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ANNUAL REPORT
2022

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ICAR-NATIONAL INSTITUTE FOR PLANT BIOTECHNOLOGY
Lal Bahadur Shastri Centre, Pusa Campus, New Delhi-110012 (India)

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आमुख

वर्ष 2022 में, विश्व के विभिन्न हिस्सों में बाढ़, सूखे और गर्म तापमान के रूप में जलवायु परिवर्तन के पदचिह्न स्पष्ट रूप से दिखाई पड़े। ये प्राकृतिक आपदाएं, विश्व की निरंतर बढ़ती आबादी के भरण-पोषण के लिए जलवायु अनुकूल फसलें विकसित करने की आवश्यकता को बार-बार दोहराती हैं। अपने प्रमुख अधिदेश के साथ, भा.कृ. अनु. प.- रा. पा. जै. सं. निरंतर रूप से जीनोमिक्स, आणविक प्रजनन, पराजेनिक और जीनोम संपादन के माध्यम से फसल सुधार के कार्य में लगा हुआ है और साथ ही पादप जैव प्रौद्योगिकी के क्षेत्र में आधुनिक जैव प्रौद्योगिकी उपकरण (टूल्स) और तकनीक भी विकसित कर रहा है। मुख्य रूप से हमने अपना ध्यान जैविक और अजैविक दबाव सहिष्णुता और अनाज की गुणवत्ता बढ़ाने के साथ-साथ उत्पादकता में सुधार लाने पर केंद्रित किया है। वर्ष 2022 के दौरान इस संस्थान की उपलब्धियां निम्नानुसार रही हैं।

जीनों की पहचान करने और जीनोमिक संसाधनों को विकसित करने के लिए, 1.8 एमबी के एन50 के साथ 456 कॉन्टिग्स वाले एक उच्च गुणवत्ता वाले जीनोम अनुक्रम को चने के वन्य प्रजाति *सिसर एरीटिनम* से उत्पादित किया गया था। *सिसर क्यूनेटम* का एक संपुर्ण लंबाई वाला क्लोरोप्लास्ट जीनोम, चने के एक वन्य प्रजातिसे अनुक्रमित किया गया था, और इसका डि-नोवो संयोजन और व्याख्या की गई। इसके अतिरिक्त, तना सड़न के कारक एजेंट, एस. स्क्लेरोटियोरम (ईएसआर-01) के भारतीय विलगनक के 129x कवरेज वाला एक प्रारूप जीनोम अनुक्रम उत्पन्न किया गया था। यह ~41 एमबी जीनोम है जिसमें 9469 प्रोटीन-कोडिंग जीन और 57 प्रभावकारक मुख्य जीन हैं।

जीनोमिक दृष्टिकोण के माध्यम से, अरहर में पुष्पण के दिनों के लिए कुल 8 क्यूटीएल, निर्धारण हेतु 6 क्यूटीएल और क्लिस्टोगैमी के लिए 5 क्यूटीएल की पहचान की गई। रा. पा. जै. सं. ने भा.कृ. अनु. प.-- रा. चा. अनु. सं., कटक के सहयोग से जीवाणविक झुलसा प्रतिरोधी और जलमग्नता सहिष्णु चावल किस्म रानी धान के विकास में योगदान दिया। आईआर 29 और अफ्रीकी चावल से प्राप्त RIL पोप्युलेशन में लवण सहिष्णुता के लिए नवीन क्यूटीएल की पहचान की गई। केएएसपी (KASP) चिह्नकों (मार्करों) को, NKSWR 173 के 75 बीसी$_1$एफ$_2$ परिवारों में अनाज की पैदावार, पादप ऊंचाई और पौद-अवस्था में लवण प्रतिबल सहिष्णुता के लिए चार क्यूटीएल की पहचान करने हेतु तैयार किया गया। सांबा मसूरी और प्राप्तकर्ता जनक के उच्च दाना संख्या प्रति पुष्पगुच्छ (पैनिकल) (जीएनपी) में, तुलनात्मक ट्रांस्क्रिप्टोम विश्लेषण के आधार पर, दो प्रमुख जीनों, एंट-कौरेन सिंथेस और प्रोटीन काइनेज की पहचान की गई और उन्हें उच्च अनाज संख्या क्यूटीएल लोकस qAG 4.1 में स्थानीयकृत पाया गया। भारतीय चाय के विकास और वर्चस्व के इतिहास को समझने के लिए, चाय के 150 जीनप्ररूपों का एसएनपी आधारित विश्लेषण किया गया, जिसमें से असम और चाइना प्रकार की चाय के स्वतंत्र वर्चस्व का पता चला। चाय

अनुसंधान समुदायों के लिए टीआईजीईआर (TIGeR) वेब संसाधन विकसित किया गया।

जड़ गांठ सूत्रकृमि ग्रसन को नियंत्रित करने के लिए, एराबिडोप्सिस में एक डीएसआरएनए मध्यस्थता वाली आरएनएआई-आधारित रणनीति का मूल्यांकन किया गया था। आरएनएआई वंशक्रम ने पादप व्रण (गाल) निर्माण में 61% तक की कमी दिखाई और इसलिए यह संकेत मिलता है कि आरएनएआई, एम. इनकॉग्निटा ग्रसन को नियंत्रित करने के लिए एक आशाजनक रणनीति है। काइमेरिक बीटी जीन क्राय1एसीएफ को अभिव्यक्त करने वाले तंबाकू पराजेनिक वंशक्रमों ने फॉल आर्मीवॉर्म (एफएडब्ल्यू) के विरूद्ध

72-80% लार्वा मृत्यता के साथ महत्वपूर्ण प्रतिरोध दर्शाया, जो एक पॉलीफैगस और अत्यधिक आक्रामक नाशीजीव है।

उपापचय (मेटाबोलॉमिक) विश्लेषण से पता चला कि फ्लेवोनोइड्स जैसे द्वितियक मेटाबोलाइट्स गेहूं में सूखे की सहिष्णुता को बढ़ाने में योगदान करते हैं और अरहर के वन्य प्रजाति काजानस प्लैटिकार्पस में herbivory को कम करते हैं। चावल की लवण सहिष्णुता में एलांटोइन की भागीदारी पर विधिवत विश्लेषण किया जा रहा है। गेहूं में, इसकी जड़ में सबसे अधिक अभिव्यक्त TaNRT2.1 जीन में से एक का लक्षण-वर्णन, एराबिडोप्सिस के atnrt2.1 परिवर्ती के पूरक द्वारा किया गया। साइटोकाइनिन के साथ गेहूं प्रजाति HD2967 बीज की प्राइमिंग के बाद एक ट्रांसजेनरेशनल ताप दबाव मेमोरी विश्लेषण किया गया।

अल्टरनेरिया प्रतिरोधिता के लिए alien प्रतिरोधिता वाले जीनों को लाने के लिए ब्रैसिका की वन्य प्रजातियों के साथ व्यापक संकरण द्वारा अंतर्गमन वंशक्रम विकसित किए गए हैं। ब्रैसिका में सफेद रतुआ प्रतिरोधिता के लिए विविध जननद्रव्यों की जांच की गई और आशाजनक जीन प्ररूपों की पहचान की गई।

चना (जीआरएएस), अरहर (एआरएफ), गेहूं (ब्रेविस रेडिक्स, एनआरटी2, एनएआर2) के जीन परिवारों के लिए जीनोम व्यापी विश्लेषण किए गए और चावल (वॉन विलेब्रांड फैक्टर ए डोमेन युक्त (vWA) जीन) और लक्ष्य विशेषकों के लिए मुख्य जीनों की पहचान की गई। अधिदेषित फसलों में विभिन्न विकासात्मक, जैविक और अजैविक प्रतिबल प्रतिक्रियाओं के लिए जीन और जीन सम्बंधित पथ विश्लेषन (पाथवे) की पहचान करने हेतु ट्रांसक्रिप्टोमिक संसाधन उत्पन्न किए गए।

फील्ड स्तर पर पुष्पगुच्छ (पैनिकल) प्रस्फुटन प्रतिरोधिता की जांच के लिए एक सरल और कुशल सिरिंज इनोल्युलेषन विधि विकसित की गई। रागी जीनप्ररूप जीएन-5 की पुनरूत्पत्ति के लिए एक प्रोटोकॉल का मानकीकरण किया गया है। एनआईपीबी आनुवंशिक संलग्नता परीक्षण के लिए परामर्श (रेफरल) केंद्र है। वर्ष 2022 में, कुल 1935 नमूनों का परीक्षण किया गया और अमरूद के पादपों की आनुवंशिक संलग्नता के परीक्षण के लिए एसओपी भी विकसित किए गए।

ब्रेसिका और चावल में पैदावार संबंधी विशेषकों और जैविक दबाव प्रतिरोधिता के लिए जीनोम संपादन उपकरणों (टूल्स) का उपयोग किया जा रहा है। किसानों के सरसों के खेतों में उभरती हुई समस्या ब्रूमरेप (ओरोबैंकि) का फील्ड और प्रयोगशाला स्तर पर अध्ययन शुरू कर दिया गया है। पोषण-विरोधी कारक और डेटा विश्लेषण में अंतर करने के लिए कृत्रिम आसूचना (आर्टिफिशियल इंटेलिजेंस) और मशीन लर्निंग की नई पहल की गई है।

एनआईपीबी अपनी स्थापना के बाद से ही आईएआरआई के आणविक जीवविज्ञान और जैव प्रौद्योगिकी (एमबीबी) के एक विषय-क्षेत्र के रूप में कार्य करता है। वर्तमान में, एमबीबी के विषय-क्षेत्र में 43 पीएच.डी और 9 एम.एससी. के छात्र पंजीकृत हैं। वर्ष 2022 में, दो पीएच.डी. के छात्रों और सात एम.एससी. के छात्रों को आईएआरआई के 60वें दीक्षांत समारोह में उपाधियां (डिग्री) प्रदान की गई। संस्थान ने दो प्रशिक्षण आयोजित किए, एक अंतर्राष्ट्रीय संगोष्ठी सह पीटीसीए की 44वीं वार्षिक बैठक और एक शैक्षणिक -उद्योग जैव प्रौद्योगिकी सम्मेलन का आयोजन किया। एनआईपीबी ने एससीएसपी के तहत 4 कार्यक्रम भी आयोजित किए और आईएआरआई में आयोजित पूसा कृषि मेले में अपने उत्पादों और प्रौद्योगिकी का प्रदर्शन किया। इसके अतिरिक्त, हिन्दी चेतना मास, सतर्कता जागरूकता सप्ताह, अंतर्राष्ट्रीय महिला दिवस, स्वच्छ भारत अभियान जैसे कई कार्यक्रमों का भी आयोजन किया।

मैं, संस्थान की विभिन्न गतिविधियों में अपना योगदान देने के लिए आईसीएआर-एनआईपीबी के सभी वैज्ञानिक, तकनीकी, प्रशासनिक कर्मचारियों, छात्रों, अनुसंधान अध्येताओं और संविदात्मक सहायक कर्मचारियों को अपना हार्दिक धन्यवाद देना चाहती हूं। मैं डॉ. मोनिका दलाल, डॉ. अमोलकुमार सोलंके, डॉ. निम्मी एमएस. डॉ. संध्या शर्मा, डॉ. युवराज आई. और सुश्री मेघा को इस रिपोर्ट के संकलन और संपादन में सहायता करने के लिए धन्यवाद देती हूं।

मैं, डॉ. एच. पाठक, सचिव, डेयर और महानिदेशक, आईसीएआर, डॉ. टी. महापात्रा, पूर्व सचिव, डेयर और महानिदेशक, आईसीएआर, डॉ. टी.आर. शर्मा, उप महानिदेशक (फसल विज्ञान) और डॉ. डी.के. यादव, सहायक महानिदेशक (बीज), आईसीएआर, की संस्थान के समग्र कामकाज में निरंतर समर्थन और सहायता करने के लिए आभारी हूं।

अनीता ग्रोवर
24/02/23

(अनीता ग्रोवर)

दिनांक: 24.02.2023

PREFACE

In the year 2022, the footprints of climate change were evident in the form of floods, drought and warmer temperatures in different parts of the world. These natural disasters reiterate the urgency to develop climate resilient crops to feed the ever increasing world population. With the mandates in focus, ICAR-NIPB is continuously engaged in crop improvement through genomics, molecular breeding, transgenics and genome editing and also developing modern biotechnology tools and techniques in the area of plant biotechnology. The focus has been to improve the productivity along with biotic and abiotic stress tolerance and enhancing the grain quality. The achievements of the institute during the year 2022 are as follows

To identify genes and develop genomic resources, a high-quality genome sequence consisting of 456 contigs with an N50 of 1.8 Mb was produced from wild relative of cultivated chickpea *Cicer arietinum*. A full-length chloroplast genome of *Cicer cuneatum,* a wild relative of the domesticated chickpea was assembled *de novo* and annotations were carried out. Furthermore, a draft genome sequence with 129x coverage of an Indian isolate of the *S. sclerotiorum* (ESR-01), causal agent for stem rot, was generated. It is a ~41 Mb genome with 9469 protein-coding genes and 57 effector candidates.

Through genomic approaches total 8 QTLs for days to flowering, 6 QTLs for determinacy and 5 QTLs for cleistogamy were identified in pigeon pea. NIPB contributed in the development of bacterial blight resistant and submergence tolerant rice variety Rani Dhan in collaboration with ICAR-NRRI, Cuttack. Novel QTLs for salinity tolerance were identified in RIL population derived from IR 29 and African rice. KASP markers were designed to identify four QTLs for grain yield, plant height, and seedling stage salt stress tolerance in 75 BC_1F_2 families of NKSWR173. Based on comparative transcriptome analysis in high grain number per panicle (GNP) NIL of Samba Mahsuri and the recipient parent, two key genes, encoding for ent-kaurene synthase and protein kinase were identified and found to be localized in the high grain number QTL locus qAG4.1. To decode the evolution and domestication history of Indian Tea, SNP based analysis of 150 genotypes of tea was carried out which revealed independent domestication events of Assam and China type. The TIGeR web

resource was developed for the Tea research communities.

For controlling root knot nematode infestation, a DsRNA mediated RNAi-based strategy was evaluated in *Arabidopsis*. The RNAi lines showed up to 61% reduction in gall formation and hence indicating RNAi to be promising strategy to control *M. Incognita* infestation. Tobacco transgenic lines expressing a chimeric *Bt* gene *cry1AcF* showed significant resistance with 72-80% larval mortality against Fall armyworm (FAW) which is a polyphagous and very invasive insect pest.

Metabolomic analyses revealed that secondary metabolites such as flavonoids contribute to enhanced drought tolerance in wheat and reduce herbivory in *Cajanus platycarpus,* a wild relative of pigeon pea. Mechanistic insight on role of allantoin involvement in salinity tolerance in rice is also being analyzed. In wheat, one of the highest expressing *TaNRT2.1* genes in root, was characterized by complementation of *atnrt2.1* mutant of *Arabidopsis.* A transgenerational heat stress memory analysis was carried out after seed priming of wheat cv. HD2967 seeds with cytokinin.

To bring the alien resistance genes for Alternaria resistance, introgression lines have been developed by wide hybridization with wild species in *Brassica*. Diverse germplasm was screened for white rust resistance in *Brassica* and promising genotypes were identified.

Genome wide analyses were carried out for gene families in chickpea (*GRAS)*, pigeon pea (*ARF),* wheat *(Brevis Radix, NRT2*, *NAR2*) and rice (von Willebrand factor A domain containing (vWA) genes) and candidate genes for target traits were identified. Transcriptomic resources were generated for identifying genes and pathways for different developmental, biotic and abiotic stress responses in mandate crops.

An easy and efficient syringe inoculation method was developed for screening for panicle blast resistance at the field level. A protocol for regeneration of a finger millet genotype GN-5 has been standardized. NIPB is the Referral Centre for Genetic Fidelity Testing. In 2022, total 1935 samples were tested and also SOP for testing genetic fidelity of guava plants was developed.

Genome editing tools are being exploited for yield related traits and biotic stress resistance in brassica and rice. Field and lab studies on Broomrape (*Orobanche*) which is emerging problem in farmer's mustard fields have been initiated. New initiatives in Artificial intelligence and Machine learning have been taken for discriminating anti-nutritional factor and data analysis.

NIPB serves as a discipline of Molecular Biology and Biotechnology (MBB) of IARI since its inception. Currently 43 Ph.D. and 9 M.Sc. students are registered in the discipline of MBB. In the year 2022, two Ph.D. students and seven M.Sc. students were awarded degrees in the 60th Convocation of IARI. Institute conducted two trainings, one International symposium cum 44th annual meeting of PTCA and one Academia-Industry Biotechnology Meet. NIPB also conducted 4 programs under SCSP and exhibited its products and technologies at Pusa Krishi Mela held at IARI. In addition, several activities such as Hindi chetna maas, Vigilance awareness week, International women's day, Swachh bharat abhiyan were celebrated.

I would like to extend my sincere thanks to all scientific, technical, administrative staff, students, research fellows and contractual supporting staff of ICAR-NIPB for their contributions in various institute activities. I thank Dr. Monika Dalal, Dr. Amolkumar Solanke, Dr. Nimmy MS, Dr. Sandhya Sharma, Dr. Yuvaraj I. and Ms Megha for their help in compilation and editing of this report.

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Anita Grover 24/2/23

Date: 24.2.2023 (Anita Grover)

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Executive Summary

विशिष्ट सारांश

अपने अधिदेशों पर ध्यान केंद्रित करते हुए, आईसीएआर-एनआईपीबी निरंतर पादप जैव प्रौद्योगिकी के क्षेत्र में आधुनिक जैव प्रौद्योगिकी उपकरणों (टूल्स) और तकनीकों को विकसित करने और जीनोमिक्स, आणविक प्रजनन, पराजेनिक और जीनोम संपादन का उपयोग करते हुए फसल सुधार के लिए उनके अनुप्रयोगों में लगा हुआ है। यह संस्थान, भारतीय कृषि अनुसंधान संस्थान के स्नातकोत्तर छात्रों के शिक्षण और प्रशिक्षण के लिए आणविक जीव विज्ञान और जैव प्रौद्योगिकी प्रभाग के रूप में भी कार्य करता है। अपने संस्थान स्तर के प्रशिक्षण कार्यक्रमों के अतिरिक्त, एनआईपीबी अनुसूचित जाति उप योजना (एससीएसपी), दीर्घकालिक प्रशिक्षण कार्यक्रमों और सम्पूर्ण भारत से छात्रों के नियमित दौरों के माध्यम से किसानों के साथ-साथ महाविद्यालय और विद्यालय के छात्रों के लिए कार्य कर रहा है। हमारा संस्थान मुख्य रूप से चना, अरहर, चावल, गेहूं और भारतीय सरसों पर काम कर रहा है। इन फसलों के साथ-साथ, यह जीनोमिक संसाधनों के विकास और इन फसलों की प्रमुख समस्याओं के जैव-प्रौद्योगिकीय समाधान खोजने के लिए राष्ट्रीय और अंतर्राष्ट्रीय संस्थानों के साथ सुदृढ़ सहयोग के माध्यम से अन्य फसलों पर भी काम करता है। संस्थान द्वारा वर्ष 2022 में छह नए पेटेंट दायर किए गए और 2 पहले दायर किए गए पेटेंट और 4 कॉपी राइट को स्वीकृति मिली। इस वर्ष के दौरान एनआईपीबी की महत्वपूर्ण उपलब्धियों का सारांश नीचे दिया गया है।

- वन्य प्रजातियां, कृषि संबंधी महत्वपूर्ण विशेषकों के लिए समृद्ध आनुवंशिक संसाधन हैं। साइसर एरीटिनम, घरेलू चने का एक वन्य प्रजाति और निकटतम पूर्वज, का एक उच्च गुणवत्ता वाला जीनोम अनुक्रम पैक बायो हाईफाई (PacBio HiFi) रीड्स का उपयोग करके तैयार किया गया। इसमें 1.8 एमबी के एन50 के साथ 456 कॉन्टिग्स शामिल थे। साइसर क्यूनेटम चने का एक अन्य वन्य प्रजाति, के पूर्ण लंबाई वाले क्लोरोप्लास्ट जीनोम को अनुक्रमित किया गया, और डि-नोवो को संकलित किया गया और इसकी व्याख्या की गई। इसका आकार लगभग 124.3 केबी है, जो कि खेती की गई प्रजातियों, साइसर एरीएटिनम (125.5 केबी) से लगभग एक किलोबेस छोटा है। सर्कुलर आरएनए (circRNAs), जिसमें नोन-कोडिंग आरएनए का एक बड़ा वर्ग शामिल है, विभिन्न जैविक प्रकियाओं में महत्वपूर्ण भूमिका निभाते हैं। सिरिक्यावंट (CIRIQUANT) और क्लियर (CLEAR) एल्गोरिदम का उपयोग करके, चने में कुल 1377 सरकुलर आरएनए की पूर्व सूचना दी गई।
- चना फली बेधक (हेलिकोवर्पा आर्मिगेरा), जड़-गांठ सूत्रकृमि, और फ्यूसेरियम आक्सिस्पोरम चने के उत्पादन में प्रमुख जैविक बाधाएं हैं। फली बेधक प्रेरित जीन की पहचान करने के लिए, लार्वा के साथ 24 घंटे की चुनौती के बाद सहिष्णु और अतिसंदेनशील जीनप्ररूप का तुलनात्मक प्रतिलेखन विश्लेषण किया गया। ज्ञात रक्षा जीनों के अतिरिक्त कीटों के खिलाफ रक्षा प्रतिक्रियाओं से जुड़े कुछ नए मुख्य जीनों की भी पहचान की गई। फाइटोनेमेटोड्स को नियंत्रित करने के लिए एक टिकाऊ और लक्ष्य-विशिष्ट विकल्प के रूप में आरएनएआई प्रौद्योगिकी उभर कर आई है। इसलिए, जड़-गांठ सूत्रकृमि (आरकेएन) प्रतिरोधिता का विश्लेषण करने के लिए, एराबिडोप्सिस में परपोषी-प्रदत्त (होस्ट-डिलीवर) आरएनएआई (एचडी-आरएनएआई) का उपयोग करते हुए एमआई-एमएसपी10 और एमआई-एमएसपी23 डीएसआरएनए कैसेट का कार्यात्मक मूल्यांकन किया गया। एचडी-आरएनएआई का लक्षणरूपी प्रभाव, आरएनएआई वंशक्रमों में व्रण (गाल) संरचना में अधिकतम 61% की कमी के साथ दिखाई दिया। जीन अभिव्यक्ति विश्लेषण ने आरएनएआई वंशक्रमों पर भरण-पोषण करने वाली एम. इनकोगनिटा मादाओं में प्रतिलेखन स्तर में महत्वपूर्ण कमी दर्शायी, जिससे प्रभावी जीन साइलेंसिंग का और साक्ष्य प्राप्त हुआ। फ्यूजीरियम मुरझान प्रतिरोधिता से जुड़े जीनोमिक क्षेत्रों की पहचान करने की तलाश में, सभी क्यूटीएल का मेटा-विश्लेषण किया गया और परिणामों से पता चला कि क्रोमोसोम 2 फ्यूजेरियम प्रतिरोधिता के लिए एक हॉटस्पॉट है।
- चने के लिए अत्यधिक सूखा, सबसे अधिक विनाशकारी अजैविक दबाव कारकों में से एक है। हमारे अध्ययन में, चना जीनोम में 46 जीआरएएस प्रतिलेखन कारक जीन की पहचान की गई। CaGRAS जीन का जीन अभिव्यक्ति विश्लेषण सूखा सहिष्णुता विशेषक अर्थात् आईसीसी 4958 (सूखा सहिष्णु) और आईसीसी 1882 (सूखा संवेदनशील) के विपरीत चने की दो किस्मों में किया गया था, जहां CaGRAS12 (एससीआर) को सूखा-प्रतिक्रियाशील पाया गया।
- चना भारत की अधिकांश आबादी के लिए प्रोटीन का एक महत्वपूर्ण स्रोत है। संवर्धित जीन प्ररूपों की प्रोटीन सामग्री में अत्यधिक भिन्नता है। इसलिए, चने के दस चयनित जीनप्ररूपों की प्रोटीन सामग्री और प्रोटीन प्रोफाइलिंग का विश्लेषण किया गया। अध्ययन किए गए जीनप्ररूपों की प्रोटीन सामग्री में 18% से 28% तक की भिन्नता थी। बीज भंडारण प्रोटीनों को एन्कोड करने वाले जीन भी उच्च और निम्न प्रोटीन युक्त चने के जीनप्ररूपों के बीच भिन्न रूप में व्यक्त पाए गए।
- फलियों में फली भित्ति (वाल) की भूमिका अत्यंत महत्वपूर्ण है। इसलिए, फली भित्ति के विकास को विनियमित करने वाले तंत्र को जानने के लिए और फली भित्ति विशिष्ट जीनों की पहचान करने के लिए भी, चने की फली के विभिन्न विकास चरणों के दौरान RNAseq विश्लेषण किया गया।
- अरहर में जल्दी फूल आना और स्थिर होना इसके वांछनीय लक्षण हैं। अरहर में जल्दी फूल आने और निर्धारकता लक्षणों

के लिए जिम्मेदार क्यूटीएल का मानचित्रण करने के लिए जीनोमिक्स का उपयोग किया गया। इन क्यूटीएल को संस्थान में मौजूद प्रतिलेखन डेटा के साथ-साथ फूलों से संबंधित जीनों के लिए विशिष्ट फ्लोरिड डेटाबेस का उपयोग करके भिन्न रूप से व्यक्त जीनों हेतु स्कैन किया गया। डीटीएफ के लिए कुल 8 क्यूटीएल और निर्धारकता के लिए 6 क्यूटीएल 6 अलग-अलग गुणसूत्रों में फैले हुए थे।

- अरहर में क्लिस्टोगैमी की ओर ले जाने वाले आणविक तंत्र की जांच की गई। इस अध्ययन के लिए नियंत्रण के रूप में उपयोग में लाई गई तीन किस्में आईसीपी87154 (क्लिस्टोगैमस), यूपी26 (आंशिक क्लिस्टोगैमस), और आशा (चैस्मोगैमस) थीं। इसके लिए जीबीएस आधारित एसएनपी की पहचान के बाद एफ2 आबादी का क्यूटीएल अनुक्रमण (सेक) विश्लेषण किया गया और फिर मुख्य जीन तक पहुंचने के लिए प्रतिलेखन (ट्रांसक्रिप्टोम) का उपयोग किया गया। मानचित्रण कार्य से 5 क्यूटीएल के एक सेट की पहचान की गई। इनमें एलएनसीआरएनए, एबीसी ट्रांसपोर्टर जी परिवार के सदस्य 31-लाइक, ई3 यूबिकिटिन-प्रोटीन लाइगेज, यूबिकिटिन कार्बोक्सिल-टर्मिनल हाइड्रॉलेज 18 आदि सहित जीन की एक सूची शामिल थी। इसके बाद प्रतिलेखन (ट्रांसक्रिप्टोम) विश्लेषण से कई डीईजी का पता चला जिसमें एनएसी डोमेन युक्त प्रोटीन, एमएडीएस-बॉक्स प्रतिलेखन कारक 1, बीईएल 1 – जैसे होम्योडोमैन प्रोटीन जैसे जीन शामिल हैं।
- अरहर में एआरएफ जीनों की तथाकल्पित भूमिका के बारे में गहरी जानकारी हासिल करने के लिए, क्यूआरटी-पीसीआर द्वारा एआरएफ जीन का अभिव्यक्ति विश्लेषण किया गया। दस एआरएफ जीनों ने विभिन्न विकास चरणों में गतिशील अभिव्यक्ति पैटर्न दर्शाए। तीन जीन, सीसीएआरएफ 2, सीसीएआरएफ 3 और सीसीएआरएफ 18 को बीज विकास और अंकुरण के दौरान सकारात्मक विनियामक पाया गया क्योंकि उनकी अभिव्यक्ति, प्रति फली में अधिक बीज संख्या वाले जीनप्ररूपों में परिपक्वता की ओर बढ़ती है।
- लवण दबाव सहिष्णुता के लिए अरहर के जीनप्ररूपों की जांच की गई, और एफ2 आबादी (250) को पूसा 992 (लवण सहिष्णु) x पूसा अरहर 16 (लवण के प्रति संवेदनशील) के साथ विकसित किया गया और पौदों की उत्तरजीविता का विश्लेषण किया गया, जिसमें सामान्य आवृत्ति वितरण पैटर्न का अनुपालन किया गया। एक तुलनात्मक प्रोटिओमिक विश्लेषण से लवणता दबाव सहनशीलता के लिए सी. कजान में ग्लाइसिन बीटाइन संश्लेषण, जीन कोलीन मोनोऑक्सीजिनेज (सीसीसीएमओ) की पहचान होती है।
- फॉल आर्मीवर्म (एफएडब्ल्यू), स्पोडोप्टेरा फ्रुगिपरडा एक महत्वपूर्ण लेपिडोप्टेरान पॉलीफैगस कीट-नाशीजीव है जो 350 से अधिक पादप प्रजातियों से आहार लेता है। एक काइमेरिक, Cry1Ac और Cry1F जीनों के क्षेत्र (डोमेन) की अदला-बदली करके विकसित बीटी जीन Cry1AcF को एफएडब्ल्यू के विरूद्ध प्रभावी दर्शाया गया था। एफएडब्ल्यू के विरूद्ध Cry1AcF तम्बाकू पराजेनिक वंशक्रमों की प्रभावशीलता का परीक्षण करने के लिए अलग पत्ता आहार आमापन (लीफ फीडिंग बायोएसे) आयोजित किया गया। चार पराजेनिक वंशक्रमों में रोग वाहक (वेक्टर) नियंत्रण (6.67% मृत्यु दर) की तुलना में 96 घंटे के लगातार भोजन लेने के बाद 72-80% मृत्यु दर देखी गई।
- फ्लेवोनोइड जैव संश्लेषण संबंधी मार्ग की जीन अभिव्यक्ति और मेटाबोलाइट्स पर हेलिकोवर्पा आर्मिगेरा के ग्रसन के प्रभाव का अध्ययन अरहर के वन्य संबंधी, काजानस प्लैटिकार्पस में किया गया था। इसने शाकाहार के दौरान कड़ाई से एक गतिशील प्रतिक्रिया दिखाई। कई गौण मेटाबोलाइट्स जैसे क्वेरसेटिन, काएम्फेरोल, पेलार्गोनिडिन डेल्फिनिडिन, एपिकैटेचिन-3-गैलेट, आदि ने वन्य संबंधी में निरंतर शाकाहार के दौरान गतिशील वृद्धि दर्शायी। जब खुराक-ओवरले परख द्वारा मूल्यांकन किया गया तो मेटाबोलाइट्स के अत्यधिक संचय से कीट आहार, वृद्धि और विकास में बाधा उत्पन्न हुई।
- अफ्रीकी चावल (ओरिजा ग्लैबेरिमा स्टुड) के नवीन लवणता सहिष्णु क्यूटीएल की पहचान और मानचित्रण किया गया। दो विपरीत जनकों आईआर 29 और अफ्रीकी चावल (एक्सेशन संख्या टीकेएम 239) के संकरण द्वारा एक द्वि-जनकीय एफ 6:7 आरआईएल आबादी विकसित की गई। अंकुरण अवस्था में मानचित्रण वाली आबादी के लक्षण प्ररूपण में से 16 अत्यधिक सहिष्णु, 38 मध्यम सहिष्णु, 19 मध्यम संवेदनशील और 6 अत्यधिक लवण-संवेदनशील वंशक्रमों का पता चला। उच्च लवणता वाले प्लॉट की आबादी में दृश्यमान लवणता-दबाव के लक्षणों के साथ कुछ लवण-सहिष्णु वंशक्रमों की पहचान करने के लिए प्रजनन चरण में स्क्रीनिंग प्रयोग, उच्च लवणता (ईसी ~ 9.77 डीएस/एम) और कम लवणता वाले भूखंडों (ईसी ~ 2.50 डीएस/एम) में भी किया गया था। इसके अलावा ओ. ग्लोबेरिमा, आईआर64 और आईआरजीएसपी में क्रमश: 5357, 5865 और 6109 गुणवत्ता-फिल्टर किए गए द्वि-एलील एसएनपी की पहचान करने के लिए दो जनकों और 209 आरआईएल के पत्ती के नमूनों का अनुक्रमण के माध्यम से जीनप्ररूपण (जीनोटाइपिंग बाय सीक्वेंसिंग) (जीबीएस) दृष्टिकोण द्वारा विश्लेषण किया गया।
- पादपों की लवण सहिष्णुता में एलांटोइन की भागीदारी के विस्तृत तंत्र का अभी तक अच्छी तरह से अध्ययन नहीं किया गया है। हमने एब्सिसिक एसिड (एबीए) और ब्रैसिनोस्टेरॉइड (बीआर) जैवसंश्लेषण मार्गों की शुरूआत करके चावल और एराबिडोप्सिस में लवणता सहिष्णुता प्रदान करने में बहिर्जात आपूर्ति के साथ-साथ अंतर्जात एलांटोइन दोनों की भूमिका का प्रदर्शन किया। लवण संवेदनशील चावल जीनप्ररूप आईआर-29 की अल्पकालिक सहिष्णुता की मध्यस्थता वाले एमआईआरएनए पर एलांटोइन के बहिर्जात अनुप्रयोग के प्रभाव को समझने के लिए, लवणता

दबाव के तहत 10 लवणता अनुक्रियाशील एमआईआरएनए की सापेक्ष अभिव्यक्ति की जांच की गई। परिणाम से पता चला कि कई miRNAs (ओएसए-एमआईआरएनए393ए, ओएसएएमआईआरएनए414, ओएसए-एमआईआरएनए530ए और ओएसए-एमआईआर818ए) ने वलणता के तहत एलांटोइन की उपस्थिति में अपने अभिव्यक्ति पैटर्न को बदल दिया।

- भारत के उत्तर-पूर्वी क्षेत्र (एनईआर) के सुगंधित परन्तु कम एंथोसायनिन युक्त जोहा चावल के साथ अत्यधिक एंथोसायनिन युक्त काले चावल की तुलना करते हुए उत्पत्ति, घरेलूपन और एंथोसायनिन जैवसंश्लेषण मार्गों को समझने के लिए पैन-जीनोमिक प्रतिलेखन (ट्रांसक्रिप्टोमिक) और एमआईआरएनए विश्लेषण आधारित अध्ययन किया गया था। गहरे पानी के उपचार के तहत इंटरनोड बढ़ाव से सम्बद्ध जीन/क्यूटीएल (एस) की पहचान के लिए जीनोम व्यापी सहबद्धता मानचित्रण (वाइड एसोसिएशन मैपिंग) का प्रयास सहिष्णु गहरे पानी के चावल की भूप्रजाति (लैंड रेस), नेघेरी बाओ के साथ उच्च पैदावार वाले, गहरे पानी के दबाव के प्रति संवेदनशील चावल की कृषि-जोपजाति, रंजीत के एक संकरण का द्विजनकीय मानचित्रण आबादी का उपयोग करते हुए, किया गया। 488 एफ 2:3 वंशक्रमों से सहिष्णु और संवेदनशील वंशक्रमों की जांच करने के लिए गहरे पानी के चावल उपचार की जांच भी पूरी कर ली गई है।
- केएएसपी (KASP) चिह्नकों (मार्करों) को, लवण दबाव सहिष्णुता के लिए एनकेएसडब्ल्यूआर173 के 75 बीसी1एफ2 परिवारों में अनाज की पैदावार, पादप ऊंचाई और अंकुरण चरण की लवण दबाव सहिष्णुता के लिए चावल में चार क्यूटीएल की पहचान करने हेतु तैयार किया गया था। लवण दबाव के तहत पैदावार के लिए प्रमुख क्यूटीएल, एनकेएसडब्ल्यूआर 173 द्वारा योगदान किए गए सकारात्मक एलील के साथ क्यूएसटीवाई 11.1, एक अत्यधिक सहिष्णु बीसी1एफ2 वंशक्रम, को आईआर64 में और साथ ही सांभा मसूरी के साथ बैकक्रास किया गया था। संकरण (क्रॉस) के बीज बोए गए और इससे बीसी 2 एफ 1 बीज प्राप्त किए गए। क्यूटीएल क्षेत्र के किनारे स्थित मार्करों का उपयोग करते हुए अंतर्गमन की पुष्टि की जाएगी।
- miRNAs, एमआईआर397 (सूखा, कम तापमान आदि में शामिल) और एमआईआर408 (तांबा, प्रकाश और यांत्रिक दबाव जैसे पर्यावरणीय दबावों की अनुक्रिया में भिन्न रूप से व्यक्त) की पहचान चावल और इसके छह वन्य पूर्वजों में की गई, जिसमें 11 जीनोम शामिल थे ताकि इन लोकी के आसपास उनके सामुदायिक विकास का एक व्यापक दृष्टिकोण प्राप्त किया जा सके। अध्ययन से पता चला कि दोनों परिपक्व एमआईआरएनए अत्यधिक संरक्षित हैं। गहन तुलनात्मक जीनोमिक्स से पता चला कि एमआईआरएनए के आसपास के 100 केबी क्षेत्र की माइक्रोसिन्टेनी केवल ओरिजा में संरक्षित थी; ज्वार, मक्का और गेहूं में बाधित थी; और यह एराबिडोप्सिस में पूरी तरह से समाप्त हो गई।
- साम्बा मसूरी और प्राप्तकर्ता जनक की आइसोजेनिक लाइन के पास अधिक दानों की संख्या प्रति पुष्पगुच्छ (जीएनपी) के विभिन्न विकासात्मक चरणों के युग्मवार तुलनात्मक प्रतिलेखन (ट्रांस्क्रिप्टोम) विश्लेषण से दो प्रमुख जीनो, LOC_Os04g52210 (OsKS3) और LOC_Os04g52590, एंट-कौरिन सिंथेज और प्रोटीन काइनेज के लिए क्रमश: एन्कोडिंग करते हुए, की विभेदात्मक अभिव्यक्ति का पता चला जो अधिक दानों की संख्या वाले क्यूटीएल लोकस qAG4.1 में एकत्रित थी।
- 83 नमूनों के अंकुरण चरण वाले सूक्ष्म क्रम विन्यास (माइक्रोएरे) डेटा के साथ नेटवर्क विश्लेषण का उपयोग करते हुए, चावल में कई अजैविक दबाव सहिष्णुता (ओरिजा सैटिवाएल.) के लिए सूखे, लवणता और ताप दबाव से संबंधित 6657 जीन, 17 संभावित मुख्य जीनों की पहचान की गई। इनमें से 15 जीनों को 4 परीक्षण जीनप्ररूप के एक पैनल और प्रत्येक दबाव प्रतिक्रिया के लिए मानक जांच जीनप्ररूपों के एक युग्म का उपयोग करते हुए अभिव्यक्ति विश्लेषण द्वारा विधि मान्य किया गया। दिलचस्प बात यह है कि सभी 15 जीनों ने सभी दबावों के तहत और सभी जीनप्ररूपों में अपनियमन दिखाया, जिससे पता चलता है कि वे वास्तव में अजैविक दबाव प्रतिक्रिया के लिए महत्वपूर्ण हैं। और अधिक प्रासंगिक रूप से 17 जीनों में से आठ को दबाव सहिष्णुता क्यूटीएल क्षेत्रों के साथ सह-स्थानीयकृत पाया गया।
- एनआईपीबी ने रानी धान के विविध विकास में योगदान दिया, जहां जीवाणविक झुलसा प्रतिरोधिता और जलमग्नता सहिष्णुता शामिल है। सहबद्धता मानचित्रण (एसोसिएशन मैपिंग) के माध्यम से चावल में सुपरऑक्साइड डिसम्यूटेज और एंटीऑक्सीडेंट गतिविधि को नियंत्रित करने के लिए एक क्यूटीएल की पहचान की गई। जैवसंश्लेषण के लिए एक जीन, एंटीबायोटिक गुणों वाला एक यौगिक ''फ्लोरोग्लुसीनॉल'' को सफलतापूर्वक क्लोन किया गया और इसे पादप विकास को बढ़ावा देने वाले जीवाणु (बैक्टीरिया) स्यूडोमोनास प्रजातियों से अलग किया गया।
- चावल में जीवाणविक पत्ता झुलसा रोग के विरूद्ध व्यापक स्पेक्ट्रम प्रतिरोध को सक्षम बनाने के लिए, जीवाणविक झुलसा संवेदनशीलता जीन Xa5 (ओएसटीएफआईआईए γ 5) में 39वें स्थान पर वेलिन को, अप्रभावी प्रतिरोधिता एलील xa5 के रूप में ग्लूटामिक एसिड में बदलने के लिए CRISPR-प्राइम vector एन्कोडिंग 2 बीपी प्रतिस्थापन उत्पन्न करने हेतु एसडीएन2 रणनीति को परिनियोजित किया गया है। हमने अपनी प्रयोगशाला में चावल प्रोटोप्लास्ट विलगनक और रूपांतरण के लिए एक कार्यात्मक प्रोटोकॉल स्थापित किया, जिसका पहले एक स्क्रीन करने योग्य मार्कर जीएफपी के साथ परीक्षण किया गया। एम्प्लिकॉन पाचन के आधार पर संपादन के लिए जांच की गई चावल की किस्म एमटीयू1010 में संपादन निर्माण को क्षणिक रूप से व्यक्त किया गया था।
- दो डायजोट्रॉफिक बैक्टीरिया ग्लूकोनासेटोबैक्टर डायजोट्रोफिकस (जीएबी) और ब्रैडीरिजोबियम जाप्पोनिकम (बीआरएच) की

कम नाइट्रोजन वाले माध्यम में चावल और सोयाबीन के साथ प्रारंभिक परस्पर क्रिया का अध्ययन किया गया। परस्पर क्रिया के आधार पर, यह अनुमान लगाया जा सकता है कि चावल और सोयाबीन की अनुकूलता क्रमश: जीएबी और बीआरएच के साथ अधिक है। चावल प्रारंभिक प्रतिक्रिया से डायजोट्रोफ को एक लाभकारी सूक्ष्मजीव के रूप में पहचानने में असमर्थ है और पीआर प्रोटीन के साथ अतिसंवेदनशील-संबंधी प्रतिलेख व्यक्त करता है। यह अध्ययन लाभकारी सूक्ष्मजीवों के प्रति परपोषी प्रतिक्रियाओं की बुनियादी समझ पर प्रकाश डालेगा।

- जल की कमी वाले सहिष्णुता तंत्र में फ्लेवोनाइड जैवसंश्लेषण मार्ग में जीनों के संबंध का पता, एक अच्छी तरह से ज्ञात जल की कमी वाली सहिष्णु प्रजाति नगीना22 (एन22) की एक संवेदनशील प्रजाति पूसा सुगंध 2 (पीएस2) के साथ तुलना करके लगाया गया था। परिणामों से पता चलता है कि सूखे की सहिष्णुता, फ्लेवोनोइड जैव संश्लेषण जीनों के बढ़े हुए प्रतिलेखन और अधिक फ्लेवोनोइड सामग्री के साथ सकारात्मक रूप से सहसंबद्ध है, जिससे पता चलता है कि सूखे के दबाव के लिए चावल में फ्लेवोनोइड चयापचय के आनुवंशिक नियंत्रण में अंतर हो सकता है।
- वॉन विलेब्रांड कारक डोमेन ए (वीडब्ल्यूए) युक्त जीनों का मनुष्यों में अच्छी तरह से लक्षण-वर्णन किया जाता है लेकिन पादपों में उनकी सबसे कम खोज की गई है। वीडब्ल्यूए जीनों की नवीनता और महत्वपूर्ण भूमिका को ध्यान में रखते हुए, 40 वीडब्ल्यूए जीन वाले चावल में सुपरफैमिली की पहचान की गई और उसका लक्षण-वर्णन किया गया। वीडब्ल्यूए जीन के अभिव्यक्ति विश्लेषण ने हार्मोन संबंधी प्रतिक्रिया और संकेतन (सिग्नलिंग) सहित जैविक और अजैविक दबाव प्रतिक्रियाओं में उनके संभावित कार्य का सुझाव दिया। 3000 चावल जननद्रव्य (जर्मप्लाज्म) जीनोम डेटा के वीडब्ल्यूए जीनों में ट्रांसपोसॉन सम्मिलन की आवृत्ति नगण्य पाई गई, जिससे इस बात पर जोर दिया गया कि ये जीन कार्यात्मक रूप से बहुत महत्वपूर्ण हैं। यह किसी भी पादप प्रजातियों में वीडब्ल्यूए जीन परिवार का लक्षण-वर्णन करने का पहला प्रयास था।
- फील्ड स्तर पर पुष्पगुच्छ प्रस्फुटन प्रतिरोधिता की जांच के लिए एक सिरिंज टीकाकरण विधि विकसित की गई। वन्य चावल जननद्रव्य और नागिना 22 के 45 ईएमएस प्रेरित उत्परिवर्ती (म्युटेंट) की जांच करते हुए इस विधि को सफलतापूर्वक विधिमान्य किया गया। पांच नए प्रस्फुटन रोग प्रतिरोधी वन्य चावल जीनप्ररूप और 15 उत्परिवर्ती की पहचान की गई थी जिनका उपयोग प्रस्फुटन प्रजनन कार्यक्रमों में किया जा सकता है। चावल के बड़े जननद्रव्य में पुष्पगुच्छ विस्फोट की जांच के लिए यह विधि आसान, मजबूत, विश्वसनीय और अत्यधिक कुशल है।
- ताप के दबाव का गेहूं के उत्पादन पर हानिकारक प्रभाव पड़ता है, जिससे गेहूं की पैदावार और गुणवत्ता में कमी आती है। इसे देखते हुए हमने गेहूं के ताप दबाव प्रतिक्रियाशील प्रतिलेखन (ट्रांसक्रिप्टोम) डेटा का विश्लेषण किया। 4483 ऊपर-विनियमित (अप-रेगुलेटेड) और 3507 नीचे-विनियमित (डाउन-रेगुलेटेड) जीनों सहित कुल 7990 डीईजी की पहचान की गई। केईजीजी विश्लेषण से 814 डीईजी वाले 146 मार्गों का पता चला। एमवाईबी और कई अन्य प्रतिलेखन कारक जैसे बीएचएलएच, डब्ल्यूआरकेवाई, एनएसी, ईआरएफ, डीईजी में काफी प्रचुर मात्रा में निर्धारित किए गए।
- साइटोकिनिन के साथ गेहूं प्रजाति एचडी2967 के बीजों की प्राइमिंग के बाद एक ट्रांसजेनरेशनल ताप-दबाव मेमोरी विश्लेषण किया गया। ब्रैसिनोस्टेरॉयड रिसेप्टर और प्रतिलेख (ट्रांसक्रिप्ट) के स्तर पर एक सकारात्मक विनियामक के प्रवर्तन के आधार पर, उच्च तापमान होने की स्थिति के तहत शीर्ष जड़ आरम्भ होने की अवस्था में गेहूं के अंकुरण में आणविक स्तर पर पादप हार्मोन ब्रैसिनोस्टेरॉयड की एक उत्साहजनक नियामक भूमिका देखी गई। एक ब्रैसिनोस्टेरॉयड संकेतन मार्ग विनियामक को आरएजे 3765, एक ताप सहिष्णु कल्टीवार से अलग किया गया और कार्यात्मक विश्लेषण के लिए इसका उपयोग किया जा रहा था।
- गेहूं (ट्रिटिकम एस्टीवम) के ब्रेविस रेडिक्स जीन परिवार का जीनोम व्यापी विश्लेषण किया गया और उनकी अभिव्यक्ति का अजैविक दबावों और हार्मोन उपचारों के तहत विश्लेषण किया गया। जीवे (इन-विवो) परस्पर क्रिया विश्लेषण से पता चला कि टीएबीआरएक्स प्रोटीन होमोटाइपिक के साथ-साथ हेटरोटाइपिक पारस्परिक क्रियाओं में भी सक्षम हैं, जो प्रोटीन-प्रोटीन इंटरैक्शन में बीआरएक्स डोमेन की भूमिका की पुष्टि करता है।
- ब्रेड बनाम ड्यूरम गेहूं में दाने के विकास के दौरान विभिन्न असहिष्णु प्रोटीन (आईपी) के लिए जीन एन्कोडिंग के तुलनात्मक अभिव्यक्ति विश्लेषण का अध्ययन प्रतिलेखन (ट्रांसक्रिप्टोमिक्स) दृष्टिकोण के माध्यम से किया गया। परिणामों ने इस परिकल्पना का समर्थन किया कि टेट्राप्लोइड ड्यूरम गेहूं हेक्साप्लोइड ब्रेड गेहूं की तुलना में कमजोर आबादी के लिए कम असहिष्णु और उपभोग के लिए बेहतर है।
- प्रतिलेखन कारक डीओएफ1, टीसीए चक्र में कार्बन (सी) चयापचय को नियंत्रित करता है और पादप में नाइट्रोजन उपयोग दक्षता को नियंत्रित करता है। हमारे अध्ययन में, एक फील्ड प्रयोग में नाइट्रोजन दबाव के प्रति विभिन्न प्रतिक्रिया वाले चार विविध गेहूं जीनप्ररूपों का चयन किया गया। परिणाम यह दर्शाते हैं कि गेहूं के जीनप्ररूपों में टीएडीओएफ1 की भूमिका भिन्न होती है, और यह भिन्नता संभवत: एन-समामेलन और अंतत: पैदावार के साथ-साथ नाइट्रोजन उपयोग दक्षता को प्रभावित करने वाले कारकों में से एक है।
- नाइट्रोजन दबाव के तहत जड़ प्रणाली स्थापत्य में भिन्नता का पता लगाने के लिए, गेहूं के द्विगुणित पूर्वजों (एजिलॉप्स-ट्रिटिकम प्रजाति) और खेती किए गए गेहूं के जीनप्ररूप (दो ड्यूरम और दो ब्रेड) के 6 एक्सेशनों का विश्लेषण किया गया। हमने यह पाया कि नाइट्रोजन दबाव के तहत खेती किए गए गेहूं की जड़ प्रणाली सबसे अधिक प्रभावित हुई।

