18.

National Agricultural Science Fund

The 'National Agricultural Science Fund' with an outlay of ₹ 500 crore during the XII Plan, supports basic and strategic research in agriculture. The main objective of the scheme has been to build capacity for basic, strategic and cutting edge application research in agriculture and address issues which can be solved by intensive, basic and strategic research jointly by team of organizations/ institutions. Underlying this objective are the following aims: (i) Foster research and a research culture that will use and advance the frontiers of scientific knowledge to effectively meet the present, anticipated and unanticipated problems of agriculture through various modes and critical investments in research projects; (ii) build the capability of the National Agricultural Research System through development of wide partnerships in science through projects; (iii) build a storehouse of advancement of knowledge in science related to agriculture and awareness of the national importance of basic and strategic research in agriculture; (iv) to provide policy support to the decision makers for use of basic and strategic research in agriculture, and; (v) organization of workshops, seminars, conferences etc. to create awareness, prioritization, scientific popularization and related issues. The scheme has already funded 111 projects, mostly in consortium mode out of which 79 are on-going projects and 65 are multi-institutional in nature.

Besides supporting, reviewing, monitoring and evaluation of the ongoing projects during the year 2015-16, NASF initiated for funding of new projects which were in the process of evaluation. From the last advertisement made, a total of 50 'Concept Notes' (CNs) were selected. The full proposals submitted by the PIs were evaluated by the Expert Committees and the Empowered Committee. A total of 13 projects were approved and sanctioned during 2015-16. Another eight projects were observed to be revised and resubmitted for further consideration.

Advertisement for new call (Call VI) were made in September 2015 to invite fresh proposals. A total of 996 CNs were received. They are in the process of being evaluated by respective DDGs and Expert Committees of ICAR. NASF was also engaged in creating awareness for the need and nature of the basic research for agriculture among institutions within and outside the traditional NARS, inviting new project proposals.

Monitoring of ongoing projects: The ongoing projects are being monitored at three levels. Each project has an 'Advisory Committee' which intensively reviews and monitors the projects. Besides, the projects are being reviewed by the 'Expert Committees' and the 'Empowered Committee'. More than 25 advisory committee meetings were held in 2015-16 to mentor, monitor and evaluate the projects. Similarly, seven Annual Review meetings were held in the month of February 2015 to review the ongoing projects by the Expert Committees. Two mega projects of national importance, viz. 'Phenomics of moisture deficit and low temperature stress tolerance in rice' and 'Stock characterization, captive breeding, seeds production and culture of hilsa (*Tenualosa ilisha*)' were monitored by the Empowered Committee on April 20, 2015 separately. Besides, the 4th Annual Workshop of NASF was held on 28-29, May 2015. The workshop was attended by the Chairman and members of Empowered Committee, Director General, ICAR, Deputy Director Generals, ICAR, members of Expert Committees, Chairmen, Advisory Committees, CPIs and CCPIs of the projects. A total of 19 projects were presented and reviewed. Besides, all the policy issues were deliberated in details and decisions were taken for the smooth functioning of NASF.

The projects under the NASF on the whole have started giving desired results. In addition to the results in terms of high impact publications, patents and technologies, a strong and sustainable platform for developing scientific capacity and culture that encompasses the extended NARS is being established. This will ensure continuous flow of knowledge, ideas and working together among different stakeholders in the basic, strategic and frontier areas for solving problems in agriculture and also forming science policy in agriculture.

SALIENT ACHIEVEMENTS

During 2015-16, besides having 109 publications in reputed journal, NASF had five patents and 38 technologies. The research highlights of some selected projects are as follows:

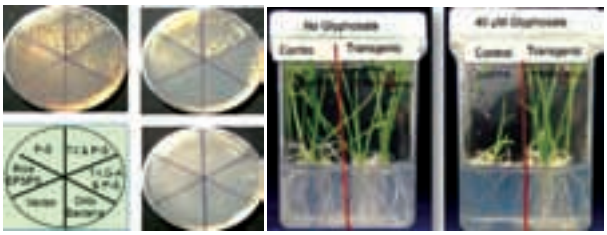
Phenomics of moisture deficit and low temperature in rice

Hyperspectral reflectance based models which can predict relative water content with high accuracy were developed for high throughput non-destructive phenotyping drought tolerance of rice. Further, to differentiate rice genotypes, hyperspectral method based separability index was developed. Twenty-five candidate genes from rice have been cloned and functional validation is in progress. Analysis rice F-box protein genes *OsFBX257* and *OsFBK10* and homeodomain

protein gene *OsHOX22* in *Arabidopsis* showed that these genes are negative regulators of stress tolerance. Transgenic analysis showed that ABA receptor (*OsABAR6*) gene confers enhanced drought tolerance to rice.

