

ICAR-NRCE



ICAR- National Research Centre on Equines
भाकअनुप - राष्ठीय अश्व अनुसंधान केन्द्र



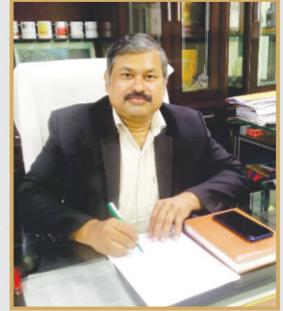
NEWSLETTER

VOL. 26 (Issue-I) JANUARY- JUNE 2024



FROM DIRECTOR'S DESK

It gives me immense pride and great pleasure to present this edition of the *ICAR-NRC Equines Newsletter* to the nation, capturing the phenomenal progress made by the institute in the field of veterinary diagnostics, health and production during the period from January to June 2024. I also take this opportunity to express my warm regards and sincere thanks to all the well-wishers of ICAR-NRC on Equines, whose constant encouragement and support continue to inspire us in our journey of scientific excellence. The past six months have indeed been remarkable, marked by several groundbreaking achievements and scientific accomplishments of Team NRCE, whose commitment and innovation continue to bring national recognition.



During this period, the institute attained milestone with the recognition of the 'Bhimthadi' Horse as the eighth registered indigenous equine breed of India. In yet another significant advancement, NRCE successfully produced a 'Zanskari foal', a native of the Zaskar Valley in Ladakh, through the combined application of artificial insemination and embryo transfer, demonstrating our strength in reproductive biotechnology. Equally noteworthy is the development of a 'DIVA vaccine against Lumpy Skin Disease', a devastating epidemic in cattle, which will now enable differentiation of vaccinated from infected animals and provide a powerful tool for effective disease control.

These achievements highlight the efforts of 'Team NRCE', who continue to work with passion and dedication towards the betterment of animal health and productivity. I extend my heartfelt congratulations to the entire NRCE team and express confidence that our collective efforts will continue to drive innovations and contribute significantly to the livestock and equine sector of the nation.

Jai Hind.

(T. K. Bhattacharya)

Recognition of *Bhimthadi* as 8th Indigenous Horse breed

ICAR-National Research Centre on Equines (NRCE), Hisar, has achieved yet another milestone in conserving India's rich equine genetic diversity. The *Bhimthadi* (Deccani) horse, once considered on the verge of extinction, has now been officially recognized as the 8th indigenous horse breed of the country. Earlier listed alongside the Chumarti and Sikang breeds as highly endangered, the *Bhimthadi* horse has been brought back to recognition through dedicated efforts of the Centre. The Centre took the initiative to find out the present status of the breed. Extensive survey of the area resulted into finding out the pure animals of the breed with the Nomadic Tribe - C of the state and distribution of the breed was found in the area between Bhima and Nira River of the Maharashtra state. The breeding tract encompassed Pune, Ahmednagar, Satara, Sangli, Solapur and Kolhapur districts with an area of about 74329 Km²

between 15.7469° N to 19.9897° N and 76.414° E to 73.3249° N and the population of the breed has been estimated to be 5134 heads in the year 2023. The breed is now recognized as the 8th breed of indigenous horse in the country and it has been assigned the Accession No. INDIA_HORSE_1100_BHIMTHADI_07008.



Bhimthadi Stallion

Raj-Zanskar: India's First Zanskari horse foal produced through embryo transfer technology

Zanskari, a native pony breed of Leh-Ladakh in Trans-Himalayan region of India is well adapted to high altitude regions. This breed of horses is known for their ruggedness, ability to withstand extreme cold climates, work tirelessly, and carry loads at high altitudes. Continuing their success in producing the foals through Embryo transfer, Scientists at Equine Production Campus, Regional Station of ICAR-National Research Centre on Equines, Bikaner, Rajasthan for the first time in the country have produced a Zanskari horse foal using Embryo transfer technology..

For this achievement, fresh semen from a Zanskari stallion was used for artificial insemination. The embryo was recovered through uterine flushing at 6.5

days post-ovulation and transferred into an estrus-synchronized surrogate mare. The surrogate successfully delivered a healthy female foal on 23rd April 2024, weighing 28 kg at birth.



