EXPRESSION OF INTEREST FOR TECHNOLOGIES DEVELOPED

Contents

ABOUT GUJARAT BIOTECHNOLOGY RESEARCH CENTRE

Gujarat Biotechnology Research Centre is established as an autonomous society under Department of Science and Technology, Government of Gujarat for tackling societal problems of state priority through cutting-edge Biotechnological intervention. The organization is well equipped with advanced research infrastructural facilities related to genomics, bioinformatics, Biobanking, and bioprospecting. The focus of GBRC is not only to undertake conduct cutting-edge research in the frontier areas of biotechnology but also conducting translational research leading to the development product/ prototype/ process development with application in healthcare, agriculture, environment, marine etc. GBRC is also evolving as a state-of-art shared laboratory facility by extending its infrastructure to outside organization/Institutions/Industries/Students for their research purposes.

ABOUT TECHNOLOGY

GBRC has developed a few in-house technologies which have been approved for licensing. These technologies are expected to be better and cost effective compared to those available on the market. The following technologies available for licensing:

1. Overproduction and purification of recombinant Serratiopeptidase
2. A probiotic bacterium to enhance milk production in lactating cattle
3. Overproduction and purification of active recombinant tissue Plasminogen Activator (tPA)
4. Molecular methods for identification and discrimination of *Ocimum tenuiflorum* syn sanctum and *Ocimum basilicum*
5. Molecular methods for identification and discrimination of *Piper sp*. and *Carica papaya*
6. Molecular methods for identification and discrimination of *Bacopa monnieri L*. and *Centella asiatica L*.
7. Developing probiotic formulation to treat endometritis in bovines
8. Kit for sex determination in date palm
9. HLA Typing Kit Based on Targeted Exon Sequencing
10. Varietal identification and seed genetic purity testing in wheat

Detailed explanation about the technologies are in the following pages.

1. Overproduction and purification of recombinant Serratiopeptidase

Production of Serratiopeptidase by engineered Escherichia coli strain for therapeutic application

Background

Serratiopeptidase is one of the important proteases which belong to the serine protease family. It is an extracellular zinc-containing metalloprotease that is produced by Serratia marcescens having molecular weight of about 53kD. It has shown therapeutic (anti-inflammatory, anti-fibrinolytic and analgesic) as well as industrial applications (detergents, food processing, leather, paper and brewing etc.). The evolution of *Serratia marcescens* as an opportunistic pathogen associated with various infections has led researchers to think and develop an alternate strategy for its industrial production.

Serratiopeptidase is also taken as a supplement to augment the overall health of the cardiovascular system. It has also shown to reduce the cancer metastasis by thinning its extracellular matrix as well as to solubilize non-living tissues such as mucous, plaques and blood clots. It has the potential to cure and treat several disorders such as atherosclerosis, arthritis, bronchitis, carpal tunnel syndrome, fibrocystic breast disease, Crohn's disease, leg ulcers, traumatic swelling, fibromyalgia, breast engorgement, migraine, Alzheimer's disease, sinusitis, hepatitis, lung disorders, arthritis, diabetes, carotid artery blockage, thrombosis, uterine fibroids.

Currently the industrial production of serratiopeptidase makes use of natural S. marcescens strain. However, many challenges and issues have arisen to use this strain because of pathogenic nature, lack of genetic tools, unclear mechanism of the utilization of cheaper carbon and nitrogen sources, industrial -scale fermentation and process. The bacteria is also reported to cause pneumonia, septicemia, and as well is associated with cystic fibrosis. Therefore, a pressing need has arisen to find an alternative approach for the production of active and efficient serratiopeptidase

Technology

GBRC make successful cloning, expression and purification of active serratiopeptidase, using Escherichia coli BL21 [DE3] and pET SUMO vector. Further it also optimize synthetic media and culture conditions for enhanced serratiopeptidase production. Initial optimization of physical parameters was done followed by a screening of different carbon and nitrogen sources. The significant media components for serratiopeptidase production as shown by factorial screening experiment were subjected to Response Surface Methodology (RSM) based optimization.

