study: A, *Cry1Ac* for cytoplasmic localization and without any organelle targeting signal; B, *Cry1Ac* with Endoplasmic Targeting Peptide (ETP) from soybean vegetative storage protein gene *vspA* (VSPαS) for ER targeting; C, ***Cry1Ac* with Chloroplast Transit Peptide (CTP) from *Nictoinia tabaccum* Rubisco small subunit for chloroplast targeting**

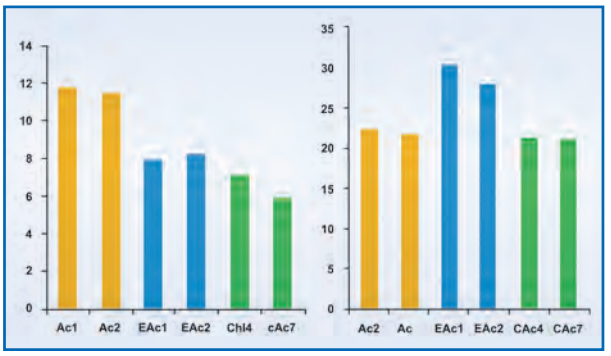


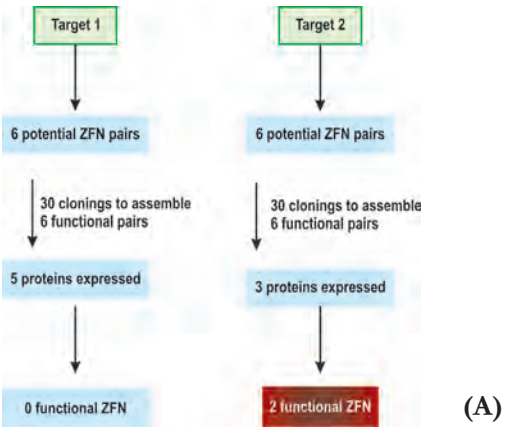
Fig. 2.2: Comparative expression analysis of *Cry1Ac* RNA and protein in different targeted lines. A: q-PCR showing *Cry1Ac* RNA level and B: quantitative ELISA indicating Cry1Ac protein level. Cytoplasmic targeted lines were shown in yellow, while ER and chloroplast – targeted lines are shown in blue and green, respectively

Targeted gene integration in rice

Using *in silico* approach a zinc finger nuclease (ZFN) pair that recognizes and cleaves a specific nucleotide sequence in *UGT* (UDP-glucosyl transferase) gene in Crossa rice genome was designed and synthesized. Binary vector pGreen/pSoup was constructed with two ZFN gene constructs driven by 35S promoter and *cry1Ac* as a gene that is to be targeted along with *HPTII* to assist in selection of rice transformants. *GUS* gene was also introduced into the vector outside the recognition sequences of ZFN but inside the T-DNA borders to aid in visual detection of the targeted events. *UGT* gene, which is a rate limiting enzyme in anthocyanin biosynthesis may impede anthocyanin levels and make it visually distinct from the non targeted plants. Also, transformation with pCAMBIA1304-*cry1Ac* was carried out in IR64 variety. A total of nine independent lines were developed in Crossa variety and none of them were found to be targeted plants.

The inability of the ZFNs was looked into the *in vitro* levels. The proteins were synthesized by *in vitro* translation and the ability of the designed ZFNs to recognize and cut the target DNA was tested. At different conditions of proteins/ DNA molar concentrations the ZFNs failed to recognize the DNA with the target sequence.

ZFN proteins were redesigned using the new and more precise software's available to detect and design ZFNs. Critical evaluation and validation of 12 potential ZFN pairs recognizing two different target sequences involving several assembly steps resulted



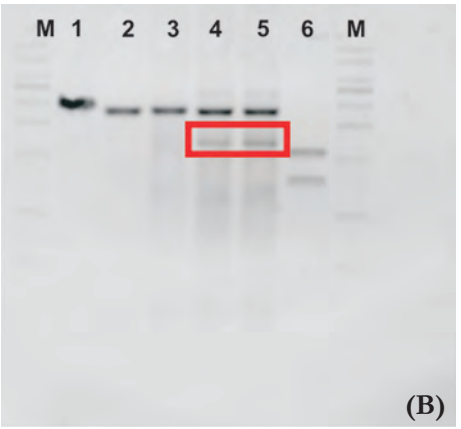


Fig. 2.3: Panel A: Summary of design and *In vitro* analysis of ZFN pairs designed and tested for two target sequences in *UGT* gene. Panel B: Site specific *In vitro* cleavage assay for validation of ZFN specificity. If the ZFN cleaves the site it is engineered for, in the plasmid fragments of size 2.2 kb and 1.8 kb are expected. Lanes: 1, Linear plasmid DNA containing *UGT* sequence (substrate); 2, substrate with buffer only; 3, substrate with control protein and buffer; 4, substrate incubated with ZFN pair1; 5, substrate incubated with ZFN pair2; 6, *Nco*I digested plasmid harboring the target sequence with nearly similar expected size (200 bp difference) of cleavage

Fig. 2.4: The genes were cloned in pGreenI0000 binary vector for plant transformation. ZFN on the same vector increases the chance of its expression and thereby targeting frequencies

in two validated ZFN pairs (Fig. 2.3) for a single target site. Constructs for stable transformation were made (Fig. 2.4) and rice transformation is in progress.

Validation of a cotton promoter for high expression in bolls

A promoter of putative senescence protein that confers high expression in boll tissues of cotton was identified and isolated using suppressive subtractive library construction. The 1.1kb promoter was used to drive the expression of *mGFP* gene in pCAMBIA1302 for its validation in model plant systems.

The strength of the promoter was tested by stable transformation in *Arabidopsis* (Fig.2.5), tobacco and by transient expression in cotton bolls (Fig.2.6). The promoter was able to confer consistent and high *GFP* expression among the plant systems and tissues tested.

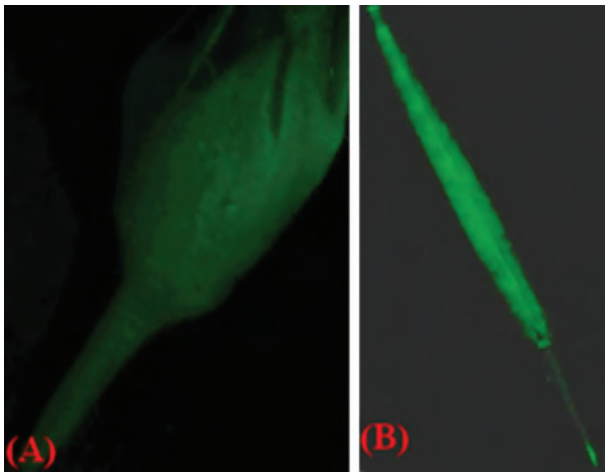


Fig. 2.5: GFP expression in *Arabidopsis* (A) Floral bud and (B) Silique. pCAMBIA1302 with *mGFP* under the promoter was used for transformation

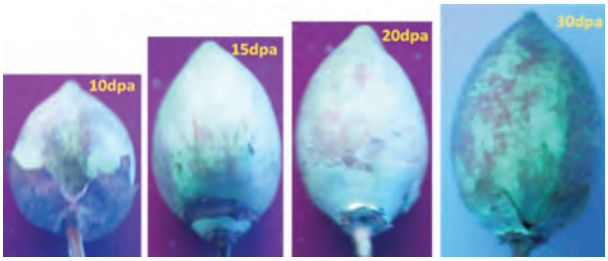


Fig. 2.6: Analysis of transient expression in cotton bolls of different ages. The *mGFP* expression was consistently high in all the ages of the boll tested. pCAMBIA1302 with *mGFP* under the promoter was used for transient expression

This promoter is now being used to drive the expression of *cry2Aa* gene for genetic engineering of cotton for insect resistance.

Characterization of cuticle related gene and its promoter

Selection of suitable promoters for desired level of transgene expression is important for deployment of insect resistance in crops plants. To facilitate high expression of Bt-ICP genes in cotton, a cuticle related gene having highest expression in leaves was selected for characterization (Fig. 2.7). *AKS1* gene encodes a transmembrane domain bearing protein of 127 amino acids. The gene was isolated and cloned from cotton. Sequencing of six colonies from both sides revealed two homeologous genes. Genome walking method was used to determine full-length promoter sequence. Promoter sequences obtained of around 800 bp downstream to ATG. Second round of genome walking is under process. This promoter will be characterized by expressing GUS and comparing it with 35S CaMV and Rubisco small subunit (RbcS) promoters.

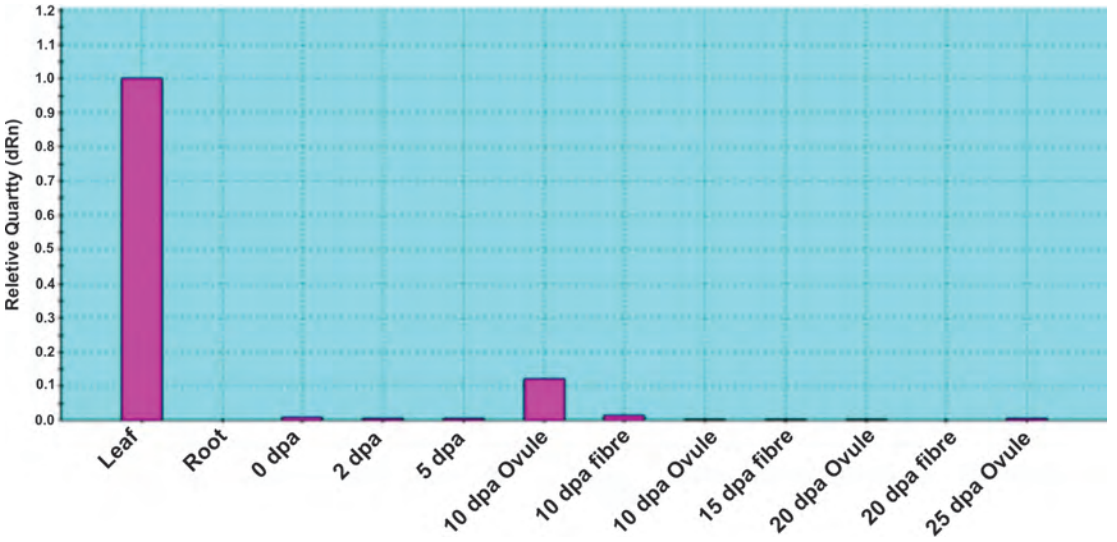


Fig. 2.7: qRT-PCR analysis reveals maximum expression of *AKS1* gene in leaf tissue

Genomics of cotton fibre development

Cotton fibres are single celled seed trichomes developed from ovule epidermal cells. Fuzzless-lintless mutants (Fig.2.8B) are ideal source to understand the molecular mechanisms implicated in fibre development by comparing with fibre bearing ovules of wild-type (Fig.2.8A). A lintless-fuzzless mutant of *Gossypium hirsutum* cv. MCU5 was used to understand the molecular mechanisms implicated in fibre development. The genome wide transcriptome analysis was carried out at fibre initiation (0 dpa), elongation (5, 10 and 15) and secondary cell wall (SCW) synthesis (20 dpa) stages by comparing the fuzzless-lintless mutant with its isogenic wild-type line using Affymetrix cotton GeneChip genome array. Analysis of transcriptome data revealed that number of down-regulated transcripts is more as compared to up-regulated transcripts at various stages analyzed and the percentage of down-regulated transcripts is high at fibre initiation (90.0%) and elongation stages (91.5%). These down-regulated genes may be having crucial role in fibre cell differentiation and early elongation (Fig.2.8C).

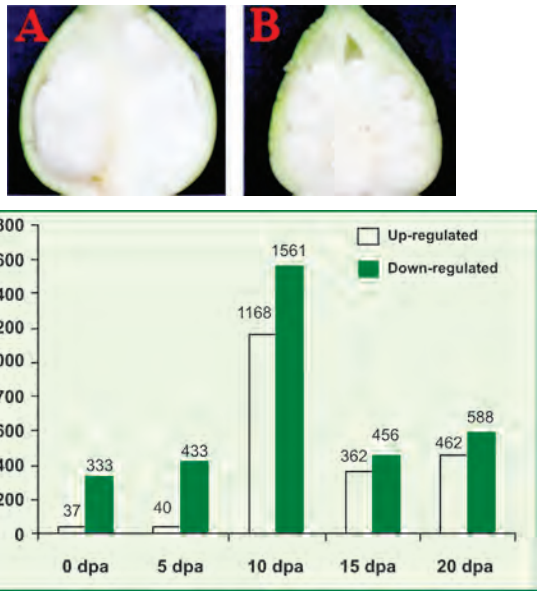


Fig. 2.8: (A) Boll and ovule at 10 dpa showing normal fibre development in wild-type; (B) Fuzzless-lintless seeds in MUT; (C) Number of up- and down-regulated transcripts in MUT as compared to WT during fibre development stages

Functional analysis of differentially expressed transcripts revealed down-regulation of many genes involved in ethylene, auxin and BR biosynthesis, and signalling pathways at fibre initiation (0 dpa) and elongation (5 and 10 dpa) stages. Further, genes involved in calcium signalling were highly down-

regulated at 0 and 10 dpa. Putative transcription factors belonging to AP2-EREBP, WRKY, NAC and C_2H_2 family were completely down-regulated at fibre initiation and early elongation stages. More numbers of transcripts encoding MYB family transcription factors were down-regulated at 10 dpa followed by 0 dpa. The genes involved in Calvin cycle and mETS (mitochondrial electron transport system), which are crucial for synthesis of cell wall biosynthesis and membrane lipids, were down-regulated at 10 dpa suggesting reduction of cytosolic sugar pool and mitochondrial energy supply in MUT. Many key genes involved in synthesis of cell wall precursors, VLCFAs (very-long chain fatty acids) and phospholipids were down-regulated during elongation stage. Further, large number of genes related to primary cell wall biosynthesis and elongation such as xyloglucanases, pectinases, expansins and arabinogalactans and cytoskeleton-related genes implicated in fibre cell elongation were highly down-regulated at 10 dpa, whereas cellulose synthases involved in secondary cell wall biosynthesis were down-regulated at 15 dpa. Transcripts encoding cupin superfamily storage proteins and heat shock proteins were highly down-regulated at 20 dpa. Large numbers of biotic and abiotic responsive genes involved in ROS scavenging, protein stabilization, ion-homeostasis and regulation of cell osmotic potential were highly down-regulated at various stages studied suggesting their role in fibre development. This comprehensive transcriptome data provides valuable information on genes implicated in fibre cell development.

Development of glyphosate tolerant rice

Popular Indian rice cultivar, IR-64, was transformed with codon optimized synthetic *CP4-EPSPS* gene encoding an EPSPS enzyme having less affinity towards glyphosate via *Agrobacterium*-mediated genetic transformation technique to develop transgenic rice tolerant to glyphosate. The gene with chloroplast targeting signal peptide and under transcriptional control of *Zea mays* ubiquitin promoter was delivered into IR-64 using binary vector pCAMBIA1301. A total of 21 putative transgenic plants were developed. Out of which 15 plants of three events were found positive for *EPSPS* and *HPTII* gene in PCR analysis. Transformants were screened by Southern blot analysis and *EPSPS* expression in selected transgenic rice plants was measured by q-PCR. EPSPS protein expression was confirmed by using Roundup Ready Immunostrip test.

Southern hybridization analysis of T_0 transformants, obtained from *Agrobacterium* mediated transformation, was done by digesting 10ìg of genomic DNA with *HindIII*, and the blot was probed with 1.3 kb *EPSPS* fragment. Five transgenic lines had two copies of *EPSPS* gene, while eight lines carried single copy of the gene (Fig. 2.9).

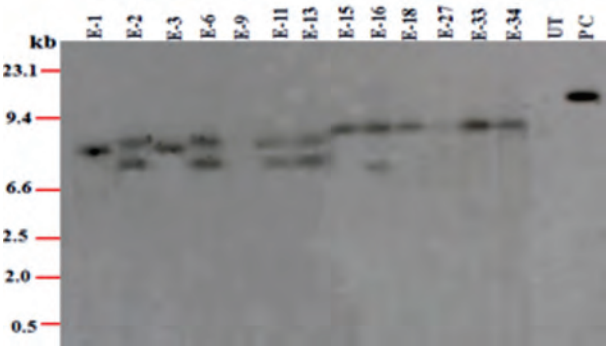


Fig. 2.9: Southern hybridization analysis of rice *EPSPS* transgenic lines. Lanes: UT, Untransformed control; PC, Linearized plasmid as positive control; E-1 to E-34, Putative transgenic lines. Numerals on the left indicate size of the DNA fragments of ladder (λ DNA, *HindIII* digest)

The qualitative protein expression in the transgenic lines was tested with Roundup Ready Immunostrips and six transgenic lines were found positive. Herbicide bioassay test confirmed that the transgenic rice can tolerate up to 1% commercial Roundup, the dose used to kill rice weeds under field condition (Fig. 2.10).

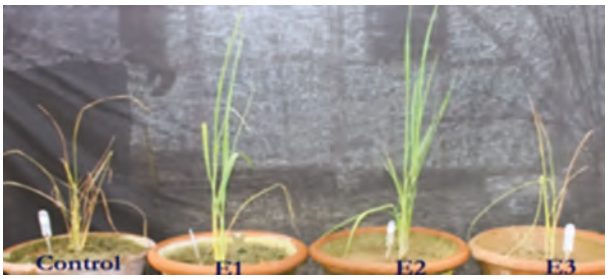


Fig. 2.10: Bioassay of *EPSPS* rice transgenic lines. Photograph was taken on fourth day after 1% roundup (Monsanto) treatment

Isolation and characterization of quorum quenching genes from *Bacillus thuringiensis* and other bacteria

Different bacterial species produce and respond to different quorum-sensing signals, which have crucial role in controlling a range of activities implicated in pathogen-host interaction and microbe-microbe competition. Signal-degrading enzymes from one bacterial species can interrupt signaling among other pathogenic bacterial species thereby preventing infection of the host. N-acyl homoserine lactones (AHLs) present in many gram-negative bacteria are well-characterized quorum-sensing signals and are inactivated by degrading enzymes AHL-lactonases. Degradation of quorum-sensing signal, known as quorum-quenching, is a potential approach for control of bacterial diseases. Since many *Bacillus thuringiensis* (Bt) isolates produce strong AHL-lactonase activity, these can be used for control of bacterial diseases. Therefore, native Bt isolates recovered from diverse habitats in India as well as Bt type strains have been screened for the presence of *aiiA (autoinducer inactivation),* AHL-lactonase genes by PCR using specially designed gene-specific primers.

Screening of 2 native Bt isolates and 3 Bt type strains obtained from Bacillus Genetic Stock Centre, Ohio State University, USA (BGSC), used as reference, for the presence of N-Acyl Homoserine Lactonases (*aiiA)* genes by gradient PCR with the primer set designed as per Dong *et al.* (2002) was carried out.

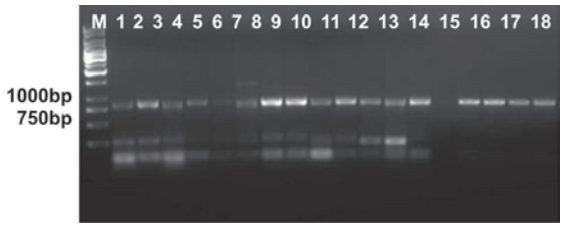


Fig. 2.11: Gradient PCR for the amplification of AHL gene from Bt reference strains and native Isolates. Lane 1-6:4J2; Lane 7-12: HD1; Lane 13-18: native isolate 722. M= 1 kb ladder

The *aii* gene was amplified from Bt subsp. *aizawai* and *Bt subsp. kurstaki* and cloned into pGEM-T Easy vector (Fig. 2.11). Sequence determination of the *aiiA* genes was done by primer walking and the 753 bp sequence was deposited in GenBank under accession no. JN 820132 and JN 820133, respectively.

An additional set of primers as designed by Pan *et al.* (2008) was also used for screening of 19 native Bt isolates and 11 type strains for the presence of *aiiA* genes by PCR using genomic DNA as template. Gradient PCR in the range of 40ºC to 55ºC with increment of 3ºC was performed to optimize the most appropriate annealing temperature for amplification. Out of six different annealing temperatures analyzed, 49ºC and 52ºC temperatures were found to be the optimum temperatures for the amplification of *aiiA*-genes (Fig. 2.12a,b,c). The *aiiA* gene was amplified from a Bt type strain Bt subsp. *galleriae* and 7 native Bt isolates (Table 2.1). The PCR products from SK-105, SK-301 and Bt subsp. *galleriae* were cloned into pGEM-T vector (Fig. 2.13). Sequence determination and analysis is in progress.

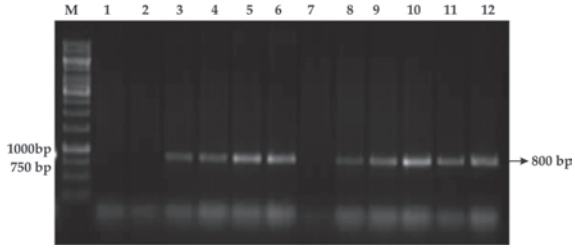


Fig. 2.12a: Gradient PCR for the amplification of AHL gene in Bt type strain 4G6 and native Bt isolate SK105. Lane 1-6: Bt subsp. *galleriae* (4G6); Lane 7-12: SK-63, at different temperatures in the range 40ºC-55ºC. M= 1Kb Ladder

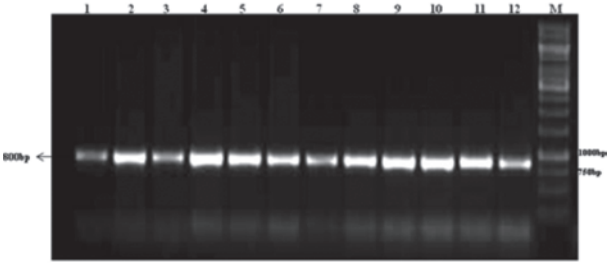


Fig. 2.12b: Gradient PCR for the amplification of AHL gene in native Bt isolates. Lane 1-6: SK-229; Lane 7-12: SK-223, at different temperatures in the range 40ºC-55ºC. M= 1Kb Ladder

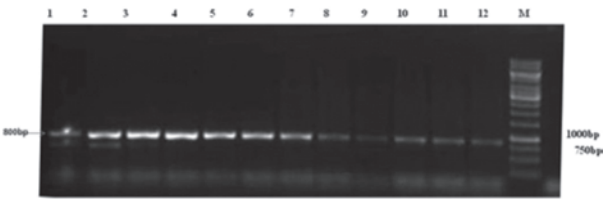


Fig. 2.12c: Gradient PCR for the amplification of AHL gene in native Bt isolates. Lane 1-6: 301; Lane 7-12: SK-105, at different temperatures in the range 40ºC-55ºC. M= 1Kb Ladder

Table 2.1: Screening of native *Bt* isolates for the presence of *aiiA* genes by gradient PCR with primers as designed by Pan *et al.* (2008)

S.No	Isolate	Source	PCR product*
1	SK- 63	Wheat field, Guntur, Andhra Pradesh	+
2	SK-88	Grain Dust, Bhareri, Shimla, Himachal Pradesh	-
3	SK-105	Chickpea field, IARI, New Delhi	+
4	SK-223	Phyllosphere, Pigeonpea field, IARI, New Delhi	+
5	SK-229	Phyllosphere, Pigeonpea field, IARI, New Delhi	+
6	SK-301	Nematodes infected field soil, IARI, New Delhi	+
7	SK-671	Kitchen garden soil, Allahabad, Uttar Pradesh	-
8	SK-701	Bengal Gram field soil, Lam, Guntur, Uttar Pradesh	+
9	SK-722	Cotton field soil, Lam, Guntur, Andhra Pradesh	+
10	SK-741	Cotton Seed VarLK689, Lam, Guntur, Andhra Pradesh	_
11	SK-791	Chilly seeds Warehouse, Nallapadu, Guntur, Andhra Pradesh	_
12	SK-938	Desert soil, Location 3, Khyaliwala, Sriganganagar, Rajasthan	_
13	SK-944	Desert soil, Location 3, Khyaliwala, Sriganganagar, Rajasthan	_
14	SK-954	Cotton field 1, Khyaliwala, Sriganganagar, Rajasthan	_
15	SK-1009	Insect infected wheat field , Sriganganagar, Rajasthan	_
16	SK-1034	Barren land, Sriganganagar, Rajasthan	_
17	SK-1036	Soil sample, Baramulla, J&K	_
18	SK-1056	Maize field, Village-Motali, Udaipur, Rajasthan	_
19	SK-1067	Maize field, Village-Bankoda, Dungarpur, Rajasthan	_

*+and_denote the presence and absence of amplified product.

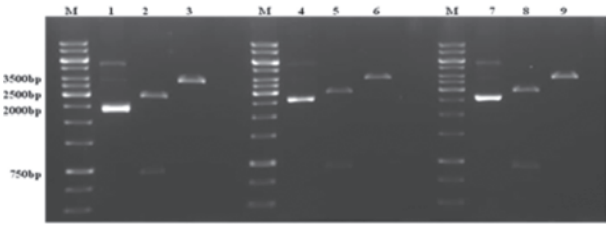


Fig. 2.13: Restriction analysis of three recombinant clones carrying AHL gene. Lane 1: Clone RKN-301 uncut plasmid DNA; Lane 2: RKN-301 restricted with Not1; Lane 3: RKN-301 restricted with Sal1; Lane 4: RKN-105 uncut Plasmid DNA; Lane 5: RKN-105 restricted with Not1; Lane 6: RKN-105 restricted with Sal1; Lane 7: RKN-4G6 uncut plasmid DNA; Lane 8: RKN-4G6 restricted with Not1; Lane 9: RKN-4G64G6 restricted with Sal1

Construction of a novel insecticidal *cry* gene of *Bacillus thuringiensis* (*Bt*) for enhanced pod borer (*Helicoverpa armigera*) resistance in transgenic crops

PCR screening using both single temperature and gradient PCR and cloning and sequencing of *cry*1- and *vip*3A-type genes from native Bt isolates and type strains was carried out (Table 2.2). A synthetic plant codon-optimized *cry1Ac34* (truncated form of *cry1Ac18* gene) gene was constructed and cloned in binary vector pBinAR.

