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Editor: Y. K. Bhardwaj

Editorial

Food is a most essential need of human beings for survival. However significant world population is grappling with widely prevalent food insecurity. Efforts are on to eliminate food insecurity through twin approach of increased production and reduced post harvest losses. I am sure this bulletin, guest edited by Dr. S. J. Jambhulkar and Dr. P. S. Variyar, both very accomplished names in their respective fields will cover various aspects of mutation breeding of crops and post-harvest management of food products.

I sincerely thank Dr. S. J. Jambhulkar and Dr. P. S. Variyar for agreeing to be the guest editor of this bulletin. It is because of their efforts that this bulletin has been possible.

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From President's Desk

Dear Members

Greetings to IANCAS family with best wishes for a scientifically enriching and professionally productive year 2019 ahead.

IANCAS strives for disseminating information and knowledge on various topics related to nuclear and allied sciences. In pursuit of its designated aims, it conducts outreach programmes, workshops, seminars, theme meetings and brings out thematic bulletins. I am delighted that present IANCAS thematic bulletin is on very relevant topic in today's context.

Food is among primary needs of human beings. However burgeoning population has put forth global challenge of food security. Also factors like climate change, industrialization, water scarcity, decreased arable land have further depleted resources for food production. Thus it is imperative to adopt an integrated approach for production and management of food products. Increased availability and accessibility to food can be ensured by Increasing production, reducing losses and improving distribution.

During the past few decades, food production has increased many folds through efficient use of fertilizers, good agricultural practices, using modern farming methods and developing improved crop varieties through mutation breeding. Mutation breeding is rigorous exercise involving handling of mutated population, identification, evaluation and selection of breeding lines, genetics, physiology, pre-field or field screening, field demonstration to arrive finally at the strain of desired interest. The bulletin gives a nice flavor of several success stories on mutation breeding of crops in India particularly those developed through radiation induced mutations. Combating constrains of increasing production and growing crops has been comparatively easier than the other aspect of food security, that is, post harvest losses. Post-harvest loss refers to measurable qualitative and quantitative food loss in the post-harvest system. Quality losses include factors that affect the nutritional value of food, the acceptability and the edibility of product. This chain comprises interconnected activities from harvesting through crop processing, marketing and food preparation to the final decisions made by consumer for its consumption or otherwise. The bulletin brings to readers several methods adopted for controlling/preventing post harvest losses.

I am sure the bulletin will help in enhancing the understanding of various aspects of food security of esteemed readers.

(B. S. Tomar)

From Secretary's Desk

India is the second most populated country of the word with huge manpower resource. Utilization of this huge manpower resource for sustained growth and development requires sustained availability of food to the population at all times. Thus, food security is one of the big challenges of such a populous country and requires efforts from all possible directions. Radiation processing is an important part of the efforts made towards achieving food security by improving the crop yield and increasing the shelf life through genetic modifications of various plant species and post-harvest processing.

Significant progress has already been made in the area of radiation processing which has contributed towards enhancing the agriculture produce. In addition to the genetic modifications of seeds and post-harvest processing, developments in other areas such as biosensors, polymer sorbents and genetically modified microbes are also helping towards improving the agriculture production.

In order to highlight the recent progress in this area, IANCAS is bringing out a bulletin on "Mutation Breeding and Post-Harvest Processing for food Security". The bulletin includes fifteen articles covering food processing, preservation, genetic mutations, development of plant beneficial genetically modified microbes and eco-friendly insect pests. On behalf of IANCAS, we thank all the authors who have contributed to this bulletin. We also thank Dr. S. J. Jambhulkar & Dr. P. S. Variyar for being guest editor of this bulletin.

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Mutation Breeding and Post-Harvest Processing for Food Security

Guest Editors

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Guest Editorial

Dr. S. J. Jambhulkar & Dr. P. S. Variyar

Delivering the benefits of nuclear science and technology for food security is one of the major mandates of DAE. In pursuit of this mandate two pronged approach has been adopted by DAE. First is the development of methods and technologies to enhance production and second is development of technologies and protocols to avoid spoilage of food products and agricultural produce. Nuclear Agriculture and Biotechnology Division (NA&BTD) and Food Technology Division (FTD) of BARC have carried out extensive research in these two areas and have left a huge impact.

For enhancing production, application of nuclear energy for crop improvement, fertilizers and nutrient uptake, fate and persistence of pesticides, sterile insect techniques and micro-organism for sustainable agriculture and horticultural crops have been proven beyond doubt. Crop yield improvement through mutation breeding is among the most impactful measures in this regard. It has advantages over other techniques of in-situ genetic manipulation. As well as activation of transposable elements from genomic repository leads to generate the large spectrum of novel variability strengthening the germplasm or improving otherwise high yielding popular varieties for specific traits. Intensive research and development activities on mutation breeding programme in BARC have resulted in the development of 44 high yielding varieties in oilseeds, pulses and cereal crops.

As far as area of enhancing shelf life and reducing spoilage of agricultural produce and food products is concerned radiation processing of food for preservation and hygienization has been highly successful. Radiation processing of food or food irradiation is a physical process in which food commodities, in bulk or pre-packaged are exposed to controlled doses of ionizing radiation such as γ-rays or X-rays to achieve different technological objectives. These technological objectives include extension of shelf-life, destruction of storage and quarantine insect pests, and killing of parasites, pathogens and spoilage microorganisms. Despite significant progress in understanding of the radiation effects on food products use of radiation processing of food technology, within the country is limited. Thus the major thrust in future should be on bringing greater visibility of irradiated food in market place. This would involve understanding the interests of those in the food supply chain and in bringing them to a common platform with food irradiation facilities.

In view of the progress achieved so far in the area of mutation breeding and food irradiation the need for providing further impetus to these programmes, there is a need to disseminate knowledge on radiation technology for enhancing food production and preservation among public and industry. This timely thematic issue containing various aspects of mutation breeding and food irradiation brought out by India Association of Nuclear Chemist and Allied Sciences (IANCAS) will provide a greater impetus in achieving the mandate of DAE in the peaceful application of radiation processing for ensuring food security of the nation.

It has been a great honour to be guest editors of the bulletin. We sincerely thank all the esteemed authors who are acclaimed experts in their areas of research for taking time from their busy schedule to contribute their valuable articles to the bulletin. Our sincere thanks to Dr. V. P. Venugopalan, Associate Director, Bio-science Group (A) & Head, Nuclear Agriculture and Biotechnology Division (NA&BTD) and Dr. S. K. Ghosh, Head, Food Technology Division (FTD) for their constant support and encouragement. We express our sincere thanks to Dr. Y. K. Bhardwaj, Editor, IANCAS for inviting us to be the guest editors and for his guidance.

Current advances in food processing and preservation

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ABSTRACT

Food processing is conversion of agricultural products into various forms of value added food products. Several techniques are used during food processing depending on the types of raw material used and the expected finished products. Food researchers are constantly innovating and upgrading the food processing techniques to minimize food loses due to deterioration and to keep the food microbiologically safe. This review discusses recent scientific advances of nanotechnology, high quality drying and role of spices in food processing and preservation.

INTRODUCTION

The transformation of agricultural products into food, or of one form of food into other forms is termed as food processing. Various processes are used during the conversion of food from raw ingredients to the finished products. These techniques influence the physical and chemical, sensory, safety and shelf life properties of the foods. Mostly, food processing leads to positive attributed in food such as, enhanced digestibility, improved organoleptic properties and importantly increased shelf life. However, depending on the technique used the processing may also trigger side reactions that cause unwanted changes in the physicochemical, sensory, and nutritional characteristics of foods [1].

Researchers from the academia and food industry are responding to the consumer's desire for microbiologically safe, yet minimally processed foods by developing different advanced technologies. Advanced research is being performed on the thermal based technologies, such as aseptic processing, ohmic, microwave & radio-frequency heating and non-thermal processing methods, such as irradiation, high pressure, pulsed electric field & UV processing [2].

Furthermore, food spoilage is a major issue for the food industry, leading to food waste, substantial economic losses for manufacturers and consumers. It is estimated that approximately one-third of all food produced for human consumption is either lost or wasted [3] and quantum of this is estimated to be 1.3 billion tons each year as per Food and Agriculture Organization of the United Nations [4]

This mini review tries to summarise some of the recent advanced in the area of food processing and preservation. The three major techniques discussed are nanotechnology, high quality drying and role of spices in food preservation. Firstly, the importance of nanotechnology in enhancing the food organoleptic properties is discussed followed by role of nanotechnology in augmenting nutritional value and shelf life of food is summarised. Second, the use of high quality drying which includes, freeze drying, infrared drying, and microwave drying in food processing is analysed. Finally, the use of spices in food preservation is reviewed. Due to space constraints, the papers discussed here should be considered as representative and not all-inclusive.

NANOTECHNOLOGY

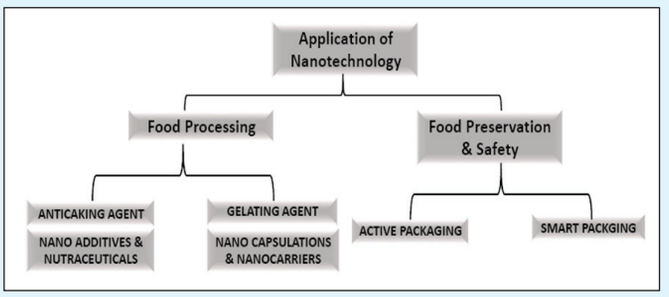

Fig. 1: Application of Nanotechnology in food processing and preservation.

Nanotechnology provides a wide range of food-related applications and it offers complete food solutions from food manufacturing, processing to packaging (Fig.1). The significance of nanotechnology in food processing can be

assessed by analyzing its role in the enhancement of food products in their appearance, texture, taste, nutritional value, and shelf-life. In employing nanotechnology, a specific type of nanomaterial is incorporated into a specific food product to develop certain desired properties for that food product [5]. These nanomaterial have been found to be stable at high temperature and pressures [6] and are in great demand in the food industry as many of them contain essential elements and also found to be non-toxic [7]. Several studies validate that these nanomaterials can effectively improve food safety by improving the efficacy of food packaging, shelf-life, and nutritional value as additives without altering the taste and physical characteristics of food products [8, 9]. Nanomaterials bring positive changes not only in the food quality and safety but also in health benefits that food delivers. Numerous organizations, researchers, and industries are coming up with novel techniques, methods, and products that have a direct application of nanotechnology in food science [10].

Nanotechnology - Food Organoleptic Properties

Nanotechnology provides a variety of options to improve the texture, taste and appearance of food. Nanoencapsulation techniques have been largely used to improve the flavor release and retention [11]. The delivery of lipid soluble bioactive compounds using nanoemulsions is widespread. As these nanoemulsions can be made using natural food ingredients by means of easy production methods, and may be designed to enhance bioavailability and water-dispersion [12]. SiO_2 nanomaterials are one of the most used food nanomaterials as carriers of fragrances or flavors in food products [13].

Nanotechnology - Nutrition Value of Food

The macro and micro nutrients such as lipids, proteins, carbohydrates, and vitamins are digested by the high acidic environment and enzyme activity of the stomach and duodenum. Some of these nutrients have bioactivity and encapsulation of these enables them to resist such adverse conditions and also allows them to assimilate readily in food products. Nanoparticles-based products with the aim to improve delivery of the sensitive micronutrients in the daily foods are being created to provide substantial health benefits [14,15]. Techniques such as nanocomposite, nano-emulsification, and nanostructuring are being applied to encapsulate and effectively deliver nutrients for targeted health and nutritional benefits.

Nanotechnology – Enhanced Shelf Life

The functional components of functional foods are generally sensitive and lose activity over time due to degradation. Nanoencapsulation of these bioactive components extends the shelf-life of the products by slowing down the degradation processes or prevents degradation. For example, curcumin, the most active and least stable bioactive component of turmeric (*Curcuma longa*), showed reduced antioxidant activity and found to be stable to pasteurization and at different ionic strength upon encapsulation [16]. In addition, the edible nano-coatings on several food materials could provide a barrier to moisture and gas exchange and deliver functional components and could also increase the shelf life of manufactured foods, even after opening the package [17,18].

HIGH QUALITY DRYING

The process of drying or dehydration is one of the oldest and most prevalent processes used for food preservation. Removal of the moisture prevents the growth of microorganisms, which causes a reduction in the moisture-mediated deterioration reactions [19]. Furthermore, drying brings about a minimization of the packing, storage, and shipping costs due to the reduction in the weight and volume.However, there are few drawbacks to drying food products. Depending on the method of drying, it may alter the characteristics of food products which suffer from several defects [20]. These faults are loss of flavour and aroma volatiles, deterioration of colour and texture, and an overall decrease in nutritional value. Due to the demand by consumers for higher quality dried products that maintain more characteristics of the fresh product, high quality drying of food is becoming increasingly important. Food high-quality drying can be defined as a special drying technology, which protects qualities of fresh foods such as colour, flavour, nutrients, rehydration, appearance, and uniformity during drying process [21]. Although there are many high quality drying techniques, here only techniques such as freeze drying, infrared drying and microwave drying will be discussed.

Freeze Drying

In freeze drying the water is removed from material by sublimation [22]. In this process of drying there are three distinct stages. The first stage is of pre-freezing the wet material, the second is the primary drying stage in which sublimation of frozen solvent takes place under vacuum, and finally the secondary drying stage where there is desorption of residual bound water from material matrix [23,24]. As this process generates negligible changes in colour, flavour, chemical composition and texture, the product quality is considered as the highest of any drying techniques [25]. Freeze drying is a time consuming and expensive process; hence industry has to put efforts to maximize process efficiency [26].

Infrared Drying

In recent years infrared drying has become more popular due to its advantages, such as, it's low drying time, the reasonable quality of the final dried product, its greater energy savings capability and its lower cost when compared to microwave and vacuum drying methods [27]. Medium and far Infrared radiation sources (wavelengths of 2–100 mm) have been studied for drying agricultural products and have been considered as prospective method for achieving production of high-quality dried foods [28, 29]. The infrared radiation could inhibit enzymatic reactions and kill the microorganism in the dried food resulting in better quality food products [30]. The infrared radiation heating can be combined with other drying methods such as hot air, microwave, freeze drying, and vibration for efficient and useful drying [27].

Microwave Drying

Foods are typically made up largely of water hence the molecular friction mechanism is mainly responsible for heating of foods by microwaves [31]. The microwave energy at 2450 MHz can be absorbed by water containing materials and can be converted to heat [32]. Microwave energy is rarely used alone in drying processes and is typically combined with a flow of hot air instead of using microwave in a static air flow chamber [33].For many year microwave-vacuum drying has been considered as a good solution for improving physical damage caused during microwave drying, such as off-colour production, scorching, and uneven heat distribution [34]. Furthermore, to combat local heat building during microwave drying, the application of microwave energy during spouted or fluidized bed drying is emerging field of research. This ensures the proper mixing of the sample and more even distribution of the applied energy throughout the sample load. Microwave-assisted fluidized bed drying has been studied with many products, including carrot [35] garlic [36] and soybeans [37] and many more.

SPICE AND FOOD PRESERVATION

Since ancient time, spices have been used as nutritional agents [38]. Spices have been mainly used as flavouring and colouring agents but their preservative properties have created wide applications in the food industry [39]. The natural antioxidant properties present in spices can be used to prevent food spoilage. Spices show antioxidant property by scavenging free radicals, chelating transition metals, quenching of singlet oxygen, and enhancing the activities of antioxidant enzymes [40]. The relationship between antioxidant properties of spices and food spoilage has been well-documented. Some studied antioxidants are gingerol from ginger, curcumin from turmeric, capsacin from red chilli, piperine from black pepper and many more [41, 42].

In addition, many compounds isolated from spices have shown antimicrobial activity against most common microorganisms that affect the food quality and shelf life [43]. Several studies have shown the *in vitro* antimicrobial activities of the spices. The spice essential oils and the active components either alone or in combinations have been studied for their antimicrobial activities. [44-46]. Spices show *in vitro* antimicrobial activity against vide variety of Gram-negative, Gram-positive bacteria, yeast and moulds [47]. In addition, the spice components such as essential oils and extracts have also shown anti-fungal activities *in vitro* [48,49]. However, as the physical and chemical properties of the food changes in the whole food, the *in vitro* studies represent only one part of the use of active compounds as preservatives in food. Hence, *in vivo* studies have been performed on real foods to assess antimicrobial potentials of spices and spice related compounds [50,51]. Although several studies have shown applications for spices and spice derived component as food preservatives, only few of them are currently applied on the market. Rosemary for example is already employed for its preservative properties in meat products. Rosemary essential oil has been used for its antimicrobial and antioxidant activity in addition to its flavouring application.