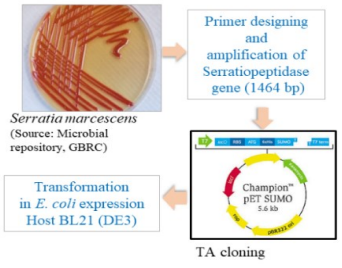
Salient features: In Bioreactor study, final yield of 2.5 ± 0.764 g L-1 of protein was obtained having 8382±291 U mg-1 of specific caseinolytic activity.

Potential Applications and Benefits

The success of the application of a statistical model for designing an optimized media for enhanced serratiopeptidase production also suggests a new insight for the scale up of serratiopeptidase towards industrial applications.

Technology Status

Technology is ready and the optimized media yielded 2.5 ± 0.764 g L-1 of protein was obtained having 8382±291 U mg-1 of specific caseinolytic activity.



2. A probiotic bacterium to enhance milk production in lactating cattle

A probiotic bacterium which enhances milk production in dairy cattle

Technology

The growing concerns towards antibiotic resistance have nudged producers to use natural options – the lactic acid bacteria (LAB) remain one of the most utilized strains in the poultry feed, impacting the probiotics in animal feed market size. Furthermore, several customers are looking for antibiotic free products. Therefore, probiotics are becoming more popular in livestock farming. Probiotics were enhanced the growth of many domestic animals improved the efficacy of forage digestion and quantity and quality of milk, meat and egg (https://doi.org/10.1186/s13099-018-0250-0).

Technology

A probiotic bacterium, GBRC-TATVAM-ISO20 has been isolated from the **rumen liquor**. The specific advantage of this culture is, it is isolated from the same environment where its application is anticipated. Based on plates assay, the bacterium is also able to hydrolyse xylan, pectin, starch, cellulose, and phytate, making it a potential candidate for animal feed supplement. The cell-free supernatant of the bacterial culture was tested for cytotoxicity on Caco-2 cells using the MTT assay, which revealed no toxicity. Animal toxicity on rats with a dose 10^9 CFU/day for a month demonstrated no toxicity of the isolated bacterium. A field level pilot study (12 animals, 6 in each control and test group) revealed ~15% increase [9.87±1.78 (0^{th} day) to 11.43±1.52 (30_{th} day) L/day] in the milk yield during late lactation period in Kankrej cattle.

Milk yield production

A feeding trial was conducted at Sardarkrushinagar Dantiwada Agricultural University. Six lactating Kankrej cattle were fed with 1^10 CFU/day and 6 animals were control. A significant increase in the milk yield was observed in the cattle fed with probiotic bacterium (Figure 1).

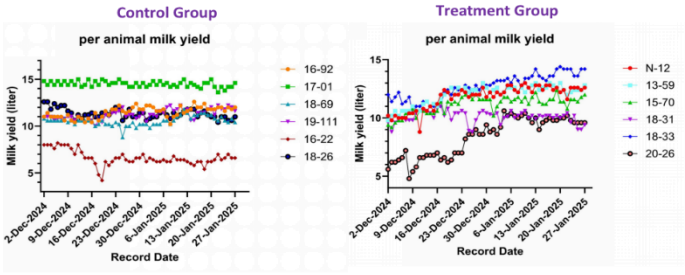


Figure 1: The results of the feeding trial in lactating cattle showing increase in milk yield in the treatment group. Control group fed with TMR and Treatment group fed with 1^10 CFU/day of GBRC-TATVAM-ISO20.

Salient features: A dose of 1^10 CFU/day of GBRC-TATVAM-ISO20 enhance milk yield in dairy cattle

Potential Applications and Benefits

GBRC-TATVAM-ISO20, a probiotic isolated from the rumen, improves feed utilization by breaking down complex plant materials, leading to better nutrition from low-cost feed. Its safe use and observed ~15% increase in milk yield can directly benefit poor dairy farmers by enhancing productivity and income without requiring costly inputs

Technology Status

Technology is ready to serve dairy and animal husbandry sector to increase animal productivity and thus farmer's income.

