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National Fund for Basic, Strategic and Frontier Application Research in Agriculture

The National Fund for Basic, Strategic and Frontier Application Research in Agriculture's (NFBSFARA) main objective is capacity-building in basic, strategic and cutting-edge application research in agriculture. It aims at: fostering research and research culture in advancing frontiers of scientific knowledge in agriculture to resolve effectively present, anticipated and unanticipated problems; strengthening of the National Agricultural Research and Education System through wide partnerships in science; developing a storehouse of knowledge in science for advancement of agriculture and for awareness of nationally important basic and strategic research; providing support to decision-makers for basic and strategic research; and in organizing workshops, seminars and conferences for creating awareness. It has already funded 102 projects, mostly in a consortium mode, out of which 72 are on-going projects; 65 being multi-institutional in nature.

Feedback: For improvement a questionnaire was given to Principal Investigators (CPIs, CCPIs) of the ongoing projects to assess their perception on the procedure followed by the NFBSFARA for its different activities related to selection of new projects, monitoring and reviewing. More than 90% of the PIs indicated satisfaction level of more than 80%. The other inputs received from them would also help in improving selection methodology further.

During 2014–15, the NFBSFARA has been in the process of funding new projects. Concept notes were invited, and out of the total of 723 'Concept Notes' (CN) submitted, 675 were evaluated by the 'Expert Committees', and 50 CN were selected for developing into full proposals. The CPIs and CCPIs of these 50 CNs were trained in a three-day workshop at the NAARM, Hyderabad, to develop winning research proposals. The full proposals based on these CNs are being processed.

Monitoring ongoing projects: Each project is reviewed individually by an 'Advisory Committee', and later all projects are reviewed by 'Expert Committees' and the 'Empowered Committee'. The following two mega projects are monitored directly by the Empowered Committee — 'Development of pod-borer resistant transgenic pigeonpea and chickpea' and 'Phenomics of moisture deficit and low temperature stress tolerance in rice'. During the year, more than 20 meetings of the 'Advisory Committees' were held.

SALIENT ACHIEVEMENTS

Pod-borer resistant transgenic pigeonpea and chickpea

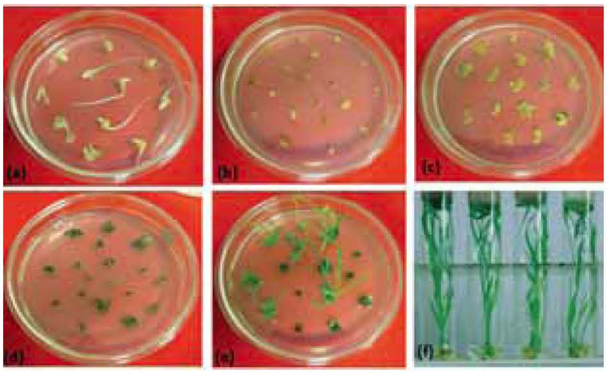
In chickpea, more than 75 new transgenic lines, using existing and new gene constructs, have been generated. Molecular characterization was done with AraSSU driven *Cry*2Aa lines, and one line (BS72C2) was noticed containing a single copy of the transgene that segregated at 3:1 ratio. This line has been found homozygous for the transgene, and gave 100% resistance against pod-borer. Another line generated using full length *Cry1Ac* gene also conferred near-complete protection against pod-borer.

Insect bioassays: *Cry*1Ac line of chickpea with undamaged leaves (*top*); damaged leaves of control (*bottom*)

In pigeonpea, 153 PCR positive *Cry* IAc plants were generated; of which 45 plants exhibited positive for Western blot and/or Strip test. Randomly selected 25 plants from these were subjected to Southern blot and 20 were found positive. A set of selected plants are in T_1 generation.