CONCLUSION

The idea of this review was to discuss the current developments in food processing and preservation technologies. Although the advances in food processing technologies such as nanotechnology and high quality drying are paving new paths day by day, there still persist many challenges and opportunities to improve the current technologies. For example, there are issues about the consequences of nanotechnology that need to be addressed in order to address the consumer's concerns. In addition, there is lot of potential for technology such as microwave-based drying techniques in the food industry. However, now it is important to perform scaling up and tackling the engineering issues that will be encountered during its implementation, and not just research. Finally, the studies of spices in food preservation can be explored further for developing novel solutions against spoilage microorganisms.

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Post Harvest Packaging of Food Products for Enhancing Food Quality and Safety

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ABSTRACT

Rising population, growing demand for food and decreasing cultivation land are the leading problems for India's food security. Enhancing the availability through coating and radiation is well proven techniques to improve shelf life of food material. However, it is not applicable to all food products. With proper knowledge of storage, the short fall in the traditional technique can be replaced by advanced packaging like active and intelligent packaging in addition to modified atmosphere packaging. This helps us for enhancing and ensuring food quality and safety for prolonged time.

Keywords: *Post-harvest loss, active packaging, intelligent packaging, edible coatings, food storage.*

INTRODUCTION

India being an agro-based economy ranks second in the world for agricultural land available with approximately 179.9 million hectares under cultivation. Agriculture accounted for 14% of GDP and 11% of India's total exports in 2015[1]total food grains production in India reached an all-time high of 263.3 million tonnes (MT. However India is also facing a main global challenge of food security for ever-growing population, while ensuring long term sustainable development. Trends like increasing population and industrialization have compressed the land available for farming. Moreover, climate change, developing life style and declining fresh water availability have applied great stress on food resources. In this situation, we need to develop an integrated and innovative approach to ensure sustainable food production and consumption. After combating these constrains and growing crops; an important stage of post-harvest comes into play. To study the loss, the term "post-harvest loss" (PHL) have been coined that refers to measurable qualitative and quantitative food loss in the post-harvest system. Quality losses include those that affect the nutritious value of food, the acceptability and the edibility of a given product. This system comprises interconnected activities from the time of harvest through crop processing, marketing and food preparation to the final decisions made by consumer to eat or discard the food. PHL is now recognized as part of an integrated approach to harness agriculture's full potential to meet the demands[2]. Research and development so as to produce transgenic crops[3] can never be successful without proper post-harvesting measures being carried out.

POST-HARVEST LOSS (PHL)

India, due to its wide range of topography shows a huge variety of crops being cultivated. A huge variety of pulses and cereals, staple crops like maize, rice, wheat, etc., spices, fruits, vegetables, oilseeds, other crops like jute, hemp, cotton, beverages and many more are available.

Many agricultural products like cassava, strawberries, etc. have very short life i.e. they start showing post-harvest loss (PHL) within only 24 to 72 hours of harvest. Short shelf-life corresponds to discouraged stakeholders, limited market potential and lower reliability industrially. PHL is a complex process that is associated with enzymatic stress response on harvesting, involving changes in gene expression, protein synthesis and accumulation of secondary metabolites which is influenced by environmental factors, physiological state of the plant, etc. various techniques have been implemented for monitoring the PHL. Post-harvest loss persist not only during harvesting and storage but also during processing, packaging, sales and consumption and the probable factors responsible for the loss [4].

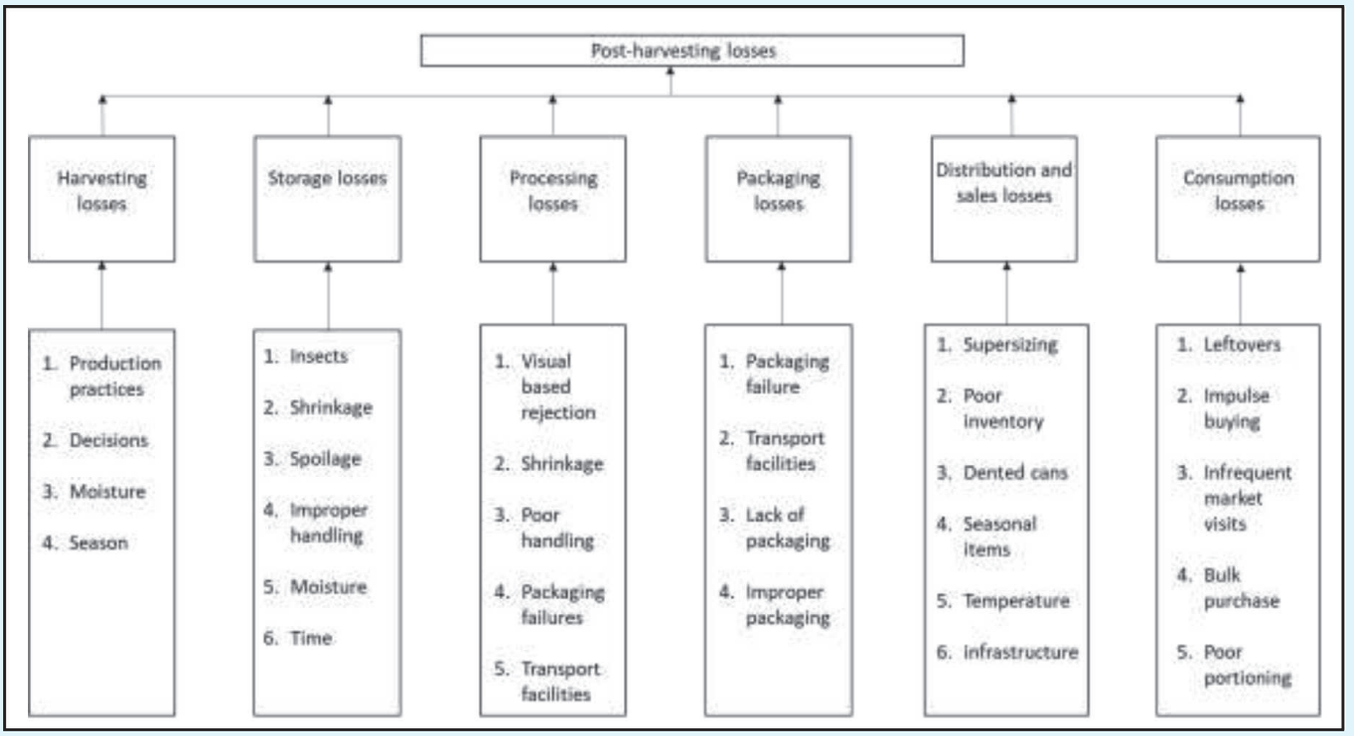

Figure 1: Post-harvest losses [4]

STORAGE/ PACKAGING

There lies a huge chain of industries and market based on agriculture which is much complex due to the variability it faces since food is perishable at one or more stages across this chain. Other factors include seasonality and uncertainty in harvest yield. Noteworthy, not all agric-chains are affected to the same degree by these complication factors. In particular, products differ in the rate of quality decay. Soybeans can for instance be stored for several years without any risk that they perish. Palm oil, on the other hand, must be processed in 24 hours after harvesting to avoid decay. Also, in the case of olive oil, the olives must be pressed within 48 hours of collection to achieve the highest quality of the final product[5]. The losses occurring during these stages are subset of PHL. Storage is an important part of post-harvest activities as it is associated with food supply for longer period of time. Figure 2 illustrates the general idea for storage requirement in agro-chain activity. Food being perishable needs attention and research related to storage of food have been carried out widely[6–10]. The type of storage being implied depends on the end use of food material. Choices like whether it is for direct consumption; will be used for new plantings the preceding year or need to be stored for very long time like more than 6-8 months need to be considered before selecting the storage type.

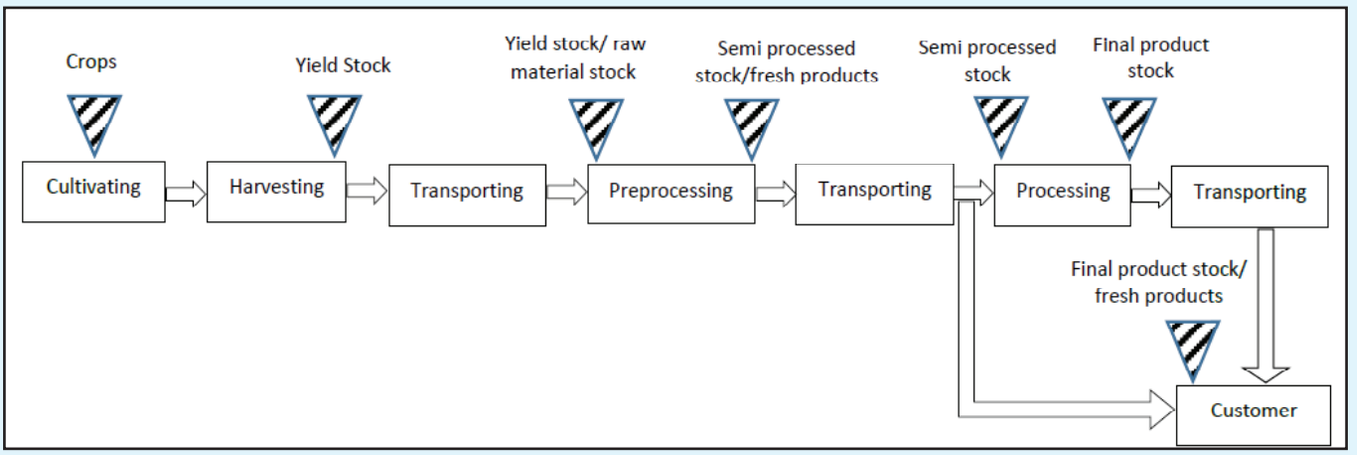

Figure 2: Agri-chains activities and possible storage points[5]

The major functions of both storage and packaging are to preserve freshness, extending the shelf life, quality, taste and deliver the food products until consumption. It should be noted that despite the fact that food borne pathogens are entirely eradicated by the efficient thermal treatment. The microbial recontamination of the food surface might take place. Consequently the reduction of food shelf life is observed and the food borne diseases is widely accelerated. Incorporation of antibacterial supplies into food products with the intention to extend its preservation time is presently being investigated and utilized. Considering the fact that microbial infection is a surface phenomenon, direct application of antimicrobial elements onto foods have limited advantages as they could be neutralized over time. The use of packaging containing antimicrobial agents is more efficient that direct surface application on to food. Polymers have the potential to replace conventional materials like glasses, paper, metals etc due to its better transparency, flexibility, chemical inertness, light weight and are economically cheaper[11].

TYPES OF STORAGE/PACKAGING TECHNIQUES

The various packaging technology implemented for improving shelf life and quality of food for longer period are classified as mentioned in Figure 3. These technologies have been explained in following appropriate sections.

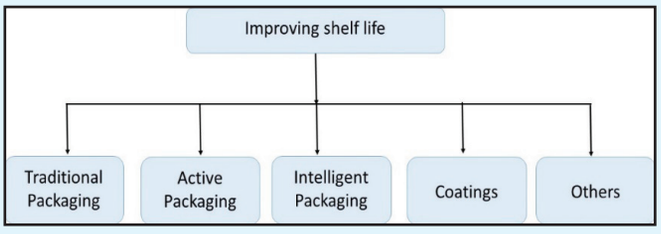

Figure 3: Classification of Food Packaging

1. Traditional storage techniques

Traditional storage techniques uses metal silos, woven bags, etc. for food packaging[12]. Packaging materials normally used for food packaging are glass, metal, paper and cardboard, wood, and plastic, as well as packaging material from mixed substances with respect to raw materials used for their production, although all the aforementioned materials possess advantages and disadvantages. For example glass which requires very high temperature for fabrication with the risk of breakage during handling and transportation, metals which are heavy and costly as compared to current packaging materials, paper which despite of having very low cost have low strength and thus limits application, etc.[13] indicate freshness, exhibit information of qualities, and improve safety. Conversion of nonbiodegradable plastic to biodegradable plastic by altering its chemical structure and molecular weight eventually lowers the production cost through bioengineering. Bioengineered food packaging materials are closely related with active, intelligent, and smart packaging. A packaging material that provides an active function apart from inert containment and protection to the product is referred to as active packaging. Intelligent and smart packaging usually incorporates the capacity to sense or quantify the attributes of the produce, the inner environment of the package, or the transport atmosphere. Apart from a number of conventional varieties of packaging, the terms are used for technologically advanced systems, such as microelectronics, computer application, and nanotechnology. This chapter will discuss the definition, basic principles, technological development, and application of antimicrobial, active, smart, intelligent packaging, including time–temperature indicators, integrity or gas indicators, freshness indicators, barcodes, and radio frequency identification tags (RFIDs. The food is sun-dried prior to storage to inhibit fungal growth along with the application of certain chemicals like boric powder to further retard the fungal or pest attack. Besides most widely used plastics include PE, PP, PVC, PS and PET [14][15]. Comparative study of the efficiency of various packaging methods has been evaluated by many researchers. H. Sudini et al. have explained the efficacy of triple layer bags that comprises of two inner HDPE bags and one outer woven PP bag; over cloth bags in terms of retention of seed weight, germinability and oil content [16].

MAP (modified atmosphere packaging) reduces the ripening rate, maintaining its color and firmness, reduces chilling injury and loss of nutrients and water during refrigeration. It also retards microbial growth ensuring safety and commercial value [17][18]. However, Inadequate atmospheres can cause the accumulation of fermentative metabolites including ethanol, etc. which leads to odours and decay off the food being packed. Recycled plastic like LDPE have been used so as to create MAP and its effect have been analysed recently [19]. MAP involves reduction of the respiration rate of fruits and vegetables by lowering the O_2 and CO_2 concentration of the storage environment; thereby increasing shelf-life. The product has to be stored at an optimal concentration of O_2 and CO_2; otherwise, its storage life would be adversely affected. The optimal

concentration of O_2 and CO_2 varies with commodity; their variety; storage temperature etc. It was reported that the weight loss of banana was reduced from around 20% to 8.4% and 8% by packing it using LDPE and HDPE films respectively[20].Similar studies have been carried out by Céline Matar et al. wherein MAP have been utilized to enhance the shelf life of strawberries [21].

2. Active packaging

Active packaging refers to the packaging technique wherein the packaging material helps controlling and maintaining the MAP through the use of certain additives present in the packaging material. Table 1 shown below gives the examples of such additives along with the information of commercial product and manufacturer.

3. Intelligent packaging

A food supply chain is complex, time-critical, and dynamic. An intelligent packaging is the one that 'speaks' to the customer. It senses change in the internal or external package environment information to enable a user to offer benefits such as more convenience, increased safety, or higher quality (or better retention of quality). They are used in the form of labels, printed layers, tablets, or are laminated in the polymer film. Examples of intelligent packaging include:

- Time-temperature indicators that can imply/signal the user about the quality of the packaged product.
- A biosensor, in theory, which can inform the user of the growth of microorganisms or even a specific microorganism in the package.
- A bar code to help communicate information for more precise reheating or cooking of the contained food in an appliance.
- An ethylene sensor, probably for the ripeness of fresh fruit.
- Nutritional attributes of the contained food.
- Gas concentrations in modified atmosphere packages[24].