Raj-Zanskar' – the first Zanskari foal produced in India through Embryo Transfer technology

Standardization of Isothermal RPA Assay for the Detection of ILTV and BCoV

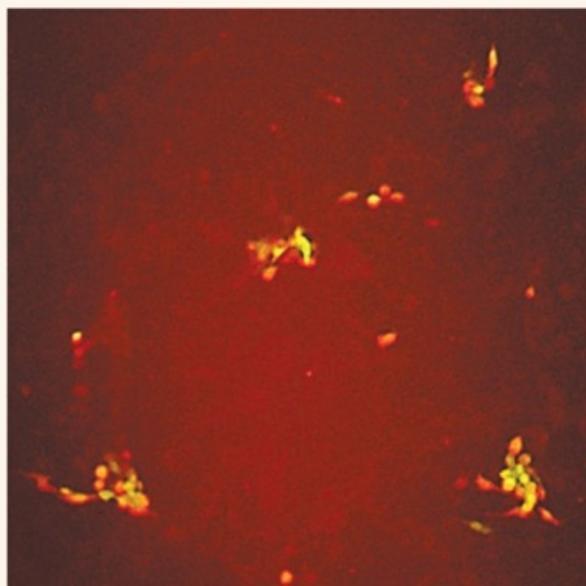
An isothermal Recombinase Polymerase Amplification (RPA) assay was standardized for the rapid detection of Infectious Laryngotracheitis Virus (ILTV) and Bovine Coronavirus (BCoV). For ILTV, primers and probes targeting highly conserved regions of the viral genome were designed and synthesized. The isothermal amplification was successfully carried out at 39 °C for 20 minutes using a thermal block, and

the expected amplicon was confirmed by gel electrophoresis. Similarly, multiple sets of primers and probes targeting conserved genomic regions of BCoV were designed and evaluated. The assay was optimized under the same isothermal conditions (39 °C for 20 minutes), resulting in successful amplification of the target regions, as evidenced by the presence of the expected bands in agarose gel electrophoresis.

Development of combined vaccine candidates for equine influenza and EHV1

ICAR-National Research Centre on Equines has made a breakthrough by developing recombinant constructs of Equine Herpesvirus-1 (EHV-1) engineered to carry the hemagglutinin (HA) gene of both Clade 1 and Clade 2 equine influenza viruses. To evaluate the performance of these recombinant viruses, growth kinetics and plaque size assays were conducted, which revealed distinct replication patterns of the mutant strains compared to the parent virus.

Further, Indirect immunofluorescence assay test (IFAT) confirmed protein expression of the inserted gene, indicating its suitability as vaccine candidate. High OD values observed with positive serum in the ELISA indicate strong binding between antibodies in the hyperimmune serum and the EHV1 Δ IR6/gE-HA(FC2) viruses. Following these confirmations, bulk propagation and purification of recombinant viruses were successfully carried out. This achievement marks an important step towards the development of a novel, effective recombinant vaccine against equine influenza.

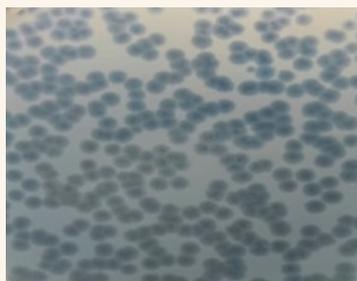


Protein expression studies of EHV1 with –Clade 2 EIV at 36hpi. PK-15 cells were infected with recombinant virus and Equine Influenza serum was used as primary antibody and secondary conjugate goat anti-horse TRITC was used. Exposure of fluorescent used GFP at 400ms and TRITC at 30ms. Magnified at 200X

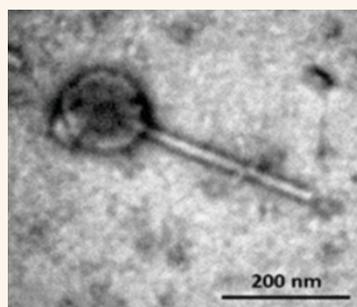
Isolation of a novel bacteriophage against MDR *Salmonella enterica* and its potential use in food biocontrol