3. Overproduction and purification of active recombinant tissue Plasminogen Activator (tPA)

Scale up production of important biopharmaceuticals e.g. recombinant tissue Plasminogen Activator, Reteplase (rPA)

Background

Thrombolytic therapy is used extensively and successfully to treat cardiovascular and cerebrovascular diseases, like acute myocardial infarction (AMI) and stroke, which are substantial contributors to illness and death globally. Thrombolytics such as plasminogen activators catalyze the conversion of plasminogen to plasmin, and plasmin's fibrinolytic properties break up intractable fibrin clots. Tissue-type plasminogen activator (tPA) being the first drug approved by Food and Drug Administration (FDA) from this category. Reteplase is a 355 amino acid single-chain non-glycosylated deletion mutant of tPA. It consists of only Kringle II and protease domains. Because of the absence of Kringle I and EGF domains, rPA fails to bind with human hepatocellular specific receptors, and this prolongs the half-life of reteplase in blood. It has a 600-fold higher affinity towards fibrin than tPA. Recombinant tPA is being marketed with the brand names such as Alteplase, Reteplase and Tenecteplase out of which Alteplase has similar structure as human tPA. Tenecteplase has modification at three sites in comparison to human tPA and Reteplase is truncated and non –glycosylated version of tPA. Most of the commercially available rPA is non-glycosylated, purified from inactive inclusion bodies; over expressed in *Escherichia coli*.

Technology

GBRC has achieved successful cloning, expression, and purification of active recombinant tissue Plasminogen Activator, Reteplase (rPA) utilizing *E. coli* Rosetta gami2 strain from Novagen®. Further culture conditions under flask and bioreactor level were optimized for enhanced rPA production. Initial optimization of extraction and purification of active rPA from Inclusion Bodies done by various physical and chemical parameters. Refolding of proteins followed by activity assays for clot lysis and Fibrinolytic activity of rPA analyzed by in vitro assays.

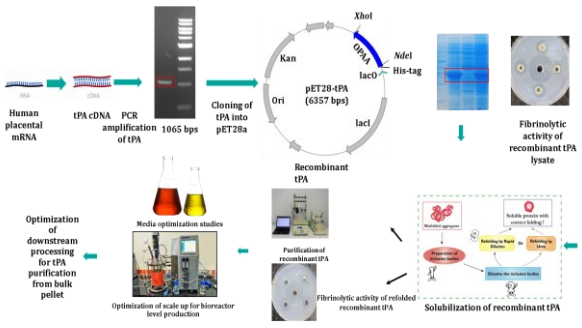
Salient features: In Bioreactor study, final yield of 85.2 gm/lit of active protein was obtained.

Potential Applications and Benefits

The success of the application of a statistical model for designing an optimized media for enhanced rPA production also suggests a new insight for the scale up of rPA towards industrial applications.

Technology Status

Technology is ready and the optimized media yielded of 85.2 g L-1 of protein was obtained.



4. Molecular methods for identification and discrimination of *Ocimum tenuiflorum* syn sanctum and *Ocimum basilicum*

Background

O. basilicum L. (sweet basil) and *O. tenuiflorum* L. syn *O. sanctum* (holy basil) are the two economically and medically significant species. *O. basilicum* L. is mainly used as a fragrance enhancer in the food and perfume industries due to its aromatic secondary metabolites. Moreover, *O. basilicum* L. has prominently displayed antibacterial, anticonvulsant, anti-inflammatory, antioxidant, antidiabetic, antistress, antihyperlipidemic, anticancer, hepatoprotective and immunomodulatory properties. Hence, it is usually used in Unani and Siddha medicinal systems. On the other hand, Tulsi (*O. tenuiflorum* L.) is one of the most sacred plants in Ayurvedic and Indian tradition. It is referred to as an 'Elixir of Life' and the 'Queen of herbs' and is widely used for therapeutic as well as cosmetic purposes. Both the species have higher trade value globally. Trade volume of *O. basilicum* seeds is 100-200 MT/year and *O. tenuiflorum* is one the most cultivated medicinal plant with market demands of 2000–3000 MT/year (http://www.rcfceast.org/wp-content/uploads/2019/03/chapter11.pdf; Last accessed on 04/06/2024). However, as both plants have similar morphology, sometimes it is difficult to discriminate them. The primary requirement for any valuable herbal product is an authentic plant species used in its formulation. Therefore, we have developed a simple and cost-effective PCR method for the identification of *Ocimum basilicum* and *Ocimum tenuiflorum*.