Table 1: Various additives and their examples employed in active packaging [22] [23]

Sr. No.	Additives	Examples	Commercial product and manufacturer
1	Antimicrobial agents	Silver, silver zeolite, glucose oxidase, triclosan, chlorine dioxide, natamycin, sulphur dioxide, allylisothiocyanate. Metal oxides nanoparticles like TiO_2, ZnO_2, MgO. Essential oils, certain plant extracts, polysaccharides (e.g. chitosan), bioactive compounds like thymol and carvacrol, certain peptides (e.g., nisin, lactoferrin), enzymes (e.g., lysozyme), etc.Quarternary ammonium salts, ethylenediaminetetraacetic acid, propionic acid, sorbic acid, etc.	Aglon®, Agion Technologies, USA Bactiblock®, NanoBioMaters, Spain Bioka, Bioka Ltd., Finland Biomaster®, Addmaster Ltd., UK Biomaster®, Linpac Packaging Ltd., USA Food-touch®, Microbeguard Co., USA
2	Carbon dioxide emitters	Sodium bicarbonate and citric acid, Sodium bicarbonate and ascorbic acid, Metal carbonates (e.g., FeCO3)	Ageless G, Mitsubishi Gas Chemical, JapanCO_2® Fresh Pads, CO_2 Technologies, USA Freshpax®, Multisorb Technologies, USA
3	Antioxidants	Natural- Cellulose derivatives, chitosan, alginate, gelatin, Synthetic-butylatedhydroxytoluene, butylatedhydroxyanisole, tertbutylhydroquinone	-
4	Oxygen scavengers	Iron, cobalt, palladium, platinum), organic acids (e.g., ascorbic and gallic acid), photosensitive dyes (e.g., eosin, curcumin), unsaturated hydrocarbon dienes, enzymes and bacterial spores or yeasts,, pyrogallol, alpha-tocopherol)	ATCO®, Laboratories STANDA Ageless G, Mitsubishi Gas Chemical, Japan, OxyRx®, Mullinix Packages Inc., USA OxyCatch®, Kyodo Printing Co., Ltd., Japan
5	Ethylene scavengers	KMnO4, TiO_2 nanoparticles, halloysite nanotubes, nano-zeolite, etc.	-

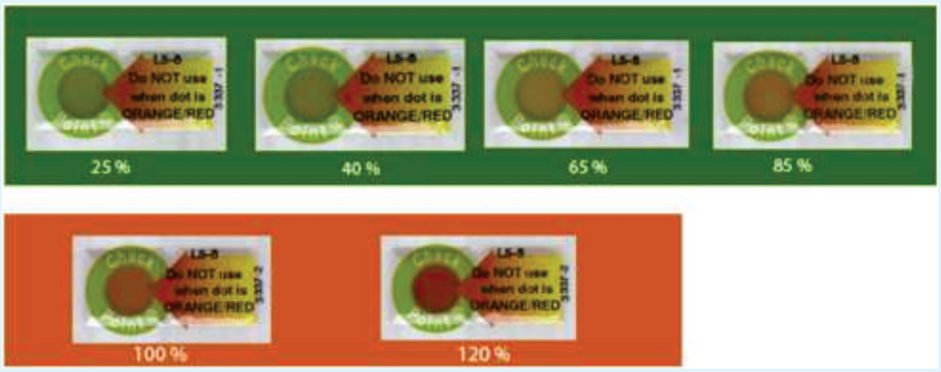

Figure 4: Colour changes of a VitsabCheckPoint® Time-Temperature Indicator label [23]

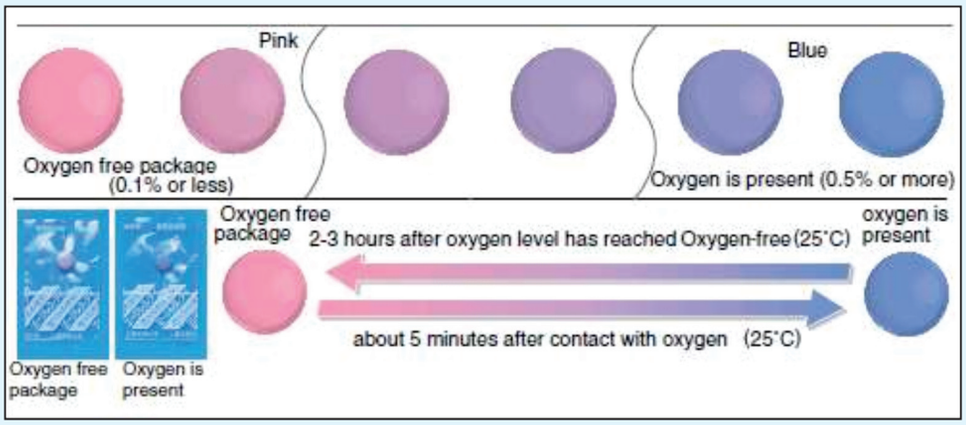

Figure 5: Ageless Eye® oxygen indicator[23]

Extensive literature on these materials have already been carried out [25][26]. A myoglobin-based indicator for modified atmosphere–packed poultry meat has been developed, which can indicate spoilage by detecting the presence of hydrogen sulphide (H2S) formed upon spoilage. For monitoring fish spoilage, Pacquit et al. developed a colorimetric dye-based sensor that detects the presence of total volatile basic nitrogen, a product of spoilage. Salinas et al. studied the use of an array of chromogenic indicators with different chemical recognition properties to follow the evolution of fresh pork sausage and boiled marinated turkey aging [27].

Economic Aspect of Other Countries

Due to the technology involved, the cost attributed to intelligent devices is estimated to be ~ 50–100% of the whole cost of the final package. However, for most food products the packaging cost should not exceed 10% of the total cost of the goods placed on the shelves, provided that the claimed benefits are unambiguously demonstrated to outweigh the possible extra expenses arising from the new technology. This mismatch between the new technology and market penetration eventually results into a negative cost/benefit analysis [28].

Table 2: List of commercial indicators for intelligent packaging

Indicators	Trade name	Manufacturer	Chemical integrity
Time-temperature indicators	OnVu®	BIZERBA, North America	Based on benzylpyridines
	CheckPoint®	VITSAB	Based on irreversible reaction between lipase enzyme with its substrate
	Fresh-Check®	TEMPTIME corporation	Based on diacetylene monomers
	eO®	CRYOLOG	Based on lactic acid
Freshness indicators	FreshTag®	Cox Technologies Recorders	-
	RipeSense®	Jenkins Group Ltd	-

4. Coating

The multi-dimensional research being carried out to search all possibilities for food security led to the emergence of various coatings which are applied to the food directly. They are usually applied over vegetables and fruits. These coatings are usually bio-degradable polymer based edible films from food-grade proteins, polysaccharides, and lipids that could potentially serve as edible packaging materials [29,30]. Edible coatings are applied by dipping, spraying or brushing to create a modified atmosphere onto the food [31]. The filmogenic solution mainly consists of a binder material which can be carrageenans, starch, chitosan, polyhydroxyalkanoates, etc. These binders act as the carriers of functional ingredients including certain antimicrobial, antioxidants, nutraceuticals and color and flavour ingredients. Incorporation of beewax, mineral oil, vegetable oil, surfactants, acetylated monoglycerides, other waxes strongly affect the permeability of coatings which ultimately affects the quality of food. Such studies were carried out by Deba Das et al. wherein they have studied the effect of starch-based edible coating onto the perishability of tomatoes for 20 days. It was concluded that the addition of lipid and antioxidant improved the surface morphology. Addition of lipid and antioxidant into the formulation delayed the tomato ripening with antimicrobial property [32]. However, these coatings are water soluble and get easily washed off which limits its duration to protect the food item. This can be avoided by adding hydrophobic agent as explained by V. R. L. Oliveira et al wherein beewax was added to coating solution. 10% addition of beewax helped improving water vapour transmission rate of the biofilm. An 80% increase in elasticity and 15% decrease in solubility indicated resistance against unfavourable environmental conditions [33]cassava starch (2%. Wax containing coating also improves the resistance to chilling injury. Pomegranate when coated with putrescine wax and carnauba wax prior to cold storage at $2^{O}C$, showed negligible symptoms of chilling injury. Chilling injury refers to development of brown discoloration of skin, surface pitting, and weight and firmness loss during storage. All these undesirable changes were significantly delayed by wax treatment [34]. Other waxes like candelilla wax and beewax have been tested for its performance to enhance shelf life of food material [35]. Chitosan is another important biopolymer that finds application in such edible coatings [36,37].

5. Others

5.1 Hydrogels

Use of hydrogels as food packaging material is an innovation brought about by extensive research. In recent years, significant improvement of their biodegradability, swelling properties, and their mechanical and thermal properties has been reported. Rejane Batista et al. through their concise review explained the use hydrogels for food packaging. According to Rejane, hydrogels are 3D, hydrophilic, polymeric networks linked through physicochemical bonding. They are known for moisture control and as bioactive agents [38]. Hydrogels are known to control humidity generated by food products with high water content during transpiration other physicochemical changes [39]. The reduced water activity reduces the growth of mould, yeast, and other bacteria on food [40]. Depending upon the application, hydrogels can be in the form of nanoparticles, microparticles, coatings and films [41].

5.2 Food Storage by Irradiation

Irradiation is a vital process for phytosanitary conservation and maintenance to combat losses, by increasing shelf-life, reducing risk of food borne diseases. Generic doses of 0.15 and 0.4kGy are approved to control many classes of insects [42][43]. Irradiation inhibited polygalactouronase activity in papaya, which slowed the process of ripening and help improving its shelf life [44]. Since ripening is related to enzymatic activity; experiments carried out shows that this can be influenced by irradiation. However, it is noteworthy that the impact of irradiation is complex and highly variable depending on type of fruit or vegetable and its maturity stage [45]. Milton Filho et al. have evaluated the changes in properties occurred within strawberries by gamma irradiation at 1, 2, 3, and 4kGy. It was concluded that even though irradiation reduces ascorbic acid content, firmness and weight; it markedly reduced the growth of moulds and yeasts enhancing the life of food. Further, it was suggested to use the 2 kGy dose as an alternative for strawberry conservation, since only minor changes were observed in the product during the 12 days of storage and it was the treatment that obtained better microbiological quality and maintenance of sensory acceptance during the storage period studied [46].Gamma irradiation have also

been used for drumstick wherein 1kGy dose of irradiation resulted in quality product even after 12 days of storage at 10^{O}C [47]. Irradiation dose beyond certain extent proved to be detrimental for its ingredients. Irradiation above this limit reduces the nutrient level along with loss of firmness as compared to control samples according to the studies conducted by Kun Ago et al. Thus it is recommended to use lower irradiation dose coupled with appropriate temperature during storage for extending the shelf-life [48].

Apart from gamma irradiation, electron beam have also been used for its assessment of efficiency. Electron beam irradiation coupled with vacuum impregnation yield longer storage life of blueberries. The study evaluates the feasibility of vacuum impregnation technology as a strategy to minimize quality degradation of irradiated food. This impregnation technique doesn't have any negative impact on moisture content, water activity, pH, sugar content and other ingredients of fruit. Impregnation with 4% (w/w) calcium lactate solution proved to be best under 160 mm Hg vacuum pressure [49].Apart from fruits and vegetables, irradiation have also been used to store meat for longer period [50,51].

CONCLUSION

The chapter deals with various aspects of PHL from harvesting to customer consumption. Deterioration of food in terms of its loss of nutrients, physical appearance, etc. makes the food unacceptable to consumers, which highly impacts the economic domain. With the knowledge of physical and chemical changes occurring into food after harvesting and during storage we can develop certain storage and packaging techniques that ensures the enhancement of its shelf-life. Use of "active" and "intelligent" packaging is one of them. These modern packaging solutions not only help to deliver quality product but also evaluates the freshness of food being packed within it. Other techniques to improve shelf-life include coating the surface of food item to reduce the rate of its evapo-transpiration. Other techniques include irradiation which sanitizes the food limiting the spread of food borne diseases.

In developing countries like India, coating of food can be encountered easily as compared to active and intelligent packaging. One major reason is that use of these packaging solutions increases the price of final product by almost 50-100% which is not accepted as it is considered to be around 10%.

Table 3: Goals and characteristics of hydrogels in food packaging [38]

Goal	Main characteristic	Hydrogel composition
Food freshness indicator	Generated information regarding food freshness Based on metabolites production of in the food Detection changes in the pH, chemical degradation or microbial growth	Poly(N,N-dimethyl acrylamideco-Methacryloylsulfadimethoxine) (poly(DMAco-SDM)) hydrogels incorporated with methacryloyl sulfadimethoxine monomer (SDM) with a pH-responsive gr.
Stability and retention of volatiles substances	Flavor encapsulation (nanoemulsions in hydrogels) Controlled release occurs by pH modification (e.g., in contact with saliva)	Flavorednanoemulsions incorporated in low methoxyl (LM) pectin and whey protein isolate (WPI) at pH 4.0 Orange oil, medium-chain triglyceride (MCT) oil, and WPI
Improvement of bioavailability of lipophilic compounds	Incorporation of lipophilic bioactive compounds (e.g. β-carotene) in food matrix, improving their bioavailability	Hydrogels based on polysaccharides (starch and xanthan gum) to incorporate β-carotene emulsion
Method for aflatoxin B1 detection	Detection of aflatoxin B1 in food sample when the hydrogelcauses collapse of the network, and occurs the release of ureaseinto the analyzed solution. Then, the released urease can catalyze the hydrolysis of urea and result in the rise of pH value. The change of pH value has a direct relationship to the concentration of aflatoxin B1.Main advantages: Cheap, available, and well-established pH testing system with the robust performance of DNA cross-linked hydrogel.	DNA hydrogel

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Radiation technology: A non-thermal technique for food preservation

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Introduction

Public awareness and regulations on food safety is increasing worldwide. Growing per-capita income in combination with increasing health consciousness and to avoid food-borne diseases, consumers now demand for safe, fresh, quality and chemical/preservative free foods for their consumption. Also, a large group of consumers are now preferring for minimally processed food like ready-to-cook (RTC) and ready-to-eat (RTE) products. As these products are processed or handled at several stages, chances of contamination by microorganism increases. The hot and humid climate is also favourable for the growth of numerous insects and microorganisms which destroy stored crops and cause spoilage of food. Conventional food processing/preservation relies on thermal treatment either to kill food borne pathogens (insects, parasites, bacteria, fungus and virus) or to inactivate the enzymes that degrade the food material to make it safe or palatable. For many foods, thermal methods are very effective. However, several foods are not suitable for thermal processing because they lose fresh like character or some of the pathogenic organisms are resistant to thermal treatment. Non-thermal processing methods that neutralizes pathogenic organisms, maintains freshness, retain nutrients and sensory attributes are therefore gathering importance to food industries as well as researchers. Conventional thermal processing operations including pasteurization, sterilization, drying and evaporation involve the use of heat. It is a common practice by many food industries to assure the microbiological safety of their products to the consumers. Though popular, thermal processing has several technological problems including uniformity in treatment and many food products are not suitable for the process. Therefore, efforts are on for other technologies/ processes to address the consumer demands for quality food. A few alternative to thermal processing methods [1,2] are now under active consideration by industries which include ionizing radiations [3], high pressure [4], pulsed electric field [5], modified atmosphere, cold plasma, UV-visible light [6] and chemical sanitizers/ preservatives. These processing methods have the ability to inactivate microorganisms at near-ambient temperatures avoiding thermal degradation of the food components and consequently preserving the safety, sensory and nutritional quality of the food products. Many food items treated with non-thermal process are safer to eat than untreated products and many products still require refrigeration to delay spoilage. In the changing scenario of world trade such as banning the use of chemical fumigants and stricter quarantine requirements especially for fruits, vegetables and cut flowers, switching over to non-thermal techniques such as radiation processing of food assumes great importance. The use of non-thermal processes in combination with other preservation technologies opens up opportunities and would potentially benefit food preservation. This article provides an overview of the use of ionizing radiation especially the use of γ-ray as non-thermal technology that is effective alternative or complementary to thermal techniques to maintain safety and quality of the food.

Preservation of food by ionizing radiation

Particulate radiation such as electron beam (EB) and electromagnetic radiations like X-ray and γ-ray are used for food irradiation. They are called ionizing radiations because they can ionize the medium through which they pass. Radiation processing of food provides mechanism to control these food losses significantly [7]. It involves controlled application of energy from ionizing radiations using radioisotopes (Cobalt-60 and Caesium-137), electron beam (up to 10 MeV) and X-rays (up to 5 MeV). The energy of these radiations is below the threshold limit to destabilize the nucleus of the elements present in food materials. The dose of radiation is measured in unit called gray (Gy) which is equivalent to 1 Joule of absorbed energy per kilogram of material. Irradiation can directly interact with the biological molecules or indirectly by interacting with water present in food material to produce first the radiolytic products like highly reactive hydrogen, hydroxyl and superoxide

radicals and molecular species like H_2O_2. Irradiation thus works directly or indirectly by disrupting the biological processes such as deactivation of enzymes in the food material that lead to decay. Similarly, these radiolytic species can cause the damage (see, Fig. 1) in the cell of the insects and microorganism present in the food leading to their elimination. The reactions with DNA cause the death of microorganisms and insects and impair the ability of potato and onion to sprout.

The treatment helps in inhibition of sprouting of tubers and bulbs, disinfestations of insect pests in agricultural commodities, delay in ripening and senescence of fruits and vegetables, destruction of microbes responsible for spoilage, and elimination of pathogens and parasites (see, Fig. 2) [8]. The applications of gamma radiation processing of the food have been classified in three broad categories, *viz*. Low dose applications (0.25-1 kGy) which serve purpose of sprout inhibition in bulbs and tubers, insect disinfestations in grains and delayed ripening of climacteric fruits; Medium dose applications (1-10 kGy) for microbial decontamination and destruction of pathogenic microorganisms in different food commodities and high dose applications for sterilization of food products.