Table 2.2: Details of *cry*1- and *vip*3A-type genes

Gene isolated	Source	Location	GenBank Acc. No.
*cry1Aa*20	SK-798	Chilli seeds warehouse, AP	JF340156
*cry1Ac*34	SK-783	Sorghum grain dust, AP	HQ230364
*cry1Ac*35	SK-784	Sorghum grain dust, AP	JF340157
cry1Ac36	SK-958	Desert soil 1, RJ	JN387137
cry1Ac37	SK-793	Chilli seeds warehouse, AP	JQ317685
cry1Ia30	Bt. *aizawai*	BGSC	JQ317686
cry1Ib7	SK-935	Desert soil 2, RJ	JN571740
vip3Aa44	*Bt. thuringiensis*	BGSC	HQ650163

Effect of enhanced level of farnesyl diphosphate(FDP) on plant-aphid interaction:

All insects use semiochemicals as olfactory messengers for interaction with the host plant as well as among themselves. Profile of sesquiterpene semiochemicals constitutes a major part in determining specificity of host emitted volatiles. It remains interesting to hypothesize if any alterations in sesquiterpene profile of the plant significantly affect its interaction with its insect pest. Farnesyl diphosphate (FDP) is a common substrate for many C15-sesquiterpenes which act as semiochemicals (Fig. 2.14). Arabidopsis FPS2 gene (AtFPS2) codes for a prenyl transferase FDP synthase which mediates the cytosolic synthesis of FDP through head to tail condensation of IPP (C5) and its allylic isomers. AtFPS2 gene was PCR amplified and cloned in a binary vector pEG 100 using GateWay cloning.

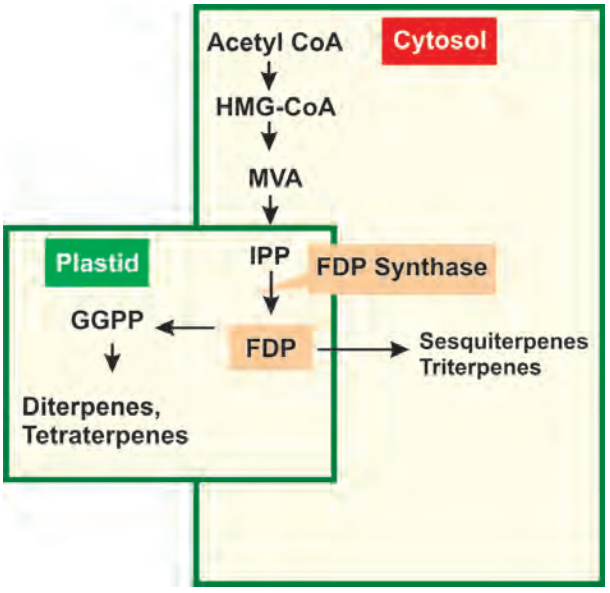


Fig. 2.14: Biosynthetic pathway of plant terpenoides

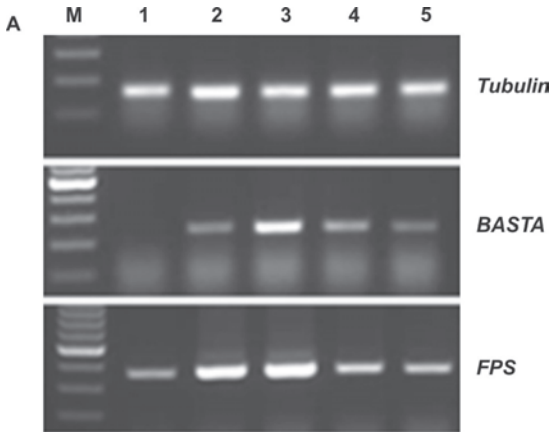
The FPS2 coding sequence was inserted under a CaMV 35S promoter either with a transit peptide (TP) sequence or without it and two binary construct *pEG:TP FPS and pEG: FPS* respectively were

generated. In case of *pEG:TP FPS* the frame of the fusion protein was confirmed by sequencing four positive clones. The binary constructs were mobilized into Agrobacterium strain for plant transformation.

Analyses of transgenic *Arabidopsis* plants constitutively expressing FPS2 gene:

Wild type Arabidopsis plants were transformed through floral dip method and several transgenic lines were initially recovered which progressed to F2 generation. Initially the transgenic plants were analyzed by PCR using a pair of primers specific to selectable marker gene BASTA and thus the plants scored positive were analyzed by RT-PCR to estimate the transgene expression level (Fig. 2). For construct *pEG:FPS* and construct *pEG:TP FPS,* gene specific internal primers were used for RT-PCR amplification which produced 363bp amplicon (Fig. 2.15A).

Both the non-transformed control plants and transgenic plants gave amplicon for FPS gene but the transcript level of FPS gene was relatively more in transgenics in comparison to control plants. To ascertain the level of transgene expression in independent transgenics primers specific to selectable marker gene viz. BASTA 1F / BASTA 1R were also used for RT-PCR amplification which generated 242bp amplicon. Different transgenic lines demonstrated varied expression of BASTA resistance gene while no expression was obtained in control line. The 157bp amplification of tubulin gene which



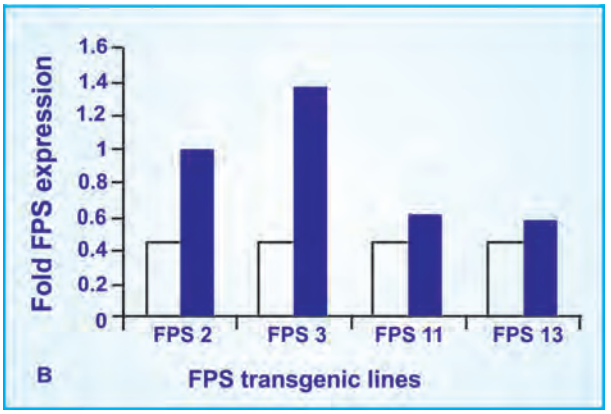
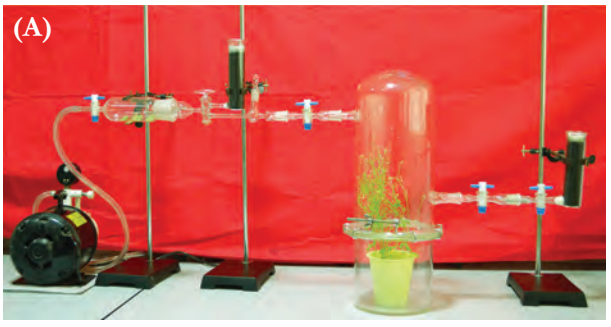


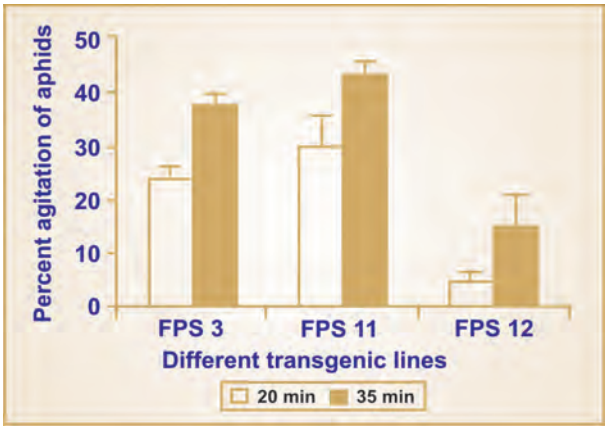
Fig. 2.15: RT-PCR analyses of the FPS-transgenic lines expressing farnesyl diphosphate synthase gene targeted to cytosol. (A) RT-PCR analyses of the FPS-transgenic plants with tubulin specific primer (Upper panel), antibiotic specific primer (middle panel) and FPS2 gene specific primers (lower panel). M: 100bp ladder; lane 1: Control plant; lane 2-5: independent transgenics. (B) Densiometric analysis of the RT-PCR amplicon as a masure of relative gene expression

was used as internal control showed similar intensity of amplification in the transgenics and wild type plants. The relative expression level of FPS gene in different transgenic lines and the fold increase in the expression of FPS gene in comparison to control plant is documented in Fig. 2.15B.

Insect bioassay with FPS transgenics

A known amount of plant material was distilled through hydro-distillation in a clavenger apparatus to emulsify its volatile components, which were then extracted in n-hexane. The hydro-distillate extracts of FPS and TP-FPS transgenic lines as well as from control plants transformed with empty vector was used in insect bioassay.





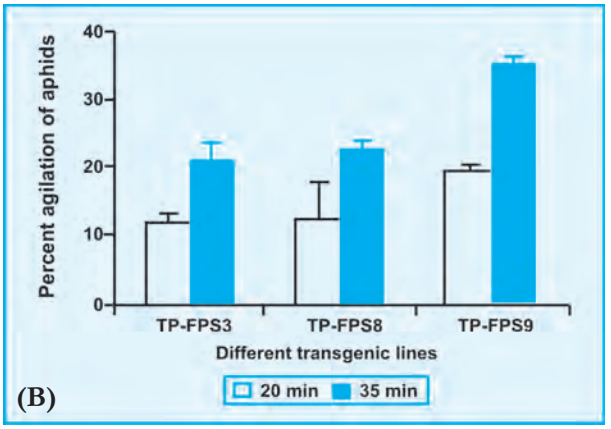


Fig. 2.16: Insect bioassay with FPS transgenics using head space volatiles. (A) A custom designed glass apparatus used for aphid bioassay with head space volatiles. (B) Nymphs (3-5 day old) of *M. persicae* were exposed to collected headspace volatiles and the agitation was recorded over a time period of 35 minutes. The data recorded was compared on the basis of percent agitation induced by per g of transgenic tissue (dry weight)

Nymphs of *M. persicae* (3-5 day old) settled and feeding on a single leaf were exposed to the volatiles emanating from 100ul of individual hydro-distillate extract in a specially designed glass tube apparatus.

Alternatively, the similar assay was performed using the head space volatiles of the FPS-transgenics (Fig. 2.16). The volatiles from the headspaces of 3-week old transgenic plants were collected by entrapment in a customized flat bottom bell-jar type glass apparatus of size $6_{1/2}$" X 18" with stop cock for 40 hours (Fig. 2.16A). Several nymphs (hundred/assay) of *M. persicae* were exposed to the collected headspace volatiles and their agitation was recorded over a time period of 35 minutes. The data recorded for all the lines were compared on the basis of percent agitation induced by per g of transgenic tissue (dry weight) at 20th and 35th minute of the assay (Fig. 2.16B). Induced agitation per g of transgenic tissue at 35th minute varied from 15.09 to 43.44 for FPS lines and from 21.46 to 35.48 for TP-FPS lines.

GC-MS profiles of volatiles extracted from FPS-transgenics

The volatiles emitted from the hydro-distillate extract of 3-week old plants were analyzed by GC-MS. The individual GC-MS profiles of FPS and TP-FPS lines identified either increase in the constituent sesquiterpenes ($C_{15}H_{24}$) or formation of some of the novel sesquiterpenes viz. β-bisabolene, β-sesquiphellandrene, α-zingiberene, γ-cadinene, epi-bicyclosesquiphellandrene, Z-β-farnesene. The component peaks were identified by comparison of their retention times and mass spectra in accordance with NIST (National Institute of Standards and Technology) library.

3. Biotechnology and Climate Change

Foodgrain productivity needs to be increased tremendously so as to meet the food demands of the burgeoning human population. Unfortunately, most of the land with agricultural potential is in areas with harsh environments. Furthermore, over 60 percent of all yield losses from crops are due to environmental stresses such as drought, temperature extremes or soil salinization. With the changing global climate these environmental stresses are becoming of paramount preposition because of their potential adverse impacts on agriculture and food security.

Most of the genetic studies in plant have been directed at limited range of species, selected either because of their status as model organisms or because of their economic importance. Very few plant species have been targeted for gene discovery on the basis of their enhanced tolerance to abiotic stresses. Many plants have evolved multiple, interconnected strategies that enable them to survive thermotolerance stress. Plants as Bajra (*Penniseteum glaucum*), Khejri (*Prosopis cineraria*) , Ber (*Zizyphus sp.*) are a few of the plants that have remarkable ability to tolerant different environmental stresses as drought, high temperature, low fertility, high salinity and low pH conditions. Therefore, a detailed molecular study of these plants will lead to the identification and isolation of novel genes/better performing alleles associated with environmental stress. Further, the isolated genes will be validated in model systems and the useful genes will be deployed for development of transgenics for environmental stress tolerance.

To look for robust allele of the drought responsive genes primers were designed based on heterologous sequence information available in the database for the candidate genes for moisture stress tolerance using ClustalW and Primer 3 software. Using PCR based strategy, amplification of the candidate genes were carried out in *Penniseteum glaucum*. Successful amplification was obtained with gene specific primers for *Pyrroline 5-carboxylate synthetase(P5CS*) in *Pennisetum glaucum*. Sequence analysis of the obtained amplicons (518bp) showed homology of more than 90% with same known gene in other crops (Table 3.1). Successful amplification for P5CS gene was obtained in 35 *Penniseteum glaucum* breeding lines (varying in tolerance to drought stress) from IARI farm (Fig 3.1). The nucleotide sequence of the 518bp amplicon has three exon regions of a total of 273bp. Sequence analysis revealed presence of nine nonsynonomous changes which are distributed in five breeding lines (Fig 3.2).

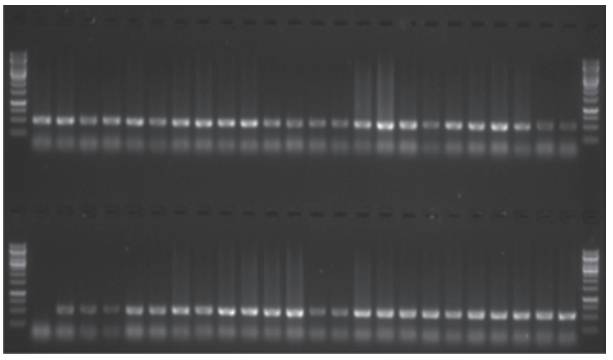


Fig. 3.1: Amplification of partial P5CS gene from gDNA of different breeding line of *Pennisetum glaucum*

Table 3.1: Per cent homology of nucleotide and amino acid sequences of P5CS gene (JQ406950) identified in 73-74 breeding line of *Pennisetum glaucum* with other accession of different crops.

Sl. No.	Crop	Accession no	% Homology	
			Nucleotide	Amino acid
1	*Pennisetum glaucum*	FJ827591	99	99
2	*Saccharum arundinaceum*	EU113257	83	88
3	*Zea mays*	BT083588	87	90
4	*Sorghum bicolor*	GQ377720	86	89
5	*Saccharum officinarum*	EF155655	82	86
6	*Oryza sativa*	D49714	85	87
7	*Triticum aestivum*	AY888045	84	83
8	*Hordeum vulgare*	AK249154	84	84

Amplification of P5CS gene in other cultivars/ genotypes/species/ breeding lines is being attempted and successful amplification obtained. Based on the sequence of the amplicons obtained for P5CS genes, RACE primers were designed and 5'-3' RACE carried out to amplify the full length gene in *Penniseteum glaucum*. A 1276bp nucleotides of P5CSwas amplified and sequenced. The partial CDS sequence of P5CS of *Penniseteum glaucum* is submitted in genBank and the Accession number obtained : JQ406950.

Drought Stress was induced in one month old seedlings by growing them in half MS media (without sucrose) and 35% PEG6000 for different time period. RNA was isolated from stressed seedling samples and suppressive cDNA library was constructed. A total of 139 clones obtained which had good quality insert having an insert of more than bp size. Annotation of sequencing data reveals presence of 24 % ESTs showing homology with genes related to biotic and abiotic stresses.

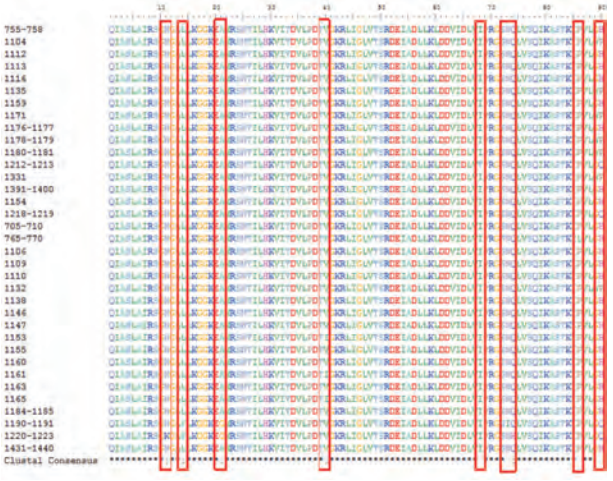


Fig. 3.2: Variation with respect to amino acid sequence in different breeding lines of *Penniseteum glaucum*

Functional analysis of rice annexin6 (*AnnOsI6*) promoter in tobacco under abiotic stress

Genes can be expressed in most plant tissues during most phases of growth and development with constitutive promoters such as CaMV35S, taking advantage of limiting temporal and spatial regulation which is suitable for proof-of-concept experiments. However constitutively active promoters are not always desirable for plant genetic engineering because constitutive over expression of transgenes may compete for the building blocks that are required for plant growth under normal conditions. It may also result in homology-dependent gene silencing, particularly when the promoter is also highly active. The use of stress inducible promoters is expected to be optimal for driving candidate abiotic stress tolerance genes.

Annexins are soluble, multifunctional, lipid binding proteins encoded by large multigene family spread throughout the eukaryotic and prokaryotic domains. They are capable of linking Ca2+, redox and lipid signaling to coordinate development with response to the biotic and abiotic environment. Last year transient expression analysis of upstream sequence of Annexin6 gene *(AnnOsI6)* from a rice drought tolerant variety Nagina22 in tobacco was reported. A 587 bp upstream region from the initiation codon and a 210bp of the coding region were amplified and deletion constructs made (P1-5$_{AnnOsI6}$) by fusing with reporter gene GUS in binary vector pBI121. The fragment harbored several known cis-elements that respond to hormone treatment and regulate stress induced gene expression. Native pBI121 harboring CaMv35S promoter was used as a control. Transient histochemical assay of GUS in leaf explants showed expression in case of all the truncated constructs. Maximum expression was observed in case of P4$_{AnnOIs6}$ (447bp) and P5$_{AnnOsI6}$ (347bp).

For long term analysis transgenic tobacco were developed with the P4$_{AnnOsI6}$ construct. The putative transformants were confirmed by PCR, RTPCR and DNA blot analyses. The fully developed transgenic tobacco plants were exposed to various stress treatments and GUS histochemical assay carried out to study promoter derived expression (Fig 3.3). Constitutive expression was observed in control plants transformed with pBI121.Differential expression of GUS was observed in various plant organs such as leaf, stem, stem–root junction and root. In *AnnOsI6* promoter driven GUS plants ABA and dehydration induced maximum expression of GUS in all organs whereas under NaCl expression was observed in leaf, root and root-stem junction. Expression of Gus was not seen under cold temperature stress at 4°C. H_2O_2 and high temperature induced GUS expression in leaf and stem only. These analyses provide promising results for an active functional promoter under abiotic stress which is being further investigated. Quantitative estimation of GUS was done by MUG assay. As annexin is known to be expressed in fruits a transient assay was carried out in different crops.

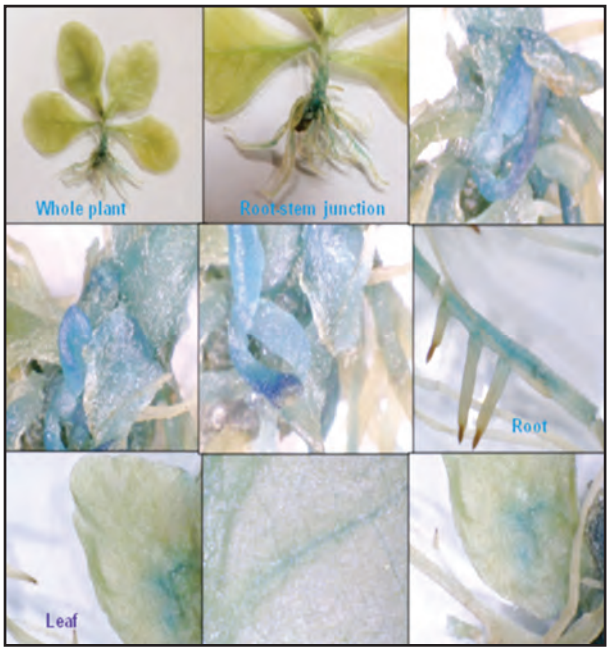


Fig. 3.3: GUS localization, expression and intensity in vegetative parts of transgenic tobacco driven by P4$_{AnnOsI6}$ after treatment with 200mM NaCl for two hours

*Csp*A and *CspB* are the major cold shock protein which are induced during cold shock in the bacteria. Csp proteins are known for their function in stress adaptation. These proteins act as RNA chaperons and they play an important role in stimulating growth following stress acclimation and during periods of high metabolic activity. These proteins regulate stress responses through a post transcriptional mechanism. The bacterial csp genes have been shown to improve the tolerance to various stresses in plants. Allels of these genes can be successfully used to improve tolerance of crop plants to various abiotic stresses.

Microbes isolated from soil were screened for tolerance to cold temperature (4^0C). Five isolates which were able to grow at low temperature have been identified on the basis of their 16SrDNA gene sequence BLAST homology analysis. Bacteria were identified as belonging to genera *Pseudomonas* and

Providencia. Full length *Csp*A gene was amplified from these microbes using primers CSPAF 5´ ATGTCCGGTAAAATGACTGG3' CSPAR -5' TTACAGGCTGGTTACGTTAC 3'. Partial *Csp*B gene was also amplified using primer CSPB1F 5'CAAGTGGTTCAACTCGGAGA3' CSPB1R 5' CCCGATTCCCTTCGACTATC3'(Fig 3.4). These genes have been sequenced and sequences submitted to NCBI Genbank. Cloning of *CspA* genes in binary vectors is underway.

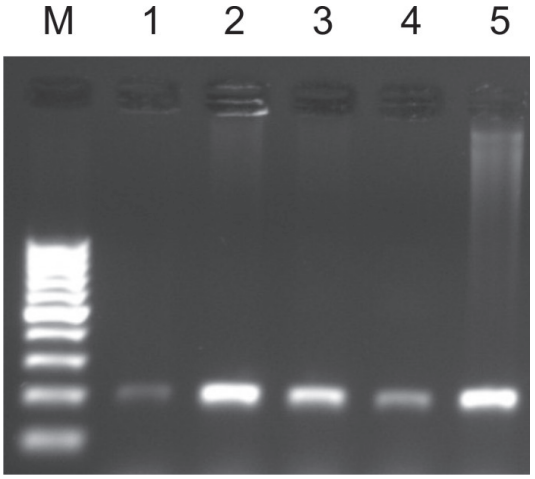


Fig. 3.4: Gel electrophoresis of PCR amplified full length *CspA* gene from cold tolerant microbial isolates M: 100 bp ladder, 1-5 cold tolerant microbial isolates

For improving the tolerance of plants to high temperatures attempts are being made to bioprospect bacterial genes responsible for tolerance to high temperature. Soil and water samples were collected from hot water springs of Tattapani (Himachal Pradesh), Bakreshwer (West Bengal) and Talvriksha (Rajasthan). Temperatures of the springs were 45°C, 62°C and 72°C respectively. Collected water samples were characterized on the basis of pH, Electric Conductance (EC) and Total Dissolved Salts (TDS). Water and soil samples were serially diluted in saline and spread plated on various thermophilic mediums in duplicates. Isolates growing above the temperature of 55 °C were selected for further analysis and identification of genes responsible for tolerance to high temperature.

The problem of soil salinity is a limiting factor, which hinders the realization of plants genetic potential of crop plants in many parts of the world, in arid and semi arid areas, similarly poses a threat to food security and thus the survival of human population in those areas. It will have an important realistic significance to exploit and utilize the saline soil, because of the limited cultivated lands and the increasing population. Salt tolerance of the plants is a complicated process involving changes at biochemical and molecular levels. Tolerance to salt in plants is determined by a series of genes that have direct or indirect effects to form a complex regulating network. For this increasing number of halophytes are being studied. Salicornia brachiata is one such halophyte successfully. *Salicornia brachiata* is a stem succulent annual halophyte, which is widely found growing in saline mud flats, degraded mangrove areas and aquafarms. This plant accumulates salt in its pith and survives even at 2 M NaCl under field condition. Genes and promoter related to salinity tolerance. For isolation of promoter of salt stress responsive gene sos1. Universal Genome walker kit was used to clone 5' upstream region of SOS1 gene. Amplified fragment after secondary PCR of Genomic DNA library corresponding to *Dra* I was ligated into pGEMT Easy vector and transformed into *E. coli* DH5α cell. Nearly 800 bp long upstream sequence of *SOS 1* gene from

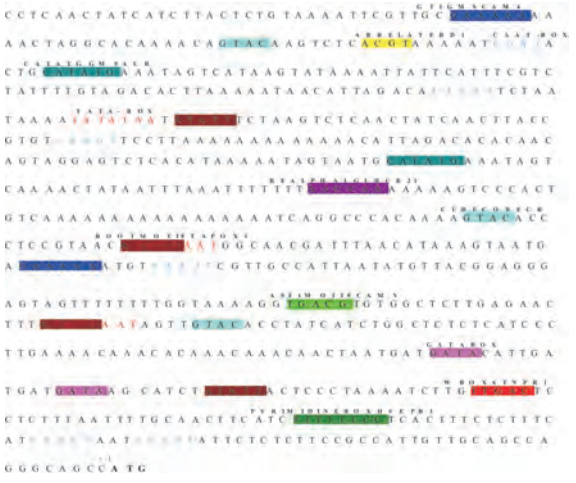


Fig. 3.5: Cloning of upstrem sequence of SOS1 from *Salicornia*

halophytic plant *Salicornia brachiata* has been cloned and sequenced. The sequence has been analysed to identify cis regulatory element using online database PLACE (Fig. 3.5). The sequence has been submitted to Genbank under accession no. JQ658427. Functional validation of the promoter is underway.

Hydrilla verticillata (L. f.) Royle is an aquatic angiosperm belonging to family Hydrocharitaceae. It has distinction with respect to photosynthesis in higher plants, as it switches to a C4 cycle from C3-cycle state at high temperature, and also lacks specialized Kranz type leaf anatomy that is found in terrestrial C4 species. The inducible C4 photosynthesis system in *H. verticillata* challenges our established notion about photosynthesis. This system provides the prospect to study the essential biochemical factors to operate a C4 photosynthetic system, which might be required to alter a C3 crop plant. C3 and C4 states of *H. verticillata* also facilitates to look at the relevant genes expression and their regulatory elements. The plants of *H. verticillata* were incubated at 34°C for 10 days to induce C4 photosynthesis, and 22°C to maintain the C3 state (Fig. 3.6) Five forward subtracted libraries were generated from leaves of plants incubated at 22^0C and 34^0C for 2 4,6,8 and 10 days. The forward subtracted and enriched DNA fragments were directly cloned into T/A cloning vector. Electrocompetent cells of *E. coli* DH5α were transformed. Nearly one thousand clones have been picked and sequenced. Bioinformatic analysis is underway.