Technology

We have designed primers specific to *O. tenuiflorum* and *O. basilicum*. A multiplex PCR has been developed and optimized for unequivocal identification of these species in herbal raw material as well as in dried powder. The primers and PCR assay have been validated using standard mixtures of both the species along with *Vitex negundo*. Results revealed specificity and selectivity of the primers for both the species. The minimum detection limit of detection of *O. tenuiflorum* is 25% when present in mixture with *O. basilicum*. The sensitivity of primers was further improved up to approximately 10–100 times with digital PCR assay.

Potential Applications

The rapid and cost-effective PCR-based approach developed in this study could be used to detect *O. tenuiflorum* and *O. basilicum* in herbal formulations and their discrimination.

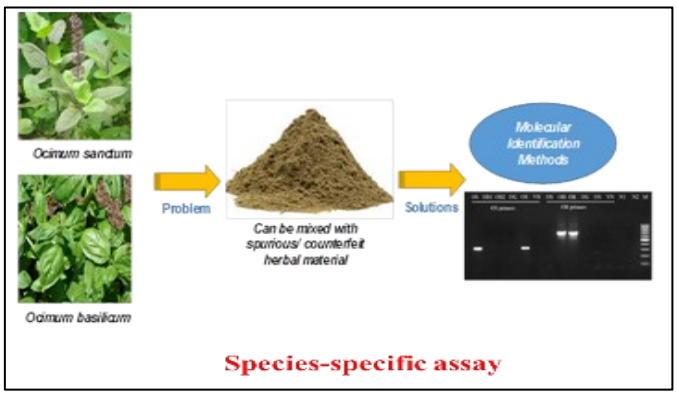
Salient features:

PCR based single tube assay for the detection of *O. tenuiflorum* and *O. basilicum* in herbal powder formulations.

Technology Status

Technology is ready to use and has been validated with standard and market herbal formulations.

Indian patent application No.: 202021056915).



5. Molecular methods for identification and discrimination of *Piper sp.* and *Carica papaya*

Background

The spice made from the fruits of *Piper nigrum* has economic and medicinal importance. It is a phenomenal species with an immense and immemorial presence in food and cuisine around the world, with the entitlement of "The King of Spice". Other than as a spice, it can be used as an antioxidant, anti-inflammatory, antibacterial, antifungal, and antitumor agent. Estimated annual demand of *P. nigrum* as herbal raw drug in Indian market is 1000-2000 MT (http://www.rcfceast.org/wpcontent/uploads/2019/03/chapter11.pdf; Last accessed on 04/06/2024). Due to the high demand, as well as export and trade, the quality is threatened by the mixture of cheap and morphologically similar materials, mainly *Carica papaya* seeds. Therefore, we have developed a simple and cost-effective PCR method for the identification of *Piper nigrum* and mixing of *Carica papaya* with *Piper nigrum*.

Technology

We have designed primers specific to *Piper spp*. and *Carica papaya*. A multiplex PCR has been developed and optimized for unequivocal identification of these species in raw material as well as in dried powder. The primers and PCR assay has been validated using standard mixtures of both the species. Results revealed specificity and selectivity of the primers for both the species. The minimum detection limit of *Carica papaya* seed is 1% when present in mixture with *P. nigrum* in the tested compositions. The primers for PN and CP were found to be specific and sensitive enough to amplify the target sequence down to 0.1 ng of total genomic DNA. Simplex, duplex, and dPCR assays were able to detect 1.0% (w/w) adulteration of CP seeds in a simulated blended formulation. dPCR assay has further improved senility of assay.