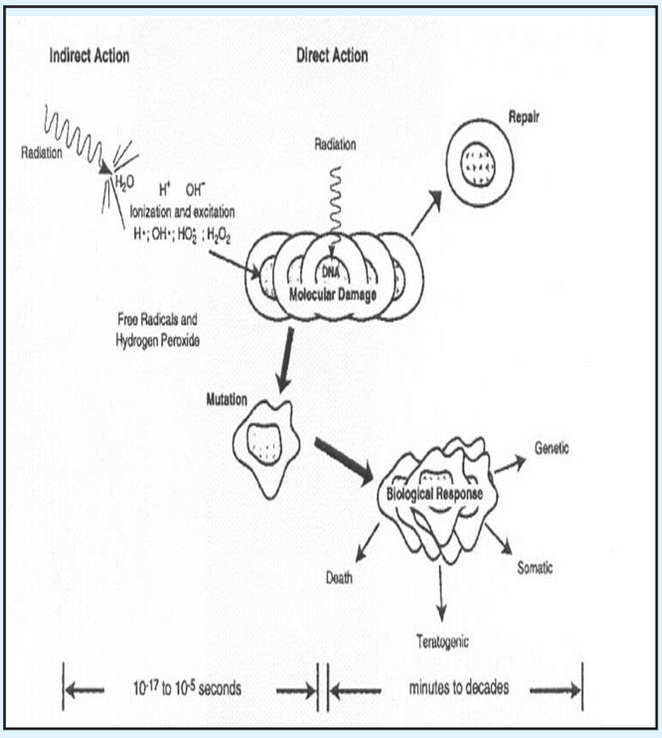

Fig. 1 Effect of ionizing radiation on living cell

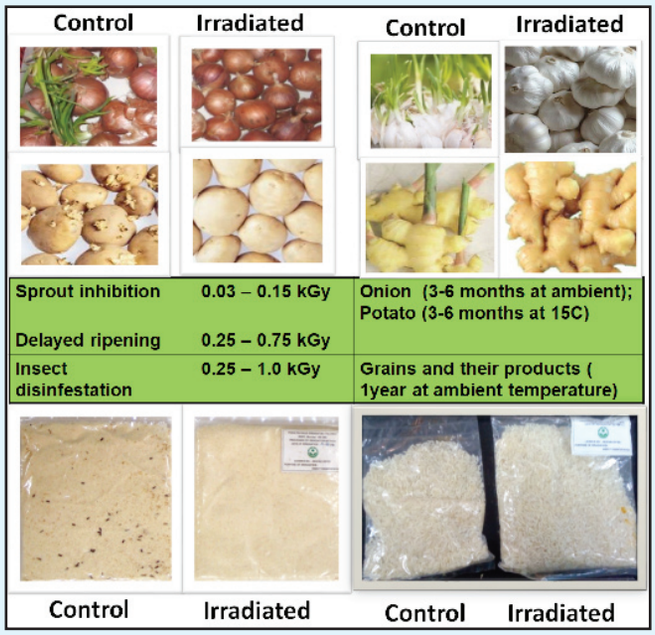

Fig. 2 Radiation processing of agricultural produce

Radiation processing is a single process but multiple applications (see, Fig. 3). Radiation processing of food is carried out in an irradiation chamber (see, Fig. 4) shielded by concrete walls of 1.5-1.8 m thickness. Food, either pre-packed or in bulk in suitable containers is sent into the irradiation chamber on an automatic conveyor that goes through a concrete wall labyrinth to prevent radiation from reaching the work area and operator room. The radiation sources Cobalt-60 (gamma energy 1.17 MeV, and 1.33 MeV) or Cesium-137 (gamma energy 0.66 MeV) are stored under 6 metre deep water when the facility is not in use. The water shield does not allow radiation to escape into the irradiation chamber, thus permitting free access for personnel to carry out plant maintenance. For the irradiation of food, the source is brought to the irradiation position above the water level after activation of all safety devices and restricting human entry. The goods in carriers or tote boxes are mechanically sent inside, positioned around the source rack and turned around their own axis, so that the contents are irradiated on both sides. The absorbed dose is determined by the residence time of the carrier or tote box in the irradiation position [9]. The absorbed dose is checked by placing the dosimeters at various positions in a tote box or carrier.

Like conventional processes, radiation processing has advantages and limitations. There are a number of additional advantages when it comes to radiation processing for sterilization of certain commodities. Gamma radiation has high penetrative power and hence

can penetrate deeper into tissues. No area of the product or packaging is left untreated. The materials can be treated in pre-packed conditions. Therefore, no chances of recontamination happen unless the packaging is opened. It is highly effective, non-residue forming and safe to workers & environments. Being a cold process, loss in volatile constituents is not significant [10].The primary limitations are that all commodities may not be amenable to the process, capital intensive, difficult to detect the treatment and reluctance by both the industry and consumer in adopting the technology. Irradiation produces little or no chemical changes in food. It maintains the wholesomeness of the food [11]. The physical properties of food are also not affected by irradiation. The majority of changes due to radiation processing of food are similar to those by other preservation methods like such as the thermal (heat) food processing. The radiolytic products and free radicals produced in the irradiated food are identical to those present in the foods processed by cooking and canning. None of these changes known to occur have been found to be harmful. The safety and wholesomeness of the technology was endorsed in the early nineties by the international statutory bodies including the World Health Organization, Food & Agricultural Organization, International Atomic Energy Agency, the Codex Alimentarius Commission, and the Food Safety and Standards Authority of India (FSSAI).

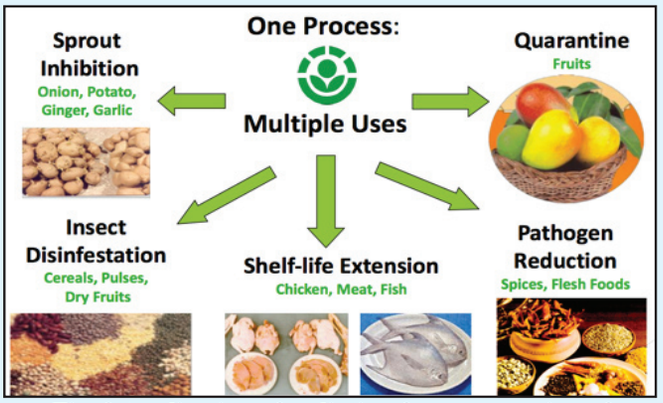

Fig. 3 Application of radiation for preservation of various foods

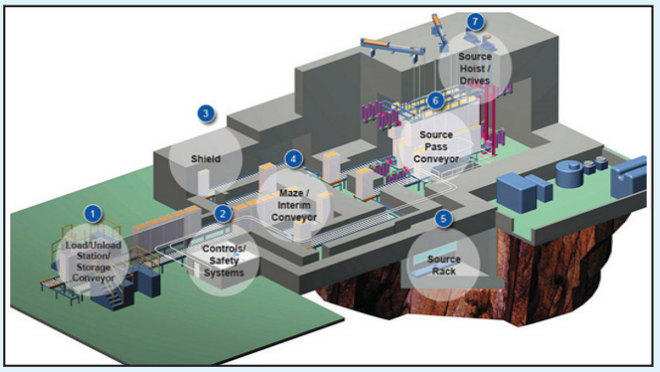

Fig. 4 A Schematic view of a radiation processing plant for food

Radiation processing does not make food radioactive as the energy of the radiations used are much lower than the energy needed for destabilizing atomic nuclei. Irradiated food never comes into direct contact with the radioactive material and there is no evidence to suggest that free radicals or radiolytic products affect safety of the radiation-processed food.

Though several packaging materials like glass, cellulose, metals and organic polymers are available for this purpose, plastics offer unique advantages over the conventionally used rigid containers. Multi-laminate packaging structure of polymers like nylon, polyvinyl chloride (PVC), cellophane, polyethylene and polyester are used as a prominent barrier material in packaging of irradiated food.

Radiation processing of food has been approved by various national and international organizations to ensure 'Food Security & Safety', and overcome 'Technical barrier to International Trade'. In countries, such as France, Belgium, The Netherlands, South Africa, USA, Thailand, Vietnam and China, the radiation-processed foods such as strawberries, mango, banana, shrimp, frog legs, chicken, spices, and fermented pork sausage are sold on regular basis on the market shelf. More than 60 countries are irradiating food for processing industries and institutional catering. These radiation-processed food items are labelled with the Radura logo to indicate the irradiation treatment and its purpose. In India currently 15 irradiation plants are functional which includes two plants set by Government of India and one each by Maharashtra government and Gujarat government. Volume of food irradiated in India has been steadily increasing. A total of more than 20,000 tons of produce is being irradiated annually in India. Irradiated mango is being exported to USA since 2007 [12]. Recently harmonization of food irradiation rules with international regulation has taken place in India through class wise clearance of irradiated food items by the Atomic Energy in 2012 and by FSSAI in 2016.

Conclusion and future outlook

Many novel non-thermal technologies are still in their early stages of development. Irradiation is the only non-thermal method that has granted the industrial status as about one million ton of various food products have been processed across the globe in 2015. The technology is safe and its use alone can guarantee the safety of

foods. Currently for setting up a commercial irradiation facility the estimated cost comes in the range of Rs. 15-20 crores excluding land cost. A 5-10% increase in cost is normally expected due to the radiation processing charges. But this additional expense is nothing compared to the benefits it provides. The costs could be brought down in a multipurpose facility treating a variety of products around the year. Processing brings benefits to the consumers in terms of availability, storage life, distribution, and improved hygiene of food. Radiation processing will be moving fast to the status of a 'wonder technology' to satisfy the sanitary and phytosanitary requirements of the importing countries [13]. Effective combinations of radiation processing with more non-thermal preservation hurdles has great potential for improving the safety and quality of foods, retaining the freshness although many technological and regulatory barriers still need to be overcome before the food supply can receive these benefits.

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Ensuring Food and Nutritional Security through Postharvest Processing

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Introduction

In the emerging scenario of the prospect of feeding an estimated population in excess of 9 billion by 2050 with diminishing natural resources and threatening climate change coupled with serious food safety issues associated therewith, a call for collaborative, trans disciplinary approach to the science of food security with a focus on enabling technologies within a context of social, market and global trends to achieve food and nutritional security is urgent, timely, and possibly existence of many life forms, including humans, too (Figure 1). These issues are compounded by the huge food wastage, extensive uses of plastics and engineered nanomaterials that finds their way along with other contaminants in to the marine environment which ultimately lands up in human body through the food chain.

Food wastage and nutritional security

The gap between food production and food availability is huge and varies with geographical regions around the world (Figure 2).

As per latest estimates by the Associated Chambers of Commerce of India, India loses approximately INR 926 bn (US$ 14.33 bn) on account of post harvest losses. It is worth noting that crops worth approximately US$ 19.4 mn is wasted in India on a daily basis only due to rejection at the farm gate and delays in the distribution process. A country-wide study measuring crop losses revealed that 3.9% - 6% cereals, 4.3%-6.1% pulses, 2.8%-10.1% oilseeds, 5.8%-18.1% fruits, and 6.9%-13% vegetables were lost during harvesting, post-harvest activities, handling and storage (1). Post-harvest losses

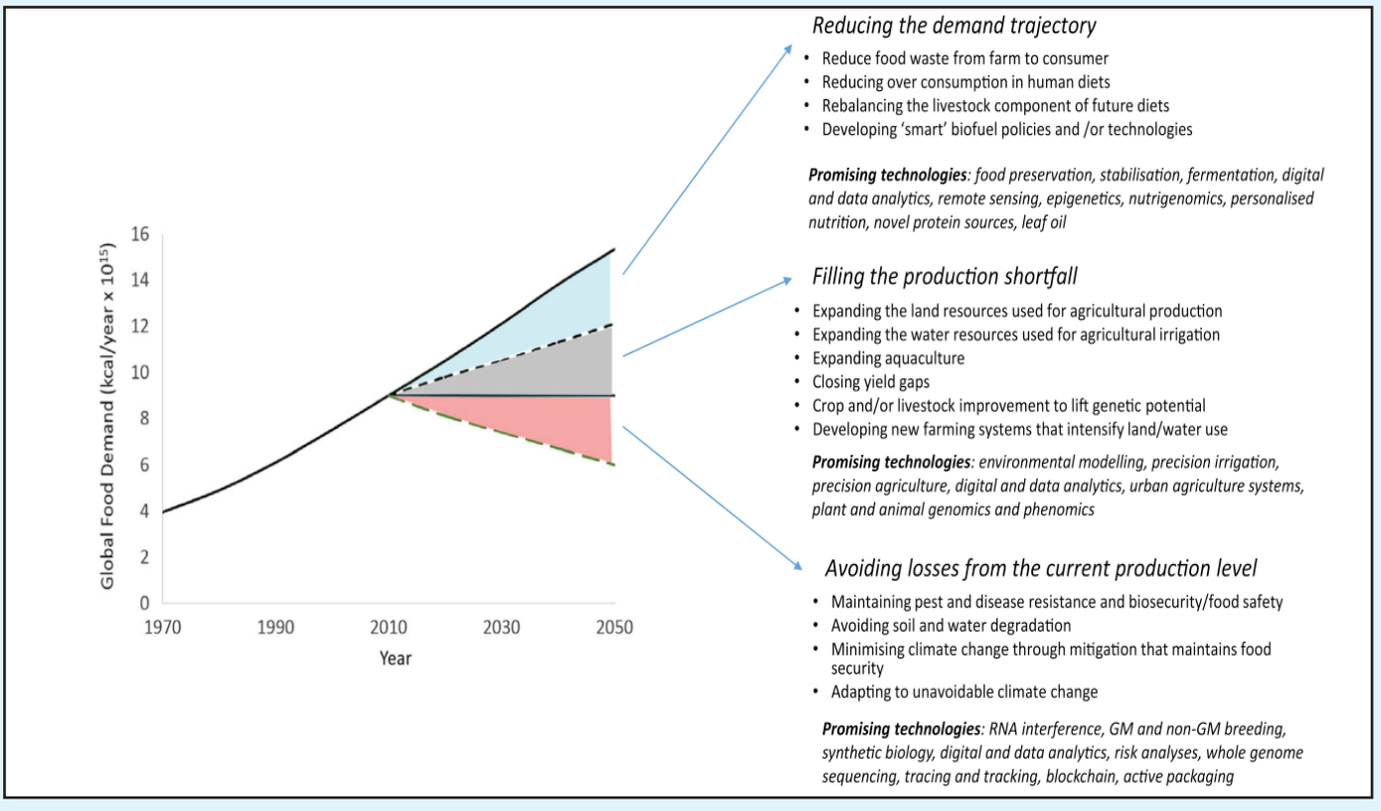

Figure 1: Food wedges framework linking food demand to likely stabilizations and promising technologies

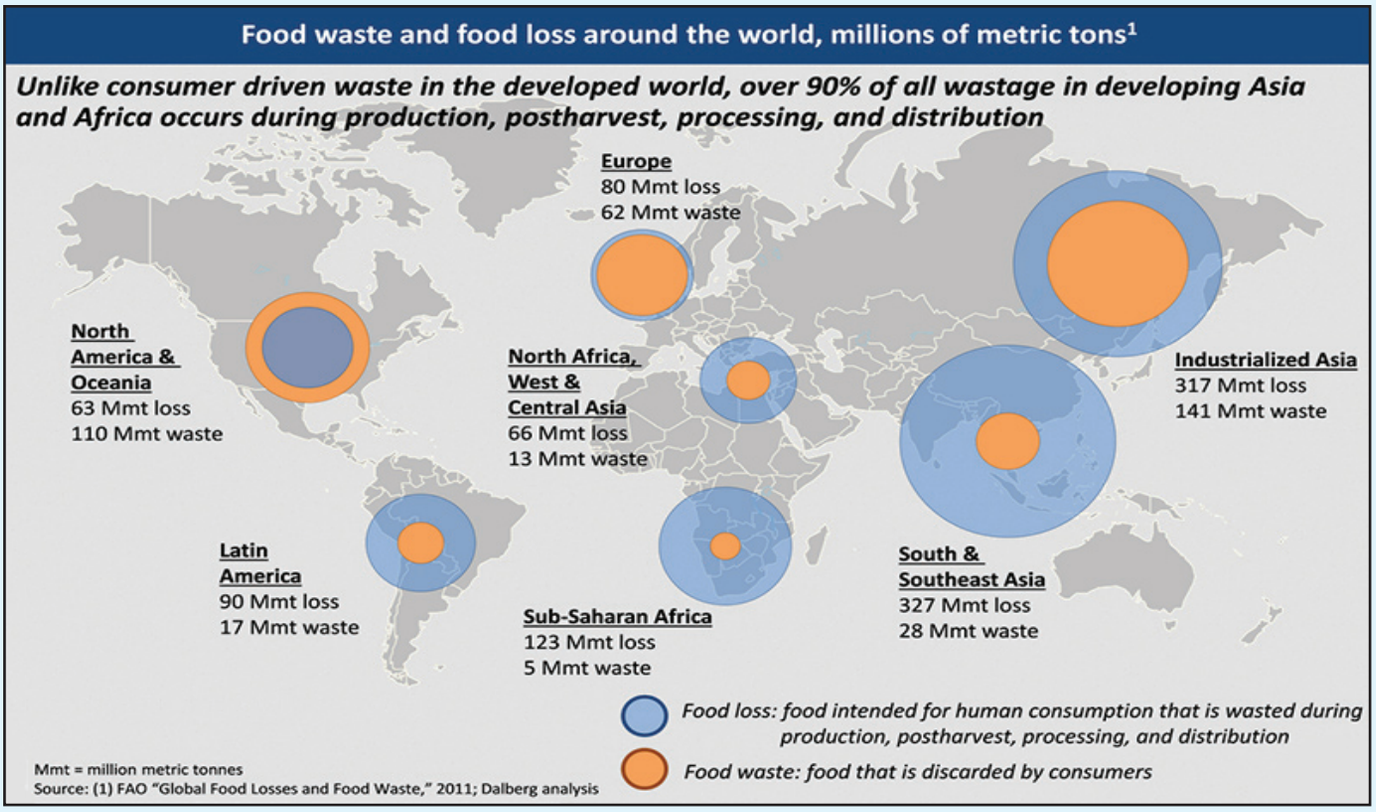

Figure 2: Food waste and food loss around the world, in millions of metric tonnes

in India stem from a range of factors (Figure 3) including lack of post-harvest infrastructure, limited technical know-how on good agricultural practices, imperfect market knowledge, and inadequate market access (1). The study estimated that an annual value of harvest and post-harvest losses of major agricultural produces at the national level was to the order of Rs 92,651 crore, calculated using production data of 2012-13 at 2014 wholesale prices.