- हमने ब्रेड गेहूं के अत्यधिक समानता वाले नाइट्रेट ट्रांसपोर्टरों के सभी 46 TaNRT2 और 8 TaNRT2 परिवार के जीनों को, उसी जीनोम, गुणसूत्र स्थान, होमोलॉग समूह, आदि पर स्थित जीनों के बीच अनुक्रम समानता के आधार पर पैरालॉग स्तर, और प्रमुख कार्यकी चरणों में 15N प्रवाह और एक व्यापक प्रतिलेख अभिव्यक्ति पर आधारित, क्रमश: पांच और तीन अलग-अलग वर्गों में, अर्थात् TaNRT2.1 - TaNRT2.5 और TaNAR2.1 - TaNAR2.3 में वर्गीकृत और प्रस्तावित किया है। इसके अलावा, हमने सीमित नाइट्रेट स्थिति के तहत नाइट्रेट अवशोषण में हानि वाले एराबिडोप्सिस के एटीएनआरटी2.1 उत्परिवर्ती (टी-डीएनए उत्परिवर्ती) को पूरक करके जड़ में उच्चतम अभिव्यक्ति TaNRT2.1 जीनों में से एक, यानी TaNRT2.1-B6 को कार्यात्मक रूप से चित्रित किया है।
- भारतीय सरसों की फसल-पैदावार में बार-बार होने वाले हानि में योगदान देने वाले प्रमुख जैविक दबावों में अल्टरनेरिया पत्ती धब्बा रोग, एफिड संक्रमण, सफेद रतुआ (अल्बुगे कैंडिडा) रोग, तना सड़न रोग और ब्रूमरेप (ओरोबैंच) संक्रमण शामिल हैं। अल्टरनेरिया प्रतिरोधिता के लिए विदेशी प्रतिरोधी जीन को, खेती की गई सरसों के जीन पूल में लाने के लिए, वन्य प्रजातियों के साथ व्यापक संकरण द्वारा अंतर्गमन वंशक्रम विकसित किए गए हैं। अन्य प्रयोग में, ए. ब्रैसिका द्वारा संक्रमण के बाद बी. जंसिया, एस. अल्बा और सी. सैटिवा का आरएनएसेक आधारित प्रतिलेखन विश्लेषण किया गया। परिणामों से पता चला कि एस. एल्बा में मध्यम और सी. सैटिवा में बी. जंसिया की तुलना में ए. ब्रैसिका के विरूद्ध अंतर्निर्मित रक्षा तंत्र की क्षमता है। एफिड्स के प्रति प्रतिरोधिता विकसित करने के लिए, एफिड्स के प्रभावकों का आणविक स्तर पर अध्ययन किया जा रहा है, जो मुख्य रूप से सरसों में जन्मजात सुरक्षा को क्षीण करते हैं और पादपों को पूरी तरह से संवेदनशील बनाते हैं। बी जंसिया में सफेद रतुआ (अल्बुगो कैंडिडा) प्रतिरोधिता के लिए आनुवंशिक और जीनोमिक संसाधनों का विकास करने के लिए, 453 ब्रैसिका प्रजाति के जननद्रव्य की, ए कैंडिडा के ग्यारह प्रचलित विषाणु विलगनकों के खिलाफ जांच की गई। 350 ब्रैसिका एक्सेशनों में ईसी766192 और ईसी766193 ने पादपों के बीजपत्र और वास्तविक पत्ती विकास दोनों चरणों में रोगजनक के 6-7 विलग्नकों (आइसोलेट्स) के खिलाफ प्रतिरोध दर्शाया। 192 अंतर्गमित वंशक्रमों (बीसी$_2$ एफ$_{12}$) और 127 पुनर्संश्लेषित वंशक्रमों (एस$_8$) में से; ईआरजे 39, 40 और आरबीजे 18 ने 3-7 विलगनकों (आइसोलेट्स) के खिलाफ प्रतिरक्षा प्रतिक्रिया दर्शाई। ब्रैसिका के वन्य संबंधियों को छह विलगनकों (आइसोलेट्स) जैसे एसी-डेल, एसी-एलडीएच, एसी-पीएनटी, एसी-एम्ब, एसी-आरएनसी और रोगजनक ए कैंडिडा के एसीडब्ल्यूएलटीएन के प्रति प्रतिरक्षित पाया गया। इसके अतिरिक्त, हरियाणा के चरखा दादरी जिले में भारतीय सरसों के किसानों के खेतों में ब्रूमरेप (ओरोबैंकी) समस्या की गंभीरता को समझने के लिए सर्वेक्षण किया गया। पी. रामोसा जो कि बी जंसिया को संक्रमित करता है, टमाटर की फसल को भी संक्रमित करता पाया गया। बी संसिया के प्रकाशित जीनोम में स्ट्रिगोलैक्टोन जैव संश्लेषित मार्ग जीनों का भी अध्ययन किया गया था। तना सड़न के कारक एजेंट एस. स्क्लेरोटियोरम ''ईएसआर-01'' के भारतीय विलगनक (आइसोलेट) के 129x कवरेज के साथ एक ड्राफ्ट जीनोम अनुक्रम उत्पन्न किया गया। यह 9469 प्रोटीन-कोडिंग जीनों और 57 प्रभावकारी मुख्य जीनों के साथ ~41 एमबी जीनोम है। एस स्क्लेरोटियोरम में द्वितिय उपापयच (मेटाबोलाइट) के अध्ययन से पता चला कि एक्सेनिक परिस्थितियों में उपापचयों (मेटाबोलाइट्स) का स्राव रोगजनक की वृद्धि और विकासात्मक चरणों पर निर्भर करता है और यह रोगजनक के विषाणु से स्वतंत्र होता है।
- वर्ष 2022 के दौरान प्रारंभिक पीढ़ी के सिंथेटिक ब्रैसिका जंकिया वंशक्रमों का रूपात्मक लक्षणों के लिए चित्रण किया गया था और आणविक चिह्नकों (मार्करों) का उपयोग करते हुए आनुवंशिक विविधता का पता लगाया गया। माइटोटिक विश्लेषण से इन सिंथेटिक बी जंसिया वंशक्रमों के 36 गुणसूत्रों का पता चला। इसके अलावा, एक बहुस्थानीय प्रयोग में प्रति सिलिका विशेषक बीज संख्या के लिए जीनप्ररूपों के एक पैनल का मूल्यांकन किया गया था और आगे के आणविक जीवविज्ञान अध्ययनों के लिए विषम वंशक्रमों की पहचान की गई थी।
- बी जंसिया में अगुणित प्रेरक (हैप्लोइड इंड्यूसर) वंशक्रम विकसित करने के लिए हमने सीईएनएच3 पैरालॉग को लक्षित करते हुए सीआरआईएसपीआर/सीएएस9 रोगवाहक को विकसित किया है। चूंकि सीईएनएच3 नॉकआउट भ्रूण के लिए घातक है, इसलिए इन सीईएनएच3 नॉकआउट वंशक्रमों को बचाने के लिए एक जीएफपी-टेलस्वैप रोगवाहक को तैयार किया गया था। इन रोग-वाहकों के सह-परिवर्तन द्वारा, हमने पराजेनिक वंशक्रम विकसित किए हैं। इन वंशक्रमों का आणविक विश्लेषण किया गया और अगुणित (हैप्लोइड) प्रेरण क्षमता की जांच करने के लिए इनका वन्य प्रकार के वंशक्रमों से संकरण किया गया।
- पादपों में सेस्ट्रम डायर्नम की पत्तियां विटामिन डी का एक संभावित स्रोत हैं। हालांकि, सी. डायर्नम में नॉर्निकोटीन भी होता है, जो इसे विटामिन डी के आहार स्रोत के रूप में उपयोग करने के लिए एक नकारात्मक गुण है। हमने नॉर्निकोटीन जैव संश्लेषण जीनों की पहचान करने के लिए सेस्ट्रम डायर्नम प्रतिलेखन का डी-नोवा संयोजन और व्याख्या (एनोटेशन) की है। सी. डायर्नम में तीन प्रतिलेख बीबीएल जीनों के लगभग समरूप माने गए।
- भारतीय चाय (कैमेलिया असामिका किस्म मास्टर्स प्रजाति टीवी-1) के जीनोम के विकास और वर्चस्व के इतिहास को समझने के लिए असम और चाइना चाय के प्रकार की स्वतंत्र घरेलू घटनाओं को प्रकट करने के लिए सम्पूर्ण विश्व के चाय के 150 जीनप्ररूपों के एसएनपी आधारित विश्लेषण के साथ एक अध्ययन का प्रयास किया गया था। कैमेलिया प्रजातियों के कैफीन और गैर-कैफीन समूह के बीच वर्चस्व की घटनाओं के दौरान कुल 512 जीन सकारात्मक रूप से चयनित मुख्य जीन

के रूप में पाए गए। टीआईजीईआर वेब संसाधन को, अनुसंधान समुदायों के अध्ययन के तहत उत्पन्न विभिन्न संसाधनों की मेजबानी और इन्हें प्रदान करने के लिए विकसित किया गया था। अजैविक दबाव और चाय के विभिन्न विकास चरणों के तहत 77 जीनों वाले हिस्टोन संशोधन (एचएम) जीन परिवार और उनके अभिव्यक्ति पैटर्न की एक जीनोम-व्यापी पहचान की गई। तीन अलग-अलग अजैविक दबावों (ठंड, निर्जलीकरण और लवणता) के तहत चाय के पौधे के पांच अलग-अलग विकासात्मक ऊतकों के क्यूआरटी-पीसीआर ने 26 अलग-अलग रूप से अभिव्यक्त एचएम जीन पाए हैं।

- अफ्रीकी रतालू (एवाईबी) (स्फेनोस्टिलिस स्टेनोकार्पा (होचस्ट ईएक्स ए रिच) हार्म्स) दोहरे लाभ, खाने योग्य कंद और साथ ही फलियों वाली, अफ्रीका की एक महत्वपूर्ण फसल है। छह अलग-अलग फलीदार फसलों की प्रजातियों से प्राप्त कुल 206 माइक्रोसैटेलाइट्स मार्करों का एवाईबी में परीक्षण किया गया और आनुवंशिक दूरस्थ अध्ययन को सक्षम बनाने के लिए एवाईबी में प्रवर्धित 81 मार्करों का उपयोग, इंटरनेशनल इंस्टीट्यूट ऑफ ट्रॉपिकल एग्रीकल्चर (आईआईटीए), नाइजीरिया से प्राप्त एवाईबी के 92 एक्सेशनों में किया गया है।
- एनआईपीबी, डीबीटी के माध्यम से सरकार द्वारा संचालित कार्यक्रम, नेशनल सर्टिफिकेशन सिस्टम ऑफ टिश्यू कल्चर रेज्ड प्लांट (एनसीएस-टीसीपी) के तहत आनुवंशिक विश्वस्तता परीक्षण के लिए सम्पर्क केंद्र है। इस कार्यक्रम के तहत हमने ऊतक संवर्धन (टिशू कल्चर) से उगाए गए पादपों की आनुवंशिक विश्वस्तता परीक्षण के लिए मानक परिचालन प्रोटोकॉल (एसओपी) विकसित किए और 5 अलग-अलग एटीएल प्रयोगशालाओं से प्राप्त 5% यादृच्छिक नमूनों का परीक्षण भी किया। वर्ष 2022 में कुल 1935 नमूनों का परीक्षण किया गया और अमरूद के पौधों की आनुवंशिक विश्वस्तता के लिए एसओपी भी विकसित किया।
- बदलती हुई जलवायु परिस्थितियों को देखते हुए, रागी में दबाव सहिष्णुता प्रदान करने के लिए, त्वरित और कुशल जीनोमिक्स रणनीतियों की आवश्यकता है। समुचित रूपांतरण प्रोटोकॉल विकसित करने से पहले, हमने रागी जीनप्ररूप जीएन-5 के लिए पुनरूत्पादनीय पुनर्जनन प्रोटोकॉल स्थापित किया है। इसके अलावा, इस प्रोटोकॉल को CRISPR-Cas9 आधारित जीन सम्पादन रोग वाहक के रूपांतरण के लिए मानकीकृत किया जाएगा।
- जड़-गांठ सूत्रकृमि मेलोइडोगाइन इनकॉग्निटा के साथ पोलियान्थेस ट्यूबरोसा के परपोषी-रोगजनक की परस्पर क्रिया (इंटरैक्शन) के आणविक तंत्र का अध्ययन करने के लिए, प्रारंभिक, मध्य और देर से संक्रमण होने की अवस्था के दौरान असंक्रमित और एम. इनकॉग्निटा-संक्रमित रजनीगंधा (ट्यूबरोज) पादपों की तुलनात्मक प्रतिलेखन प्रोफाइल तैयार की गई है। नियंत्रण और संक्रमित पादपों के बीच आमतौर पर व्यक्त 8,289 सीडीएस में से 256 को काफी हद तक विनियमित किया गया था और 129 को संक्रमित पादपों में काफी कम विनियमित किया गया था। हमारे परिणाम, जड़-गांठ सूत्रकृमि एम. इनकॉग्निटा के साथ अपनी सहबद्धता के दौरान रजनीगंधा में एक व्यापक जीन अभिव्यक्ति के परिवर्तन को बताते हैं।
- पोषण-विरोधी कारक प्रोटीन और पोषण संबंधी प्रोटीन में अंतर करने के लिए, विभिन्न मशीन लर्निंग एल्गोरिदम जैसे सपोर्ट वेक्टर मशीन, रैंडम फॉरेस्ट और लॉजिस्टिक रिग्रेशन आदि के प्रदर्शन का विश्लेषण किया गया। एसवीएम आधारित एल्गोरिदम ने 5962 प्रोटीन (सकारात्मक डेटासेट: 3614 और नकारात्मक डेटासेट: 2348) के डेटासेट में अन्य की तुलना में पोषण-विरोधी कारक प्रोटीन का अंतर दर्शाने में 92% की बेहतर सटीकता के साथ प्रदर्शन किया है।
- एनआईपीबी ने कच्ची अवस्था में एनजीएस डेटा कवरेज का पता लगाने के लिए एक नवीन कृत्रिम आसूचना एकीकृत पाइपलाइन विकसित की है। कम्प्यूनेशनल पाइपलाइन को सभी आवश्यक पूर्व-प्रसंस्करण अवस्थाओं (जैसे गुणवत्ता जांच, एडाप्टर ट्रिमिंग, गुणवत्ता फिल्टरिंग इत्यादि) के साथ एकीकृत किया गया है और संरेखण के लिए हैश-टेबल-आधारित मानचित्रण एल्गोरिदम का उपयोग किया है। पाइपलाइन पाठ्य और ग्राफिकल प्रारूप में कवरेज गहराई, कवरेज चौड़ाई और गुणवत्ता अंक सहित कवरेज प्रोफाइलिंग संबंधी जानकारी प्रदान करती है।
- यह संस्थान अपने आरम्भ से ही आईएआरआई के एमबीबी के एक प्रभाग के रूप में पादप आणविक जीवविज्ञान और जैव प्रौद्योगिकी के क्षेत्र में मानव संसाधन विकास में सक्रिय रूप से कार्यरत है। वर्तमान में 43 पीएच.डी. और 09 एम.एससी. छात्र केंद्र में आणविक जीवविज्ञान और जैव प्रौद्योगिकी के विषय-क्षेत्र में पंजीकृत हैं। वर्ष 2022 में, 60वें दीक्षांत समारोह में दो पीएच. डी. के छात्रों को डॉक्टरेट की डिग्री और सात एम.एससी. के छात्रों को मास्टर की डिग्री से सम्मानित किया गया।
- सीएएफटी और आनुवंशिक विश्वस्तता परीक्षण प्रशिक्षण के अलावा, इस वर्ष हमने प्लांट टिश्यू कल्चर एसोसिएशन (भारत) की 43वीं वार्षिक बैठक के साथ-साथ पादप जैव प्रौद्योगिकी और पोषणिक सुरक्षा में प्रगति-2022 पर एक अंतर्राष्ट्रीय संगोष्ठी आयोजित की है। हमने, एनआईपीबी के अनुसंधान कार्यक्रमों/उपलब्धियों के बारे में उद्योगों से चर्चा करने और इनका मूल्यांकन करने के लिए एकेडेमिया-उद्योग जैव प्रौद्योगिकी सम्मेलन की व्यवस्था की। हमने महाराष्ट्र, ओड़िशा, केरल और उत्तराखंड में 4 एससीएसपी कार्यक्रम भी आयोजित किए जहां 800 से अधिक किसानों को आईसीएआर प्रौद्योगिकियों से लाभ मिला। हमने आईएआरआई मेला मैदान में आयोजित पूसा कृषि मेले में अपने उत्पादों और प्रौद्योगिकियों का भी प्रदर्शन किया। इसके अलावा, हिन्दी चेतना मास, सतर्कता जागरूकता सप्ताह, अंतर्राष्ट्रीय महिला दिवस, स्वच्छ भारत अभियान जैसे कई कार्यक्रमों को आयोजित किया।

Executive Summary

With focus on its mandates, ICAR-NIPB continuously engaged in developing modern tools and techniques in the area of plant biotechnology and their applications for crop improvement using genomics, molecular breeding, transgenics and genome editing. This institute also functions as the Division of Molecular Biology and Biotechnology for teaching and training of post graduate students of Indian Agricultural Research Institute. Besides its institute level training programs, NIPB is serving for the farmers as well as college and school students through Scheduled Castes Sub Plan (SCSP), long term training programs and regular visits by students from all over India. This institute is mainly working on chickpea, pigeonpea, rice, wheat, and Indian mustard. Along with these crops, it also works on other crops through strong collaborations with national and international institutions for developing genomic resources and finding biotechnological solutions to the major problems in these crops. While working in 2022, six new patents had been filed and 2 previously filed patents, and 4 copy rights were granted. The significant achievements of the NIPB during this year are summarized below.

- Wild species are rich genetic resources for agronomically important traits. A high-quality genome sequence of *Cicer reticulatum*, a wild relative and immediate progenitor of the domesticated chickpea was produced using PacBio HiFi reads. It consisted of 456 contigs with an N50 of 1.8 Mb. A full-length chloroplast genome of *Cicer cuneatum,* another wild relative of chickpea was sequenced, and *de novo* assembled and annotated. It is approximately 124.3 Kb in size, which is about one kilobase smaller than the cultivated species, *Cicer arietinum* (125.5 Kb). Circular RNAs (circRNAs) comprise a large class of non-coding RNAs play a considerable role in various biological processes. By using CIRIQUANT and CLEAR algorithms, a total of 1377 circular RNAs were predicted in chickpea.
- Gram pod borer (*Helicoverpa armigera*), Root -Knot nematodes, and *Fusarium oxysporum* are major biotic constraints for chickpea production. To identify pod borer induced genes, comparative transcriptome analysis of a tolerant and susceptible genotypes after 24 h of challenge with the larvae was carried out. In addition to known defence genes, new candidate genes associated with defence responses against insects were also identified. RNAi technology has emerged as a sustainable and target-specific alternative to control phytonematodes. Therefore, for analysing root-knot nematodes (RKNs) resistance, functional evaluation of Mi-msp10 and Mi-msp23 dsRNA cassettes were performed using host-delivered RNAi (HD-RNAi) in *Arabidopsis*. The phenotypic effect of HD-RNAi was evident with a maximum of 61% reduction in gall formation in RNAi lines. The gene expression analysis showed a significant decrease in the transcript level in *M. incognita* females feeding on RNAi lines, providing further evidence of effective gene silencing. In the quest to identify the genomic regions associated with *Fusarium* wilt resistance, a meta-analysis of all the QTLs was carried out and the results revealed that chromosome 2 is a hotspot for Fusarium resistance.

- Terminal drought is one of the most devastating abiotic stress factors for chickpea. In our study, 46 GRAS transcription factors genes were identified in the chickpea genome. The expression analysis of *CaGRAS* genes was done in two chickpea varieties contrasting for drought tolerance i.e., ICC 4958 (drought tolerant) and ICC 1882 (drought sensitive) where CaGRAS12 (SCR) was found to be drought-responsive.
- Chickpea is an important source of protein for vast majority of Indian population. There is a huge variation in protein content in the cultivated genotypes. Hence protein content and protein profiling of ten selected genotypes of chickpea was analysed. The protein content of studied genotypes varied from 18% to 28%. The genes encoding the seed storage proteins were also found to be differentially expressed between high and low protein containing chickpea genotypes.
- The role of pod wall in legumes is extremely important. Hence in order to unravel mechanisms regulating pod wall development and also in identification of the pod wall specific genes, RNAseq analysis during various pod developmental stages in the chickpea was carried out.
- Early flowering and determinacy are desirable traits in pigeonpea. Genomics approaches were used to map the QTLs responsible for early flowering and determinacy traits in pigeonpea. These QTLs were scanned for differentially expressed genes using in-house transcriptome data as well as florid database specific for flowering related genes. A total of 8 QTLs for DTF and 6 for determinacy were spread over 6 different chromosomes.
- The molecular mechanism that leads to cleistogamy was investigated in pigeonpea. Three varieties used for this study were ICP87154 (cleistogamous), UP26 (partial cleistogamous), and Asha (chasmogamous) as the control. For this GBS based SNPs identification followed by QTL Seq analysis of F2 population and then transcriptome was used to arrive at candidate genes. From the mapping work a set of 5 QTLs were identified. These included a list of genes including a lncRNA, ABC transporter G family member 31-like, E3 ubiquitin-protein ligase, ubiquitin carboxyl-terminal hydrolase 18, etc. Subsequently transcriptome analysis revealed a number of DEGs that include genes like the NAC domain-containing protein, MADS-box transcription factor 1, BEL1-like homeodomain protein.
- To gain an insight into the putative role of ARF genes in pigeonpea, expression analysis of ARF genes was carried out by qRT-PCR. Ten ARF genes showed dynamic expression patterns in different development stages. Three genes, CcARF2, CcARF3 and CcARF18 were found to be positive regulators during seed development and germination as their expression increases towards maturity in the genotypes with higher seed number per pod.
- Pigeonpea genotypes were screened for salt stress tolerance, and the F_2 population (250) was developed with PUSA992 (salt tolerant) X Pusa Arhar 16 (salt sensitive) and survival of seedlings was analyzed which followed a normal frequency distribution pattern. A comparative proteomic analysis leads to the identification of the glycine betaine synthesis gene Choline Monooxygenase (*CcCMO*) in *C. cajan* for salinity stress tolerance.
- Fall armyworm (FAW), *Spodoptera frugiperda* is an important Lepidopteran polyphagous insect-pest that feeds on more than 350 plant species. A chimeric *Bt* gene *cry1AcF* developed by swapping the domains of *cry1Ac* and *cry1F* genes was shown to be effective against FAW. Detached leaf feeding bioassay conducted

to test the effectiveness of *cry1AcF* tobacco transgenic lines against FAW. Four transgenic lines showed 72-80% mortality after 96 hr of continuous feeding compared to that of vector control (6.67 % mortality).

- The effect of *Helicoverpa armigera* infestation on the gene expression and metabolites of the flavonoid biosynthesis pathway was studied in pigeonpea wild relative, *Cajanus platycarpus*. It showed a dynamic response tightly linked during herbivory. Many secondary metabolites like quercetin, kaempferol, pelargonidin, delphinidin, epicatechin-3-gallate, etc. showed dynamic increase during continued herbivory in the wild relative. Over accumulating metabolites when assessed by a diet-overlay assay interfered in insect feeding, growth and development.
- The identification and mapping of novel salinity tolerant QTLs of African rice (*Oryza glaberrima* Steud.) was done. A bi-parental $F_{6:7}$ RIL population was developed by crossing two contrasting parents IR 29 and African rice (Accession no. TKM 239). Phenotyping of the mapping population in seedling stage revealed 16 highly tolerant, 38 moderately tolerant, 19 moderately sensitive and 6 highly salt-sensitive lines. The screening experiment at reproductive stage had also been conducted in high salinity (EC ~9.77 dS/m) and low salinity plots (EC ~2.50 dS/m) to identify a few salt-tolerant lines with visible salinity-stress symptoms in the population in high salinity plot. In addition, the leaf samples of two parents and 209 RILs were analyzed by Genotyping by Sequencing (GBS) approach in order to identify 5357, 5865 and 6109 quality-filtered bi-allelic SNPs in *O. glaberrima*, IR64 and IRGSP, respectively.
- The detail mechanisms of allantoin involvement in salinity tolerance in plants are not yet studied well. We demonstrated the role of both exogenously supplied as well as endogenous allantoin in rendering salinity tolerance in rice and *Arabidopsis* via induction of abscisic acid (ABA) and brassinosteroid (BR) biosynthesis pathways. To understand the effect of exogenous application of allantoin on miRNAs mediated short-term tolerance of salt sensitive rice genotype IR-29, the relative expression of 10 salinity responsive miRNAs were investigated under salinity stress. The result demonstrated that several miRNAs (osa-miRNA393a, osamiRNA414, osa-miRNA530 and osa-miR818a) changed their expression pattern in the presence of allantoin under salinity.
- A pan-genomic transcriptomic and miRNA analyses based study was performed to understand the origin, domestication and anthocyanin biosynthesis pathways by comparing the high anthocyanin containing black rice with aromatic but low anthocyanin containing Joha rice from the North-Eastern Region (NER) of India. Genome wide association mapping for identification of genes /QTL(s) associated with internode elongation under deepwater treatment was attempted using biparental mapping population of a cross of high yielding, deepwater stress sensitive rice cultivar, Ranjit, with the tolerant deepwater rice landrace, Negheri bao. The screening for deepwater rice treatment has been also completed to check the tolerant and sensitive lines from 488 $F_{2:3}$ lines.
- The KASP markers were designed to identify four QTLs in rice for grain yield, plant height, and seedling stage salt stress tolerance in 75 BC_1F_2 families of NKSWR173 for salt stress tolerance. The major QTL for yield under salt stress, *qSTY11.1* with positive allele contributed by NKSWR173, a highly tolerant BC_1F_2 line, was backcrossed to IR64 and simultaneously with Sambha Masuri. Seeds of the cross were planted and BC_2F_1

seeds were obtained. The introgression will be confirmed using the markers flanking the QTL region.

- miRNAs, MIR397 (involved in drought, low temperature etc) and MIR408 (differentially expressed in response to environmental stresses such as copper, light, and mechanical stress) were identified in rice and its six wild progenitors comprising 11 genomes to obtain a comprehensive view of their community evolution around these loci. The study showed that both mature MIR are highly conserved. Deep comparative genomics revealed the microsynteny of the 100 kb region surrounding MIRNAs was only conserved in Oryza; disrupted in Sorghum, maize, and wheat; and completely lost in Arabidopsis.
- Pairwise comparative transcriptome analysis of different developmental stages of high grain number per panicle (GNP) Near isogenic line of Samba Masuri and the recipient parent revealed differential expression of two key genes, LOC_Os04g52210 (OsKS3) and LOC_Os04g52590, encoding for ent-kaurene synthase and protein kinase, respectively, collocated in the high grain number QTL locus qGN4.1.
- Using network analysis with the seedling stage microarray data of 83 samples, 6657 genes pertaining to drought, salinity and heat stresses, 17 potential candidate genes were identified for multiple abiotic stress tolerance in rice (*Oryza sativa* L.). Out of these 15 genes were validated by expression analysis using a panel of 4 test genotypes and a pair of standard check genotypes for each stress response. Interestingly, all the 15 genes showed upregulation under all stresses and in all the genotypes suggesting that they are indeed important for abiotic stress response. More pertinently, eight of the 17 genes were found to be co-localized with the stress tolerance QTL regions.
- NIPB contributed in the variety development of Rani Dhan where bacterial blight resistance and submergence tolerance is incorporated. Through association mapping a QTL for controlling superoxide dismutase and antioxidant activity in rice was identified. A gene for biosynthesis of a compound "Phloroglucinol" with antibiotic properties was successfully cloned and characterized from plant growth promoting bacteria *Pseudomonas* spp.
- To enable broad spectrum resistance against the bacterial leaf blight disease in rice, SDN2 strategy is deployed to generate a CRISPR-Prime construct encoding 2 bp substitutions to change the Valine at 39th position in bacterial blight susceptibility gene *Xa5* (*OsTFIIAγ5*) to Glutamic acid as in the recessive resistance allele xa5. We established a functional protocol for rice protoplast isolation and transformation and tested with a screenable marker GFP. The edit construct was transiently expressed in rice variety MTU1010 screened for edit based on amplicon digestion.
- The early interaction of two diazotrophic bacteria *Gluconacetobacter diazotrophicus (GAB)* and *Bradyrhizobium japonicum (BRH)* with of rice and soybean under low nitrogen medium were studied. Based on the interaction, it is inferred that the compatibility of rice and soybean is more with GAB and BRH, respectively. Rice is unable to identify the diazotroph as a beneficial microorganism from an early response and expressed hypersensitivity-related transcripts along with PR proteins. This study will shed light on the basic understanding of host responses to beneficial microorganisms.
- The relationship of genes in the flavonoid biosynthesis pathway in water deficit tolerance mechanism was explored by comparing a well-known water deficit tolerant cv. Nagina22 (N22) with a sensitive cv. Pusa Sugandh 2 (PS2). The

results suggests that drought tolerance is positively correlated with an enhanced transcription of flavonoid biosynthesis genes and higher flavonoid content suggesting that there could be differences in the genetic control of flavonoid metabolism in rice for drought stress.

- von Willebrand factor domain A (vWA) containing genes are well characterized in humans but they are least explored in plants. Considering the novelty and important role of vWA genes, the superfamily was identified and characterized in rice with 40 vWA genes. Expression analysis of vWA genes suggested their probable function in biotic and abiotic stress responses including hormonal response, and signaling. The frequency of transposon insertion in vWA genes in 3000 rice germplasm genome data found to be negligible, emphasizing that these genes are functionally very important. This was the first attempt to characterize vWA gene family in any plant species.
- A syringe inoculation method was developed for screening for panicle blast resistance at the field level. The method was successfully validated by screening the wild rice germplasm and 45 EMS induced mutants of Nagian 22. Five novel blast disease-resistant wild rice genotypes and 15 mutants were identified which can be used in blast breeding programs. The method is easy, robust, reliable, and highly efficient for screening the large germplasm of rice for panicle blast.
- Heat stress has detrimental impact on wheat production leading to decrease in yield and quality of wheat. In view of this, we analysed heat stress responsive transcriptome data of wheat. A total of 7990 DEGs, including 4483 up-regulated and 3507 down regulated genes were identified. KEGG analysis revealed 146 pathways involving 814 DEGs. MYB and many other transcription factors as bHLH, WRKY, NAC, ERF, were determined to be quite abundant in the DEGs.
- A transgenerational heat stress memory analysis was carried out after seed priming of wheat cv. HD2967 seeds with cytokinin. An encouraging modulatory role of plant hormone brassinosteroid was observed at the molecular level in wheat seedlings at crown root initiation stage under high temperature exposure, based on induction of brassinosteroid receptor and a positive regulator at the transcript level. A brassinosteroid signaling pathway regulator was isolated from RAJ 3765, a heat tolerant cultivar and being utilized for functional analysis.
- Genome wide analysis of *BREVIS RADIX* gene family from wheat (*Triticum aestivum*) was carried out and their expression was analysed under abiotic stresses and hormone treatments. *In-vivo* interaction analysis revealed that *TaBRX* proteins are capable of homotypic as well as heterotypic interactions which corroborated with the role of BRX domain in protein-protein interaction.
- The comparative expression analysis of the genes encoding for different intolerant proteins (IP) during grain development in bread vs. durum wheat were studied throughtranscriptomicsapproach.Results supported the hypothesis that tetraploid durum wheat is less intolerant and better for consumption for the vulnerable population than that of hexaploid bread wheat.
- The transcription factor Dof1 regulates Carbon (C) metabolism in the TCA cycle and controls the nitrogen use efficiency in the plant. In our study, four diverse wheat genotypes with varied response to N stress in a field experiment were selected. The results showed that role of TaDof1 varies among the wheat genotypes, and this variation is probably one of the factors

affecting N-assimilation and ultimately yield as well as Nitrogen use efficiency.

- To detect variation in root system architecture under nitrogen stress, 6 accessions from diploid progenitors of wheat (Aegilops–Triticum species) and cultivated wheat genotypes (two durum and two bread) were analysed. We observed that cultivated wheat had the most affected root system under nitrogen stress.
- We have classified and proposed the names for all 46 TaNRT2 and 8 TaNAR2 family genes of high affinity nitrate transporters of bread wheat based on sequence similarity among genes located on the same genome, chromosome location, homeolog group, etc., up to in-paralog level, and also based on 15N influx and an extensive transcript expression at key physiological stages, into five and three different classes, respectively, viz., TaNRT2.1 – TaNRT2.5 and TaNAR2.1 – TaNAR2.3. Further, we functionally characterized one of the highest expressing TaNRT2.1 genes in root, i.e., TaNRT2.1-B6, by complementing *atnrt2.1* mutant (T-DNA mutant) of *Arabidopsis* having impairment in nitrate uptake under limiting nitrate condition.
- The major biotic stresses contributing to recurrent loss in crop yield in Indian mustard include Alternaria leaf spot disease, aphid infestation, white rust (*Albugo candida*) disease, stem rot disease and broomrape (*Orobanche*) infestation. To bring the alien resistance genes for Alternaria resistance into the gene pool of cultivated mustard, introgression lines have been developed by wide hybridization with wild species. In another experiment, RNAseq based transcriptome analysis of *B. juncea, S. alba* and *C. sativa* after the infection by *A. brassicae* was carried out. The results revealed *S. alba* have moderate and *C. sativa* have potential in built defense mechanism against *A. brassicae* compared to *B. juncea.* For developing resistance to aphids, the effectors of aphids which primarily lead to attenuation of innate defense in mustard and make the plants completely susceptible are being studied at the molecular level. To develop genetic and genomic resources for white rust (*Albugo candida*) resistance in *B. juncea,* 453 *Brassica* species germplasm was screened against eleven prevalent virulent isolates of *A. candida*. Among 350 *Brassica* accessions EC766192 and EC766193 showed resistance against 6-7 isolates of the pathogen at both cotyledonary and true leaf growth stages of the plants. Out of 192 introgressed lines (BC_2F_{12}) and 127 resynthesized lines (S_8); *ERJ 39, 40* and RBJ 18 showed immune reaction against 3-7 isolates. *Brassica* wild relatives were found immune against six isolates namely *Ac-Del, Ac-Ldh, Ac-Pnt, Ac-Amb, Ac-Rnc* and *Ac-Wltn* of the pathogen *A. candida.* Further towards understanding the severity of broomrape (*Orobanche*) problem on farmers' fields in Indian mustard in Charkha Dadri district of Haryana were surveyed. *P. ramosa* which infest *B. juncea* was also found to infest tomato crop. Strigolactone biosynthetic pathway genes were also studied in the published genome of *B. juncea*. A draft genome sequence with 129x coverage of an Indian isolate of the *S. sclerotiorum* "ESR-01" causal agent for stem rot was generated. It is a ~41 Mb genome with 9469 protein-coding genes and 57 effector candidates. The studies of the secondary metabolite in *S. sclerotiorum* revealed the secretion of the metabolites under axenic conditions depends on the growth and developmental stages of the pathogen and is independent of the virulence of the pathogen.
- During 2022 the early generation synthetic *Brassica juncea* lines were characterized for morphological traits and genetic diversity was accessed using the molecular markers. The mitotic

analysis revealed 36 chromosomes in these synthetic *B. juncea* lines. Also, a panel of the genotypes was evaluated for the seed number per siliqua trait in a multilocation experiment and the contrasting lines were identified for the further molecular biology studies.

- For developing haploid inducer line in *B. juncea* we have developed CRISPR/Cas9 vector targeting *CENH3* paralog(s). Since *CENH3* knockout is embryo lethal therefore a GFP-tailswap vector was designed to rescue these *CENH3* knockout lines. By co-transformation of these vectors, we have developed transgenic lines. Molecular analysis of these lines was done and these were crossed with wild type to check the haploid induction ability.
- *Cestrum diurnum* leaves are a potential source of vitamin D in plants. However, *C. diurnum* also contains nornicotine, which is a negative trait for utilizing it as a dietary source of vitamin D. We have performed *De novo* assembly and annotation of Cestrum *diurnum* transcriptome to identify the nornicotine biosynthesis genes. Three transcripts were considered potential homologues of the BBL genes in the *C. diurnum*.
- A study to decode the evolution and domestication history of Indian Tea (*Camellia assamica* var. Masters *cv.* TV-1) genome was attempted with SNP based analysis of 150 genotypes of tea across the world to reveal the independent domestication events of Assam and China type. A total 512 genes were found as positively selected candidate genes during the course of domestication events between caffeine and non-caffeine group of Camellia species. The TIGeR web resource was developed to host and provide the different resources generated under the study to the research communities. A genome-wide identification of Histone Modification (HM) gene family of 77 genes and their expression patterns under abiotic stress and different developmental stages of tea was performed. The qRT-PCR from five different developmental tissues of tea plant under three different abiotic stresses (cold, dehydration and salinity) has found 26 differentially expressed HM genes.
- African yam bean (AYB) (*Sphenostylis stenocarpa* (Hochst. ex A. Rich) Harms) is an important crop of Africa with dual benefits, edible tuber as well as beans. A total of 206 microsatellites markers obtained from six different leguminous crops species were tested in AYB and to enable the genetic distance studies 81 markers amplified in AYB have been used in 92 accessions of AYB sourced from the International Institute of Tropical Agriculture (IITA), Nigeria.
- NIPB is the Referral Centre for Genetic Fidelity Testing under National Certification System of Tissue culture Raised Plant (NCS-TCP), a program run by Govt. of India through DBT. Under this program we developed standard operating protocols (SOP) for genetic fidelity testing of tissue culture raised plants and also test 5% random samples received from 5 different ATL labs. In 2022, total 1935 samples were tested and also SOP for genetic fidelity of guava plants was developed.
- In view of changing climatic conditions, to impart stress tolerance in finger millet, quick and efficient genomics strategies are needed. Before developing proper transformation protocol, we have established reproducible regeneration protocol for a finger millet genotype GN-5. Further this protocol will be standardized for transformation of CRISPR-Cas9 based gene editing vectors
- To study the molecular mechanism in the host-pathogen interactions of *Polianthes tuberosa* with root-knot nematode *Meloidogyne incognita*, the comparative transcriptome profiles of uninfected and

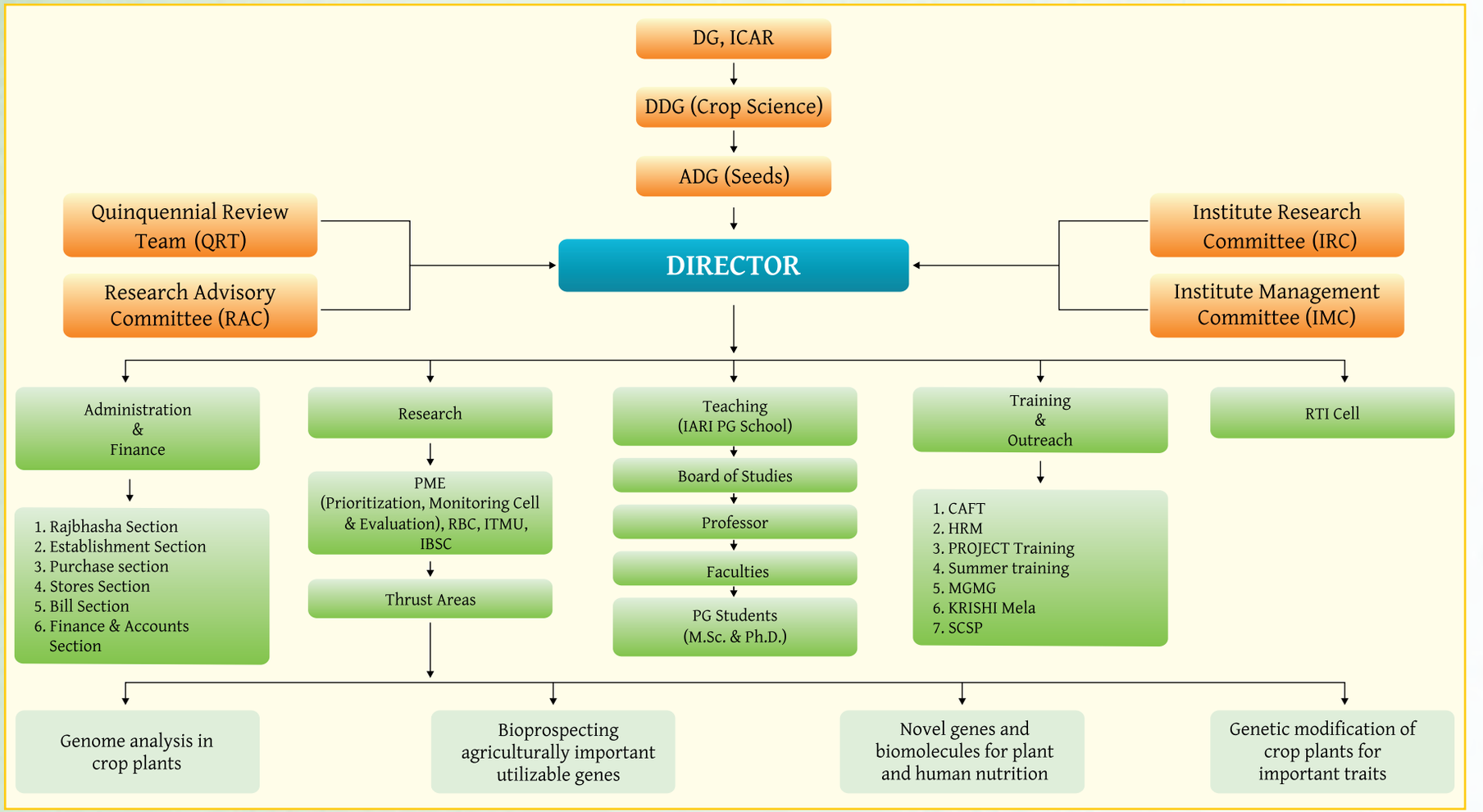
M. incognita-infected tuberose plants, during early, mid, and late infection stage has been generated. Of the 8,289 CDS commonly expressed between the control and infected plants, 256 were upregulated and 129 were downregulated significantly in the infected plants. Our results provide a comprehensive gene expression changes in tuberose during its association with root-knot nematode *M. incognita*.

- For discriminating the anti-nutritional factor proteins and nutritional proteins, the performance of different machine learning algorithms such as support vector machine, random forest, and logistic regression, etc were analysed. The SVM based algorithm has performed with better accuracy of 92% in discriminating anti-nutritional factor proteins than others in a dataset of 5962 proteins (positive dataset: 3614 and negative dataset: 2348).
- NIPB has developed a novel artificial intelligence integrated pipeline to detect the NGS data coverage at the raw stage. The computational pipeline is integrated with all the required preprocessing stages (such as quality check, adapter trimming, quality filtering, etc.) and used the Hash-Table-based mapping algorithm for alignment. The pipeline provides coverage profiling information including coverage depth, coverage breadth, and quality score in textual as well as graphical format.
- This institute is actively engaged in Human Resource Development in the area of Plant Molecular Biology and Biotechnology as a Division of MBB of IARI since its inception. Currently 43 Ph.D. and 09 M.Sc. students are registered in the discipline of Molecular Biology and Biotechnology. In the year 2022, two students were awarded with Doctoral degree and seven M.Sc. students were awarded with Master's degrees in the 60th Convocation 2022.
- In addition to CAFT and Genetic fidelity testing trainings, this year we have conducted an International Symposium on Advances in Plant Biotechnology and Nutritional Security-2022 along with 43rd annual meeting of Plant Tissue Culture Association (India). We arranged Academia-Industry Biotechnology Meet to discuss and appraise the industries about the research programs/ achievements of NIPB. We also organized 4 SCSP programs in Maharashtra, Odisha, Kerala and Uttarakhand, where more than 800 farmers got benefited with ICAR technologies. We also showcased our products and technologies at Pusa Krishi Mela held at IARI Mela ground. In addition, several activities such as Hindi Chetna Maas, Vigilance Awareness week, International Women's Day, Swachh Bharat Abhiyan were celebrated.

About the Institute

- NIPB AND THE MANDATE
- FINANCIAL STATEMENT
- PERSONNEL
- STAFF POSITION
- RESOURCE GENERATION

ORGANOGRAM OF ICAR-NIPB



ICAR-NIPB

National Institute for Plant Biotechnology (NIPB) is the premiere research institution of the Indian Council of Agricultural research (ICAR), engaged in plant 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). In February 2019, NRCPB was elevated to the level of National Institute for Plant Biotechnology (ICAR-NIPB). This brings us much greater national responsibility to conduct basic and applied research, and human resource development in plant biotechnology. NIPB has acquired an excellent infrastructure in terms of equipment and other physical facilities and also a high degree of scientific competence. Research at NIPB involves structural and functional genomics, molecular mapping and marker development, transgenics and genome editing for improving the agronomic traits, biotic -abiotic stress tolerance and nutritional quality in crops. The institute mainly works on five major crops, namely rice, wheat, pigeonpea, chickpea and Indian mustard. Being the premiere institute of plant biotechnology of ICAR, NIPB works on other agricultural and horticultural crops through strong collaborations with national and international institutions for developing genomic resources and finding biotechnological solutions to the major problems in these crops. In addition to research, the institute contributes significantly to development of human resource by offering regular M.Sc. and Ph.D. programmes in partnership with PG School, IARI. The institute also imparts short and long term trainings to students and scientists from other universities and institutions.