Salmonella enterica is a major food borne pathogen, and the emergence of multidrug-resistant (MDR) strains poses a growing public health concern. As antibiotic efficacy declines, bacteriophages offer a promising alternative for controlling MDR bacterial infections, particularly in food safety applications. In this study, a broad-host-range lytic bacteriophage, VTCCBPA219, was isolated from pond water against MDR *Salmonella enterica* serotype Paratyphi. The phage exhibited strong lytic activity against 28 out of 30 tested *S. enterica* strains, indicating a wide host range. Notably, VTCCBPA219 remained active under acidic conditions (pH 3) and high temperatures (up to 70°C), suggesting its stability in harsh environments. Transmission electron microscopy (TEM) classified the phage within the class Caudoviricetes. Whole genome sequencing revealed a genome size of approximately 146 kb with 44.5% GC content, encoding 250 predicted coding sequences (CDSs) and 25 tRNAs. The absence of lysogeny-associated

genes confirms its strictly lytic nature. Furthermore, the phage demonstrated the ability to eradicate *S. enterica* biofilms formed on borosilicate glass surfaces, highlighting its potential application as a biocontrol agent in food processing and sanitation.



Plaque characteristics of phage VTCCBPA19



TEM photograph of phage VTCCBPA19

Development of an ORF 154-based DIVA ELISA for differentiating LSDV-infected and vaccinated animals:

Mass vaccination to achieve disease-free status requires a serological test to differentiate infected and vaccinated animals (DIVA) towards the end of the control program. The lumpy skin disease (LSD) vaccines currently in use (licensed) are not DIVA-compatible. India recently approved a new live-attenuated LSD vaccine derived from the local Ranchi strain. Unlike field strains of the LSD virus (LSDV), the Ranchi strain has a distinct 801-nucleotide deletion in its inverted terminal repeat (ITR) region, affecting ORF003/ORF154. In this study, we successfully cloned and expressed LSDV ORF154 into pET28a and purified the His-tagged protein using Ni-NTA affinity chromatography. SDS-PAGE and Western blot analyses confirmed the presence of a ~28 kDa protein, consistent with the predicted molecular weight. An optimized antigen concentration of 500 ng/well and serum dilution of 1:50, and at a positivity cut-off of 22%, the assay showed high sensitivity (96.125%) and

specificity (95.77%), effectively distinguishing infected from vaccinated cattle. These findings demonstrate the potential of an ORF154-based ELISA as a reliable serological diagnostic tool for LSDV surveillance and disease control programs, making the Ranchi strain-based LSD vaccine the first DIVA-compatible vaccine.

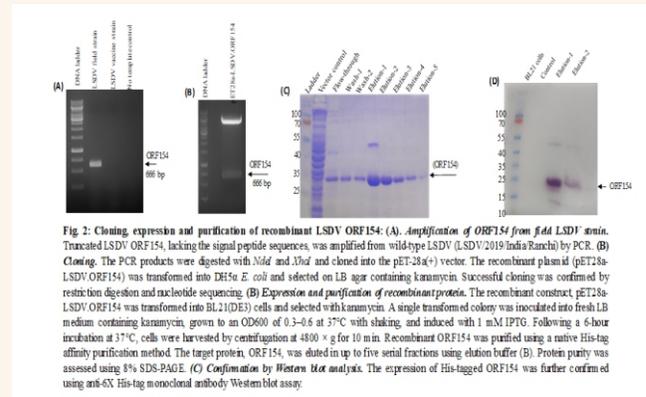


Fig 2: Cloning, expression and purification of recombinant LSDV ORF154: (A) Amplification of ORF154 from 544 LSDV strains. Truncated LSDV ORF154, lacking the signal peptide sequences, was amplified from wild-type LSDV (LSDV/2019/India/Ranchi) by PCR. (B) Cloning. The PCR products were digested with *Nde*I and *Xba*I and cloned into the pET28a(+) vector. The recombinant plasmid (pET28a-LSDV ORF154) was transformed into DH5α *E. coli* and selected on LB agar containing kanamycin. Successful cloning was confirmed by restriction digestion and raxletide sequencing. (C) Expression and purification of recombinant protein. The recombinant construct pET28a-LSDV ORF154 was transformed into BL21(DE3) cells and selected with kanamycin. A single transformed colony was inoculated into fresh LB medium containing kanamycin, grown to an OD₆₀₀ of 0.3–0.6 at 37°C with shaking, and induced with 1 mM IPTG. Following a 6-hour incubation at 37°C, cells were harvested by centrifugation at 4800 × g for 10 min. Recombinant ORF154 was purified using a native His-tag affinity purification method. The target protein, ORF154, was eluted in up to five serial fractions using elution buffer (B). Protein purity was assessed using 8% SDS-PAGE. (D) Confirmation by Western blot analysis. The expression of His-tagged ORF154 was further confirmed using anti-6X His-tag monoclonal antibody Western blot assay.