Double herbicide tolerant transgenic rice for weed management

The rice EPSP synthase (P173S) and acetolactate synthase (P171S) were mutated by site directed mutagenesis to confer tolerance to herbicide glyphosate and sulfonylurea, respectively. The mutated EPSP synthase (P173S) and ALS (P171S) genes were transformed simultaneously and stable rice transgenic rice lines with moderate tolerance to glyphosate and sulfonylurea were developed without any fitness cost.



Validation of EPSPS mutants for conferring tolerance to glyphosate

Selective multisite-compensating mutations in rice EPSPS by molecular docking were generated. The double (P173S and T169I) and the triple (P173S, T169I and G168A) compensating rice EPSPS mutant encoding genes were cloned and expressed in *E. coli*. Both the double and triple mutants produced functionally active EPSP synthase in *E. coli* and confer higher tolerance to glyphosate compared to wild type or single amino acid substitution (P173S) rice EPSPS mutant. Glyphosate-metabolizing strategy in transgenic lines by constitutively overexpressing codon optimised IgrA gene from *Pseudomonas* to degrade glyphosate into glycine and phosphate in transgenic rice plants to enhance glyphosate tolerance and minimize crop injury was utilized.

Development of transgenic pigeon pea and chickpea

Emphasis was given on generating large number of transgenic chickpea lines using the following constructs; 35S promoter regulated *Cry2Aa*; AraSSU promoter regulated *Cry2Aa*; and 35S regulated *Cry1Ac-full* genes. The idea of using different versions of *Bt* constructs was to obtain lines with optimum level (30 – 80 ng/mg of protein) of *Bt* toxin having complete protection against pod borers. In all, 20 independent events were obtained with 35S-cry2Aa gene and 13 independent events with AraSSU-Cry2Aa gene. A total of 45 putative independent events were generated using the chimeric 35S-cry1Ac-full length gene. Out of these 45 lines, only four lines were found to have high expressing for Cry1Ac toxin. T_1 plants of the high expressing line are currently being grown for segregation analysis.

Transgenics in pigeon pea were developed with two *Bt* ICPs, *cry1AcF* and *cry2Aa*. The progeny of 28 T_1 plants of *cry1AcF* events and progeny of 54 T_1 plants of *cry2Aa* were advanced into T_2 generation. Based on the PCR analysis using various primers, 74 plants with *cry2Aa* gene and 45 plants with *cry1AcF* gene were tested positive. Complete characterization of transgenic events generated in the first phase was achieved. Besides, emphasis was also given for the generation of large number of transgenic lines using the constructs; 35S promoter regulated *cry1Ac* and 35S regulated *cry2Aa*. Three hundred and thirty eight primary transformants carrying *cry*1Ac and 283 primary transformants carrying *cry*2Aa gene have been established in pigeon pea and seeds have been harvested from 190 primary transformants carrying *cry*1Ac and 216 primary transformants carrying *cry2Aa*. Twenty seeds each from these primary transformants have been sown in pots and screening for the presence of transgene is in progress.

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

Techniques for plant infection and multiplication of uredospores under controlled conditions and their visualization on/in plant surface were standardized. Uredospores were found germinating on host (wheat) and non-host (rice) surfaces alike as revealed in fluorescence and scanning electron microscopic analysis. Surface hydrophobicity and medium influenced uredospore germination. Predominant *Magnaporthe oryzae* causing rice blast was identified as O_2 type, using MLST based genotyping; rice infecting O_2 type and a non-O_2 type was found infecting wheat also at conducive environmental conditions. One each of O_2 and O type was genetically transformed for green fluorescence protein expression that was used for deciphering their interaction with plants. qPCR based absolute quantitation of *M. oryzae* was optimized. A genomics-guided approach was developed to identify NHR genes in wheat and to map their homologs in rice. Thirty full-length candidate NHR genes and 60 phytohormone signaling genes were identified in wheat and further mapped to rice genome by combining homology search, EST assembly and mapping to the draft genomes. EDS1 and NPR1 were identified as two strong NHR candidates in wheat-blast-rice-rust system. Homologs of these genes had not been previously identified in wheat. qPCR based assays have been developed for evaluating gene expression in wheat and are being translated to rice.