Mandate

1. To undertake basic plant molecular biology research for understanding molecular mechanisms underlying basic biological processes
2. To develop capabilities of devising tools and techniques of biotechnology and genetic engineering for crop improvement
3. To use the knowledge gained and technologies developed for advancing agriculture development
4. To serve as a national lead centre for plant molecular biology and biotechnology research and to create trained manpower in the areas of plant biotechnology and genetic engineering

Staff Strength of the Institute

	Sanctioned	Filled	Vacant
Scientific	36+1	35	02
Technical	14	07	07
Administrative	21	13	08
Skilled Supporting Staff	02	-	02
Total	**73+1**	**55**	**18**

Financial Statement 2021-22

(Rs. in Lakhs)

Institute Grant		
	Allocation	**Utilization**
Capital	129.44	117.56
Revenue		
Establishment	1136.09	1135.96
Pension & Other Retirement Benefits	62.79	62.69
Travelling Allowances	9.50	9.44
Research and Operational Expenses	403.50	403.22
Administrative Expenses	433.20	432.57
Miscellaneous Expenses	7.30	7.18
Total	**2182.32**	**2168.62**

Resource Generation

Sales of Farm Produce	0
License Fee	0
Leave Salary and Pension Contribution	0
Interest Earned on Short Term Deposits	10.87
Income Generated from Internal Resource Generation	17.10
Miscellaneous Receipts	23.86
Total	**51.83**

Network projects on transgenic crops (NPTC)

	Allocation	**Utilization**
Capital	61	32.93
Travelling Allowances	0	0
Research and Operational Expenses	155	154.3
Total	**216**	**187.23**

Fund Received through Externally Funded Projects

Externally Funded Projects	612.20
Consultancy Projects	0.00
Total	**612.20**

Scientific Staff

Director
Dr. Ajit Kumar Shasany

National Professor B. P. Pal Chair
Dr. Nagendra Kumar Singh

Principal Scientist	Senior Scientist	Chief Technical Officer
Dr. Sarvjeet Kaur Dr. Anita Grover Dr. Rekha Kansal Dr. Jasdeep Chatrath Padaria Dr. Ram Charan Bhattacharya Dr. Debasis Pattanayak Dr. Pranab Kumar Mandal Dr. Pradeep Kumar Jain Dr. Kishor Gaikwad Dr. Sharmistha Barthakur Dr. Kanika Dr. Monika Dalal Dr. Tapan Kumar Mondal Dr. Ashish Kumar Dr. Prasanta Kumar Dash Dr. Rhitu Rai Dr. Vandana Rai Dr. Subodh Kumar Sinha Dr. Rohini Sreevathsa Dr. Anil Kumar Singh	Dr. S.V. Amitha Charu Rama Mithra Dr. Amolkumar U. Solanke Dr. Navin Chandra Gupta Dr. Ramavatar Nagar Dr. Nimmy M.S. Dr. Mahesh Rao	Dr. Rohit Chamola Dr. Pankaj Kumar
	Scientist (S.S.)	**Technical Officer**
	Dr. Deepak Singh Bisht Dr. Anshul Watts Dr. Sandhya Sharma	Dr. Rampal Singh Niranjan
	Scientist	**Technical Assistant**
	Dr. Joshitha Vijayan Dr. Shbana Begam Dr. Yuvaraj Iyyapan	Mr. Deepak Kumar Rathore Ms. Rita Ms. Megha Mr. Anshul Kumar Verma

Administrative Staff

Senior Administrative Officer	Sr Finance & Account Officer	Assistant Administrative Officer
Mr. Sumit Singh	Mr. Rahul Kumar	Mrs. Sangeeta Jain Mr. Vipin Kumar
Assistant	**Upper Division Clerk**	**Lower Division Clerk**
Mr. Mohit Sikka Mr. Sudarshan Kumar Jha Ms. Nidhi Nailwal Mrs. Rekha Chauhan	Mr. Rajesh Kumar Pal Mr. Kunal Maan	Mrs. Priyanka Nair Mr. Mitravesh Choudhari

Research Achievements

1. GENOME ANALYSIS IN CROP PLANTS
2. BIOPROSPECTING AGRICULTURALLY IMPORTANT UTILIZABLE GENES
3. NOVEL GENES AND BIOMOLECULES FOR PLANT AND HUMAN NUTRITION
4. GENETIC MODIFICATION OF CROP PLANTS FOR IMPORTANT TRAITS
5. NATIONAL PROFESSOR-B. P. PAL CHAIR

1. GENOME ANALYSIS IN CROP PLANTS

Genomic analysis of early flowering/ determinacy in pigeon pea

Kuldeep Kumar, Sandhya Sharma, Durgesh Kumar, Kishor Gaikwad*

Pigeonpea (*Cajanus cajan* (L.) Millsp.), is a highly nutritious grain legume. Although it is a perennial plant, but primarily cultivated as an annual crop with sowing to flowering duration ranging between 60 to 180 days which is highly dependent on the photoperiod. The main target trait for developing varieties with a specific duration is the number of days to flowering. Therefore, in the current study, an attempt was made to map the QTLs responsible for early flowering and determinacy traits in pigeonpea.

A super early flowering determinate accession (ICPL 20338) was crossed with a late flowering indeterminate variety (Malvi 3). The F1 seeds from all the crosses were selfed to develop F2 mapping populations. The F2 populations (574 individuals) were assessed alongside their parents and F1s for days to flowering and determinacy trait. Interestingly, phenotypic data reflected the prevalence of linkage between the flowering time and determinacy trait as all early flowering lines were DT while late flowering were IDT in nature. These were bulked for QTL Seq (4 bulks for DTF (days to flowering) and DT (determinacy) and data amounting to 30X genome coverage was generated on an Illumina platform. Upon data analysis, overlapping QTL regions were identified for both traits on chr 3 comprising of about 457 SNPs/Indels in about 500 genes which were subsequently narrowed down to 87 and finally 52 genic and high confidence SNPs/ indels (Fig. 1.1). Selected candidate genes (13) belong to a diverse family including ARF18, PPRs, TFL-1, Reville, HUA transporter, shoot gravitropism, serine threonine phosphatases, cytokinin etc. Parallely, 96 F2 plant representing all flowering intervals were selected for biparental mapping. GBS based approach was used for genotyping of these F2 individual along with both parents. Reference based assembly and variant calling identified a total of 241198 SNPs out of which 7987 SNP markers were grouped into 11 linkage groups. A total of 8 QTLs for DTF were identified with LOD cut off of 2.5. The QTLs for DTF were scattered on 6 different chromosomes, with chromosome 2 possessing 3 QTLs. Similarly, a total of 6 QTLs were identified for determinacy traits, which were scattered on six different chromosomes (chr 1, 2, 3, 7, 8, and 10). Interestingly an overlapping interval for DT traits as identified through QTLseq was identified by this approach, further supporting the results obtained.

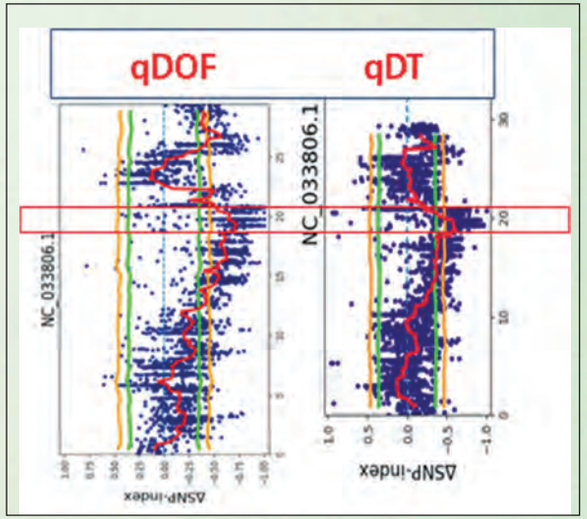


Fig. 1.1: QTL Seq in pigeonpea for early flowering/ determinacy

Cleistogamy (Delayed flower opening) in pigeonpea

Kuldeep Kumar, Sandhya Sharma, Durgesh Kumar, Kishor Gaikwad*

Cleistogamy is a process where the flower opens after fertilization which promotes self-fertilization/prevents outcrossing. This feature helps in maintaining the seed purity, ensures self-fertilization, and also prevents gene escape which can mitigate the ethical issues associated with GMOs. Little information is known about the molecular mechanism behind this feature except in barley. Thus an attempt was made to unravel the molecular mechanism that leads to cleistogamy in pigeonpea. 3 varieties were taken for this study ICP87154 (cleistogamous), UP26 (partial cleistogamy), and Asha (chasmogamous) as the control (Fig. 1.2). For Identification of genes/markers, GBS based SNPs identification followed by QTL Seq analysis of F2 population and then transcriptome data was used to arrive at candidate genes. From the mapping work a set of 5 QTLs were identifed. These included a list of genes including a lncRNA,ABC transporter G family member 31-like,E3 ubiquitin-protein ligase,ubiquitin carboxyl-terminal hydrolase 18,zinc finger CCCH domain-containing protein 30 etc. Subsequently transcriptome analysis revealed a number of DEGs, that include genes like the NAC domain-containing protein, MADS-box transcription factor 1, BEL1-like homeodomain protein, E3 ubiquitin ligase RF12, transcription factors like MYB59 like, BHLH and APL all of which are involved in flower development and found to be downregulated in floral buds of Asha as was also observed in the transcriptome data. The downregulation of these genes indicates their potential role in the development of cleistogamous type flowering.

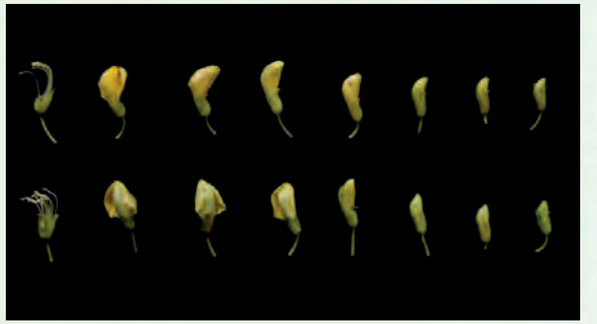


Fig. 1.2: Cleisto vs normal pigeonpea flowers showing diadelphous stamens

Genome-wide exploration of Auxine Response Factor (ARF) gene family and their expression profile in pigeonpea genotypes contrasting for seed number per pod

Sandhya Sharma*, Kumari Arpita, Harsha Srivastava, Kishor Gaikwad

ARF gene family members are the key regulator of auxin response that confers specificity to the auxin mediated response. In this study, we have performed a comprehensive characterization of ARF gene family in pigeonpea. The analysis revealed the presence of 12 ARF genes in pigeonpea (*C. cajan*) distributed across the chromosomes (Fig.1.3). Gene structure analysis indicated that the number of exons ranges from 1-4, most of them possessing single exon. The phylogenetic analysis revealed that ARF proteins of *C. cajan* are more similar to that of *Glycine max*. Based on the expression atlas data it was found that several CcARFs were expressed in tissue-

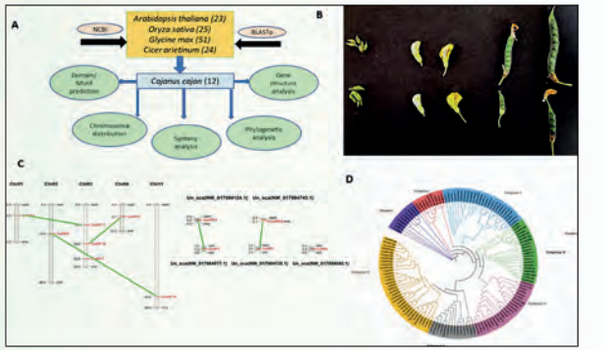


Fig 1.3: A). Schematic workflow to identify ARF genes in *Cajanus cajan*. B) Developmental stages of genotype contrasting for seed number per pod C) Chromosomal distribution map of ARF genes in pigeonpea and D) Phylogenetic and synteny analysis of ARF family genes from Chickpea, Soybean, Pigeonpea, Rice and Arabidopsis.

specific manner. Strikingly, CcARF8 were found to be expressed in hypocotyl, bud and flower tissue whereas CcARF7 in immature and mature pod tissue highlighting their role in flower and pod development respectively.

Tissue specific expression of ARF genes were analysed in four developmental stages viz. germination stage, seedling stage, vegetative stage and reproductive stage. Most of ARFs have found to be critically important for the development at early stages such as; CcARF17 plays an essential role during the initial development of radical and embryo, the expression of CcARF8 has been found to be upregulated in the hypocotyl region while CcARF7 has been found to be upregulated during the development of vegetative meristem. From the expression atlas data (Pazhamala et al., 2017), it can be concluded that ARFs are dynamic in their expression as the same ARF gene controls the development at different stages, as it has been observed in case of CcARF18 which regulates the development of vegetative root and leaf during the initial stages; however, at later stages it is involved in the development of immature seed and reproductive leaf. Similarly, ARF7 has also been found to regulate the shoot meristems as well as in pod development (Fig 1.4)

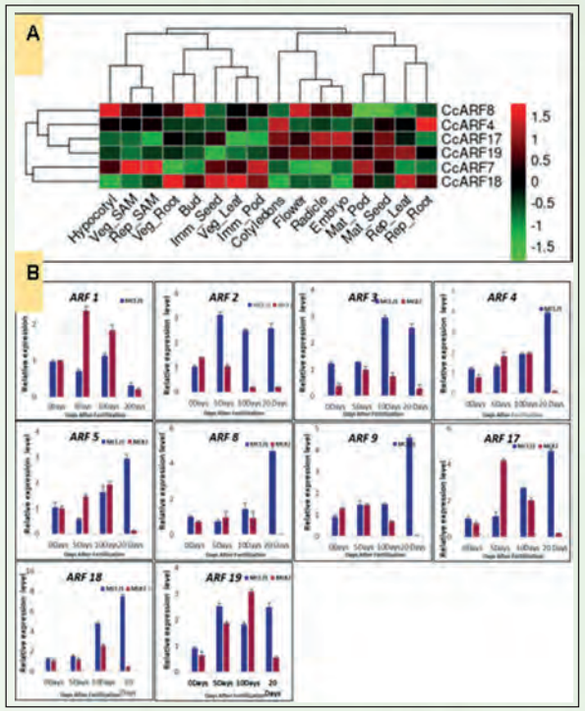


Fig. 1.4: A) *In-silico* expression atlas: Heatmap representing the expression profile of ARF genes in the vegetative and reproductive tissues of pigeonpea. B) qRT-PCR analysis of CcARF genes in different embryo developmental stages in pigeonpea. Data are means of three biological replicates (6 pooled plants each), and error bars denote SE. Tubulin-6 gene was used as an internal control.

Expression of secondary metabolite genes in various pod developmental stages in cluster bean

Sandhya Sharma, Harsha Srivastava, Ajit Shasany, Kishor Gaikwad*

A total of 27, 29 and 35 genes involved in phenylpropanoid biosynthesis, flavonoid biosynthesis and terpenoid biosynthesis were identified in guar genome, respectively. *De novo* sequencing assembly led to the identification of a total of 209525, 375595 and 255401 unigenes at 25, 39 and 50 days after flowering respectively, with an average length of 201 bp. Based on the expression values secondary metabolites genes from phenyl propanoid, falavoinoid and terpenoid biosythetic pathways were selected. The real time expression analysis of these genes were carried out in various pod deveoplmental stages in RGC-936 genotypes carrying high gum content. Most of the gene such as Neomentholdehydrogenase, phenyl amonia lyases, mannitol dehydrogenase,

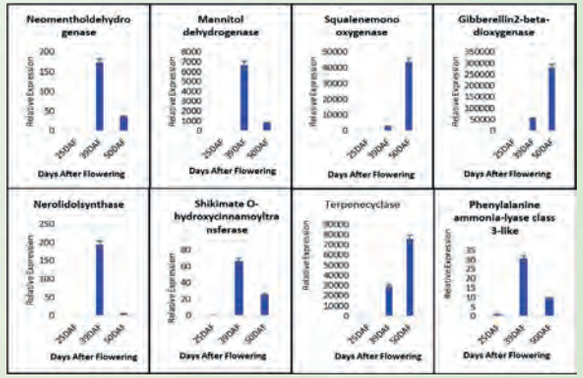


Fig. 1.5: Real-time expression of secondary metabolite genes involved in galactomannan biosynthesis during various pod developmental stages in clusterbean

siikimatate-o-hydroxccinamoyltrasferase were found to be expressed more at 39 DAF (39 DAF reported to have high galactomannan accumulation) (Fig. 1.5).

Development of broad spectrum and durable bacterial blight resistant variety through pyramiding of four resistance genes in rice

Prasanta K Dash, in collaboration with ICAR-NRRI, Cuttack

Bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* is a major biotic constraint on obtaining higher grain yields in rice. Marker-assisted backcross breeding (MABB) was performed by the pyramiding of *Xa4*, *xa5*, *xa13* and *Xa21* resistance genes in the popular variety, Ranidhan. A foreground selection in BC_3F_1 progenies detected all the target genes in 12, 7 and 16 progenies by using the closely linked markers (Fig. 1.6). The screening of the BC_3F_2 progenies for the four target genes resulted in eight plants carrying all the four target genes. A bioassay of the pyramided lines conferred very high levels of resistance (Fig. 1.6A). In addition, these pyramided lines were similar to Ranidhan in morpho-quality traits viz. panicles/plant, grain length, grain breadth, grain weight.

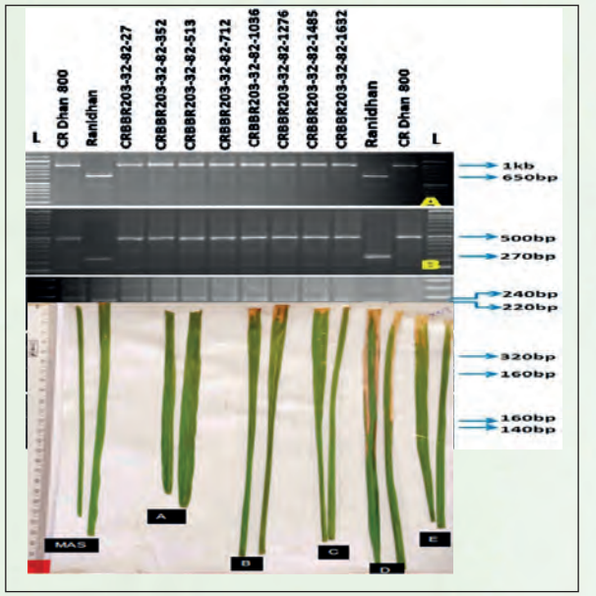


Fig. 1.6: BC_3F_2 derivatives of Ranidhan for introgression of four BB resistance genes, *Xa21, xa13, xa5* and *Xa4,* using molecular markers.

Molecular breeding for incorporation of submergence tolerance and durable bacterial blight resistance into the popular rice variety 'Ranidhan'

Prasanta K Dash, in collaboration with ICAR-NRRI, Cuttack

Ranidhan is a popular late-maturing rice variety of Odisha state, India. The farmers of the state suffer heavy loss in years with flash floods as the variety is sensitive to submergence. Bacterial blight (BB) disease is a major yield-limiting factor, and the variety is susceptible to the disease. BB resistance genes *Xa21*, *xa13*, and *xa5*, along with the *Sub1* QTL, for submergence stress tolerance were transferred into the variety using marker-assisted backcross breeding approach (Fig 1.7). Screening of the BC_3F_1 progenies using markers detected 12 plants carrying the target genes. Foreground selection in the BC_3F_2 progenies detected four plants carrying the target genes in the homozygous condition. The bioassay of the

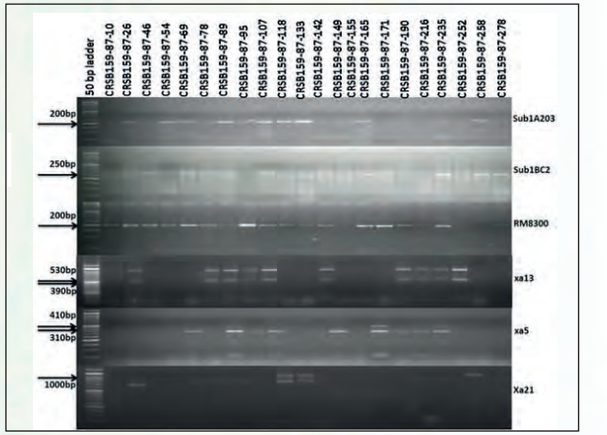


Fig 1.7: Representative electropherogram of BC_3F_1-derived lines of Ranidhan using submergence tolerance markers (Sub1BC2 and Sub1A203) and markers for bacterial blight resistance genes *Xa21*, *xa13*, and *xa5*.

pyramided lines conferred very high levels of resistance to the predominant isolates of bacterial blight pathogen. These BB pyramided lines were submergence-tolerant and similar to Ranidhan in agro-morphologic quality traits; hence, they are likely to be adopted by farmers.

Analysis of homologous regions of small RNAs *miR397* and *miR408* reveals the conservation of microsynteny among rice crop-wild relatives

Prasanta K Dash* and Payal Gupta

miRNA are small non-coding RNAs that play important roles in a wide range of biological processes in plant growth and development. *MIR397* (involved in drought, low temperature, and nitrogen and copper (Cu) starvation) and *MIR408* (differentially expressed in response to environmental stresses such as copper, light, mechanical stress, dehydration, cold, reactive oxygen species, and drought) belong to conserved *miRNA* families that either negatively or positively regulate their target genes. In the present study, we identified the homologs of *MIR397* and *MIR408* in *Oryza sativa* and its six wild progenitors, three non-*Oryza* species, and one dicot species. We analyzed the 100 kb segments harboring *miRNA* homologs from 11 genomes to obtain a comprehensive view of their community evolution around these loci in the farthest (distant) relatives of rice. Our study showed that mature *miR397* and *miR408* were highly conserved among all *Oryza* species (Fig 1.8A). Comparative genomics analyses also revealed that the microsynteny of the 100 kb region surrounding *miRNA* was only conserved in *Oryza* spp.; disrupted in *sorghum,* maize, and wheat; and completely lost in *Arabidopsis*. There were deletions, rearrangements, and translocations within the 100 kb segments in *Oryza* spp., but the overall microsynteny of the region was maintained (Fig 1.8B). The phylogenetic analyses of the precursor regions of all *MIRNAs* under study revealed a bimodal clade of common origin. This comparative analysis of miRNA involved in abiotic stress tolerance in plants provides

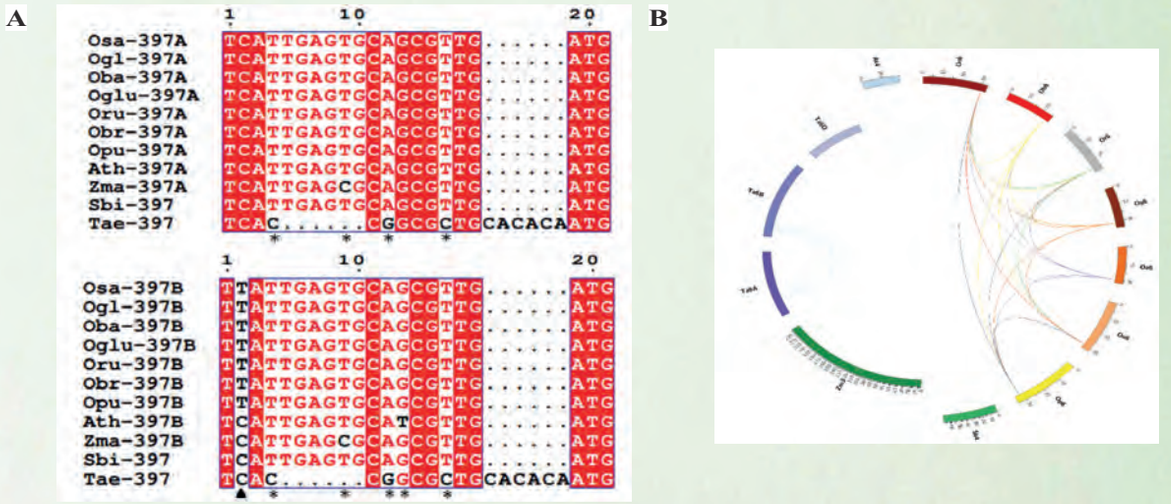


Fig. 1.8: (A) Multiple sequence alignment of mature *MIR397* to detect the region of conservation and divergence. * denotes position of Single nucleotide polymorphism. Osa- *Oryza sativa*, Oba- *Oryza barthii*, Ogl- *Oryza glaberrima*, Oglu- *Oryza glumaepatula*, Oru- *Oryza rufipogon*, Obr- *Oryza brachyantha*, Op1- *Oryza punctata*, Sbi- *Sorghum bicolor*, Zma- *Zea mays*, Tae- *Triticum aestivum*, Ath- *Arabidopsis thaliana*. (B) Diagrammatic representation of microsynteny analysis of 100kb genomic segments flanking *miR397A* across different poaceae members. Circular plot showing patterns of synteny and collinearity. os6- *Oryza sativa* chr 6, ob6- *Oryza barthii* chr 6, og6- *Oryza glaberrima* chr 6, ou6- *Oryza glumaepatula* chr 6, or6- *Oryza rufipogon* chr 6, oa6- *Oryza brachyantha* chr 6, op6- *Oryza punctata* chr 6, sb4- *Sorghum bicolor* chr 4, zm3- *Zea mays* chr 3, ta6A- *Triticum aestivum* chr 6A, ta6B- *Triticum aestivum* chr 6B, ta6D- *Triticum aestivum* chr 6D, at4- *Arabidopsis thaliana* chr 4.

a powerful tool for future *Oryza* research. Crop wild relatives (CWRs) offer multiple traits with potential to decrease the amount of yield loss owing to biotic and abiotic stresses. Using a comparative genomics approach, the exploration of CWRs as a source of tolerance to these stresses by understanding their evolution can be further used to leverage their yield potential.

Association Mapping for QTL controlling Superoxide Dismutase and Antioxidant Activity in Rice

Prasanta K Dash, in collaboration with ICAR-NRRI, Cuttack

Antioxidant-rich rice is a cheaper way to solve stress-related disorders and other health benefits for the global rice-eating population. Multiple antioxidant traits *viz.* superoxide dismutase, flavonoids, etc. activity were mapped using a representative panel population through association mapping. Potential landraces carrying multiple antioxidant compounds were identified from the population. The population showed linkage disequilibrium for the studied traits based on the Fst values. A total of 14 significant marker–trait associations were detected for these antioxidant traits. The study validated the QTLs, *qANC3* and *qPAC12-2* for anthocyanin content will be useful in marker-assisted breeding. Chromosomal locations on 11 and 12 were detected as antioxidant hotspots. These strongly associated QTLs will be useful in the antioxidant improvement programs in rice.

Cloning and molecular characterization of the *phl*D gene involved in the biosynthesis of "Phloroglucinol", a compound with antibiotic properties from plant growth promoting bacteria *Pseudomonas* spp.

Payal Gupta, Prasanta K Dash* and Rhitu Rai

*phl*D is a novel kind of polyketide synthase involved in the biosynthesis of non-volatile metabolite phloroglucinol by iteratively condensing and cyclizing three molecules of malonyl-CoA as substrate. Phloroglucinol or 2,4-diacetylphloroglucinol (DAPG) is an ecologically important rhizospheric antibiotic produced by pseudomonads; it exhibits broad spectrum anti-bacterial and anti-fungal properties, leading to disease suppression in the rhizosphere. Additionally, DAPG triggers systemic resistance in plants, stimulates root exudation, as well as induces phyto-enhancing activities in other rhizobacteria. Here, we report the cloning and analysis of the *phl*D gene from soil-borne gram-negative bacteria—*Pseudomonas*. The full-length *phl*D gene (from 1078 nucleotides) was successfully cloned and the structural details of the PHLD protein were analyzed (Fig 1.9) in-depth via a three-dimensional topology and a refined three-dimensional model for the PHLD protein was predicted. Additionally, the stereochemical properties of the PHLD protein were analyzed by the Ramachandran plot, based on which, 94.3% of residues fell in the favored region and 5.7% in the allowed region. The generated model was validated by secondary structure prediction using PDBsum. The present study aimed to clone and characterize the DAPG-producing *phl*D gene to be deployed in the development of efficient biocontrol of rhizospheric pathogens.

Draft genome sequencing and secretome analysis of *S. sclerotiorum*

Gupta NC*, Yadav S, Arora S, Mishra DC, Budhlakoti N, Gaikwad K, Rao M, Prasad L, Rai PK, Sharma P

The polyphagous nature and ubiquitous presence of the *S. sclerotiorum* pathogen

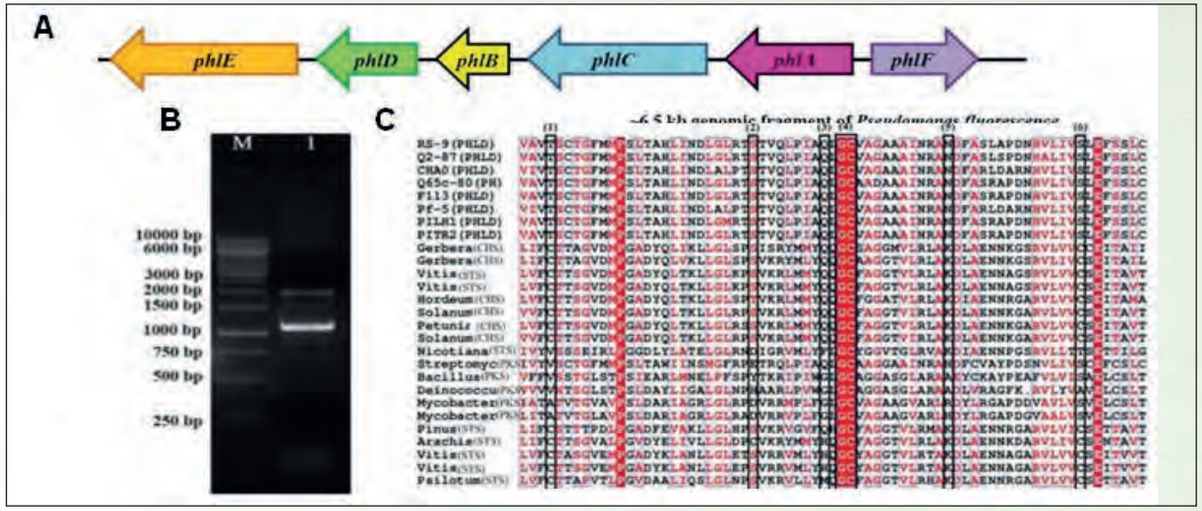


Fig. 1.9: (A) Schematic representation of a ~6.5 kb genomic fragment of *Pseudomonas* harboring the genes responsible for the biosynthesis of 2,4-DAPG by *phl* operon. *phl* operon comprises four genes *phl*A, *phl*B, *phl*C, and *phl*D. The operon is flanked on either side by *phl*E and *phl*F genes that are separately transcribed and coded for the putative efflux and regulatory (repressor) proteins, respectively. (B) PCR amplification of the *phl*D gene from genomic DNA of *Pseudomonas fluorescens.* Lane M: 1 kb DNA ladder; Lane 1: ~1078 kb amplicon of the *phl*D gene. (C) Alignment of predicted amino acid sequences for PHLD from various strains of *Pseudomonas* and type III polyketide synthase (PKS) from gram-positive bacteria and plant chalcone synthases/Stilbene synthase (CHS/STS). Conserved residues are indicated by boxes.

make it major biotic stress for more than 500 plant species including oilseed *Brassica*. The virulence of the pathogen usually varies with their isolates of different origins, interacting hosts, and environmental factors. In the present study,a draft genome sequencing and secretome analysis of *S. sclerotiorum* (ESR-01) was carried out. A paired-end shotgun library with a mean fragment size of 478 bp yielded ~6.9 GB, while a mate-pair library with a mean fragment size of 818 bp yielded ~3.5 GB of clean reads. The filtered high-quality reads, paired-end (PE) and mate-pair (MP) read of 23,191,545 and 11,569,965, respectively, were *de novo* assembled into 328 scaffolds (BioProject PRJNA722876; WGS Accession No. JAGTAE010000000), with an N50 scaffold size of ~ 447.13 kb using SOAPdenovo (v2.04). Assembly resulted in a total size of 40.98 Mb with an overall coverage of 129X (Table 1.1). Around 80% of the scaffolds were larger than 5 kb including 35% of scaffolds larger than 50 kb. In total 9469 protein-coding genes were predicted from assembled scaffold against *Botrytis cinerea* using the AUGUSTUS-3.2.1 gene prediction program. Assembly and gene statistics features of *Sclerotinia sclerotiorum* are given in Table 1.1. Functional annotation with the BLASTx program (NCBI-blast-2.3.0+ standalone tool) has shown the homologous sequences for 9412 genes against NR (non-redundant protein) database whereas the remaining 57 genes were without any blast hits. Gene ontology (GO) mapping was carried out by using the Blast2GO PRO and that revealed 4514 genes involved in biological processes, 4677 genes in molecular processes, and 4151 genes were assigned to cellular functions. Further, the potential involvement of the 2994 predicted genes in different KEGG pathways was established.

The effector plays an important role in the pathogen's virulence while interacting with the host during the disease establishment. We have developed the effector prediction pipeline (Fig. 1.10) and out of 9469 predicted proteins in the ESR-01 genome, 1006 were annotated as classical secretory proteins by SignalP versions 2.1 and 4.1 (Fig 1.10a). After filtering the secretory proteins with

Table 1.1 Assembly and gene statistics features of Sclerotinia sclerotiorum isolate 'ESR-01'

Features	S. sclerotiorum isolate "ESR-01"
Coverage	129 X
Assembly size (Mb)	40.98
Scaffold N50	447128
GC content (%)	37.71
Predicted protein-coding genes	9469
Average gene length (bp)	1587
Gene density (number of genes per Mb)	231
Unique genes	57
GC content in predicted genes (%)	45.88
tRNA genes	74
rRNA gene	11
Transposable elements	647
Secreted proteins	944
SNPs	27450
SSRs	3844

TMHMM (for transmembrane proteins), GPI-SOM (Glycosylated proteins), BLASTN, ESTs databases, PFAM, and EffectorP, a total of 57 effector candidates were predicted out of which 27 were the annotated (Fig. 1.10 b). The involvement of these predicted 57 effectors in the biochemical pathway was deciphered by KEGG analysis. The results showed only 4 secretory effectors were involved in the distantly related pathway of pathogenesis and those were related to purine metabolism, glyoxylate and dicarboxylate metabolism, drug metabolism, and T cell receptor signaling pathway along with riboflavin and thiamine metabolism. The functional correlation of the predicted 369 secretory proteins in the *S. sclerotiorum* 'ESR-01' isolate in pathogenesis was further evidenced by the presence of CAZymes in 164 proteins.

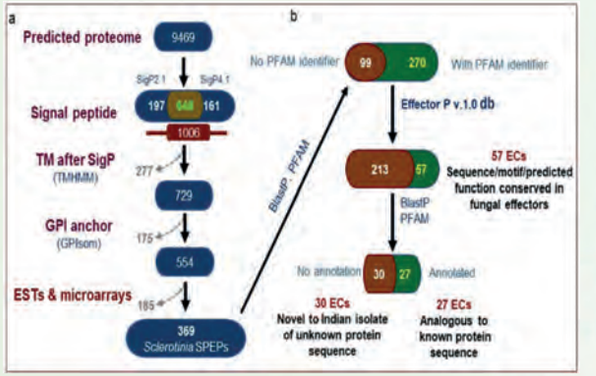


Fig 1.10: *S. sclerotiorum* secretome prediction, and effector identification. (a) Out of the 554 predicted secreted proteins, 369 had experimental evidence for expression *in planta*. The number of proteins filtered out is indicated with dotted arrows, the number of selected proteins is given within boxes, bioinformatics tools and resources used are indicated by the boxes, (b) identification of effector candidates (ECs) based on sequence, motifs, or protein domains conserved in fungal effectors.

Based on the functional relevance of the *S. sclerotiorum* secretory effector proteins (SSEPs) to pathogenesis, five of them were selected for qRT-PCR (Fig. 1.11) which showed the isochorismatase hydrolase (gene 70), and oxalate decarboxylase (gene 2387) was induced gradually in due course of infection whereas alpha, beta-hydrolase (gene 3672), and protease propeptide inhibitor (gene 7551) were induced at the early stage (up to 8 hr) and downregulated at the later stage (16 hr) of infection. One of the SSEPs with hydrolytic function (gene 5446) has shown neutral to the different stages of infection. Three of the effector candidates (gene 70, gene 3672, gene 5446) among 27 annotated effectors *viz.* isochorismatase, alpha beta-

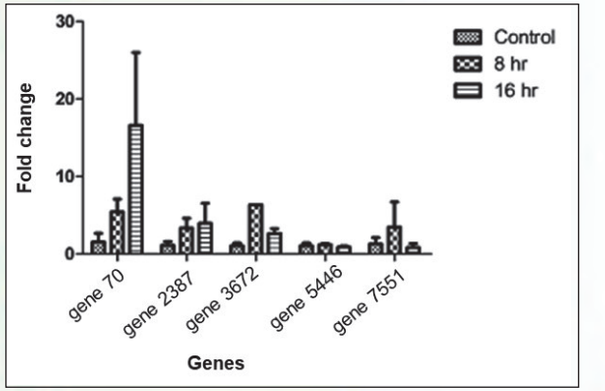


Fig. 1.11: Expression analysis of the selected secretory effector genes by qRT-PCR in the *S. sclerotiorum* infected leaves of *B. juncea* after 8- and 16- hrs of infection.

hydrolase, and one another class of hydrolase revealed hydrolytic activity which is also evidenced to be important for virulence via differential expression in *S. sclerotiorum* transcriptome on *B. napus* infection.

UPLC-Q-TOF-MS-based untargeted studies of the secondary metabolites secreted by *Sclerotinia sclerotiorum* under the axenic condition

Gupta NC*, Arora S, Kundu A, Sharma P, Rao M, Bhattacharya RC

Secondary metabolites are known to play a vital role in host-pathogen interaction wherein little is known about the secondary metabolites secreted by *S. sclerotiorum* in axenic culture. In this study, we predicted the genomic repertoire of the metabolites in an Indian isolate 'ESR-01' of the *S. sclerotiorum* and analysed the secondary metabolites secreted by pathogenically diverse Indian isolates of the *S. sclerotiorum* in axenic culture through untargeted high-resolution UPLC-QTOF-ESI/MS. The total ion chromatogram (TIC) of ethyl acetate extract obtained through UPLC-QTOF-ESI-MS of the six different *S. sclerotiorum* isolates revealed various characteristic peaks (S1-S6) corresponding to their molecular and adduct ions. With the manual stimulation of the UPLC-MS peaks, ten different metabolites namely, (i) sclerin, (ii) sclerotinin B, (iii) sclerone, (iv) bostrycoidin, (v) botcinin D, (vi) botcinin A, (vii) gliovirin, (viii) melanin, (ix) scleramide, and (x) botcinic acid were putatively identified (Fig. 1.12). Four of the metabolites sclerin, sclerone, melanin, and sclerotinin B have already been known in *S. sclerotiorum* whereas the six other metabolites have been found and reported for the first time secreted by *S. sclerotiorum*.

A high-quality, contiguous genome assembly of *Cicer reticulatum*, the immediate progenitor of domesticated chickpea, *Cicer arietinum*

Ramawatar Nagar*, Nimmy MS, Sarvjeet Kaur, Kishor Gaikwad, Ajit Kumar Shasany, Pradeep Kumar Jain

Chickpea is one of India's most produced

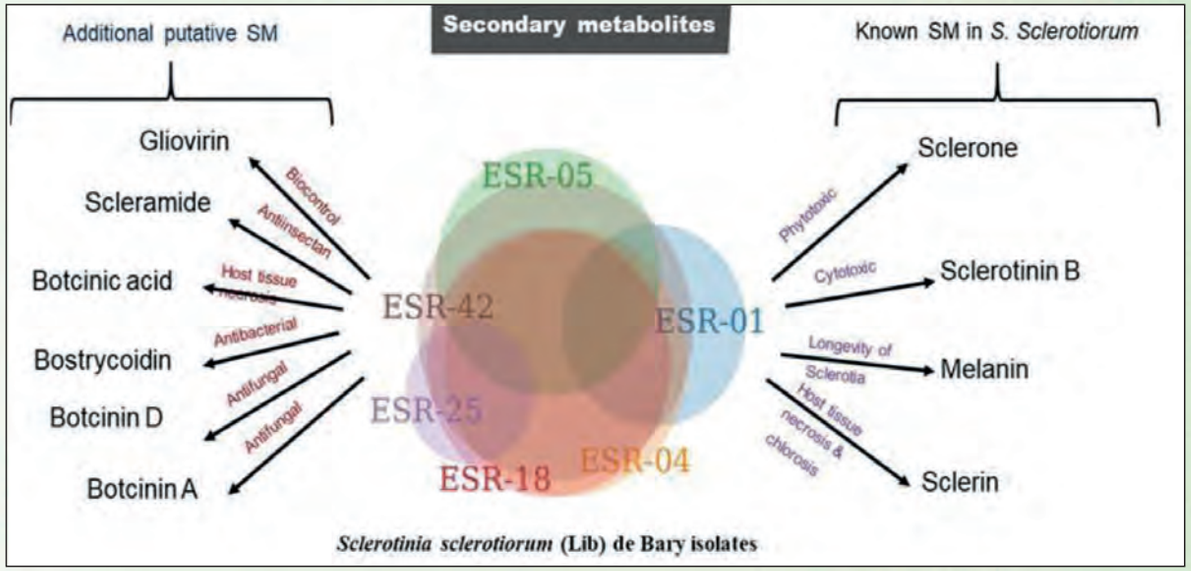


Fig. 1.12: Schematic representation of the secondary metabolites identified by LCMS analysis of the liquid culture of the *S. sclerotiorum* pathogen. The left panel enlists the six additional putative SM and the right panel shows the known SM identified under the axenic condition.

and consumed pulse crops. Its demand continues to increase with the growing population. Wild relatives of cultivated crops, such as *Cicer reticulatum*, constitute a vast reservoir of genetic diversity that can be exploited for breeding initiatives to meet the challenge of global food security. *Cicer reticulatum* is believed to be the immediate progenitor of cultivated chickpea and the only cross-compatible wild relative. This cross-compatibility enables the easy transfer of important agronomic traits to the cultivated chickpea through traditional breeding methods. A high-quality contiguous genome of *Cicer reticulatum* can enable the identification of genomic changes during domestication.

We produced a high-quality genome assembly of the *Cicer reticulatum*, genotype ILWC-237. The genome was sequenced using both short Illumina and long PacBio reads technologies. The PacBio latest Sequel IIe platform was used to produce HiFi reads. The Illumina sequencing was done in two libraries, yielding a cumulative output of 102.8 Gb data, translating to ~120 X coverage of the chickpea genome. The PacBio sequencing run produced ~56 Gb CCS reads, which were translated into ~21 Gb HiFi reads. The HiFi reads were *de novo* assembled using the Hifiasm assembler, resulting in a highly contiguous genome assembly of ~723 Mb in size (Table 1.2). The assembly was composed of 456 contigs with an N50 of 1.8 Mb. To assess the completeness of the assembly, BUSCO (benchmarking of single copy orthologs) analysis was performed, which yielded a score of 99%, indicating a highly complete genome assembly. The assembly will be scaffolded and placed into chromosomes with the help of Hi-C in subsequent analysis.

Table 1.2: Assembly state of *Cicer reticulatum*, genotype ILWC-237

Assembly features	State
Name of the genotype	ILWC-237
Contigs Generated	456
Max. Contig Length (Mb)	52.5
Min. Contig Length (Mb)	15,595
Average Contig Length (Mb)	15.86
Total Contigs Length (Mb)	723
N50 values (Mb)	18.7
BUSCO coverage	99 %

Meta-analysis of the Fusarium wilt resistance QTLs revealed chrmososme2 is a hostspot for wilt resistance.

Ramawatar Nagar*, Nimmy MS, Pradeep Kumar Jain

Fusarium oxysporum f. sp. *ciceris* (Foc), the causal agent of the Fusarium wilt disease, has evolved into multiple races. Similarly, chickpea has evolved race-specific resistance to the Fusarium wilt pathogen which has been successfully used for breeding wilt-resistant varieties. Several QTLs have been identified for Fusarium wilt resistance against many races (Race0, Race1, Race2, Race3, Race4, and Race5). However, the gene(s) associated with Fusarium wilt resistance has not been identified so far. To find the candidate Fusarium resistance genes, a meta-analysis of all reported chickpea Fusarium wilt resistance QTLs was conducted. The sequences of markers (SSR/SCAR) flanking to Fusarium resistance QTLs were retrieved from the literature. A short query nucleotide blast analysis was performed to find hits of SSR/SCAR markers. The most hit turned out to be on chromosome 2 revealing that it is a hotspot for Fusarium resistance in chickpea. About 60% of the chromosome has been flanked by the many QTLs reported for the different races of Fusarium (Race 0, Race 1, Race 2, Race 3, Race 4, and Race 5) (Fig. 1.13). Using reference genome annotation information, gene sequences of all major resistance gene classes have been extracted and further bioinformatic analysis is underway to assess their association with Fusarium wilt resistance in chickpea.

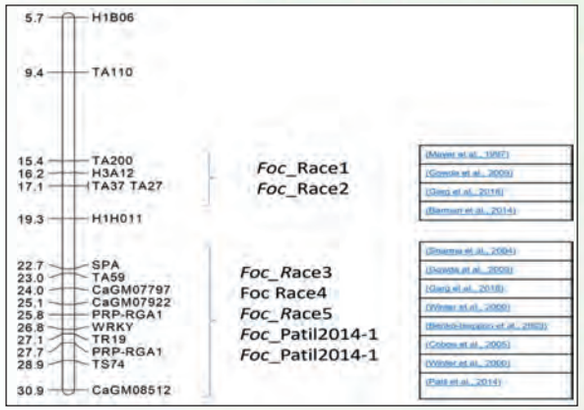


Fig. 1.13: Physical map of chickpea chromosome2 with the location on chickpea Fusarium wilt resistance associated markers.

De novo assembly and annotation of the chloroplast genome of the annual wild relative of chickpea, *Cicer cuneatum*

(Ramawatar Nagar*, Nimmy MS, Pradeep Kumar Jain)

The domesticated chickpea, *Cicer arietinum*, has eight wild relatives. These have been classified into primary, secondary, and tertiary gene pools. One of the eight wild relatives of chickpeas is *Cicer cuneatum*, which is one of the tertiary gene pool species. Chloroplast genomes are frequently used in systematics and phylogeography because of their simple circular structure, their predominantly clonal inheritance along the maternal line, and their high copy number in the cell. The chloroplast genome of *Cicer cuneatum* had not been reported in the public domain until now. To assemble the chloroplast genome of *Cicer cuneatum*, whole-genome Illumina sequencing data of the *Cicer cuneatum* genotype ICC20215 (SRR13566169) was downloaded from NCBI. The raw paired-end reads were quality-processed and subsetted to 20% of the whole reads randomly using the seqtk tool. For chloroplast genome assembly, the GetOrganelle tool was used, which produced a 124298 bp contig which was annotated for coding and non-coding gene content using the GeSeq web browser, https://chlorobox.mpimp-golm.mpg.de/geseq.html.The chloroplast genome size of *Cicer cuneatum* is approximately 124.3 Kb, which is about one Kb smaller than the cultivated species, *Cicer arietinum* (125.5 Kb). The chloroplast genome contains all the essential protein-coding and non-coding genes that are present in typical plant chloroplast genomes. These genomes contain 111 different functional genes, including 78 protein-coding genes, 27 tRNA genes, and four rRNA genes (Fig. 1.14). The annotation of the genome will be further manually curated and submitted to the NCBI database for public access. The full-length chloroplast genome of *C. cuneatum* can provide valuable information about the evolutionary history and genetic diversity of this species, as well as its relationship to other members of the genus Cicer.