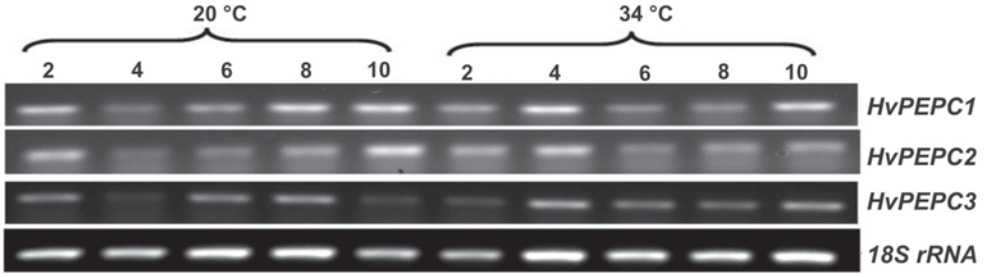


Fig. 3.6: Gene expression of *PEPC* (isoforms *1, 2* and *3*) in leaves of *H. verticillata* at 20 °C and 34 °C, using semi-quantitative RT-PCR. 18S rRNA was used as internal control

4. Biotechnological Approaches for Increasing Productivity

Heterosis breeding in mustard

Thirteen hybrids based on *Moricandia arvensis* CMS system were evaluated in replicated field trial along with the checks Pusa Jai Kisan, Varuna and the hybrid DMH1. Only two hybrids namely, Hybrid 23 and Hybrid 2007A showed 18-30% superiority for seed yield over the check variety Varuna (Table 4.1). It is worth noting that Hybrid 2007A was found to out yield the check in the previous year also. However, Hybrid 23 which did not show superiority in the previous year trial outperformed all other entries during the current year. These results show significant influence of environment on yield of hybrids and point to the need for multi-location, multi-year testing to identify consistently better yielding hybrids.

Table 4.1: Performance of *Moricandia arvensis* CMS-based hybrids

Hybrid	Mean yield (Q/ha)	% superiority over best check
Hybrid-23	36.42**	29.7
Hybrid-2007A	33.33**	18.7
Hybrid-10	27.47	-
Yybrid-22-04 Y	27.16	-
Hybrid-19-04	26.85	-
Hybrid-8A	26.23	-
Hybrid-22-04 Br	25.68	-
DMH-1(C)	25.31	-
Pusa Jai Kisan(C)	23.39	-
Varuna (C)	28.09	-
$CD_{5\%}$	**3.65**	

Isolation and genetic characterization of albino silique mutant of *Brassica rapa*

In *Brassica*, physiological studies have shown that silique wall photosynthesis makes major contribution to the seed reserves. Hence, the significance of leaf photosynthesis to seed yield is remains a mystery. We have identified an albino silique mutant in *B. rapa* (Fig. 4.1), which provides a unique opportunity to answer this question.

Chlorophyll content of silique wall was estimated spectrophotometrically after extracting with DMSO. Chlorophyll A, chlorophyll B and total chlorophyll content of albino silique were 10.6, 15.8 and 26.3 µg/g as compared with 251.8, 89.8 and 341.6 µg/g, respectively, in green silique. This clearly shows that photosynthesis is severely impaired in silique wall of the albino mutant.

To understand the genetic nature of the mutation, crosses were made between the albino mutant and wild type, and F_1, F_2, and BC_1 generations were raised and silique phenotypes were recorded. Albino silique was dominant over green silique and segregated as a monogenic trait. No linkage was detected between this triat and seed colour or locule number (Table 4.2). Whitesilique plants showed normal seed set. Seed test weight and oil content of F_2 plants with albino or green silique were comparable. These results are at variance with the current view regarding the contribution of silique photosynthesis to seed yield and further studies are planned to find the source of photosynthates contributing to seed development. The albino silique mutation showed pleiotropic effect, especially on petal colour.

Table 4.2: Inheritance of silique colour and locule number in *Brassica rapa*

Generation	Albino silique	Green silique	χ^2 (3:1 or 1:1)	Bilocular locular	Tetra	χ^2 (15:1)
Green x Albino (F1)	90	-	-	90	-	
F2	294	96	NS	359	31	NS
BC1F1 (testcross)	167	171	NS			

Fig. 4.1: Albino and green silique of *Brassica rapa* showing normal seed set

Cloning of CENH3 and Histone 3 genes for generating haploid-inducer lines of *B. Juncea*

Centromere histone proteins play pivotal role in chromosome segregation during cell division and genetic engineering of CENH3 has been shown to be useful in creating haploid-inducer lines in *Arabidopsis*. Since such haploid-inducer lines could accelerate breeding, we have initiated work to develop haploid-inducer lines in *B. juncea* by substituting native CENH3 proteins with engineered CenH3 tailswap protein. As a first step towards this goal, we have amplified and cloned CenH3 genomic DNA and cDNA from *B. juncea*, *B. rapa*, *B. nigra*, *Diplotaxis erucoides* and *Orychophragmus violaceous*. Likewise, Histone 3 gene was cloned from *B. juncea* for use in construction of Tail swap vectors. These are being used to construct CenH3 silencing and Tailswap vectors.

Microarray analysis of citrus ovule transcriptome to identify genes associated with polyembryony

In continuation of our earlier efforts aimed at isolation of genes governing nucellar polyembryony in citrus, we have conducted a microarray analysis of citrus ovule transcriptome. RNA isolated from various stages of developing ovules starting from pre-anthesis to early fruit set stage were used to probe 34 K citrus Affymetric chips. The raw signal intensities were processed using RMA algorithm in GenespringGX software and normalized signal intensities of all three technical replicates were individually analyzed and plotted as Principal Component Analysis (PCA) plot (Fig. 4.2). A strong difference in gene expression was found between the pre-anthesis stages of poly- and mono-embryonic cultivars. Whereas post-anthesis and initial fruit set stages of mono- and polyembryonic varieties did not

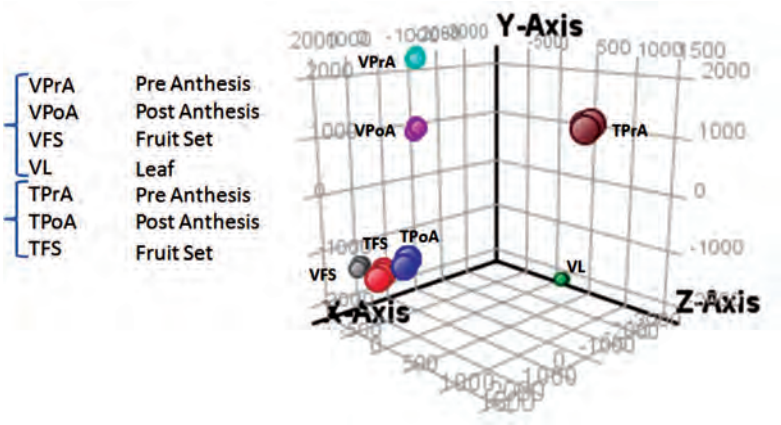


Fig. 4.2: Principal Component Analysis (PCA). Three dimensional (3D) plot shows positions and distances of different ovule developing stages of mono- (Temple –T) and poly-embryonic (Vaniglia Sanguigno- V) cultivars and leaf tissue (L) on the basis of normalized signal intensities of Affymetrix citrus array chip processed using RMA algorithm

exhibit strong transcriptional differences. As expected, transcripts of leaf tissue showed major differences when compared with transcripts of the ovule tissues. As proliferation of nucellar embryo initials starts at the pre-anthesis stage in the polyembryonic cultivar (Vaniglia Sanguigno), these transcript differences between poly- and mono-embryonic cultivars suggests that mining the microarray data might uncover the key genes involved in polyembryony.

We analyzed the type of transcripts differentially expressed during pre-anthesis stage of ovule development (Fig. 4.3). Using cutoff P-value d"0.05 and e"3.0 log fold change, we identified a total of 347 and 174 transcripts up-regulated (blue circle) in pre-anthesis stages of poly- (VPrA) and mono-embryonic (TPrA) cultivar, respectively. Among these, only 111 and 83 transcripts were exclusively up-regulated. To identify transcripts related to polyembryony, we attempted to identify common differentially expressed genes between VPrA and pre-anthesis stage of mono-embryonic cultivar (TPrA), and VPrA and post-anthesis stage of poly-embryonic cultivar (VPoA). The upper intersection of Blue and green circle (blue round) represents such transcripts. Functional annotation and target description of these transcripts indicates that majority (20%) of these transcripts belong to HSPs and also includes the

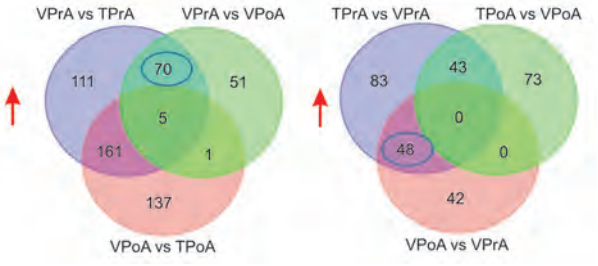


Fig. 4.3: Venn diagram depicting number of transcripts showing up-regulation (≥3.0 fold and cutoff P-value ≤0.05) in citrus ovules between various stages of seed formation

transcripts we identified in our previous SSH study. Thus, results of both SSH and microarray approaches were congruent and supplemented each other.

Phytohormone homeostasis and yield enhancement in mustard

Changes in growth of crop plants obtained by mutations in genes controlling hormonal status have contributed significantly to increase yield and were the basis for the green revolution. In this regard modulation of phytohormones concentration in mustard by genetic engineering to increase yield was attempted. Genes governing cytokinin homeostasis in plants such as cytokinin oxidase/dehydrogenase (CKX) was isolated from mustard by designing PCR based primers using *Arabidodpsis* gene homology.

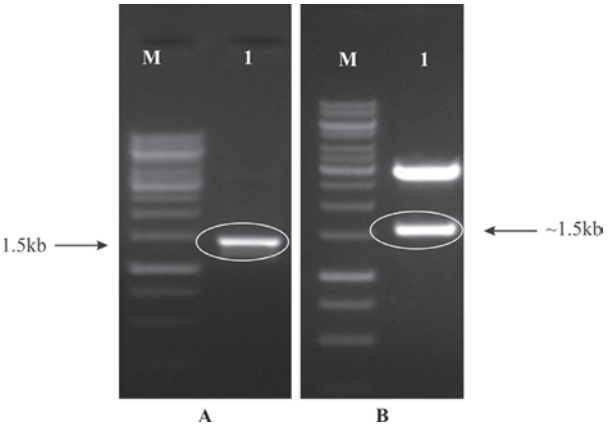


Fig. 4.4: Amplification and cloning of Mustard *ckx5* in pGEMT-Easy vector. A) Lane M- 1kb DNA ladder, lane 1: 1.5 kb amplicon of mustard *ckx5*. B) Lane M: 1kb DNA ladder, lane 1: Restriction digestion of *ckx5* in pGEMT-Easy with *EcoR1*

However, small amplicons of different sizes did not represent the full length gene. Thus, genome walker strategy was standardized to create genome walker library of mustard and full length *ckx5* (1.5kb) gene was isolated by PCR (Fig 4.4). BLAST analysis of nucleotide sequence (Figure 4.5) confirmed that the gene cloned is *ckx5* as it showed 99% similarity to the *ckx5* of *Arabidopsis thaliana*. The amino acid sequence of *ckx5* is shown in Fig. 4.6. The multiple alignment of the amino acid sequence of mustard *ckx5* with various other species showed >88% homology.

5'ATGATAGCTTACATAGAGCCATACTTCTTAGATAACGAC
GGTGAAGCCGCCTCCGTCACCGGACAATCTTTTGATGGCG
TCTCCGAGTCACTCGACATCCAGGGAGAAATCTTATGCGG
CGGTGCTGCGGCGGATATCGCCGGAAAAGACTTCGGTGGC
ATGAACTGCGTGAAGCCTCTTGCGGTGGTGAGACCCGTGG
GACCGGAGGATATCGCCGGAGCGGTGAGAGCAGCTCTGA
GGTCCGATAAACTCACCGTGGCGGCGCGTGGAAACGGCCA
TTCTATCAACGGCCAGGCCATGGCGGAAGGAGGACTCGTC
GTCGATATGCGTTCCACGGCGGAGAATCATTTCGAGGTTG
GTTTTTTACCCGGCGGTGCGTTTGTCGACGTCTCCGGAGG
GGCATTATGGGAAAACGTGTTGAAACGGTGCGTTTCGGAG
TATGGCTTGGCGCCTCGGTCGTGGACTGATTACCTCGGGT
TGCGGTAGGCGGGACGTTGTCGAATGCCGGCGTTGGTGGA
CAAGCGTTCCGTTACGGACCACAGACGTCAAATGTAACGG
AGTTAGATGTGGTGACGGGAAATGGGGACGTCGTTACTTG
CTCGGAGATGGAGAACTCGGAGCTCTTCTTCTCCGTTTTAG
GTGGTCTTGGCCAGTTCGGTATCATCACCAGAGCTAGGGT
TTTGCTACAGCCAGCACCAGATATGTCATCCGGCTCGGTTC
TTTACTGCCTTGAACTAGCTCTCCACTACCGAGATTCCGAC
TCTAACTCAACCGTCGACAAGAGAGTGGAGAGATTGATCG
GACGGCTGAGATTTGTTGAGGGGTTGAGGTTCGAGGTGGA
ACTGCCTTACGTTGACTTTTTACTGAGGGTAAAACGAGCTG
AAGAGATCGCAAAGGTGAACGGCACGTGGGAAACGCCGC
ACCCTTGGCTTAACCTCTTCGTGTCGAAACGTGACATCGGG
GAATTTGATCGGACGGTTTTCAAGGAACTTGCCAAGAACG
GAGTCGGAGGGCCCATGTTGGTCTACCCACTCTTGCGAAG
CAGGTGGAATGACCGTACATCCGTGGTTTTACCTGAAGGA
GAGATATTCTACATTGTGGCATTGCTTCGATTCGTGCCGAC
GTGTGCTAAACCTTCGTCGGTCGAGAAAATGGTGGCTCAA
AATCAGGAGATTGTTCAATGGTGTGTCAGAAAGGGAATAG
ATTTCAAGCTATATTTTCCTCACTACAAGTCTCGAGAGGAA
TGGATTCGTCATTTTGGGAACCGATGGTCGAGGTTCGTTG
ACAGGAAAACAATGTATGATTCCATGGCTATCCTTTCACCG
GGTCAAAAGATTTTCAATAGGGCTCCTTGA 3'

Fig. 4.5: Nucleotide sequence of *ckx5* from mustard (1355bp)

MIAYIEPYFLDNDGEAASVTGQSFDG
VSESLDIQGEILCGGAAADIAGKDFGG
MNCVKPLAVVRPVGPEDIAGAVRAAL
RSDKLTVAARGNGHSINGQAMAEGG
LVVDMRSTAENHFEVGFLPGGAFVDV
SGGALWENVLKRCVSEYGLAPRSWT
DYLGLTVGGTLSNAGVGGQAFRYGPQ
TSNVTELDVVTGNGDVVTCSEMENSE
LFFSVLGGLGQFGIITRARVLLQPAPD
MMVRWIRVVYTEFDEFTQDAEWLVS
QKNESSFDYVEGFVFVNGADPVNGW
PTVPLHPDHEFDPTRLPSSGSVLYCLE
LALHYRDSDSNSTVDKRVERLIGRLR
FVEGLRFEVELPYVDFLLRVKRAEEIA
KVNGTWETPHPWLNLFVSKRDIGEFD
RTVFKELAKNGVGGPMLVYPLLRSRW
NDRTSVVLPEGEIFYIVALLRFVPTCA
KPSSVEKMVAQNQEIVQWCVRKGID
FKLYFPHYKSREEWIRHFGNRWSRFV
DRKTMYDSMAILSPGQKIFNRAP

Fig. 4.6: Amino acid sequence of *ckx5* from mustard (517aa)

For efficient expression of *ckx* gene exclusively in roots a root specific promoter was isolated from *Arabidopsis* by PCR based strategy. This promoter mediates a developmentally regulated gene expression that becomes specific to the roots in advanced developmental stages. A database search revealed that it contains regulatory sequence elements that have been shown to possess functional *cis*-sequences that impart specificity. Suitable primers were designed to amplify 1.5 kb promoter DNA sequence (Fig. 4.7) and amplified product was cloned in pGEMT easy vector.

Sequencing of the clone revealed 100% homology to reported sequence without any mutation/deletion (Fig. 4.8). Currently, cloning of *ckx5* under root specific promoter is under progress.

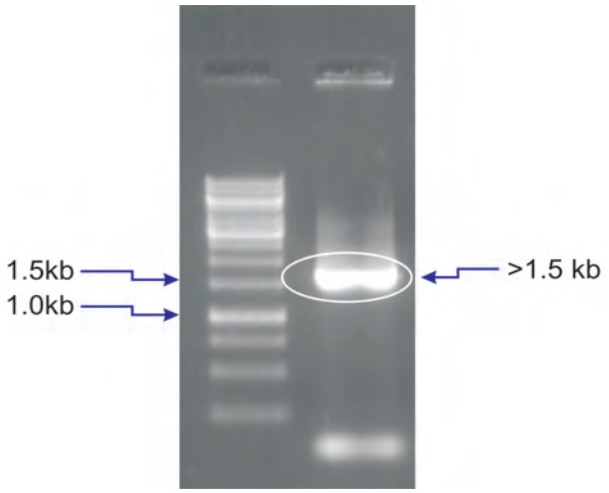


Fig. 4.7: Amplification of root specific promoter from *Arabidopsis*. A) Lane M- 1kb DNA ladder, lane 1: 1.5 kb amplicon of *pyk10* promoter

5'CTCCCATATGGTCGACCTGCAGGCGGCCGCGAA
TTCACTAGTGATTGACCCGGGACTGCAACGAAGT
GTACCAACAACTTGACTAGGATTCTAAGTTCTTTT
ATGTATAGGATGTCTATATTAAACTACCATGACTAA
CATATATATAGTAGTTCCATATGCTCGATAAACTATG
ATAGATCAACAATTTTAAACATATAGTTTAACACTA
TTTATTTGTTCAACGTCAATAGTTTATAGTTACGCA
TGCGCTCGGCTTAGATTTGGTCCCCAACAGTCGA
AATTGTCAAATAATATAAAATAAAAGTTTCATTGTT
AGGATTCATTTATTCTTCGGGTGGTTATTGTAATAA
AAGGCAAAAGAAAAAGAAGAACAAAATTCACA
AGTAAAAAAAAAAGATAACATCATTCTTTTAGTCGA
CAAAAAAAAAAAAAAAATCAAAAAGATTTATTCAG
TACTACAGTTTAATATTGTTTTGACTTTTTTCTTTTT
CTTTATATTATCTGAAAATTCTAGACTGCAGCTGAA
ACATGTGATATGGATTAAAGGCGTATCCAGTATCC
ACATAAAGAGGAGTGGTGTCGCTCACCCAGTCAC
CCTTGTTACTTGTTAGATAGCATTAATACATTTGTA
AGCAACAGCTTATCTGATAGACATGTCTTAATTGG
GAAATATGCTCTAAGATGATACAACCATGGTTCCA
ACTGTTGACCACCATAGCTGATAACATGTTGATTA
CATTTTTTCTTTTC3'

Fig. 4.8: Nucleotide sequence of root specific promoter

5. Genomics and Molecular Markers

A. Genomics

Pigeonpea Genome

Pigeonpea (***Cajanus cajan***) is an important grain legume of the Indian subcontinent, South-East Asia and East Africa. More than eighty five percent of the world pigeonpea is produced and consumed in India where it is a key crop for food and nutritional security of the people. In Indian Pigeonpea Genomics Initiative, we have sequenced the genome of popular pigeonpea variety "ASHA". The estimated size of pigeonpea genome packed in 11 chromosomes is 858 Mbp. It plays important role in food and nutritional security because it is a rich source of proteins, minerals and vitamins. This is the first plant genome sequence completed entirely through a network of Indian institutions led by the Indian Council of Agricultural Research and provides a valuable resource for the pigeonpea variety improvement.

Sequencing and Assembly

High quality genomic DNA was isolated from the leaves of a single plant of variety 'Asha' using CTAB method. Sequencing of 19 plates of whole genome shotgun libraries of short DNA fragments was carried out using GS-FLX Phase D chemistry and 3 plates of paired end sequences from a library of 20 Kb long fragments of pigeonpea genomic DNA using GS-FLX Titanium chemistry. Filtered high quality sequence reads were assembled using "Newbler GS De Novo assembler version 2.5.3" (Roche Inc. Germany). A total of 25,489,474 sequence reads with sequence information of 10,101,433,318 bp was generated. The primary sequence assembly included 21,102,008 sequence reads (82.79%) with 9.48 Gb sequence data, >10× coverage of the pigeonpea genome in 332,766 sequence contigs with consensus sequence of ~548 Mb. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/ GenBank under the accession AFSP00000000. The contigs were arranged into 59,681 scaffolds with the help of paired end sequences of 20 kb fragment library, covering ~458 Mb of genome sequence with average scaffold size of 7,679 bp, N50 size of 13,989 bp and the largest scaffold size of 177,971 bp. The large sequence contigs, representing about 60% of the estimated 858 Mb size of the pigeonpea genome were used for the analysis of genes and repeat contents of the genome and mining of SSR loci.

Gene Content of the Pigeonpea Genome

The 454 GS-FLX large sequence contigs containing ~511 Mb of high quality sequence were used for gene prediction using FGENESH software (Table 5.1). Total 59,515 genes were predicted out of which 47004 were protein coding genes and 12511 genes were TE related. All protein coding genes were further classified into several functional categories (Fig. 5.1).

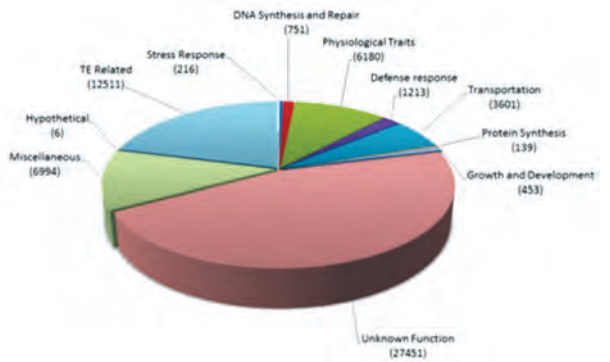


Fig. 5.1: Functional Classification of Genes

Table 5.1: Summary of gene prediction statistics from the pigeonpea genome

Software Used	MolQuest FGENESH
Reference Species	*Arabidopsis thaliana*
Total number of input sequences (All contigs)	191705
Total number of sequences in which genes have been predicted	88555
Total number of GENES predicted (> 0.5kb)	59515
Largest GENE Size	11523 bases
Smallest GENE Size	501 bases
Average GENE Size	1170 bases
Maximum number of genes predicted in a single sequence	11
Total number of EXONS	233560
Largest EXON Size	6555 bases
Smallest EXON Size	4 bases
Average EXON Size	~300 bases
Maximum number of Exons in a gene	54
Total number of INTRONS	~180000
Largest INTRON Size	4884 bases
Smallest INTRON Size	30
Average INTRON Size	~350 bases
EST Matches with bit score more than 100	99.9%

Anchoring of Pigeonpea Sequence Scaffolds to Genetic Map

We developed a high density intra-species reference genetic map of pigeonpea based on 366 genic-SNP markers (unpublished data). The 59,681 sequence scaffolds of pigeonpea genome were compared with the sequences of mapped genic- SNP markers and 347 (99.3%) of these showed matches with an equal number of scaffolds, covering total sequence of ~7.42 Mb (Fig. 5.2). The anchored scaffolds provide genome wide nucleation points for the finishing of the pigeonpea genome and creation of large pseudomolecules for its eleven chromosomes. The 347 scaffolds were assigned to the eleven linkage groups of pigeonpea. We predicted 1,041 genes in the anchored scaffolds, 63 of these genes were identified as TE-related genes and 26 genes did not show any hit in the database. Out of the 7,424,371 bp of anchored scaffolds 1.697 Mb (23%) were RE which was less than half of the 63.95% RE in the whole pigeonpea genome, indicating that the anchored scaffolds represented gene-rich regions of the genome. Anchored scaffolds represented only ~1.6% of the total ~458 Mb of assembled scaffolds, but

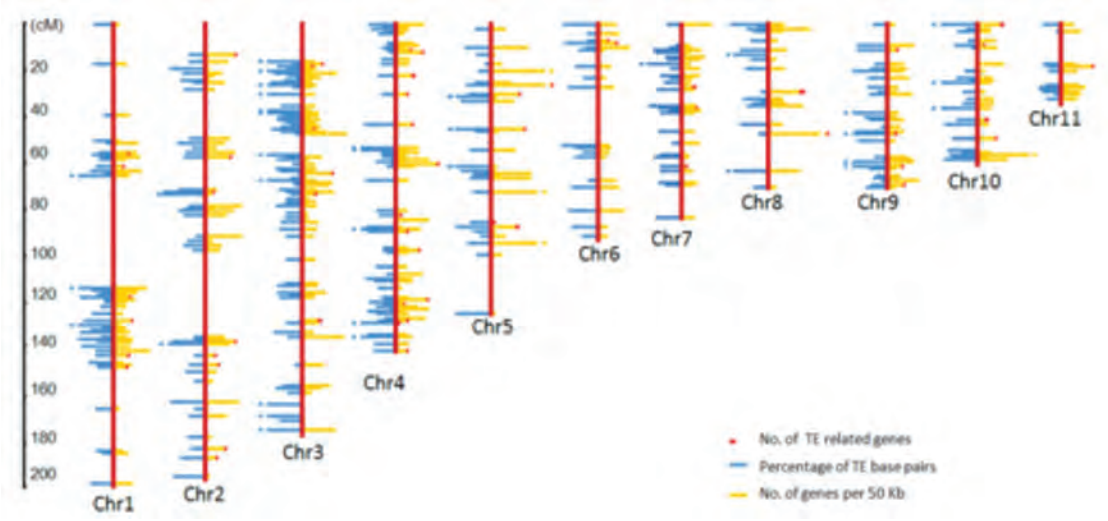


Fig. 5.2: Density of genes and TE repeats in the 347 anchored scaffolds on the 11 chromosome of pigeonpea. The orange bars on right side of the chromosomes represent gene density and blue bars on the left represent TE density. The red color highlighted at the end of orange bars represent number of TE-related genes in that particular scaffold. The discontinuous blue bars indicate highest TE density of more than 40%.

they do provide a random sample of the genome and large number of nucleation points for the finishing of the genome. The average number of genes per 50 kb of scaffold sequence in the entire genome was 7.01. The gene density in the scaffolds was expected to be inversely proportional to the repeats density, which was true for many of the anchored scaffolds.