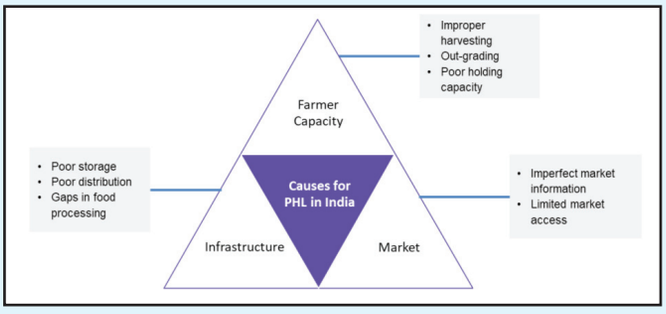

Figure 3: Causes of postharvest losses (PHL) in India

Food processing and nutritional security

However, reducing food losses throughout the supply chain from production to consumption and sustainable enhancements in preservation, nutrient content, safety and shelf life of foods, enabled by food processing will also be essential. Since prehistoric times, food processing has been a key aspect of the food production chain that links agricultural production with the provision of food to people in the form and at the time it is required. Some of the benefits of food processing include destruction of food-borne microbes and toxins, improved bioavailability of nutrients, extension of shelf life, improved sensory characteristics and functional properties (2). Food processing also encompasses the use of food additives and food packaging which are essential in achieving the objectives of food processing. Table 1 provides selected examples of the impacts of traditionally used common food processing operations.

These are essentially based on either application of heat (smoking/canning/drying/baking/frying/extrusion) or withdrawal of heat (cooling/freezing) both of which reduce the free moisture in food that causes spoilage due to microbial or enzymatic activity. Another approach where the water activity or free water is reduced is by use of intermediate moisture foods. Yet another approach is based on the use of microbes which generate compounds and conditions that prevent spoilage and extend shelf life with desirable attributes (the very concept of fermentation). Selected examples of processes for converting food materials into final products are summarized in Table 2.

Table 1 : Benefits and impacts of food processing operations

Technique	Examples	Outcomes & benefits	Impact
Preservation	• Pasteurization of milk or juice • Fermenting dairy into cheese or yogurt • Pickling or canning produce • Salting meats	• Distributors can ship products over greater distances • Retailers can stock products longer • Consumers can keep foods longer	• A range of local and non-local foods remain available over a longer time frame
Processing for food safety (cleaning, sterilization)	• Washing, pasteurizing, cooking, salting, drying, refrigerating, freezing	• Food-borne pathogens and contaminants are removed or minimized, meaning that consumers are at a lower risk of foodborne illness	• A greater proportion of the population has access to safe food
Processing to change flavour, texture, aroma, color or form	• Milling grains • Mixing ingredients • Adding flavors and colors • Molding foods and ingredients into shapes	• Manufacturers may gain higher profits and a foothold in a competitive market • Consumers have access to a wider variety of products	• Adds value to food products
Processing to reduce preparation times and make food more portable	• Ready-to-serve meals • Fast foods • Convenience foods: Bottled drinks, meat jerky, cakes, cookies, breakfast cereal bars, frozen pizzas, baby food	• Manufacturers may gain higher sales by responding to consumer demand for convenience food • Consumers can eat virtually anywhere, at any time, with minimal effort	• Access to safe (and preferably nutritious) foods for time-poor consumers
Processing to restore and/ or raise nutrient levels in food	• Fortifying milk with vitamin D, salt with iodine, and grains with B vitamins, iron and folic acid	• Manufacturers can use fortification as a selling point, potentially generating greater sales • Consumers are at lower risks for chronic nutrient deficiencies	• Adds value and nutrition density to food, can improve bioavailability and population health implemented as public health policies

Ref: Augustine et al., 2016

Table 2 : Examples of food products prepared with different processing methods

Materials	Processes	Processed food products
Beef, lamb, pork, poultry & fish	• Slaughtering, cutting up, boning	• Frozen, refrigerated in bulk or retail packs
	• Comminuting, fermentation, extrusion, drying	• Small goods such as salami, bologna, sausages, jerky, cured dried meat/fish products, surimi
	• Cooking, pasteurization, sterilization, high pressure processing	• Ready to eat meal, meal components, luncheon or canned meat/fish products
Grains, cereal & legumes with may need dairy and other ingredients	• Grinding, sifting, milling	• Flour, milled rice, oat bran/grain
	• Rolling, steaming, puffing, drying, extrusion, frying	• Breakfast cereal, crispy snack foods, meat analogues
	• Cooking, steaming, sterilization, baking, fermentation, kneading	• Baked goods e.g. cake, bread, ready to eat grains e.g. precooked rice, beer, wine other healthy grain beverages
Dairy products	• Pasteurization, sterilization, separation, homogenization, high pressure processing, pulse electric field	• Liquid whole cream, skim and flavored cold pasteurize, pasteurized and UHT milks, cream
	• Fermentation, agitation, shearing and mixing	• Yoghurt, cheese, butter, whipped cream
	• Evaporation, sterilization, drying, separation	• Evaporated milk, condensed milk, milk powder, whey protein concentrate, whey, protein isolate
Fruits and vegetables	• Crushing, maceration, vacuum concentration, pasteurization, UHT, high pressure processing, pulse electric field	• Various concentrates, juices and juice mixes
	• Fermentation, picking, drying	• Kimchi, jams, dried and other form of pickled or preserved fruits and vegetables
	• Freezing, sterilization	• Frozen and canned fruits and vegetables products
	• Minimally processed	• Fresh produced

Ref: Augustine et al., 2016

While the traditional methods continue to be used for food processing, there are quite a few emerging technologies which have been commercialized to varying extents. These include gamma irradiation, high pressure processing, UV irradiation, cool plasma, ultrasound processing, pulsed electric field among many others, each having their own mechanism of extending the shelf life of raw and processed foods.

Other possible solutions include advancements in food processing technologies, nanotechnology, innovative food formulations and the use of genomic approaches manifested in examples such as alternative protein sources, insect flour, nutrigenomics, 3D food printing, biomimicry, food engineering and merging technology.

Conclusion

Processed food contributes to both food security (ensuring that sufficient food is available) and nutrition security (ensuring that food quality meets human nutrient needs). There is a common misconception that processed foods in general are "less healthy" or "junk foods" However many processed foods can offer equal, or in some more rare cases greater nutritive value, for instance, better absorption of lycopene from stewed canned tomatoes vs. regular whole tomatoes. Processing enables addition of many important nutrients that are deficient in the diet of general population in a specific geographic location. Food safety has improved dramatically in the last few decades due to modern food processing. New packaging technologies, use of preservatives, and innovations in functional ingredients have allowed delicious foods to stay fresh from farm to fork!

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Creating novel genetic variability in sugarcane using induced mutations

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Abstract

As a major cash crop, sugarcane occupies a prominent position in Indian agriculture scenario. Despite its seminal place, limitations such as complex genome, narrow genetic base, poor fertility, susceptibility to biotic and abiotic stresses and long duration pose significant challenges for crop improvement programmes. Both conventional and biotechnological approaches have been exploited in improving crop productivity. However, in vitro culture combined with mutation induction offers special advantages for sugarcane improvement. At BARC, we have made significant progress in the area of radiation induced mutagenesis in vitro and established protocols for mutant development. A wide spectrum of induced mutants with agronomically desirable traits has been isolated for evaluation under field conditions. In addition, the mutants have also served as a genetic resource for biochemical, physiological and molecular investigations into abiotic stress responses and agronomic traits. This article presents an overview of the in vitro mutagenesis based system that has evolved as a simple, efficient method for use in sugarcane improvement.

Introduction

Sugarcane (*Saccharum* spp.) is a commercially important crop contributing to major global sugar production and as the raw material for sugar producing and allied industries. Globally, India occupies second place as the largest single producer of sugar next to Brazil**,** whereas it is the single largest sugar producing country in Asia. Most of the important sugarcane varieties are hybrid clones from crosses of *Saccharum officinarum* L., and *S. spontaneum* with a few genes incorporated from, *S. barberi* Jesw., *S. sinense* Roxb. and to a limited extent *S. robustum* Brandes (SBI 2014). Breeding efforts have contributed significantly to the development of several improved sugarcane varieties. However, the crop improvement efforts are often constrained by crop's narrow gene pool, higher polyploidy, complex genome, poor fertility, and the long breeding/selection cycle. Changing environmental conditions adversely affect crop productivity, necessitating the development of improved crop varieties with higher tolerance to biotic and abiotic stresses. Thus there exists an immediate requirement to identify and/or develop novel sugarcane germplasm with agronomic traits, stress tolerance and improved productivity (Fig. 1).

In the past few decades, mutation breeding has contributed significantly towards crop germplasm improvement and several mutant varieties have been developed through induced mutations (Jain 2005, Suprasanna et al. 2015). The significant impact of Mutation breeding on food production, quality enhancement and economics has been globally recognized (Ahloowalia et al. 2004). The International Atomic Energy Agency (FAO/IAEA),

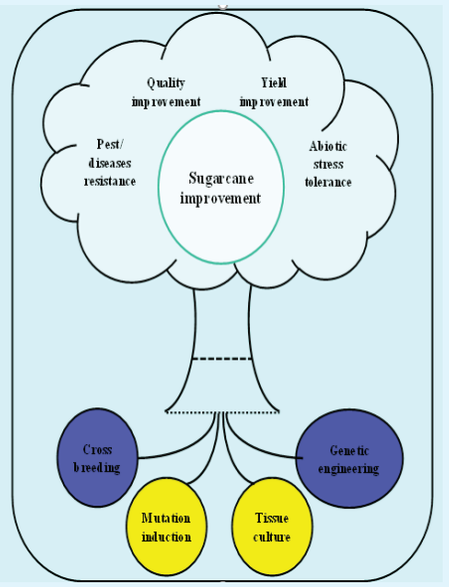

Fig. 1

Vienna, Austria, maintains a mutant variety database (MVD) with more than 3200 listed varieties and all the information on crop mutant varieties, mutagen used and improved traits in different plant species. Their data shows that mutagenesis in seed-propagated crops is more practised with a high proportion (77 %) of mutants as compared to 23 % in vegetative propagated crops (Suprasanna et al. 2015). As of now, thirteen mutants of sugarcane are listed on FAO/IAEA MVD by using gamma rays and *in vivo* vegetative propagates (Table 1).

In vitro mutagenesis

In vitro selection at cellular level is a useful tool for the isolation of mutants for desirable traits (Koch et al. 2012, Suprasanna et al. 2012). It is based on the premise that *in vitro* selection pressure is applied either through path toxins or agents related to pathological condition or by exposure with sodium chloride, polyethylene glycol, and manifold for selecting salt or drought tolerance (Suprasanna et al. 2012). The whole process of *in vitro* mutagenesis involves a series of important steps (Fig 2). Briefly it entails mutation induction by treating explants or *in vitro* cultures (protoplasts, cells, tissues and organs) with a mutagen, followed by screening/selection and characterisation of mutants.

For mutation induction, different plant propagules [seeds in the case of seed-propagated crops and plant parts such as stem cuttings, twigs, buds and tubers in vegetatively propagated plants] can be exposed to physical or chemical mutagenic agents. Physical mutagens like gamma rays, ion beams and chemical mutagens like ethyl methanesulfonate (EMS) and sodium azide can be used and successful protocols of mutagenic treatments have been developed for different crop plants (Mba et al. 2009, Suprasanna et al. 2010). In case of crop plants, *in vitro* mutagenesis and selection (IVMS) has been successfully applied for the improvement in agronomic traits like salinity and drought tolerance.

Table 1. Sugarcane mutants listed on FAO/IAEA MVD

Mutant variety	Parent variety	Year of release	Country	Target tissue	Mutagen used (dose)	Trait(s) improved
Co6608	Co449	1966	India	Vegetative propagules	Gamma rays (30-50 Gy)	Red rot resistance
Co997 mutant	Co997	1967	India	Vegetative propagules	Gamma rays (30-50 Gy)	Red rot resistance
Nanei	Ni 1	1981	Japan	Vegetative propagules	Chronic gamma rays (420 Gy)	Better tillering, longer and thicker cane, higher yield and sugar
Co8153	Co 6304 x Co 6806	1981	India	Seed	Gamma rays (150 Gy)	Improved juice quality and yield
Co 85017	Co 740	1985	India	Vegetative propagules	Gamma rays (150 Gy)	Resistance to *Ustilago scitaminea*, better cane yield and sucrose%
Co 85035	Co 740	1985	India	Vegetative propagules	Gamma rays (150 Gy)	Resistance to *Ustilago scitaminea*, better cane yield and sucrose%
Guifu 80-29	Guitang 72-28	1989	China	-	Gamma rays (80 Gy)	Late maturity, high sugar, small stem
CCe 10582	C 87-51	1990	Cuba	-	-	Improved cane yield
Yutangfu 83-5	Yuetang 71-210	1992	China	-	-	-
CCe 183	C 87-51	1993	Cuba	-	-	Resistance to eyespot
CCe 283	C 87-51	1993	Cuba	-	-	Resistance to eyespot
CCe 483	C 87-51	1993	Cuba	-	-	Resistance to eyespot
Guitang 22	Xintaitang 1	2005	China	Vegetative propagules	Gamma rays (80 Gy)	Higher sugar yield, higher tonnage

IVMS is of special significance for vegetatively propagated crops (VPCs) and several successful examples in different VPCs have demonstrated the combination of *in vitro* culture with selection as a relatively inexpensive, simple and efficient process for crop improvement. A large number of uniformly growing *in vitro* cultures can be generated through synchronization of growth cycle permitting efficient segregation of chimeras.

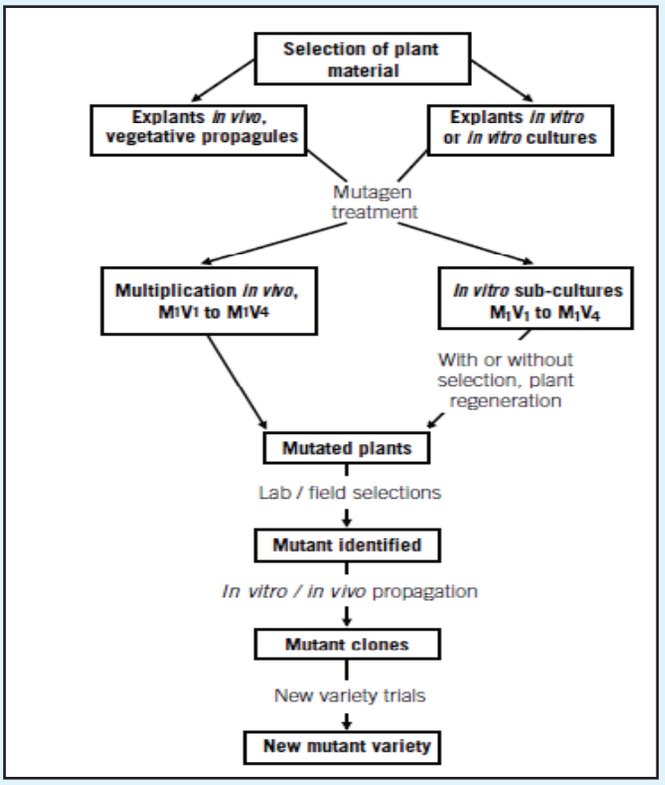

Fig 2. Process of in vitro mutagenesis in plants

Compared to mutagenesis using *in vivo* material, *in vitro* explants are better suited for mutagenesis and screening *in vitro* enables handling of large populations within a small laboratory space and time. (Suprasanna et al. 2010). With a long-duration vegetatively propagated crop like sugarcane as case in point, *in vitro* mutagenesis demands special notice. Conventional breeding programs are based on crossing between two parents with contrasting characters, trait segregation and phenotypic selection for desired character, followed by repeated backcrossing, ultimately yielding an improved variety. For long duration crops like sugarcane this procedure can take as much as 10-12 years. Further complications arise from inherent factors such as poor seed viability (half life is 35 min), limited flowering and parental incompatibility. Complex nature of apical meristems and propagating materials also pose a problem in vegetatively propagated plants; however, it is possible to overcome its effects with suitable handling of irradiated materials and to some extent by using the *in vitro* culture techniques (Suprasanna et al. 2010aWith the generation of useful mutants, characterization of causal mutations becomes the next point of consideration. Historically, molecular marker based on various genomic features, such as RAPD, SSR, AFLP SSCP etc. have been utilized for detection of genetic variation and mapping. The advances in sequencing platforms and the associated minute resolution has largely replaced these methods in crops with sequenced genomes, though these continue to be utilized in utilized in crops with poor genomic resources. While characterizing induced mutants, the type of genomic lesions should also be taken into consideration. For example transposable elements constitute sizable part of most crop genomes, and are known to be activated under stress, be it radiation or tissue culture (Negi et al. 2016). Thus, a genome wide assay of transposon movement, such as that offered by SSAP, IRAP or REMAP can be useful for crops with large sized genomes. Given that TEs are known to play functional roles in stress response and certain types (e.g. MITEs) tend to be clustered in genic regions (R gene clusters), TE based screening appears especially useful. In case of a close association with loci of interest, these markers can be utilized for large scale screening of natural variants, landraces and wild relatives, followed by map based cloning and introgression of these loci into elite cultivars.