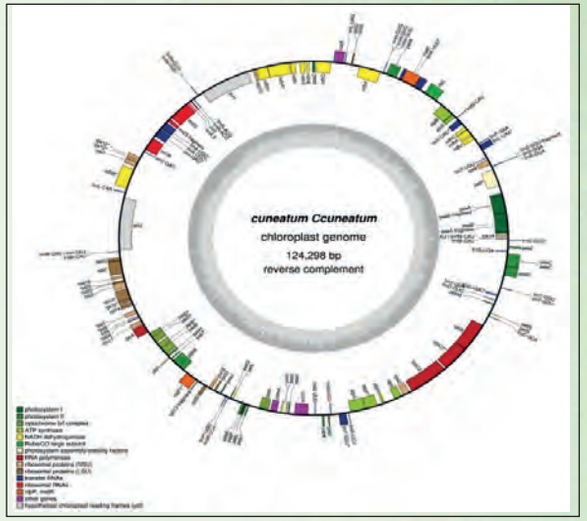


Fig. 1.14: Map of the *Cicer cuneatum* chloroplast genome. Genes inside the circle are in the positive orientation while the genes outside the circle are in the negative orientation. The colour scheme represents various classes of genes. Genes with similar functions are shown in the same colour. The inner grey circle indicates the GC content of the genome.

Pod borer (*Helicoverpa armigera*) induced transcriptional response in chickpea (*Cicer arietinum* L.)

Nimmy M.S*, Ramawatar Nagar, Sagar D, Vinod Kumar, Sanjay Singh,

Chellapilla Bharadwaj, Rohini Sreevathsa and Pradeep Kumar Jain

Gram pod borer or *Helicoverpa armigera* is one of the major pests of chickpea which cause damage to leaves as well as pods leading to significant yield loss. Till now there is no cultivar which has ever reported with stable resistance to this pest. In a field study conducted in IARI by our group in 2020-21, based on percent pod damage, we selected 3 chickpea genotypes which showed differential tolerance to gram pod borer. The genotype ICC 3137 shown maximum percentage of pod damage and hence classified as susceptible, ICCL 86111 with least percent pod damage has been classified as tolerant and RSG 959 as moderately tolerant. We reconfirmed the relative level of tolerance of these genotypes in lab by detached leaf assay. Terminal branch of three to four fully expanded leaves were weighed and embedded in 3% agar-agar in a petri plate and infested with a single third instar larva with 3 replications each per genotype (Fig. 1.15). The initial and final larval weight were recorded before and after 6h and 24 h feeding period. The results revealed that ICC 3137 was affected maximum compared with the other two genotypes under study. The larvae consumed maximum amount of leaf in ICC 3137 gaining maximum larval weight. The whole plant bioassay was also carried out by exposing the whole plant to larval feeding using a single larva per plant. The percent damage caused at the end of 6 h and 24 h feeding period as well as the percent weight gained by the larva was calculated.

We aim to understand the mechanism of resistance by identifying the genes differentially expressed in response to the insect attack which may be contributing in defence response. To identify the pod borer induced genes, transcriptome analysis was carried out using the identified tolerant and susceptible chickpea genotypes uninfested and infested for 24 h after challenge with the larvae. Differential gene expression analysis was performed using DESeq2 package in which 0 h time point was used as baseline and 24 h as pod borer infested for both tolerant and susceptible. From the differentially expressed genes we shortlisted those with >2 fold expression. Among the genes highly upregulated in tolerant cultivar include genes involved in cell wall modification xyloglucan endotransglycosylase/hydrolase, Transcription factors like ethylene responsive factors (ERFs), WRKY, MYB, DREB and bHLH, and a few uncharacterized genes were also present. The genes with probable role in insect resistance have been short listed for validation. The results of this study will provide an insight into the pod borer induced transcriptional changes in chickpea.

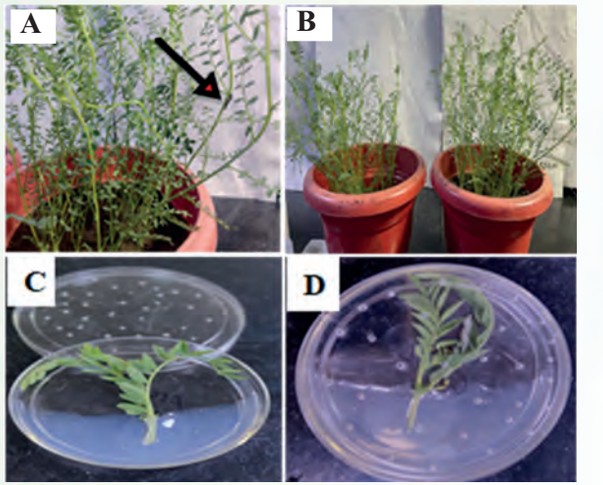


Fig. 1.15: (A) Pod borer larvae feeding on tender shoot (B) Mechanical damage caused by *Helicoverpa armigera* after 6 h and 24 h infestation. Damage after 24 h was more evident (observed dropping shoot) (C) Chickpea shoot fixed in 3% agar- agar (D) Larva feeding on detached chickpea shoot which is fixed in agar.

Identification of pod wall specific gene (s) in chickpea (*Cicer arietinum* L.)

Nimmy MS*, Ramawatar Nagar, Vinod Kumar, Chellapilla Bharadwaj, Monika Dalal, Kishor Gaikwad and Pradeep Kumar Jain

Chickpea (*Cicer arietinum* L.) is an important legume widely cultivated in India. Its seeds are rich in protein. The chickpea seeds develops inside the pod. Pod wall protects the seeds from pathogens and insect pests. The pod wall of legumes is important as it has role in photosynthesis and it transfers the photosynthate to seeds and hence has role in grain filling at the later stage of the pod growth. Also, the pod wall minimizes the CO_2 losses to the atmosphere and helps in the assimilation of storage product in the seeds. Considering the physiological importance of pod wall, identification of the genes which are either unique to the pod wall or expressed more in the pod wall is significant. Detailed profiling of pod wall specific gene expression will help to gain understanding of role of different classes of genes in pod developmental stages of chickpea. Hence, we aim to identify genes that are highly up-regulated in the chickpea pod wall using RNA sequencing. Chickpea cultivar JG11 was grown in the field at IARI. The flowers were tagged on the day they opened completely and also the unopened flower also tagged for sample collection. We could observe after 5th day of anthesis (DAA) the flower drops and there is formation of pod with developing seeds inside. We collected pod wall by separating seeds from 6 DAA, 8DAA, 10 DAA and 12 DAA (Fig. 1.16). At least three biological replicates were collected for each sample. Total RNA was isolated from tissue samples (pooled from three biological replicates). We target to identify transcripts unique to the pod wall to gain a better understanding of gene expression in the pod wall tissues.

Study of changes in growth parameters under salt stress in chickpea genotypes

Nimmy M.S*, Ramawatar Nagar, Vinod Kumar, Kirti Bala, Palak Jain, Chellapilla Bharadwaj and Pradeep Kumar Jain

Chickpea is an important food legume, known for its high nutritional value, and generally considered as relatively salt sensitive crop. The harmful effect of salt stress on the growth of the plant is due to osmotic, ionic stress, or nutritional imbalance. Availability of large genetic variation provides opportunity to identify the saline tolerant genotypes in chickpea. Assessing the effect of salinity on growth characteristics of chickpea including root traits is important in understanding the molecular mechanisms of stress tolerance and improving crop stress tolerance by gene manipulation. We have used seeds of six chickpea genotypes both tolerant and

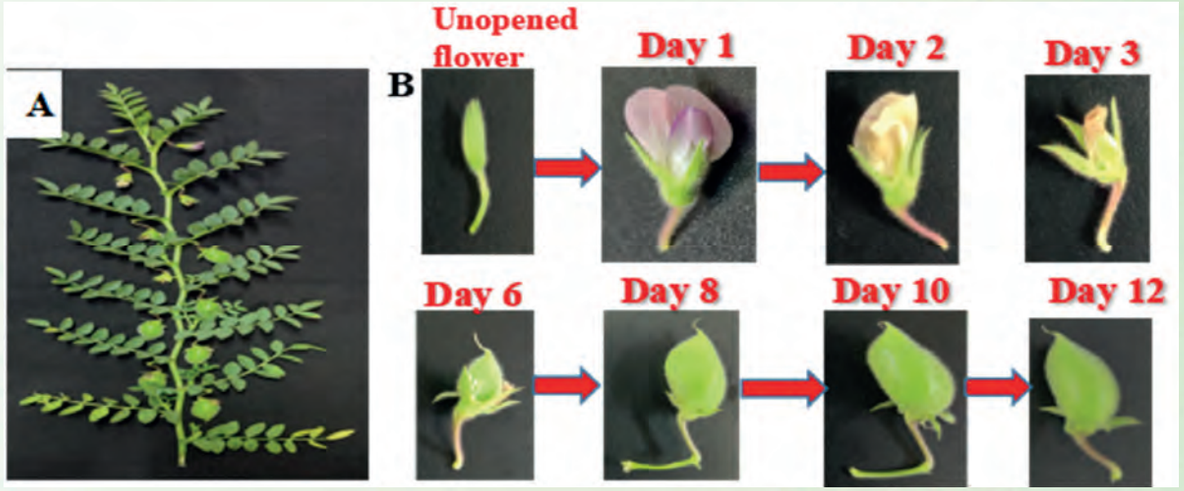


Fig. 1.16: (A) & (B) Shows different developmental stages of pod from unopened flower to 12day old pod in chickpea cultivar JG11 (single plant)

susceptible to salinity procured from Genetics Division, IARI, New Delhi for this study. The physiological experiment was set-up in a Random Complete Block Design (RCBD) comprising three biological replicates of each genotype and 18 day old seedlings were subjected to salt stress (100mM NaCl) in the glass house. CSG 8962 (Karnal Chana-1) was used as salt tolerant check. Unstressed plants were kept as control for comparison (Fig. 1.17). At least three biological replicates of each tissue sample were harvested. Sampling was done after 1, 3 and 5 days of treatment. Effect of Salt (NaCl) treatments on the shoot and root fresh and dry weight (g) of various chickpea genotypes of 18-days-old after 1, 3 and 5 days of treatment was measured (Fig.1.18). We also analyzed the root system architecture of the six genotypes. In both control and salt stressed conditions, total root length, which is the total length of all types of roots of the seedling, was higher in KWR 108. Genotype CSG 8962 exhibited the root system with the smallest overall length in control conditions while JG62 had shown the root system with the smallest overall length under salt stressed conditions. In case of lateral root total length, we observed that in both normal and salt stressed conditions, KWR 108 has the longest lateral root length. It is the least for ICCV 10 in a control situation and JG 62 in salt stressed condition. In case of lateral root density in the control sample the genotype CSG 8962 has the highest lateral root density, however in the salt stressed condition, the genotype JG 315 has the highest. We aim to identify potential candidate genes involved in the response of roots in saline condition in chickpea.

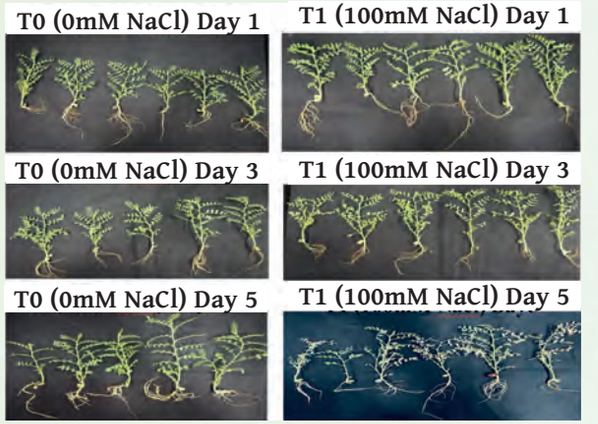


Fig. 1.17: Effect of Salt (100 mM NaCl) treatment on different chickpea genotypes

Estimation of seed protein content and gene expression analysis in selected chickpea genotypes

Tanya Jain, Yashwant Yadava, Rita Singh and Nimmy MS*

Chickpea is an important legume crop which serves as an excellent source of protein for the Indian population. The crude protein

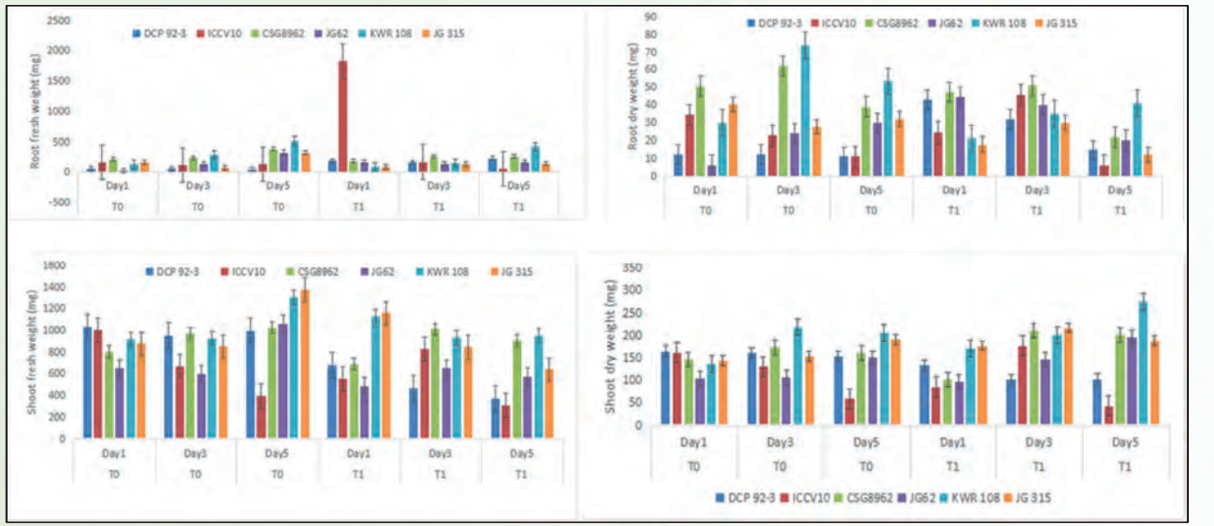


Fig. 1.18: Effect of Salt (NaCl) treatments on the shoot and root fresh and dry weight (g) of various chickpea genotypes of 18-days-old after 1, 3 and 5 days of treatment

content in all the cultivated varieties ranges from 12.6- 30.5%. There is huge variation in the protein content for all the genotypes and varieties which are grown at large scale globally. Hence, there is an urgent need to understand the protein content and genetics of seed protein content traits for improving its nutritional value. The information obtained will help in developing high yielding cultivars with better protein content in chickpea. Therefore, we made an attempt to estimate the seed protein content in selected genotypes of chickpea and to carry out the expression analysis of marker genes associated with seed protein content. Ten chickpea genotypes were evaluated for seed protein content. We could observe the protein content of studied genotypes vary from 18-28%. Based on this we picked four genotypes for further study. The seed proteins were isolated and resolved on SDS-PAGE gel. The majority of the protein bands ranged from 5-100 kDa. As many as about 19 protein bands were clearly visible in all the protein samples and comparison with the available literature indicates that these protein bands belong to globulin, glutelin and albumin classes of seed storage proteins (Fig. 1.19). The genes encoding seed storage proteins (SSPs) were found to be differentially expressed in genotypes with high- and low-protein content in our study. The expression of globulin, glutelin, albumin and vicilin genes was 8-fold, 3.5-fold, 40-fold and 4-fold upregulated respectively in genotype with high protein content as compared to the genotype with low protein content.

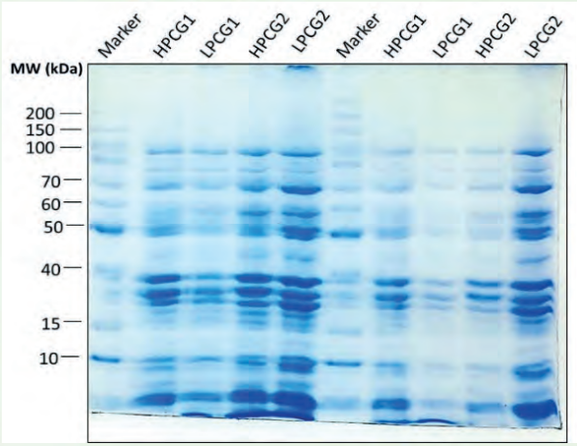


Fig. 1.19: Resolution of seed proteins on SDS-PAGE gel (12 %). The protein bands of high protein content genotypes 1 and 2 and low protein content genotypes 1 and 2 were resolved on PAGE gel in replicate.

Transcriptome profiling in wheat (*Triticum aestivum* L.) genotype Raj 3765 for deciphering the genes and pathways involved in thermo-tolerance

Mawuli K.Azameti and Jasdeep Chatrath Padaria*

Wheat, one of the most widely consumed staple food crops globally, is relatively vulnerable to high temperature-induced heat stress. To address the increasing demand for wheat grain and also to safeguard its production, it is imperative to understand the comprehensive molecular mechanisms underlying thermo-tolerance and subsequently develop wheat cultivars tolerant to heat stress. In view of this, we analysed heat stress responsive transcriptome data of wheat to determine its gene expression level under heat stress. RNA-seq data from wheat genotype Raj 3765 flag leaf exposed to high temperature (42 °C) for 6 h (SRR16347581, and SRR16347579) were analysed. A total of 237,586 trinity transcripts were generated with GC percentage of 47.53. A total of 7990 significant DEGs, comprising 4483 up-regulated and 3507 down-regulated genes, to heat stress were identified. Volcano plots were employed in the visualization of the number of transcripts that were significantly regulated during heat treatment (Fig. 1.20). The results indicated that there were more upregulated genes than the downregulated genes in flag leaf of wheat genotype Raj 3765 after 6 h of heat treatment. Heatmap analysis of the first 30 up and down regulated DEGs is represented in Fig. 1.21.

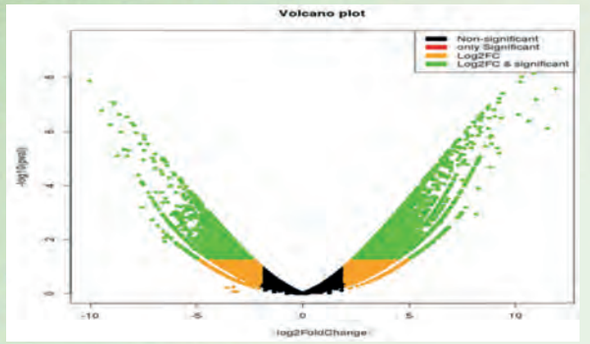


Fig. 1.20: Analysis of differentially expressed genes (DEGs) in wheat flag leaf represented by volcano plot. The negative values indicate down regulation while the positive values represent the up regulation. The significantly up and down regulated DEGs are shown in green dots according to the criteria of |log2FC| ≥ 2 and p-values (p < 0.05).

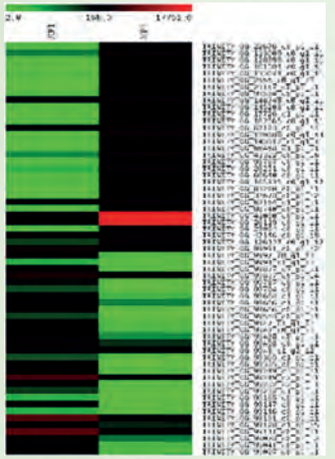


Fig. 1.21: Heatmap analysis of the up and down regulated DEGs between the control and the heat-stress treated wheat genotype Raj 3765. The upper 30 genes are the up-regulated DEGs while the lower 30 genes are the down-regulated DEGs.

Gene ontology analysis showed that 48.93% (3910) of the DEGs were functionally categorized into different ontology families. Out of this, 988 were found to be playing roles in biological processes, 1545 for cellular component and 1377 genes performing molecular functions. The KEGG analysis revealed 146 pathways playing roles in different stress tolerance, involving 814 DEGs (Fig.1.22). Out of this, 133 DEGs were involved in metabolic pathways, 69 DEGs in secondary metabolites biosynthesis path- way, 37 DEGs involved in Plant-pathogen interaction pathway, and 27 DEGS playing roles in protein processing in endoplasmic reticulum.

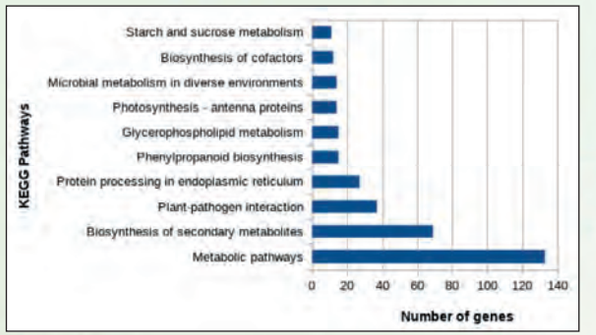


Fig. 1.22: Graphical representation of the top 10 pathways of DEGs during heat stress treatment

Various transcription factors (TFs) were identified in the DEGs obtained. A total of 1909 transcripts encoding different TFs were identified. Among the differentially expressed TFs, MYB, bHLH, WRKY, NAC, ERF, C3H, and C2H2 were most prevalent (Fig 1.23). Verification of few selected DEGs using RT-qPCR produced expression levels similar to the transcriptome data. These results could be helpful in enhancing our understanding of the mechanism underlying thermotolerance in wheat.

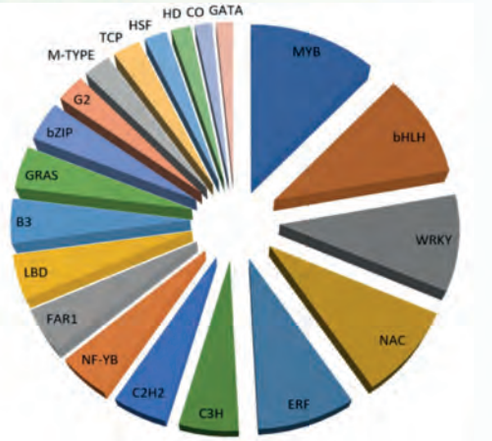


Fig. 1.23: The first twenty transcription factors (TFs) identified in the DEGs

Machine learning approaches for discriminating anti-nutritional proteins from nutritional proteins

Yuvaraj Iyyappan*, Sanchita Naha, Sarvjeet kaur and Ajit Kumar Shasany

Anti-nutritional elements are compounds that hinder the absorption of nutrients from plant products used as human food. These anti-nutritional factors can bind with nutrients and act as a major concern because they reduce the digestibility of protein, and nutrient bioavailability which leads to impaired gastrointestinal functions and metabolic performance. To increase the nutrition value we need to study the anti-nutritional factor proteins and to develop strategies for elimination and/or reduction of anti-nutritional factors in plants. The major antinutritional proteins present in plants are saponins, tannins, phytic acid, gossypol, lectins, trypsin inhibitors, protease inhibitors. In this study we have analyzed the performance of different machine learning algorithms such as support vector machine, random forest, and logistic regression, etc. for discriminating the anti-nutritional factor proteins and nutritional proteins. The study was performed to calculate the accuracy of different machine learning algorithms in discriminating anti-nutritional factor proteins than the others in a dataset of 5962 proteins (positive dataset: 3614 and negative dataset: 2348) (Table 1.3). The features used were amino acid composition, Di-peptide composition (DPC), Pseudo amino acid composition (PAAC), Composition-transition-distribution (CTD) and auto -correlation function. This study may be helpful to better understand the anti-nutritional factor proteins and to develop strategies for elimination and/or reduction of anti-nutritional factors in plants. The SVM based algorithm has performed with better accuracy of 92% in discriminating anti-nutritional factor proteins than the others in a dataset of 5962 proteins (Table: 1.3).

Table 1.3: Comparative analysis of performance of various machine learning methods such as Support Vector Machine (SVM), Logistic regression (LR), Random Forest (RF) in discriminating anti-nutritional proteins from nutritional proteins.

	Method	Sensitivity	Specificity	Accuracy	Precision	Matthews correlation coefficient
AAC	SVM	100	80	91	86	83
DPC	SVM	91	83	87	83	74
PAAC	SVM	96	82	89	85	78
CTD	SVM	83	87	85	81	70
AAC	LR	85	87	85	79	71
DPC	LR	90	92	91	87	82
AAC	RF	90	91	90	86	80
DPC	RF	93	90	91	84	81

2. BIOPROSPECTING AGRICULTURALLY IMPORTANT UTILIZABLE GENES

An insight about the evolution and domestication history of Indian Tea (*Camellia assamica* var. Masters *cv.* TV-1) genome

Hukam C. Rawal, Megha Rohilla, Abhishek Mazumder, Tilak Raj Sharma, Nagendra Kumar Singh, Tapan Kumar Mondal*

The origin and evolution of Assam type tea i.e. TV1 was studied with SNP based analysis of 150 genotypes of tea across the world to reveal the independent domestication events of Assam and China type (Fig. 2.1). Among the total predicted 30,039 high-confidence genes, 512 genes were found serving as positively selected genes during the course of domestication events between caffeine and non-caffeine group of Camellia species. The pan-genome analysis has detected several core and variable genes among 40 Camellia genotypes collected in India. A genome-wide study of four Camellia genomes has provided 400 fusion genes with an estimated rate of around 93 fusion genes per species per million years and no shared recent fusion events between Chinary (CSS) and Assam type tea (TV1). To make available the generated omics data of Indian tea, TV1 and Indian tea germplasm data to the research communities, we have developed the TIGeR web resource to host and provide the respective links of these different resources including miRNAs, lncRNAs, SSRs, TF, InDels, MITEs, ILPs, etc. The TIGeR was designed and implemented using HTML and CSS scripts along with MySQL database and Apache web server on an Ubuntu platform. Conclusively, this work provides the insight about the evolutionary history, domestication, gene evolution by fusion and genetic variation of Indian tea genome which will be immensely helpful to the tea breeders for marker assisted breeding.

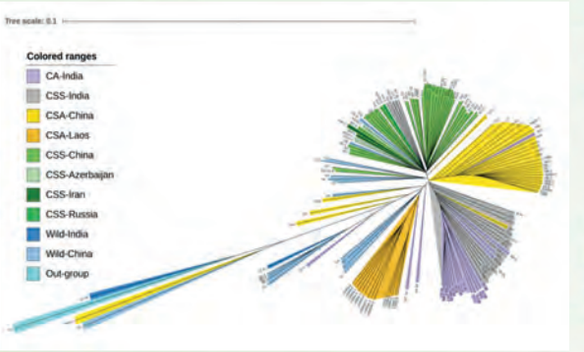


Fig. 2.1: SNP based evolutionary study across 150 tea genotypes clustering wild genotypes, Assamica type and China type tea into separate groups.

Identification and mapping of novel salinity tolerant QTLs of African rice (*Oryza glaberrima* Steud.)

Abhishek Mazumder, Megha Rohilla, Tapan Kumar Mondal*

Salinity is a major abiotic stress factor affecting the productivity of rice worldwide. With increasing land degradation and sea water intrusion in the low lands, there is a need to improve agricultural productivity from saline soils. With this background, a bi-parental $F_{6:7}$ RIL population had been developed by crossing two contrasting parents i.e. IR 29/African rice (Accession no. TKM 239) and advancement of the F_1 hybrid upto $F_{6:7}$ generation. Phenotyping of the mapping population (79 RILs, GKS-188-8-$F_{6:7}$) in seedling stage in hydroponics Yoshida solution (Yoshida, S et al. 1976) with 100 mM NaCl (EC ~11.65 dS/m) stress

and control (EC ~0.8 dS/m) at 25 DAS reveals that a total of 16 lines were highly tolerant (SES = 3), 38 lines moderately tolerant (SES = 5), 19 lines moderately sensitive (SES = 7) and 6 lines were highly salt-sensitive (SES = 9) (Fig. 2.2A). Different parameters related to seedling salinity had been recorded viz. root and shoot length, root and shoot fresh and dry weight, chlorophyll content from leaf, sodium (Na), potassium (K), Na/K ratio from the salt-stressed and control seedlings grown in hydroponics. Also, salinity screening experiment at reproductive stage had been conducted in high salinity (EC ~ 9.77 dS/m) and low salinity plots (EC ~ 2.50 dS/m) at ICAR-CSSRI, RRS, Canning town, WB (Fig. 2.2 B). Salinity-stress symptoms were visible in the population in high salinity plot. A few salt-tolerant lines had been identified.

In addition, 209 RILs were subjected to Genotyping by Sequencing (GBS) approach to identify SNPs and to construct a coarse genetic linkage map. After quality check with FastQC tool, a total of 83.24 Gb clean reads had been generated. Sequence reads were demultiplexed, and aligned using BWA tool with three reference genome assembly viz. Nipponbare (IRGSP), IR64 and *Oryza glaberrima* from NCBI database. The total number of filtered SNPs obtained by GATK pipeline were 5357, 5865 and 6109 in case of *O. glaberrima*, IR64 and IRGSP respectively. These filtered SNPs will be anchored in three different linkage maps corresponding to each reference assembly by JoinMap (v 4.1) software. The three linkage maps will further be used to construct a consensus map which will be used to locate different trait related QTLs across the genome of salt-tolerant African rice.

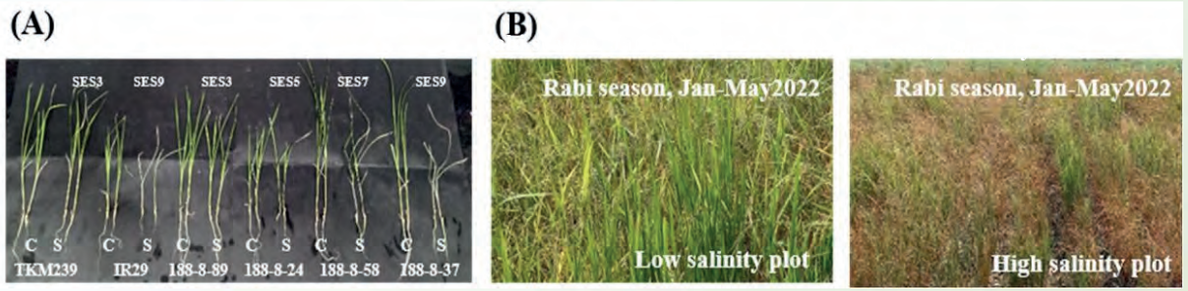


Fig. 2.2: Phenotyping of the mapping population (population size – 209) (A) Salinity screening at seedling stage in Yoshida solution. (B) Screening at reproductive stage in saline soil in experimental plot at ICAR-CSSRI, Canning town, WB. C- control, S – stress, SES for control – 1. SES – standard evaluation system for rice (IRRI)

Allantoin a purine metabolite involved in salinity tolerance of monocot *(Oryza sativa)* and dicot *(Arabidopsis thaliana)* through synergic activation of phytohormones

Soni Chowrasia, Jyoti Nishand, Rekha Mahato, Kanti Kiran, Nitasana Rajkumari, Hukam C. Rawal, Tapan Kumar Mondal*

Allantoin is a purine metabolite involved in nitrogen metabolism in plants, yet several reports in recent time indicate its involvement for various abiotic stress including salinity. Allantoin is chemically known as (1-2,5-dioxoimidazolidin-4-yl) urea or 5-ureido hydantoin and formed from the xanthine in peroxisome in the presence of key enzyme uricase. However, the detailed mechanisms of allantoin involvement in salinity tolerance in plants are not studied well. For the first time, we demonstrated the role of both exogenously supplied as well as endogenous allantoin in rendering salinity tolerance in rice and Arabidopsis via. induction of abscisic acid (ABA) and brassinosteroid (BR) biosynthesis pathways. Exogenous application of allantoin resulted in short-term salt-tolerance in salt-sensitive rice genotype (IR-29). Transcriptomic data of allantoin treated IR-29 under salinity stress induced ABA and BR biosynthesis genes (Fig. 2.3). Further, the

key gene of allantoin biosynthesis pathway i.e., *uricase* of the halophytic species *Oryza coarctata* was also found to induce ABA and BR biosynthesis genes when over-expressed in transgenic *Arabidopsis* after salt treatment. Thus, indicating that ABA and BR biosynthesis pathways are involved in allantoin mediated salinity tolerance in both monocot and dicot. Additionally, it has been found that several biochemical parameters were also associated with allantoin-mediated salinity tolerance in transgenic *Arabidopsis* (Fig. 2.4). These findings depicted the functional conservation of allantoin for salinity tolerance in both plant clades.

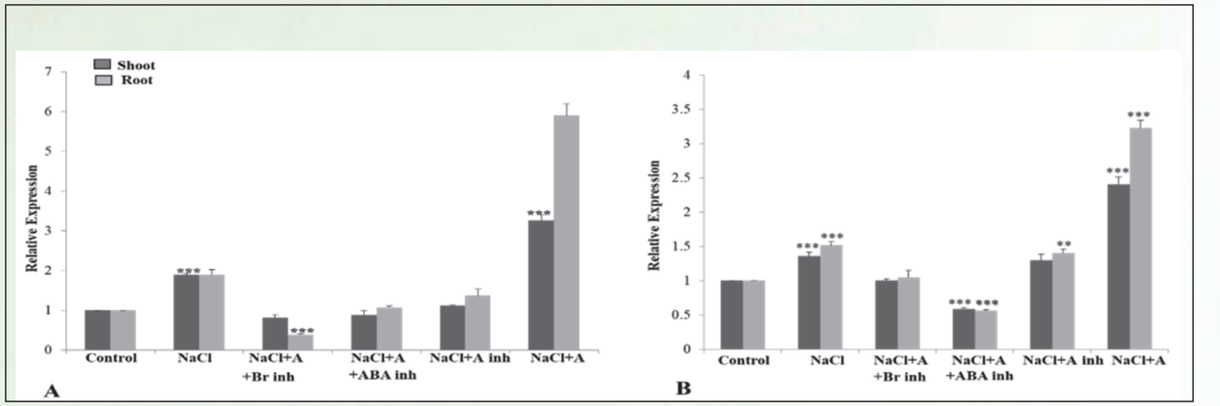


Fig. 2.3: Relative expression of A) Brassinosteroid (Br), and B) Abscisic acid (ABA) biosynthetic genes in shoot and root tissues. Each column represents mean value of three biological replicates and bars indicate SEs. *** and ** represents statistically significant differences as compared to control at value of *P < 0.0001* and *P<0.005* respectively (A- Allantoin and inh-inhibitor)

Allantoin mediated regulation of miRNAs for short term salinity stress tolerance in *Oryza sativa* L. cv. IR-29

Jyoti Nishad, Alok Kumar Panda, Soni Chowrasia, Chongtham Nirmala, Tapan Kumar Mondal*

Allantoin is a nitrogenous compound derived from purine catabolism that contributes to nitrogen recycling in plants. In rice, accumulation of allantoin in response to salinity stress has been reported. But the role of allantoin under salinity stress is not elucidated till now. To understand the effect of exogenous application of allantoin on miRNAs mediated short-term tolerance of salt sensitive rice genotype IR-29, the relative expression of 10

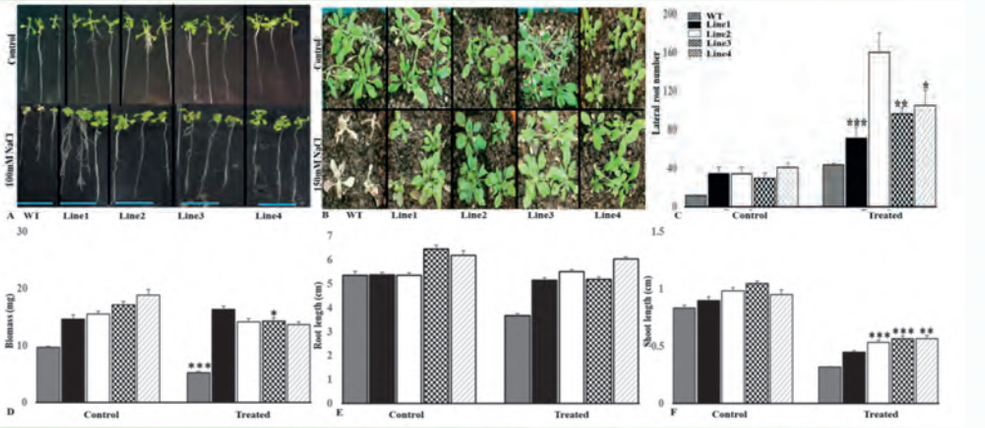


Fig. 2.4: The stress response of *Arabidopsis* transgenic plants overexpressing *Oc_urc* gene under salinity stress condition (100 mM-NaCl). A) Phenotyping of wild type (WT) *Arabidopsis* and *Oc_urc* transgenic lines in seedlings stage after 7 days of salinity treatment B) Growth response of 3 weeks old wild type *Arabidopsis* and *Oc_urc* transgenic lines after 10 days of salinity treatment C) Determination of lateral root growth D) Biomass determination E) Analysis of relative root length and F) Determination of relative shoot length. Each column represents mean value of three biological replicates and bars indicates SEs. ***, ** and * represents statistically significant differences as compared to control at value of *P< 0.0001*, *P< 0.001* and *P< 0.01*

salinity responsive miRNAs were investigated under salinity stress. The result demonstrates that several miRNAs changed their expression pattern in presence of allantoin under salinity (Fig. 2.5). The miRNAs such as osa-miRNA393a, osamiRNA414, osa-miRNA530 and osa-miR818a were down-regulated under salinity condition whereas all these four miRNAs were up-regulated under salinity along with allantoin treated condition indicating that this differential expression under allantoin may play an important role for salinity tolerance of IR-29.

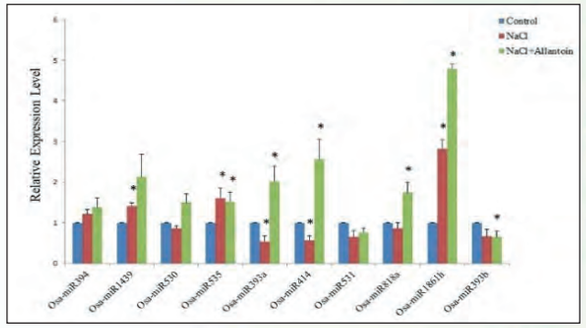


Fig. 2.5: (A) Expression analysis, using qRT-PCR, of selected salt responsive miRNAs in root tissue of salt sensitive rice genotype IR-29. (* indicates statistically significant change ($p < 0.05$) in expression).

Pan-genomic, transcriptomic, and miRNA analyses to decipher genetic diversity and anthocyanin pathway genes among the traditional rice landraces

Hukam C. Rawal, Alok Kumar Panda, Tilak Raj Sharma, Tapan Kumar Mondal*

Black rice is famous for containing high anthocyanin while Joha rice is aromatic with low anthocyanin content from the North-Eastern Region (NER) of India (Fig. 2.6 A-B). The present study was aimed to understand the origin, domestication and anthocyanin biosynthesis pathways in Black rice using the next generation sequencing approaches. With the sequencing data, various analyses were carried out for differential expression and construction of a pangenome. Protein coding RNA and small RNA sequencing analysis aided in determining 7415 and 131 differentially expressed transcripts and miRNAs, respectively in NER rice. This study will aid in better understanding for decoding the theory of high or low anthocyanin content in different rice genotypes. With this study, not only the genome assembly of North-Eastern region of India rice landraces has been made available in the public domain but also the comparative pan-genomic analysis of 8 NER genomes along with 100 other genotypes of Indian origin comprising all 4 types of rice such as Aromatic, Aus, Indica and Japonica has provided a gene pool of NER specific genes along with genes specific to pigmented and non-pigmented NER rice seeds. This is the first

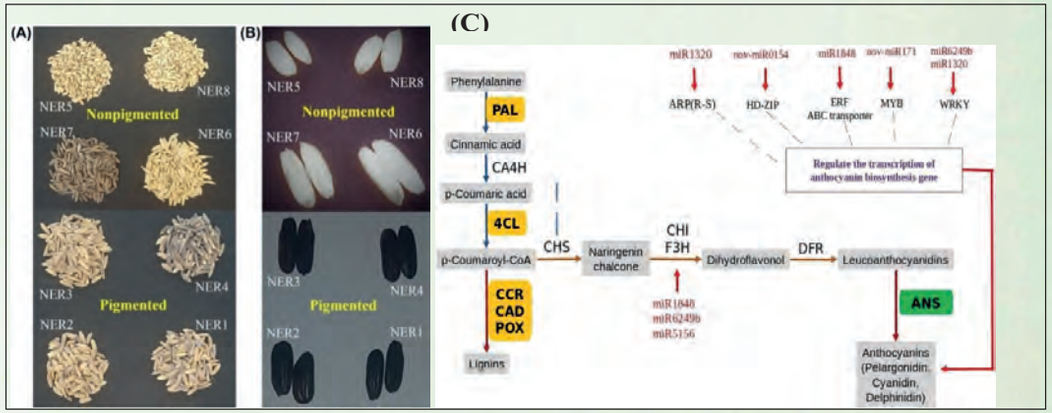


Fig. 2.6: Morphology of seeds of eight NER rice genotypes. (A) seeds' colors, and (B) kernels' colors. (C) Anthocyanin and lignin synthesis pathways in Manipur Black rice. The enzymes found differentially expressed in our dataset are represented in bold within colored boxes. Solid arrows are assigned to represent direct steps while contiguous lines are assigned for possible intermediate steps via some other genes. Enzymes abbreviations: PAL, Phenylalanine ammonia-lyase; CA4H, Cinnamic acid 4-hydroxylase; 4CL, 4-Coumaroyl CoA ligase; CHS, Chalcone synthase; CHI, Chalcone isomerase; F3H Flavanone-3-hydroxylase; DFR, Dihydroflavonol 4-reductase; ANS, Anthocyanin synthase; CCR, Cinnamoyl CoA reductase; CAD, Cinnamyl alcohol dehydrogenase; POX, Peroxidase.

report on the identification and expression analysis of miRNAs and their target genes that are regulating anthocyanin biosynthesis of black rice. These findings could further provide the functional characterization of miRNAs and targets in regulating anthocyanin biosynthesis.

Genome wide association mapping for identification of genes /QTL(s) associated with internode elongation under deepwater treatment using biparental mapping population of Ranjit and Negheri bao

Megha Rohilla, Abhishek Mazumder, Tapan Kumar Mondal*

Assam is a north-eastern state of India which gets flooded every year during monsoon season due to excessive rainfall. Therefore, farmers prefer to cultivate traditional, tall, photosensitive deepwater rice because of its unique internode elongation ability under water. Deepwater rice genotypes have poor yield performance under stressful environment. Therefore, discovering gene responsible for internode elongation and introgression in high-yielding rice cultivars to make sensitive varieties to elongating ones are important concern. We have made a cross of high yielding, deepwater stress sensitive rice cultivar "Ranjit" with the tolerant deepwater rice landrace "Negheri bao" for making RIL population. First, we have developed F_1 plant and from F_1, advancement of the generation upto 488 F_2 lines and further advancement of the F_2-derived F_3 population and seeds of the F_4 population have been harvested. The screening for deepwater rice treatment has been also completed to check the tolerant and sensitive lines from 488 $F_{2:3}$ lines (Fig. 2.7).

Genomewide identification of Histone Modification (HM) gene family and their expression patterns under abiotic stress and different developmental stages of tea (*Camellia assamica*)

Hukam C. Rawal, Jyoti Nishad, Tapan Kumar Mondal*

Histone modifications (HMs) play important roles in the regulation of gene expression in every domain of life. Tea (*Camellia assamica*), a woody perennial plant, is exposed to various stresses during its different developmental stages and HMs are expected to play crucial roles in its adaptation under different stress conditions. Characterization of tea HM genes is still unexplored, therefore in the present study a complete genome-wide characterization of HM genes was performed. Around 77 HM genes representing 10 subfamilies were identified. Further, detailed characterization of gene structures, identification of conserved motifs, and domains, phylogenetic analysis, identification of *Cis* elements and localization in the chromosomes were carried out. Comparative analysis has showed that *C. assamica* had nearly half of the HM genes

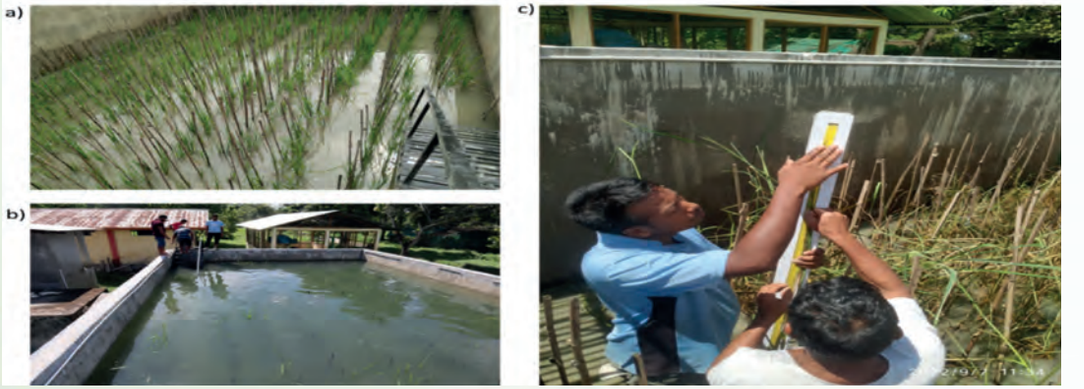


Fig. 2.7: Screening of RIL population. a) Transplanting of 488 lines of $F_{2:3}$ population in deepwater tank b) Deepwater treatment at 6 leaf stage in deepwater tank c) Data collection for various phenotypic traits after 14 days of deepwater treatment

(77) as compared to the number found in China type tea, i.e., *C. sinensis* (151). Protein–protein interactions showed that HM genes of *C. assamica* participated in different plant developmental processes, seed dormancy, phytohormone signaling and biotic as well as abiotic stresses. Gene expression analysis through qRT-PCR from five different developmental tissues of tea plant and under three different abiotic stresses (cold, dehydration and salinity) showed that 26 family members had differential expression indicating their involvement in various developmental stages or responses under different abiotic stresses in tea plant.

Introgression of salinity tolerance QTL in Rice mega variety IR64 and *Sambha Masuri*

Amit Singh, Nivesh Kumar, Nitin, Krishnmurthy S. L., Deepak Singh Bisht, Nagendera K. Singh, Vandna Rai*

75 BC_1F_2 families were phenotyped for salinity tolerance (SES score) in Phytotron and yield under stress in field microplots. A significant and novel QTL for yield under salt stress, *qSTY11.1* with positive allele contributed by NKSWR 173, was mapped on chromosome 11, explaining 22 percent of the phenotypic variance (Fig. 2.8). For introgression of QTL, a highly tolerant BC1F2 line was backcrossed to IR64 and simultaneously with Sambha Masuri. Seeds of the cross are planted during this year. The introgression will be confirmed using the markers flanking the QTL region. Allele specific KASP markers were designed for the four QTLs (Table 2.1) for grain yield, plant height, and for seedling stage salt stress tolerance (Fig. 2.9). The KASP study is underway now.