Comparative Analysis of Pigeonpea and Soybean Genomes

Pigeonpea and soybean belong to the same clade Millettieae of the plant family Fabaceae . Pigeonpea is a shrub grown as annual crop that has high seed protein and starch contents but minimal oil content. Soybean on the other hand is an annual herb with seeds rich in oil and protein but low in carbohydrates. The 47,004 protein coding genes of pigeonpea were compared with 42,094 protein coding genes of soybean using BLAST search with default parameters. Total 31,937 (67.94%) of the pigeonpea genes showed matches with soybean genes, whereas 9,067 genes were unique to pigeonpea. Similarly, out of 42,094 genes predicted in soybean 40,392 showed significant matches with pigeonpea genes, whereas 1,702 genes were unique to soybean. This shows that pigeonpea has significantly higher number of unique genes that differentiate it from soybean. Conservation of synteny between pigeonpea and soybean was analyzed on the basis of 347 genetically anchored scaffolds of pigeonpea. There are total 1,041 genes in the anchored scaffolds of which 512 are single copy genes. Genes on each of the pigeonpea chromosomes showed matches with multiple soybean chromosomes but some soybean chromosomes showed significantly higher number of matches, and therefore are likely be syntenic. A clear conservation of synteny was observed only with chromosomes 1, 3, 4 and 9 of pigeonpea with chromosomes 2, 5, 7, 8, 12, 13, 15 and 17 of soybean (Fig. 5.3). Chromosomes 2, 5, 6, 7 and 10 did not show clear synteny with any soybean chromosomes. Chromosomes 8 and 11 of pigeonpea did not show more than 10 matches with any of the soybean chromosomes.

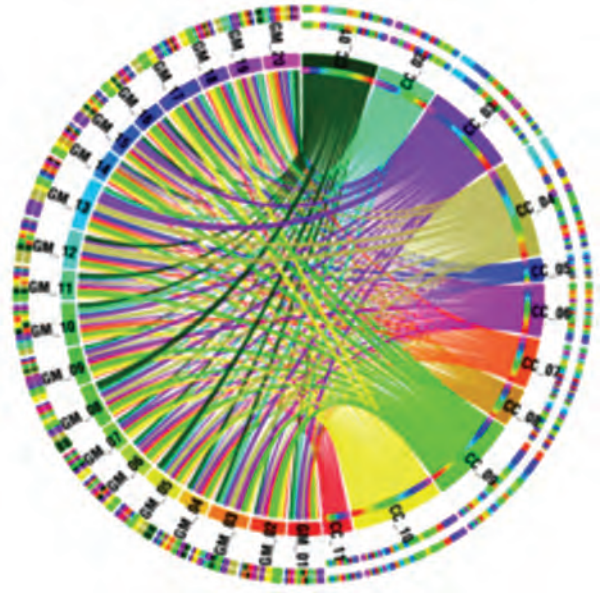


Fig. 5.3: Circular map of syntenic relationship between 11 pairs of pigeonpea chromosomes with 20 pairs of soybean chromosomes based on 512 single copy genes in the genetically anchored scaffolds of pigeonpea genome. The outer circles depict soybean chromosome bars showing proportion of gene matches with different chromosomes of pigeonpea and vice versa.

Low level of synteny between pigeonpea and soybean suggests that they might have only one genome in common and both are ancient amphiploids. Their genomes have highly evolved after speciation from a common ancestral species; hence there is limited conservation of synteny between the two. This is in contrast to high conservation of macro synteny between rice and wheat, which separated about 50 mya.

Development and Validation of Hyper Variable HASSR Markers for Pigeonpea

Pigeonpea genome was analyzed to identify 1,89,895 SSR loci comprising of 100,373 mono-nucleotide, 49,325 dinucleotide, 18,505 tri-nucleotide, 2,217 tetra-nucleotide, 512 penta-nucleotides, 815 hexa-nucleotide and 18,148 compound repeats (Table 5.2). Overall there is one SSR locus for every 2.88 kb of the pigeonpea genome sequence. Search for class

I SSRs (n ≥ 20 bp,) and hyper variable HASSRs (n ≥ 50 bp,) revealed that class I SSRs are most prevalent in the dinucleotide category, whereas HASSRs are most abundant in the compound SSR category (Table 5.2).

We observed higher validation success rate of 81.92% for these genomic-SSR markers as compared to 72% success with genic-SSR markers described earlier. HASSR markers showed 40.8% polymorphism (Fig. 5.4), which is three times higher than 12.9%

Table 5.2: Frequency of different types of SSR loci in the pigeonpea genome

Type of SSR	Total No. of SSR loci	Class I SSR(n> 20 bp)	Hyper variable SSR (n >50 bp)
Mononucleotide	1,00,373	987	0
Dinucleotide	49,325	18,000	203
Trinucleotide	18,505	5,822	515
Tetranucleotide	2,217	2,217	17
Pentanucleotide	512	512	15
Hexanucleotide	815	815	70
Compound	18,148	18,148	10,891
Total	**189,895**	**46,501**	**11,711**

Based on the SSR length criteria 46,501 loci were classified as class I SSR and 11,711 of these were HASSR. All the SSR loci belonging to tetra-, penta, hexa- and compound category were of class I SSR, while more than half (10,891) of the compound SSRs was of HASSR type. For wet lab validation we attempted to design PCR primers for 1,220 HASSR loci, taking 300 loci from the compound SSRs and all the loci from the remaining categories. Total 358 primer pairs amplified a single PCR product of expected size and these were screened for polymorphism in a set of eight pigeonpea genotypes. polymorphism observed with type I genic-SSR markers on the same set of eight genotypes. The HASSR polymorphism was much higher than the earlier reported 28.40% polymorphism for BAC-end sequence derived genomic SSR markers obtained using high resolution capillary electrophoresis. This underlines the high potential utility of the HASSR markers in pigeonpea molecular breeding.

Wheat genome

Total ~238 GB Sequencing data (6 Paired End + 2 Single End) was received from TGAC, BBSRC

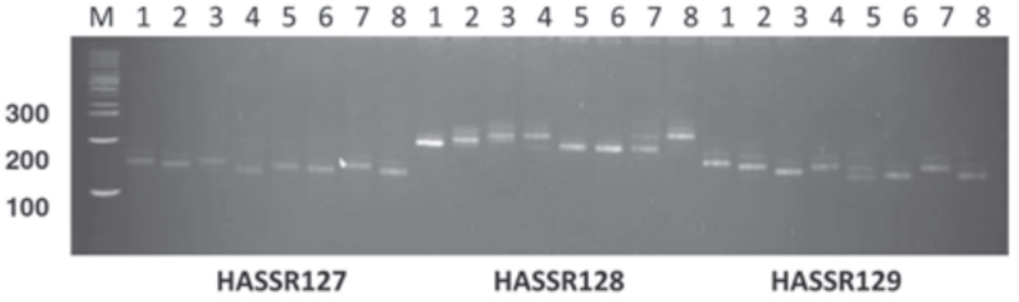


Fig. 5.4: Agarose gel showing allelic variation in PCR product size with three different HASSR markers (HASSR27, HASSR28, HASSR29) in a set of eight pigeonpea varieties. 1 Asha, 2 UPAS 120, 3 HDM 04–1, 4 Pusa Dwarf, 5 H2004-1, 6 Bahar, 7 Maruti, 8 TTB7; M=100 bp DNA size marker

(Biotechnology and Biological Sciences Research Council), Norwich, UK. Quality check was performed by removing those bases from the raw sequenced reads whose quality score below Q20 (Phred –Score) using software package sickle, followed by trimming the Wheat 2AS specific single /paired end adapters, single/paired end sequencing primer and PCR primer from end of the reads using software CLC Genomic Workbench. The trimmed data was exported in the fastq format so that data will be accepted by different assembly software.

From the 14 illumina raw files a total of 1,116,247,283 numbers of reads was generated which was used for the quality check, after removing the bases below Q 20. Finally a total of 1,069,164,366 reads were left after trimming adapter, PCR primer and sequencing primer which was used for the de-novo assembly. A total no. of 56832 BAC clones were obtained from Czech Republic. Out of these, BAC DNA of 768 samples has been isolated and are being fingerprinted using SNaPShot Technology.

B. High throughput mapping and genotyping for important agronomic traits in rice and wheat

Mapping QTLs for drought tolerance and associated traits in wheat

A mapping population of 206 recombinant inbred lines (RILs) derived from a cross between drought sensitive high yielding wheat variety WL711 and drought tolerant traditional wheat variety C306 was used for this purpose. For shoot and root related traits phenotyping, 206 RILs and both parents were grown in PVC pipes (Fig. 5.5). Ten traits selected for phenotyping included six above ground traits and four below ground traits (Fig. 5.6). A well saturated framework genetic linkage map was constructed using these RILs with 173 molecular markers, including 169 SSR, 2 CAPS, 1 EST-STS and 1 SNP, randomly distributed over the 21 wheat chromosomes. Total thirty seven QTLs were identified for ten drought related traits included six above ground traits and four below ground traits at eighteen different chromosomal locations but most of these showed small inconsistent effects. A consistent QTL for drought susceptibility index (*qDSI.4B.1*) was mapped on the short arm of chromosome 4B, which was also co-located with QTLs for grain yield per plant, harvest index and root biomass under drought.

Fig. 5.5: Both parents and 206 RILs were grown in PVC pipes for phenotyping

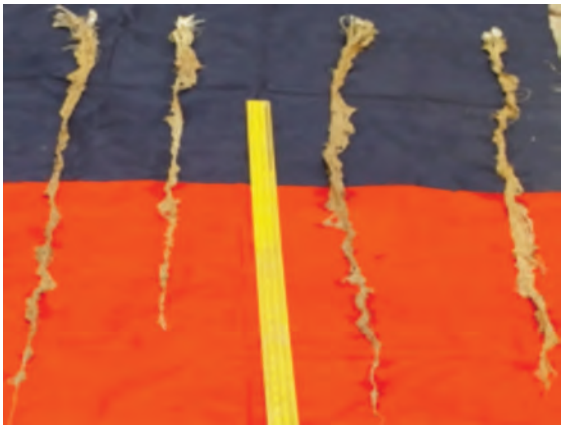


Fig. 5.6: Variation in root growth of the parental lines of RIL population under control and drought conditions

We identified a number of candidate genes for drought tolerance using a combination of QTL mapping and microarray expression profiling approaches. Transcriptome profiling of the parents and RIL bulks with extreme phenotypes revealed five genes (Table. 5.3)

Table 5.3: Number of differentially expressed gene probes at $P < 0.01$ and log fold change of >2.0 between drought tolerant and drought sensitive genotypes under control and drought stress conditions [a]Taking signals from the sensitive parent WL711 as base

Genotypes/Genes	Control condition			Drought condition		
	Up	Down	Total	Up	Down	Total
WL711	-	-	-	1814	3896	5710
C306	-	-	-	1297	2088	3385
Sensitive RIL bulk	-	-	-	2467	2500	4967
Tolerant RIL bulk	-	-	-	1764	1361	3125
WL711 versus C306[a]	962	2251	3213	818	1315	2133
Sensitive versus tolerant RIL bulk[b]	774	754	1528	574	671	1243
Common in tolerant parent /tolerant bulk	-	-	-	76	86	162
Genes in the *qDSI.4B.1* QTL interval or short arm of 4B	-	-	-	1	4	5

Taking signals from the sensitive RIL bulk as base underlying this QTL that were differentially expressed between the parents as well as the two RIL bulks, suggesting that they are likely candidates for drought tolerance. Syntenic genomic regions of rice, sorghum and maize were identified that also harbour genes for drought tolerance (Fig. 5.7). The identified robust QTL for drought tolerance in wheat variety C 306 spans about 10 cM and is tightly linked with the *Rht1b* gene for plant height in wheat, therefore it will be necessary to develop fine mapping population to narrow down the QTL region for the identification and cloning of the genes underlying this QTL. The consistent QTL identified in this work will be useful for marker assisted breeding for drought tolerance in wheat.

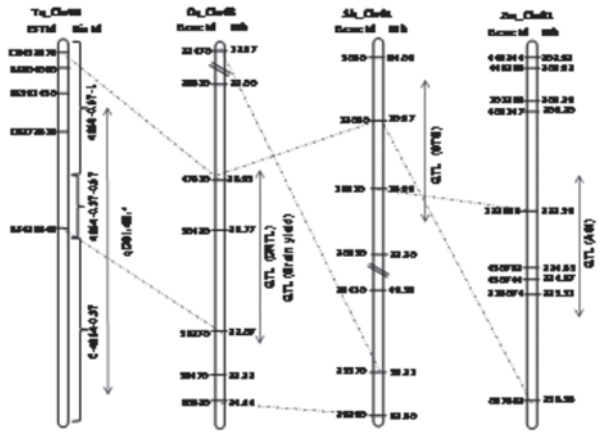


Fig. 5.7: Colinearity of genes and QTLs in the *qDSI4B.1* region of wheat chromosome 4B with syntenic regions of rice, maize and sorghum. Dotted lines connect positions of orthologous genes. Arrows indicate positions of QTLs. Only last digits of the respective gene Id are shown

Characterization of thirty one NILs for seed storage proteins in wheat

Thirty one near isogenic lines comprising different alleles of HMW, LMW, gliadins, albumins and triticin were evaluated for bread making quality (Table 5.4). Quality evaluation was carried out from material harvested from the field of IARI in replicated field trials using several technological tests: grain protein content (Prot), grain hardness (GH), grain moisture, thousand kernel weight (TKW), SDS sedimentation test, farinograph (dough stability, water absorption, dough development time).TKW, GH was evaluated by SKCS (single kernel characterisation system). Prot was done by near infrared reflectance spectrometry (NIRS). Bread making test was performed for end use quality and loaf volume was measured by rapeseed displacement method. Statistical analysis of data was

Table 5.4: Details of NILs having different alleles from different seed storage proteins of wheat

Seed storage proteins	Alleles of wheat
HMW Glutenin	*Glu A1b, Glu B1b, Glu B1b*, Glu B1i, GluD1d, GluA1a*
LMW Glutenin	*GluD4a, Glu A3c, GluA3 e, GluA3a, Ks, GluA3d*
Gliadin	*Upper Band, Lower Band, GliB1a, GliB1c*, GliA1g, GliA1h, GliB1b, GliB1d, γ- gli mb, γ- gli mc, γ- gli md, γ- gli me*
Albumin	*mc, mf, mb, mg*
Triticin	*TriD1a and TriA1a*

performed by software SPSS by one way variance analysis (ANOVA). Glutenin alleles *GluD1d, GluB1b, GluA3d* and triticin alleles Tri*D1a,* Tri*A1a* shows consistent positive effect on dough stability which was determined by farinograph. Comparison of dough stability among *GluD1d, TriD1a* and *HD2329* shows a constant increase of stability in *TriD1a and GluD1d. GluD1d, GluD4a, GluB1b, GluB1b*, GluB1i, TriD1a* and *TriA1a* show consistent positive effect on SDS sedimentation volume (Table 5.2). *GluA3c, GliA1g, GliB1c*, GliA1h* alleles shows negative effect on dough stability and *GluA3a, GluA3c, GliB1a, GliB1c*, GliA1g,* and *GliA1h* shows negative effect on SDS sedimentation volume. Gliadin alleles have no significant effect on bread making quality except *GliB1c*, GliA1g,* and *GliA1h* which shows negative effect on SDS sedimentation volume and loaf volume. *GluD1d, TriD1a, TriA1a* alleles shows consistent positive effect on farinograph dough stability, SDS sedimentation and loaf volume (Table 5.5).

A contrasting behaviour of different alleles of seed storage proteins was seen towards end products quality (Fig.5.8). NILs of *GluD1d, GluD4a, GluB1b, TriD1a* and *TriA1*a shows consistent positive and *GluA3c, GliB1c*, GliA1g,* and *GliA1h* shows consistent negative effect on bread making quality when compared with HD2329. These alleles are of potential value in breeding programmes designed to improve bread-making quality.

Identification of candidate rice aroma genes using bulk segregant analysis

Aroma, a quantitative trait, is one of the most important quality attributes of basmati rice. The complex inheritance of this trait makes it difficult for the identification of genes responsible for aroma using any one single analytical approach. The total number of genes expressed in the aroma QTLs aro3.1, aro4.1 and aro8.1 mapped on chromosomes 3, 4 and 8 respectively is quite large. A bi-parental segregating

Table 5.5: Behavior of different alleles as compared to HD2329

Traits	Allleles with positive effect	Alleles with negative effect as compared to HD 2329
Dough stability (min.)	*Glu D1d* (6), *GluB1b* (3), *GluA3d* (4),*TriD1a* (5), *TriA1a* (4)	*GluA3c*(3), *GliA1g*(5) ,*GliB1c**(6), *GliA1h* (4)
SDS Sedimentation volume (mL)	*GluD1d*(7), *GluD4a*(5), GluB1b(4), GluB1b*(5), *GluB1i*(4), *TriD1a* (6)	*GluA3a*(3), *GluA3c* (4), *GliB1a* (4), *Gli B1c**(6), *GliA1g*(3) , *GliA1h*(5)
Loaf volume (cc)	*GluA1a*(30), *GluD1d*(60), *GluD4a* (40), *GluB1b**(40), *GluB1i*,(40) *GluA3d*(30), *TriD1a* (50), *TriA1a*(40)	*GliB1c **(30), *GliB1b* (20), *GliA1h*(30)

population of recombinant inbred lines (RILs) has been used for narrowing down the number of differentially expressed genes using the bulk segregant analysis (BSA). There are thousands of differentially expressed genes between the aromatic parent (Pusa 1121) and the non-aromatic parent (Pusa 1342). It is a highly complicated task to analyze any significant data from such a large number of genes; moreover, all of these genes cannot be responsible for aroma. Hence, two extreme bulk groups were made comprising of six highly aromatic and six non-aromatic RILs selected from among the mapping population through sensory analysis of aroma detected by soaking the seeds in 1.7% potassium hydroxide solution. RNA isolated from fresh leaves of each segregating line were pooled together and used for making cDNA copies through in vitro hybridization. The differential expression of genes was analyzed through the Affymetrix Rice genome array containing probe sets designed from 48564 *japonica* and 10260 *indica* gene sequences. Pooling together RILs expressing a particular phenotype significantly reduced the number of differentially expressed genes to about 160. 16 differentially expressed genes between the RIL bulks are found to be co-located in the QTL region aro4.1, whereas 2 genes have been identified to be differentially expressed in the QTL region aro3.1. Hence the focus has now been shifted to these genes, increasing the probability of hitting the gene(s) which are directly or indirectly responsible for the expression of aroma in rice. Proteomics approach is now being employed to further validate these results/genes.

Single Nucleotide Polymorphism (SNP) Based High density molecular map in rice

A molecular map in rice solely based on SNPs from a biparental recombinant inbred population was developed from the parents Nagina22 and IR64, which are drought and heat tolerant and susceptible cultivars respectively. 282 recombinant inbred lines were genotyped along with three technical replicates of the parents using infinium assay technology (Illumina Inc) at 5246 loci. Of these, 1512 loci were polymorphic (28.2%). Chromosome wise distribution of polymorphic markers along with the number of loci genotyped is given in Fig. 5.8. Chromosome one had the highest number of polymorphic markers (203) followed by chromosome 3 (172) whereas chromosome nine had the least number of polymorphic markers (66). This map (Fig. 5.9) will be very helpful in identifying genomic regions (quantitative trait loci-QTLs) associated with heat and

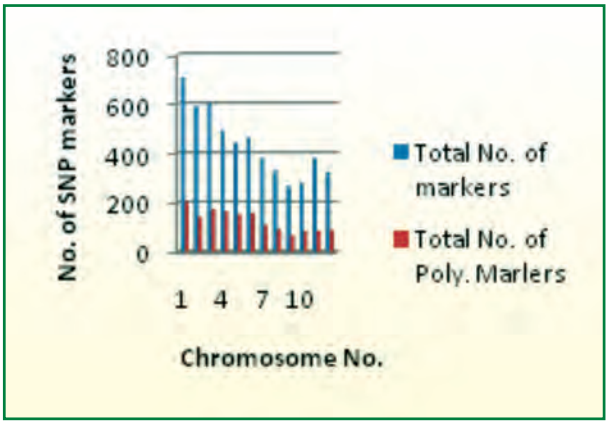


Fig. 5.8: Chromosome wise distribution of genotyped and polymorphic loci in N22/IR64 mapping population

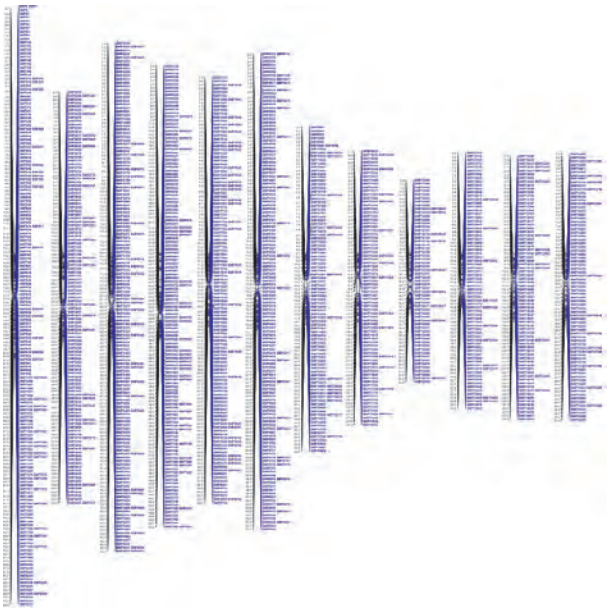


Fig. 5.9: High density molecular map in N22/IR64 mapping population in rice

drought tolerance once appropriate phenotypic data sets are available.

Microsatellite distribution in monocot and dicot species

A total of 797,863 SSRs were identified for the six genomes including three of each monocot (Brachypodium, Sorghum and Rice); and dicot (Arabidopsis, Medicago and Populus) plant species. Among the six genomes analysed, maximum number as well as frequency of SSR was observed in Populus followed by Medicago whereas least for the Sorghum genome. The frequency of SSR was considerably higher in dicots as compared to monocot species. In monocots, rice genome has near about twice the frequency of SSR than Sorghum and Brachypodium genomes. These species belong to very diverse groups of monocots and dicots, and the distribution pattern of SSR motifs with particular sequence identified in these genomes was not uniform however the overall pattern of SSR motifs with particular length was similar. Mono- nucleotide repeats were dominating over the other type of repeats in all the six plant species. However, the frequency of SSR was decreasing stepwise with the increased motif length (mono- to hexa- nucleotide repeat) except for Brachypodium where the frequency of tri-repeats was higher than the di-repeats (Fig. 5.10). The mono-repeats were found to be minimum (43%) for Sorghum and maximum (79%) for Medicago genome. Mostly the mono-, di-, and tri-nucleotide repeats contributes major proportion of SSRs and very little share was contributed by tetra-, penta-, and hexa-nucleotide repeats. Maximum of 5.4 % contribution of tetra-, penta-, and hexa- nucleotide, was observed for Sorghum genome. Similar trend was obtained for other genomes studied in present investigations.

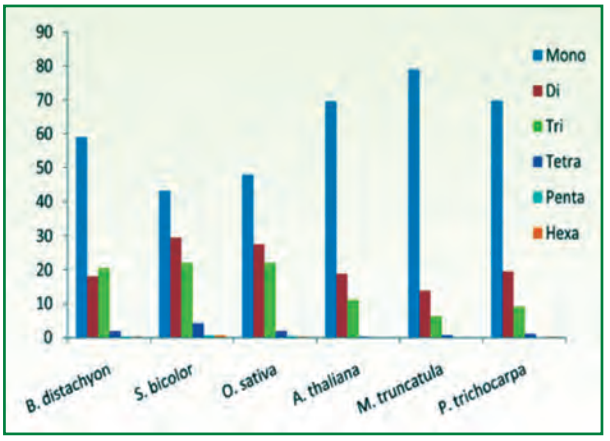


Fig. 5.10: Genome-wide distribution of microsatellites in monocot and dicot plant species with respect to motif length. Microsatellites were identified with criteria of mono- to hexa-nucleotides motifs using MISA software tool, and the minimum repeat unit was defined as 10 for mono-, 6 for di-, 5 for tri-, tetra-, penta-, and hexa-nucleotides

Mapping of loci underlying spot blotch (*Bipolaris sorokiniana*) resistance in wheat

Spot blotch of wheat caused by *B.sorokiniana* is a destructive disease of wheat particularly in the warm and humid parts of India and is slowly spreading into cooler traditional areas of wheat also. The main aim of the project is identifying the loci underlying spot blotch resistance so as to incorporate durable resistance into the high yielding popular varieties of India. Fresh crosses were effected between the susceptible parent HD2932 and the resistant donors SW89-5422 and Chirya1 in Rabi 2010-2011 and the F1s were advanced in summer 2011 in the National phytotron facility of IARI. Currently the 2 F2 mapping populations HD2932/SW89-5422 (259 lines) and HD2932/Chirya1 (154 lines) are evaluated for spot blotch resistance in polyhouse and are being genotyped for polymorphic markers. Briefly, the F2 populations were inoculated with a monoconidial culture of the highly virulent strain 'BS 75' of *B. sorokiniana* at a concentration of 10^4 spores/ml of water at 3 stages *viz.,* tillering stage, flag leaf emergence and the anthesis stage i.e., GS20, GS37 and GS65 on Zadok's scale and disease evaluation was done on the flag leaves at GS77 i.e, late milking stage. The disease severity percentage showed a continuous distribution

but there is an indication of segregation of 1 or 2 major genes (depending upon the cut off point for resistants) in both of the populations. The F1s of the crosses HD2932/ SW89-5422, HD2932/Chirya1 and HD2932/'Milan/Shanghai7' were also evaluated for spot blotch disease thus giving a score of 3-4 on a scale of 1-9 of Saari and Prescott (1975), however the F1s showed a prolonged chlorosis till a score of 5-6.

A screening of the susceptible parent HD2932 and the resistant parents with a total of 226 primers that included mostly SSRs including known linked markers to spot blotch resistance and a few EST primers resulted in a total of 87, 89 and 91 polymorphisms between the susceptible parent HD2932 and the resistant parents SW89-5422, Chirya1 and Milan/Shanghai7 respectively. Currently genotyping of the F2 populations is in progress and the segregation of the marker *Xgwm67* known to be linked to the spot blotch resistance gene in HD2932/ SW89-5422 population is shown in Fig. 5.11 below. The segregation of the marker is yet to be verified for its linkage to the trait.