Potential Applications

The rapid and cost-effective PCR-based test developed in this study could be used to detect mixing *Carica papaya* seeds with product containing *Piper spp.*

Salient features:

PCR based single tube detection method for identification of *Piper spp* and to check the presence of papaya seeds in piper powders.

Technology Status

Technology is ready to use and have been validated with standard and market herbal formulations.

6. Molecular methods for identification and discrimination of *Bacopa monnieri L.* and *Centella asiatica L.*

Background

Brahmi is herbal therapeutics used in Indian traditional medicinal systems for treating neurological and psychiatric disorders like memory loss, cognitive deficits, and impaired mental function. It is widely sold in the market, with the name "Brahmi'' being used to describe both *Bacopa monnieri* and *Centella asiatica*. These two species are a classic illustration of confusing and misrecognized species because of parallel evolving knowledge systems, currently used polynomial nomenclature in Sanskrit, varying perceptions in various communities, and vernacular equivalent. Due to overlapping therapeutic properties, both Brahmi are described as Medhya Rasayana (brain tonic), however in Charaka Samhita *C. asiatica* is included in medhya rasayna, and over the period, *B. monnieri* is also used for the same. In Indian markets the annual demand of *B. monnieri* is 1000-2000 MT whereas for *C. asiatica* annual demand is 500-1000 MT (http://www.rcfceast.org/wpcontent/uploads/2019/03/chapter11.pdf; Last accessed on 04/06/2024).

Technology

We have designed primers specific to *Centella asiatica*. PCR assays have been developed and optimized for unequivocal identification of *Bacopa monnieri* and *Centella asiatica* in herbal raw material as well as in dried powder. The primers and PCR assay has been validated using standard mixtures of both the species. Results revealed specificity and selectivity of the primers for both the species. Detection of both the species when present in 25% in mixture was detected. It can be further tested at lower levels of adulteration.

Potential Applications

The rapid and cost-effective PCR-based test and approach developed in this study could be used to detect *Bacopa monnieri* and *Centella asiatica* in herbal raw material as well as in dried powder.

Salient features:

- PCR based detection methods for detection of *Bacopa monnieri* and *Centella asiatica* in brahmi based herbal

Technology Status

Technology is ready to use and have been validated with standard and market herbal formulations.

7. Developing probiotic formulation to treat endometritis in bovines

Cure Bovine Endometritis with Key Probiotics

Background

Uterine diseases cause infertility and repeat breeding conditions in bovines (cattle and buffaloes in Indian scenario) leading to decreased reproductive efficiency followed by effect on production and increased odds of early culling of animals from herd. This phenomenon ultimately ends up with astonishing economic losses to the dairy industries. During transition period it is common that cattle and buffalo have nutritional requirements that exceed their dietary intake potential. This leads to a state of negative energy balance during which, body tissue reserves mobilize to provide energy. The Calving process, on the other hand, allows environmental and opportunistic microorganisms to move, transit, remain, and invade the reproductive system. The traditional broad spectrum antibiotic therapy has limited success considering non-specific polymicrobial infection during the postpartum period and also develops antimicrobial resistance. This phenomenon made researcher to think on alternative approaches. Of late, in human use of LAB gave promising results. However, in bovines no such data is available on the role of LAB in vaginal microbiota as well its effect on animal health. Here in this work we have isolated various probiotics cultures from healthy uterus of buffalo and cow and screened them for their potential probiotic activities. From these isolates after detailed In-vivo and In-vitro testing, potential consortia had been formulated and tested in the field trial.

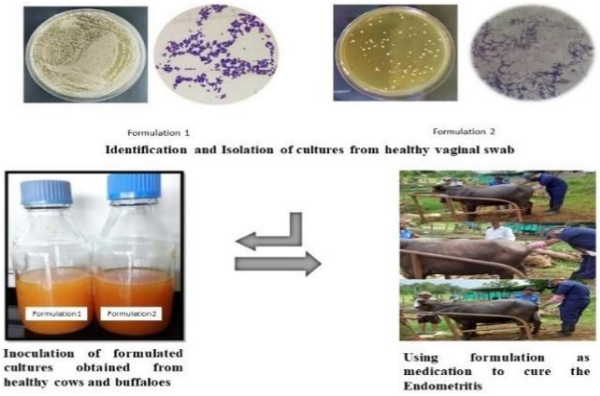
Technology

We have developed a probiotic formulation, which were originally isolated from vaginal microflora of healthy buffalo and cow. Their probiotics characteristics were studied phenotypically and genotypically. Applications of these probiotic consortia was done on animals with endometritis.