Generation of a genetically engineered BHK-21 cell line for scale up of vaccine production:

Foot-and-mouth disease (FMD) is a highly contagious disease of ruminants and pigs, caused by an Aphthovirus that belongs to the family Picornaviridae. It is currently endemic in India and country is running a FMD control program through mass vaccination campaign. In order to generate herd immunity, ~500 million animals need to be vaccinated twice a year as the protective immunity of FMD vaccine lasts only for 6 months. Production of 1000 million vaccine doses is a difficult task and the manufacturers are barely able to meet this requirement. Therefore, technological interventions are required to scale up vaccine production. For vaccine production, FMD virus (FMDV) is propagated in BHK-21 cells and then inactivated and mixed with adjuvant. The amount of vaccine doses produced depends of the amount of virus being produced. Recent progress using transcriptomics, genome-wide siRNA and CRISPR screens have enabled the identification of numerous host factors regulating virus replication. Some cellular proteins serve as host restriction factor

and therefore interfere with the virus replication. In this invention, we generated genetically engineered BHK-21 cells lines deficient in two of the cellular proteins Cav1 (Caveolin-1) and HDAC6 (histone deacetylase 6) that serve as host restriction factor in FMDV life cycle. The knockout cells generated ~10-times higher virus titre as compared to the wild type (normal) BHK-21 cells, and therefore, it has high commercial interest to economize the cost of vaccine.

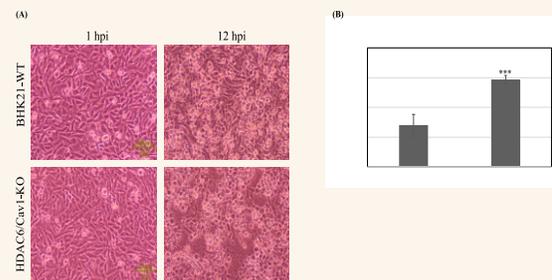


Fig. 6: Growth of FMDV in HDAC6/Cavi-KO and WT BHK21 cells: Confluent monolayers of HDAC6/Cavi-KO and WT BHK21 cells in triplicates, were infected with FMDV at MOI of 0.1. (A) The images showing cytopathic effects were taken at 1 hpi and 12 hpi (scale = 20 μm). The virus particles released in the infected cell culture supernatants at 12 hpi were quantified by plaque assay (B). Error bars indicate SD. Pair-wise statistical comparisons were performed using Student's t test. (NS=non-significant difference, *** = P<0.001). Values are means ± SD and representative of the result of at least 3 independent experiments

Technology Transfer

Sr No	Name of the technology	Recipient/Beneficiary	Revenue generated
1.	Semen collection and cryopreservation in indigenous horses	Mr Raghavendra Singh Ji , Dundlod, Jhunjhunu, Raajsthan	59,000/-
2.		Mr. Rajyavardhan Rathore , Fateh Stud Farm Kelwa, Udaipur, Rajasthan	59,000/-
3.		Mr Navaneet Equine Breeding India Agrahara Main Road, Bangalore, Karnataka.	59,000/-
			1,77,000/-
4.	“Customized AV for semen collection from Stallions”	Mr Raghavendra Singh Ji , Dundlod, Jhunjhunu, Raajsthan	17,700/-
5.		Mr Jaykumar Desai Gujarat	17,700/-
6.		Mr Navaneet Equine Breeding India Agrahara Main Road, , Bangalore , Karnataka	17,700/-
			53,100/-

Capacity Building of Technology Recipients on Equine Semen Collection and Cryopreservation”



Hands on Training for Stakeholders in Semen Collection and Cryopreservation of Indigenous Horses