Mutant development in sugarcane

At BARC, significant progress has been made to integrate tissue culture with radiation induced mutagenesis in the improvement of sugarcane (Suprasanna 2008, Patade and Suprasanna 2008) and a work-flow has been established (Fig. 3). In our studies with popular sugarcane cv. CoC-671, we have used *in vitro* mutagenesis in combination with cellular selection for salt tolerance in (Patade et al. 2008). Young-leaf roll derived embryogenic callus cultures were subjected to 0-50 Gy gamma irradiation and radiosensitivity was assessed. The cultures were then cellular selection with 0.5 to 2% NaCl followed by different physiological and biochemical analyses including cell viability, osmolytes like free proline and glycine betaine, electrolyte leakage, lipid peroxidation, sodium-potassium content and antioxidant defense in stressed and control (unstressed) callus cultures. Results showed that the evaluated attributes are crucial in alleviating salt stress effects and in improving salt tolerance in sugarcane. Subsequent to irradiation, low gamma ray doses (<20 Gy) maintained good

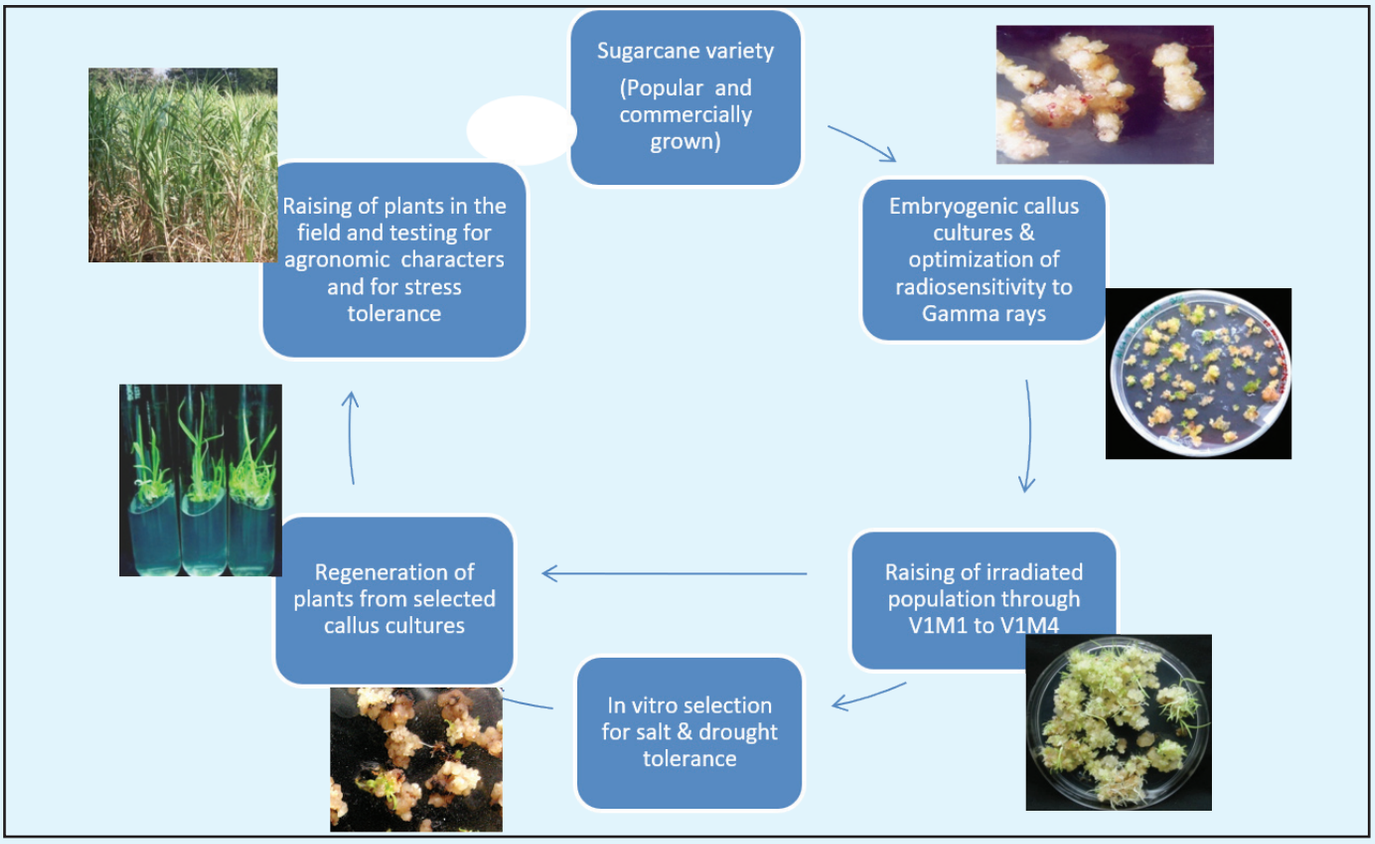

Fig. 3. Radiation mutagenesis followed by *in vitro* selection in sugarcane

regeneration response than doses >20 Gy. Doses beyond 30 Gy showed low frequency of plant regeneration. Lower regeneration response at higher radiation doses is attributed to the toxic effect of gamma radiation on cells / tissues and/or less competitiveness of these cells and their progenies. In this regard, we have used culture treatments to stimulate somatic embryo differentiation and plant regeneration response by subjecting high-dose irradiated embryogenic cultures to partial desiccation for 4-6 hrs (Suprasanna et al., 2008).

The above R & D activities in sugarcane have provided a focused support to the DAE-BRNS funded collaborative research projects on the application of radiation mutagenesis for the isolation of useful mutants in sugarcane which include, programmes at Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola and Vasantdada Sugar Institute (VSI), Pune. Under a collaborative project on mutant development in sugarcane was taken up (under DAE-BRNS) with Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola. IVMS studies were conducted with three sugarcane cultivars (Co 671, Co86032 and Co 94012) for generating gamma-ray irradiated plant population and more than 9000 plants were assessed at field level for different agronomic and quality traits (Suprasanna et al. 2010). The results have indicated a broad range of mutations for morphological, quality and yield contributing characters and that mutation spectrum. The spectrum of mutations was broader in the Co 94012 for morphological characters whereas, for quality and yield characters, cultivar Co 86032 exhibited wider range of mutations. Mutations observed were 0.16 to 0.48% for quality characters like high brix (0.04 %) and low Brix (0.008 %). Mutations for early maturity were observed in Co 86032 (0.17 %) and for mid late maturity in CoC 671 (0.16 %). Range of mutations obtained for cane yield attributing characters was 0.09 to 0.38 % at different doses of gamma rays and cultivars. In case of yield attributing characters, maximum mutations obtained for cane weight per plant (0.023 %) followed by cane diameter and cane height. More than 40 mutant clones (AKTS, AKola Trombay Sugar) were found promising for different desirable agronomic traits over respective checks for average cane weight and H.R. Brix.

The field assessment of stable sugarcane mutants showed significant changes in agronomic traits. The mutants AKTS-01 and AKTS-02 were found superior for total plant height, millable cane height and number

of internodes, & cane juice quality (brix%, sucrose%, CCS%) and cane yield attributes (cane yield and CCS yield) respectively. The juice sucrose % had significant positive correlation with NMC, brix % and purity %. AKTS-01 exhibited highest chlorophyll content across all the growth stages followed by AKTS-13. Reducing sugars were found significantly higher in the mutants, AKTS-16, AKTS-13 and AKTS-06 during 8th, 10th and 12th month of cane growth, respectively (Mirajkar et al. 2016). The studies imply that *in vitro* mutagenesis is an effective tool for the induction of genetic variability in sugarcane for agronomic and juice quality traits. Such induced mutants with superior attributes will be useful in building pre-breeding and high sucrose content genetic stocks.

Towards this endeavour of applying plant mutation breeding in sugarcane, the strategy of IVSM was continued under a collaborative research with Vasantdada Sugar Institute (VSI), Pune (Maharashtra). Embryogenic cultures of two high-yielding cultivars (Co86032 and Co 740) were irradiated with gamma rays (10, 20 and 30 Gy), selected *in vitro* with NaCl and the regenerated plantlets were transplanted to field after hardening. Co 86032, also referred to as 'wonder cane', is the most popular sugarcane cultivar in tropical sugarcane cultivating regions. The field selection of promising clones based on agronomic (quantitative and qualitative) characters resulted in eighteen mutant clones of Co 86032 and seventeen mutant clones of Co 740 (Nikam et al. 2014). Significant variation in phenotypic characters was observed in the selected mutants with respect to respective parents, while many showed improvement in one or more agronomic characters. One mutant, S-4209, was of special interest. Not only it showed comparable yield under control conditions, but a significant yield improvement under natural field salinity was also observed. After a 3-year field stabilization, seedlings derived from single eye bud sets were screened for salt tolerance by exposure to 50 and 200 mM NaCl for 15 days with or without recovery phase. This strategy permits assessment of both aspects of salt tolerance-continued growth and biomass accumulation under mild stress, as is generally observed under field conditions, and survival and resumption of growth under severe stress. The salt stress treatment to 6-leaf stage plants of 4209 showed significantly better photosynthetic- and water use efficiency (WUE), sodium exclusion and sustained cellular redox homeostasis relative to parent (Negi et al. 2017). This improved mutant trait was found to be stable under normal field and natural saline field (EC 3-8 dS/m). It can be speculated that these improved features result in a stablilized yield and quality related parameters under salinity. Detailed molecular characterization is currently in progress but preliminary results suggest stress inducible nature of this genetic changes, i.e. the changes in gene expression are activated under salinity, thus crossing out any potential negative effects on yield which may occur in case of constitutive expression. As mentioned before, the parent Co 86032 accounts for about 70% of total cultivation in the largest sugarcane cultivating zone. Therefore a mutant which maintains the agronomic attributes of this variety under normal field conditions and shows improved traits under salinity is highly suitable for adaptation by farmers in these regions. Thus, our results on IVSM strategy successfully demonstrated that the improvement of 'difficult to breed' long-duration, vegetatively propagated crops can be augmented by radiation mutagenesis. The study also puts forth successful implication of radiation energy for development of sustainable sugarcane agriculture through generation of inexhaustible and wealthy genetic resource.

Conclusions

In vitro mutagenesis in case of vegetatively propagated crops like sugarcane is a simple, feasible alternative towards crop improvement. Considerable progress has been made in mutant development, field evaluation, and characterization at physiological and biochemical levels in sugarcane. The techniques of induced mutagenesis have occupied an important place for the plant breeder in providing a 'rapid and relatively low-cost' approach to generate mutant resource, novel alleles and phenotypes. The advanced genomics tools are making the mutation discovery more feasible towards development of successful mutant varieties. For ex. techniques such as cDNA- AFLP, single strand conformational polymorphism (SSCP), serial analysis of gene expression (SAGE), microarray, differential display, TILLING, high-resolution melt (HRM) analysis, etc., are being used for rapid and in-depth global analysis of mutational events. The advent of Next-generation sequencing technologies can be used to generate information on mutation density and spectra. To sum up, the creation of new mutant germplasm will have to be continued to develop and screen for novel mutants for use in crop improvement.

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Mutation breeding for genetic improvement of mustard, wheat and sesame

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Introduction

Crop improvement is a key route to ensure continued benefits arises from food and plant products. However, seed yield in most of the crop pants has reached to stagnation. Wide range of genetic, biochemical and metabolic variation needs to be generated for effective crop improvement. Induced mutations provide a powerful means of creating new and useful variability in crop plants both in qualitative and quantitative characters. The technique can also be applied for rectifying specific defects of adapted varieties. Mutation breeding is an appropriate methodology to fulfill the present plant breeding needs for sustainable crop yield enhancement. It has been successfully employed to enhance the production and productivity of crop plants [1-5]. An overview on the modifications in morphological, biochemical and yield attributes through mutagenesis and their direct and indirect use to develop high yielding varieties in mustard, wheat, and sesame crops has been presented in this article.

Indian mustard (*Brassica juncea*):

Brassica juncea called as an Indian mustard is most important oilseeds crops in India and being cultivated in around 6mha with the productivity of 1100kg/ha. Demand of edible oil is ever increasing due to increasing population and their health consciousness. Therefore production, productivity and quality of oilseed crops need improvement. Besides, there is need to develop climate resilient varieties due to changing climate. Existing germplasm of Brassica crops has limitation of exploitable variability to develop high yielding varieties with biotic and abiotic stress tolerance with desirable oil and food quality. Mutation breeding methodology has better option to evolve desirable variability to achieve the target of enhanced seed and oil yields. We have isolated of large spectrum of variability for morphological, biochemical and yield and yield contributing characters though induced mutagenesis. These mutations are being evaluated at genetic, biochemical and molecular levels and used extensively in hybridisation to develop high yielding genotypes/varieties which has been briefly summarized here.

Dwarf: Plant height of presently released varieties is around 2-2.5m long. However, yield attributes mainly number of siliqua and seeds per siliqua are not proportionate to biomass of the plant and lot of energy is being wasted on biomass production. Dwarfing genes could be useful in *Brassic*a species to increase seed yield by reducing lodging and increasing harvest index through effective source-sink relationship. A gamma ray induced dwarf mutant was isolated from parent variety "Varuna'. Plant height of dwarf mutation is 85-90cm compared to 165-170cm of the parent and maturity reduced to 70 days which is half of parent variety. However, number of siliqua are 277 which is similar to parent variety (273). The seed yield per plant of the mutant is 14g/plat which similar to parent (13g/plant). This indicated that by reduction in the height, biomass could be fully exploited for effective sink. Besides dwarfness, additional three characters like early, yellow seed-coat and reduced erucic acid have been mutated in the same plant. It could be due to pleiotropic effect. This is the first report on isolation of dwarfing genes in *Brassica juncea*. This mutation has been extensively used in crossing programme and large number of early and high yielding genotypes has been developed. These genotypes are being evaluated for seed yield potential.

Yellow seed coat: In general, seeds of *Brassica* species are brown/black in colour. Yellow seeded rapeseed-mustard are more desirable than brown/black seed because it has thinner seed coat, higher oil content, high protein and lower fiber content than brown seeded varieties within same genetic background. It has improved nutritive value of the meal after oil extraction. Till late 1960s all *B. juncea* genotypes available in the germplasm collection had brown or black seed coat. Induced mutation to isolate yellow seed coat was initiated at BARC, Mumbai, India. Using ^{35}S radioisotope, two yellow seed coat mutants (*YSM1* and *YSM2*) were

isolated from blackish brown seed variety Rai5 but could not published. New yellow seeded mutant was isolated from the same variety Rai5 using ^{32}P radioisotope [6] and named as Trombay Mustard 1 (TM1). Using this mutant in cross breeding programme, improved high yielding genotypes were developed .The yellow seed coat mutants and their derivatives were extensively used in cross breeding programme throughout the India and large number of high yielding bold seeded genotypes has been developed [7] In recent years, we have also isolated yellow seed coat mutant (TM50, TJD1) from most popular variety 'Varuna' using gamma rays. These mutants are being used in cross breeding to develop high yielding varieties.