Dominant nuclear male sterility system in rice for hybrid seed production

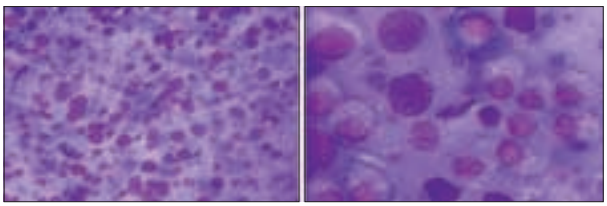
Efforts were made to develop a dominant nuclear male sterility (NMS) system using *Syn orfB* gene in different genetic backgrounds and evaluation of fertility restoration system using *Cre-lox* mediated excision, RNAi-mediated down regulation of the male sterility inducing *Syn orfB* gene. Tapetum specific RTS2 promoter was cloned from IR64 and characterized in transgenic tobacco. The Real-time PCR and histochemical study confirmed higher expression of *Gus* gene in anther as compared to leaf and root. The transformed plantlets having *Cre* gene

Transgenic IR 64 with *orf* B

construct were regenerated. Based on standard heterosis, the best heterotic combinations identified are Reeta/ CR3854, CR3813-2-2-5-1-1/R261 and Improved Tapaswini/MTU1010.

Infertility in crossbred bulls and early prediction of fertility

Safe extraction of spermatogenic and sertoli cells from the testis of live bulls using percutaneous needle aspiration biopsy (PNAB) method was standardized. Methodology for *in vitro* culture of spermatogenic cells and sertoli cells was standardized. Among the several proteins that were differentially expressed between the high and low fertile bulls, MDH2, ENO1, RIBC1, CAPN7, ATP5D, GLB1L2, NCAPD3, DECR1, GCNT2, GDI2, TOP, and USP12 were found over expressed in high fertile spermatozoa, whereas DST, TMEM43 and BSP1 were over expressed in low fertile spermatozoa.

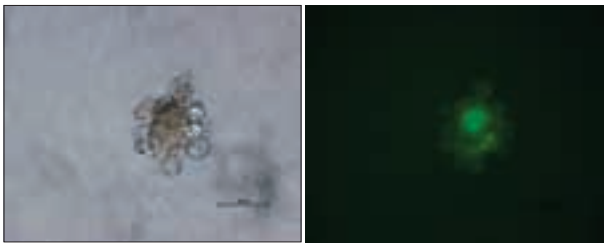

Testicular cells isolated from live bulls using PNAB method

Transgenic goat for production of human lactoferrin

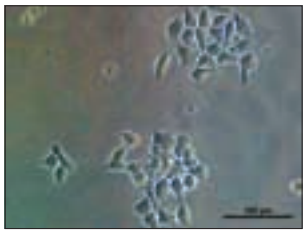
The human lactoferrin gene construct was developed with highest promoter activity under 6.5 kb fragment of beta-casein. Simultaneously, goat mammary epithelial cell line was established and characterized. Subsequently, the construct prepared was transfected into goat mammary epithelial cell line by nucleofection. The cells were observed for the expression of GFP as the expression vector contains GFP as the reporter marker. Lactoferrin expression in the transgenic cell line was also confirmed by western blotting. The embryos were transferred in the uterine lumen by means of a long micropipette (conditioned in 10-20 µl PBS medium).

Parthenogenetic goat from embryonic stem cells

The overall cleavage rate and blastocyst production in TRIS + Heparin (37.67% and 13.8%) were comparatively higher as compared to sperm capacitated in TALP + Heparin (35.07% and 10.07%). The overall cleavage rate, morula and blastocyst production in mCR_2aa and mCR_2aa+cysteamine were 36.39, 21.62 and 4.95% and 31.72, 30.89 and 8.98%, respectively. The mechanical activation failed to induce cleavage in *in vitro* matured goat oocytes whereas chemical activation of intracytoplasmic sperm injected *in vitro* matured goat oocytes (29.66%) showed significantly higher ($p<0.05$) cleavage rate as compared to non-activated oocytes (8.14%). The blastocyst production was significantly higher following activation of *in vitro* matured oocytes by 5 µM calcium ionophore and 6-DMAP as compared to ethanol activation. IVF derived embryos were selected at the 2-cell stage between 32 and 48 h post-insemination. Altogether electro-fusion of two cell stage IVF derived embryos at 1.25kV/cm for 99 µsec tend to show more developmental competence than other fusion parameters as it reached up to 8 cell stage.