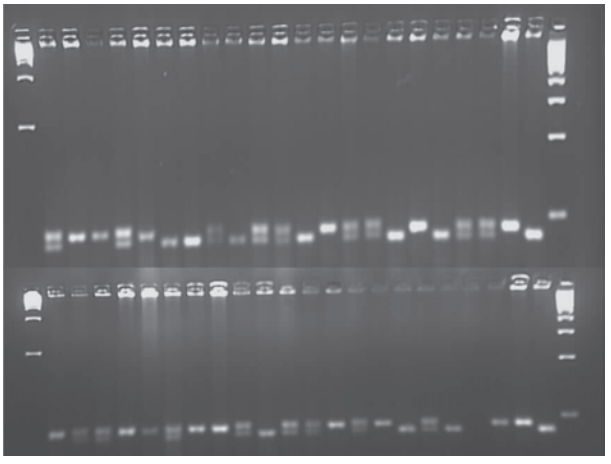


Fig. 5.11: The segregation of the marker *Xgwm67* known to be linked to the spot blotch resistant locus in the F2 population HD2932/SW89-5422. The first and the last lanes are 1 Kb ladder and 100 bp ladder. The 2 lanes preceding the last lane (100 bp ladder) are the susceptible parent HD2932 and the resistant parent SW89-5422

C. Functional genomics

Evaluation of transgenic rice carrying *AtDREB1A* gene for cold tolerance

In the previous year, two independent homozygous transgenic lines of Pusa Sugandh2 carrying *AtDREB1A* gene were identified and screened for their response to drought and cold. This year, cold stress tolerance was tested on T_4 plants of these two homozygous transgenic events along with control in growth chamber (Fig. 5.12). At the end of the stress cycle, leaves of both transgenic and control seedlings exhibited leaf rolling. However after 5 days of recovery significant level of tolerance in both the events where as the control plants exhibited 100% mortality. After 15-days of stress, the transgenic plants showing tolerance response had higher cell membrane stability (95.4% and 97.01%) as compared to control (73.7%) (Fig. 5.13). RT- PCR analysis was carried out using gene specific primers. Up-regulated expression of the *DREB* gene was observed in the cold treated transgenic plants over control (Fig. 5.14).

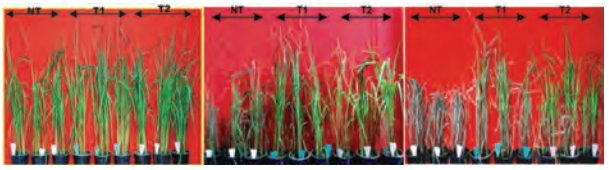


Fig. 5.12: Cold stress analysis of homozygous lines; NT-Non-transgenic plant, T1-transgenic event-1, T2- transgenic event–2

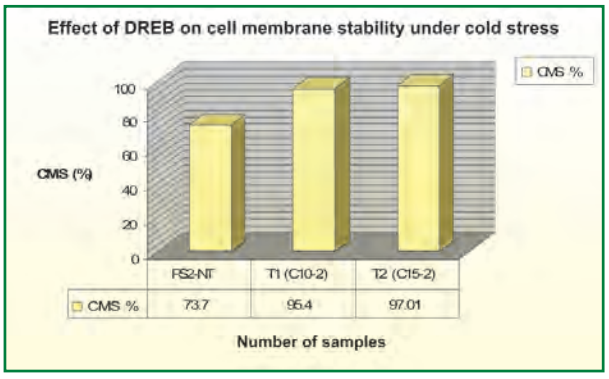


Fig. 5.13: Cell Membrane stability (CMS) in NT-Non-transgenic plant and transgenic events (T1 and T2)

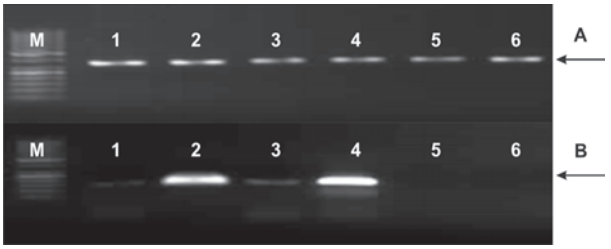


Fig. 5.14: RT-PCR analysis of DREB gene expression under cold stress: A. Lanes 1-6 - 280 bp amplicon of tubulin, B. Lanes 1& 3 - transgenic (T1, T2) non-stressed, 2 & 4 – transgenic (T1, T2) -stressed, 5 – Non-transgenic non-stressed, 6 – Non transgenic stressed, M- 50 bp ladder DNA marker. Arrows show 150 bp amplicon of *DREB* gene

Functional genomics of *Alternaria* blight resistance in Brassica

A total of 456 clones were sequenced from the suppression subtractive hybridization (SSH) library of cv. *Varuna*. Sequences of these clones were subjected to BLAST analysis with EST database of *Arabidopsis*, which revealed 248 differentially expressed genes belonging to different functional categories. Besides 176 ESTs which did not have any match in the *Arabidopsis* sequence database were also identified. The gene ontology (GO) classification of these transcripts is given in Fig. 5.15.

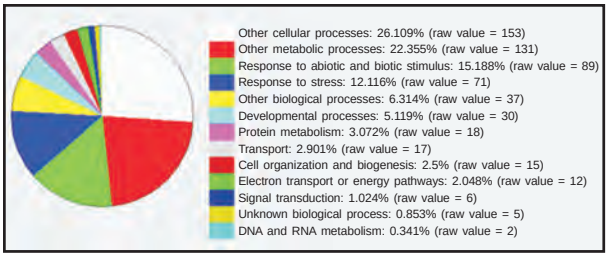


Fig. 5.15: Functional categorization of transcripts obtained from SSH library of *Varuna*

A SSH library was also constructed using 29 day stressed plants of *S. alba*. A total of 750 recombinant clones containing the differentially regulated transcripts were obtained and sequenced from this library which revealed 144 candidate genes, which are homologous to transcription factors and genes belonging to other metabolic pathway. Validation of differentially regulated genes was done by Semi qRT-PCR. Two candidate genes namely NAC14 (944bp) and NAC19 (1171) were cloned into pDrive Cloning vector from Brassica cv. *S.alba, RGN73, Varuna, RLM619, BEC144 and BIOYSR*. Constructs were designed for genetic transformation of susceptible mustard variety using NAC14 isolated from *S. alba* (P_{rd29A}: NAC14: NOS-Ter) into pCAMBIA2300 binary vector (Fig. 5.16).

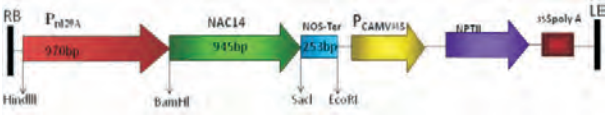


Fig. 5.16: Transformation construct of NAC 14

Phenomics of moisture deficit and low temperature tolerance in rice

A network project on "Phenomics of moisture deficit and low temperature tolerance in rice" has been initiated in the current year with funding from National fund, ICAR wherein a phenomics facility is to be established and operations standardized to evaluate genotypes under moisture deficit and low temperature tolerance. In this project, publically available transcriptome data generated from various experiments at different developmental stages of rice, grown either in field or pot cultures were analyzed to identify drought responsive genes. A total of 82 uncharacterized expressed genes were found to be regulated by drought stress from this analysis of which six genes showed high levels of expression in drought tolerant Nagina22 as compared with drought susceptible IR-64 under drought conditions in the microarray data generated from our study (Fig. 5.17). Out of these six genes, five had more than 2 fold increase (Log 2) in expression in Nagina22 seedlings under drought. These genes were cloned and sequenced from Nagina22.

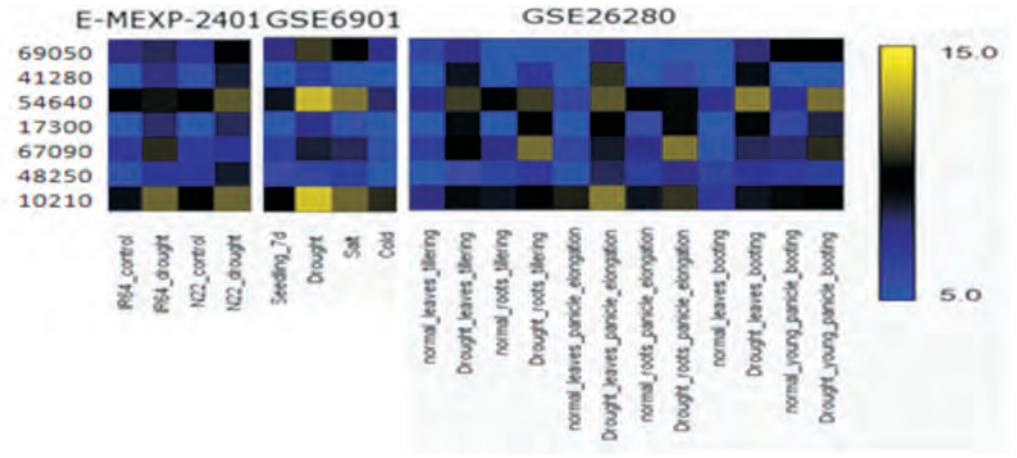


Fig. 5.17: Expression map of uncharacterized differentially expressed proteins during drought condition in different experiments

To confirm the expression of selected candidate genes, Nagina 22 seedlings were grown in pot cultures. After 14 days, stress was imposed by withholding water. Total RNA was isolated from leaves of both the control (N22C) and drought stressed (N22S) plants.

Semi quantitative analysis (Fig. 5.18) of these selected expressed genes were found to be in alignment with the microarray data of E-MEXP-2401 which was generated from Nagina22 under drought condition. The genes were amplified and cloned in cloning vector pGEMT. These genes were sequenced

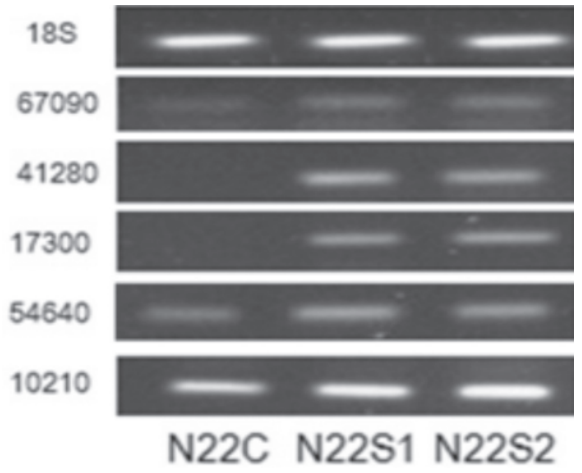


Fig. 5.18: Semi quantitative analysis of selected uncharacterized differentially expressed proteins during drought

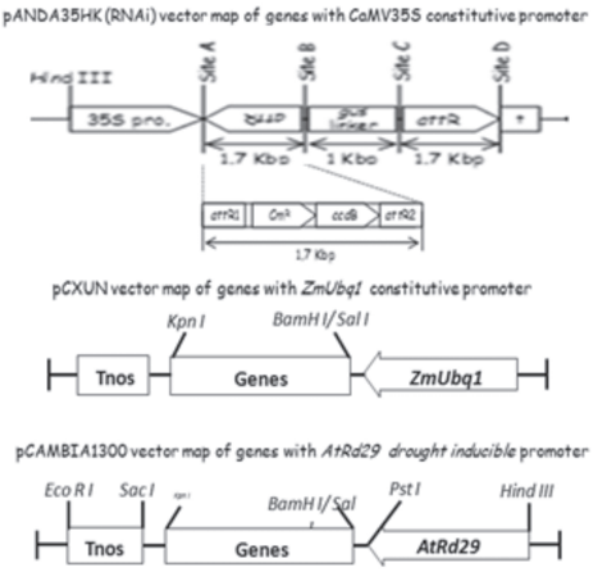


Fig. 5.19: Construct maps for functional validation of expressed protein encoding genes

and subcloning was done in plant expression vector under drought inducible *AtRD29A* promoter and constitutive *ZmUbq1* promoter for functional validation. *A. tumefaceins* (EHA105) harbouring pCAMBIA 1305.1 GUS vector was used for the optimization of callus transformation. Transformation of above mentioned gene constructs into IR-64 and N-22 is in progress (Fig. 5.19). The sequencing data of these expressed genes showed significant differences in translated proteins between Nagina 22

and the reference genome, Nipponbare. For the first time callus transformation of N-22 was optimized, and only 10 days old callus is sufficient to produce maximum number of transformation event.

Genetic transformation of rice using *cry1Aabc*

The vector pCaMV35S*: cry*1Aabc: *Nos*T pC1200 construct was validated (Fig. 5.20) for the transformation of elite *indica* rice variety Pusa Sugandh 2. A total of 2200 mature embryo derived calli were used for biolistic transformation in five batches. After three round of hygromycin selection (15 days each) the resistant calli were transferred to regeneration medium (Fig. 5.21). The regenerated plantlets were transferred to rooting medium. After root development the plantlets were kept in liquid hardening medium ($1/10_{th}$ MS). A total of 23 putative transformants were obtained. The transformants were analysed for the presence of transgene through PCR using *cry*1Aabc gene specific primers. Six putative transformants were confirmed positive for the PCR amplification of transgene (Fig. 5.22). The plants are hardened in soilrite at culture room condition. The plants will be transferred to phytotron glass house for further molecular analysis.

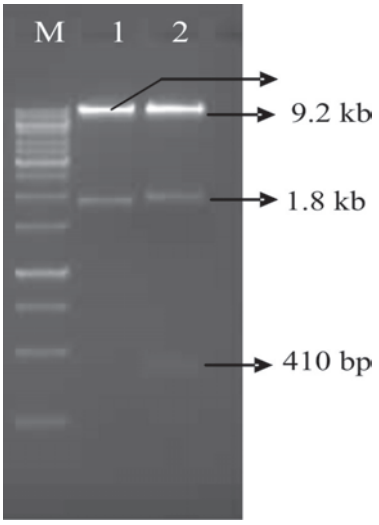


Fig. 5.20: Validation of pC1200:*cry*1Aabc construct: M-1kb ladder, Lane 1-*Bam*H1 digestion releases 1.8 kb *cry*1Aabc gene fragment, Lane 2- *Hind*III and *Eco*RI digestion releases 1.8 kb *cry*1Aabc gene fragment, and 410 bp of 35S promoter fragment

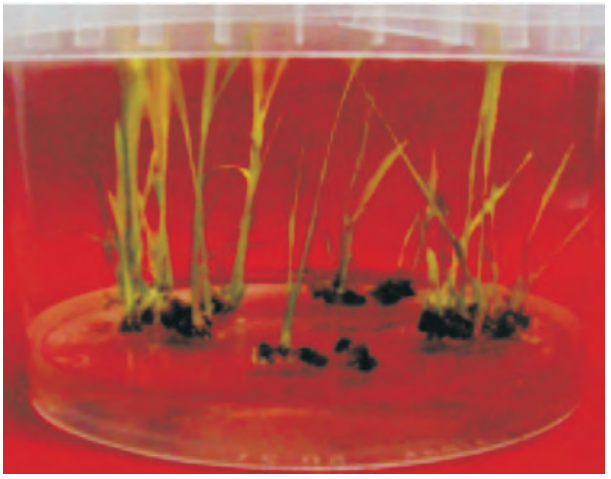


Fig. 5.21: Putative PS2 transformants on regeneration medium

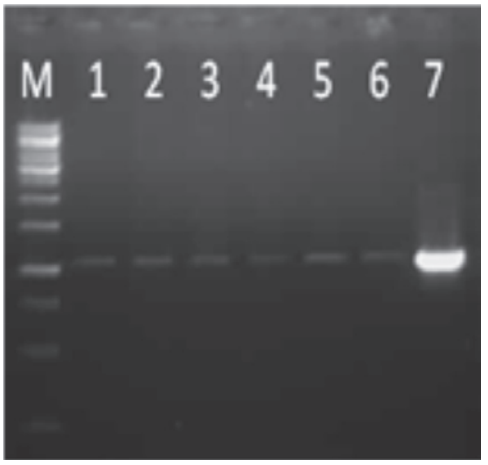


Fig. 5.22: PCR confirmation of *cry*1Aabc transformants. Lane M – 1 kb ladder, Lane 1-6 (1.2 kb) *cry*1Aabc gene specific amplification from transformants, and Lane 7 positive control

Expression analysis of *Pi-k^h* (*Pi54*) gene and co-regulated genes in transgenic rice lines

In order to study the nature of gene expression and confirm the involvement of upstream elements in regulating the expression of *Pi-k^h* (*Pi 54*) gene in blast resistant transgenic lines (Fig.5.23), quantitative Real Time RT-PCR (qRT-PCR) was performed using

Fig. 5.23: Phenotype of Tansgenic line containing *Pi54* blast resistance genes

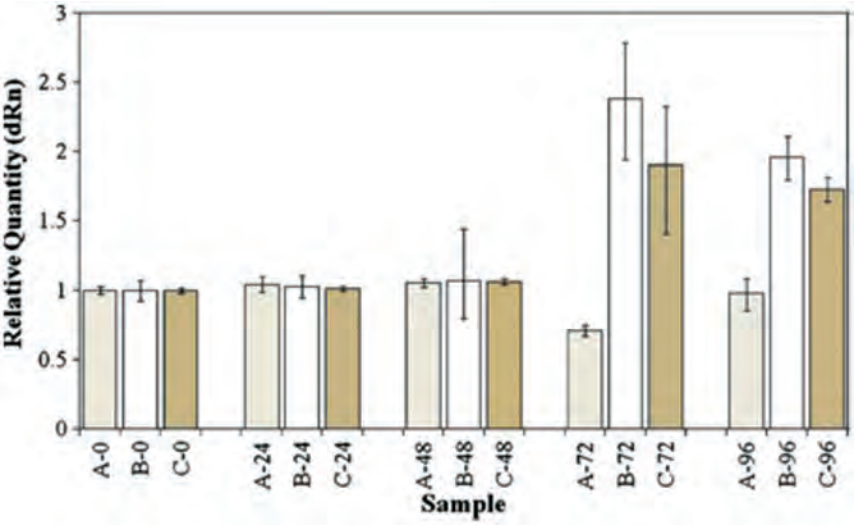


Fig. 5.24: Relative expression of *Pi54* gene upon pathogen challenge in two transgenic lines using real time PCR analysis A: Non-Transgenic TP309; B: Transgenic Line TP-Pi54-2; C: Transgenic Line *TP-Pi54-15*; Error bars represent the standard deviation of the mean of three biological replicates. dRn (or delta Rn) refers to baseline corrected normalized fluorescence

Pi54 exon specific primers. RNA was isolated using Trizol reagent from blast inoculated leaves of transgenic (T3) plants as well as non-transgenic control plants. After 72 h of inoculation of *M. oryzae* spore suspension, changes in transcript abundance in rice leaves was obtained by qRT-PCR (Fig. 5.24). Expression of *Pi54* gene after 0 to 96 h post inoculation (hpi) of TP-*Pi54*-2, TP-*Pi54*-15, and Taipei 309, was compared. To normalize the qRT-PCR data, each sample was compared with the Elongation factor 1- α specific transcript. *Pi54* gene was found to be expressed 2.31 fold higher in TP-*Pi54*-2, 1.5 fold higher in TP- *Pi54*- 15 after 72 hpi. In contrast, the susceptible allele was found to express lower in Taipei 309.

Microarray techniques was used to determine the extent of *Pi-k^h* (*Pi54*) gene expression in resistant transgenic lines in relation to their reaction to the blast pathogen and also to find out genes co-regulated with *Pi-k^h* gene during *M. oryzae*- rice interaction. In this study, we investigated and compared the global gene expression changes happening in response to the infection by *M. oryzae* at 72 hpi, in resistant TP-*Pi54* and susceptible wild Taipei 309 (TP309) using microarray based transcriptome analysis.

Pi54 gene (*Pi-k^h Oryza sativa*-Tetep.) confers high degree of resistance to diverse strains of fungus *M. oryzae*. In order to understand the genome wide co-expression of genes in the transgenic rice line containing *Pi54* gene, microarray analysis was performed at 72 h post inoculation of *M.* oryzae strain PLP-1. Expressed genes were categorized as per their roles on the basis

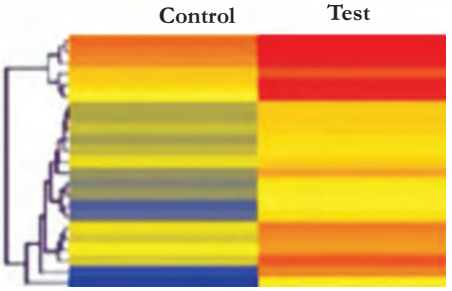


Fig. 5.25: Expression pattern of genes for stimulus response as represented by the heat map profile of mock-inoculated vs. TP-*Pi54* rice lines inoculated with *M. oryzae.* Colour changes show the level of gene expression in their respective clusters. Yellow: Mildly expressed genes; Blue: Highly suppressed genes; Red: Highly expressed genes

of Gene Ontology (GO) score. Various defense response genes like Callose, Laccase, PAL, Peroxidase, transcription factors like NAC6, Dof zinc finger, MAD box, bZIP, WRKY and other important categories of genes were found to be up regulated in transgenic lines. The result of Microarray based co-regulation of defense response genes influenced by *Pi54* mediated resistance is given in Fig. 5.25.

D. Allele mining

Allele mining for blast resistance gene *Pita* in land races of rice

Rice blast caused by *Magnaporthe oryzae* is the most destructive disease affecting rice production worldwide. For the management of this disease many blast resistance genese are available. Of these *Pita* is dominant blast resistance genes which is effective under Indian conditions. We mined alleles of this genes in land races of rice collected from different parts of the country (Fig. 5.26). Besides, *Pi-ta* orthologue sequences of 220 rice accessions belonging to wild and cultivated species (*O. rufipogon*, *O. barthii*, *O. glaberrima*, *O. meridionalis*, *O. nivara*, *O. glumepatula* and *O. sativa*) were retrieved from the database and included in the analysis. Based on nucleotide polymorphism, 64 haplotypes have been identified, with major haplotypes forming eight main haplogroups. *Pi-ta* orthologues of Indian land races were found scattered in most of the major haplotypes indicating its heterogenous nature (Fig. 5.27) . Five unique and novel Pi-ta protein variants were identified from the Indian land races. The detail molecular analysis revealed high degree of inter- and intra-specific relationship amongst the alleles cloned from *Oryza* species, and helps selection of better alleles for future breeding experiments.

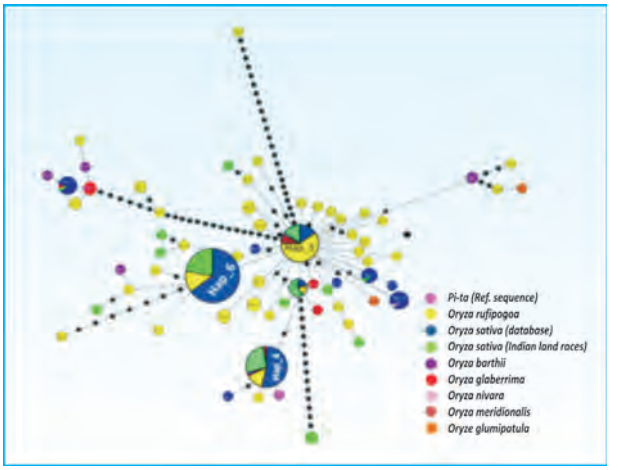


Fig. 5.27: Haplotype network based on nucleotide polymorphism of the *Pi-ta* coding region of 268 accessions

Bioprospecting of genes and allele mining for abiotic stress tolerance in rice

In the network project on "Bioprospecting of genes and allele mining for abiotic stress tolerance" funded by NAIP of ICAR, to identify a minicore reference set in rice, 7000 genotypes constituting the core set were collected, multiplied and phenotyped in the last two years. These accessions were genotyped at 36 microsatellite marker loci, uniformly distributed across all the 12 rice chromosomes, using an automated fragment analysis system in the current year. SSR Genotyping yielded a total of 440 alleles

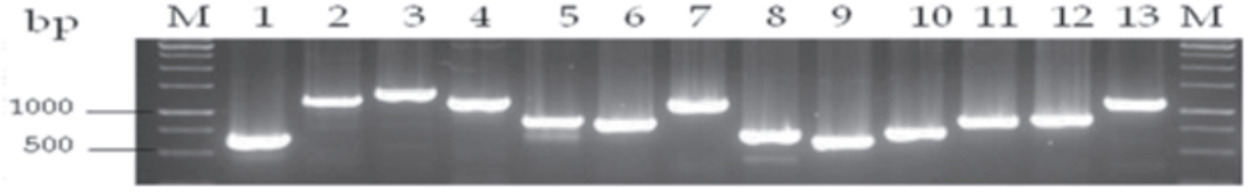


Fig. 5.26: An example of PCR amplification of different fragments of *Pi-ta* allele of rice land race, Mesebatta. bp- Base pairs, M- Molecular weight marker and lanes 1-13, PCR fragments obtained from overlapping fragments of *Pi-ta* allele

with an average of 12.57 alleles per locus. Polymorphism Information Content (PIC) ranged from 0.0881 (RGNMS190) to 0.8619 (RM552). Using both genotypic and phenotypic data sets, a minicore constituting 228 genotypes was identified representing all 440 alleles, having the same proportion of phenotypic characters that are present in the core set as well. The identified minicore set is further being genotyped for a selected set of SNPs (5246) present in abiotic responsive genes using the high throughput SNP genotyping facility (Illumina inc.,) established in the previous year. Gene expression profiling was carried out at booting stage of landrace Nagina 22 after imposing heat, which revealed 1977 up regulated and 2930 down regulated genes at a minimum of two fold change. Twenty of them were confirmed by RT-PCR and 150 by semi qRT-PCR.

Generation, Characterization and Use of EMS Induced Mutants of Upland Variety Nagina-22 for Functional Genomics in Rice

This year, a total of 507 selected mutant lines were grown in *Kharif* 2011 under irrigated conditions of which 382 were phenotypically characterized according to DUS descriptors. DNA Fingerprinting of Nagina22 and these 382 Mutants was carried out with a selected set of 60 SSRs to screen admixtures from the real mutants. F2 and F3 analysis of the seven mutants, for which inheritance studies were initiated in the last two years, revealed monogenic inheritance for all of them. A gain of function mutant for moisture stress tolerance was identified from screening of 500 mutants in 25% PEG6000 stress under hydroponic nutrient culture, which survived for 7 days of stress where as the wild type dried in 48 hrs. This mutant was found to be morphologically and genetically similar to the wild type, Nagina 22. It showed increased relative water content (RWC), cell membrane stability and chlorophyll content under stress condition over the wild type control. Scanning Electron Microscope (SEM) study revealed higher number of partially opened and completely closed stomata in the mutant as compared to Nagina 22. Comparative microtomic dissection of root depicted altered size, number and spatial arrangement of central meta-xylem and phloem in the mutant. Microarray analysis revealed that differentially expressed genes for moisture stress tolerance of mutant involved in various metabolic pathways like flavonoid, phnenylalanine, tyrosine and tryptophans biosynthesis pathways (Fig 5.28). TILLING with candidate genes revealed SNP sites in 32, 26 and 8 pools for PTF1, HD3a and PHO1 gene respectively (Fig. 5.29). Sequencing of the PHO1 mutants showed mutation at 19 different nucleotide sites and overall, one mutation per 102 kb was observed with PHO1 gene.