Non-Locular Siliquae: Keeping number of siliquae and seed weight constant**, i**ncreasing seed number per siliqua will directly help in increasing seed yield. In existing germplasm, siliquae has two locules with 13-17 seeds. Two mutations for non-locular siliquae/replumless mutation were isolated from stable Trombay mustard genotypes. Number of seeds/siliqua is in the range of 19-28 with enhanced siliqua density.

Light green leaf: Indian mustard has dark green leaf canopy in general. A light green leaf mutant was isolated using gamma rays. It segregates into three distinct phenotypic classes i.e. dark green, light green, and yellow leaf plants indicating single gene control with incomplete dominance. Fresh and dry matter was more in dark green leaf plant but seed yield was more in light green leaf plant indicating efficient source-sink relationship. This phenomenon could be exploited in heterosis breeding.

Disease resistance: TM1 which was reported as yellow seed coat mutation was also found resistant to powdery mildew disease under field condition during multilocation evaluation. It was also found club root resistant under endemic condition. *Alternaria* leaf spot is one of the major diseases of Brassicas but so far no reliable resistant source has been reported. We have isolated the dwarf mutation with yellow seed coat (TM277) which was found resistant to *Alternaria* leaf spot under field condition.

Abiotic stress tolerance: Weather aberrations are the major factors affecting the production and productivity of rapeseed-mustard in India. More than 50% cultivation of Indian mustard is carried out under the rain-fed farming system. High average surface soil temperature during the month of October imposes severe limitations on germination pattern, seedling establishment, and thus yield of Indian mustard. Loci associated with thermo-tolerance at seedling stage in oilseed Brassicas have not been identified yet. Large number of Trombay mustard high yielding genotypes derived through induced mutagenesis was evaluated for seedling stage thermo tolerance under field and controlled conditions (25°C and 42°C). Based upon yield and germination index we have identified TM106, TM108, TM134 and TM256 as high temperature tolerant genotypes at seedling stage. Further confirmation is being conducted at biochemical and transcriptomic levels.

Altered fatty acid composition: Edible oil is an important component of the human diet and provides energy. In vegetable oils, the major part of the fatty acids is represented by oleic acid (C18:1), linoleic acid (C18:2) and α linolenic acid (C18:3) along with other fatty acids like palmitic and stearic acids. Traditional rapeseed-mustard oil contains high proportion (~50%) of erucic acid (C22:1) and thus it is an exception to other vegetable oils. Mutation breeding has been successful in tailoring oil crops for desirable fatty acid composition [8] because oil crop plants tolerate wide range of fluctuation in fatty acid composition without losing viability and single mutation can result into desirable oil composition. Two mutations namely TPM1 and TJD1 have reduced erucic acid up to 25%. These mutations are being evaluated for genetic control of reduced erucic acid and development of zero erucic acid recombinants.

Development of high yielding genotypes/varieties: Utilization of induced mutations in Brassica crop improvement programme has resulted into the development of 31 high yielding varieties (12 in *B. juncea*, 14 in *B. napus*, 2 in *B. rapa*, and 3 in white mustard) worldwide. Fifteen varieties have been developed using gamma rays and 4 by X-rays. Remaining varieties were developed using chemical mutagen and mutants used in hybridization. In BARC, desirable mutations like dwarf, early, yellow seed coat, non-lolcular siliquae, bold seed size, long main fruiting axis, more primary and secondary branches, reduced erucic acid were extensively used in hybridisation and large number of high yielding genotypes has been developed. Three high yielding varieties namely TM2, TM4 and TPM1 are released for

cultivation. Among them TM2 and TPM 1 are direct mutants whereas TM4 is derivative of mutant used in hybridisation. TM4 and TPM1 are yellow seed coat varieties. In recent years, five genotypes namely TM204, TM172, TM106, TM43 and TM179 are in advance stage of yield evaluation/identification for release.

Wheat (*Triticum aestivum*)

Wheat is second important food crop of India. It is cultivated on 30.6 million hectares area with a production of 98.4 million tons in the year 2016-17. Wheat production in India showed consistent improvement after green revolution. However, stagnation of yield experienced in present breeding programs is due to maximal utilization of genetic variability through recombination and selection. This situation demands the creation of new genetic variability through induced mutagenesis. Continued development of agronomically superior wheat varieties, improved nutrition and processing quality, tolerance to biotic and abiotic stresses is needed to harvest stable yields for ensuring food security. Genetic variation is the foundation for improvement in any crop. Mutation breeding is an important tool to generate desired variability. The major objective of programme is to improve traits like rust resistance, drought tolerance, short stature, early maturity, herbicide tolerance and pre harvest sprouting tolerance. Specific traits and the mutant isolated are discussed here

Rust resistance: Stripe, leaf and stem rust are the major biotic stresses of wheat. The losses due to rusts can be very large and vary from year to year and region to region. Therefore, the hunt for new resistance genes continues against incessantly evolving rust pathogen races. Mutation breeding programme to isolate resistant mutants against rust diseases has been started in variety HD2967. Screening of M_2 population in stripe rust epiphytotic conditions resulted in isolation of two stripe rust immune and seven resistant mutants. These mutants are being evaluated for yield and resistance to other rusts.

Drought tolerance: Drought stress is a major constraint to harvest stable yields in all wheat growing zones of India. Wheat genotypes capable of giving higher yield under moisture stress condition are considered desirable. An attempt has been made to isolate drought tolerant mutants in M_2 population of HD2967. The population was evaluated under moisture stress conditions. Eight drought tolerant mutants were confirmed in M_3 generation based on yield and yield contributing traits over parent HD2967. These mutants will be further confirmed for physiological and biochemical traits.

Dwarf: Plant height is an important feature determining the lodging tolerance, fertilizer responsiveness and harvest index of wheat. Green revolution genes, *Rht-B1b* and *Rht-D1b* known for height reduction and were found in more than 95% of released wheat cultivars. Studies were carried out to identify new dwarfing genes in bread wheat to exploit in breeding programs [9]. A mutant genes conferring short height in tetraploid wheat were characterized [10]. However the initiative to isolate dwarf mutants in *Triticum aestivum* cultivars is lacking. A series of dwarf mutants with height ranging from 65 to 85cm as compared to 95cm height of parent HD2967 have been isolated. These mutants will be characterized for the modifications in dwarfing locus and evaluated for seed yield.

Early maturity: Early maturity is desirable trait in the areas where the wheat crop is affected by terminal heat stress. Flowering in wheat is regulated by three set of genes i.e. photoperiod genes (*Ppd*), vernalization genes (*Vrn*) and earliness per se genes (*eps*). These genes are present in three copies on chromosomes of three genomes of wheat. Early mutants were identified and confirmed in M_3 generation of two cultivars, PBW677 & HD2967. These early mutants will be utilized for genetics and molecular studies to decipher the nucleotide alterations responsible for earliness.

Herbicide tolerance: Glyphosate is an active ingredient in widely used brand name "Round up ready" and many other brands of herbicides. This herbicide controls broad spectrum of unwanted plant species in crop fields. The mode of action of glyphosate is to inhibit aromatic amino acid biosynthesis pathway with 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase as its primary target [11]. The consequence of inhibiting EPSP is to impede biosynthesis of aromatic amino acids. Initial studies were carried out to isolate mutants tolerant to Glyphosate in wheat. A gamma ray induced putative herbicide tolerant mutants were isolated in M_2 generation and will be confirmed in subsequent generations.

Pre-harvest sprouting (PHS) tolerance: Climate change is an inevitable risk to wheat yield and quality and calls for development of climate smart cultivars. Unpredictable rains during maturity experience total loss of yield and end use quality due to sprouting of wheat in the spike. PHS is a complex trait and largely influenced by environmental conditions. Putative mutants for PHS have been isolated from parent varieties WSN109-4 and HD2967. Their validation shall be confirmed in subsequent generations.

Seasame (*Sesamum indicum*)

Sesamum indicum L. commonly known as sesame is one of the very ancient crops in India. It is grown in all the major states of India and is known by various names such as til, gingelly, simsim, gergelim, rasi, etc. Sesame oil is highly prized for its quality, nutritive value and pharmaceutical use. The oil contains about eighty percent of monounsaturated fatty acid composed of mainly oleic acid and linoleic acid. Sesame oil has two antioxidants namely sesamin and sesamol. These are present in the unsaponifiable fraction of the oil. They are responsible for high stability at room to frying temperature.

The breeding objectives in sesame are increasing yield, resistance to disease and pest and indehiscence of capsule, uniform maturity or determinate habit, better harvest index, shorter duration, potential in response to higher inputs, higher oil and protein content, more sesamin and sesamoline production, drought tolerance and less photosensitive. Induced mutation could contribute to the creation of required character that are not available in the germplasm of sesame. Mutation breeding in sesame was started in BARC in early 80s [12]. Twenty true breeding morphological mutants using gamma rays. Three mutants are submitted to National Bureau of Plant Genetic Resources (NBPGR), New Delhi. These are polypetalous corolla mutant (INGR no-7030), stiff stem mutant (INGR no-2018), and long seedling mutant (INGR NO-7029).

In the recent mutation breeding experiments carried out in two popular varieties namely Phule Til (PT-1) & Roma using gamma ray and sodium azide (SA), high mutation frequency was observed in combined dose of gamma rays and SA. Wide range of variation was observed for morphological characters. Chlorophyll mutations consisting of albino, xantha, chlorina were isolated. Morphological mutation includes tricotyledonary seeds, monostem, fascinated stem, extremely dwarf, drooping type, wrinkled leaf, long epicotyls. Among the prominent mutations, extreme dwarf has only 18cm plant height compared to 122cm of the parent and is being reported first time. Fasciations mutation looks like fusion of 2-3 plants and possesses large number of pods up to 100. Long epicotyls mutation has double length of epicotyls than parent. All these mutations have novelty and are being characterised at genetic, biochemical and molecular levels. Development of white seed coat with bold seed size (4gm/1000seed) genotypes has demand in confectionery industries as well as in export. A total of 20 high yielding Trombay Bold Sesame (TBS) genotypes have been developed through mutation breeding and using mutations in cross breeding. These genotypes shall be tested in yield trials at ICAR and SAU.

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Induced mutation for genetic enhancement of the plant beneficial fungi *Trichoderma* species

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Intoduction

Trichoderma spp. are filamentous fungi commonly occurring in soil, on tree bark, on wild mushrooms and, as endophytes. These fungi are widely used in agriculture as biofungicides and in industry as source of plant biomass degrading enzymes (Mukherjee et al. 2013). *Trichoderma* spp. can kill other fungi through the production of fungal cell-wall lysing enzymes like chitinases, glucanases and proteases, and are called mycoparasites. These fungi also produce a plethora of bioactive secondary metabolites that are antimicrobial (and some are also anti-cancer) (Mukherjee et al. 2012). Trichoderma spp. can colonize roots of higher plants and induce a systemic defense response against invading pathogens (Mukherjee et al. 2013). Several hundred *Trichoderma*-based formulations are available in India and elsewhere for agricultural use. In BARC, we have developed *Trichoderma* formulations for application as a biofungicide, and also for degradation of plant biomass. Here is a brief account of approaches taken for genetic improvement of these fungi using induced mutagenesis as a viable tool.

Strain improvement for enhanced enzyme production

Trichoderma spp. can actively degrade plant biomass and are useful as source of enzymes like cellulases and hemicellulases, and also for cleaning-up of plant biomass wastes like crop residues. *Trichoderma reesei* is the most important industrial source of cellulases. Mandels et al. (1971) used a linear accelerator to mutagenise *T. reesei* QM6a and isolated a mutant with two-fold cellulase secretion.

The same strain was subjected to a serial mutagenesis, both physical and chemical (combination of UV and nitrosoguanidine) which resulted in isolation of a mutant RUT C30 that has several fold improvement in cellulase biosynthesis and protein secretion, and till date, this is the major industrial source of cellulases (Montenecourt and Eveleigh 1977). Bailey and Nevalainen (1981) used gamma-irradiation to obtain stable cellulase over-producing mutants of *T. reesei*. Li et al. (2010) used microwave and ultraviolet radiation to enhance cellulase production of *Trichoderma viride*. Recently, electron and (12)C(6+)-ion beams have been used to improve cellulase production in *T. viride* (Li et al. 2016).

Strain improvement for enhanced biocontrol properties

Several biocontrol strains of *Trichoderma* spp. have been subjected to induced mutagenesis in order to enhance biocontrol properties. The main issue being a suitable selectable marker for antagonism/biocontrol, and hence, most selections have been indirect. Papavizas and Lewis (1981, 1983) used UV-ray mediated mutagenesis to improve *Trichoderma* spp. The selection was for tolerance to a fungicide (carbendazim) that is highly inhibitory to *Trichoderma*. Interestingly, the mutants differed from the wild type strains in appearance, growth habit, survival in soil, antibiosis and disease control potential. Ahmad and Baker (1988) used nitrosoguanidine to mutagenise *Trichoderma* spp. The mutants selected on benomyl (a fungicide) were efficient colonizers of rhizosphere, and hence had improved biocontrol potential. Using a two-step mutagenesis involving UV-radiation and gamma-radiation, we obtained stable benomyl-tolerant phenotypic mutants of *T. pseudokoningii* (later identified as *T. harzianum*). The mutants differed from wild type in antibiosis and a few were superior to wild type in biocontrol potential (Mukherjee et al. 1999). Using UV-radiation, Szekeres et al. (2007) obtained mutants of *T. harzianum* with improved chitinolytic activities and had significantly higher biocontrol index. Benomyl resistant mutants with increased mycoparasitic activity was reported by Olejnikova et al. (2010). Using gamma-ray, we obtained a mutant of *Trichoderma virens* that differs from wild type in colony morphology, is a hyper-producer of secondary metabolites including the antimicrobial viridin, and is having higher disease control potential.

Strain improvement for enhanced stress tolerance

Being living microbes, *Trichoderma* spp. when applied in agricultural settings are subjected to biotic and abiotic stresses. Enhancing tolerance to stress like fungicides, metals, extreme pH, temperature, salinity, etc., can be achieved by induced mutagenesis. Troutman and Matejka (1978) used gamma-ray induced mutagenesis to obtain benomyl-tolerant mutants of *T. viride*. Idea of generating fungicide- tolerant mutants is to combine fungicide and *Trichoderma* in the context of integrated disease management. Benomyl-tolerant mutants of *T. viride* were generated by chemical mutagenesis for controlling Botrytis gray mould of chickpea (Mukherjee et al. 1997). Jayaraj and Radhakrishnan (2003) developed UV-induced carbendazim resistant mutants of *T. harzianum* and used these mutants for control of *Rhizoctonia solani* in cotton. Carbendazim-resistant mutants of *T. asperellum* were generated and evaluated in combination of half the recommended dose of the fungicide for management of dry root rot of chickpea; the integrated treatment significantly improved disease control (Ramanagouda et al. 2016). Mohamedi and Haggag (2006) reported induction of two stable salinity-tolerant mutants of *T. harzianum* for control of tomato wilt. Kredics et al. (2001) obtained metal-tolerant mutants by UV-mutagenesis. More efforts are needed to obtain mutants of *Trichoderma* spp. for inducing tolerance to other stresses like high pH, low and high temperature and salinity stress.

Muta-genomic approach for identification of novel genes and gene functions

Mutants, both "loss-of-function" and "gain-of-function", are invaluable tools for genetic studies. Combining classical mutations with high throughput omics' studies is a powerful tool for discovery of novel genes and gene functions. *T. reesei* cellulase hyper-producing mutant RutC30 was sequenced using massively parallel DNA sequencing and the assembled genome sequence was compared with that of the wild type strain QM6a (Le Crom et al. 2009). This study led to the identification of 223 SNPs, 15 small indels and 18 large deletions totaling about 100 kb. Among the deletions was the carbon catabolite repressor gene *creA*, resulting in constitutive expression of many hydrolytic enzymes. In our laboratory, we have studied one mutant of *T. virens* that does not sporulate or produce hydrolytic enzymes, and is downregulated in secondary metabolism. We used the SSH tool to identify genes that are not expressed in the mutant, and this study led to the discovery of the "vir" cluster responsible for biosynthesis of volatile sesquiterpenes. This happens to be the first gene cluster having been discovered in any *Trichoderma* sp. (Mukherjee et al. 2007).

Conclusion

Strain improvement for biocontrol and industrial/ environmental applications of *Trichoderma* spp. hold great promise, but most often it has been directed towards obtaining fungicide tolerant mutants, with a few classical examples on improvement of hydrolytic enzyme production. With several fungicides being phased-out, and hardly any new molecule being introduced, future agriculture will depend heavily on biological pesticides, and in the climate change scenario, microbial pesticides having tolerance to abiotic stresses with improved biocontrol potential will be required. Creating novel strains using genetic engineering is a possibility, but chances are remote that these GMOs will clear regulatory hurdle and be allowed to be applied in field. Mutation will thus play a bigger role to create superior strains of *Trichoderma* and other microbial pesticides for wider acceptance as alternative to chemicals.