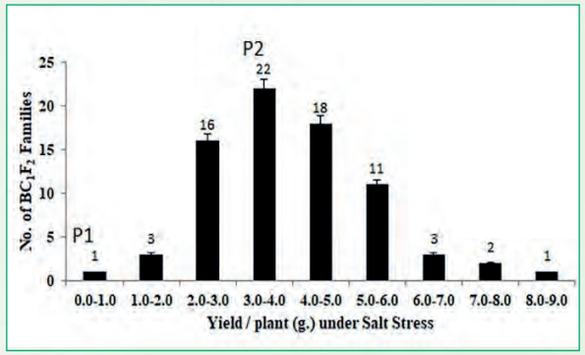


Fig. 2.8: The frequency distribution of yield and related traits in BC1F2 families was evaluated under reproductive stage salinity stress in soil microplots. P1:I R64 (Did not survive), P2: NKSWR 173

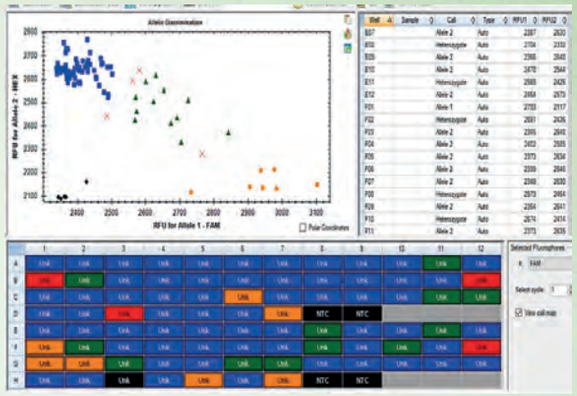


Fig. 2.9: KASP assays for markers of *qPHT8.2* and *qSIS3.2*

Identification of QTL(s)/gene(s) for salt stress tolerance in pigeonpea

Nivesh Kumar, Keshav Tiwari, Pragya Mishra, Deepak Singh Bisht, R. S. Raje, Nagendera K. Singh, Vandna Rai*

C. *cajan* genotypes were screened for salt stress tolerance and tolerant and susceptible genotypes were selected which were further employed for generation of recombinant inbred lines population (Fig. 2.10 A). An

Table 2.1 QTLs and KASP markers designed for fine mapping.

Trait	QTL	Peak Marker	Affy ID	Chr
Grain yield under salt stress (from NKSWR173)	*qSTY11.1*	SCR200 – Os11g32720	AX-95930579	11
Seedling stage salinity tolerant QTLs from IR64	*qSIS1.1*	CSCWR - Os01g48720	AX-95942413	1
Plant Height (from IR64)	*qPHT8.2*	CSCWR – Os08g39380	AX-95930579	8
Seedling stage salinity tolerant QTLs from IR64	*qSIS3.2*	CSCWR - Os03g07870	AX-95962416	3

F_2 population (250) was developed with PUSA992 (salt tolerant) X Pusa Arhar 16 (salt sensitive). The seeds of F2 population (250) were sown of which 140 were germinated. The germinated seedlings were further subjected to 150 mM NaCl stress. Survival of the seedlings was analyzed (Fig. 2.10 B).

Fig. 2.10: Effect of salt stress on three genotypes of pigeonpea Pusa Arhar 16 (sensitive), Asha (moderately tolerant), and Pusa 992 (tolerant) (A), screening for salt stress tolerance of the 250 recombinant inbred lines (RILs) developed using Pusa Arhar16 X Pusa 992

Identification of genes for salt stress tolerance in pigeonpea

Keshav Tiwari, Nivesh Kumar, Pragya Mishra, Deepak Singh Bisht, R. S. Raje, Nagendera K. Singh, Vandna Rai*

A comparative proteomic analysis led to the identification of glycine betaine synthesis gene Choline Monooxygenase (CcCMO) in *Cajanus cajan* for salinity stress tolerance. Synthesis of glycine betaine (GB) and choline were quantified after NaCl stress and with precursors glycine, serine and choline to further investigate the pathway. GB and choline showed significant increase when seedlings were grown with NaCl and serine; NaCl with choline (Fig. 2.11). The result confirmed the presence of choline to glycine betaine synthetic pathway in *C. cajan.* qRT-PCR was done for the genes present in GB pathway: betaine aldehyde dehydrogenase (BADH), choline monooxygensae (CMO), phosphoetahnol amine phosphatase (ChoPh), choline ethanol amine kinase (Ethkin), serine hydroxy methyl trasnferase (SHMT), and methionine synthase (SAMS) (Fig. 2.11).

After identifying the glycine betaine synthesis pathway in *C. cajan*, choline monoxygensae (*CcCMO*) gene sequence was extracted for *C. cajan* database and primers were designed for gene amplification (Fig. 2.12). *CcCMO* was cloned and sequenced and further transformed to protein expression vector. The studies are going on to check the expression of protein and salt tolerance of *CcCMO.*

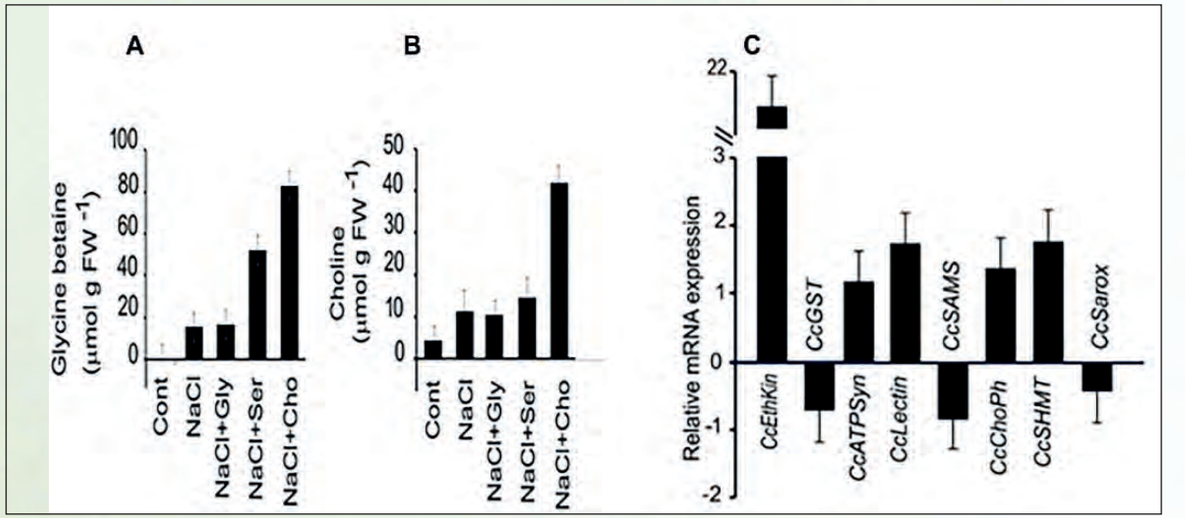


Fig. 2.11: Effect of salt stress on the glycine betaine (A), choline (B), and relative expression of genes (C) in control and salt-stressed seedlings of *C. cajan*

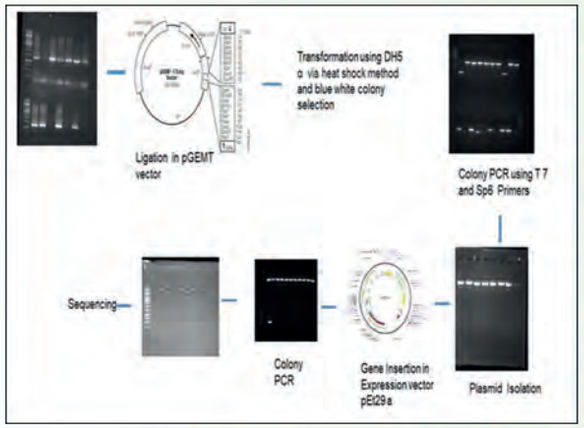


Fig. 2.12: Cloning of choline monooxygenase (CcCMO) gene from pigeonpea.

Design of pegRNA (prime editing guideRNA) and cloning to generate CRISPR Prime based constructs for editing *TFIIAγ5* in rice

Pyla Bhuvaneswari, Naveen C Gupta, Akshay Taluqdar, Prasanta Dash and Rhitu Rai*

CRISPR-Prime editing gains an edge over other genome editing techniques due to its ability to bring about all kinds of base alterations, besides small insertions and deletions. Out of the four major susceptibility genes facilitating *Xanthomonas oryzae* pv *oryzae* in rice to cause bacterial blight disease, three are sucrose transporters (SWEETs) and function in a race specific manner. Editing the SWEETs in their promoter region by deletions results in resistance derived from lack of susceptibility. The fourth S gene is a Transcription factor, TFIIAγ5. A single amino acid change in the coding region (V39E) of this TF does not disrupt its function as TF but disables its function of a chaperone for TAL effectors, thus leading to non race specific broad spectrum resistance. Our work involves use of PE technology for editing OsTFIIAγ5 (Xa5) by specific consecutive two base pairs swap to change Valine at 39th position to Glutamic acid and thus result in resistant allele of OsTFIIAγ5 (xa5). Prime editors involve optimized selection of three components viz. primer binding site, reverse transcriptase template and the sgRNA in context of the target site, unlike only sgRNA in CRSIPR-Cas. For better stabilization of RNA-DNA complex, a PBS of 30C is recommended, but screening the OsTFIIAγ5 target site, PBS lengths of minimum 32C were available, therefore same was selected. An 18 nucleotide long RT template was chosen for higher homology needed for a bp swap. Glutamic acid is encoded by GAA and GAG. In our pegRNA design, we included the codon GAG to enable detection of edit by amplicon digest as GAG created a site for enzyme *Smo*I (Fig 2.13a).

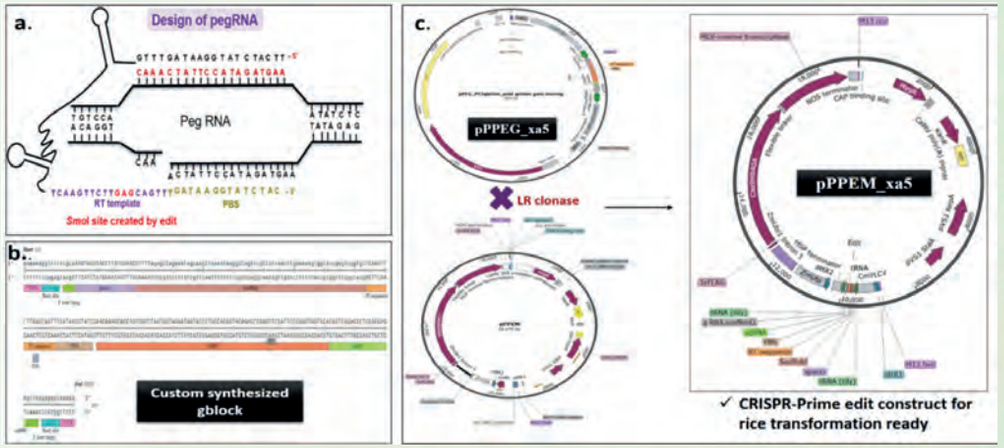


Fig. 2.13: Design and generation of prime edit construct for 2bp swap in *OsTFIIAγ5*. a) Schematic representation of pegRNA design bound to target site in *OsTFIIAγ5* genomic DNA sequence. b) Custom synthesized DNA sequence for pegRNA-scaffold-RT-PBS-tRNA- sgRNA, flanked on both ends with *Bsa*I site and 4bp compatible ends for cloning into entry vector pPPEG by Golden Gate method. c. Generation of PE binary vector construct by Gateway cloning.

We chose PE3 strategy which employs two sgRNAs, other one for making nick on the 2nd strand also. The two sgRNAs were expressed under the CmYLCV Pol II promoter as a single polycistronic gene separated by t-RNA, so that the endogenous tRNA processing enzymes process the polycistronic transcript to release individual gRNAs efficiently (Fig 2.13b). The Golden Gate and Gateway cloning techniques were utilized to generate entry (for expression of pegRNAs) and destination (binary vector expressing PE enzymes and the pegRNAs) constructs (Fig 2.13c).

To validate the constructs, we isolated good quality and high yield of protoplasts (10^8/ml) from rice variety MTU1010. First using a screenable marker, GFP, we transformed rice protoplasts (PEG mediated) at moderately high efficiency levels (Fig. 2.14a) thus establishing a functional protocol for rice protoplast isolation and transformation in our lab.

For testing the efficacy of design and efficiency of construct, a 414bp long amplicons flanking the edit site were generated from edit construct transformed rice protoplasts and subjected to *Smo*I digestion, expected to yield an additional band of 200 bp if edited (Fig. 2.14b). However, only the undigested original allele band of

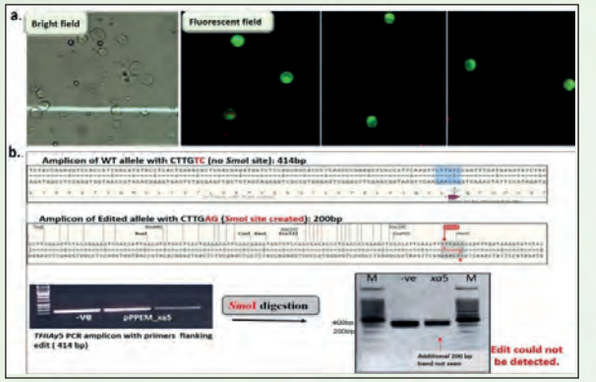


Fig. 2.14: Transient expression in rice protoplasts. a) Evaluation of protoplasts for quality and quantity using hemocytometer under microscope in bright field and GFP transformed protoplasts. b) Amplicon digest based screening for edit in *OsTFIIAy5* gene.

414 bp was visible on the agarose gel, with no signs of any additional band (Fig. 2.14b). The edit could not be detected by amplicon digest. The failure in detecting the edit by amplicon digest can be attributed to many factors including possibly low levels of editing efficiency.

Comparative transcriptome analysis deciphered variable defense response in *Brassica juncea* L., *Sinapis alba* L. and *Camelina sativa* L. after infection by *Alternaria brassicae*

Sandesh Waghmare and Anita Grover*

A. Brassicae is most serious and widely spread pathogen of *Brassica* and it leads to about 60% yield loss at field level. There is no source of resistance against *A. brassicae* among the sexually compatible relatives of *B. juncea.* However higher resistance to *A. brassicae* is shown in *C. sativa*, a wild relative of Indian mustard and moderate resistance is observed in *S. Alba.* The molecular mechanism underlying activation of plant defense responses are complex and understanding how plants defend themselves against a range of pathogen is therefore of crucial importance for crop improvement.

Alternaria disease progression was observed in infected *B.juncea*, *S. alba* and *C. sativa* plants and mock inoculated control plants from 0 hr to 15 days. In infected *B. juncea* chlorosis started after 3dpi and disease symptoms appeared after 6dpi. Clear necrotic lesions developed after 8dpi and 9dpi in *S. alba* and *C. sativa* respectively. After 9dpi the diameter size of lesion was 1.4 cm in *B. juncea*, 9 mm in *S. alba* and 7 mm in *C. sativa*. Tissue necrosis was estimated by tryphan blue staining. In tryphan blue stained leaves, necrosis was observed after 24 hpi in *B. juncea*. In *S. alba.* it was observed after 48hpi while in *C. sativa* it appeared after 72hpi. Highest level of necrosis was observed in *B. juncea* after 8dpi. In mock inoculated plants of three species

necrotic lesions were completely absent (Fig. 2.15). RT- PCR showed the differential expression of PR1 and PR12 gene at different time point interval in *B. Juncea* and *C. sativa*. In *B. juncea* PR 1 was induced but in *C. Sativa* PR1 was not expressed significantly. PR 12 was not induced significantly in *B. juncea* but higher levels of expression were seen in *C. Sativa* (Fig. 2.16). RNAs of samples extracted from stage 24 hpi, 48 hpi and 96 hpi were pooled together and RNA sequencing was done. Analysis of transcriptome data gave differentially expressed genes in *B. juncea, S. alba* and *C. sativa*. In *B. juncea* 50 defense related genes were activated after *A. brassicae* but in *S. Alba*, 298 genes and in *C. sativa* 282 genes were activated with significant fold value compared to uninfected plants. These findings suggest that *S. alba* have moderate and *C. sativa* have potential in built defense mechanism against *A. brassicae* compared to *B. juncea.*

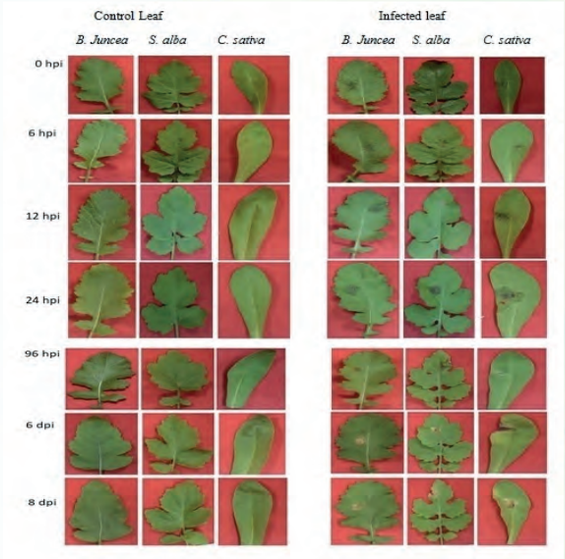


Fig. 2.15: Artificial inoculation of A.brassicae to B.juncea, S.alba and C.sativa

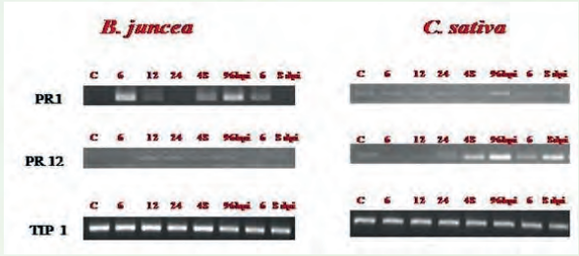


Fig. 2.16: Expression of PR1 and PR12 gene in *B.juncea* and *C.sativa* after infection of *A.brassicae*

Genome wide scan for vital insecticidal genes in different Brassica species

Deepanshu Jayaswal, Rekha Kansal*

The plant origin based genes especially from leguminous family viz. chickpea lectin (CHPL) and urdbean protease inhibitor (UPI) are known for their insecticidal activities. The whole genome survey was conducted to find the CHPL and UPI homologous genes in the Brassica genome. The *Brassica rapa* genome (annotated) was used as reference. The conserved domain of both the insecticidal genes was scanned in *Brassica rapa, B. nigra* and *B. juncea* genome. The UPI domain containing homologs was not found in all the three Brassica genome. On the other hand, 98, 387 and 636 candidate genes were found to possess conserved domain of CHPL in the *Brassica rapa, B. nigra* and *B. juncea* genome respectively. Only one CHPL orthogroup was found in all the three Brassica genome. The phylogenic study revealed that the chickpea lectin is distantly related to Brassica lectin and present in different clade (Fig. 2.17).

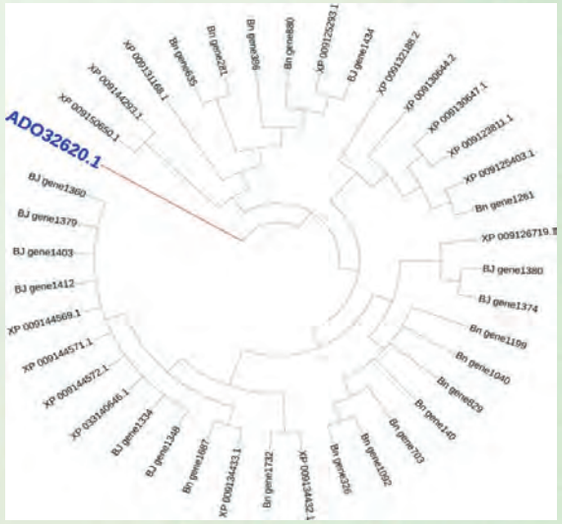


Fig. 2.17: Phylogenetic analysis of the CHPL and their orthologs found in different Brassica species

Development of F2:F3 population of *Brassica juncea* and their Aphid Bioassay

Deepanshu Jayaswal, Rekha Kansal*

The transgenics in *Brassica juncea* had been developed using CHPL and UPI genes. The lines were molecularly analyzed and the homozygous lines were used as parent and breeding was done to stack the genes for developing aphid resistant Brassica. 270 plants of *Brassica juncea* containing both the CHPL and UPI transgenes in homo or heterozygous condition were grown in the pots kept in the net house of NIPB. The screening of plants was done with gene specific PCR and non-segregating line was selected by progeny to row testing. The plants of selected line were used for Aphid Bioassay. The mortality and natality data were recorded and statistically analyzed (Fig.2.18). The F3 plants showed more than 85% mortality and there was a significant reduction in natality of *Lipaphis erysimi* compared to control plants of *Brassica juncea*.

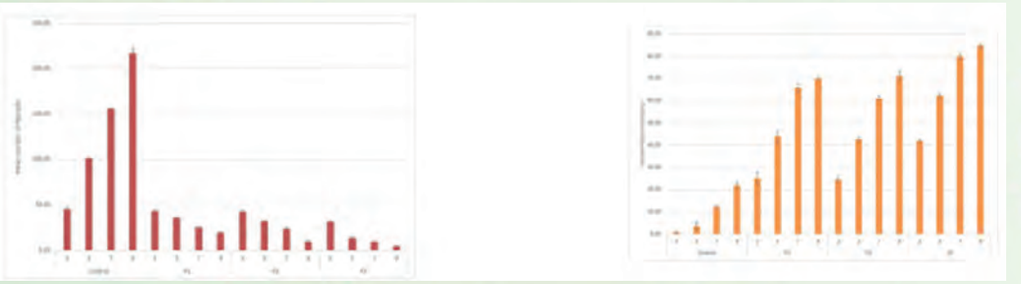


Fig. 2.18: Aphid bioassay of F3 plants of Brassica juncea (a) natality of Lipaphis erysimi after 3, 5, 7, 9 days after infestation (b) mortality of Lipaphis erysimi after 3, 5, 7, 9 days after infestation

Transgenerational analysis of heat stress memory and modulation of ubiquitin proteasome system after seed priming with cytokinin

Praful Jaiswal and Sharmistha Barthakur*
(Jaiswal et al. 2022, Plant Growth Regul)

To identify and assess the effects of cytokinin under high temperature, seeds of wheat cv. HD 2967 were primed with 50 mg L^{-1} Kinetin with and without additional heat priming at 42 °C. Primed seeds were raised in season in field for two continuous years and exposed to elevated temperature intermittently in an indigenously built heat trap chamber at anthesis for three consecutive days. Extensive phenotyping of a series of agronomic, biochemical and physiological parameters were recorded. To assess transgenerational effect of priming, in the next year, the primed harvested seeds were sown in two staggered timings in season and similar data collected. Correlational analysis showed positive correlation between chlorophyll index and grain traits ($r = 0.866$, 0.892; $p < 0.001$). Survival of plants under stress relies heavily on proteomic plasticity.

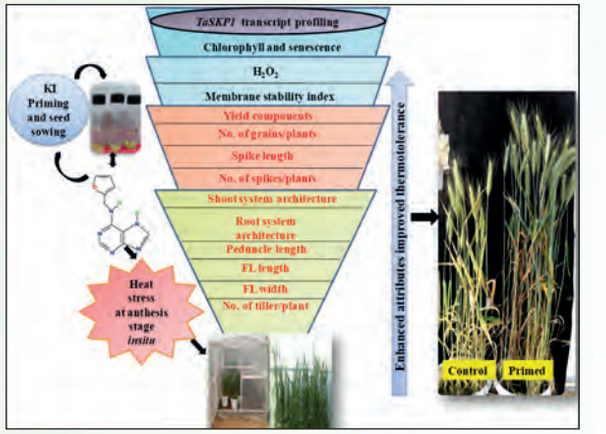


Fig. 2.19: Positive effect of Kinetin seed priming on wheat cultivar HD2967 under ambient and further *in situ* exposure in a heat trap chamber at anthesis stage in field condition. In the figure depicted in chronological order from bottom to top; plant architecture system- No. of tiller/plant, flag leaf length (FL length), Flag leaf width (FL width), Peduncle Length; Root architecture system- Root length, Root volume, Root surface area (RL, RV, RSA), Yield components-number of grains per plant, Spike length, No. of spikes/plants; physiological aspects- H_2O_2, membrane stability index, chlorophyll, flag leaf senescence and *TaSKP1* transcript profiling.

Ubiquitin proteasome system (UPS) plays a major role in maintaining this plasticity by critical interaction with plant hormones including cytokinins. Transcript expression profiling of SKP1 (S-phase kinase protein1), an essential component of SCF (SKP1-Cullin-F-Box) ubiquitin ligase, in the primed plants showed differential and positive induction indicating their involvement and reveals avenue for breeding heat tolerant wheat. The results document that seed priming with cytokinins can be utilized as an alternative strategy to ameliorate adverse effects of high temperature in wheat production towards sustainable crop productivity under changing climate scenario (Fig. 2.19).

Elucidating the role of Brassinosteroid under high temperature stress in wheat

Adil Rahim Magray and Sharmistha Barthakur*

Brassinosteroids (BRs) are steroid plant hormones that are essential for plant growth and development. This hormone controls the division, elongation and differentiation of various cell types throughout the entire plant life cycle and has the ability to mitigate various environmental stresses. Here, impact of heat stress on seedling stage of HD2967 and RAJ3765, two contrasting cultivars of wheat was studied after seed priming with 24-epibrassinolide and brassinozole-a BR inhibitor. Differential response was observed in root system architecture and chlorophyll content of the two genotypes. Transcript expression profiling of BZRI –a positive regulator of BR signaling and BRI1-a BR receptor showed upregulation under treatment of 24-epibrassinolide and heat stress whereas their response was downregulated after brassinozole priming. In general, results indicate significant role of BRs in modulating heat tolerance in wheat seedlings.

Genome wide analysis of *BREVIS RADIX* gene family from wheat (*Triticum aestivum*): A conserved gene family differentially regulated by hormones and abiotic stresses

Sneha Tiwari, Senthilkumar K. Muthusamy, Pranita Roy and Monika Dalal*

BREVIS RADIX is a plant specific gene family with unique protein-protein interaction domain. It regulates developmental processes viz. root elongation and tiller angle which are pertinent for crop improvement. In the present study, five BRX family genes were identified in wheat genome and clustered into five sub-groups. Phylogenetic and synteny analyses revealed evolutionary conservation among BRX proteins from monocot species. Expression analyses showed abundance of *TaBRXL1* transcripts in vegetative and reproductive tissues except flag leaf (Fig.2.20). *TaBRXL2, TaBRXL3* and *TaBRXL4* showed differential, tissue specific and lower level expression as compared to *TaBRXL1*. *TaBRXL5* expressed exclusively in stamens. *TaBRXL1* was upregulated under biotic stresses. *TaBRXL2* expression was enhanced under abiotic stresses including drought stress. *TaBRXL2* and *TaBRXL3* were upregulated by ABA and IAA in roots. In shoot, *TaBRXL2* was upregulated by ABA while *TaBRXL3* and *TaBRXL4* were upregulated by IAA. Expression levels, tissue specificity and response time

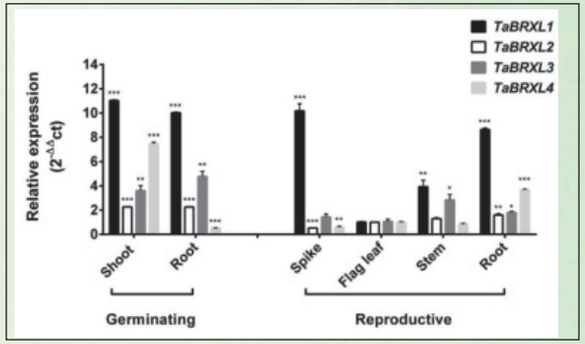


Fig. 2.20: Expression profile of *TaBRX* genes at different developmental stages. Quantitative RT-PCR analysis was performed in shoot and root tissues at germinating stage (2-days-old seedlings) and reproductive stage (full spike emergence) of wheat (*Triticum aestivum*)

under different conditions suggests distinct as well as overlapping functions of *TaBRX* genes. This was also evident from global co-expression network of these genes. Further, TaBRX proteins exhibited homotypic and heterotypic interactions (Fig.2.21) which corroborated with the role of BRX domain in protein-protein interaction. This study provides leads for functional characterization of *TaBRX* genes.

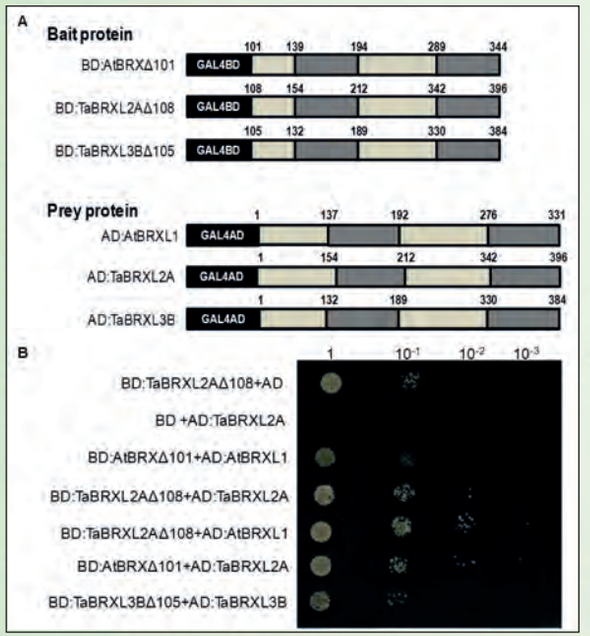


Fig. 2.21: *In-vivo* interaction between BRX proteins. (A) Schematic representation of truncated bait protein constructs and full-length prey protein constructs. (B) Homotypic and heterotypic interaction of BRX proteins

Identification and characterization of Circular RNAs in chick-pea

Gopal Kalwan, Kishor Gaikwad and Pradeep Kumar Jain*

Circular RNAs (circRNAs) comprise a large class of non-coding RNAs that lack free 3′ and 5′ ends, are covalently closed and produced by a non-canonical splicing event called back splicing (Fig. 2.22). Earlier circRNAs were regarded as junk RNAs produced due to splicing errors but recent progress in circRNA research indicated their considerable roles in various biological processes. circRNAs have also been found to be involved in various abiotic and biotic stress responses in plants. Keeping all such diverse role in mind we performed the circRNA analysis using available transcriptome data of seed protein content (SPC) contrasting genotypes. Circular RNA identification was done using two pipelines CIRIQUANT (https://github.com/bioinfo-biols/CIRIquant) and CLEAR (https://github.com/YangLab/CLEAR). A total of 1377 circular RNAs were predicted using these two algorithms, which are encoded from different genomic regions such as exonic, intronic and intergenic. Huge variation was observed in the number of circRNA predicted from these regions, exonic region showed highest number when compared with other genomic regions, which is due to use of only RNA seq data i.e., only consist of coding region. A proper understanding of the role of circRNAs in different aspects of plant development and stress response will provide novel ways to manipulate plants for their enhanced survivability and productivity in adverse climatic conditions.

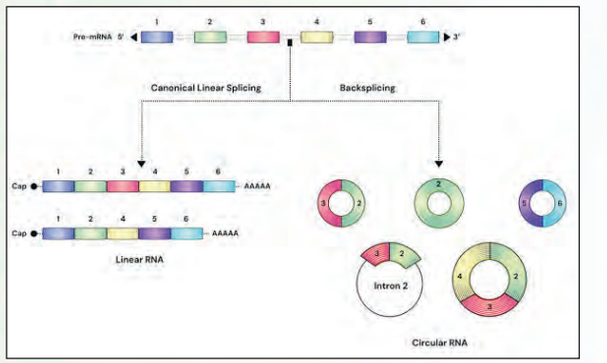


Fig. 2.22: Schematic presentation of origin of circular RNA.

Genome-wide identification and expression analysis of the GRAS gene family in response to drought stress in chickpea (*Cicer arietinum* L.)

Sheel Yadav, Yashwant Yadava and Pradeep Kumar Jain*

The GRAS (gibberellic acid insensitive, repressor of GAI and scarecrow) transcription

factors (TFs) regulate diverse biological processes in plant growth and development and also regulate gene expression in response to various abiotic stresses like cold, drought, etc. In chickpea one of the most devastating abiotic stress is terminal drought. The GRAS TF family has not been characterized in chickpea (*Cicer arietinum* L.) until now. In this study, we report 46 GRAS TF genes (CaGRAS genes) in the chickpea genome. The CaGRAS proteins were categorized into nine subfamilies based on their phylogenetic relationship with known GRAS members of *Arabidopsis* and soybean. The PAT subfamily was the largest consisting of ten CaGRAS members whereas the LAS subfamily was the smallest with only one member. Gene duplication analysis revealed that segmental duplication was the primary reason for the expansion of this gene family within the chickpea genome. The gene expression levels of CaGRAS genes were analysed using two different chickpea varieties contrasting for drought tolerance trait, i.e., ICC 4958 (drought tolerant) and ICC 1882 (drought sensitive). These two genotypes also differed in their root morphologies, under well-watered and drought stress conditions. The gene expression analysis revealed a potential role of PAT, SCR, SCL3 and SHR GRAS members in the regulation of differential response to drought, in the root tissues, for both the genotypes (Fig. 2.23). CaGRAS 12 (SCR) was identified as a drought-responsive GRAS TF gene, which could serve as a potential candidate gene for utilization in developing chickpea varieties with improved drought tolerance. This study demonstrates the drought-responsive expression of CaGRAS genes in chickpea and also describes the morpho-physiological response of chickpea plants to drought stress conditions.

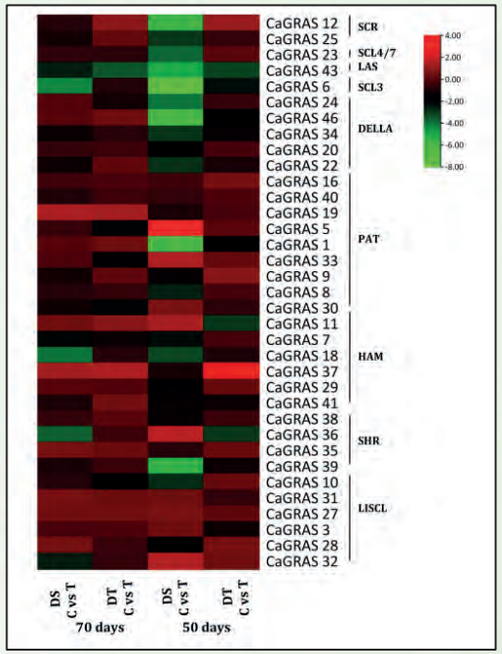


Fig. 2.23: Gene expression profiles of CaGRAS genes in root tissues in plants subjected to drought stress treatment; DT: drought tolerant variety; DS: drought sensitive variety; the FPKM values were transformed into log2FC and comparisons between the control (C) and stress (T) conditions were made for each of the genotypes.

RNAi-mediated silencing of the root-knot nematode (*Meloidogyne incognita*) effector genes, Mi-msp10 and Mi-msp23, confers resistance in *Arabidopsis* and impairs reproductive ability of the root knot nematode

Anil Kumar, Anil Sirohi and Pradeep Kumar Jain*

Root-knot nematodes (RKNs) are the most damaging pathogens severely affecting global food production. The sustainable options to minimize menace of nematode populations through economically feasible measures are limited. Thus, the development of innovative and target-specific strategies that aid in their management is imperative. RNAi technology has emerged as a sustainable and target-specific alternative to control phytonematodes. Here, we characterized two novel subventral gland and dorsal gland-specific effectors, Mi-msp10 and Mi-msp23, to determine their potential effectiveness in controlling *M. incognita*. Comparative developmental profiling using qRT-PCR revealed higher expression of both effectors in the adult nematode female. Furthermore, functional evaluation of Mi-msp10 and Mi-msp23 dsRNA cassettes was performed using host-delivered RNAi (HD-RNAi) in *Arabidopsis*. The transgenic lines were examined against

M. incognita, and the phenotypic effect of HD-RNAi was evident with a 61% and 51% reduction in gall formation in the Mi-msp10 and Mi-msp23 RNAi lines, respectively (Fig. 2.24). A significant drop in the nematode adult females by 59% for Mi-msp10 and 49% for Mi-msp23-RNAi lines was observed. Similarly, production in egg masses decreased significantly by 76% (Mi-msp10) and 60% (Mi-msp23) for the RNAi lines, which eventually decreased the reproductive factor by 92% and 75%, respectively. The gene expression

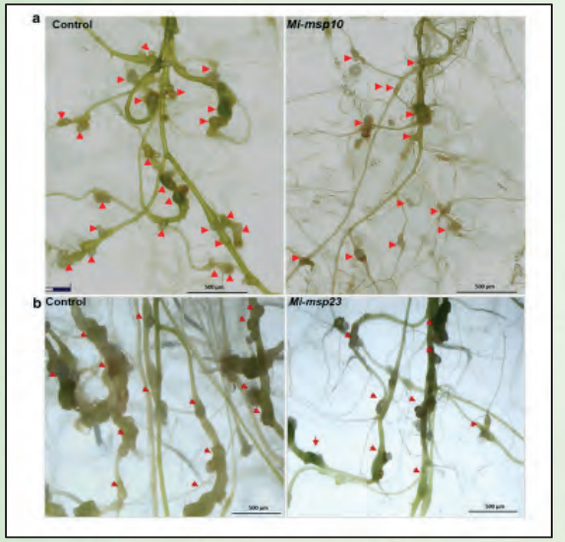


Fig. 2.24: *Meloidogyne incognita* infection assay in roots of *Arabidopsis* control plants and RNAi lines (Mi-msp10 and Mi-msp23). a) Roots of a control plant showed more galls compared to RNAi line of Mi-msp10. b) Roots of control plant showed more galls compared to RNAi line of Mi-msp23. Arrowheads indicate the galls.

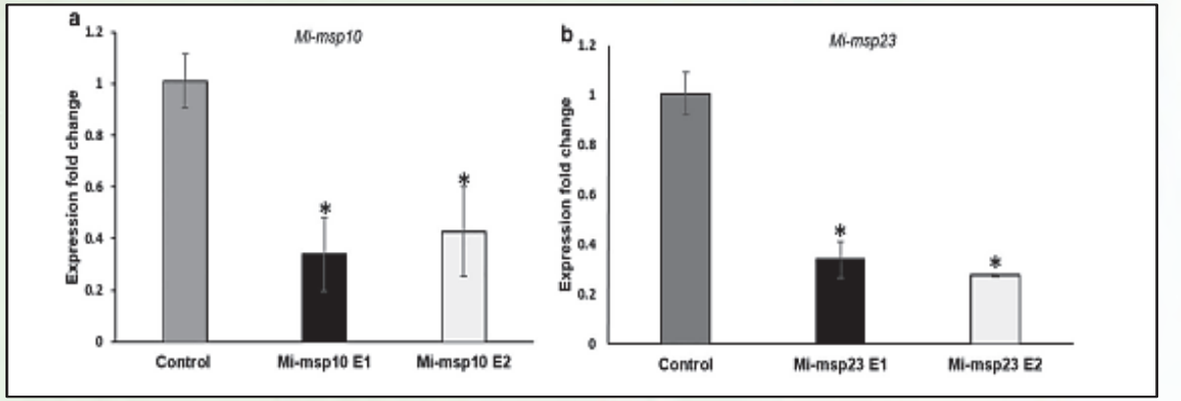


Fig. 2.25: qRT-PCR analysis of Mi-msp10 and Mi-msp23 genes expression in female nematode harvested from control and RNAi lines. a) Transcript reduction was observed in Mi-msp10 E1 and Mi-msp10 E2 as compared to control. b) Transcript reduction was observed in Mi-msp23 E1 and Mi-msp23 E2 as compared to the control. The data were normalized using *MiActin1* as a housekeeping gene. Each bar represents the mean ± SE. Asterisk denotes statistically significant differences (* $P \leq 0.05$) to control as per student's t test. All experiments were repeated twice with similar results.

analysis showed a significant decrease in the transcript level by up to 72% (Mi-msp10) and 66% (Mi-msp23) in *M. incognita* females feeding on RNAi lines, providing further evidence of effective gene silencing (Fig. 2.25). Overall, our findings provide useful information and support further development of RNAi-based strategies to control *M. incognita*.

Screening of Indian *Bacillus thuringiensis* isolates from diverse sources for the presence of candidate insecticidal genes

Rishika K.S., Dr. Sarvjeet Kaur*

Bacillus thuringiensis (Bt) is a gram-positive, ubiquitous entomopathogenic, spore-producing bacterium carrying insecticidal *cry*, *cyt*, and *vip* genes which have specific toxicity towards different insect orders. The *cry1A*-type genes have been successfully deployed in transgenic crops. Isolation of new types of toxin genes is important due to development of resistance in lepidopteran pests towards these genes. The *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F* and *cry1G*-type genes have not been fully explored. Their presence in 63 *Bt* isolates recovered from different habitats in various agro-climatic zones of India has been investigated in this study. Presence of *cry1*-type genes in native *Bt* isolates

Table 2.2: Prevalence of *cry1*-type genes in native *Bt* isolates recovered from different Agro-climatic zones of India

S. No.	Agro-climatic Zone	No. of *Bt* isolates screened	No. of positive *Bt* isolates	*cry1*-type genes present	Prevalence (%)
1.	Trans Gangetic plain region	28	7	*1B, 1F, 1C, 1E, 1D*	25
2.	East Coast Plains and Hills Region	16	4	*1B, 1F, 1C, 1D*	25
3.	Upper Gangetic Plain Region	3	1	*1G*	33
4.	Lower Gangetic Plains Region	3	1	*1C*	33
5.	Eastern Himalayan Region	5	1	*1B*	20
6.	Western Himalayan Region	5	0	-	0
7.	Gujarat Plains and Hills Region	1	0	-	0
8.	Southern Plateau and Hills region	2	0	-	0

was examined by PCR amplification using different primers from conserved regions of these genes. Presence of one or more *cry1*-type genes *viz.*, *cry1B, cry1C, cry1D, cry1E, cry1F, cry1G* and *cry1I* was observed in 13 *Bt* isolates. The *cry1C*- and *cry1F*-type genes were found to be abundant as compared with other genes (Table 2.2). One *Bt* isolate each *viz.* SK-669 and SK-13 showed presence of *cry1G* and *cry1D* genes respectively. Four *Bt* isolates showed presence of full length *cry1I*-type genes.

Evaluation of toxicity of Vip3Aa proteins towards lepidopteran pests

Rishika K.S., Sarvjeet Kaur*

The *vip3Aa44*, *vip3Aa67*, vip3Aa69 and *vip3Aa70* genes were previously cloned in pET29a vector and expressed in *E. coli* in our laboratory and were found to be toxic to *Helicopverpa armigera* (cotton bollworm, podborer). Efficacy of these toxins towards other agronomically important Lepidopteran pests such as *Plutella xylostella* (diamondback moth), *Spodoptera litura* (cotton leafworm), and *S. frugiperda* (fall army worm) was examined this year. Vip3Aa44, Vip3Aa67, Vip3Aa69 and Vip3Aa70 proteins have shown toxicity in terms of percent mortality towards *S. litura* and *P. xylostella* in laboratory bioassays at 10 ppm concentration. LC_{50} (lethal concentration 50) of Vip3Aa44, Vip3Aa67, Vip3Aa69 and Vip3Aa70 proteins towards *S. litura* was determined (Table 2.3). Growth retardation and sub-lethal effects of toxins in terms of malformed, decreased and delayed pupation and adult formation were seen at different developmental stages in the whole life cycle of *S. litura*. This study indicates potential of these *vip3Aa* genes in deployment for crop protection.

Table 2.3 Lethal concentration LC_{50} (µg/g) of Vip3Aa toxins against *S. litura* evaluated after 7 days in laboratory bioassays

Treatment	LC_{50} µg/g	95% Fiducial limit		Slope+SE	Chi square value	Degrees of freedom
		Lower	Upper			
Vip3Aa44 (Vip2)	13.23	4.09	83.05	0.467±0.11	0.481	2
Vip3Aa67 (Vip_792)	11.45	3.11	94.63	0.416±0.11	0.099	2
Vip3Aa70 (Vip_851)	20.18	5.27	272.13	0.408±0.11	0.060	2
Vip3Aa69 (Vip_986)	3.53	1.00	13.05	0.477±0.11	0.066	2

Molecular modeling of Vip3Aa proteins

Ashfak Mujawar,Yuvaraj I, Deepak kumar Rathore, Sarvjeet Kaur*

Molecular modeling of three dimensional structures of deduced amino acids sequences of vip3Aa81, vip3Aa82, vip3Aa83, vip3Aa84, and vip3Aa85 genes (Bacillus thuringiensis Nomenclature Committee www.bpprc.com, NCBI GenBank accession numbers MZ191100, MZ191101, MZ191102, MZ191103, and MZ191104) cloned from Bt isolates, revealed that amino acids substitutions were in receptor binding domains of these genes.

Machine Learning based classification and prediction of genes responsible for nutrition in rice

Shbana Begam*, Soumya Sharma and Yuvaraj I.

Rice nutritional quality related genes were collected from the public domain and preprocessing like removal of noisy and filling the missing values etc was done. By using these collected datasets a classification model for nutritional rice genes has been developed using machine learning techniques like support vector machine and random forest. Both techniques are showing prominent results but support vector machine produce more accuracy as compared to random forests. This model will help to predict the nutritional gene and will classify the further in the nutritional categories.

A novel Artificial intelligence based model for coverage detection of raw NGS data

Shbana Begam*, Samarth Godara, Hukam C Rawal

Next-generation sequencing (NGS) data have become the most prevalent forms of sequence data due to the massively increased rate and amount of sequencing being done. A successful NGS experiment depends on several predefined factors, including read length, sequencing accuracy and coverage. Here, read length refers to the number of nucleotides present in a single read and sequencing accuracy refers to the quality score of reads. Whereas the coverage of the sequenced reads is determined in two forms, i.e., the coverage depth and breadth (Fig. 2.26). The coverage depth can be defined as the number of reads that align the known reference bases and coverage breadth is a fraction of the reference genome that can be assembled by the sequence reads. To calculate the coverage details at the raw level we are working on an AI-integrated model for rapid coverage profiling of genome sequence reads. Till now a pipeline has been developed that shows the actual workflow of the system. In this entire process, we are using a novel Hash Table-based mapping algorithm.

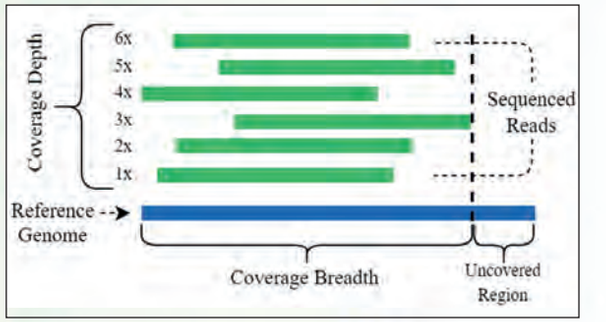


Fig. 2.26: Graphical representation of coverage profiling

3. NOVEL GENES AND BIOMOLECULES FOR PLANT AND HUMAN NUTRITION

Understanding plant-microbe interaction of rice and soybean with two contrasting diazotrophic bacteria through comparative transcriptome analysis

Manish Ranjan Saini, Latha P Chandran, Kalyani Makarand Barbadikar, Amitha Mithra Sevanthi V, Gautam Chawla, Megha Kaushik, Ekta Mulani, Amol Sarjerao Phule, Rajani Govindannagari, Bandeppa Sonth, Subodh Kumar Sinha, Raman Meenakshi Sundaram, Pranab Kumar Mandal* (Front. Plant Sci. 2022)

Understanding the beneficial plant-microbe interactions is becoming extremely critical for deploying the microbes in imparting plant fitness and achieving sustainability in agriculture. We have studied the early interaction of two diazotrophic bacteria *Gluconacetobacter diazotrophicus* (GAB) and *Bradyrhizobium japonicum* (BRH) with rice and soybean under low nitrogen medium. Root colonization of GAB in rice and in soybean were higher. Peroxidase enzyme activity increased initially but thereafter got reduced sharply in soybean and gradually in rice. The roots of rice and soybean inoculated with GAB and BRH harvested from five-time points were pooled and transcriptome analysis was executed along with control. Two pathways, 'Plant pathogen interaction' and 'MAPK signaling', were specific to RG whereas the pathway related to the nitrogen metabolism and plant hormone signalling was specific to RB in rice. Plant-diazotroph-specific transcripts *viz.* chitinase, brassinosteroid, auxin, MYB, nodulin, and NRT were common; three transcripts *viz.* NAR, thaumatin, and thionin were exclusive in rice and another three transcripts *viz.* NAC, ABA and ammonium transporter were exclusive in soybean. Based on the interaction, it can be inferred that the compatibility of rice and soybean is more with GAB and BRH respectively. It seems that rice is unable to identify the diazotroph as a beneficial microorganism from an early response, and expressed hypersensitivity-related transcripts along with PR proteins. The molecular mechanism of diazotrophic associations with rice and soybean will shed light on the basic understanding of host responses to beneficial microorganisms (Fig.3.1).