Estrous induction was found in formulation one and two at rates of 44% and 51% respectively and both the formulations showed a pregnancy rate of 60% and 68% respectively.

Potential Applications

New approach to treat endometritis in dairy animals. Probiotic bacteria produce antimicrobial peptides and organic acids that has potential to inhibit pathogens. New probiotic formulation has as probiotics can be used to prevent and/or treat bovine endometritis. There is growing concern about the potential impact of extensive antibiotics used in livestock, which is resulting in the spread of AMR (Anti-Microbial Resistant) strains. Infections caused by multi-drug-resistant strains can be combated by the use of probiotic formulation.



8. Kit for sex determination in date palm

PCR based methodology for gender identification in date palm (Phoenix dactylifera L.) of Gujarat, India

Background

The Date palm (*Phoenix dactylifera* L.) is one of the chief flowering plants included in the family of Arecaceae. In India, the area for date palm cultivation is around 18286 ha with 171522 MT production. In India, date palm is cultivated among the major parts Kutch region of Gujarat and few regions of Rajasthan. In date palm is dioecious plant in which inflorescence has male and female flowers on different plant. Traditionally, Date palm plants are produced from seed, and because of dioecious nature, seed produced plants contain approximately the same number of males and females plants. Additionally, only female plants produced fruits, and few male plants were needed only for pollination and fertilization with female ovules. Hence, the selection of female plants is the most crucial procedure for the economic cultivation of date palms. The sex of seedlings can be find out only in the flowering stage and it produces mostly at the age of 4-8 years of the plant. So, the initial gender determination of date palm is very important for farmers for increasing profits.

Technology

GBRC has developed DNA based molecular technique to detect sex of the plant at very early age. The technique very robust and based on simple PCR test. PCR based sex determination of date palm with Glycerol-3 phosphate acetyl transferase (GPAT3) male specific primer and LOX5.1 as positive control. GPAT3 plays important role in male fertility. According to results GPAT3 amplification only occur in male date palm samples, not in female. So that, if result of PCR amplification produced two bands than male and if one band than it is female.

Potential Applications and Benefits:

- The sex determination of seedlings at early stage could help to improve breeding efforts by generating experimental gender specific genetic pools that will promote date palm genetic improvement.
- It also helps to farmers for removing unwanted extra male plants at seedling stage and by which it can increase production of date palm.

Salient features: PCR based method which can detect sex of date palm.

Technology Status

Technology is ready and already tested for more than 300 date palm leaf samples collected from different areas of Gujarat, India and it can successfully distinguish male and female sex of date palm.

9. HLA Typing Kit Based on Targeted Exon Sequencing

Background

HLA typing through targeted exome sequencing (TES) is an advanced molecular technique used to identify specific alleles of Human Leukocyte Antigen (HLA) genes, which are located in the major histocompatibility complex (MHC) on chromosome 6 and play a pivotal role in immune response regulation and transplant compatibility. Unlike traditional methods such as serological typing or low-resolution PCR-based approaches, TES focuses on sequencing the exonic regions of HLA genes, providing high-resolution, allele-level data critical for precise donor-recipient matching in organ and hematopoietic stem cell transplantation. In this process, DNA is extracted, and specific HLA loci (e.g., HLA-A, -B, -C, -DRB1, -DQB1) are selectively amplified using primers designed to capture the highly polymorphic regions of these genes. The amplified DNA is then sequenced using next-generation sequencing (NGS) platforms, generating comprehensive data that is analysed with specialized bioinformatics tools to assign HLA alleles based on reference databases like the IPD-IMGT/HLA database. TES offers advantages over other methods by detecting novel alleles, resolving ambiguities in heterozygous samples, and providing detailed genotypic information, though it requires sophisticated computational pipelines and can be challenged by the complexity of HLA polymorphism and pseudogene interference. This approach is increasingly utilized in clinical settings for transplantation, disease association studies, and personalized medicine, where precise HLA typing is essential for predicting immune-related outcomes.