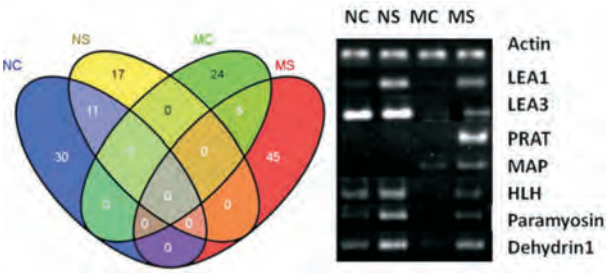


Fig. 5.28: (A): Venn diagram showing differentially expressed genes in the wild type and Nagina 22 under control and stress conditions; (B): Semi-q RTPCR of some of the identified differentially expressing genes

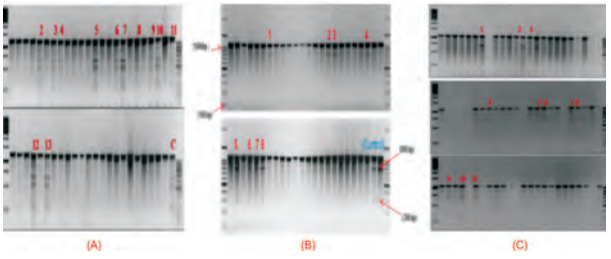


Fig. 5.29: TILLING for (A) PTF1 gene; (B) HD3a gene; and (C) PHO1 gene

E. Databases

Plant Genome Databases

We have developed the local database of genomic resources of crop plants and named it as Plant Genome database. By December , 2011 this database

consists of information for 79 plant species which include Cereals, Vegetables, Fruits, Oilseeds, Legumes, Fibers and Others (Table. 5.6). The DNA sequence categories includes cDNA, EST, GSS, HTG, STS, Genomic and Unigenes of these crops (Fig. 5.30). A total of 1,62,76,956 sequence entries are stored in Plant Genome DB (Table 5.6). These genomics resources are being downloaded from NCBI (www.ncbi.nlm.nih.gov) and the formatted sequences have been stored in plant genome DB using MySQL database server. These sequences can be obtained from www.nrcpb.org.

Table 5.6: Number of sequence entries in PLANT GENOME DB at NRCPB

Sequence Type	Vegetable	Fruits	Cereals	Oilseeds	Legumes	Fiber	Others	Total
cDNA	14895	20628	175909	5250	305	171	49	**217207**
EST	1578720	1597387	4345948	1794000	381672	274277	352912	**10324916**
Genomic	67350	41812	223811	13688	993	4029	19585	**371268**
GSS	1197804	363401	2554410	369361	287514	53582	9510	**4835582**
HTG	5450	169	20044	760	42	197	49	**26711**
STS	533	1312	112339	8050	363	1147	0	**123744**
Unigene	144445	8401	176795	47887	0	0	0	**377528**

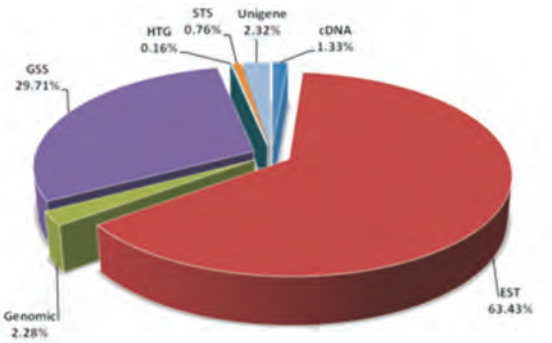


Fig. 5.30: Distribution of different types of genomic resources in plant genome database

6. Honorary Scientists' Projects

Generation, characterization and use of EMS induced mutants of upland rice variety Nagina-22 (Prof. R. P. Sharma)

Mutants generated (> 20000) across centres have been redistributed among project partners and sub-sets of these lines have been phenotyped for various important traits. This has led to identification of putative mutants for moisture stress tolerance, salt tolerance, efficient phosphate utilization and biotic stresses such as bacterial blight resistance and blast resistance. Present number of mutants showing visible phenotypic alterations as well as conditional mutants has exceeded 800 all of which have been phenotyped for DUS characteristics. The distribution of phenotypic mutants for different characteristics is given in the Fig. 6.1. Mutants for plant height and grain morphology were more frequent.

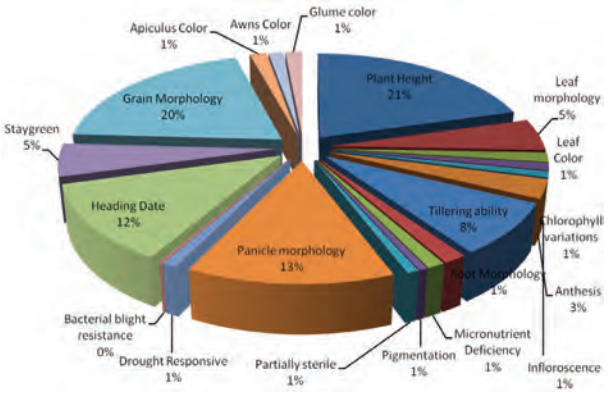


Fig. 6.1: Distribution of phenotypic nutants for different characteristics

Rice Mutant Database

A set of 382 mutants from the mutant garden were characterized at appropriate growth stages according to DUS descriptors. In addition to this, the study of inheritance was done for more than 25 mutants, most of which were found to be governed by single genes. All these data have been added to the Rice Mutant Database (RMD) (Fig. 6.2). The format of the RMD was modified for better representation and maintenance of the mutant data. The database included the information regarding the DUS characteristics, DNA fingerprint images, phenotype details and mutant photograph of all the 382 mutants. The database is being continuously updated after receiving information from the partner institutes.

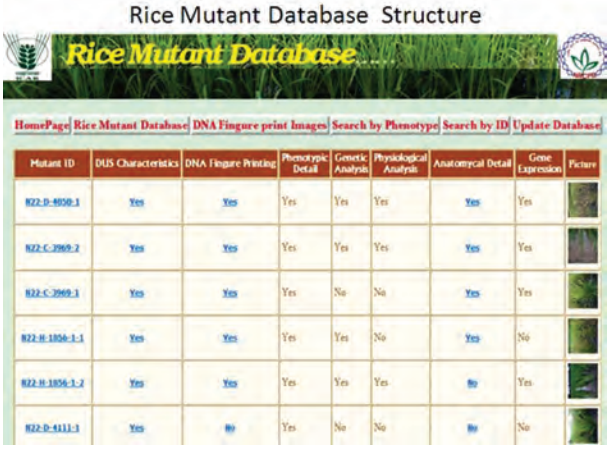


Fig. 6.2: Screen shot of Rice Mutant Database

Emeritus Scientist's Project (Dr. K.R. Koundal)

Gene stacking approach for development of aphid resistant transgenic mustard

Rapeseed mustard (*Brassica*) is the major oilseed crop in India. However insect-pests are the most limiting factor influencing oilseed products especially aphids. Although pesticides provide some relief but

these chemicals are cost effective and environmentally hazardous. To increase the crop productivity we have to use the latest technology of plant genetic transformation. The plant protease inhibitor and lectin genes have been shown to confer resistance to various insects. However aphids being sap sucking insects demand the expression of cloned insecticidal genes in the phloem. Therefore, the present strategy is designed to clone the lectin gene and protease inhibitor individually and together under the control of phloem specific promoter *rolC* in a suitable binary vector and genetic transformation of *Brassica* using these constructs for resistance to aphids.

Construction of desired gene construct consisting Chickpea protease inhibitor gene and phloem specific promoter in binary vector pCAMBIA1300 with Nos terminator

PCR amplification and cloning of Nos Terminator in pCAMBIA1300 binary vector

For PCR amplification of Nos T, primers were designed having *Xba*I and *Sal*I restriction sites incorporated in forward primer and reverse primer respectively for directional cloning keeping plasmid DNA of pBI121 as template. The eluted PCR product were restricted with *Xba*I and *Sal*I sites incorporated in their primers and were purified by PCR clean up.

Plasmid DNA of pCAMBIA1300 binary vector was isolated from the overnight grown culture and was linearised by restricting with *Xba*I and *Sal*I and were checked on gel.

Ligation was performed overnight at 16°C keeping 5:1 insert to vector molar ratio in 10µl reaction volume. Transformation was performed using *E.coli* XL1-Blue competent cells which was already prepared by $CaCl_2$ and plated on LA plate having Kanamycin (50µg/ml) as antibiotic selection marker. Two colonies which are PCR positive was also confirmed by restriction for the presence of NosT with pCAMBIA1300. (Fig. 6.3)

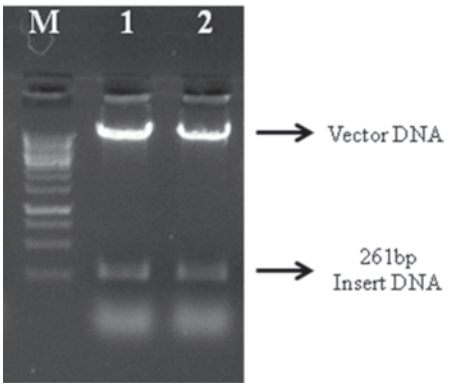


Fig. 6.3: Agarose gel electrophoresis of restricted recombinent clones of pCAM+Nos T. Lane M: 1kb DNA ladder, Lanes 1 & 2 showing the presence of desired insert

PCR amplification and cloning of Chickpea protease inhibitor gene in pCAMBIA1300.

For cloning of CPPI gene gradient PCR was performed using gene specific primers having appropriate restriction sites *Kpn*I in forward primer and *XbaI* in reverse primer incorporated into these and using plasmid of Chickpea Protease inhibitor (CPPI) gene in pCR2.1 TOPO as template**.** The eluted PCR product was restricted with *KpnI* and *XbaI* sites and were purified by PCR clean up.

Ligation was performed overnight at 16ˆC keeping 5:1 insert to vector molar ratio in 10µl reaction volume using 50ng/µl of vector DNA. Transformation was performed using *E.coli* XL1-Blue competent cells which was prepared by $CaCl_2$ and

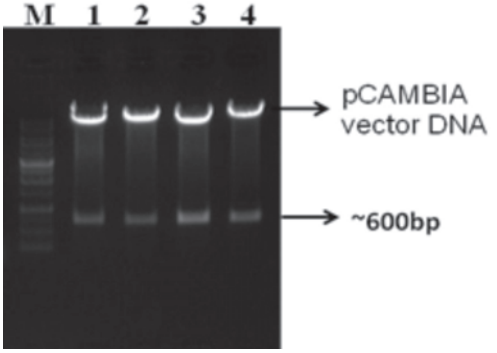


Fig. 6.4: Restriction of chimeric plasmid DNA. Lane M: 1kb Ladder, Lanes 1-4: Restriction digestion of plasmid DNA showing the presence of CPPI insert

plated on LA plate having Kanamycin (50µg/ml) as antibiotic selection marker. Four colonies which are PCR positive was also confirmed by restriction for the presence of CPPI with pCAMBIA1300. (Fig. 6.4)

Cloning of *rolC* promoter in pCAMBIA1300 binary vector construct

The *rol*C was PCR amplified with the gene specific primers having *Eco*RI in forward primer and *Kpn*I restriction site in reverse primer keeping *rolC* in pCR2.1 in TOPO as template. Restricted *rolC* promoter was gel eluted and again restricted with the *Eco*RI and *Kpn*I as the restriction site present in forward and reverse primer. Then *rolC* promoter was purified by PCR cleanup kit for directional cloning of *rolC* in linearised pCAM1300+nosT+CPPI construct.

Ligation was performed overnight at 16˚C keeping 5:1 insert to vector molar ratio in 10µl reaction volume .Transformation was performed using *E.coli* XL1-Blue competent cells which was prepared by $CaCl_2$ and plated on LA plate having Kanamycin (50µg/ml) as antibiotic selection marker. Five colonies which were PCR positive was also confirmed by restriction for the presence of rolC promoter with pCAMBIA1300 binary construct.(Fig. 6.5.)

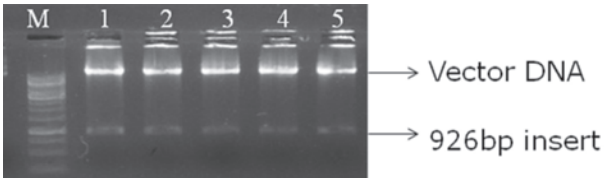


Fig. 6.5: Agarose gel electrophoresis of restricted recombinants. Lanes 1-5 showing the presence of *rolC* insert

Further more the whole construct was also confirmed by restricting plasmid with *Eco*RI and *Sal*I showing 1.8kb fragment including *rolC*+CPPI+NosT and backbone of binary vector pCAMBIA1300. (Fig. 6.6)

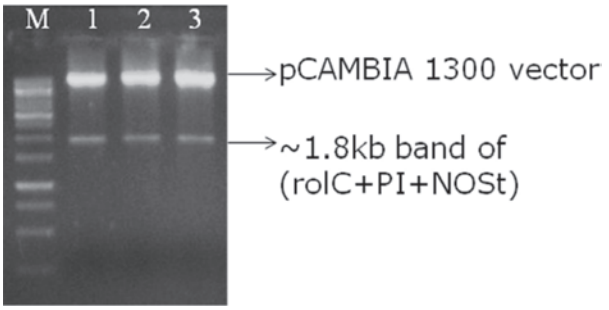


Fig. 6.6: Agarose gel electrophoresis of restriction of the recombinant plasmid with *Eco*RI and *Sal*I which released whole 1.8kb cassette. Lane M- 1kb DNA Ladder, Lanes1,2&3- showing recombinant clones releasing ~1.8kb insert

This construct is now ready for genetic transformation in *Brassica*. Similar work for cloning of lentil lectin gene in a suitable binary vector is under progress.

Ramalingaswamy Fellow's Projet (Dr. Ajay Jain)

Characterization of the genes involved in phosphate uptake and mobilization in *Arabidopsis*

Phosphorus (P) plays a central role in metabolic processes like photosynthesis, respiration, glycolysis, maintenance of redox balance, and energy synthesis and is an indispensable building block for the biosynthesis of nucleic acids and phospholipids. To modulate P homeostasis, plants must balance P uptake, mobilization, and partitioning to various organs. Among the molecular responses, Pi-starvation-induced high-affinity Pi transporters play a pivotal role in the acquisition and mobilization of Pi in plants. The role of high-affinity Pi transporter Pht1;5 in mobilization of Pi between source and sink was empirically demonstrated by functional characterization of the loss-of-function mutants and transgenic lines overexpressing this gene in Arabidopsis. The study also revealed a tangible link between Pi transporters and ethylene signaling. In addition, Glycerol-3-phosphate transporter (*GlPt*), a

sugar-phosphate/anion antiporter, has also been implicated in Pi mobilization. Using transcript analysis and promoter-reporter fusion lines, we show Pi deficiency-mediated differential spatiotemporal regulation across members of the Arabidopsis *G3Pp* family. The potential involvement of *G3Pps* in seedling ontogeny was also demonstrated. Analysis of the mutant *atg3pp4* exhibited compensation by other members within the gene family. In addition, the mutant revealed an altered root phenotype, and several Pi starvation-induced genes involved in root development and/or Pi homeostasis were up-regulated. These results suggest a pivotal roles for both Pht1;5 and GlPt in maintaining Pi homeostasis in Arabidopsis during growth under different Pi regimes. Now efforts are underway to identify transcription factors (TFs) regulating Pi homeostasis.

In planta synthesis of gold nanoparticles (AuNPs)

Manipulating matter at the nanoscale (1 to 100 nanometers) for creating new materials endowed with unique attributes is called nanotechnology. The properties of gold in "nano" form change dramatically from that of bulk gold and acts as an effective catalyst and their properties could further be engineered by manipulating their geometries for varied applications in medicine, consumer goods, as a catalyst, and the list continues to grow. Although chemical and physical techniques are being employed routinely for generating AuNPs, there are growing apprehensions on the risks of generating hazardous by-products causing environmental concerns. Therefore green synthesis of AuNPS is an environment-friendly viable alternative. Therefore, to determine the effects of varying concentrations of $KAuCl_4$ on the growth response of the root system, wild-type *Arabidopsis* seedlings were grown hydroponically under sterile condition initially for 5 d on 0.5x MS + 1.5% sucrose and maintained under standard controlled growth conditions (16-h/8-h day/night cycle at 22°C with an average PAR of 60-65 µmol m^{-2} s^{-1} provided by florescent tubes). Subsequently, the seedlings were transferred to the nutrient medium supplemented with different concentrations (0, 1, 10, 25, 50 and 100 ppm) of $KAuCl_4$ for 1 d. Seedlings were removed from the hydroponic set up for documenting different root traits. The inhibitory effects of increasing concentrations of $KAuCl_4$ were evident on the primary root growth, and the number of lateral roots. Interestingly at low conc of 10 ppm $KAuCl_4$ there was significant increase in total root length (Fig. 6.7A, B) and shoot area (Fig. 6.7C) and formed monodisperse AuNPs (Fig. 6.7D), majority of which were spherical (S) and only a few of them were triangular (T) and of exotic (E) shapes and their sizes ranged from 20 to 50 nm (Fig. 6.7 D-F). The study clearly demonstrated the feasibility of green synthesis of AuNPs. Efforts are now underway to identify "piggy-back transport" molecular mechanism(s) facilitating the bioreduction and uptake of dosage-dependent $KAuCl_4$ by roots and subsequent mobilization of AuNPs into intracellular organelles and shoots of Arabidopsis.

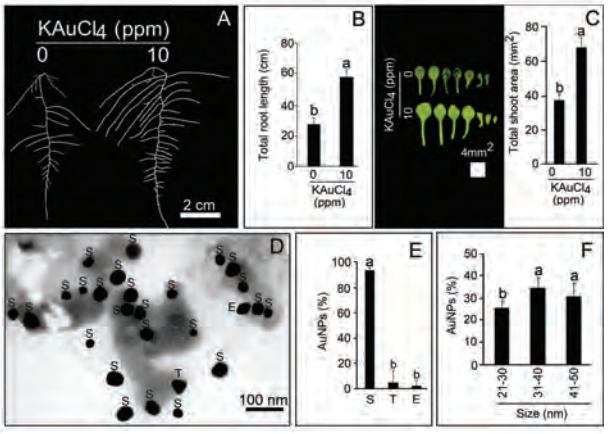


Fig. 6.7: Arabidopsis seedlings raised hydroponically and treated with 0 and 10 ppm of $KAuCl_4$ for 7 days and documented for (A) Root system architecture, (B) Total root length, (C), Shoot area, (D) AuNPs using TEM, (E) Per cent shape distribution, and (F) sizes of AuNPs

Institutional Activities

- Human Resource Development
- Personnel
- Other Activities
- Recruitments/Promotions/Retirements
- Institutional Projects
- Externally Funded Projects
- Technology Commercialization and IPR
- Institutional Award
- Awards and Honours
- Visits Abroad
- Linkage and Collaboration
- List of Publications
- Distinguished Visitors
- International Conference on Plant Biotechnology for Food Security: New Frontiers-2012

Human Resource Development

NRCPB has been actively engaged in human resource development in the area of plant molecular biology and biotechnology since its inception. Currently 36 Ph.D. and 10 M.Sc. students are registered in the discipline of Molecular Biology and Biotechnology at the Centre. In the previous year, two Ph.D. and nine M.Sc. students were awarded with doctoral and masters degrees, respectively. The list of students currently enrolled for their post graduate studies is given below:

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
Ph.D.			
01.	9441	Ms. Joshitha Vijayan	Dr. T. R. Sharma
02.	9561	Mr. N. Umakanta	Dr. Srinivasan
03.	9562	Mr. Rama Prashat G*	Dr. N. K. Singh
04.	9563	Ms. Neetu Singh Kushwah	Dr. S. R. Bhat
05.	9564	Mr. Devanna	Dr. T. R. Sharma
06.	9565	Ms. Nimmy M.S.*	Dr. Srinivasan
07.	9566	Mr. Viswanathan Satheesh	Dr. Srinivasan
08.	9567	Mr. Rajiv Kumar Singh*	Dr. P. A. Kumar
09.	9568	Mr. Vinod Kumar	Dr. K. C. Bansal
10.	9606	Mr. Ali Salari	Dr. K. C. Bansal
11.	9710	Mr. Soham Ray	Dr. T. R. Sharma
12.	9711	Ms. Deepika Singh	Dr. N. K. Singh
13.	9712	Mr. Parameswaran, C	Dr. Srinivasan
14.	9713	Mr. Deepak Singh Bisht	Dr. S. R. Bhat
15.	9715	Mr. Siddanna Savadi	Dr. S. R. Bhat
16.	9716	Ms. Charagonda Revathy	Dr. Srinivasan
17.	9766	Mr. N. M. C. Nayankantha	Dr. Anita Grover
18.	9858	Mr. Anshul Watts	Dr. S. R. Bhat
19.	9860	Mr. Shanmugavadivel P.S.	Dr. N. K. Singh
20.	9861	Mr. Senthilkumar, K.M.	Dr. K. C. Bansal
21.	9862	Mr. Chandra Prakash	Dr. Srinivasan
22.	9863	Ms. Archana Kumari	Dr. T. R. Sharma
23.	9864	Mr. Kumaraswamy H.H.	Dr. N. K. Singh

S. No.	Roll No.	Name of the Student	Chairperson, Advisory Committee
24.	9865	Ms. Suman Lata	Dr. S. R. Bhat
25.	9866	Mr. Israr Ahmad	Dr. P. A. Kumar
26.	9867	Mr. Jagannadham Prasanth Tej	Dr. Srinivasan
27.	9869	Ms. K. N. Poornima	Dr. Anita Grover
28.	9909	Ms. Hoda Hayati	Dr. K. C. Bansal
29.	10012	Mr. Neeraj Anand	Dr. N. K. Singh
30.	10013	Mr. Ravi Prakash Saini	Dr. P. A. Kumar
31.	10014	Mr. Kishor Uttamrao Tribhuban	Dr. T. R. Sharma
32.	10015	Mr. Hemant Balasaheb Kardile	Dr. K. C. Bansal
33.	10016	Mr. Chandra Shekar, N	Dr. Anita Grover
34.	10017	Mr. Chet Ram	Dr. R. C. Bhattacharya
35.	10018	Mr. Rajendra Prasad Meena	Dr. Jasdeep Padaria
36.	10059	Mr. Bhupendra Singh Panwar	Dr. Sarvjeet Kaur
M.Sc.			
01.	20077	Ms. Vidushi Rastogi	Dr. R. C. Bhattacharya
02.	20078	Mr. Donald James	Dr. Jasdeep Padaria
03.	20079	Mr. Deepak Vishwanath Pawar	Dr. Rekha Kansal
04.	20080	Mr. Rakesh Bhowmick	Dr. Kanika
05.	20081	Mr. Krishnendu Pramanik	Dr. Kanika
06.	20188	Mr. Lianthanzauva	Dr. Rekha Kansal
07.	20189	Mr. Albert Maibam	Dr. Kishor Gaikwad
08.	20191	Ms. Priya Balkrishna Danekar	Dr. Kishor Gaikwad
09.	20228	Ms. Priyanka	Dr. R. C. Bhattacharya
10.	20230	Mr. Mahesh Mohanrao Mahajan	Dr. Kanika

* Taken relief from P. G. School pending submission of thesis

Degrees awarded in the discipline of Molecular Biology and Biotechnology during the Convocation held on 20th February, 2012

S. No.	Name of the student and Roll No.	Chairperson, Advisory Committee	Thesis Title
Ph.D.			
01.	Mr. Navin Chandra Gupta (9233)	Dr. Srinivasan	Cloning and characterization of a wound-responsive regulatory element from *Arabidopsis thaliana*
02.	Mr. Giriraj Kumawat (9442)	Dr. N. K. Singh	Molecular mapping of genes for traits involved in plant architecture and earliness in Pigeonpea (*Cajanus cajan* (L.) Millsp.)
M.Sc.			
01.	Ms. Momena Khandaker (4818)	Dr. P. A. Kumar	Cloning of *Cadherin* gene fragment from *Helicoverpa armigera*
02.	Mr. Neeraj Anand (4779)	Dr. N. K. Singh	Development and validation of HvSSR marker in Rice
03.	Mr. Dhiman Chakravarty (4892)	Dr. R. C. Bhattacharya	Identification of potential target in mustard aphid (*Lipaphis erysimi*) for developing RNAi mediated resistance in host plant
04.	Ms. Sujata Kumari (4893)	Dr. R. C. Bhattacharya	Development of a plant transformation vector for host mediated delivery of siRNA molecules targeted to aphid specific genes
05.	Mr. Ravi Prakash Saini (4894)	Dr. Anita Grover	Expression analysis of defense genes in response to *Alternaria brassicae* infection and salicylic and Jasmonic acids in *Brassica juncea*
06.	Mr. Hemant B. Kardile (4895)	Dr. K. C. Bansal	Expression profiling of selected photosynthetic genes under water deficit and high temperature conditions in wheat
07.	Mr. Kishor Uttamrao Tribhuvan (4869)	Dr. P. A. Kumar	Proteome analysis of transgenic Bt and non-Bt Brinjal
08.	Ms. N. T. Thuy (4928)	Dr. Jasdeep Padaria	Development of high temperature responsive expressed sequence tag (EST) library in *Prosopis cineraria* (L.) Druce
09.	Ms. P. H. Diep (4931)	Dr. Jasdeep Padaria	Development of drought response expressed sequence tag (EST) library in *Prosopis cineraria* (L.) Druce