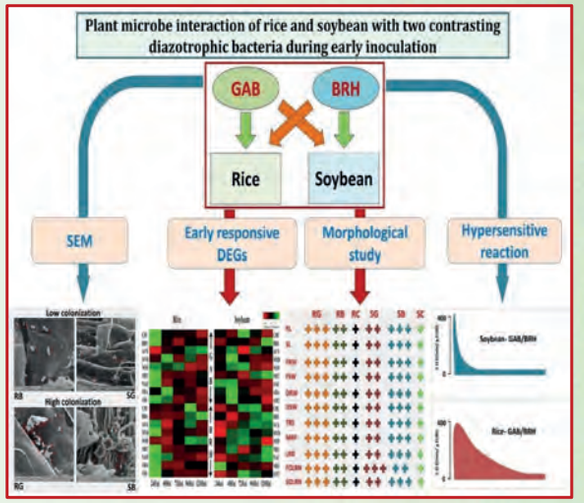


Fig. 3.1: Overall effect of BRH and GAB inoculation in rice and Soybean

Expression profiling of flavonoid biosynthesis genes in association with accumulation of flavonoid compounds in rice under water deficit stress

Karikalan Jayaraman, Amitha Mithra Sevanthi, Venkat Raman K and Pranab Kumar Mandal* (J. Environ. Biol. 2022)

To investigate whether the phenolic and flavonoid contents have any direct relation

with the water deficit stress tolerance in rice, and if so, then, which genes in the flavonoid biosynthesis pathway are involved in the tolerance mechanism was explored by comparing a well-known water deficit tolerant cv. Nagina22 (N22) along with a sensitive cv. Pusa Sugandh 2 (PS2) (Fig. 3.2 A & B). Water deficit stress was imposed on both the cultivars at the seedling stage by withholding water for 7days and the expression level of flavonoid biosynthesis genes (*OsCHS*, *OsCHI*, *OsF3H*, *OsF3'H*, *OsDFR*, and *OsANS*) and the accumulation of total phenolics and flavonoids content were investigated. qRT-PCR analysis revealed enhanced expression of all six major genes of the flavonoid biosynthesis pathway N22 compared to PS2 under water deficit stress. Accumulation of total phenolics and flavonoids compounds increased during water deficit stress in both the genotypes, but, it was significantly higher in N22 as compared to PS2. Enhanced drought tolerance is positively correlated with an enhanced transcription of flavonoid biosynthesis genes and higher flavonoid content (Fig. 3.2 C & D), suggesting that there could be differences in the genetic control of flavonoid metabolism in rice for drought stress.

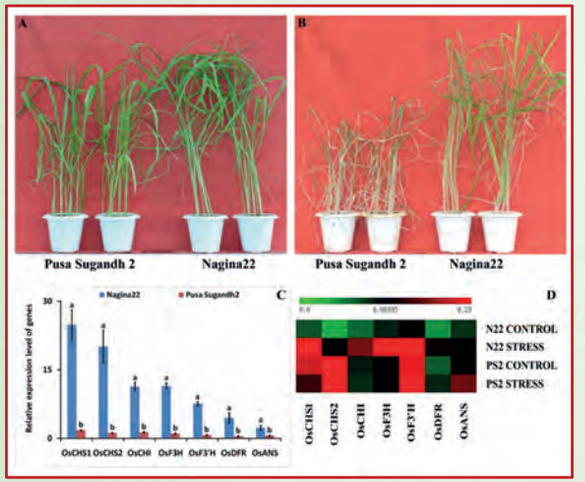


Fig. 3.2: Top: Phenotypical performance of contrasting rice (N22 and PS2) cultivar A. normal condition, B. under water deficit condition. Bottom: Expression profiling of genes involved in flavonoid biosynthesis pathway: (A) Relative expression (B) A heat map showing the transcript levels in N22 and PS2 under control and water deficit stress. The red colour in this figure indicates a high level of expression while green denotes low level of expression.

Comparative expression profile of genes encoding intolerant proteins in bread vs. durum wheat during grain development

Megha Kaushik, Ekta Mulani, Anju Mahendru, Govind Makharia, Sumedha Mohan and Pranab Kumar Mandal* (J. Plant Growth Regul. 2022)

Wheat proteins provide the nutritional requirement for human. The major concern associated with them is their intolerance among a large genetically predisposed population. These proteins include gluten, albumins like amylase/trypsin inhibitors (ATIs), serpins, thionins, and defensins. In this study, we performed a comparative (bread vs. durum wheat) expression analysis of the genes encoding for different intolerant proteins (IP) during grain development, through transcriptomics approach. All transcripts related to IPs were extracted; 146 and 133 transcripts were identified in case of bread and durum wheat respectively. However, only five IP genes were differentially expressed (DEGslog2fold>2) and all of them were found to be under the GO terms 'Molecular Function'. For comparative expression of the IP genes between the two genotypes, FPKM values of each gene were studied. These IPs, during grain development, belonged to the major categories of thionin, serpin, ATI, gliadin and glutenin. Bread wheat was expressing a greater number of IP genes and mostly they were upregulated in comparison to the durum wheat. Highest numbers of IP transcripts were mapped on the BB genome; and maximum IP genes were located in chromosome1D. IP genes with higher expression in both the genotypes were further analysed for their stage specific expression during grain development. We have also analysed the transcripts related to amylose and amylopectin biosynthesis, where durum wheat showed a high amylose:

amylopectin ratio in comparison to bread wheat (Fig. 3.3). This study is another support for the hypothesis that tetraploid durum wheat is less intolerant and better for consumption for the vulnerable population than that of hexaploid bread wheat.

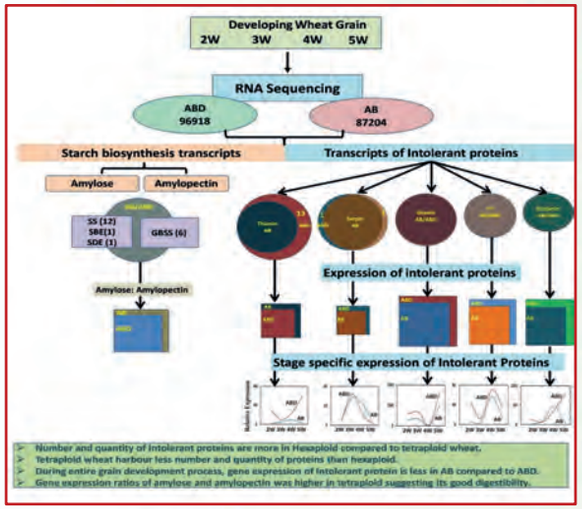


Fig. 3.3: Gene expression through RNAseq analysis followed by qRT-PCR revealed the difference in different intolerant protein and starch biosynthesis related genes between tetraploid durum wheat and hexaploid bread wheat.

Nitrogen stress induced TaDof1 expression in diverse wheat genotypes and its relation with nitrogen use efficiency

Alka Bharati, Gayatri Tehlan, Chetan Kumar Nagar, Subodh Kumar Sinha, Karnam Venkatesh and Pranab Kumar Mandal* (Cereal Res Commun. 2022)

Transcription factor Dof1 regulates Carbon (C) metabolism in the TCA cycle and also controls the NUE in the plant by ensuring optimum C-skeleton supply for N-assimilation. In this study, four diverse wheat genotypes were selected based on their varied response to N stress in a field experiment. These genotypes are subjected to N-stress in Pots in the next two consecutive years, where N stress level on year 2 was higher than year 1. Expression of *Tadof1* was studied in leaf tissue from two growth stages (30DAS: stage 1 and 30DAS: stage 2). *TaDof1* expression was found upregulated during year 1 and gradually down-regulated at stage 2 of year 2 when the N-stress was maximum. HS-277, an Efficient NUtE genotype, show relatively highest *TaDof1* expression under limited N-stress; however, the downregulation was also maximum under severe stress (Fig. 3.4). We also mesured GS2 NADH-GOGAT activity in the two contrasting genotypes HS-277 and VL-401 and results was also significantly different. Our result shows that role of *TaDof1* varies among the wheat genotypes, and this variation is probably one of the factors affecting N-assimilation and ultimately yield as well as NUE.

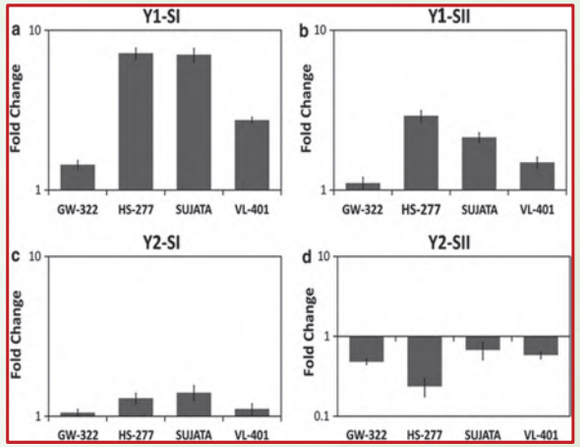


Fig. 3.4: Relative Expression of *TaDof1* gene in Y1-SI, Y1-SII Y2-SI, Y2-SII by qRT- PCR

Soil compaction affects root growth and gene expression of major N assimilating enzymes in wheat

Surajit Mondal, Shalom Christopher, Debashis Chakraborty and Pranab Kumar Mandal* (J. Soil Sci. Plant Nutr. 2022)

Soil compaction, detrimental to root proliferation and water and nutrient uptake, is difficult to detect due to its dependency on soil water. To know the primary effect on roots and subsequently, the responses of genes related to nitrogen metabolism as useful indicators a pot experiment was conducted with three soil compaction levels: no- (Bulk density: BD~1.4 g cm^{-3}), moderate- (BD~1.6 g cm^{-3}) and high-compaction (BD~1.8 g cm^{-3}); two nitrogen doses: with @150 kg N ha^{-1} and without nitrogen; and two wheat

genotypes: *Choti Lerma* (less N-responsive) and *HD-2967* (N-responsive). It was observed in seven weeks in pots that the root growth was severely restricted by compaction while genotype and N-doses had no effect. Higher compaction reduced root length density (RLD) of *Choti Lerma* by 25-41%. For the *HD-2967* genotype, compaction had no impact on RLD with +N but reduced with -N (13-25%; $p<0.05$). N-application reduced the adverse effect of high compaction on root volume density (RVD) in *Choti Lerma* compared to 28% ($p<0.05$) reduction in without N. Gene expression of N-assimilating enzymes varied in two contrasting genotypes. Under low N, *HD-2967* showed a gradual increase in gene expression related to N-assimilation, whereas, after upregulation to moderate compaction, gene expression was down-regulated in *Choti Lerma*. Genotype role was found to be greater in adjusting compaction as well as N stress (Fig. 3.5).

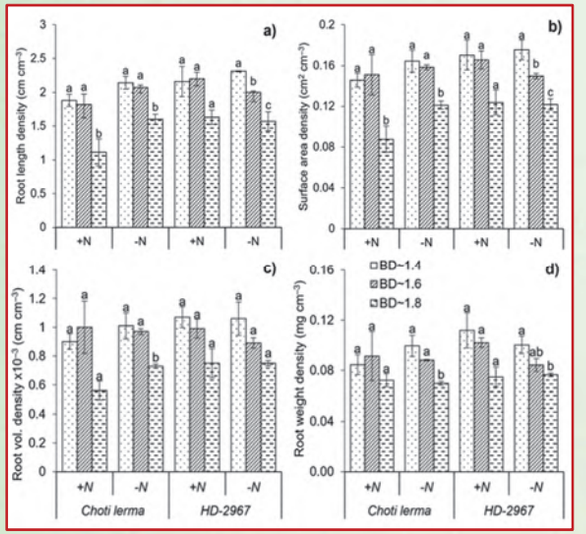


Fig. 3.5: Root morphological parameters in Choti Lerma and HD-2967 genotypes of wheat grown in pots with different soils bulk density (BD) levels and different N-doses

Variation in root system architecture in cultivated wheat and their progenitors under nitrogen stress

Gayatri, Pranita Roy and Pranab Kumar Mandal* (Plant Physiol. Rep. 2022)

Diploid progenitors of cultivated (hexaploid and tetraploid) wheat are of considerable interest in plant breeding as these have outstanding potential for resistance to both biotic and abiotic stresses. Hence, significant efforts are being made to transfer their genetic variation into bread wheat. Root system architecture is an important characteristic of crop performance to adapt under adverse environmental conditions like low nitrogen availability. Diploid progenitors along with their cultivated wheat have never been assayed for their root parameters in response to different nitrogen conditions. In this study, we selected 6 accessions from diploid progenitors of wheat (*Aegilops–Triticum* species) and tested them in the presence of cultivated wheat genotypes (two durum and two bread) under optimum and stressed nitrogen conditions for their root parameters in seedling stage. Significant variation was observed between and within the species. Of all accessions tested, *A. speltoides* (BB) got least affected root system under nitrogen stress. Moreover, cultivated wheat had the most affected root system under nitrogen stress (Fig. 3.6). These results suggest there is significant unexplored potential for the use of wheat progenitors in wheat breeding to improve the root system or to develop synthetic mapping populations to study root traits.

Fig.3.6: a) Seeds of 10 different genotypes belong to five genome species and b) Root System Architecture of 10 different genotypes belong to five genome species under N-optimum and N-stress conditions

Comparative transcriptome profiling of *Polianthes tuberosa* during a compatible interaction with root-knot nematode *Meloidogyne incognita*

Kanchan B M Singh, Pawan Jayaswal, Shivani Chandra, Jayanthi M, Pranab Kumar Mandal* (Mol. Biol. Rep. 2022)

The root-knot nematode (RKN; *Meloidogyne* spp.) is the most destructive plant parasitic nematode known to date. RKN infections, especially those caused by *Meloidogyne incognita,* are one of the most serious diseases of tuberose. To investigate the molecular mechanism in the host-pathogen interactions, Illumina sequencing platform was employed to generate comparative transcriptome profiles of uninfected and *Meloidogyne incognita*-infected tuberose plants, during early, mid, and late infection stage. A total of 7.5 GB (49 million reads) and 9.3 GB (61 million reads) of high-quality data was generated for the control and infected samples, respectively. These reads were combined and assembled using the Trinity assembly program which clustered them into 1,25,060 unigenes. The major proportions of CDS were annotated for carbohydrate metabolism, signal transduction and translation related pathways in control and infected samples (Fig. 3.7). Of the 8,289 CDS commonly expressed between the control and infected plants, 256 were significantly upregulated and 129 were significantly downregulated in the infected plants. Our results provide a comprehensive gene expression changes in tuberose during its association with RKNs and point to candidate genes that are involved in nematode stress signaling for further investigation. This is the first report addressing genes associated with *M. incognita*-tuberose interaction and the results have important implications for further characterization of RKN resistance genes in tuberose.

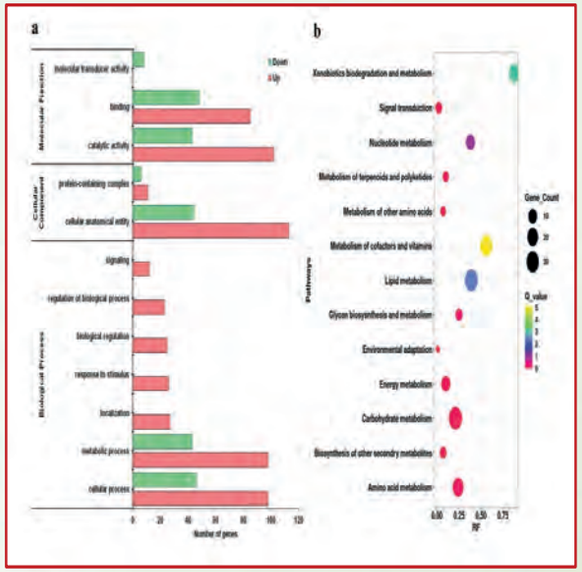


Fig. 3.7: (a) Gene ontology of the differentially expressed genes. (b) KEGG enrichment pathway analysis of the differentially expressed genes.

Classification and nomenclature of members of TaNRT2 and TaNAR2 families of high affinity nitrate transporters of bread wheat

Amresh Kumar, Pranab Kumar Mandal, Subodh Kumar Sinha* (Environ. Exp Bot. 2022)

Based on sequence similarity among genes (*TaNRT2s* and *TaNAR2s*) located on the same genome, chromosome location, homeolog

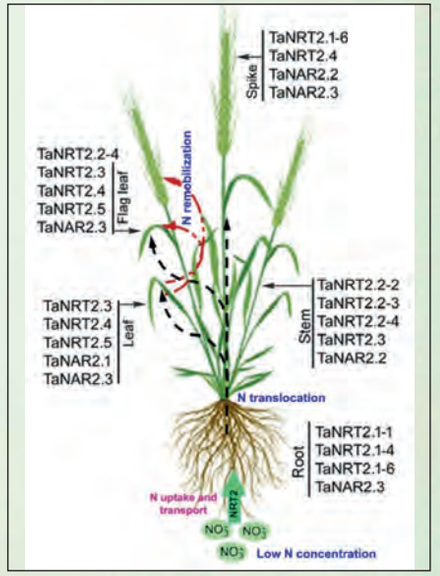


Fig. 3.8: A model representing candidate nitrate transporters (TaNRT2 and TaNAR2) present in different tissues of bread wheat vital for nitrate acquisition and transport

group, etc., up to in-paralog level, classification of TaNRT2 and TaNAR2 into five and three different classes, respectively, viz., *TaNRT2.1* – *TaNRT2.5*, and *TaNAR2.1* – *TaNAR2.3*, have been proposed that possibly involved in nitrate uptake and transport in different tissue of bread wheat. A model is proposed that represents the most potential genes involved in nitrate uptake and translocation in specific tissues (Fig. 3.8).

Differential contribution of HATS and LATS in different tissues of bread wheat at key physiological stages

Amresh Kumar, Pranab Kumar Mandal, Subodh Kumar Sinha* (Environ Exp Bot. 2022)

The contribution of HATS in all tissues (e.g., root, stem/shoot, leaf/flag leaf, and spike) of plants grown in limiting conditions is more than that of N optimally grown plants irrespective of genotypes (e.g., K9 and CL) (Fig. 3.9). However, K9, in general, showed a higher ^{15}N influx among genotypes and growth stages (e.g., GS-19, GS-39, and GS-65) in root tissues under low nitrogen condition. CL had the highest ^{15}N influx contributed by high-affinity nitrate transport system in spike tissues at GS-65 among all four genotypes, both at optimum and low N conditions. AK was found to be a poor performer in ^{15}N influx in all tissues. The overall ^{15}N influx by low-affinity nitrate transport system is comparatively lower than high-affinity nitrate transport system in all genotypes at all four stages.

Functional characterization of a high affinity nitrate transporter gene of bread wheat, *TaNRT2.1-B6*

Amresh Kumar, Pranab Kumar Mandal, Subodh Kumar Sinha* (Environ Exp Bot. 2022)

One of the highly expressed genes in root tissues, i.e., *TaNRT2.1*-B6, among 46 identified in the reference genome sequence of Chinese Spring genotype, was functionally characterized using Arabidopsis knockout mutant of *AtNRT2.1* (*At1g080900*), impaired in nitrate uptake activity under nitrate limiting condition. The results showed successful integration of *TaNRT2.1*-B6 into the Arabidopsis mutant genome, e.g., the transgene amplification in complemented lines, i.e., COML1 and COML2 only (COML1: Complemented Line1, COML2: Complemented Line2), neither in Columbia-0

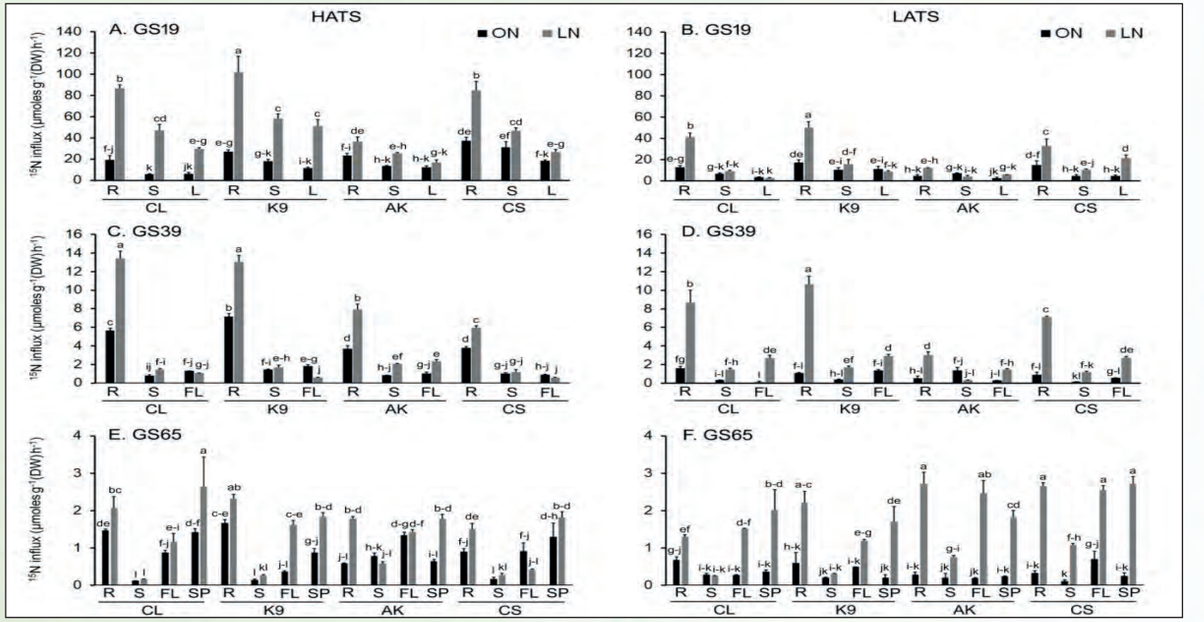


Fig. 3.9: Nitrate-^{15}N influx in different tissues (R: root; S: shoot; L: leaf; FL: flag leaf; S: spike) of four wheat genotypes (CL: Choti Lerma; K9: K9107; AK: AKAW-4732; and CS: Chinese Spring) at GS19 (A and B), GS39 (C and D), and GS65 (E and F)

(wild type) nor in mutant line (*atnrt2.1*) line (Fig. 3.10), single copy integration in both complemented lines (Fig. 3.10), the qPCR expression of endogenous (*AtNRT2.1*) in wild type, and *TaNRT2.1*-B6 in complemented line; however, no C_q in mutant line (Fig. 3.10), and finally, the ^{15}N influx analysis in complemented lines that allowed recovery of ^{15}N influx in nitrate uptake impaired line by 1.56 times 1.49 times in N+ and N- conditions respectively (Fig. 3.10).

Cloning of low affinity nitrate transporter genes of bread wheat

Muhammed Shamnas v, Akanksha Singh, Subodh Kumar Sinha*

During the reporting period, we cloned two paralogous genes in bread wheat, putatively termed, viz., *TaNRT1-I* and *TaNRT1-II*, orthologous to Arabidopsis gene responsible for root-to-shoot nitrate transport. The full-length cDNA sequence was amplified using gene-specific forward and reverse primer and cloned in pJET1.2 vector. The recombinant plasmids were confirmed by restriction digestion resulting in the expected insert size (Fig. 3.11A), which was further confirmed by DNA sequencing. Further, the

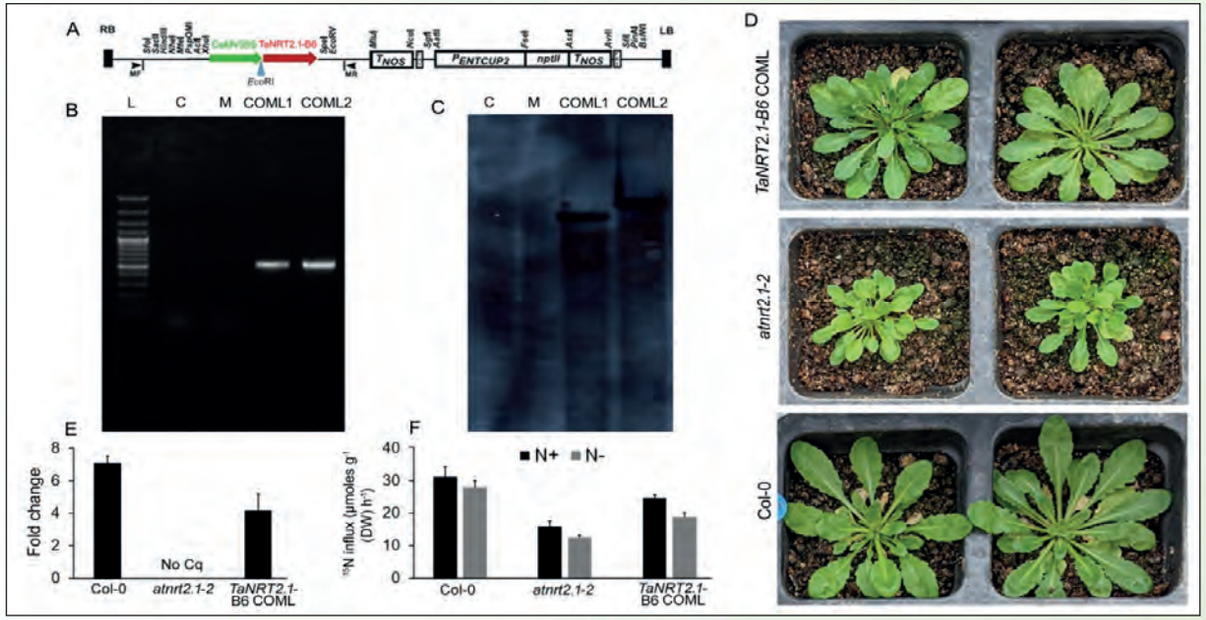


Fig. 3.10: Functional characterization of *TaNRT2.1-6B* by complementation of *atnrt2.1* mutant: PCR amplification of transgene in wild-type, mutant, and complemented lines (COML1 and COML2) (B). Southern hybridization of wild-type, mutant, and complemented lines (COML1 and COML2) (C). Wild type, mutant, and complemented lines (D). qPCR expression analysis of wild-type, mutant, and complemented line using primer pair specific for *AtNRT2.1* and *TaNRT2.1-B6* (F).

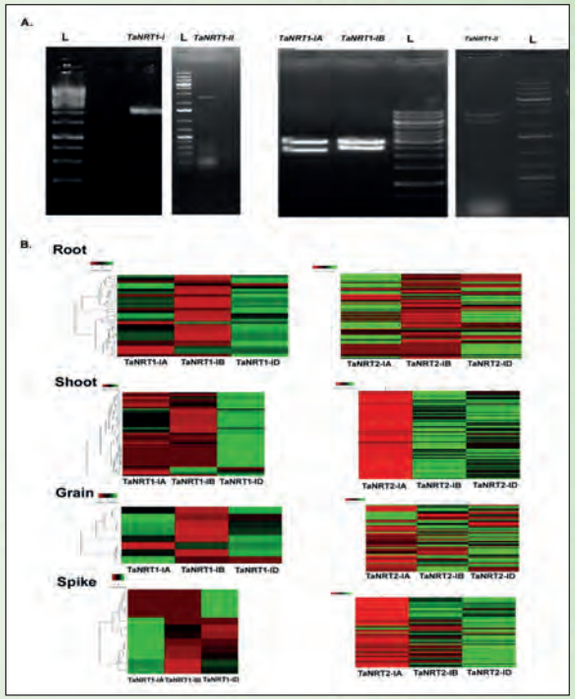


Fig. 3.11: A: Amplification of *TaNRT1-I* (~ 2.3kb) and *TaNRT1-II* (~ 2.4kb) using gene specific primers and restriction digestion of respective genes with expected band size. B: Heatmap showing the homeolog specific (A, B, and D) expression pattern of *TaNRT1-I* and *TaNRT1-II* genes in different tissues (root, shoot, grain and spike). Each row in the heatmap represents different abiotic and biotic stress treatments at different developmental stages.

in-silico differential gene expression analysis of *TaNRT1-I* and *TaNRT1-II* was performed for different tissues (e.g., root, shoot, spike, and grain) using publically available database, expVIP (an expression, visualization, and integration platform) Wheat Expression Browser (http://www.wheat-expression.com/), in terms of log2 transcript per million (TPM) and heat maps were generated (Figure 3.11B). The transcript read analysis showed that *TaNRT1-ID* expressed highly compared to *TaNRT1-IA* and *IB* in root tissue. In contrast, in the *TaNRT1-II* gene, A homeolog shows higher expression in root tissue followed by homeolog D and the least expression in homeolog B. Also, we observed differential expression in other major organs, viz., shoot, grain and spike, in all homeologs (A, B, and D) for both genes providing new insights into exploring its functional role in plant development.

Deciphering of pod borer [*Helicoverpa armigera* (Hübner)] resistance in *Cajanus platycarpus* (Benth.) offers novel insights on the reprogramming and role of flavonoid biosynthesis pathway

Shaily Tyagi, Maniraj Rathinam, Pathour Rajendra Shashank, Ajit Kumar Shasany and Rohini Sreevathsa* (Toxins 2022)

Management of pod borer, *Helicoverpa armigera* in pigeonpea (*Cajanus cajan* L.), an important legume crop, has been a pertinent endeavour globally. As with other crops, wild relatives of pigeonpea are bestowed with various resistance traits that include the ability to deter the *H. armigera*. Understanding the molecular basis of pod borer resistance could provide useful leads for the management of this notorious herbivore. Earlier studies by our group in deciphering the resistance response to herbivory through multiomics approaches in the pigeonpea wild relative, *Cajanus platycarpus*, divulged the involvement of the flavonoid biosynthesis pathway, speculating an active chemical response of the wild relative to herbivory. The present study is a deeper understanding of the chemical basis of pod borer (*Helicoverpa armigera*) resistance in a pigeonpea wild relative, *Cajanus platycarpus*, with a focus on the flavonoid biosynthesis pathway. To substantiate, quantification of transcripts in *H. armigera*-challenged C. platycarpus (8 h, 24 h, 48 h, 96 h) showed dynamic upregulation (up to 11-fold) of pivotal pathway genes such as chalcone synthase, dihydroflavonol-4-reductase, flavonoid-3'5'-hydroxylase, flavonol synthase, leucoanthocyanidin reductase, and anthocyanidin synthase (Fig. 3.12). Targeted LC-MS analyses demonstrated a concomitant increase (up to 4-fold) in naringenin, kaempferol, quercetin, delphinidin, cyanidin,

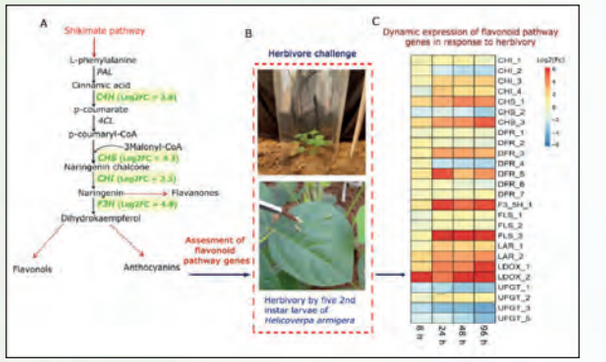


Fig. 3.12: Dynamic response of flavonoid biosynthesis pathway genes in C. platycarpus under continued herbivory

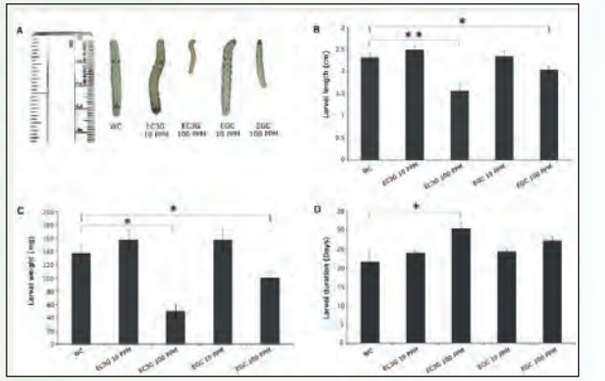


Fig. 3.13: Diet overlay assay for the validation of selected flavonoids on *H. armigera*. Response of *H. armigera* larvae to artificial diet feeding assay incorporated with water-soluble flavonoids in 10 and 100 ppm concentrations

epigallocatechin, and epicatechin-3-gallate. Interestingly, *H. armigera* diet overlaid with the over-produced flavonoids (100 ppm) showed deleterious effects on growth leading to a prolonged larval period demonstrating

noteworthy coherence between over-accumulation of pathway transcripts/ metabolites (Fig.3.13). The study depicts novel evidence for the directed metabolic reprogramming of the flavonoid biosynthesis pathway in the wild relative to pod borer; plant metabolic potential worth exploiting for pest management.

De novo assembly and annotation of *Cestrum diurnum* transcriptome and identification of nornicotine biosynthesis genes

Vandana Mathur, Ramawatar Nagar, Ajit Kumar Shasany*

Vitamin D is an essential nutrient for various bodily functions. Deficiency in vitamin D has been linked to an increased risk of various health conditions, such as cancer, Parkinson's disease, depression, dementia as well as the severity of coronavirus infection. Humans can produce vitamin D from 7-dehydrocholesterol (7-DHC) after exposing their skin to ultraviolet B (UVB) light, but dietary intake is still the primary source. Unfortunately, around one billion individuals worldwide are vitamin D deficient, and this number is increasing, mainly due to low dietary intake. One potential source of vitamin D is the leaves of certain species of solanaceae, such as *Cestrum diurnum*, which contain 1,25-dihydroxycholecalciferol-like activity. However, *Cestrum diurnum* also contains nornicotine, which is a negative trait for utilizing it as a dietary source of vitamin D. To address this issue, researchers are using CRISPR/Cas9-based techniques to knock out the berberine bridge enzyme-like (BBL) gene, which is involved in the last step of nornicotine biosynthesis. To identification of nornicotine biosynthesis genes, whole transcriptome of *Cestrum diurnum* was sequenced using Illumina RNASeq technology and de novo assembled using Trinity software. Annotation of the transcriptome was done using multiple plant genome annotation pipelines such as EggNOG and Viridiplantae (Table 3.1). To identify the nornicotine biosynthesis gene, including the BBL gene, in the *Cestrum diurnum* transcriptome, known nornicotine biosynthesis genes from closely related plant species were used as a query for protein blast (blastp) against the transcriptome. The top hits with an e-value of 0 were selected as potential homologous genes and these genes were extracted and further characterized for completeness. Six copies of the BBL have been reported in Nicotiana species, including BBLa, BBLc, BBLd.2 from *Nicotiana sylvestris*, and BBLb, BBLd.1, and BBLe from *Nicotiana tomentosiformis*. The genes BBLa and BBLc hit the same transcript, while the BBLd hit a different transcript. Three transcripts, TRINITY_DN43238_c4_g1, TRINITY_DN30276_c0_g2, and TRINITY_DN45310_c4_g3, where the e-value was 0, were considered potential homologues of the BBL genes in the *Cestrum diurnum*. The genomic region of these transcripts would be amplified to look for intron content, and then a gRNA would be designed and synthesized.

Table 3.1 Transcriptome statistics of *Cestrum diurnum*

Assembly Features	Statistics
Total trinity 'genes'	303657
Total trinity transcripts	514289
Percent GC	39.47
Contig N10	3234
Contig N20	2441
Contig N30	1938
Contig N40	1546
Contig N50	1210
Median Contig length	461
Average Contig	771.89
Total Assembled bases	397000000

4. GENETIC MODIFICATION OF CROP PLANTS FOR IMPORTANT TRAITS

CRISPR/Cas9 mediated genome editing in Indian mustard *Brassica juncea*

Muthuganeshan, Satish Kumar, Smriti Singh, Naresh Samal, Anshul Watts, R.C. Bhattacharya*

For harnessing the advantages of CRISPR/Cas system of genome editing in Brassica improvement programme, we have optimized the process and successfully demonstrated knockout of *PDS* gene in *B. juncea*. Following the successful demonstration of targeted mutation by CRISPR/Cas9 system, several target genes have been identified for developing mutants and assay for the plausible effects of the mutation on yield and quality enhancement of the mustard oil. In regulating fatty acid biosynthesis in plants, transcription factor WRI1 serves as a key regulator. WRI1 interacts with another class of TFs called TCPs, which are plant-specific TFs and play important roles in diverse biological processes, such as shoot apical meristem and leaf development, etc. TCP TF displayed strong correlation with the expression of AtWRI1 during embryo development. In order to understand the interaction between WRI1 and several TCP transcription factors, targeted mutants are being generated for the selected TCP transcription factors. For construction of the genome editing CRISPR/Cas9 vectors TCP A, TCP B, TCP Ba, TCP C protospacer sequence was amplified and cloned between U6 promoter and scaffold region by overlap extension PCR (Fig. 4.1A). All the clonings were confirmed through restriction digestion (Fig. 4.1B) and sequencing. Plant transformation with these constructs will be undertaken for generating CRISPR/Cas9 mediated genome edited lines for the TCP-TFs.

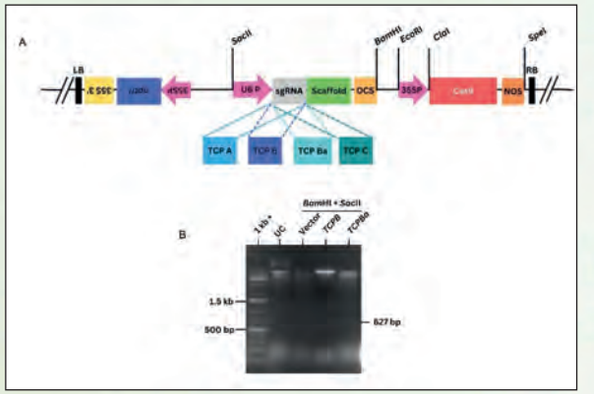


Fig. 4.1: (A) Schematic map of the CRISPR/Cas vector and (B) Cloning of TCP sgRNAs

Deciphering the genes and pathways associated with Alternaria resistance in introgression lines of Indian mustard

Sharani Choudhury, Mahesh Rao, Anamika Kashyap, Shivani Lama, Ashish Gupta, Rohit Chamola, R.C. Bhattacharya*

The productivity of Indian mustard is substantially inflicted due to a number of major biotic stresses. Developing genetic resistance against Alternaria blight in the cultivated varieties of mustard has not been possible. The major bottleneck has been unavailability of the resistance source within the crossable germplasms. Against Alternaria, no potential R gene or source of genetic resistance could be found in the gene pool of cultivated Indian mustard (*B. juncea*). In one of the previous studies in this research institute, *Diplotaxis erucoides* and seven other crop wild relatives (CWRs) of *B. juncea* demonstrated high level of genetic resistance against *A. brassicae*.

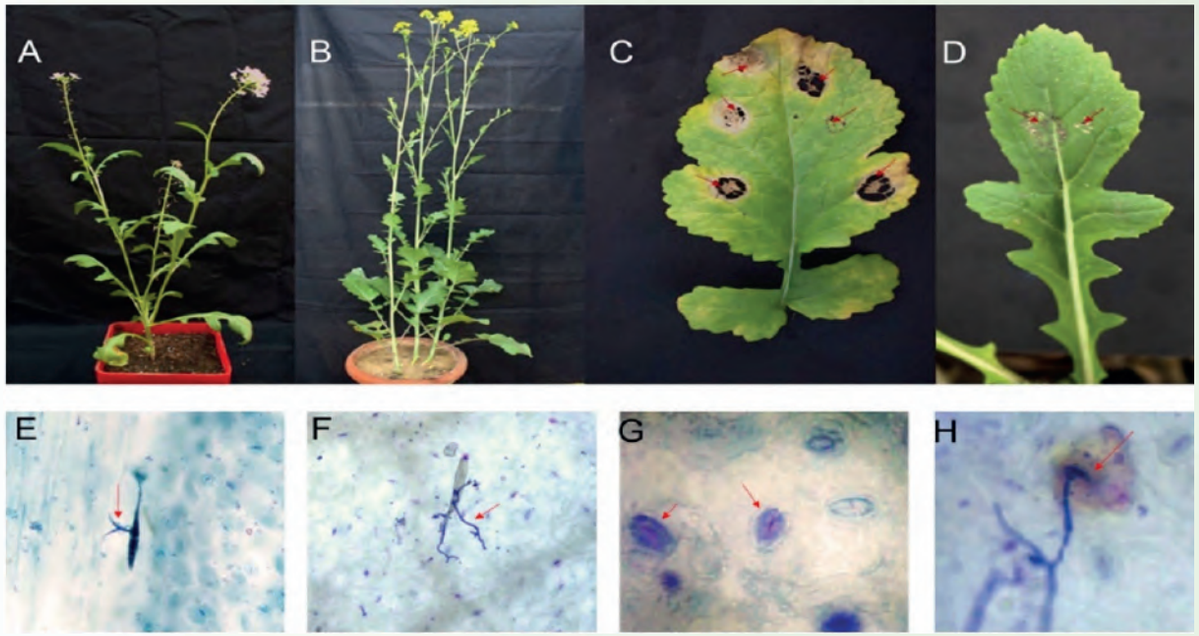


Fig. 4.2: Alternaria leaf blight disease assay. Leaves of two months old plants *D. erucoides* (A) and *B. juncea* (B) were inoculated with spore suspension of *A. brassicae* by scratch method. Rapid appearance of symptoms was observed on the infected leaves of susceptible *B. juncea* (C) whereas, no symptoms were observed on the inoculated leaves of *D. erucoides* (D) after 14 days of inoculation. Arrows (in C and D) depict the specific area of scratch and inoculation on the leaves. Arrows (in E-H) shows spore germination and emergence of hyphae of *A. brassicae* at 24 hpi in *D. erucoides* (E) and *B. juncea* (F) detected at 10X magnification. At 48 hpi no significant hyphae penetration of host cells was observed in *D. erucoides* (G) whereas, efficient hyphae penetration was detected in *B. juncea* (H) at 40X magnification.

Study of the pathogenesis by artificial inoculation of the resistant wild species *D. erucoides,* revealed that it restricted pathogenesis by *A. brassicae* starting from entry of the pathogen. In this resistant species, upon germination of the spores the hyphae were found to enter the host only through stomata whereas in case of susceptible *B. juncea* direct entry were also spotted along with stomatal entry, which makes it more prone to rapid pathogenesis (Fig. 4.2). A further gene expression study showed that, along with other pathways, Indole glucosinolate (IG) pathway significantly contributed towards Alternaria resistance in *D. erucoides*, and regulation of this pathway is positively influenced by jasmonate mediated signalling. Thus, many of the genes of IG biosynthetic pathway were transcriptionally activated by methyl jasmonate (Fig. 4.3).

For the transfer of resistance trait from *D. erucoides* to *B. juncea*, several introgression lines were developed and advanced to BC2F9 generation with recurrent selection for the resistance at 2-3 different locations including hot spot for the disease. Genomics data are being generated using these introgression lines for identifying the introgressed chromosomal segments associated with the trait. Gene expression study revealed that IGMT, ST5a, CYP83B1 gene associated with indole glucosinolate biosynthetic pathway and defense signalling related gene were significantly activated in response to inoculation by *A. brassicae* in resistant introgression lines compared to susceptible parent. To verify the role of *IGMT* gene of *D. erucoides* and thus the indole glucosinolates in conferring the resistance against Alternaria, a gene silencing construct (RNAi) was developed for transcriptional attenuation of the *IGMT* gene. Mobilization of the construct into Agrobacterium (GV3101) and further study are in progress.

Identification of effector molecules based on head transcriptome of mustard aphids

Ashakiran Loitongbam, Muthuganeshan, Shusma Sharma, R.C. Bhattacharya*

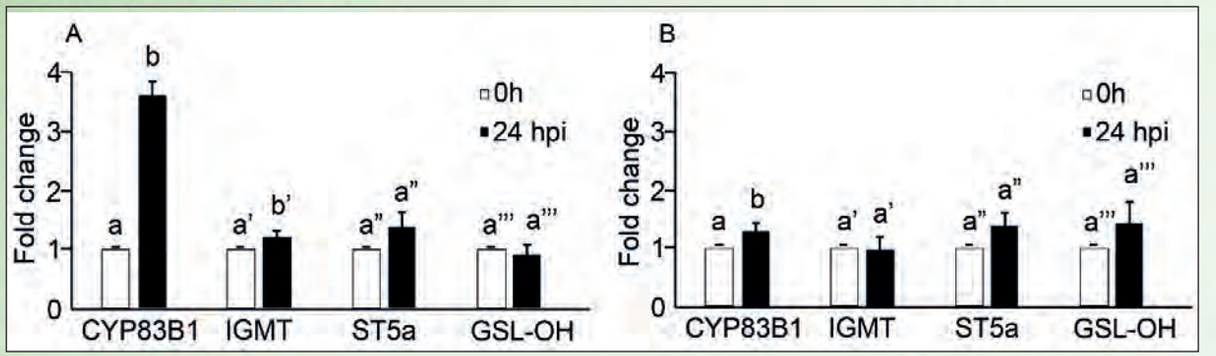


Fig. 4.3: Activation of GSL biosynthesis pathway genes in response to inoculation by *A. brassicae* in *D. erucoides* (A) and *B. juncea* (B) leaves. Leaves of 2 months old plants were inoculated with *A. brassicae* spore culture ($1x10^5$/ml) by scratch method. Total RNA was extracted from the leaves and assayed for gene-expression of GSL pathway genes *CYP83B1*, *IGMT*, *ST5a* and *GSL-OH* by qRT-PCR with *GAPDH* as an internal control. Values represent mean ± SD (*n*=3). *Different letters* indicate significantly different values.

In Indian mustard, among the biotic factors, the major yield loss caused by aphid-infestation alone is as high as 78%. Aphid colonize the mustard plants by attenuating the host defense mechanism. The known changes in host plant are cell enlargement resulting in hypertrophy, susceptibility of the host towards infection. At the molecular level, effector decreases the level of expression of jasmonate biosynthetic genes (LOX, AOC, 12-OPDR), redox genes (GST6), and other downstream defense genes (PAL, ELI3, MYR, and TPI). Till now, very few attempts have been made for identifying the major effector molecules released by the aphids in establishing a successful colonization. However, the major effector molecules, if identified, can be used to find the host interacting factors that lead to attenuation of host-defense and eventually susceptibility. For identifying the effector molecules for mustard aphid, head transcriptome data is being analysed through a pipeline of bioinformatics-based prediction (Fig. 4.4).