MoU of Customised AV technology between ICAR-NRCE and Dr. Jay Desai

VISIT OF DIGNITARIES:

A mobile app titled “mobile app on MARE-USG: application of ultrasonography in mare reproduction” and a technical bulletin titled "Practical Guide to Embryo transfer in Equines" released by Hon'ble DDG Dr Raghavendra Bhatta and Animal Husbandry commissioner Dr Abhijit Mitra on Feb 2nd 2024



INSTITUTIONAL ACTIVITIES

Training Programmes on Zoonoses:

S. No	Workshops/Trainings	Date	Participants
1	Workshop on Zoonotic Diseases has been organized at Bhiwani, Haryana	01 st March, 2024	A total of 31 Medical and Veterinary Professionals and 45 farmers from Bhiwani district
2	A 5 days training program on “One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases” at ICAR-NRCE, Hisar	12-16 Feb 2024	A total of 30 Medical and Veterinary and Wildlife Professionals from Punjab & Haryana and 3 technical staff from ICAR-NRCE, Hisar has attended this training
3	A 5 days training program on “One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases” at ICAR-NRCE, Hisar	19-23 Feb 2024	A total of 29 Medical and Veterinary Professionals from Punjab & Haryana and 6 technical staff from ICAR-NRCE, Hisar has attended this training
4	A 5 days training program on “Zoonotic Diseases Diagnosis Under One Health Approaches and Equine Husbandry Practices” at ICAR-NRCE, Hisar	18-22 March 2024	A total of 20 Veterinary Professionals from Tamil Nadu has attended this training



Release of training compendium during the 5-day program on 'One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases' at ICAR-NRCE, Hisar.



Launch of mobile app 'MARE-USG: Application of Ultrasonography in Mare Reproduction' by Hon'ble DDG (AS) Dr. Raghavendra Bhatta and Animal Husbandry Commissioner Dr. Abhijit Mitra on 2nd February 2024.”

Dr. Raghavendra Bhatta, Deputy Director General (Animal Science), ICAR, visited the Regional Station, Bikaner, on April 7, 2024. He was accompanied by Dr. AK Tomar, Director, ICAR-CSWRI, Avikanagar, and Dr. A Sahoo, Director, ICAR-NRCC, Bikaner. During the visit, the delegation toured the equine museum, animal sheds, foaling line, embryo transfer (ET) laboratory, and other scientific facilities. Dr. Mehta, Head of the Regional Station, apprised the dignitaries of ongoing activities and developments.



Training programme on **Donkey farming**: A three days training programme on **EDP on Donkey farming** 26-28th Feb 2024. This is a collaborative Online Training Program of MANAGE, Hyderabad & ICAR-NRCE, Hisar, Haryana on Entrepreneurship Development Programme on Donkey farming.

A Brain storming session on “**Stallion semen quality control and MSP guidelines and standards for the stallion semen in the country**” was organised at ICAR-NRCE, on 18 April 2024. A total of 40 delegates were presented for this meeting and shared their valuable feedback on this topic.

Equine Health Camp and KishanGosthi: An equine health camp and kishangosthy were organized at Annual Livestock Show Tilwara, Rajasthan. 35 equines were examined for illness by multidisciplinary team of scientists and technical officer. Pregnancy diagnosis was done in 4 mares by rectal palpation. Biological sample were collected from all available equines and all were provided anthelmintic bolus and mineral mixture. KishanGosthi was organized and approx. 200 farmers took part and were benefitted.

International Yoga Day Celebration: International Yoga Day was celebrated with great enthusiasm on dated 21st June, 2024 at ICAR-National Research Centre on Equines, Hisar. Yoga practice and awareness camp was organized in front of main office building of the center. Scientists, officers and staff of the center took part in the yoga day. Sh. Deepak Solanki, Yogacharya, Hisar briefed about importance of this day and called upon the gathering to adopt yoga in their daily life.