Personnel

Scientific staff	Area of interest
Dr. P. Ananda Kumar kumarpa@nrcpb.org	Main interest is in isolation, characterization, expression and protein engineering of insecticidal protein genes of *Bacillus thuringiensis*; Developed Bt transgenic tomato and brinjal. Current focus is on isolation of tissue specific promoters by genomic approaches and developing techniques for targeted gene introduction in plants.
Dr. N. K. Singh nksingh@nrcpb.org	Working on different aspects of structural, functional and comparative genomics with special emphasis on rice, wheat, pigeonpea, tomato and *Rhizobium*; Contributed to genome sequencing of rice, tomato and chickpea *Rhizobium M. ciceri*; Mapping genes for yield and quality traits in rice, wheat and pigeonpea; high resolution mapping of loci for salinity tolerance and grain characteristics in rice.
Dr. Srinivasan srinivasan53@gmail.com	Structure-function relationship in enzymes; molecular biology and genetic transformation of plants and functional genomics of drought tolerance are the main areas of interest; developed T-DNA mutagenized populations of *Arabidopsis* with promoterless GUS; isolated and characterized novel genes and tissue specific promoter elements. Current interest is on functional genomics of abiotic stress tolerance in chickpea.
Dr. S. R. Bhat srbhat22@rediffmail.com	Working on development and molecular/genetic characterization of cytoplasmic male sterility systems in *Brassica*, molecular tagging of fertility restorer genes, development of transgenic Brassica for improved seed and oil yield, wide hybridization and gene introgression, and cloning and characterization of plant promoters.
Dr. T. Mohapatra tm@nrcpb.org	The main area of interest is development and application of molecular markers and genomic tools. Designed a large set of genomic and genic microsatellite markers in rice, mustard and sugarcane; developed a Basmati rice variety by marker assisted selection; contributed to the physical mapping and genome sequencing in rice and tomato. Current research focuses on understanding and mitigating stress tolerance employing molecular genetic and genomic tools.
Dr. (Mrs.) Sarvjeet Kaur dr_sarvajeetkaur@yahoo.com	Working on Isolation and characterization of quorum quenching genes from *Bacillus thuringiensis* and other bacteria for plant disease control; also working on isolation of *cry1*- and *cry 2*-type genes from native *Bacillus thuringiensis* isolates from diverse habitats in India for development of transgenic crops.
Dr. (Mrs.) Anita Grover anitagrover@hotmail.com	Research includes exploring Plant-Fungus interaction with the system of *Brassica juncea- Alternaria brassicae* leading to the isolation of defense genes and development of transgenics of *Brassica juncea* for increased resistance to *Alternaria* blight.
Dr. T. R. Sharma trsharma@nrcpb.org	Major areas of research include structural, functional and comparative genomics of plant and plant pathogens; Concentrating on mapping, cloning and functionally

Scientific staff	Area of interest
	validating disease resistance genes and QTL in rice; Cloned rice blast resistance gene *Pi-k*^{h} (*Pi54*) from rice line Tetep and *Pi-Rh* from a wild relative of rice *Oryza rhizomatis*; Also working on development of DNA markers and various databases.
Dr. R.C. Bhattacharya ramcharan99@yahoo.com	Identification and mobilization of genes conferring resistance to insect herbivores and fungal pathogens, into the elite crop cultivars through development of transgenics is the major area of research. Long term objective is identification of defense related signal peptides which initiate the signaling pathways and the associated signaling cascades so as to fully understand systemic wound signaling in plants.
Dr. Debasis Pattanayak debasis.pattanayak@rediffmail.com	Transgenics, *Bacillus thuringiensis*, gene silencing, RNAi, precursor tRNA processing
Dr. P.K. Mandal pranabkumarmandal@gmail.com	Plant Biochemistry and Molecular Biology
Dr. (Mrs.) Rekha Kansal rekhakansal@hotmail.com	Isolating genes (lectins, protease inhibitors and amylase inhibitors) from legumes like chickpea, pigeonpea, peas and mothbean for imparting improved tolerance to sucking pests like, *Lipaphis erysimi*; development of transgenic mustard with lectin gene driven by tissue specific promoter is underway.
Dr. (Mrs.) J.C. Padaria jasdeep_kaur64@yahoo.co.in	Recently initiated work on biotechnology and climate change focusing on bioprospecting of genes and allele mining for tolerance from species such as *Penniseteum*, *Zizyphus* and *Prosopis;* identifying novel *cry* genes from indigenous *Bacillus thuringiensis* isolates collected from different unexplored regions of India. A microbe isolated from Indian soil, antagonistic towards different phytopathogens, was identified and characterized at molecular level.
Dr. Pradeep Kumar Jain jainpmb@gmail.com	Broad area of research is functional genomics including isolation of plant genes and promoters. Contributed to isolation of anther and trichome specific promoters and nematode-responsive root-specific promoter. Also involved in identification and characterization of candidate genes associated with few important traits like drought, temperature, blight and wilt in chickpea.
Dr. (Mrs.) N.K. Durga nadellak1@yahoo.com	Research areas include molecular markers- mapping of loci governing quantitative and disease resistance traits. Currently working on mapping of loci governing resistance to spot blotch (*Bipolaris sorokiniana*) in wheat.
Dr. (Ms.) S. Barthakur sbthakur@yahoo.com	Research interest is molecular biology and genetic engineering for abiotic stress tolerance in plants; Working on isolation and functional characterization of genes and promoters involved in various abiotic stress responses.
Dr. K.S. Gaikwad kish2012@yahoo.com	Contributed to the sequencing of the rice and tomato genomes; pursuing the development of genomic resources including deep transcriptome analysis in crops like sugarcane and pigeon pea; also focusing on abiotic stress particularly on salinity and thermotolerance in rice and pigeonpea.
Dr. (Mrs.) Monika Dalal monika@nrcpb.org	Genetic engineering wheat for improved drought tolerance, and functional genomics for abiotic stress tolerance especially drought and salinity stress in wheat.

Scientific staff	Area of interest
Dr. (Mrs.) Vandana Rai vandnarai2006@gmail.com	Stractural and functional genomics of different crop plants. Molecular mapping of genes for productivity quality improvement in crop plants
Mr. R.S. Jaat rsjaatnrcpb@rediffmail.com	The current area of research is Biotechnology and climate change.
Dr. (Mrs.) Kanika Kumar kanika@rediffmail.com	Research focus is on abiotic stress management in plants through the microbes associated with them by exploiting PGPRs. Currently studying the role of microbial genes involved in reducing the concentration of stress ethylene in plants; also involved in structural and functional genomics studies of *Mesorhizobium ciceri*.
Dr. Prasanta K. Dash prasanta@nrcpb.org	Worked on isolation of insecticidal genes and promoters from Indian legumes. Has isolated lectin genes from chickpea and lentil and protease inhibitor genes from mung bean. Current focus is on manipulating pathways for yield enhancements.
Dr. Rhitu Rai rhitunrcpb@yahoo.com	Research focus is on plant pathogenesis and plant disease resistance mechanisms. Aim to functionally characterize R/Avr genes, gene expression profiling of pathogen and host plant during pathogenesis and understand mechanism of pathogenicity.
Dr. Navin Chandra Gupta guptanc@nrcpb.org	Use of molecular and bioinformatics approaches to comprehend the novel genes and promoters and their function with a view to utilize them in crop improvement program; also working on development of transgenic mustard with high oil content.
Dr. S. V. Amitha CR Mithra amitha@nrcpb.org	QTL mapping specifically for grain traits and abiotic stress tolerance in rice by using SSR and SNP markers is the current area of research.
Dr. Amolkumar U. Solanke amolsgene@nrcpb.org	Main research interests are genomics and transgenic development with special emphasis on understanding biotic and abiotic stress in plants through various genomic tools; molecular biology of fruit development; development of transgenics for stress tolerance.
Sh. Ramawatar ram_nrcpb@nrcpb.org	Functional Genomics particularly understanding role of non-coding RNA in plant pathogen interaction and disease development is the current area of research.
Sh. Dinabandhu Behera aumreetam@rediffmail.com	Main research interest is in the area of structural, functional and comparative genomics of crop plants.

INSA Honorary Scientists
Dr. H.K. Das
Dr. R.P. Sharma

DBT Ramalingaswamy Fellow
Dr. Ajay Jain

ICAR Emeritus Scientist
Dr. K.R. Koundal

DST Inspire Faculty Fellow
Dr. Charu Lata

Technical staff	Administrative staff	Supporting staff
Dr. Kamlesh Batra	Sh. Chandra Prakash	Sh. Shivji Jha
Smt. Sunita Srivastava	Sh. Kishan Dutt	
Ms. Suman Bala	Sh. Mohan Singh	
Dr. Krishan Pal	Mrs. Rajinder Kaur	
Sh. H.C. Upreti	Sh A.K. Jain	
Sh. Ravinder Rishi	Sh. B.S. Dagar	
Sh. Ram Niwas Gupta	Mrs. Sangeeta Jain	
Sh. R.K. Narula	Sh. Ramchandra Jha	
Smt. Sandhya Rawat	Sh. Bachu Singh	
Smt. Seema Dargan	Mrs. Rekha Chauhan	
Sh. Rohit Chamola		
Dr. Pankaj Kumar		
Dr. R.S. Niranjan		

Other Activities

Vigilance Awarness Week

NRCPB, New Delhi, observed Vigilance Awarness Week during 31.10.11. Dr. P.A. Kumar, Project Director, NRCPB, administered the pledge to the staff.

Hindi Fortnight

Competitions in Hindi Essay writing, technical work writing, declamation, poetry recitation and noting and drafting were conducted for the staff of the NRCPB as part of Hindi month 2011 from 1-30 Sept., 2011.

Sports

The following officials and players are participated in Inter-Zonal Competition CRIJAF, Barrackpore, Kolkatta w.e.f. 16.01.2012 to 19.01.2012

1. Sh. Krishan Dutt – Chief Demision
2. Sh. B.S. Dagar, Sports Secretary
3. Dr. P.K. Dash (Event) – Short put and Discuss Throw
4. Smt. Sangeeta Jain (Event) Short Put

The sports team representing NRCPB at ICAR Central Sports meet held at CRIJAF, Kolkata

Recruitments/Promotions/Retirements

Recruitments

- Dr. R.C. Bhattacharya joined as Principal Scientist in July, 2011
- Dr. D. Pattnaik joined as Principal Scientist in July, 2011
- Dr. P.K. Mandal joined as Principal Scientist in Aug., 2011
- Dr. Vandana Rai joined as Sr. Scientist in Dec., 2011
- Dr. Monika Dalal joined as Sr. Scientist in June., 2011

Promotions

- Sh. Krishan Dutt, Assitant Promoted as A.A.O w.e.f. Dec., 2011
- Sh. Anoj Kr. Jain, P.A promotied as Private Secretary w.e.f. Sept., 2011
- Dr. Pankaj Kumar, T-6 promoted as T-7/8 w.e.f. Dec., 2010
- Sh. Rohit Chamola, T-6 promoted as T-7/8 w.e.f. Feb., 2010
- Sh. Ravinder Rishi, T-5 promoted as T-6 w.e.f. July 2007
- Dr. Kanika, Scientist (SS) promoted as Sr. Scientist w.e.f. May, 2008

Retirements

- Sh. Ram Chander Jha, UDC was retired in Dec. 2011

Institutional Projects

S. No.	Project Title	Date of Start	Date of Completion	Principal Investigator	Name of Associates
1.	Isolation of plant genes and promoters	1st April, 2009	31st March, 2014	Dr Srinivasan	Anita Grover Rekha Kansal P.K. Jain Rhitu Rai Pratibha Sharma J. Kumar C. Bharadwaj R.N. Gupta Sandhya Rawat
2.	Transgenic crops for biotic stress resistance	1st April, 2009	31st March, 2014	Dr. P. Ananda Kumar	Sarvjeet Kaur R.C. Bhattacharya A.U. Solanke Suman Bala K.P. Singh Rakesh Narula R.S. Niranjan
3.	Biotechnology and Climate Change	1st April, 2009	31st March, 2014	Dr. P. Ananda Kumar	Jasdeep Padaria Ranjit S. Jaat Sharmistha Barthakur Kanika Tara Satyawati Lata Bidisha Chakrabarti Sunita Srivastava
4.	Biotechnological approaches for increasing crop productivity	1st April, 2009	31st March, 2014	Dr. S.R. Bhat	Prasanta Dash Navin Gupta R. Chamola Seema Dargan
5.	Genomics and molecular markers in crop plants	1st April, 2009	31st March, 2014	Dr. N.K. Singh	T. Mohapatra T.R. Sharma N. Kanaka Durga Kishor Gaikwad S.V. Amitha Mithra H.C. Upreti Pankaj Kumar A.K. Singh D.K. Yadava U.D. Singh C. Viswanathan S. Raje K.K. Mondal A. Rao

Externally Funded Projects

S. No.	Funding Agency	Project Title	Principal Investigator	Amount (in Lakhs)
1.	DBT	Protein engineering of delta endotoxins of *Bacillus thuringiensis* for management of major insect pests	Dr. P. Ananda Kumar	49.28
2.	DBT	Gene stacking in Bt-cotton	Dr. P. Ananda Kumar	51.48
3.	NAIP	Genomics of cotton boll and fiber development	Dr. P. Ananda Kumar	250.81
4.	NFBSRA	Targeted gene integration in rice and cotton	Dr. P. Ananda Kumar	79.53
5.	ICAR-NPTC	Network Project –Resistance to pod borer in pigeon pea	Dr. P. Ananda Kumar	
6.	NFBSRA	Development of pod borer resistant transgenic pigeonpea and chickpea Sanctioned Budget	Dr. P. Ananda Kumar	73.90
7.	NFBSRA	Phenomics of moisture deficit and low temperature stress tolerance in Rice	Dr. P. Ananda Kumar	2926.77
8.	NAIP	Towards Development of a Single Cell C4 Photosynthetic system in Rice	Dr. Kanika	98.76
9.	DBT	Development of transgenic groundnut (*Arachis hypogaea L.*) for enhanced abiotic stress tolerance	Dr. Kanika	22.83
10.	ICAR	ICAR Network Project on Transgenics in Crops: Drought tolerance in mustard	Dr. Kanika	269.19
11.	ICAR-NPTC	Development of Aphid resistant Transgenic *Brassica* Drought tolerance in mustard	Dr. Rekha Kansal	110.76
12.	ICAR	Fusarium wilt resistance and drought tolerance in chickpea under NTPC	Dr. Srinivasan	145.92
13.	ICAR (NFBSRA)	Evaluating candidate genes towards enhancement of drought tolerance in chickpea	Dr. Srinivasan	333.44
14.	NAIP	Unraveling molecular processes involved in adventives polyembryony towards genetic engineering for fixation of heterosis	Dr. S.R. Bhat	698.00
15.	ICAR-TMC	Exploitation of apomixes and thermo sensitive genetic male sterile system in cotton hybrid seed production.	Dr. S.R. Bhat	15.00
16.	ICAR EFC-NPTC	Network project on transgenic crops-Rice Wheat	Dr. N.K. Singh	307.04

S. No.	Funding Agency	Project Title	Principal Investigator	Amount (in Lakhs)
17.	ICAR EFC-NPTC	Network project on transgenic crops- Central Facility	Dr. N.K. Singh	469.34
18.	DBT	Marker assisted breeding of aboitic stress tolerance rice varieties with major QTS_s for Drought, submergence and Salt Tolerance	Dr. N.K. Singh	297.50
19.	DBT	Molecular marker technologies for faster wheat breeding in India	Dr. N.K. Singh	14.73
20.	DBT	Physical mapping and sample sequencing of wheat chromosone 2A – International Wheat Genome Sequencing Consortium/India	Dr. N.K. Singh	156.48
21.	CSIR	Marker assisted creation of heterotic pools and diversification of male sterility and fertility restoration system for hybrid seed production in rice	Dr. T. Mohapatra/ Dr. S.V. Amitha CR Mithra	53.44
22.	DBT	Generation, characterization and use of EMS induced mutants of upland variety Nagina 22 for functional genomics in rice.	Dr. T. Mohapatra/ Dr. N. K. Singh	177.76
23.	NAIP	Bioprospecting and allele mining for abiotic resistance in rice	Dr. T. Mohapatra/ Dr. N. K. Singh	1519.50
24.	ICAREFC-NPTC	Functional genomics of Alternaria blight resistance and drought tolerance in mustard	Dr. T. Mohapatra/ Dr. S. R. Bhat	232.96
25.	ICAR EFC-NPTC	Development of transgenic rice tolerant to drought and resistant to yellow stem borer	Dr. T. Mohapatra/ Dr. D. Pattanayak	138.11
26.	DBT	Construction of novel insecticidal cry gene of *Bacillus thuringiensis* (Bt) for enhanced pod borer (*Helicoverpa armigera*) resistance in transgenic crops	Dr. S. Kaur	29.39
27.	NAIP	Diversity analysis of *Bacillus* and other predominant genera in extreme environments and its utilization in Agriculture.	Dr. J.C. Padaria	69.14
28.	DBT	Molecular cloning and functional characterization of rice blast resistance genes (Phese II)	Dr. T. R. Sharma	85.41
29.	ICAR	National Initiative for climate resilient agriculture	Dr. J.C. Padaria	1046.00
30.	NAIP	Allele mining and expressing profiling of resistance and a virulence genes in rice blast patho system for development of rice non-specific disease resistance	Dr. T. R. Sharma	618.98
31.	DBT	Isolation and Molecular analysis of Pi-kh allele for wild species of rice resistance to *Magnopothe grisea*	Dr. T. R. Sharma	09.00
32.	DBT	Puccinia triticina genomics network on De Novo genome sequencing, fitness, variation and pahtogenicity. Network Project	Dr. T. R. Sharma	571.47

S. No.	Funding Agency	Project Title	Principal Investigator	Amount (in Lakhs)
33.	ICAR-NPTC	Bioinformatics & Comparative Genomics	Dr. T. R. Sharma	326.670
34.	NAIP	Understanding plant nematodes interactions using RNAi	Dr. P. K. Jain	128.00
35.	ICAR NF	Role of small peptides in systemic defense response of Indian mustard (*Brassica juncea)* to aphids (*Lipaphis erysimi)*	Dr. R.C. Bhattacharya	110.29
36.	DBT	A transgenic approach to develop aphid resistant *Brassica juncea* plant types & their field evaluation.	Dr. R.C. Bhattacharya	87.77
37.	DST	Crop plants which remove their own major biotic constraints (Indo-Australia Collaborative in Grant Challenge Programme)	Dr. R.C. Bhattacharya	503.12
38.	PPV&FRA	Establishment of Referral Laboratory for conducting special test	Dr. R.C. Bhattacharya	73.33
39.	ICAR	Molecular breeding and functional genomics of flax (*Linum usitatisimum*)	Dr.Prasanta Dash	162.35
40.	DST	Identification,cloning and characterization of cell wall biosynthetic pathway genes from sorghum (*sorghum bicolor L Moench*)	Dr. Monika Dalal	10.11
41.	DBT	Molecular cloning and functional characterization of the annexin family genes from pearl millet under abiotic stress	Dr. Sharmistha Barthakur	45.00
42.	ICAR AP-Cess	PGPRs: An alternative genome for bacterial wilt resistance in tomato	Dr. Rhitu Rai	34.7

Technology Commercialization and IPR

The mandate of the Institute Technology Management Unit relates to registration of patents, facilitation of contract research projects and commercialization of IPR enabled technologies of the centre through Public- Private Partnership.

The following activities were undertaken by the ITMU during the year 2011-12.

I. Patents applications filed:

The following three applications were filed for patent rights by NRCPB.

1. A Novel nematode-induced gall-specific promoter from *Arabidopsis thaliana*

The present invention relates to the field of plant molecular biology. In particular, it describes the identification of a novel nematode-induced gall-specific promoter from *Arabidopsis thaliana*. Using *in silico* approach, meta-profile analysis of microarray datasets for root specificity and response to nematodes led to the identification of specific genes. AT2G18140 gene expression showed consistent up regulation at 7-day post infection (using root knot nematode, *Meloidogyne incognita*) in the gall tissues. Upstream region (1525 kb)of this gene was cloned and fused with *GUS* reporter gene. GUS assay in transgenic plants confirmed that the upstream sequence directs the expression of the *GUS* reporter gene specifically in galls in response to nematode infection. These regulatory sequences would be immensely helpful to target gene of invading nematodes (by eliciting RNAi) and thereby provide very effective control against nematode infection.

2. A Novel root-specific and nematode-responsive promoter from *Arabidopsis thaliana*:

The present invention relates to the field of plant molecular biology. In particular, it describes the identification of a novel root-specific and nematode (*Meloidogyneincognita)* responsive promoter sequence from *Arabidopsis thaliana*. Using *in silico* approach, meta-profile analysis of microarray datasets for root specificity and response to nematodes led to the identification of specific genes. AT1G74770 gene expression was significantly up-regulated at 21-day post infection with root knot nematode. The upstream region (1500 bp) was cloned and fused with the GUS reporter gene. GUS assay in transgenic *Arabidopsis* plants confirmed that the upstream sequence directs the expression of the GUS reporter gene specifically in roots only in response to nematode infection. These regulatory sequences would be immensely helpful to target gene of invading nematode (by eliciting RNAi) and thereby provide very effective control against nematode infection.

3. A strategy for genetic engineering of male sterility and transgene containment in plants

This invention covers the coding and regulatory nucleotide sequences, encoded polypeptide and constructs synthesized incorporating these sequences. Expression of orf108 gene leads to ablation of male and female gametes of plants. This invention also pertains to identification of a tissue specific promoter element AtPrx18 (*Arabidopsis thaliana* Peroxidase18 gene) that drives gene expression in male and female gametes. We show that through controlled expression of orf108under

the AtPrx18 promoter, both male and female sterile plants could be generated. Further, we demonstrate that a transgene containment could be achieved using orf108 with tissue specific promoter AtPrx18. This transgene containment strategy is particularly suitable for genetic engineering of clonally propagated plants and tree species.

II. MoUs/MTAs signed

1. Memorandum of Understanding (MoU) was signed on 13-07-2011 by NRCPB and M/s Bejo sheetal seeds Pvt. Ltd. Jalna, for transfer of *cry1Aa-B* gene.
2. Memorandum of Understanding (MoU) was signed on 27.08.2011 by NRCPB and Ankur Seeds Pvt. Ltd., Nagpur, for transfer of Bt Tomato Event 25.
3. Two Memoranda of Understanding (MoU) were signed on 19.10.2011 by NRCPB and M/s Nirmal Seeds Pvt. Ltd. Jalgaon, for transfer of *cry1Fa1 and cry1Aabc* genes.
4. Material transfer agreement (MTA) was signed on 31-05-2011 by NRCPB and Marathwada Agricultural University, Parbhani for transfer of c*ry1Ac* gene.
5. Material transfer agreement (MTA) was signed on 15-07-2011 by NRCPB and Indian Institute of Information Technology Allahabad for transfer *of Cicer areitinum* lectin, *Vigna radiate* lectin, *Vigna mungo* protease inhibitor and *Vigna radiata* protease inhibitor.
6. Material transfer agreement (MTA) was signed on 18-11-2011 by NRCPB and Biotechnology Deptt., Kurukshetra University, Kurukshetra for transfer of *Vigna acontifolia* lectin gene.
7. Material transfer agreement (MTA) was signed on 16-12-2011 by NRCPB and Navsari Agricultural University, Navsari for transfer of c*ry1Ac* gene.
8. Material transfer agreement (MTA) was signed on 31-12-2011 by NRCPB and Agri Biotech Foundation, Acharya N.G. Ranga Agricultural University, Hyderabad for transfer of c*ry1Ac, cry1Fa1, cry2Aa and cry1Ac-F* genes.
9. Material transfer agreement (MTA) was signed on 03-03-2012 by NRCPB and Sipani Krishi Anusandhan Farm, Mandsaur, M.P. for transfer of seeds of three Pigeon pea varieties viz; Shivan, Shipra and Changli.

Memorandum of Understanding (MoU) was signed by NRCPB and Ankur Seeds Pvt. Ltd., Nagpur, for transfer of Bt Tomato Event 25

Institutional Award

During the Year 2011-12 NRCPB has received two national level award namely Sardar Patel Outstanding ICAR Institution Award and Mahindra Samriddhi Krishi Sansthan Samman - 2012. Sardar Patel Outstanding ICAR Institution Award was conferred to recognize the outstanding performance made by NRCPB during the year 2010-11 . Mahindra Samriddhi Krishi Sansthan Samman is the recognition for Public Sector organizations committed to a broad policy on agriculture that has become instrumental in changing the lives of hundreds of farmers.

Sardar Patel Best ICAR Institution Award

Mahindra Samriddhi Krishi Sansthan Samman

Awards and Honours

Amolkumar U. Solanke - Awarded Jawaharlal Nehru Award 2010 for P.G. Outstanding Doctoral Thesis Research in Agricultural Biotechnology.

Monika Dalal - Awarded with the best poster for the research article titled 'Characterization of LEA3 like gene from Sorghum bicolor (L) Moench' presented in young scientist convention 2011, organized by Andhra Pradesh Akademi of Sciences, Hyderabad & Acharya Nagarjuna University, Guntur, held at Guntur, AP from Oct 27-28, 2011.

Puja Singh, Richa Shukla, Poonam Tiwari, Ram Niwas Gupta and Rekha Kansal - Awarded with the best poster for the research article titled 'Cloning of Chickpea Lectin Gene and its Efficacy against Aphids in Transgenic Mustard' presented in IUPAC sponsored second International Conference on Agrochemicals Protecting Crops, Health and Natural Environment: Role of Chemistry for Sustainable Agriculture held at New Delhi, India from February 15-18, 2012.

Sharmistha Barthakur - elected and served as member of sectional committee of the section of Agriculture and Forestry Sciences for 99th Session of the Indian Science Congress held at Bhubaneswar, January 3-7, 2012

Sharmistha Barthakur - Awarded with the best poster for the research article titled "Genetic diversity analysis for high temperature stress tolerance in pearl millet presented in the 'National seminar on Indian agriculture: preparedness for climate change 'organized by Indian society for Agricultural Sciences at NASC compex, Pusa, New Delhi, March 24-25, 2012

Visits Abroad

- Ajay Jain- Invited as a Visiting Scientist to Purdue University, USA, to work on the "*In planta* functional characterization of high affinity phosphate transporter *Pht1;5"* from July1st-August 31st 2011
- Ajay Jain- Invited as a Visiting Scientist to Western Kentucky University, USA, to obtain training on the uses of Transmission Electron Microscopy (TEM) for determining the geometry of *In planta* synthesized gold nanoparticles (AuNPs) and Energy Dispersive X-ray Spectroscopy (EDS) for validating the fidelity of AuNPs from September 1st- 30th, 2011

Linkage and Collaboration

The centre has an active and close collaboration with other ICAR institutes and State Agricultural Universities and is instrumental in providing gene constructs for transgenic development targeting biotic and abiotic tolerance and quality enhancement. It also plays a pivotal role in developing genomic resources, both functional and structural, for a variety of crop species. The scientists at the centre work in close collaboration especially with different disciplines of the Indian Agricultural Research Institute like Biochemistry, Plant Physiology, Genetics, Entomology, Pathology, Nematology, Horticulture, Microbiology and Water Technology Centre. With the ever increasing need of computational and statistical intervention in the area of modern molecular biology and biotechnology, the centre has also developed active collaboration with the Indian Agricultural Statistics Research Institute. Over the years the centre has developed working linkages with the international laboratories.