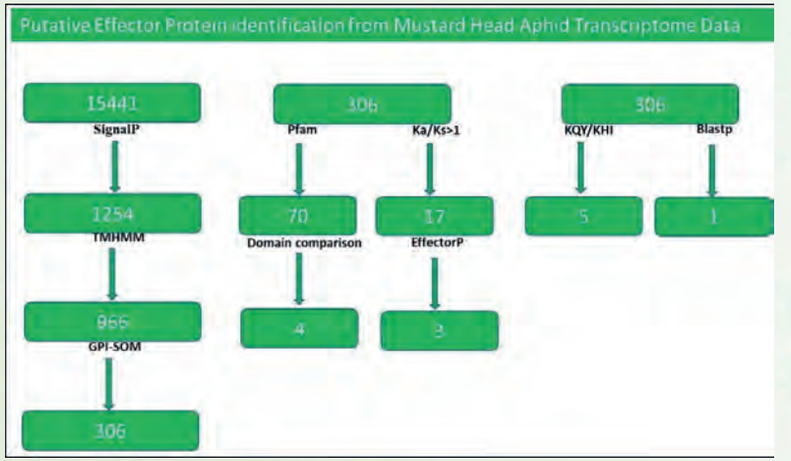


Fig. 4.4: *In silico* prediction of putative effector molecules using head transcriptome data of mustard aphid (*Lipaphis erysimi*)

Effector proteins are secretory proteins, therefore, proteins having trans membrane binding site, GPI binding site were filtered from the set of proteins encoded by the head transcriptome. From the remaining, selective proteins were filtered out using SignalP, TMHMM, GPI-SOM in a step-wise manner. From the identified proteins, 5 proteins were identified to belong to KQY and KHI family (feature present in Signal peptide region of effector protein). One of them shared 78% homology with Mp2 effector protein, and the remaining four were found to have similar kind of domain as known in other effectors like Me10, Mp0 and MpCOO2. Like other pathogen effectors, aphid effector molecules survived positive selection and evolved. Therefore, Ka/Ks ratio (Inparanoid) was calculated using previously identified aphid effector molecules. Three effectors showing Ka/Ks >1 was selected for further characterization. The putative effectors thus identified were named as Le1, Le2 etc. in subsequent characterization. Out of the putative effectors identified through *in silico*

analysis, the coding sequence for three were cloned into pET 28a vector and introduced into the host strain BL21(DE3) for *in vitro* protein expression (Fig. 4.5). Expression analysis of the effectors and bioassay for their effect on host immunity are under progress.

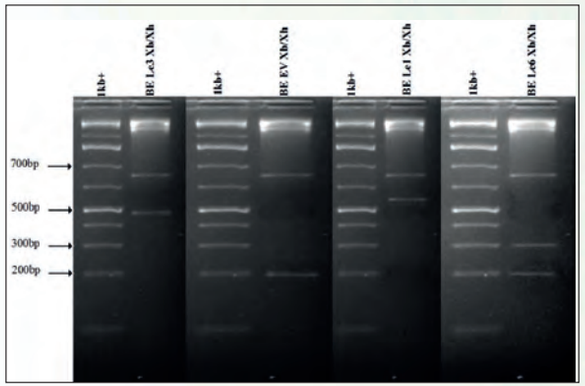


Fig. 4.5: Confirmation of the cloning of effector encoding genes in pET 28a vector by restriction analysis using *Xba1* and *Xho1*. EV (empty vector), BE Le3, BE Le1, BE Le6, effector genes.

Evaluation of *Brassica* accessions/introgressed lines/resynthesized *Brassica* lines for white rust (*Albugo candida*) resistance

Ashish Kumar*, Mahesh Rao, Jameel Akhtar, Rashmi Yadav and R. C. Bhattacharya

Brassica wild relatives namely, *B. oxyrrhina, Camelina sativa, Diplotaxis assurgens, D. catholica, D. cretacia, D. erucoides, D. muralis, D. siettiana, D. tenuisilique, D. viminea, Erucastrum lyratus, E. abyssinicum, E. canariense, E. cardaminoides* and two exotic accessions of *Crambe abyssinica viz*, EC694071 and EC694138 were screened for white rust (*Albugo candida*) resistance and found immune (PDI = 0) against the six isolates namely *Ac-Del, Ac-Ldh, Ac-Pnt, Ac-Amb, Ac-Rnc* and *Ac-Wltn* of the pathogen *A. candida*. *Brassica* accessions were screening and evaluated under artificial inoculated conditions. Phenotyping of 453 accessions of *Brassica* species obtained from ICAR-NBPGR was done against twelve isolates of *A. candida*. Amid exotic accession of *B. juncea viz*., EC766192 resulted resistant reaction for *Ac-Smt, Ac-Bpr*, Ac-*Ayo* and *Ac-Ran,* isolates. Similarly, EC766193 was found resistant against *Ac-Hsr*, *Ac-Dhd*, *Ac-Del, Ac-Ldh, Ac-Bpr, Ac-Pnt, Ac-Mrn* and *Ac-Gwl* isolates at both cotyledonary and true leaf growth stages of the crop. Further introgressed (BC_2F_{12}) and resynthesized (S_8) lines of *B. juncea* were also evaluated under artificial inoculated conditions. Among 192 introgressed lines, ERJ-39 resulted immune reaction for Ac-Bhrtpr and Ac- Del; ERJ 40 for identified as immune (PDI=0) against 8 isolates namely, Ac-Del, Ac-Ldh, Ac-Bhrtpr, Ac-*Mrt*, Ac-*Mrn*, Ac-*Rnc*, Ac-*Smt* and Ac-*Pnt* except Ac-*Gwl* and Ac-*Skn* isolates. Out of 127 resynthesized lines, RBJ 40 showed complete resistance reaction for three isolates (*Ac-Bpr, Ac-Ldh, Ac-Hsr*) and RBJ 18 showed immunity against six isolates in field as well as artificial testing. Finally, field screening of *Brassica* accessions, introgressed and resynthesized lines was carried out under natural conditions. Phenotyping of 453 *Brassica* accessions, 192 ILs, and 127 RBJ lines was done under field conditions at Research Farm IARI, Issapur farm NBPGR New Delhi. Among them two indigenous accessions, IC265495 and IC597932 and ten exotic accessions of *B. juncea*, *viz*., EC206651, EC766311, EC766313, EC766133, EC766134, EC766144, EC766145, EC766148, EC766164 and EC766193 and two accessions of *B. carinata viz*., EC206641 and EC206642 were identified as immune for white rust disease under natural field condition of Delhi. Introgressed lines; ERJ 12, ERJ 13, ERJ 40, ERJ 47 and ERJ 158 were identified as immune under natural field conditions of Delhi. RBJ lines 18, 40, 11 and 55 were found immune under both the conditions *i.e*., natural field and artificially inoculated conditions against the *Ac-Del* isolate.

Characterization of early generation synthetic *Brassica juncea* lines

Pooja Garg, Ranjeet Kushwaha, Jyoti Kumari, Shikha Tripathi, N C Gupta, R C Bhattacharya and Mahesh Rao*

Total 37 early generation synthetic *Brassica juncea* were developed and evaluated for the morphological traits which showed a wide range of variability for the different traits. The variation in siliqua length and number of seed per siliqua were observed from 3.07 to 4.65 cm and 6.5 to 18.75 seeds per siliqua, respectively. Also, the genetic diversity of accessed using the molecular markers specific to A and B genome which describe the diversity generated in the synthetic *B. juncea* lines. The fertility was analysed by pollen viability analysis which indicate the recovery of fertility almost >80 % in most of the genotypes (Fig. 4.6). The mitotic study showed 36 chromosomes in the synthetic lines which need to be further analysed for the meiotic pairing (Fig. 4.7). The stability will be done in the coming generation under multilocation evaluation and the contrasting lines will be identified for the economic traits for molecular biology studies.

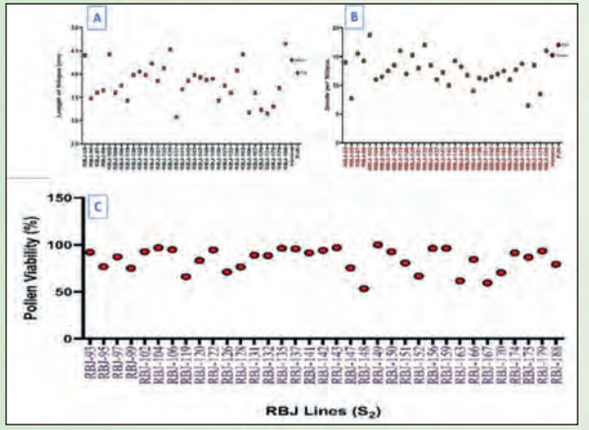


Fig. 4.6: Graph representing the variation for A). Length of siliqua (cm); B). Seed per siliqua and C). Pollen viability, in the early generation resynthesized *B. juncea* lines.

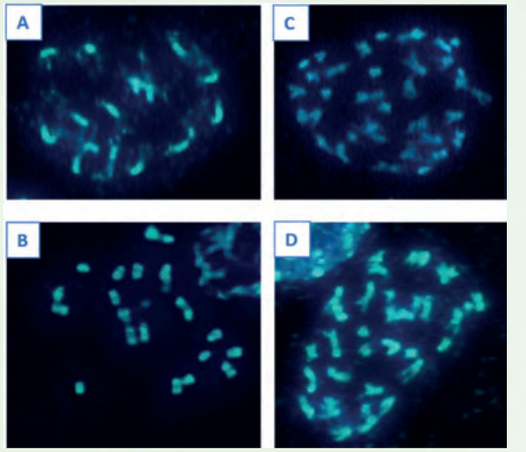


Fig. 4.7: Mitotic chromosome of the parental diploid species A). *B. nigra* (BB, 2*n*=16); B). *B. rapa* (AA, 2*n*=20); and C). & D). the synthetic *Brassica juncea* (RBJ 122 and 132) lines (AABB, 2*n*=36)

Identification of contrasting lines for the seed per siliqua

Pooja Garg, Anamika Kashyap, Sujata Kumari, N C Gupta, Rashmi Yadav, R C Bhattacharya and Mahesh Rao*

A panel of 161 Brassica juncea germplasm, including the exotic as well as indigenous collection, were evaluated in a multilocation testing during 2021-2022 for the identification of the contrasting genotype for seed per siliqua. The yield contributing data were recorded at all the locations and the contrasting lines for seed per siliqua were identified with highest and lowest seed per siliqua respectively in the panel (Fig. 4.8). The further analysis will be done for the identification of the underlying gene(s) regulating the seed per siliqua in the *B. juncea*.

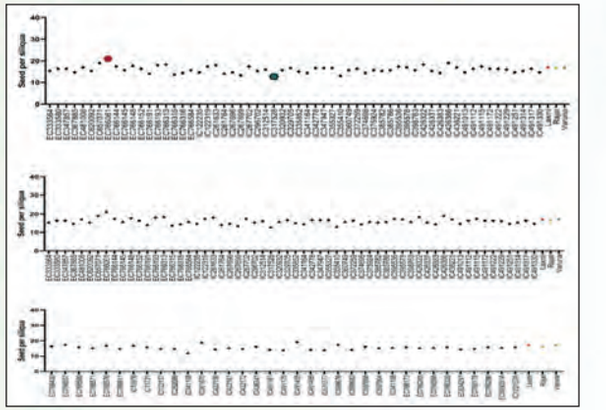


Fig. 4.8: Graphical representation of the variation of seed per siliqua in a panel of *B. juncea* genotypes.

Analysing the impact of gamma irradiation on rapeseed mustard through cytogenetic tools for the utilization in the pre-breeding program

Mahesh Rao*, Mariana Baez, Annaliese S. Mason

Under the SERB-SIRE (SERB-International Research Experience) program the work started on 'Breaking the blocks: eliminating linkage drag in introgression breeding in rapeseed mustard' is started from September 2022 with Dr. Annaliese S. Mason, Professor and Chair of Plant Breeding, The University of Bonn, Bonn, Germany for six months. The objective of the proposed work is to optimize genetic transfer of targeted loci and traits from exotic germplasm into Brassica (rapeseed and rapeseed-mustard) for crop improvement through radiation, molecular cytogenetics and chromosome painting approach. Under this seeds of the *Brassica juncea* (AABB), *Brassica napus* with B1 and B6 additional chromosome were irradiated with different doses of gamma rays (1400Gy, 1750Gy and 2000Gy) and the root sample were collected at seedling stage. The samples are being processed for the mitosis and the in-situ hybridization. The chromosomal breakage was observed during the preliminary mitotic analysis of the sample from different gamma doses (Fig. 4.9). Further detailed analysis is underway for the intergenomic interactions and translocation.

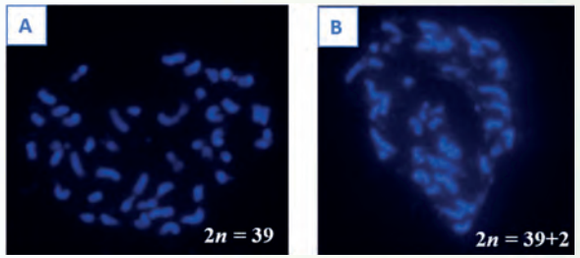


Fig. 4.9: Mitotic chromosome of the AA-94-5 AAB_1CC (Chr. No39) A). from the root of the *Un*-irradiated (control) and B). From the root of the treated with 1400 Gy

Development of haploid inducer (HI) line in *Brassica juncea*

Anshul Watts*

An *in-vivo* HI line is required in *B. juncea* to accelerate the production of pure line. CENH3, Matrilineal, DMP and Baby boom genes have been identified which can produce such haploid inducer line in different crops (Zargar et al. 2022). We are employing CENH3 mediated strategy to develop such HI line in *B. juncea*

Transgenic *B. juncea* lines have been developed by co-transformation of CENH3 CRISPR/Cas9 and GFP-tailswap cassette in *B. juncea* which were earlier developed in our laboratory. Further such transgenic lines were checked through PCR based analysis (Fig. 4.10A). In one of the events, plants were showing dwarfness, bunchy phenotype (Fig. 4.10B) and partial sterility (Fig. 4.10C). Interestingly some the flowers showed five petals (Fig. 4.10D). These lines were selfed and crossed with wild type *B. juncea* to check the haploid induction ability of these lines.

Understanding *Brassica-Orobanche* interaction system for identification of key genes responsible for *Orobanche* infestation and their functional validation

Anshul Watts*, Komal Mehta and R.C. Bhattacharya

Orobanche cernua and *Phelipanche ramosa*

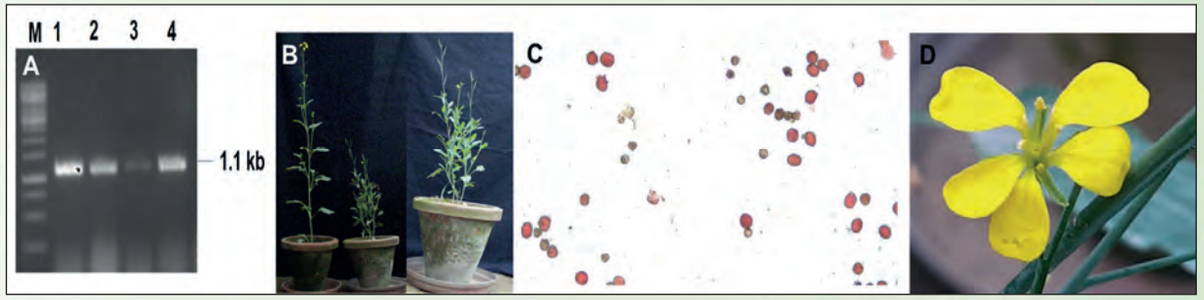


Fig. 4.10: T_0 plants of CENH3 CRISPR/Cas9 vector transformed and PCR analysis of these plants. A) PCR analysis of T_0 plants B) Comparison of wild type and CENH3 edited line of *B. juncea* and bunchy phenotype C) Partial sterility in one of the edited events D) Five petal phenotype observed in some flowers

infestation in tomato: *Orobanche cernua* and *Phelipanche ramosa* infestation were observed in the tomato fields near the villages of the Charkhi Dadri district of Haryana. The attachment to roots of both the species were studied (Fig. 4.11). Molecular marker analysis showed that it is the same *Phelipanche ramosa* species which infest *B. juncea*.

Fig. 4.11: Infestation of *Orobanche cernua* and *Phelipanche ramosa* to the roots of tomato A) *Orobanche cernua* attachment to the tomato roots B) *Phelipanche ramosa* attachment to the roots of tomato

Identification of Strigolactone biosynthetic enzymes genes in the published genome of *B. juncea*: In the published genome of *B. rapa*, *B. nigra*, *B. oleracea* and *B. juncea* strigolactone biosynthetic pathway genes were identified (Table 4.1). The copy number of these genes as well as gene structure were studied in *B. juncea*.

Table 4.1. Identification of strigolactone biosynthesis genes in the published genome of *B. rapa*, *B. nigra* and *B. juncea*.

SL biosynthesis genes	*Arabidopsis thaliana*	*B. rapa*	*B. nigra*	*B. juncea*	*B. oleracea*
9-cis/all-trans-β-carotene isomerase	AT1G03055	Bra032560	BniB036007	BjuA037233, BjuB041833	Bol018398
Carotenoid Cleavage Dioxygenase 7 (AtCCD7);	At2g44990	Bra040330	BniB013750	BjuA017469, BjuB041894, BjuB041897	Bol021707
Carotenoid Cleavage Dioxygenase 7 (CCD8)	AT4G32810	Bra011384	BniB009305	BjuA003315, BjuB014372	Bol017864
Cytochrome P450	AT2G26170	Bra000558	BniB049600	BjuA010892, BjuB041302	Bol015014
CLA methyltransferase	At4g36470	-	-	BjuA014196, BjuB040772	-
Lateral Branching Oxidoreductase (LBO1), 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	At3g21420	Bra023884, Bra031281	BniB003132, BniB014091	BjuA005709, BjuA019754, BjuB026021, BjuB007929	Bol038359, Bol018788

Identification, characterization of oil yield-related genes in *B. juncea* and their functional validation

Anshul Watts* and Ritesh Kumar Raipuria

Yellow-seeded types have thinner testa, higher oil and protein, lower fibre as compared to their black-seeded counter parts. In order to develop yellow-seeded *B. juncea* lines through CRISPR/Cas9 mediated genome editing a basic helix-loop-helix (bHLH) transcription factor was selected which can target the biosynthetic genes of flavonoid biosynthetic pathway. A single guide RNA was designed from the conserved region of both the copies of bHLH transcription factor. Further overlap extension PCR strategy was utilized to clone this sgRNA in binary vector. The developed constructed was mobilized into *Agrobacterium tumefaciens* strain GV3101 and transformed in *B. juncea* cultivar Varuna which is black seeded variety.

The chimeric *Bt* gene *cry1AcF* expressing transgenic tobacco lines effectively controls insect pest fall armyworm (*Spodoptera frugiperda*)

Manjesh Saakre, Karthik Kesiraju, Shaily Tyagi, K Venkat Raman., Sandeep Jaiswal, Jyotsana Tilgam, Krishnayan Paul, Sougata Bhattacharjee, Joshitha Vijayan, Rohini Sreevathsa and Debasis Pattanayak*

Fall armyworm (FAW), *Spodoptera frugiperda* is an important Lepidopteran polyphagous insect-pest that feeds on more than 350 plant species. In India, this invasive pest was first spotted during May 2018 on maize crops in several districts of Karnataka state. Some *S. frugiperda* species have recently developed resistance to many conventional insecticides in the field. Furthermore, lack of resistant genes in crop germplasms impedes conventional breeding for imparting resistance to FAW. Transfer of *Bt* (*Bacillus thuringiensis*) insecticidal protein (Cry) coding genes into susceptible crops is the most successful technology for combating insect resistance. In the present study, a recombinant binary vector pCAMBIA2300 harbouring *cry1AcF* gene under the transcriptional control of CaMV35S promoter (pCAMBIA2300::CaM V35SP:*cry1AcF*:NosT) was constructed and *Agrobacterium* mediated genetic transformation was carried out in tobacco.

Putative transformants were screened by gene specific PCR (Fig. 4.12a). The integration of T-DNA into the genome of transgenic lines was detected by Southern blotting using 700 bp probe of *nptII* gene fragment. Transgenic event *Bt*-7 carried a single copy of the transgene. The other four transgenic lines, *Bt*-2, *Bt*-5, *Bt*-13 and *Bt*-19, had multiple copy insertion of the transgene in their genome (Fig. 4.12b). The expression of Cry1AcF protein was validated by western blot analysis in the selected four transgenic events. In all selected transgenic tobacco plants, a single intense band of 70 kDa, corresponding to the molecular mass of Cry1AcF protein, was observed (Fig. 4.12c).

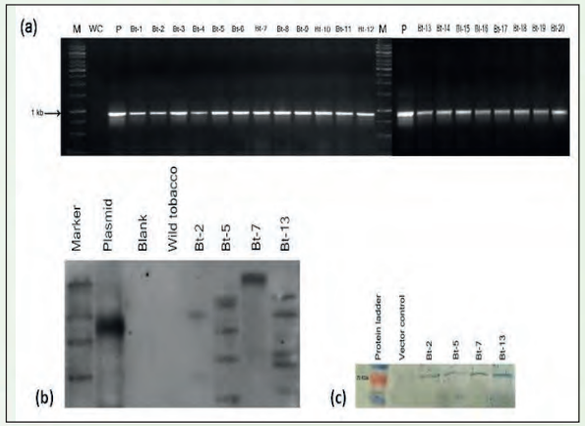


Fig. 4.12: Molecular characterization of putative transgenic events. (a) PCR amplification using gene specific primers which amplified a 900 bp region of *cry1AcF* gene. Lane M: 1 kb ladder; Lane WC: Water Control; Lane P: pC::AcF plasmid control; Lanes Bt-1 to Bt-20: Transgenic lines. (b) Southern hybridisation of selected transgenic plants using *nptII* gene as a probe. Lane M: 1 kb ladder; Lane P: pC::AcF plasmid control; Lane B: Blank; Lane WT: wild tobacco control; Lanes Bt-2, Bt-5, Bt-7 and Bt-13 are transgenic lines. (c) Western blotting of transgenic plants plants. Lanes Lanes Bt-2, Bt-5, Bt-7 and Bt-13 are transgenic protein samples detected ~70k kDa blue colour bands.

Detached leaf feeding bioassay was conducted to test the effectiveness of *cry1AcF* tobacco transgenic lines against *S. frugiperda.* Four transgenic lines (*Bt*-2, *Bt*-5, *Bt*-7 and *Bt*-13) showed 72-80% mortality after 96 hrs of

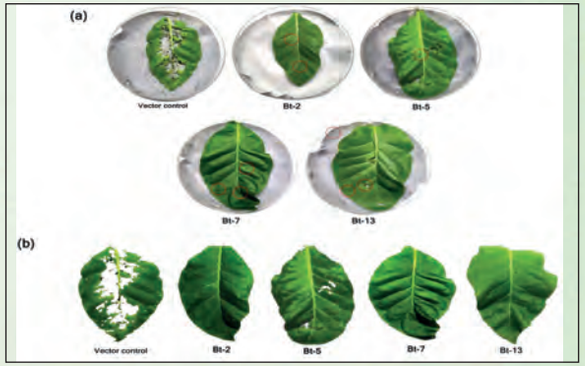


Fig. 4.13: The leaf detached insect bioassay of Cry1AcF-expressing transgenic tobacco leaves against *S. frugiperda.* (a) Leaves of vector control plant were compared with transgenic events (Bt-2, Bt-5, Bt-7 and Bt-13), dead insect larvae found on transgenic leaves were marked with red circles (b) The extent of leaf damage in transgenic lines (Bt-2, Bt-5, Bt-7 and Bt-13) compared with wild tobacco leaves.

continuous feeding compared to that of vector control (6.67 % mortality) (Fig. 4.13a). Leaf damage caused by *S. frugiperda* larvae in these four transgenic lines was also the least ranging from 5 to 10 per cent (Fig. 4.13b).

Identification of major candidate genes for multiple abiotic stress tolerance at seedling stage by network analysis and their validation by expression profiling in rice *(Oryza sativa L.)*

M. K. Ramkumar, Ekta Mulani, Vasudha Jadon[1], Sureshkumar V, Amolkumar U Solanke, N.K. Singh and Amitha Mithra Sevanthi[*] (3 Biotech, 2022)

Rice has a wealth of microarray and RNA-seq resources for abiotic stress tolerance but a very few studies have explored the mechanism behind multiple stress tolerance. In this study, we utilized 6657 abiotic stress responsive genes, identified from the seedling stage microarray data of 83 samples pertaining to drought, salinity and heat stresses to perform unweighed network analysis and identify key hub genes or master regulators of multiple abiotic stress tolerance. Our datasets were represented by seven different genetic backgrounds from indica, japonica, aus and Basmati germplasm. Of the total 55 modules identified, the top 10 modules with 8-61 nodes comprised of 239 genes. From these 10 modules, 10 genes common to all the three stresses were selected. Further, based on the centrality properties and highly dense interactions, we identified seven intra-modular hub genes leading to a total of 17 potential candidate genes. Out of these 17 genes, 15 were validated by expression analysis using a panel of 4 test genotypes and a pair of standard check genotypes for each stress response. Interestingly, all the 15 genes showed upregulation under all stresses and in all the genotypes suggesting that they are indeed important for abiotic stress response (Fig. 4.14). More pertinently, eight of the 17 genes were found to be co-localized with the stress tolerance QTL regions. The current study thus not only provided an effective approach for identifying the key genes from large datasets but provided with major candidate genes which can provide a basic machinery to plants for tackling multiple abiotic stresses, at least at seedling stage. These candidates can serve as potential source for functional validation under abiotic stress in rice as well as related species.

Fig. 4.14: Validation of the selected candidate genes in a panel of genotypes under drought, high temperature and salinity stress

Development of genomic resources in African yam bean (*Sphenostylis stenocarpa*)

Seun Cecilia David (DBT-TWAS PhD Postgraduate Sandwich Fellow) and Dr Amitha Mithra Sevanthi*

African yam bean (AYB) (*Sphenostylis stenocarpa* (Hochst. ex A. Rich) Harms) is an important crop of Africa with dual benefits, edible tuber as well as beans. The molecular resources are limited in this crop and hence developing genomic resources will be very useful for its crop improvement. The major objectives of the study are to explore microsatellite markers from other legume species and test them for cross-transferability in AYB accessions as well as develop preliminary genomic resources, transcriptomic resources using RNA-seq platforms. A total of 206 microsatellites markers obtained from six different leguminous crops species namely *Vigna unguiculata* (Cowpea), *Cyamopsis tetragonoloba* (Cluster bean), *Vigna mungo* (Black gram), *Vigna radiate* (Green gram), *Glycine max* (Soybean), and *Cicer arietinum* (Chickpea) were tested in two AYB accessions along with positive control (two genotypes each from the respective donor legume). Eighty-one primers out of the 206 SSR primers were found to be successfully amplified in AYB accessions (Fig 4.15 and Table 4.2). Among the six species tested, blackgram and cowpea SSR Primers had the highest number of amplifications in AYB accessions with 23 and 19 primers respectively, while Soybean had the least with 3 primers. To enable the genetic distance studies in AYB using these 81 markers, 92 accessions of AYB accession have been sourced from the International Institute of Tropical Agriculture (IITA), Nigeria with Import permit number 357/2022 and IQ No. 376/2022 following the laid-out import and quarantine procedure by the National Bureau of Plant Genetics and Resources (NBPGR, New Delhi). The root and shoot samples of one of the accessions, namely, TSs-364, has been subjected to genome-wide transcriptome analysis.

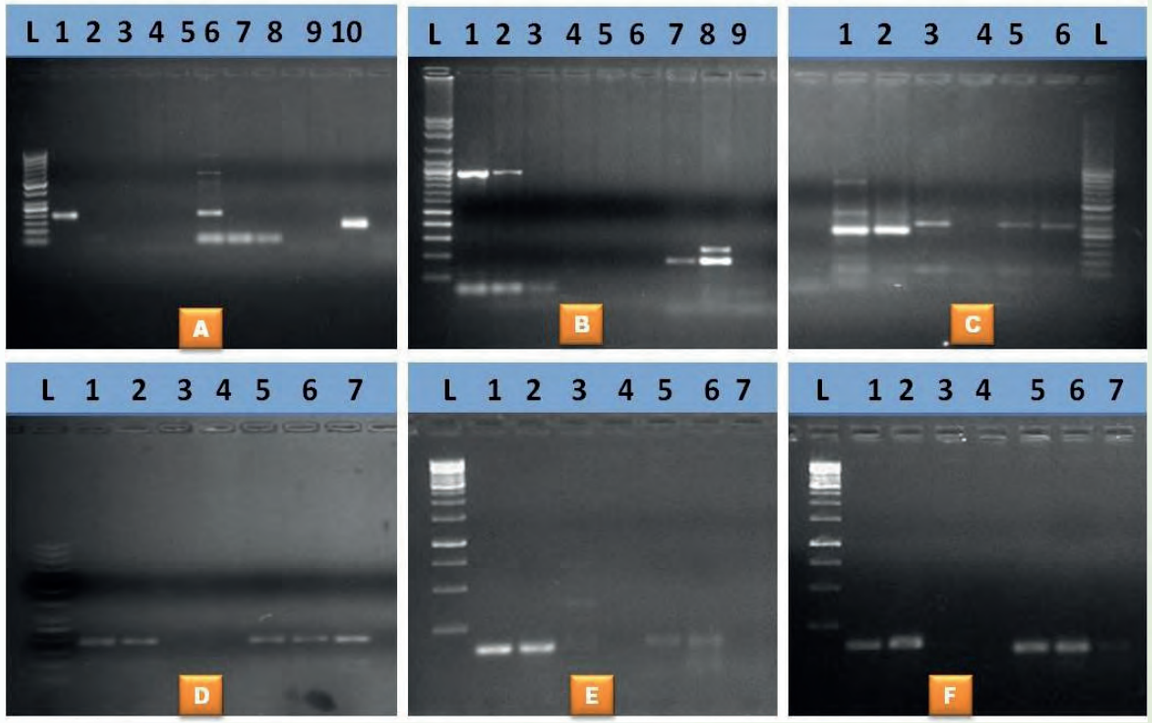


Fig. 4.15: African Yam Bean (AYB) cross-transferability analysis using SSR primers of different leguminous crops. A, B and C: Cross-transferability of chickpea SSRs in AYB; D: Cross-transferability of cluster bean SSRs in AYB; E and F: Cross-transferability of cowpea SSRs in AYB

Table 4.2: Details of the SSR primers tested from six different legumes and amplified in African Yam bean (AYB) for cross-transferability

S. No.	Crop species	Number of SSRs tested in the legume	Number of SSRs amplified in the legume	Cross-transferability (%) in AYB	
1.	*Vigna unguiculata*	23	23	19	82.61%
2.	*Cyamopsis tetragonoloba*	37	35	14	40%
3.	*Vigna radiata*	25	25	9	36%
4.	*Vigna mungo*	28	27	23	85.29%
5.	*Glycine max*	40	17	3	17.65%
6.	*Cicer arietinum*	25	25	11	44%

Genome-wide analysis of von Willebrand factor A (vWA) gene family in rice

Suhas Gorakh Karkute, Vishesh Kumar, Mohd. Tasleem, DC Mishra, Chaturvedi KK, Anil Rai, Amitha Mithra Sevanthi, Kishor Gaikwad, Tilak Raj Sharma, Amolkumar U Solanke* (Rice Science, 2022)

Resistance to blast disease in rice is a complex process involving large number of genes and different cellular pathways. The response of rice cultivars depends on the presence of resistance genes and defense regulator genes which ultimately results in immune response. Tetep cultivar of rice is highly immune to blast disease and therefore it has been explored to identify novel resistance governing genes. We have identified two novel blast responsive genes in the blast resistant Tetep cultivar having von Willebrand factor domain A (vWA). von Willebrand factor domain A (vWA) containing genes are well characterized in humans but except few BON genes, they are least explored in plants. Considering the novelty and important role of vWA genes, we have identified and characterized vWA superfamily in rice. We observed 40 vWA genes distributed across all the 12 chromosomes in rice genome. In addition to vWA domain, vWA proteins possess other different motifs or domains, such as Ubiquitin Interacting Motif which acts in protein degradation pathway, RING finger in protein-protein interaction, etc. Twenty of the 40 vWA genes were unique while the remaining twenty shared large fragment similarities with each other indicating gene duplication (Fig. 4.16). Expression analysis of vWA genes in the available expression data in RiceMetaSys, RiceMetaSysB and Genevestigator suggested that they probably function in biotic and abiotic stress responses including hormonal response, and signalling. Thus, diversity of functions carried out by vWA superfamily in plants make them one of the most important gene families. The frequency

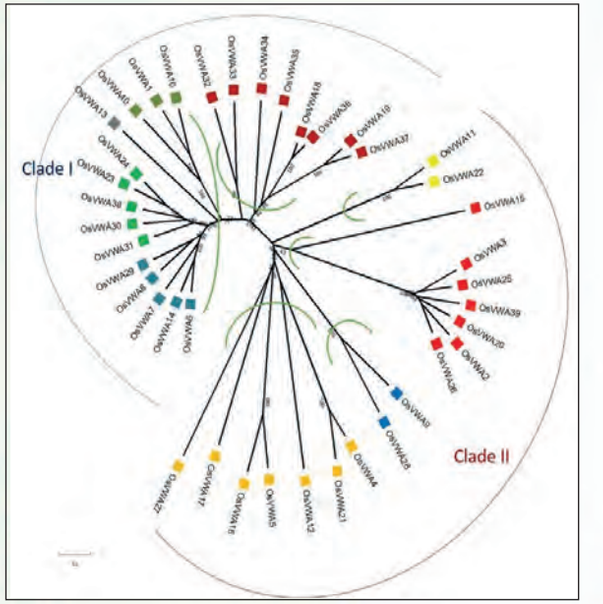


Fig. 4.16: Evolutionary relationship of vWA proteins in rice. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Poisson correction method

of transposon insertion in 31 of the 40 vWA genes in 3000 rice germplasm genome data was found to be negligible, emphasizing that these genes are functionally very important. Structural variant analysis showed that the vWA genes in a blast-resistant cultivar Tetep had huge variations compared to susceptible cultivars HP2216 and Nipponbare. qRT-PCR analysis of vWA genes in *Magnaporthe oryzae* infected rice tissues showed *OsvWA9*, *OsvWA36*, *OsvWA37* and *OsvWA18* as probable candidates for blast disease resistance (Fig. 4.17). This is the first attempt to characterize vWA gene family in any plant species.

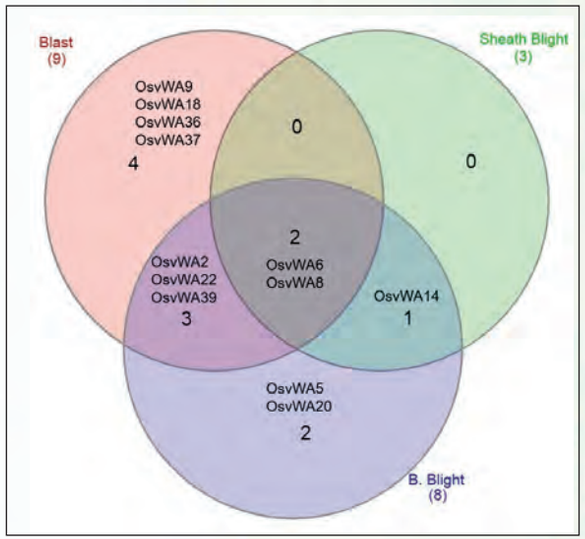


Fig. 4.17: Venn diagram showing vWA genes expressed in different biotic stresses Blast, Sheath Blight and Bacterial Blight

Development of syringe inoculation method for panicle blast infection and its utilization in identification of novel resistant resources

Vishesh Kumar, Pankaj K Singh, Suhas Gorakh Karkute, Mohd. Tasleem, Someshwar Bhagat, MZ Abdin, Amitha Mithra Sevanthi, Anil Rai, Tilak Raj Sharma, Nagendra K Singh and Amolkumar U Solanke* (3 Biotech, 2022)

Panicle blast is the most severe type of rice blast. Screening of rice genotypes for panicle blast resistance at the field level requires an efficient and robust method of inoculation. Here, we developed a method that can be utilized at both small and large-scale screening and assessment of panicle blast infection and disease reaction. The method involves inoculation of *Magnaporthe oryzae* spore culture in the neck of panicle using a syringe and covering the inoculation site with wet cotton and aluminium foil to provide the required humidity for spore germination (Fig. 4.18). We standardized the procedure where neck was infected with pathogen solution with spore concentration about 10^5 spores/ml. The suspension was then injected to the panicle using syringe just before the panicle comes out of flag leaf. The injected portion was covered with moist cotton with double distil autoclaved water to provide moist condition for the pathogen. The method was standardized by using panicle blast resistant cv. Tetep and a susceptible cv. HP2216 inoculated with three different isolates of *M. oryzae*. The method was evaluated at phenotypic as well as molecular level by expression analysis of disease responsive pathogenesis-related (PR)

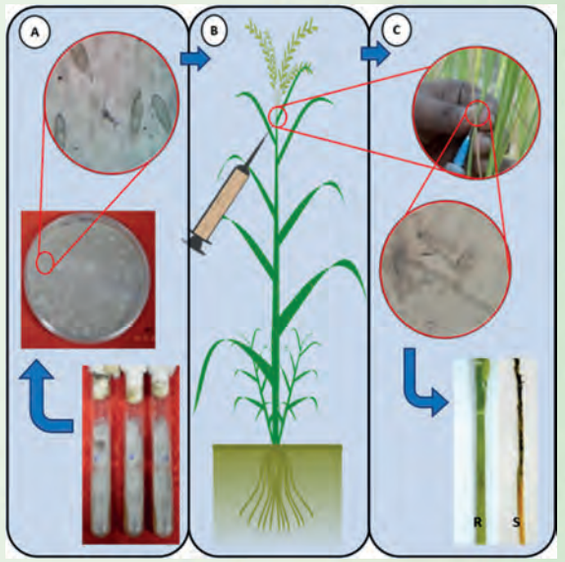


Fig.4.18: A) *Magnaporthe oryzae*, strain mo-ni-025 was cultured on potato dextrose agar for 10-15 days, followed by sporulation of fungus on Mathur's media for 10-15 days at 25°C incubation, after which plates were washed with distilled water to make spore suspension and checked under a microscope. B) The spore suspension was injected into the neck of the rice panicle for infection at 60-70% humidity and 24-25°C of temperature. C) Resistance genotype of rice shows only marks of infection while the susceptible genotypes show the effect of severe damage caused by *M. oryzae* infection.

genes. The method was further successfully validated by screening the wild rice germplasm for panicle blast response in the field using three *M. oryzae* strains and subsequently with the most virulent strain in 45 EMS induced mutants of Nagia 22 shortlisted based on field screening in a blast hotspot region. We have thus identified five novel blast disease-resistant wild rice genotypes and 15 mutants which can be used in blast breeding programmes. The method is easy, robust, reliable, and highly efficient for screening the large germplasm of rice for panicle blast.

Genetic fidelity testing of tissue culture raised plants under NCS-TCP program and development of standard operating protocol (SOP) for testing of guava

Bhuvnesh Sareen, Dipti Dhumale, Mayuri D Mahalle, Mohd Tasleem, Amitha Mithra Sevanthi and Amolkumar U Solanke*

We are Referral Centre for Genetic Fidelity Testing under National Certification System of Tissue culture Raised Plant (NCS-TCP), a program run by Govt. of India through DBT. Under this program we developed standard operating protocols (SOP) for genetic fidelity testing of tissue culture raised plants and also tests 5% random samples of received from 5 different ATL labs. In case of banana and date palm samples, UBC810, UBC818, UBC840, UBC857, UBC873 and UBC880 markers are used while in case of potato UBC807, UBC812, UBC 817, UBC822, UBC886 and UBC891 are used. In 2022, total number of samples tested are 1935.

In addition, this year we have developed SOP for genetic fidelity of guava plants. For this, the leaf samples of 10 different guava varieties (Pant Prabhat, Lucknow 49, Allahabad Safeda, Punjab Pink, Hisar Surkha V, Hisar Surkha red, Lalat, Shweta, Trichy1 and Black guava) were collected from the orchard of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi and used for the development of genetic fidelity protocol. The genomic DNA were isolated from the collected germplasm and then PCR amplification was carried out using ISSR primers. Initially, a set of 100 ISSR primers available from University of British Columbia (UBC) were screened for polymorphism with different guava germplasm. Out of the 100 (UBC801-UBC900) screened primers, amplification in different guava germplasms were observed with 39 ISSR primers. Among the amplified primers, UBC811, UBC812, UBC815, UBC816, UBC817, UBC841, UBC846, UBC855 UBC889 and UBC891 showed polymorphic bands and can be used for the genetic fidelity testing of guava samples (Fig. 4.19). A total of 35 polymorphic bands were recorded among the above 10 identified polymorphic primers.

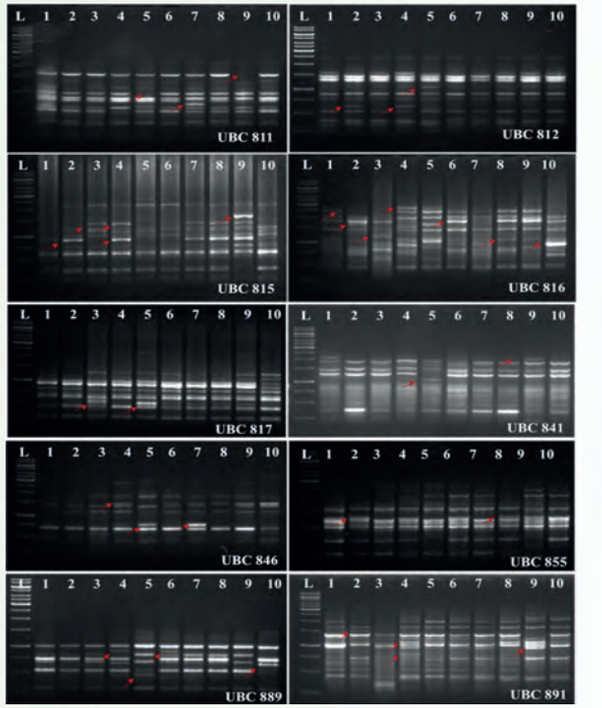


Fig. 4.19: Gel images showing polymorphic bands in guava germplasm using ISSR primers L. 1kb ladder 1. Pant prabhat; 2. Lucknow 49; 3. Allahabad Safeda; 4. Punjab pink; 5. Hisar surkha (v); 6. Hisar surkha red; 7. Lalat; 8. Shweta; 9. Trichy 1; 10. Black guava

Standardization of finger millet regeneration protocol for transformation of CRISPR-Cas9 based gene editing vectors

Ramesh Namdeo Pudake (SERB-TARE Fellow) and Amolkumar U. Solanke

Heat and drought are the major environmen-

tal constraints in finger millet production. In view of changing climatic conditions, to impart stress tolerance in finger millet, quick and efficient genomics strategies are needed. CRISPR-Cas9 based genome editing system provides ample opportunity for the study of functional aspects of genome. In this project we have selected candidate downregulated genes under the abiotic stress in finger millet, constructing CRISPR/Cas9 expression cassettes specific to the candidate genes, and further characterizing generated finger millet mutant at molecular and functional level for heat and drought tolerance. Initially seeds for susceptible cultivar of finger millet GN-5 was collected from the Indian Institute of Millets Research, Rajendranagar, Hyderabad. Further, 10 primer pairs for the candidate genes as negative regulator were designed and used for the amplification of the suitable region for gRNA design. The PCR products were cloned and sequenced (Fig. 4.20). Following candidate gene sequencing gene knockout construct pRGEB-32 with EcARM (U-box domain-containing protein); EcDREBIP2 (E3 ubiquitin protein ligase DRIP2-like); EcSTRS2 (DEAD-box ATP-dependent RNA helicase) and EcU-box22 (Protein spotted leaf 11-like) are prepared and transformed in Agrobacterium. Before developing proper transformation protocol, we have established reproducible regeneration protocol. For the callus induction, finger millet GN-5 seeds were surface sterilized and kept on the MS medium containing the different combination of auxin and cytokinin. Agar powder (0.8% w/v) was used for solidification of media and pH was adjusted (5.8) before autoclaving. For callus initiation petri dishes were kept in the dark at 27±1°C. After 5th week of culture initiation, data on callus growth were recorded. The calli were then transferred to fresh maintenance medium. After a month calli were placed on medium for shoot induction, and the proliferation response was observed after eight weeks (Fig. 4.21). Further work on Agrobacterium mediated transformation of CRISPR-Cas9 construct in the calli of finger millet is progress.

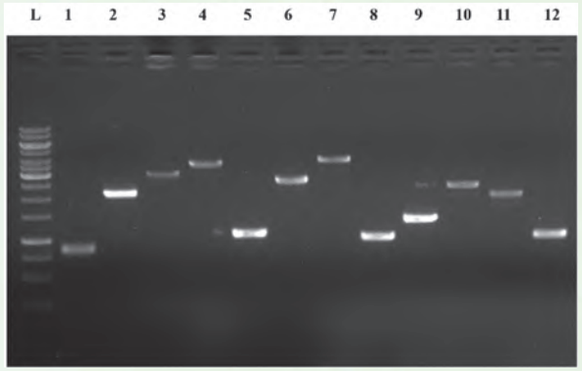


Fig. 4.20: The confirmation of amplification of candidate genes from GN-5 cultivar. L: 1 Kb Ladder, 1-12: ARM, DREB, HPT2, PP2C, RBO-F1, RBO-F2, STRS1-F1, STRS1-F1, Ubox22, WD40 upper, WD40 lower and ZF

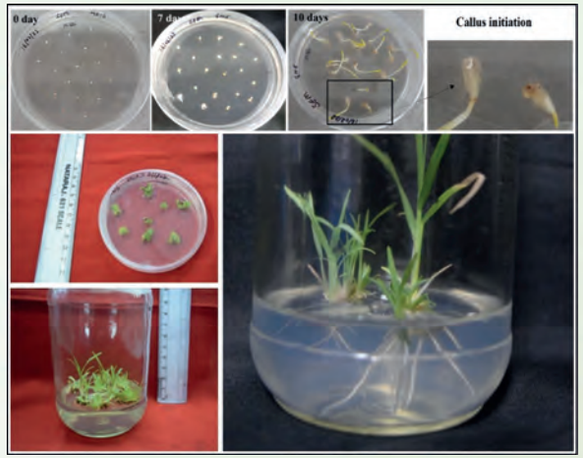


Fig. 4.21: Standardization of regeneration in Finger millet cultivar GN-5.

Dissecting the molecular network regulating high grain number phenotype in qGN4.1 QTL-NILs of rice (*Oryza sativa* L.)

Deepak Singh Bisht*, Manish Kumar, Nitin Kumar, Vandana Rai, Nagendra Kumar Singh

Optimizing plant architecture for efficient acquisition and allocation of resources has been a major focus of yield improvement programs in rice. Numerous genes and QTLs directly affecting the plant architecture have significantly increased the yield of modern-day rice varieties. Here we report a

detailed investigation of molecular genetic factors influencing the panicle architecture in rice. For this study, we have used two contrasting genotypes; rice mega variety Samba Masuri and its high-yielding near-isogenic line (SM-NILs). SM-NILs carries introgression of a high grain number qGN4.1 QTL on chromosome 4 with broader flag leaf, few tillers, high GNPP, and 25-30% more yield compared to SM (Fig 4.22). Contrasting morphological features of SM-NILs suggest that the qGN4.1 QTL enhances grain productivity in rice by modulating plants morphological traits for efficient utilization of photosynthates and other resources. To gain a detailed molecular understanding, a comparative transcriptomic analysis of SM and SM-NILs was performed at three different developmental stages (panicle initiation stage S-1, heading stage- S2, and milking stage S-3; Table 4.3). Amongst the 48 genes in the *qGN4.1* QTL region, only LOC_Os04g52210 and LOC_Os04g52590 were identified to be differentially regulated between SM-NILs and SM. LOC_Os4g52210 (OsKS3) codes for ent-kaurene synthase were significantly downregulated in SM-NIL, whereas *LOC_Os04g52590* encodes a protein kinase domain-containing protein and was upregulated in the SM-NILs (Table 4.4). The differential regulation of these two genes provides the first line of evidence for their involvement in regulating the panicle structure and high grain number phenotype of SM-NILs.