New Initiatives

Equine Ecotourism: A disconnect between general public and the equines was perceived by the analyses of equine census figures of last few decades of the country. In order to overcome this, the Equine Ecotourism has been initiated at the campus in the year 2017 with creation of several new attractions like Herbal Park, Desert Photoshoot Point, Lakadi Ka Pool and the Equine Museum, in addition to the Horse Riding, Buggy Riding and Tonga Riding. The impact of this initiative has so far been excellent as thousands of visitors, students and farmers have visited the Centre every year and got acquainted with the indigenous horses. Thus, the initiation of Equine Eco-tourism at the campus has not only supported our efforts to sustain the species *in situ* but has also acted as a source of revenue for the Institute.

Equine Museum: An equine museum has been initiated at the Campus for the tourists, farmers and students to get the scientific knowledge about the equines in the country. Comprehensive pictorial information about the world's best horses; breeds of indigenous horses; well-known warriors and their brave horses; research activities and achievements of the institute, and awards and recognition of the institute at various levels has been presented.



भ्रूण प्रत्यारोपण से अश्व के दो नए क्लोन बने

वाराणसी में भ्रूण प्रत्यारोपण में सफल, राष्ट्रीय अश्व अनुसंधान केंद्र के वैज्ञानिकों ने सफलतापूर्वक दो नए क्लोन बने



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भारत 2030 तक होगा मुंहसुर रोगमुक्त, दुग्ध उत्पादों का बढ़ेगा निर्यात, 2047 तक दूध उत्पादन 600 मिलियन टन करने का लक्ष्य

अर्थव्यवस्था विभाग के प्रधान सचिव, डॉ. राजेश कुमार ने कहा कि मुंहसुर रोगमुक्त भारत 2030 तक होगा मुंहसुर रोगमुक्त, दुग्ध उत्पादों का बढ़ेगा निर्यात, 2047 तक दूध उत्पादन 600 मिलियन टन करने का लक्ष्य

पशुओं में मुंह खुर की बीमारी से विदेशों में नहीं बिक रहे दूध से बने प्रोडक्ट : डा. राघवेंद्र

डॉ. राघवेंद्र ने कहा कि मुंह खुर की बीमारी से विदेशों में नहीं बिक रहे दूध से बने प्रोडक्ट : डा. राघवेंद्र

कोरोना, डिप्थीरिया, इन्फ्लुएंजा, हार्पिस, इबोला और माइक्रोप्लाज्मा समेत इंसान व पशुओं की 19 गंभीर बीमारियों की लैब में होगी जांच

वाराणसी में 19 गंभीर बीमारियों की लैब में होगी जांच

घोड़े में मिला ग्लैडर्स, पांच किमी क्षेत्र में कटेनेमेट जोन घोषित, नौ टीमें बनाई

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8 कंपनियों को 50 लाख तक की 50 प्रतिशत पूंजी सहायता देनी जाएगी अब गधे, घोड़े व खच्चर भी होंगे राष्ट्रीय पशुधन मिशन में शामिल, केंद्रीय मंत्रीमंडल ने दे दी मंजूरी

केंद्रीय मंत्रीमंडल ने दे दी मंजूरी

एनआरसीई और एम्सीयू का शिक्षा में सहयोग को एम्प्रोव्यू

एनआरसीई और एम्सीयू का शिक्षा में सहयोग को एम्प्रोव्यू

अनुसंधान केंद्र में हिंदी पहचान 14 तक

अनुसंधान केंद्र में हिंदी पहचान 14 तक

जागरण सिटी दिखाए

जागरण सिटी दिखाए

एनआरसीई लैब में 5 हजार पशुओं के सूक्ष्मजीवी संरक्षित मानव और पशु रोगों की वैक्सीन तैयार करने में सहायक

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देखभर में लुप्त प्राय नस्ल के रोय के 6660 आध, एनआरसीई वैज्ञानिकों ने इस्को तैयार की तककत जगत बचाई

देखभर में लुप्त प्राय नस्ल के रोय के 6660 आध, एनआरसीई वैज्ञानिकों ने इस्को तैयार की तककत जगत बचाई

भारत में जांस्करी नस्ल के घोड़े का पहला बच्चा भ्रूण स्थानांतरण तकनीक से पैदा, लेह-लद्दाख में कारगर

भारत में जांस्करी नस्ल के घोड़े का पहला बच्चा भ्रूण स्थानांतरण तकनीक से पैदा, लेह-लद्दाख में कारगर

Published by : Dr. T. K. Bhattacharya, Director, ICAR-NRCE, Hisar
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