Dominant nuclear male sterility to produce hybrid-rice seed

The *orfB* gene from CMS rice line was constructed in pCAMBIA1300 vector (loxP-*Os Ubi*1 promoter - NOS terminator-EPSPS marker-loxP) to induce sterility. And to restore fertility, *CRE* gene was constructed in pCAMBIA1300 vector (*Os Ubi*1 promoter-CREgene-NOS terminator -bar as selectable marker). Another strategy for restoration of fertility was double strand RNA of *orfB* gene to down-regulate male sterility gene expression. First construct transformed rice varieties IR 64 and Swarna and other two constructs transformed IR 64. Sterility of the transformants was confirmed by pollen sterility test. The gene stability analysis is continuing in different breeding populations.

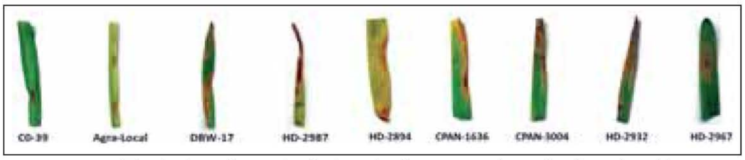


Different stages of regeneration of transgenic rice plants with syn *orfB* gene– (*a*) embryogenic calli of Swarna; (*b*) Calli after co-cultivation of 3 days; (*c*) Calli after 7 days of selection; (*d*) Calli after 45 days of selection; (*e*) Regeneration of putative transformants; (*f*) Regenerated plantlets

Mechanisms of non-host resistance against rust and blast in rice and wheat

The project aimed to compare and decipher behaviour of non-adopted pathogens, *Puccinia graminis tritici* and *Magnaporthe oryzae,* on rice and wheat.

The predominant *Magnaporthe* species observed infecting rice was identified as O2 type, based on the ACTT deletion polymorphism in *calmodulin* gene. Non O2 types such as O type and G type were also found among Indian *Magnaporthe* populations. O2 type of *Magnaporthe oryzae* was also found infecting wheat and caused nearly typical spindle- shaped lesions on wheat-leaf. The same isolate was found infecting grains. Thirty candidate NHR genes were identified in wheat, and sixty phytohormone signaling genes identified in wheat were mapped in rice. Primers for assay of gene expression have been designed and tested in wheat.



Typical and atypical plant lesions on wheat-leaf to rice blast (Check, Co.39)

Biofuel from whey by stress-tolerant metabolically-engineered yeast

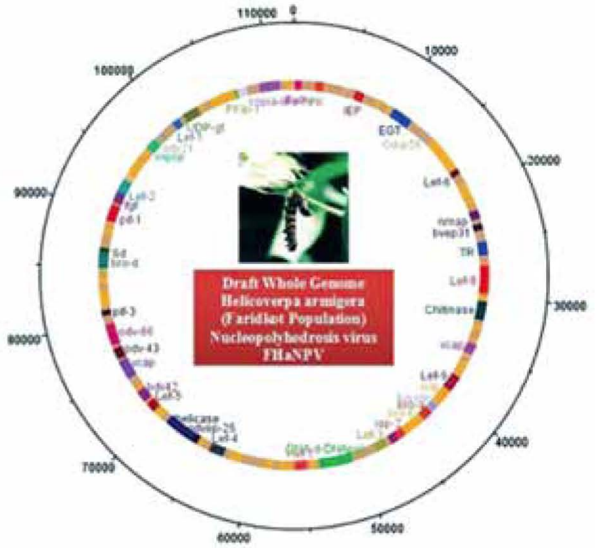
From 213 thermo-tolerant yeast (*Klyuveromyces*) isolates, nine were selected, which showed tolerance to 7.5% ethanol concentration. Among the selected isolates, 6C17 and 6C18 produced up to 8–10% ethanol in 20% sugar concentration in broth. Optimum environment for 6C17 to produce ethanol was *p*H 5, temperature 37ºC, lactose 20% and yeast extract 1.0%. 6C17 yielded 8.0% ethanol with 15% lactose broth and 7.5% with concentrated whey. *Kluyveromyces marxianus* MTCC 1389 produced 10% ethanol in 15% Yeast Peptone Lactose broth and 7.5% ethanol with concentrated whey.