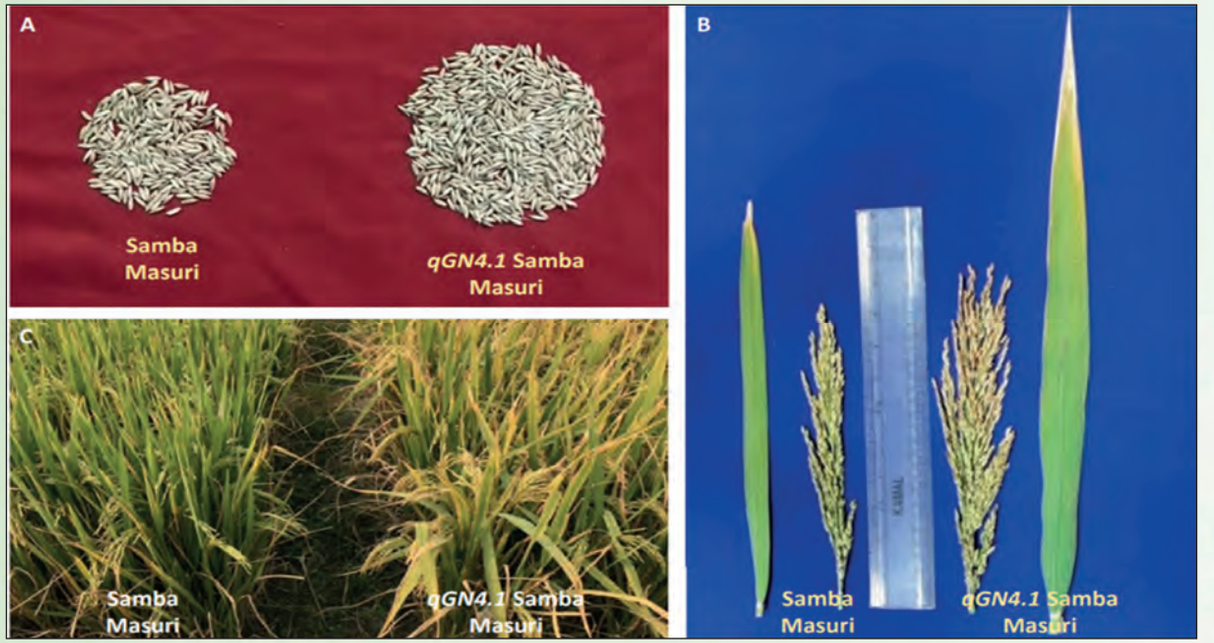


Fig. 4.22: Morphological evaluation of Samba Masuri (SM) and qGN4.1 Samba Masuri NILs (SM-NILs). A) Number of grains per panicle. B) Flag leaf length and panicle morphology. C) SM and SM-NILs planted in IARI fields

Table 4.3: Pairwise comparison at different developmental stages.

Comparison	DEG set name	Down-regulated	Up-regulated	All genes
SM-NIL-S1_ Vs SM_S1	S-1	330	306	636
SM-NIL-S2_ Vs SM_S2	S-2	391	451	842
SM-NIL-S3_ Vs SM_S3	S-3	330	306	667

SM-NIL-S1_Vs_SM-S1, SM-NIL-S2_Vs_SM-S2, and SM-NIL-S3_Vs_SM-S3 represent the comparison of SM-NIL with SM at the panicle initiation stage (S1), late booting stage (S2), and milking stage (S3), respectively.

Table 4.4: DEGs identified from different stages and collocated in the QTL region.

Gene	Annotation	S-1 Log2(FC)	S-2 Log2(FC)	S-3 Log2(FC)
LOC_Os04g51809	expressed protein	0	NS	-2.16910323
LOC_Os04g51930	retrotransposon protein, putative, LINE subclass, expressed	3.084882684	3.656810156	NS
LOC_Os04g52210	terpene synthase, putative, expressed	-10.20930353	-8.100797506	-7.804377185
LOC_Os04g52440	aminotransferase, putative, expressed	NS	NS	2.194421604
LOC_Os04g52530	A heavy metal-associated domain-containing protein expressed	NS	3.896164446	NS
LOC_Os04g52590	protein kinase domain-containing protein, expressed	2.323321291	2.403689697	3.519214824

SM-NIL-S1_Vs_SM-S1, SM-NIL-S2_Vs_SM-S2, SM-NIL-S3_Vs_SM-S3 represent the comparison of SM-NIL with SM at the panicle initiation stage (S1), late booting stage (S2), and milking stage (S3), respectively.

Internal control gene selection for quantitative reverse transcriptase PCR during sheath blight infection in rice (*Oryza sativa* L.)

Joshitha Vijayan*, Illa M Tiwari, Soham Ray, Priyanka Jain, Ram Jatan, Nitin Kumar, Manish Kumar, Nagendra Kumar Singh, Deepak Singh Bisht

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is one of the commonly used techniques to assay gene expression in molecular biology. The selection of an optimal internal control gene during sheath blight of rice, caused by the necrotrophic fungus *Rhizoctonia solani*, is necessary for the accurate estimation of expression levels of target genes under this condition. In our study, we have tested eight different potential rice internal control genes *viz.*, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), eukaryotic elongation factor - 1α (*eEF-1α*), ubiquitin C (*UBC*), Actin (*Act*), 25S rRNA (*25S*), 18S rRNA (*18S*), ubiquitin 5 (*UBQ5*) and ubiquitin 10 (*UBQ10*), based on literature survey. Quantitative reverse transcriptase PCR experiments were conducted taking two different rice genotypes namely, HP2216 (susceptible to sheath blight) and Tetep (tolerant to sheath blight), which differ in their tolerance spectrum towards sheath blight infection. We noted the C_T-values of all target genes under study at 0, 12, 24, 36 and 48 hour,-post inoculation in replicates and carried out statistical analysis to identify the most suitable gene for our purpose. As we measured the expression level based on $\Delta\Delta C_T$ method, we assumed that the C_T-value of a potential internal control gene remain constant under all the experimental conditions across all rice genotypes. Therefore, a two-factor ANOVA was done for testing equality of mean C_T ($_T$), where progressive *R. solani* infection-time becomes one factor and genotype becomes the other. Though *GAPDH* seems to be the internal control gene of choice when variable genotype and progressive infection-time are considered simultaneously, UBQ5 showed the highest expression stability under progressive sheath blight infection when a single genotype is considered (Table 4.5). To check the performance of internal control genes, we did qRT-PCR of defense gene OsCHI11 (LOC_Os06g51050) using two best (GAPDH

and UBQ5) and two worst internal control genes (18 S and 25S) which also confirmed that UBQ5 and GAPDH are the best internal control genes for rice sheath blight studies (Fig. 4.23). To the best of our knowledge, this work is the first initiative to discover an internal control gene for assaying qPCR-based gene expression in rice during sheath blight invasion.

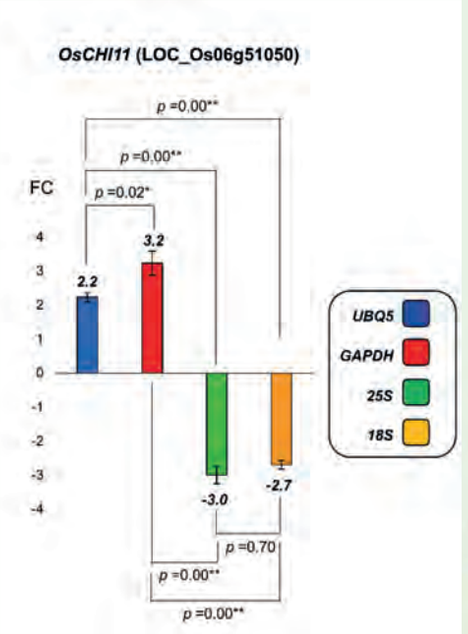


Fig. 4.23: Mean ± SE (n = 6) expression fold-change of *OsCHI11* and *OsPGIP1* in sheath blight tolerant genotype 'Tetep' calculated using four different internal control genes, *UBQ5*, *GAPDH*, *25S* and *18S* at 24 hours post inoculation compared to mock inoculated control (SE = Standard Error).

Table 4.5: Results of Fisher's F test applied over two-factor ANOVA with replication at 99% confidence intervals (1% levels of significance)

ANOVA: 2F-wR (CI = 99%)	GAPDH	eEF-1α	UBC	Actin	25S	18S	UBQ5	UBQ10
Time Point (TP)	NS	*	*	*	*	*	*	NS
Genotype (G)	NS	*	*	NS	*	*	*	NS
Interaction (TP X G)	NS	*	NS	NS	*	*	NS	*

5. NATIONAL PROFESSOR- B. P. PAL CHAIR

Allele mining for agronomically important genes in wild rice germplasm and stress tolerant landraces of rice growing in the hot spots

Maintenance and utilization of wild rice germplasm available at ICAR -NIPB

Deepak Singh Bisht, Amit Rathor, Vandana Rai, Nagendra Kumar Singh*

Maintaining and utilizing wild rice germplasm is important for conserving genetic diversity and developing new varieties with desirable traits. The ICAR-National Institute of Plant Biotechnology (ICAR-NIPB) has made significant efforts in collecting and evaluating wild rice germplasm in India. In 2021, 100 new wild rice germplasm seeds were collected from 12 out of the 15 agro-climatic zones of India

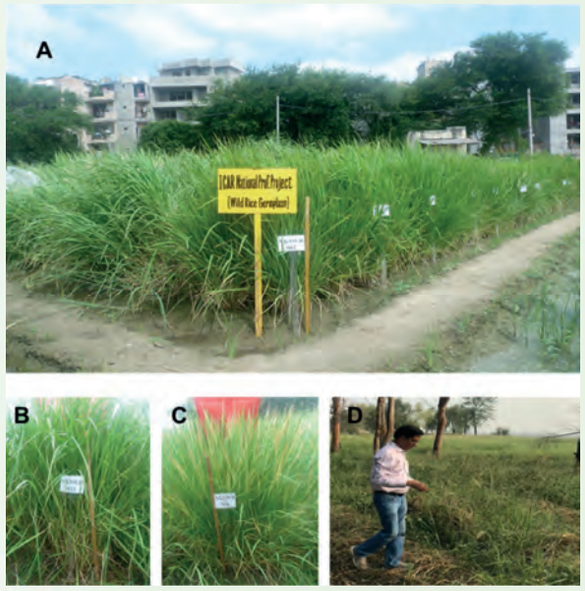


Fig. 5.1: Multiplication of wild rice germplasm in IARI research farm.

under the National Professor B.P. Pal Chair project. In 2022, these germplasms along with the previously collected accessions were thoroughly evaluated for different types of biotic and abiotic stresses (Fig. 5.1). Seeds from 354 accessions representing nine states, namely Assam, Bihar, Chhattisgarh, Goa, Gujarat, Himachal Pradesh, Odisha, Uttar Pradesh, and Uttarakhand, were submitted to the NBPGR germplasm database, and the remaining wild rice accessions are ready deposited to NBPGR. Using wild rice germplasm information on geographical location, such as village, block, district, state, longitude, and latitude, and 46 morphological characters, a web portal called the "Indian Wild Rice (IWR) Database" has been developed (nksingh.nationalprof.in:8080/iwrdb).

Human Resource Development

- POST-GRADUATE TEACHING PROGRAMME
- TRAINING AND CAPACITY BUILDING
- TRAININGS AND SYMPOSIUM ORGANIZED

POST-GRADUATE TEACHING PROGRAMME

ICAR-National Institute for Plant Biotechnology (NIPB) has been actively engaged in Human Resource Development in the area of plant Molecular Biology and Biotechnology since its inception. Currently 43 Ph.D. and 09 M.Sc. students are registered in the discipline of Molecular Biology and Biotechnology at the Centre. In the year 2022, two Ph. D. and seven M.Sc. students were awarded with Doctoral and Master's degrees in the 60th Convocation 2022, respectively.

Students on roll in the discipline of Molecular Biology and Biotechnology during the Academic Session 2022-23 is as follows.

Ph.D. Students

S No.	Name of the Student	Roll No.	Name of the Chairperson	S. No.	Name of the Student	Roll No.	Name of the Chairperson
1	Ms. Priyanka Singh	10697	Prof. N. K. Singh	23	Mr. Ankur Poudel	11646	Dr. Pranab Kumar Mandal
2	Ms. Parichita Priyadarshini	10837	Dr. Pradeep Kumar Jain	24	Ms. Mareyam Mukhtar	11817	Dr. Amol Kumar U. Solanke
3	Ms. Jyotsana Tilgam	10838	Dr. Debasis Pattanayak	25	Mr. Jeet Roy	11818	Dr. Pranab Kumar Mandal
4	Ms. Sreeshma N.	10839	Prof. N. K. Singh	26	Ms. Vibha Kamati	11820	Dr. Monika Dalal
5	Ms. Alka Bharati	10840	Dr. Pranab Kumar Mandal	27	Ms. Priya	11821	Dr. R. C. Bhattacharya
6	Mr. Kuldeep Kumar	10842	Dr. Kishor Gaikwad	28	Ms. Nitasana Rajkumari	11822	Dr. Tapan Kumar Mondal
7	Mr. Deepanshu Jayaswal	10843	Dr. Rekha Kansal	29	Mr. Anuj Kumar	11823	Dr. Subodh Kumar Sinha
8	Mr. Mahendra C.	10844	Dr. Kanika	30	Ms. Renu Kumari	11824	Dr. Jasdeep C. Padaria
9	Mr. Sougata Bhattacharjee	11069	Dr. Debasis Pattanayak	31	Ms. Priyanka Kumari	11928	Dr. Debasis Pattanayak
10	Ms. L. Ashakiran Devi	11070	Dr. R. C. Bhattacharya	32	Mr. Ahmed Mohammed Ismail	11934	Dr. Kanika
11	Ms. Bablee Kumari Singh	11071	Dr. S. V. A. C. R Mithra	33	Mr. Ashfak Siraj Mahammad Mujawar	12099	Dr. Amolkumar U. Solanke
12	Mr. Sachin	11074	Dr. Pranab Kumar Mandal	34	Mr. Machindra Sudhir Nirgude	12100	Dr. Kishor Gaikwad
13	Mr. Bipratip Dutta	11290	Dr. S.V.A.C.R. Mithra	35	Ms. Meena S.	12102	Dr. Pranab Kumar Mandal
14	Mr. Akash Paul	11293	Dr. Kishor Gaikwad	36	Ms. Mahi Baaniya	12103	Dr. Debasis Pattanayak
15	Ms. Shaziya Sultana	11367	Dr. Sharmistha Barthakur	37	Ms. Alvakonda Sheena Sabatina	12104	Dr. Jasdeep C. Padaria

S No.	Name of the Student	Roll No.	Name of the Chairperson	S. No.	Name of the Student	Roll No.	Name of the Chairperson
16	Mr. W. Sandesh Tulsiram	11370	Dr. Anita Grover	38	Ms. Sowmyapriya R.	12105	Dr. Monika Dalal
17	Mr. Krishnayan Paul	11546	Dr. Debasis Pattanayak	39	Ms. Shahina Perween	12106	Dr. Tapan Kumar Mondal
18	Mr. Muhammed Shamnas V.	11547	Dr. Subodh Kumar Sinha	40	Mr. Viraj Gangadhar Kamble	12107	Dr. Rhitu Rai
19	Mr. Naresh Kumar Samal	11548	Dr. R. C. Bhattacharya	41	Ms. Anindita Barua	12108	Dr. Ajit Kumar Shasany
20	Mr. Deepesh Kumar	11550	Dr. S. V. A. C. R Mithra	42	Mr. Kumar Nupur Hrishikeshan	12109	Dr. Kanika
21	Mr. Dhivyanandham K.	11551	Dr. Monika Dalal	43	Mr. Kanishk Milind Diwekar	12228	Dr. Subodh Kumar Sinha
22	Mr. Gopal	11552	Dr. Pradeep Kumar Jain				

M. Sc. Students

S.No.	Name of the Student	Roll No.	Name of the Chairperson
1	Mr. Tharun Kumar C J	21673	Dr. Sarvjeet Kaur
2	Mr. Ashutosh Diliprao Thakare	21674	Dr. Anil Kumar Singh
3	Ms. Soumya Chakraborty	21675	Dr. Deepak Singh Bisht
4	Mr. Balaji B	21676	Dr. N. C. Gupta
5	Mr. Sanjay T D	21677	Dr. P. K. Dash
6	Ms. Sonam Brijlal Ingle	21678	Dr. Anil Kumar Singh
7	Mr. Subhash A	21679	Dr. Sarvjeet Kaur
8	Mr. Bhanu Kumar Tiwari	21680	Dr. Deepak Singh Bisht
9	Mr. Kiran Mahavir Magdum	21681	Dr. Jayanti M.

Degrees awarded in the discipline of Molecular Biology and Biotechnology (MBB) during the 60th Convocation held in February, 2022

S No.	Student Name and Roll No.	Chairperson	Thesis Title
Ph.D. Students			
1.	Mr. Lal Bahadur Singh	Dr. P. K. Jain	Genome-wide discovery and characterization of miRNAs and target genes under water-deficit stress conditions in chickpea
2.	Mr. Kishor Uttamrao Tribhuvan	Dr. Kishor Gaikwad	Understanding the Molecular Mechanism of Flowering in response to photoperiod in pigeon pea (Cajanus Cajan (L) Millsp)
M.Sc. Students			
1.	Mr. Akash Maity	Dr. Monika Dalal	Subcellular Localization of MIZ1 (MIZU KUSSEI 1) Homologs in Wheat
2.	Ms. Rekha Mahato	Dr. Tapan Kumar Mondal	Study of allantoin mediated salinity tolerance in rice genotype IR-29
3.	Mr. Mutawar Ashfak S.	Dr. Sarvjeet Kaur	Screening of Bacillus thuringiensis isolates recovered from different agro-climatic zones in India for the presence of vip3-type genes.
4.	Mr. Mahamed Ashiq I.	Dr. Sharmistha Barthakur	In silico whole-genome analyses of MBF1 (Multiprotein Bridging Factor 1) gene and phenotypic characterization of heat stress responses in two contrasting wheat cultivars (Triticum aestivum L).
5.	Ms. Shwetha R.	Dr. Jasdeep C. Padaria	Characterization of gene CaM (Calmodulin) from Pennisetum glaucum (L.) R.Br for its role in heat stress tolerance
6.	Ms. Ankita Vilasrao Chinche	Dr. Kanika	Molecular and Biochemical analysis of traits related to salt tolerance in RIL mapping population of Wheat (Triticum aestivum L.)
7.	Ms. Anindita Barua	Dr. N. C. Gupta	Genome-wide identification and cloning of cytokinin oxidase/ dehydrogenase 5 (CKX5) gene from Brassica juncea

TRAINING AND CAPACITY BUILDING

Details of training by ICAR-NIPB staff during 2022

S. No.	Name	Subject Area	Duration	Host Institute
1	Dr. Subodh Kumar Sinha	"Metagenomic Data Analysis" (ONLINE)	Oct 18-20, 2022	ICAR-Indian Agricultural Statistics Research Institute, New Delhi
2	Dr. Mahesh Rao	"Breaking the blocks: eliminating linkage drag in introgression breeding in rapeseed mustard"	Sept 2022 - Feb, 2023	The University of Bonn, Bonn, Germany Under SERB-SIRE program
3	Dr. Anil Kumar Singh	Online Training on Prediction of non-coding RNA	Feb 16-18, 2022	ICAR-IASRI, New Delhi
4	Dr. Sharmistha Barthakur	Biosecurity and Biosafety: Policies, Diagnostics, Phytosanitary Treatments and Issues"	Aug 2-11, 2022	ICAR-NBPGR, New Delhi

HRD fund allocation and utilization

Total HRD Fund allocation for 2022 (Rs. In Lakhs)	Actual Expenditure for 2022 (Rs. In lakh)	% utilization
7.00	6.98062	99.72

TRAINING PROGRAMME ORGANIZED

43rd annual meeting of Plant Tissue Culture Association (India) & International Symposium on Advances in Plant Biotechnology and Nutritional Security-2022 (April 28-30, 2022)

The symposium was organized by NIPB, New Delhi in hybrid mode from April 28-30, 2022. The Hon'ble Chief Guests Dr. Trilochan Mohapatra, Director General, ICAR and Secretary DARE, Padma Shri Prof. Pramod Tandon, Secretary, PTCA(I), Padma Shri Prof. Sudhir Kumar Sopory, Former Vice Chancellor, JNU and Dr. Tilak Raj Sharma, DDG (CS), ICAR with Prof. R.P. Sharma, Former Director, NRCPB inaugurated the Conference. The Director, NIPB and Symposium Chair Dr. Ajit Kumar Shasany in his welcome address dealt upon the need for nutritional security and it significant progress in India. Dr. Tapan Kumar Mondal, convener of the conference presented the theme of the conference and Dr. Rekha Kansal, organizing secretary delivered the formal vote of thanks. More than 450 delegates (physical: 250 and online: 200) across the country attended the event; The physical mode of the conference comprised of 9 sessions, plenary lectures, three sessions of keynote lectures, (PTCA-I) membership presentations, (PTCA-I) memorial lectures, a workshop, annual general body meeting of PTCA(I) besides the Inaugural and Valedictory sessions. The online mode of the conference featured 186 presentations (92 oral and 94 posters) in 7 different sessions.

CAFT training

This year 4th CAFT training, "Genome Utilization and genome editing for useful traits" was organized by NIPB from 30th Nov to 20th Dec 2022. The training was inaugurated by Padma Shree R. S. Paroda, Ex DG, ICAR and lectures were delivered by 26 resource persons from 15 different institutes comprising of ICAR, CSIR, DBT, DST etc. who are the domain expert on this area. The training was attended by 25 young faculties across the country. The entire training has been divided into 4 modules. They are: Genome decoding, Genome analysis, Genome editing, Closing and interaction which consists of visit and interaction. The valedictory function at the end was graced by Prof. Anupam Verma and

Dr. Seema Jaggi, Additional Director General (HRD).

DBT-sponsored Training program for Accredited Testing Laboratories (ATLs) on Genetic Fidelity Testing and Virus Indexing of Tissue Culture-Raised Plants" 4th-16th May, 2022

A DBT-sponsored Training program for Accredited Testing Laboratories (ATLs) on "Genetic Fidelity Testing and Virus Indexing of Tissue Culture-Raised Plants" was conducted at the two referral centres: ICAR-National Institute for Plant Biotechnology, Pusa Campus, New Delhi for Genetic Fidelity Testing and Advanced Centre for Plant Virology, Division of Plant Pathology, IARI, New Delhi for Virus Indexing from 4th – 16th May, 2022. Total nine trainees participated in the training, four trainees were from, ATL NABI, Mohali; and other four trainees were from, ATL AAU, Anand and one trainee from NCS-TCP Management cell, NIPGR, New Delhi.

Extension And Outreach Activities

SCSP PROGRAMME

In the year 2022, ICAR-NIPB conducted 4 Schedule Caste Sub- Plan (SCSP) programme for socio-economic development of Schedule caste communities (SCs) in the country. The details of the programmes conducted are given as follows

	Name	Date & Place of training	Number of Beneficiary farmers	Items distributed
1	Dr. Amolkumar Solanke, Dr. SV Amitha Mithra, Mr. Anshul Kumar Verma	6 March 2022, Warora, Maharashtra	224	kit with vegetable seeds and farm tools
2	Dr. P.K. Dash, Dr A. K Shasany, Dr. PK Mandal, Mr Vipin	11 July 2022, Talcher, Odisha	178	Saplings: 356, Rice seeds kit: 3 tonnes, Mushroom spawns: 180 bottles
3	Dr. Nimmy M. S, Dr. AmolKumar Solanke, Dr. Ramawatar	16th June 2022, Vengola, Ernakulam District, Kerala	250	Neem Cake (2kg/packet), Micro food (1kg packet) Organic manure (2kg/packet), Pseudomonas(0.5 kg/packet), Organic pesticides (100 ml), Growbag (5 number/kit), Bone meal (2kg bag)
4	Dr. Deepak Bisht, Dr. Rekha Kansal, Dr. Pradeep Jain, Dr Rohit Chamola	23rd June 2022, Chkrata, Dehradun district, Uttarakhand	200	Lemon and mango saplings



KRISHI MELA

The products and technologies developed by ICAR-NIPB were showcased with appropriate charts and posters in Pusa Krishi Mela held from 9-11 March 2022 at the IARI Krishi Mela ground. Scientists and technical staff of NIPB explained the work and attended the queries of the farmers and other visitors during this period.

Other Institutional Activities

- INSTITUTIONAL PROJECTS
- EXTERNALLY FUNDED PROJECTS
- IPR, MOUS AND COPY RIGHT
- AWARDS AND HONOURS
- RECRUITMENTS/PROMOTIONS/ RETIREMENTS
- OTHER ACTIVITIES
- LIST OF PUBLICATIONS
- IMPORTANT COMMITTEES

INSTITUTIONAL PROJECTS

Project Title	Date of start	Date of Completion	Principal Investigator	Name of Associates
Genome analysis in crop plants	April 2021	March 2026	Dr. Kishor Gaikwad	Dr. Jasdeep C Padaria Dr. PK Dash Dr. Anil K Singh Dr. Navin Chandra Gupta Dr. Nimmy MS Dr. Ramavatar Nagar Dr. Shandhya Sharma Dr. Yuvaraj I
Bioprospecting agriculturally important utilizable genes	April 2021	March 2026	Dr. Tapan Kumar Mondal	Dr. Anita Grover Dr. Rekha Kansal Dr. Sarvajeet Kaur Dr. Pradeep Kumar Jain Dr. Sharmishta Barthakur Dr. Kanika Kumar Dr. Monika Dalal Dr. Rhitu Rai Dr. Vandna Rai Dr. Shbana Begam
Novel genes and biomolecules for plant and human nutrition	April 2021	March 2026	Dr. Pranab Kumar Mandal	Dr. Ajit Kumar Shasany Dr. Subodh Kumar Sinha Dr. Rohini Sreevathsa
Genetic modification of crop plants for important traits	April 2021	March 2026	Dr. Ramcharan Bhattacharya	Dr. Debasis Pattanayak Dr. Ashish Kumar Dr. SV Amitha Mithra Dr. Amolkumar Solanke Dr. Mahesh Rao Dr. Deepak Singh Bisht Dr. Anshul Watts Dr. Joshitha Vijayan

EXTERNALLY FUNDED PROJECTS

S. No.	Title of the project	Funding agency	Budget & Duration	Principal Investigator
1.	Decoding the genomes of guar and black gram for the identification of important genes	ICAR-CRP on Genomics	Rs. 145.00 Lakhs 2021-continuing	Dr. Kishor Gaikwad
2.	Molecular mapping and identification of candidate gene(s) responsible for the cleistogamous trait in pigeonpea *Cajanus cajan* Millsp	DST-SERB	Rs 36.63 Lakhs 2019-2022	Dr. Kishor Gaikwad
3.	Exploiting alien genetic resources for developing climate resilient wheat and understanding mechanism of heat tolerance (Multi-Institutional)	ICAR-NASF	Rs. 25.64864Lakhs (Aug 2018 - January 2022)	Dr. Jasdeep Chatrath Padaria (CCPI at NIPB)
4.	Identification of QTLs for subcomponent of WUE through strategic utilization of whole genome sequences and accurate phenotyping rice.	ICAR-NASF	Rs. 51.26 Lakhs	Dr. Prasanta K Dash
5.	Identification and functional characterization of the key resistance/ susceptible determinants for Sclerotinia stem rot disease in oilseed Brassica	DST/SERB/CRG	Rs. 32.00428 Lakhs Dec 2020 - 23	Dr. Navin C Gupta
6.	Identification of effector candidates from necrotrophic plant pathogen, *Sclerotinia sclerotiorum* and its target proteins in Indian mustard (*Brassica juncea*)	DBT	Rs. 38.16 Lakhs Dec 2019 - 23	Dr. Navin C Gupta
7.	Meta-Analysis and computational approach for biomolecular interactions profiling of *Sclerotinia sclerotiorum*–Brassica pathosystem	ICAR-NPABCB	Rs. 50.0 Lakhs April 2021-26	Dr. Navin C Gupta
8.	Germplasm characterization, genomics analysis and gene discovery for yield, metabolites, stress tolerance in tea	DBT	Rs. 72 Lakhs 2022-2025	Dr. Tapan Kumar Mondal
9.	Study on methylation patterns under drought stress for identification of key genes and microRNAs involved in drought stress tolerance in chickpea	DST	Rs. 38 Lakhs 2020-2023	Dr Pradeep K umar Jain
10.	Deciphering the transcriptional regulator(s) of hydrotropism in wheat	SERB	Rs. 40.70 Lakhs 2018 to 2022	Dr. Monika Dalal

S.No.	Title of the project	Funding agency	Budget & Duration	Principal Investigator
11.	Genetic modifications to improve biological nitrogen fixation for augmenting nitrogen needs of cereals	ICAR-Incentivizing Research in Agriculture	Rs. 78.56 Lakhs (for 2022-23) 2015- continuing	Dr. Pranab Kumar Mandal
12.	Development of low immunogenic wheat	ICAR-CRP on Biofortification	Rs. 22.00 Lakhs (for 2022-23) 2015-continuing	Dr. Pranab Kumar Mandal
13.	Identification and functional validation of partner proteins of two-component high affinity nitrate transporter of wheat	SERB, DST	Rs. 36.32 Lakhs 2019- 2022	Dr. Subodh Kumar Sinha
14.	Functional delineation of exitron-spliced and exitron-containing isoforms of NRT1.5 gene of bread wheat (*Triticum aestivum* L.) in context with root-to-shoot nitrate transport	DBT	Rs.41.12400 Lakhs 2022- 2025	Dr. Subodh Kumar Sinha
15.	Functionality of Methionine sulfoxide reductase gene(s) in redox homeostasis-mediated resistance to *Helicoverpa armigera* in pigeonpea wild relative *C. platycarpus*	SERB	Rs.42.00 Lakhs 2020-2023	Dr. Rohini Sreevathsa
16.	CRISPR Crop Network: Targeted improvement of stress tolerance, nutritional quality and yield of crops by using genome editing	ICAR-NASF	Rs.118.44 Lakhs 2022- 2025	Dr. Ramcharan Bhattacharya (CCPI)
17.	Genomics-led improvement of biotic and abiotic stress tolerance in mustard rape for economic and environmental sustainability	DBT	Rs.108 Lakhs 2018- 2023	Dr. Ramcharan Bhattacharya (CCPI)
18.	Development of haploid inducer line, and enhancement of seed-meal quality in *Brassica juncea* through CRISPR/Cas mediated genome editing	DBT	Rs.50 Lakhs 2018- 2022	Dr. Ramcharan Bhattacharya
19.	A way forward to developing aphid-resistance in Indian mustard (*Brassica juncea*): Host- and virus-mediated gene silencing of parthenogenetic genes in mustard aphid	SERB	Rs.38 Lakhs 2019- 2022	Dr. Ramcharan Bhattacharya
20.	An integrated omics approach to develop white rust (*Albugo candida*) resistance in *Brassica juncea*	SERB	Rs.35.2443Lakhs 2021- 2024	Dr. Ashish Kumar
21.	Discovery of novel genes and QTLs conferring resistance to ToLCNDV disease from indigenous sources, genome-wide transcriptional dynamics and allele mining of the candidate genes in Cucurbitaceous vegetables	NASF	Rs. 39.943 Lakhs 2022- 2023	Dr. Amitha Mithra SV

S.No.	Title of the project	Funding agency	Budget & Duration	Principal Investigator
22.	Referral Centre for Genetic Fidelity testing of Tissue culture raised plants (NCS-TCP)	DBT	Rs.160.33 Lakhs 2021- 2026	Dr. Amolkumar Solanke
23.	RiceMetaSys: Understanding of rice gene network for biotic and abiotic stress management through system biology approach	CABin Scheme ICAR	Rs.20 Lakhs 2017 - 2025	Dr. Amolkumar Solanke
24.	In-vivo maternal haploid induction system in *Brassica juncea* through CRISPR/Cas9 mediated genome editing of pollen specific DMP gene	SERB	**Rs.**30.27 lakhs 2021-2024	Dr. Anshul Watts
23.	Strengthening cultivar diversity of rapeseed mustard to manage climate related risks and foster productivity in stress prone areas of Central and Western India	Bioversity international-CIAT alliance	Rs.31.45 Lakhs 2021- 2024	Dr. Mahesh Rao
24.	Induced mutation of synthetic *B. juncea* haploid for isolation of climate resilient genotypes	DAE-BRNS	Rs.31.45 Lakhs 2020- 2023	Dr. Mahesh Rao
25.	Broadening genetic diversity of Indian mustard (*Brassica juncea*) through resynthesis of amphidiploid genome by crossing parental diploid species	SERB, ECRA	Rs.33.64Lakhs 2018- 2022	Dr. Mahesh Rao
26	Breaking the blocks: eliminating linkage drag in introgression breeding in rapeseed mustard" with Dr. Annaliese S. Mason, Professor and Chair of Plant Breeding, The University of Bonn, Katzenburgweg 5, 53115, Bonn, Germany	SERB-SIRE	Rs. 16.50 Lakhs 2022 - 2023	Dr. Mahesh Rao

IPR, MoUs, COPYRIGHT AND GERMPLASM REGISTRATION

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 institute through Public- Private Partnership. The following activities were undertaken by the ITMU during the year January 2022 to December 2022.

I. Patent Granted:

S. No.	Title	Application No./Patent Number	Date of filing/Granting	Name of the Inventor/ Scientist
1	*M. oryzae* polynucleotide associated with Blast resistance and uses thereof	397653 398/DEL/2013	25-05-2022	Dr. T.R. Sharma

II. Patent Filed:

S. No.	Title	Application No./ Patent Number	Date of filing/Granting	Name of the Inventor/Scientist
1.	Innovative Elisa Based Assay For Multiplexed assessment of RuBisCo(MARIE) content in the water stressed rice	202211076990	30-12-2022	Dr. P. K. Dash
2.	A polynucleotide sequence from *Oryzacoarctata* confers multiple abiotic stress tolerance	202211074347 Complete	21-12-2022	Dr. T. K. Mondal
3.	A polynucleotide sequence from *Oryzasativa* indica confers multiple abiotic stress tolerance	202211074345 Complete	21-12-2022	Dr. T. K. Mondal
4.	A polynucleotide sequence of rice confers heat stress tolerance	202211074365 Complete	21-12-2022	Dr. T. K. Mondal
5.	A Rice Blast Disease Resistance Governing Gene Containing A Von Willebrand Factor Domain	202111056668 Complete	05-12-2022	Dr. Amol Kumar U Solanke
6.	A novel DUF740 Polynucleotide associated with multiple stress tolerance from rice	202111038665 Complete	26-8-2022	Dr. P.K Mandal

III. MTA/MoUs signed:

S.No.	Name of University/Dept.(ICAR or Non-ICAR)	Date	Purpose
1.	M.S. Swaminathan Research foundation, Chennai	29.04.2022	Promotion and cooperation in various fields like Scientist and technologists visits, exchange of germplasm, breeding and literature
2.	National Academy of Agriculture Research Management, Hyderabad	14.05.2022	MOA for Association for innovation Development of Entrepreneurship in Agriculture a-IDEA
3.	NationalAgri-Food Biotechnology institute, Mohali, Punjab	24.06.2022	Promotion and acceleration of the research and training in various disciplines of agricultural research
4.	CSIR- Central Institute of Medicinal and Aromatic Plants, Lucknow, UP	06.07.2022	Promotion and cooperation visits of Scientists and technologists, exchange of Germplasm/ breeding material and technologies etc.
5.	GangadharMeher University, AmrutaVihar, Sambalpur, Odisha	29.11.2022	Collaboration in areas of mutual interest

IV. Copy right was granted to following databases/softwares during 2022

S. No.	Description	Copyright diary number
1.	TEAMID: A comprehensive data base of simple sequence repeat markers of tea	SW-15148/2021
2.	TEA HAPMAP-1:A first- generation haplotype map of tea	SW-15745/2022
3.	TLNC: A comprehensive data base of long non coding RNAS (LNCRNAS) of tea	SW-15746/2022
4.	TENGEXA: An R package based tool for tissue enrichment and gene expression analysis	SW-15146/2021

V. Registration of germplasm:

Germplasm IC128335, a drought tolerant wheat genotype (INGR22116) developed by Kumar S, Sareen S, Mishra KK, Potdhukhe NR, Upadhyay D, Meena BK, Budhalakoti N, Kumari J, Singh AK, Bansal R, Padariya JC, Tyagi BS and Singh GP was registered at ICAR-NBPGR, New Delhi on December 08, 2022.

AWARDS AND RECOGNITION

1. Dr. Amitha Mitra SV. received Nanaji Deshmukh ICAR Award for Outstanding Interdisciplinary Team Research in Agricultural and Allied Sciences, 2021.
2. Dr. PK Mandal - Adjunct faculty of Uttar Banga Krishi Viswavidyalaya, Coochbehar.
3. Dr. PK Mandal - IBSC member of BAYER Crop Science Limited
4. Dr. PK Mandal - Editorial Board Member for SAARC Journal of Agriculture
5. Dr. PK Mandal - Editorial Board Member Journal of Plant Biochemistry and Biotechnology
6. Dr. PK Mandal - Institute Management Committee member of ICAR-IASRI, New Delhi; ICAR-NRRI, Cuttack; ICAR-SBI, Coimbatore.
7. Dr. PK Mandal - Departmental Promotion Committee Member (as an expert) for the promotion of Scientists of Biochemistry, Plant Scientist of ICAR- NIASM, Baramati, ICAR-IGFRI, Jhansi.
8. Dr. Monika Dalal - Member IBSC of ICAR-Indian Institute of Maize research, Ludhiana
9. Dr. Monika Dalal - DBT nominee in IBSC of Gautam Budhha University, G. Noida, UP
10. Dr. SK Sinha - external Member, Committee for Course for academic session 2021-22 to 2022-23. Department of Biochemistry, Faculty of Agriculture, Sri Karan Narendra Agriculture University, Jobner, Rajasthan.
11. Dr. AK Gupta received a Best Poster award for the poster entitled 'Phenotyping of *Brassica* germplasm for identification of resistant sources against *Albugo candida* by Kumar V. et al., during National Symposium on 'Recent trends in Phytopathology to address emerging challenges for achieving food security' organized by the ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Amora on February 20-21, 2022
12. Dr. NC Gupta received a Best oral presentation award for the poster: Amino acid substitution causes variation in kernel row number determined by fea4 gene in tropical maize. *In National Conference on Maize for Resource Sustainability Industrial Growth and Farmers Prosperity,* pp06 held on 23-25 Feb 2022, MPUAT, Udaipur, Rajasthan.
13. Dr. NC Gupta received a Best oral presentation award for the poster: Inheritance and validation of molecular markers for kernel row number in tropical maize. *In National Conference on Maize for Resource Sustainability Industrial Growth and Farmers Prosperity,* pp05 held on 23-25 Feb 2022, MPUAT, Udaipur, Rajasthan.

PROMOTIONS / RECRUITMENTS / RETIREMENTS/ TRANSFERS

Promotions:

1. Dr. S.V. Amitha Mitra, Senior Scientist was promoted to the post of Senior Scientist (S.S) w.e.f. 15-12-2021.
2. Dr. Amolkumar U. Solanke, Senior Scientist was promoted to the post of Senior Scientist (S.S) w.e.f. 15-12-2021.
3. Dr. Navin Chandra Gupta, Senior Scientist was promoted to the post of Senior Scientist (S.S) w.e.f. 23-06-2022.
4. Dr. Nimmy M.S., Scientist (S.S) was promoted to the post of Senior Scientist w.e.f. 15-12-2020.
5. Dr. Mahesh Rao, Scientist (S.S) was promoted to the post of Senior Scientist w.e.f. 15-09-2021.
6. Sh. Ramawatar, Scientist (S.S) was promoted to the next higher grade of Scientist w.e.f. 11-05-2020.
7. Dr. Sandhya Sharma, Scientist was promoted to the post of Scientist (S.S.) w.e.f. 01-01-2020.
8. Sh. Rajesh Kumar Pal, LDC was promoted to the post of UDC w.e.f. 28-06-2022.
9. Sh. Kunal Maan, LDC was promoted to the post of UDC w.e.f. 28-06-2022.

Recruitments: NIL.

Retirements:

1. Smt. Seema Dargan, Ex-CTO was superannuated from ICAR services w.e.f. 31st Aug, 2022.
2. Sh. Anoj Kumar Jain, Ex-Private Secretary from ICAR services w.e.f. 31st Aug, 2022.

Transfers: NIL

Visit Abroad:

Name	Visit	Period
Dr. Mahesh Rao, Senior Scientist	Germany	30-08-2022 to 02-03-2023
Dr. Nimmy M.S., Senior Scientist	USA	11-10-2022 to 24-10-2022
Dr. Amolkumar U. Solanke, Senior Scientist (S.S)	USA	11-10-2022 to 24-10-2022

OTHER ACTIVITIES

Academia-Industry Biotechnology Meet

ICAR-NIPB, New Delhi organized Academia-Industry Biotechnology Meet on 12th January 2022 (on-line mode). The aim was to discuss and appraise the Industry about the research programs/achievements of NIPB. Importantly it was an attempt to bring all the major stakeholders-industry, research organization and policy planners at one forum to discuss and to understand the expectations of Industry partners from the biotechnology institutes in the coming days. The meet was graced by dignitaries including Dr T. Mohapatra, Secretary (DARE) and Director General (ICAR), Dr TR Sharma (Deputy Director General, Crop Sciences) and Dr DK Yadava (Assistant Director General, Seeds). All the scientific staff of NIPB and representatives from 16 companies (all across the country and involved in area of agricultural biotechnology) participated in the meet. Dr AK Shasany (Director, NIPB) presented salient achievements of the Institute to give an idea of the areas of research and products in hand and others which are likely to be available in the near future. An excellent panel discussion on, "Biotechnological intervention in agriculture through PPP mode", was moderated by Dr KK Narayanan (Director & CEO, Agrigenome Labs Private Ltd) and eminent panelists included Dr AK Shasany, Dr DK Yadava, Dr M Ramasami (Chairman, Rasi Seeds), Dr Sudha Mysore (Chaiperson, Agrinnovate India ltd), Mr Ram Kaundinya (Director General FSII), Dr. Shivendra Bajaj (Executive Director FSII) and Mr Paresh Verma (CEO, Shriram Bioseed Research).

HINDI CHETNA MAAS

To celebrate Hindi chetna maas (14th Sept. to 13th Oct. 2022) competitions such as Hindi essay writing, debate competition, poetry recitation, noting and drafting were conducted for the staff of NIPB.

Hindi workshops

Four Hindi workshops were conducted during the year 2022.

S. No.	Quarter & Date	Guest Speaker	Topic
1	Jan-March 2022 (30-03-2022) (Online mode)	Dr. Aravind Chaturvedi, SSP (Vigilance), Lucknow, Uttar Pradesh	"महाकवि सूर्यकांत त्रिपाठी 'निराला' की प्रथम कविता "जन्मभूमि पर परिचर्चा"
2	April-June 2022 (29-06-2022)	Ms Sunita Kumari, Assistant Director-Rajbhasha ICAR-IARI, New Delhi	"राजभाषा के रूप में हिंदी"
3	July- Sept 2022 (14-09-2022)	Dr. Ajit Kumar Shasany Director, ICAR-NIPB, New Delhi	"हिंदी दिवस"
4	Oct-Dec 2022 (20-12-2022)	Dr. N.K. Singh, National Professor ICAR- ICAR-NIPB, New Delhi	"विदेशों में हिंदी की स्थिति"

INTERNATIONAL WOMEN'S DAY CELEBRATION

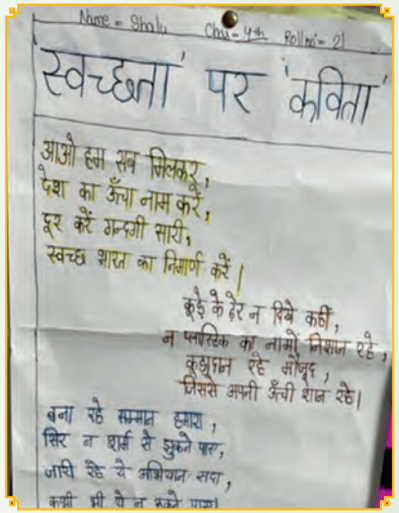
ICAR-NIPB observed International Women's Day on March 8, 2022. Chairperson of the ICC/Women cell Dr. Saravjeet Kaur welcomed everyone and apprised the gathering about the importance and history of the day. Dr. Sharmistha Barthakur introduced the chief guest, Shrimati Mridula Thakur Pradhan, Chairperson of Vikas Foundation Trust; and the guest speaker, Shrimati Kalpana Mohapatra, executive president Pusa Institute Ladies Association. Mrs. Mohapatra, in an eloquent speeh talked about Indian way of life and traditions and the role of women in the family and society over the ages. The chief guest Mrs Thakur Pradhan elaborated upon changing times and the need for adaptation and innovation in thinking and mindset of both the genders towards an equitable and inclusive society in line with the 2022 year's theme "Gender equality today for a sustainable tomorrow". Dr. Ajit Shasany, Director, NIPB also addressed the NIPB staff. On this occassion a "Wellness room" was also inaugurated by the Chief Guest.

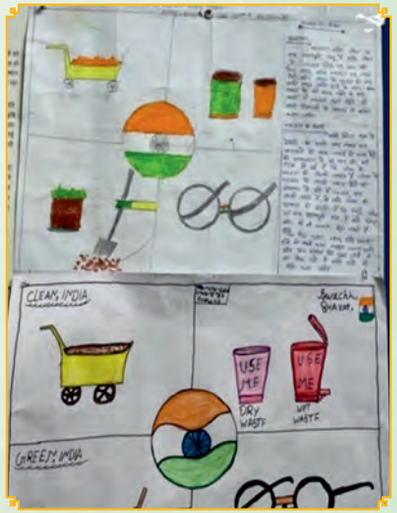
VIGILANCE AWARENESS WEEK

The Vigilance Awareness Week was observed from October 31 to November 06, 2022. The theme for this year was "Corruption Free India for a developed Nation" (भ्रष्टाचार मुक्त भारत–विक्सित भारत). The Director with the staff took the pledge of integrity on October 31, 2022. On this occasion a lecture was organized on November 03, 2022. The speaker for this event was Sh. G.C. Prasad, Comptroller, Bihar Animal Science University, Patna, Bihar. He emphasized the importance of knowing the Do's and Don'ts of the system while doing our duties and advocated us to practice preventive vigilance and refrain from being a predator.

SWACHH BHARAT ABHIYAN

Two cleanliness drives namely, Swachhta Special Campaign 2.0 (02-31 October, 2022) and Swachhta Pakhwada (16-31 December, 2022) were organized by ICAR-NIPB. Under 'Swachhta Special Campaign 2.0 all the labs, corridors and surrounding areas were cleaned. The old and unserviceable instruments were sent to stores for auction. During Swachhta Pakhwada, Swachhta pledge was administered by Director, NIPB and plantation was done. The activities were planned at the Institute, School, nearby residential areas and market places. A workshop was organized to make people aware to generate wealth from the waste. Drawing completion was held involving children from the School.





LIST OF PUBLICATIONS

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