A. Inter-Institutional Linkages

- Delhi University, New Delhi
- Jawaharlal Nehru University, New Delhi
- Chaudhary Sarwan Kumar Vishwavidyalaya, Palampur
- Central Rice Research Institute, Cuttack
- Directorate of Medicinal and Aromatic Plants, Anand
- University of Agricultural Sciences, Dharwad
- Institute of Himalayan Bioresources and Technology. Palampur
- National Bureau of Plant Genetic Resources, New Delhi
- Bose Institute, Kolkata
- International Centre for Genetic Engineering and Biotechnology, New Delhi
- International Crops Research Institute for Semi-Arid Tropics, Hyderabad
- Twenty three ICAR institutes under the Network project on Transgenics
- Thirty five ICAR institutes, SAUs and others like IITs in NAIP Mega Project entitled "Bioprospecting of genes and allele mining for abiotic stress tolerance"

B. International Linkages

- Division of Plant Pathology, UC Davis, California

List of Publications

Research Article

1. Barthakur, S. (2011). Response of thylakoid bound ascorbate peroxidase under abiotic stress in two wheat genotypes, Annals of Agricultural Research New Series Vol 32**.** No 3 & 4:130-134
2. Bhat, S.R. (2011). Rationalizing investment and effort in whole genome sequencing for harvesting applied benefits. Current Sci. 100: 1633-1637
3. Bhat, S.R. and Bandyopadhyay, A. (2011). Reinvigorating plant breeding to meet the challenges of food production in the 21st century. Ind. J. Genet. 71: 95-101
4. Bhatia, V., Uniyal, P.L. and Bhattacharya, R.C. (2011). Aphid resistance in *Brassica* crops: Challenges, biotechnological progress and emerging possibilities, *Biotechnology Advances* 29: 879-888
5. Chelliah, A., Gupta, G.P., Karuppiah, S. and Kumar, P.A. (2011). Chimeric ä-endotoxins of *Bacillus thuringiensis* with increased activity against *Helicoverpa armigera. Science.* International Journal of Tropical Insect Science, Vol. 31: 59-68
6. Choudhary, P., Khanna, S.M., Jain, P.K., Bharadwaj, C., Kumar, J., Lakhera, P.C. and Srinivasan, R. (2012). Genetic structure and diversity analysis of primary gene pool of chickpea using SSR markers. Genet. Molecular Research.11: 891-905
7. Deokar, A.A., Kondawar, V., Jain, P.K., Karuppayil, S.M., Raju, N.L., Vadez, V., Varshney, R.K. and Srinivasan, R. (2011). Comparative analysis of expressed sequence tags (ESTs) between drought-tolerant and -susceptible genotypes of chickpea under terminal drought stress. BMC Plant Biol. 11:70
8. Dutta, S., G.Kumawat, ..., Gaikwad, K., Sharma, T. R., Raje,R.S., ...Cook, D.R. and Singh,N. K. 2011. Development of genic-SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanus cajan* (L.) Millspaugh] (2011) BMC Plant Biology, 11:17
9. Gahloth, D., Shukla, U., Birah, A., Gupta, G.P., Kumar, P.A., Dhaliwal, H.S. and Sharma, A.K. (2011). Bioinsecticidal activity of *Murraya koenigii* miraculin-like protein against *Helicoverpa armigera* and *Spodoptera litura*. Archives of Insect Biochemistry and Physiology, Vol. 78(3):132–144
10. Grover, A. (2012). Plant chitinases: Genetic Diversity and Physiological roles. Critical reviews in Plant Sciences,31:57-73
11. Gupta, S.K., Rai, A.K., Kanwar, S.S., Chand, D., Singh, N.K. and Sharma, T.R. (2012). The single Functional blast resistance gene Pi54 activates a complex defence mechanism in rice. J Exp Bot. 2012 Jan; 63(2):757-72
12. Gupta, N.C., Jain, P.K., Bhat, S.R. and Srinivasan, R. (2011). Upstream sequence of fatty acyl-CoA reductase (FAR6) of *Arabidopsis thaliana* drives wound-inducible and stem-specific expression. Plant Cell Report. 31:839-850
13. Gupta V., Natarajan C., Kanika and Prasanna R. (2011). Identification and characterization of endoglucanases for fungicidal activity in Anabaena laxa. J. App. Phycol., 23(1):73-81

14. Hiremath, P.J., Farmer, A., Cannon, S.B., Woodward, J., Kudapa, H., Tuteja, R., Kumar, A., BhanuPrakash, A., Mulaosmanovic, B., Gujaria, N., Krishnamurthy, L., Gaur, P.M., KaviKishor, P.B., Shah, T., Srinivasan, R., Lohse, M., Xiao, Y., Town, C.D., Cook, D.R., May, G.D. and Varshney, R.K. (2011). Large-scale transcriptome analysis in chickpea (*Cicerarietinum* L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. Plant Biotechnology Journal, 9: 922–93

15. Kadam, S., Singh, K., Shukla, S., Goel, S., Vikram, P., Pawar, V., Gaikwad, K., Khanna-Chopra. R. and Singh, N. (2012). Genomic associations for drought tolerance on the short arm of wheat chromosome 4B. Funct Integr Genomics. 2012 Apr 5

16. Katara, J.L., Deshmukh, R., Singh, N.K. and Kaur, S. (2012). Molecular typing of native *Bacillus thuringiensis* isolates from diverse habitats in India using REP-PCR and ERIC-PCR analysis. *Journal of General and Applied Microbiology* 58 (2) (In press)

17. Kudapa, H., Bharti, A.K., Cannon, S.B., Farmer, A.D., Mulaosmanovic, B., Kramer, R., Bohra, A., Weeks, N.T., Crow, J.A., Tuteja, R., Shah, T., Dutta, S., Gupta, D.K., Singh, A., Gaikwad, K., Sharma, T.R., May, G.D., Singh N.K. and Varshney, R.K. (2012). A Comprehensive Transcriptome Assembly of Pigeonpea (Cajanus cajan L.) using Sanger and Second-Generation Sequencing Platforms. Mol Plant. Jan 18

18. Kumar, N., Jhang, T., Satyavir and Sharma, T.R. (2011). Molecular and Pathological Characterization of *Colletotrichum falcatum* Infecting Subtropical Indian Sugarcane. J. Phytopath. 159:260-267

19. Kumar, P., Vasupalli, N., Srinivasan, R. and Bhat, S.R. (2012). An evolutionarily conserved mitochondrial *orf108* is associated with cytoplasmic male sterility in different alloplasmic lines of *Brassica juncea* and induces male sterility in transgenic *Arabidopsis thaliana*. J. Expt. Botany, 63 , doi:10.1093/jxb/err459)

20. Kumar, P.A., Mohapatra, T., Sharma, T.R., Bhattacharya, R.C., Dash, P., Gupta, N.C. and Solanke A.U. (2011). Biotechnology and Crop Improvement. The Indian Journal of Agricultural Sciences, 81(9): 1-14

21. Kumar, V., Thakare, D.R., Saha, D.N., Jajoo, A., Jain, P.K., Bhat, S.R. and Srinivasan, R. (2012). Characterization of upstream sequences of the peroxidase gene, Atprx18 of Arabidopsis thaliana. J. Plant Biochem. Biotechnology, 21, 121-127

22. Majumder, K., Sairam, R.K. and Bhattacharya, R.C. (2012). Differential expression of salt overly sensitive pathway genes salinity determines stress tolerance in Brassica genotypes. Plant Physiology and Biochemistry 51: 90- 101

23. Meena, R.K., Kumari, G.G.K, Alpana, Gujar, G.T. and Kaur, S. (2012). Screening of *Bacillus thuringiensis* Isolates Recovered from Diverse Habitats in India for the presence of *cry*1A-Type Genes and Cloning of a *cry*1Ac33 Gene Toxic to *Helicoverpa armigera* (American bollworm). Asian Journal of Biotechnology, 4: 53-69 (*DOI:* 10.3923/ ajbkr2012)

24. Nadella, K.D., Marla, S.S., and Kumar, P.A. (2012). "Metabolomics in Agriculture" OMICS: A Journal of Integrative Biology. April 2012, Vol. 16, No. 4: 149-159

25. Nagarajan, V.K., Jain, A., Poling, M.D., Lewis A.J., Raghothama, K.G. and Smith, A.P. (2011) Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. Plant Physiology 156: 1149-1163

26. Padaria, J.C. and Kapoor, V. (2011). Plasmid borne gene of *Bacillus pumillus* MTCC7615 responsible for fungal antagonism towards *Rhizoctonia solani*. Indian Journal of Biotechnology, 10:316-320

27. Padaria, J.C., Soren, K.R. and Singh, A. (2011). Cysteine proteinase-like gene has role in symbiotic properties of *Mesorhizobium ciceri* TAL620. Journal of Ecofriendly Agriculture, 6:131-136

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30. Pandit, A., Rai. V., Sharma, T.R., Sharma, P.C. and Singh N.K. (2011). Differentially expressed genes in sensitive and tolerant rice varieties in response to salt stress. J. Plant Biochem. Biotechnol. 20 (2):149-154

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37. Rana, B. Saharan., Joshi, M., Prasanna, R., Kanika and Nain, L. (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann. Microbiol., *61(4):893-900*

38. Saha, D., Kumar, V., Bhat, S.R. and Srinivasan, R. (2011). Characterization of Upstream Sequences of the LOJ Gene Leads to Identification of a Novel Enhancer Element Conferring Lateral Organ Junction-Specific Expression in Arabidopsis thaliana. Plant Mol. Biol. Rep. 29, 265–277

39. Sarkar, D., Tiwari, J.K., Sharma, S., Sharma, Poonam S., Gopal, J., Singh, B.P., Luthra, S.K., Pandey, S.K. and Pattanayak, D. (2011). Somatic

hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun.: Molecular, phenotypic and late blight resistance characterization. Plant Cell and Tissue Organ Culture 107: 427-440

40. Sharma, A., Sonah, H., Gupta, N.K., Singh, N.K. and Sharma, T.R. (2011). Analysis of Genetic Relationship among Earthworm Species using Morphological and Molecular Markers. Zoological Sciences,28:25-31

41. Shrivastava, D., Dalal, M., Nain, V., Sharma, P.C. and Kumar, P.A. (2011). Targeted Integration of *Bacillus thuringiensis* delta-Endotoxin *cry1Fa1* in Brinjal (*Solanum melongena* L.). Current Trends in Biotechnology and Pharmacy, Vol. 5 (2): 1149-1156

42. Singh (2011) Cysteine proteinase-like gene has role in symbiotic properties of *Mesorhizobium ciceri* TAL620. Journal of Ecofriendly Agriculture, Vol 6, 131-136

43. Singh, A., Chamail, A., Chand, D., Chakrabarti, S.K. and Pattanayak, D. (2011). pUC-IVT, a modified pUC19 based *in vitro* transcription vector. Journal of Plant Biochemistry and Biotechnology 21: 60-65

44. Singh, A.K., Gopalakrishnan, S., Singh, V.P., Prabhu, K.V., Mohapatra,T., Singh, N.K, Sharma, T.R., Nagarajan, M. and Vinod K.K. (2011). Marker assisted selection: a paradigm shift in Basmati breeding. Indian J. Genet :71:1-9

45. Singh, N.K., Gupta, D.K., Jayaswal, P.K., Mahato, M.K., Dutta, S., Singh, S. Bhutani, Dogra , S., Singh, V., Kumawat ,B.P., Pal, G., Pit , J.K., Singh, A., Rawal, A., Kumar, H., Prashat, A., Khare, G. R., Yadav, A., Raje, R., Singh, R.S., Datta, M.N., Fakrudin, S., Wanjari, B., Kansal, K.B., Dash, R., .Jain P.K., Bhattacharya, P.K., Gaikwad, K., Mohapatra, T., Srinivasan, R. and Sharma, T.R. (2011).The first draft of the Pigeonpea genome sequence. J of Plant Biochem and Biotechnology. 21:98-112

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47. Sonah, H., Deshmukh, R.K., Singh, V.P., Gupta, D.K., Singh, N.K. and Sharma, T.R. (2011). Genomics Resources in Horticultural crops: Status, Utility and Challenges. Biotechnology Advances 29: 199-211

Popular Articles

1. Dash, P.K. and Rai, R. (2011). Chromosomes, Cancer and Cure. Science reporter, 48(11), pp 19-21.

2. Dash, P.K. and Rai, R. (2012). Bionic bacteria to fight disease and global warming. Science reporter, 49(3), pp18.

3. Dash, P.K. and Rai, R. (2012). Retaining muscle strength. Science reporter, 49(2), pp45-47.

4. Dash, P.K. and Rai, R. (2011). Artificial leaf: Solution to the world's energy crisis. Science reporter, 48(6), pp16.

5. Kumar, P.A. (2011). Biosefty of Bt crops. Pharma Bioworld 9: 40-43.

6. Kumar, P.A. (2011). Bt cotton: A revolution. Commodityindia.com 11: 28-29.

7. Kumar, P.A. and Pattanayak, D. (2011). Public-private partnership. Indian Seed and Planting Material 4 (2): 62-64.

Book Chapters

1. Dalal, M., Mayandi, K., and Chinnusamy, V. (2012) Sorghum: Improvement of Abiotic Stress Tolerance. *In* Improving Crop Resistance to Abiotic Stress. Vol II Part IIIA, Ed. Tuteja N., Gill S. S., Tiburcio A. F., and Tuteja R. by Wiley-VCH Verlag GmbH & Co. KGaA.

2. Deka, S., Barthakur, S. and Pandey, R. (2011) Potential effects of climate change on insect pest dynamics In: Climate change and crop productivity Ed: Dr Madan Pal *et al*, New age Publications, New Delhi

3. Kaur, S. (2012) Risk assessment of Bt transgenic crops. In: *Bacillus thuringiensis Biotechnology, Ed.* Estibaliz Sansinenea, Springer Publishers. Dordrecht, Netherlands. Heidelberg. p. 41-86. ISBN 978-94-007-3020-5.

4. Kaur, S. (2012). Metagenomics: progress, opportunities and challenges. In: *Advances of Biotechnology*. Ed. Behera, K.K., New India Publishing Agency. New Delhi. ISBN No: 978-93-81450-26-0 (In press).

5. Nain V, Sahi, S and Kumar, P.A. (2011) In silico identification of regulatory elements in promoters. In: Computational biology and applied bioinformatics. (Eds. Lopes HS and Cruz LM), InTech, pp. 47-66.

6. Padaria, J.C. and Kumar, A. (2011) Bioprospecting of genes for changing global climate. in "Climate Change: Impacts and Adaptations in crop plants" (Eds:Madan Pal, Sangeeta Khetarpal, Renu Pandey and Pramod Kumar) , Today & Tommorrow's Printers and Publishers, IARI, New Delhi, pp:103-118

7. Sharma, M., Kansal, R., Kothari, S.I., Shrivastava, M.B. and Gupta, V.K. (2011) Tissue culture of recalcitrant crops: Problems and solutions. In: Biotechnology: A New Approach Eds P.C.Trivedi Agribios: pp31-46.

Success Stories

1. Six success stories in English and Hindi compiled and edited by Dr. P.K. Mandal and Dr. R.S. Niranjan on:
   i. Improved Pusa Basmati-1
   ii. Pusa Jai Kisan
   iii. Pusa Hara chana
   iv. *Bt* Brinjal
   v. Hybrids in Mustard
   vi. *Bt* Tomato

2. NRCPB PROFILE in English and Hindi compiled & edited by Dr. P.K. Mandal, Dr. P.K. Dash, Dr. R. Rai and Dr. R.S. Niranjan

Paper in Seminar/Symposium/Conference

1. Arul, B.P., Chauhan, H., Choudhary, M., Tarafdar, A. and Padaria, J.C. (2012). Callus formation from immature embryo for genetic transformation of Indian elite wheat cultivars. In: Proceeding of International Conference on Plant Biotechnology for Food Security: New Frontiers, organized by the Society for Plant Biochemistry and Biotechnology, NRCPB, Pusa, New Delhi-110012, February 21-24th, 2012, pp: 36.

2. Barthakur , S. and Khomdram, S. (2012). Engineering proline metabolism and transport to develop climate resilient crop plants, National seminar on Indian agriculture: preparedness for climate change organized by Indian society for agricultural sciences at NASC complex, Pusa, New Delhi, March 24-25 2012

3. Barthakur, S., Pandey, N. and Kumar, R. (2011). Analysis of an environmental stress related annexin promoter from rice in transgenic tobacco, ICER 2011 Surat, December 2011

4. Barthakur, S. (2012) Molecular prospecting of Pearl millet (*Pennisetum glaucum*) for thermotolerant genes, BBCON ,Siliguri, February 2012

5. Bhatt, D., Raipuria, R.K., Prabu, R.C., Biswas, K. and Padaria, J.C. (2012). Identification of stress-induced genes from the thermo tolerant Indian wheat (*Triticum aestivum* cv. Raj 3765) through analysis of subtracted cDNA libraries. In: Proceeding of International Conference on Plant Biotechnology for Food Security: New Frontiers, organized by the Society for Plant Biochemistry and Biotechnology, NRCPB, Pusa,

New Delhi-110012, February 21-24th,2012 , pp: 126.

6. Biswas, K., Raipuria, R.K., Prabhu, R.C., Bhatt, D., Narang, P., Padaria, J.C. (2012). Analysis of heat responsive SSH libraries during anthesis in wheat (*Triticum aestivum* l.). **In:** Proceeding of International Conference on Plant Biotechnology for Food Security: New Frontiers, organized by the Society for Plant Biochemistry and Biotechnology, NRCPB, Pusa, New Delhi-110012, February 21-24th, 2012, pp: 135.

7. Chhapekar, S., Raghavendra, Rao S., Phanindra, M.L.V., Pavan, G., Ramakrishna, Ch., Singh, V.K., Dhandapani, G., Solanke, A.U., Pattanayak, D. and Kumar, P.A. (2012). Development of glyphosate tolerant rice. International Conference on Plant Biotechnology for Food Security: New Frontiers held at New Delhi from February 21-24, 2012.

8. Choudhary, M., Tarafdar, A., Chauhan, C., Arul, B.P. and Padaria, J.C. (2012). *Agrobacterium tumefaciens* mediated transformation for development of thermo tolerant wheat (*Triticum aestivum* l.). In: Proceeding of International Conference on Plant Biotechnology for Food Security: New Frontiers, organized by the Society for Plant Biochemistry and Biotechnology, NRCPB, Pusa, New Delhi-110012, February 21-24th, 2012, pp: 137.

9. Dalal, Monika. (2012). International conference on Plant Biotechnology for food security: New frontiers" Organized by Society for Plant Biochemistry and Biotechnology, Feb 21-24, 2012 at New Delhi

10. Deka, S., Kumar, R. and Barthakur, S. (2012). Cloning and functional analysis of Annexin2 (*AnnOsI2*) from a drought tolerant rice cultivar, International Conference on Plant Biotechnology for Food Security: New Frontiers, New Delhi Feb 21-24, 2012

11. Deka, S. and Barthakur, S. (2012). Intervention of biotechnology as IPM component to mitigate the impact of climate change on insect population – an analysis, National seminar on Indian agriculture: preparedness for climate change organized by Indian society for agricultural sciences at NASC complex, Pusa, New Delhi, March 24-25 2012

12. Deka, S., Arora, Kanika and Barthakur, S. (2012). Expression analysis of annexin genes under biotic stress , IUPAC-2nd International Conference on agrochemicals Protecting Crops, Health and natural environment, New Delhi, 15-18 February, 2012

13. Deokar, A.A., Kohli, D., Kondawar, V., Jain, P.K. and Srinivasan, R. (2012). Identification and characterization of CarERF3: a drought responsive gene in chickpea. Poster presentation in International Conference on Plant Biotechnology for Food Security: New Frontiers held at New Delhi, India from 21-24 Feb (ST36, Pg. no. 123).

14. Devi, B.M. and Rai, R. (2012). ABC transporter influences the production of 2,4 Diacetyl PhloroGlucinol. International Conference on Plant Biotechnology for Food Security: New Frontiers, NASC, New Delhi, India ST-55, 132pp.

15. Dhatwalia , D., Satyawathi, C.T. and Barthakur, S. (2012). Transcript expression profiling of annexin multigene family in Pennisetum glaucum, International Conference on Plant Biotechnology for Food Security: New Frontiers, New Delhi Feb 21-24, 2012

16. Dogra T., Priyadarshini A., Kanika, Kumar A.,. Singh N.K. (2011). Genetic and Molecular characterization of gene (s) associated with salt tolerance in *Mesorhizobium ciceri* Ca181. In: Proceedings of National Conference on Environment and Biodiversity of India held on 30-31 December in New Delhi. Pp-71

17. Gardea, J. and Jain, A. (2012). Chairpersons for Session V (Nanobiotechnology and Diagnostics)

at the International Conference on Plant Biotechnology for Food Security: New Frontiers, February 21-24, 2012, NASC Complex, New Delhi.

18. Gupta, N.C., Jain, P.K., Bhat, S.R. and Srinivasan, R. (2011). Poster (Abs # FG-12) "Molecular cloning of a wound-inducible promoter from a promoter tagged population of *Arabidopsis thaliana"* International Symposium on Plant Biotechnology towards Tolerance to Stresses and Enhancing Crop Yield". Sep 28th – Oct 1st, Ranchi, Jharkhand.

19. Jain, P.K. (2012). RNA interference and its applications in crop biotechnology with a case study on HD-RNAi. Invited talk at National Conference on Biotechnology and Biodiversity held at Rewa, MP from 13-14th March, 2012.

20. Jain, P.K., Deokar, A.A., Kondawar, V., Jagannadham, P.T.K. and Srinivasan, R. (2012). Identification and characterization of *CarNAC*: a drought responsive candidate gene in chickpea. Oral presentation in IUPAC Sponsored 2nd International Conference on APCHNE: Role of Chemistry for Sustainable Agriculture held at New Delhi, India from 15-18 Feb 2012 (OP36, Pg. no. 88).

21. Kakrana, A., Kumar, A., Sirohi, A., Srinivasan, R. and Jain, P.K. (2012). Isolation and characterization of a novel root-specific and nematode responsive promoter from Arabidopsis thaliana. Poster presentation in International Conference on Plant Biotechnology for Food Security: New Frontiers held at New Delhi, India from 21-24 Feb (ST48, Pg. no. 129).

22. Kanakachari, M., Dhandapani, G., Padmalatha, K.V., Liji, C., Kumar, S., Patil, D.P., Das, A., Leelavathi, S., Katageri, I.S., Reddy, M.K., Solanke, A.U., Siva, Reddy V and Kumar, P.A. (2011). Genome wide transcriptome analysis of fuzzless-lintless mutant cotton (*G. hirsutum* L. cv. MCU5) during fibre elongation. World Cotton Research Conference-5, held at Mumbai from November 7-11, 2011.

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Distinguished Visitors

01.04.2011	Visit Dr. Diana Arias and Mr. Ernst Vrancken from the Global Technology Development-Rice, USA
14.04.2011	Visit of Dr. Jerome Verdier , Global Brassica , Unit Head
14.04.2011	Visit of Sjaak Van der Ploeg , Head of Cabbage Breeding
18.07.2011	Visit of Mr. Ed. Porter , Director, New Technology Division (US Dept of Agricultural)
15.10.2011	Visit of His Excellency U Thien Sein , President of Myanmar
25.10.2011	Visit of Mr. Ravinder Singh from Molecular, Cellular and Development biology, USA
28.10.2011	Visit of Dr. Chris Barker and Dr. Raju Datla from TUFGEN, Canada
28.10.2011	Visit of Dr. Patrick Schnable from Lowa State University, USA
28.11.2011 to 30.11.2011	Visit of Prof. Maurice Moloney from Director and Chief Executive, UK
28.11.2011 to 30.11.2011	Visit of Dr. Angela Karp from Director of Rothamsted Center for Bioenergy and Climate change, UK
28.11.2011 to 30.11.2011	Visit of Prof. Keith Goulding from Director of the Center for Soils and Ecosystem Fund and Head of the Dept for Sustainable Soils Grassland Systems, UK
28.11.2011 to 30.11.2011	Visit of Prof. Peter R. Shewry from Associate Director of Rothamsted Research and Director of the Center for Crop Genetic Improvement, UK
28.11.2011 to 30.11.2011	Visit of Prof. Martin A. J. Parry from Head of the Dept. of Plant Sciences at Rothamsted Research, UK
24.01.2012	Visit of Dr. Meena K. Sakharkar from University of Tsukuba, Japan
10.02.2012	Visit of Dr. Senthil Kumar Muthappa from The Samuel Roberts Noble Foundation, Ardmore, USA
18.02.2012	Visit of Dr. Mickelbart Michael V from Dept. of Horticulture and Landscape Architecture, Purdue University,USA
09.03.2012	Visit of Dr. Autar Mattoo and Mr. Mathew Jones from USDA Sustainable Agricultural Systems Laboratory, USA

International Conference on Plant Biotechnology for Food Security: New Frontiers-2012

The "International Conference on Plant Biotechnology for Food Security: New Frontiers-2012" was organized by the Society for Plant Biochemistry and Biotechnology and NRCPB in association with IARI, New Delhi from 21-24 Feb, 2012 at NASC complex, New Delhi-12. The conference was attended by 700 delegates of whom 50 delegates were from abroad. The inaugural session was chaired by Prof. MS Swaminathan and Prof. V.L. Chopra was guest of honour. Prof G. S. Khush, in the inaugural talk on "Role of Biotechnology in World Food Security" pointed biotechnology can play key role in food security in three important steps namely raising the yield ceiling, closing the gap between yield in experimental and farmers fields, and sustaining the current yield levels. He advised that media should report science, and scientists should educate the public about the science of biotechnology. Prof. Swaminathan concluded that emphasis should be given to nutritional security and the biotechnology.

The technical programme of the conference consisted of four plenary lectures, fourteen technical sessions of oral presentations and two poster sessions. The 14 technical sessions were organized on diverse areas of biotechnology and its application in agriculture. In these sessions, 14 lead lectures and 52 invited lectures were delivered by eminent scientists from abroad and India. The technical sessions were on the following topics: developmental biology, genomics and bioinformatics, molecular plant breeding, biochemistry and metabolic engineering, biotic and abiotic stress management, plant microbe-interactions, nanotechnology and diagnostics, biotechnology education, biosafety and novel perspectives. The conference has also organized two poster sessions with a total of 370 poster presentations.

The overall major recommendations emerged from the conference are as follows:

- A combination of genetic engineering, molecular breeding and conventional plant breeding is necessary for food and nutritional security, profitability of farmers and environmental safety.
- The application of biotechnology such as Bt-cotton has enhanced livelihood security of farmers, besides its environmental benefits. Hence many more technologies should be generated.
- We needs to apply genetic engineering and molecular breeding for development of C4 rice, nitrogen fixation in cereals, crops with enhanced resource use efficiency, climate resilient agriculture and biofortification.
- Exploitation of indigenous germplasm diversity to generate genomic resources, sequence based genotyping, and genomics-assisted crop improvement.
- Current emphasis is mainly on understanding the function of genes in isolation. However, the poor understanding of complex interplay between genes limits translational research. Hence, systems biology approach should be adapted.

- We need to develop trained human resource in the area of bioinformatics.
- Biosafety regulations need to be more efficient to enhance the pace of biotechnology product development and delivery to the end user.
- Public-private partnership is necessary for biotechnology product development.
- Biotechnology education needs to be strengthened and the need for stand alone universities in agriculture was emphasized.

Inaugural Session of ICPBFS - 2012

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