Abiotic stress tolerance in *Catla catla*

Supplementation of selected amino acids and vitamins resulted in reduction of stress owing to high temperature in *Catla catla*. *TLR5* gene expression was induced significantly in liver, kidney, spleen and blood, and *TLR* 22 expression was mostly up-regulated in liver, following cold and heat stresses. The results indicate that the major carp *Catla catla* can be a potential species for aquaculture under climate change regime.

Nuclear polyhedrosis viruses to control *Helicoverpa armigera*

From the collection of *Helicoverpa armigera* available, one least virulent and one most virulent NPV populations were identified based on the LC_{50} values, and both were subjected to whole genome sequencing using Illumina Hiseq2000, TrueSeq Library. The known proteins and hypothetical proteins were annotated separately, and contigs have been submitted to GenBank. Both the genomes have been identified as SNPV and MNPV; most virulent strain being the Single Nuclear polyhedrosis virus and the least virulent is the Multiple Nuclear polyhedrosis virus. Ten genes each were selected from both the populations for homology and found that none of the genes were similar. It may be concluded that single capsid or SNPV is most virulent as compared to multi capsid or MNPV.



DNA plotter for gene arrangement in *Helicoverpa armigera* nuclear polyhedrosis virus of most virulent strain, whole genome (113,631bp) (Source: http://www.icar.org.in/en/node/8064).

Parthenogenetic goat from embryonic stem cells

Following transfer of *in vivo* and *in vitro* fertilized embryo, offspring were obtained in goat. However, with parthenogenetic embryo transfer, pregnancy could be sustained only up to 34 days. Overall 2-cell, 4-cell, 8-16-cell, morula, blastocyst and hatched blastocyst production from parthenogenetic goat embryos in the RVCL and mCR2aa media were 16.84 *vs* 10.08, 19.76 *vs* 28.57, 29.60 *vs* 39.49, 28.72 *vs* 21.84, 3.11 *vs* 0.00 and 1.94 *vs* 0.00% respectively. Similarly, overall 2-cell, 4-cell, 8-16-cell, morula, blastocyst and hatched blastocyst production from *in-vitro* fertilization of matured oocytes was 21.40%, 25.81%, 30.71%, 15.84%, 5.55% and 0.98%, respectively. The fertilized goat embryos through ICSI were produced with a cleavage rate of 34.28% following activation. The putative parthenogenetic embryo from goat was produced for further investigations.

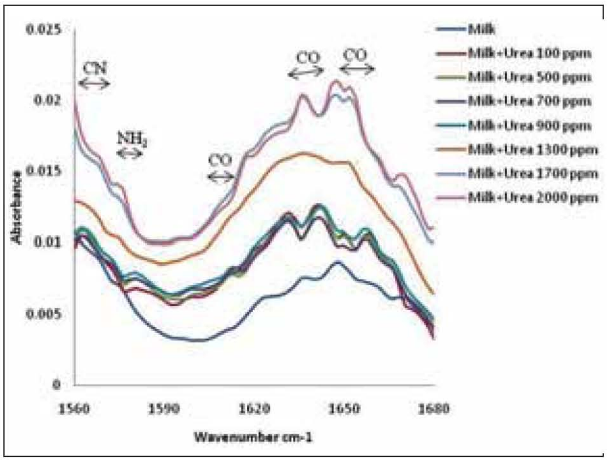
Microbial ethanol production from agricultural biomass

Parthenium and *Eichhornia* are potential sources for lignocellulosic (LC) ethanol production. Indigenous cocktail of crude enzyme from *Phoma exigua* or *Myristica cinnamomea* improved total sugar release from paddy-straw and *Parthenium* biomass, which indicates its potential for saccharification of lignocellulosic biomass.