Technology

GBRC has developed targeted exon sequencing based technique for HLA typing The kit enables targeted exon sequencing of 11 HLA loci using NGS. Applications include clinical HLA typing and immunogenetics research.

Covered HLA Loci:

- MHC Class I: HLA-A, B, C
- MHC Class II: HLA-DPA1, DPB1, DQA1, DQB1, DRB1, DRB3, DRB4, DRB5

Potential Applications and Benefits:

- Transplantation: Ensures precise HLA matching for organ and stem cell transplants, reducing rejection risk.
- Disease Research: Identifies HLA allele associations with autoimmune disorders, infections, and cancers.
- Personalized Medicine: Supports tailored immunotherapy and predicts drug hypersensitivity reactions.
- Vaccine Development: Informs vaccine design by analyzing HLA-related immune responses.
- Population Genetics: Studies HLA diversity for insights into ancestry and evolution.

Salient features: Sequencing based HLA typing for clinical and non-clinical samples.

Technology Status

Technology is ready and already tested for few samples.

10. Varietal identification and seed genetic purity testing in wheat

Background

Varietal identification and seed genetic purity testing in wheat are critical processes in agriculture to ensure the authenticity and quality of wheat varieties, which directly impact crop performance, yield, and market value. Wheat (*Triticum* spp.) exhibits significant genetic diversity, with numerous varieties developed for various traits and environmental adaptability. Varietal identification involves distinguishing between wheat cultivars using morphological, biochemical, or molecular markers, such as DNA-based techniques like SSR (Simple Sequence Repeats) or SNP (Single Nucleotide Polymorphism) analysis, to confirm the identity of a variety. Seed genetic purity testing assesses whether a seed lot conforms to the genetic characteristics of its declared variety, detecting contamination or admixtures from other varieties or off-types. These processes are essential for quality control in seed production, certification, and trade, ensuring farmers have access to reliable seeds that maintain desired agronomic traits and support food security. Molecular methods, particularly those leveraging next-generation sequencing, have become increasingly prevalent due to their precision and ability to handle the complex polyploid genome of wheat. Traditionally, varietal identification relies on the Grow-Out-Test (GOT), a time- and resource-intensive process requiring 6–8 months, extensive field conditions, and trained field personnel. With the advancement of genomics and molecular diagnostics, molecular marker-based methods offer a precise, rapid, and reproducible alternative for varietal testing.

Technology

GBRC has developed a novel invention which presents a time-saving and cost-effective PCR-based assay for the identification and differentiation of wheat varieties of Gujarat. The innovation leverages high-throughput Next-Generation Sequencing (NGS) to discover unique molecular markers and validates them using PCR. The assay for seed genetic purity testing significantly reduces the time, cost, and subjectivity associated with traditional GOT-based methods. It is highly accurate, specific, and suitable for large-scale testing, with potential use in both research and regulatory contexts.

Potential Applications and Benefits:

- Seed Certification: Quick and accurate varietal verification for seed agencies/traders.
- Breeding Programs: Fast varietal confirmation in wheat variety development programs.
- IPR Protection: DNA fingerprinting for Plant Variety Protection.
- Quality Control: Seed companies can implement in-house varietal tracking.
- Research and Germplasm Management: Useful for genotype validation and database creation.
- Time and Cost Efficiency: Reduces testing from months to days, and per-sample cost.

Salient Feature:

- Rapid varietal identification and seed genetic purity testing within 6-8 days instead of months of GOT trials.
- Eliminates environmental influence and human error associated with morphological method i.e. GOT.
- Cost effective.
- Tailored specifically for wheat varieties cultivated in Gujarat region.

Technology Status:

Technology is ready for varietal identification and already tested for seed genetic purity testing of few varieties.