Transgenic goat embryo at morula stage expressing hLF-GFP protein

Regulation of fatty acid synthesis by RNAi in pig


Porcine induced pluripotent stem (iPS) cell colonies

The project aims to produce designer pork with reduced fat using transgenic induced pluripotent stem (iPS) cells. The siRNA against SCD1 gene in porcine mesenchymal stem cells were screened. Of the three siRNA sets, one is shown to down-regulate expression of SCD1 in a dose dependent manner. From mesenchymal stem cells, induced pluripotent stem (iPS) cells were produced for transgenesis. Mesenchymal stem cells (MSC) were transfected three sets of siRNAs designed against porcine SCD1. One of these sets was found to down-regulate the SCD1 gene in a dose dependent manner.

Genetically engineered vaccines against poultry viral diseases

The IBDV VP2 gene cassette inserted into the final cDNA clone of the NDV virus generated at sites, SacII (position 2354) and AvrII (position 5251). The final clone had an expected size of 21,344 bp. Transfection was done using the full length clone of the virus along with the support plasmids and recombinant virus was rescued. The

demonstration of recombinant virus was done by RT-PCR using gene specific primers for both NDV and IBD VP2. The rescued virus was pelleted, run on SDS-PAGE and reacted with NDV and IBDV specific antisera on a western blot. Specific bands in relation to VP2 protein (44 kDa) and NDV proteins were observed in the blot.

Adaptive mechanisms and captive breeding in hilsa

Hilsa grown in freshwater pond (161 g)

Assessment of the population characters of hilsa from Hooghly estuary and near shore areas showed over exploitation of the stock, as current exploitation level exceeded maximum sustainable yield levels; exploitation of spawning stock biomass is 40% more than the level of sustainability. Major habitat parameters and their favorable range in nature for the fish has been identified for juveniles and adults. Size-wise natural food items, feeding habits, reproductive biology, sex ratio, breeding seasons and location of availability of brooders were established. Genetic analysis showed definite differentiation between east and west coast samples (F_{st} value 0.51225 to 0.94259 $p<0.05$). Hilsa of Hooghly was found genetically distinct from that of Padma and Brahmaputra rivers. Juvenile hilsa showed higher branchial NKA activity prior to seaward migration indicating physiological preadaptation to face salt water. Plasma estradiol and 17α 20α-dihydroxy progesterone (DHP) in the fish showed biannual surge, linked with growth and maturation of oocytes. Attempts on artificial breeding resulted in 30 to 95% fertilization of eggs and 38-98% hatching. Management of live feed and water quality management could improve hatchery-stage larval survival up to 40% and nursery- stage up to 30%. Culture trials in freshwater ponds resulted in average weight gain of 160g/252 mm in 13 months with 20% survival; in brackish water ponds, hilsa attained average weight gain of 250 g in 13 months with 30% survival. Freshwater pond reared specimens showed ovary growth up to stage IV.

Green fishing systems for tropical seas

Designs of 79 trawl nets, 31 gillnets, 11 purse seines, 4 ring seines, 14 hook and lines, and 2 dolnets were collected and documented. Measures to reduce drag of trawl nets were identified. Two new trawl nets, one fish trawl and one shrimp trawl each were fabricated incorporating drag reduction measures such as increased mesh size and reduced twine size. A modified method (mathematical formula) for calculating the sinking speed of seine nets has been derived. The *in situ* validation of the derived formula has been conducted at Lakshadweep by trained project staff with assistance from Scuba divers. A design for an optimized gillnet with new generation materials, Sapphire (7x3) and STAR (No.8) with mesh size 150 mm for targeting large pelagic species was developed.