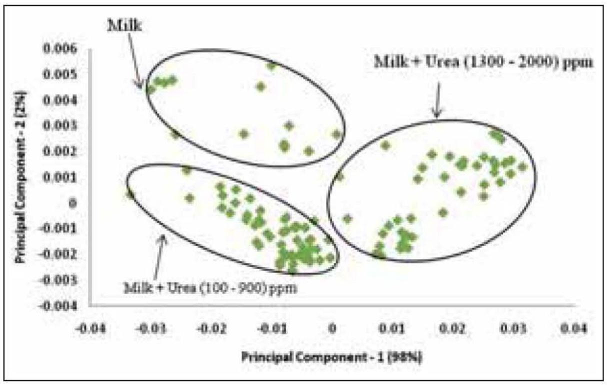
A novel psychrotolerant fungi, *Aspergillus niger* SH3, was found to produce cold-active cellulase, which resulted in high saccharification efficiency at 40°C, thereby reducing energy requirement during saccharification. Thirty-one pentose-fermenting isolates have been identified and characterized to be used for fermentation of saccharified biomass.

Detection and quantification of adulterants and contaminants in fruit-juices and milk

Model for quantitative prediction of soymilk in milk with coefficient of determination of 0.99 and 0.92 for calibration and validation, respectively, has been developed. Similarly, a detection model for added urea (as low as 100 ppm) in milk at 5% significance level has been developed. Six sets of primers for Loop Mediated Isothermal Amplification (LAMP) assay have been designed for detection of *Escherichia coli* targeting *tuf* gene (elongation factor tu).



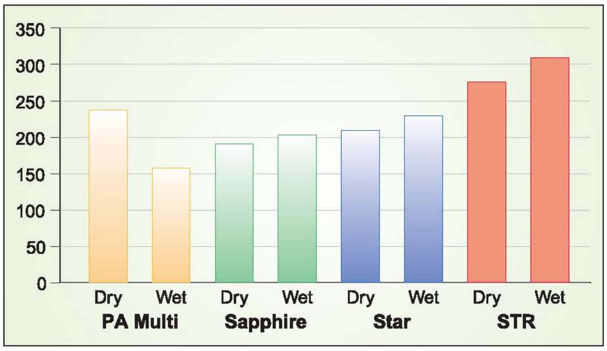
Typical spectra of milk and urea-mixed milk in selected spectral rage 1,680–1,560/cm



Principal component scores plot depicting clusters of milk and urea-mixed milk in the wave number range of 1,670–1,564/cm

Green fishing systems for tropical seas

Annual fuel consumption by mechanized and motorized fishing fleet of India has been estimated at 1,220 million litres, which is about 1% of the total fuel consumption in India. Significant improvements in operational savings of fuel can be achieved by optimizing vessel and machinery designs. An all India survey has been completed, and data of craft and gear digital designs have been compiled. The design of 19.80-m energy efficient fishing vessel has also been finalized. The designs for high sinking speed purse seine, low drag trawl, optimized large meshed new generation gillnets have been finalized, and fabrication has been initiated.



Break load comparison of diffrerent new generation gillnet webbings (N)

Identification of plant and nematode genes involved in disease development

Novel cuticle collagens col-5 and Dpy-31 have been identified and characterized in *M. incognita.* Six dsRNA constructs were prepared for the following genes — *col-5*, *col-180* and *Dpy-7* (IARI); *ram-4*, *Lag-1* and *Dpy-31*(NRCPB)— through gateway technology. Four dsRNA constructs, col-5, col-180, Dpy-7 and col-1 in tomato var. Pusa Ruby and three constructs ram-4, Lag-1 and Dpy-31 in *Arabidopsis thaliana* have been mobilized.

Jute-based biocomposites

Jute fabric with two designs (twill/sateen) in different constructions was developed in handloom as reinforcement. The fabric was modified with nano-polysiloxane polymer as coupling agent to improve interfacial adhesion between jute and polyester resin in a biocomposite.

Polypropylene blend yarn was developed for making compression moulded composite products using DREF friction spinning process by varying twists and proportions of jute-yarn.

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