Defense genes of tiger shrimp against bacteria and white spot syndrome virus

2-D gel analysis was carried out with respect to different time point intervals of WSSV infection. Different spots were observed in white spot syndrome virus (WSSV) infected shrimps. The spots were excised and MALDI-TOF analysis revealed differentially expressed proteins. At 72 h post-infection, two of the proteins, viz. Tropomyosin and Arginine Kinase were overexpressed in WSSV infected tissues indicating specific role of these host proteins. Both conventional RT-PCR and real time analysis for differentially expressed genes with respect to WSSV infection were carried out. Expression analysis was carried out for about 22 defense genes. While it was difficult to differentiate the expression pattern by conventional RT-PCR, real time PCR could differentiate clearly the differences at different time point intervals. Immune genes involved in the Toll pathway of shrimp, such as Spatzle (extracellular ligand of Toll), myeloid differentiation factor 88 (MyD88), tumor necrosis factor receptor-associated factor 6 (TRAF6), cactus (mammalian IkB homologue) and dorsal (mammalian NF-kB homologue) were characterized. Full gene sequencing of myeloid differentiation factor 88 (MyD88), tumor necrosis factor receptor-associated factor 6 (TRAF6) and spatzle, and partial gene sequencing of cactus (mammalian IkB homologue) and dorsal (mammalian NF-kB homologue) was done. The akirin gene that showed maximum upregulation during infection was amplified, cloned and expressed. Recombinant clones of akirin (AKN) were sequenced.

Diversity and synthesis of immunoglobulins in the Indian major carps

Immunoglobulin (Ig) Z and IgM expression has been analyzed in rohu and catla following argulus infection and the qRT-PCR data revealed significant induction of IgM in skin, muscle, gill and kidney of rohu. In catla, IgZ gene expression was enhanced in blood and gill followed by intestine and spleen. These data suggests important role of IgM and IgZ in parasite infection in fish. The B cell activating factor (BAFF) has been cloned and sequenced in rohu. Full-length BAFF–cDNA has been amplified through RACE, and its structural domains has been characterized.

Microalgal triacylglycerols (TAGs) as source of biodiesel

A locally isolated microalga *Scenedesmus obliquus* (Turpin) Kützing (GA 45) was grown successfully using the low-cost farm-fertilizer (UZn) medium in closed raceway pond system. The maximum biomass and lipid yield were recorded as 1.10 g/L and 115 mg/L, respectively, for 30 cm culture depth. The maximum areal biomass productivity was recorded as 11 g/m^2/day with lipid productivity of 1.15 g/m^2/day. Under biphasic N-starved condition, lipid productivity was doubled up to 2.28 g/m^2/day. Harvesting techniques involving pH-

Closed raceway pond system for cultivation of microalgae

induced flocculation and dissolved air floatation (DAF) with alum were found to be suitable for large scale testing of microalgal harvesting. Oven, tray and solar drying protocols were fully standardized for the microalgae.

Decision support system for enhancing productivity of grapes

A dynamic simulation model for growth, development, biomass and yield of grape vines was designed to run at daily step (for 20 years cycle) and to provide outputs on phenology, growth and yield parameters taking into account the effects of water and nutrient (nitrogen) stresses. This DSS is available to farmers for use and validation. A total of 40 growers were registered and trained on DSS software usage.

Polymeric nano-materials for microencapsulation of nutraceuticals

Shelf life of guava dices stored in the egg shell could be extended up to 14th day compared to only 6 days in the macro-perforated package. The same for papaya was up to 20th day in the egg shell compared to only 4 days under macro-perforated package. Different modifications of native starch and incorporation of plasticizers and functional compounds were tried to arrive at a suitable biodegradable film for subsequent use. Hydrophilic OH-MMT was prepared by simple ion exchange reaction method between THAC and Na^+-MMT. The good degree of exfoliation achieved can be attributed with the strong interactions between the modified silicate layers and polymer matrix through efficient hydrogen bonding.

Biodegradable electrospun fibre mat for packaging of fresh perishable agricultural material

Multi-phase electrospinning setup was fabricated for production of electrospun nano fibre mat. The machine has multi-axial arrangements with adjustable nozzle geometry that can orient the needle to desired angle; automated linear motion and multiple parallel needles. The machine has the advantage of forming multi-layer fibre and produce mat with even surface. The parameters for electrospining process, viz. voltage, distance, flow rate and duration were optimized. The optimized parameters were 25 kV voltage, 15 cm distance, 0.04 ml/min flow rate and 15 min duration. Volatile gas profile of the Alphonso mango during the ripening stage was mapped by non-destructive method. Analysis revealed major gases that are coming from the mango fruit during ripening were 3-methyl furan, α-pinene, α-ocimene, P-mentha-1,4-diene, tetramethyl cyclohexadiene, dimethyl octatriene, ethyl octanoate, ethyl-trans-4-decenoate, ethyl decanoate and caryophyllene.

Colorimetric sensor for mango ripening