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Dr. Prasun K. Mukherjee, Scientific Officer H, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, joined BARC on completion of his Ph.D. (Plant Pathology) degree from GB Pant University of Agriculture and Technology, Pantnagar, under a Dr. KS Krishnan DAE Research Fellowship. Dr. Mukherjee has been University topper throughout his career and is also all-India topper, Agricultural Research Service (Plant Pathology- 1991). He is an elected fellow of National Academy of Sciences, India, and National Academy of Agricultural Sciences (FNASc and FNAAS). He has been a Visiting Scientist at Israel Institute of Technology and a Fulbright Visiting Scholar at Texas A&M University. He is a recipient of Homi Bhabha Science and Technology Award, DAE Group Achievement Award (twice, as Group Leader) and VASVIK award. An internationally acclaimed *Trichoderma* biologist, he has published 75 research papers/reviews, some in high impact journals like Nature Reviews Microbiology, Annual Review of Phytopathology and Genome Biology, and he is a highly cited author. He has edited two books published by Springer and CABI (UK), and is an Editor of three international journals from Elsevier and Springer. In addition, three of his technologies have been transferred to several companies, reflecting a perfect blend of basic and applied research that Dr. Mukherjee has been successfully pursuing.

An Overview on Super Absorbent Polymer and its Application in Agriculture

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Abstract

Super Absorbent Polymer (SAP) materials are a class of synthetic macro molecular network that can absorb and retain large amount of water. Application of SAP hydrogel in the soil is preliminary deemed as an inexpensive mode of water restoration for extended period of time that acts like a mini water reservoir. These hydrogels are sometime also referred as magic water crystals that supply the reserved water to the plants and allow their unprecedented growth under arid conditions. Over the past one decade research on SAP materials and advancement in their preparation have been reported for augmenting the health of soil and controlled delivery of plant nutrients. The application of SAP hydrogels is an established water conservation technique in agriculture and is widely used in different parts of the world. Although, the SAP kingdom is focused towards its hygiene application (>80% of its total production), the water scarcity, growing population and increased demand for food in developing nations are the key factors driving demand of fastest-growing SAP market for agriculture application, which can be broadly categorized into gardening, horticulture, floriculture, forestry, landscaping, and golf-course. This article will briefly summarize the background, advancement and future prospect of SAP hydrogel including the safety, environmental issues and challenges for agriculture application.

Keywords: *Super absorbent polymer, soil health, controlled irrigation, agriculture*

1. Super Absorbent Polymer (SAP) Hydrogels:

The Origin and History

Since time immemorial, water absorbing materials have been used ubiquitously by mankind. Majority of these materials are of natural origin with distinct nature of water absorption and retention capacity. In the early day's cellulose or fibber based materials were used as the water absorbing materials. The most viable example of absorbing material is a sea sponge that was used for hygiene application dates back to the ancient Greece [1, 2]. However, the water retention capacity of these materials is poor being only approximately 20-30 times of their weight which includes tissue paper, cotton, sponge, and fluff pulp [3-7]. After the invention of polyester in 1941 and polyurethane foam in 1952, spongy absorbing materials were manufactured synthetically [8, 9]. Since then, applications of these absorbing materials have evolved as per the requirement of society. Understanding the behaviour of polymer precursors depending upon the nature of accessible functionality and possible amelioration in its native structure allows their fabrication in to a highly hydrophilic or hydrophobic absorbing material. In recent years, we have witnessed a dramatic growth in the development and industrial production of absorbing materials in multiple formats with wide applicability that vary from decorative gels to multi-functionalized oil-sorbers.

The first highly absorbing polymer material named 'Super Slurper' was introduced by the United States Department of Agriculture (USDA) in the early 1960s and it was proclaimed that it could improve water retention in soils for agricultural use [10]. Super-slurper resin was produced by the grafting of acrylonitrile polymer onto

the backbone of starch which showed water absorption greater than 400 times its dry weight. The retention of water remains intact in the polymer network of gel as do fibber based absorbers. Due to the substantial potential of Super-slurper, The USDA gave permission to several USA companies for its further development [11-15]. Since then wide range of grafting combinations with different co-monomers such as acrylic acid (AA), polyvinyl alcohol (PVA), polyethylene oxide (PEO) and natural fibers like starch and cellulose have been used to manufacture highly water absorbing polymer materials, which have been termed as 'superabsorbent polymers' (SAPs) [16-28]. The advancements in the SAPs using derivatized acrylic acid replaced former superabsorbent materials and it became the primary material used in SAP products. In general, SAPs are partially crosslinked three dimensional polymer networks that enlarge when they come in contact with water and are having the ability to absorb water up to several hundred times of their own dry weight.

In 1966, Bashaw and Harper demonstrated the first practical application of crosslinked potassium polyacrylate as a water immobilizing agent in fire-fighting [29]. Later, in 1968, Harper and co-workers claimed in their patent the use of crosslinked polyacrylate in diapers [30]. In another patent, Harper and co-workers [31] and Harmon [32] also claimed the use of similar type of materials in medical and personal care products in the US. However, the first commercial production of SAPs material for feminine napkins was initiated by Japan in 1978 [33], and witnessed was a great success. Thereafter, its application in baby diapers was first introduced by European countries in 1980. In the beginning, small amount of SAP (1-2 gm) in diapers made them fluffy and bulky, however, later Japanese manufacturer fabricated thinner diapers using higher SAPs amount i.e. ~4-5 grams for better comfort [34]. This idea succeeded very well and resulted in the growth of superabsorbent polymer industry producing about 414,000 metric tons per year in the initial phase [35]. Subsequently, applications of SAPs have grown in various fields.

2. Chemistry behind Superabsorbent Polymers

Many theories and models have been developed to explain the mechanism of the water retention process [36-39]. Considering poly(acrylic acid) as a model system of SAP, it structure contains ionizable carboxylic acid groups on each repeating unit (Fig. 1), due to this polymer backbone of the SAPs is hydrophilic.

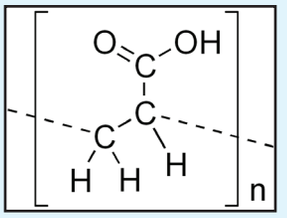

Figure 1. Repeating units of poly(acrylic acid)

Therefore in the presence of water, polymer and solvent interactions are developed by hydration and the formation of hydrogen bonds. Figure 2 exemplify inside of a SAP network. The backbone is generally crosslinked with hydrophilic crosslinkers to avoid complete dissolution.

The overall neutrality in the SAP particle is developed by balancing the negative carboxylic groups by positive counter ions. Thereafter, the counter ions hydrate rapidly during SAP to water interaction. Thus, these hydrated counter ions increase the osmotic pressure within the polymer network. A weak attraction of counter ions to the carboxylic groups does not allow counter ions to move into the surrounding solution from the polymer network. Due to this, osmotic imbalance generated inside SAP is balanced by diffusion of more water molecules inside the polymer. Notably, ionic solutions may lower the osmotic pressure difference which cause reduced swelling of SAP. The repulsion of charged groups inside the polymer is another factor that also helps to expand the polymer and contribute to the swelling capacity. Poly(acrylic acid) polymer proves to exhibit the best performance versus cost ratio.

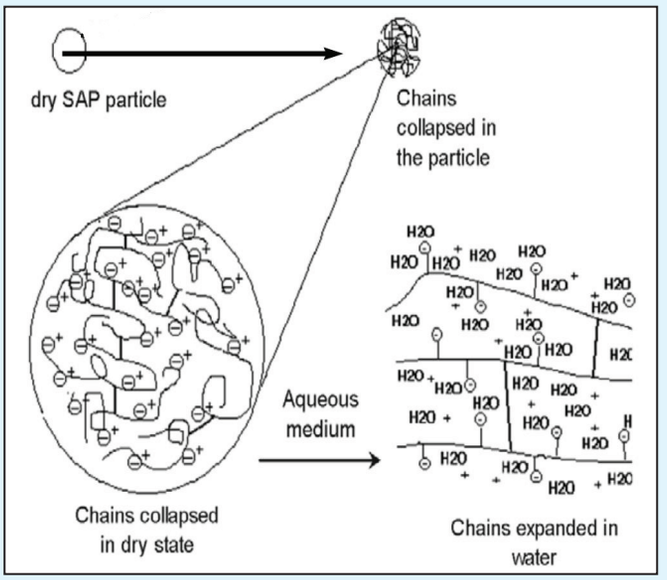

Figure 2. Swelling of SAPs (*reproduced from Zohuriaan-Mehr et. al., Irani. Polym. J. 17, 2008, 451*)

3. Synthesis of superabsorbent polymers

Superabsorbent polymers are commonly made from the polymerization of acrylic acid in the presence of an initiator to form a poly-acrylic acid. At present, this polymer is the most commonly used for SAP synthesis globally. Other materials used to make a superabsorbent polymer are polyvinyl alcohol, polyethylene oxide, grafted starch, carboxymethylcellulose, ethlyene maleic anhydride, polyacrylonitrile, and polyacrylamide to name a few. The synthetic monomer grafted starch is one of the oldest SAP forms, which was one of the most popular commercial SAPs productions for the first few decades. However, this type of SAP was gradually discontinued in the late 1990's and particularly all commercial SAPs are now made without starch. Today superabsorbent polymers are made using three primary methods: (1) suspension polymerization, (2) solution polymerization or (3) gel polymerization, by radiation or chemical crosslinking. Each process has certain advantages over the others and there are trade-offs between them. All yield a consistent quality of product.

(A) Suspension Polymerization: Suspension polymerization requires a higher degree of production control and product engineering during the polymerization step, due to this only a few companies are using this technique for SAP production. This process suspends the water-based monomers in a hydrocarbon-based solvent like cyclo-hexane. The net result of this process is to generate primary polymer particle in the reactor rather than mechanically in post-reaction stages. Crosslinkers can be added during or just after the reaction stage for enhancing the performance. This process allows the synthesis of true spherical particles with large intercalated void volume that affords fast fluid uptake and even the ability to handle more viscous liquids in certain cases.

(B) Solution polymerization: This is a commonly used process today for co-polymer SAP production particularly using acrylamide monomer. This process is efficient and generally requires a lower capital cost. A water based monomer solution is used to produce polymerized gel, wherein the polymerization's own reaction energy (exothermic) is used, thus, reducing the manufacturing cost. The obtained bulk amount of polymer gel is then chopped, dried and ground to a particular size of granules. Any treatments to enhance performance characteristics of the SAPs are usually accomplished after the final granule size is created.

(C) Gel Polymerization: In a water solvent system, a mixture of glacial acrylic acid (GAA) with crosslinker and UV initiator are blended and placed either on a moving belt. Passing of this mixture through a series of strong UV lights chamber causes the polymerization and crosslinking reactions. The resulting sticky gel containing 60-70% water is further shredded and placed in the dryers for obtaining granular/powder of SAP. Sometimes, additional surface cross-linking is performed for increasing the ability of SAP to swell under load (a property knows as 'absorbency under load' or absorbency against pressure). This method is currently the most popular approach for the synthesis of sodium polyacrylate SAPs used in baby diapers and other disposable hygienic products. The SAP materials are often synthesized by polymerization of monomers and/or grafting of monomers on polymer chain. Polymerization and grafting involves a free-radical process that can be initiated using chemical, photo-chemical or radiation procedures. Use of ionization radiations for grafting/tethering of chemical moieties is commonly referred as 'radiation-grafting'. Interaction of ionizing radiation to the material causes the liberation of electron from an atom or a molecule due to the kinetic energy of photons present in ionizing radiations e.g. X-rays, gamma radiation, beta and alpha particles or machine accelerated particles. Recently, synthesis of superabsorbent polymer materials using radiation procedure have gained much attention due to several advantages like low-temperature operation, high purity, high efficiency, simple process and being environmental friendly. This process is a promising technology having several potential applications. Studies around the globe have reported radiation-graft synthesis of SAP materials for several applications.

4. Applications of Superabsorbent Polymers

Superabsorbent polymer materials has been explored for various diverse applications in personal care, health care, agriculture/ horticulture, automotive, and other fields, which are classified in Table 1.

Table 1: Major application areas of superabsorbent polymer materials

Personal Care	Healthcare	Agriculture	Others
Adult Incontinence Products, Sanitary Napkins, Baby Diapers, Nappy Pads, Urinary Bag	Wound Dressing, Medical Waste Solidification, Super absorbent Mat	Seed Coating, Root Dipping, Soil Broadcasting, Flower beds, Ornamental gardens	Automotive, Construction, Packaging, Entertainment and Industrial Water

5. Markets of Superabsorbent Polymers

A recent market survey has estimated the global market for super absorbent polymer as USD 7.47 billion in 2017 with major market in Europe and North America (Fig. 3) and is expected to rise at a compound annual growth rate (CAGR) of 6.2% during the forecast period up to 2025 [40]. The SAP kingdom is its hygiene application which consumes more than 80% of its total production. Another recent report published by 'Research and Markets' suggested agriculture drives demand for SAPs will grow at a CAGR of 5.8%, and global market may reach up to the value of 10 billion USD up to the forecast period up to 2022. It is assumed that Asia Pacific region will register a significant growth in next five years due to high demand from developing economies such as India and China.

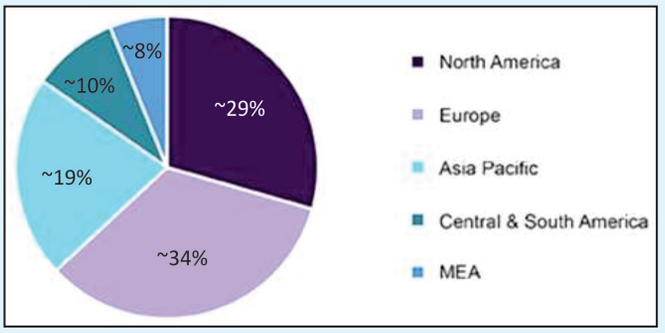

Figure 3. Global superabsorbent market by region in 2017 (*MEA=Middle East & Africa*)

Baby diapers are experiencing upswing in the demand on account of rising disposable income, growing population, and increasing awareness regarding baby health. Other countries such as Indonesia, Vietnam, and Singapore are also projected to exhibit increase in demand for adult diapers and female hygiene products, thereby triggering the growth of the regional market. Due to the high global market demand of SAPs, companies are taking strong measures (like multiple branding, network strengthening, fervent outreach marketing, and superior quality product development) to gain a foothold in emerging economies by increasing their market penetration. Some of the key companies are listed below which are actively engaged in R&D and manufacturing of high quality superabsorbent polymer materials;

1. BASF SE,
2. Nippon Shokubai Co., Ltd.,
3. Formosa Plastics Corporation,
4. LG Chemical Ltd.,
5. Sumitomo Seika Chemicals Co., Ltd.,
6. SDP Global Co., Ltd.,
7. Songwon Industrial Co., Ltd.,
8. KAO Corporation,
9. Evonik Industries AG,
10. Sanyo Chemical Industries

6. Agriculture Application: Mode of Action

Drought is the largest abiotic stress that obstructs the growth of plant and its productivity. The severity and frequency of drought leads to change in climate affecting agriculture practices. Beside arid conditions, the three most common soil conditions reducing agriculture productivity are 1) low water holding capacity, 2) high evaporation and transpiration, and 3) leaching of soil moisture. However, factors like reprehensible irrigation approaches, soil salinity majorly in coastal regions, low or overuse of synthetic fertilizers and pesticides also rigorously affect the growth of plants. Sometimes these factors even lead to permanent damage to the soil-biota. Moreover, a shortage of water availability for routine irrigation is also an issue for many countries. Therefore, the optimization of the use of water resources is tactical for the long-term competitiveness in the agriculture practices. Indeed, the shortage of water has become a serious problem especially in the areas those are facing progressive desertification. Management of water use is anticipated to be one of the prime challenges in the future for agriculture industry. A study suggested that the requirement of water is supposed to become 50% higher by 2030 [41].

Development of innovative agriculture practices is apparently an important need that requires promoting of the technologies for optimum use and exploitation of water resources to guaranteed delivery of appropriate amount of water to the plants timely and effectively. Besides this, one of the ways is the change in soil management that can affect the quantity of deep drainage replenishing ground and sub-surface water. The introduction of good soil management practices will decrease the proportion of rainfall that is lost as runoff, which will increase base flows and decrease peak flows in soil and thus reduce the incidence of flooding as well. Conversely, an improvement of nutrient management in soil will lead to higher grain and foliage production.

The use superabsorbent polymer (SAP) is one of most attractive approaches of nourishing the soil and its uses may vary depending upon region, environment and crops. SAPs are prepared using monomeric /polymeric

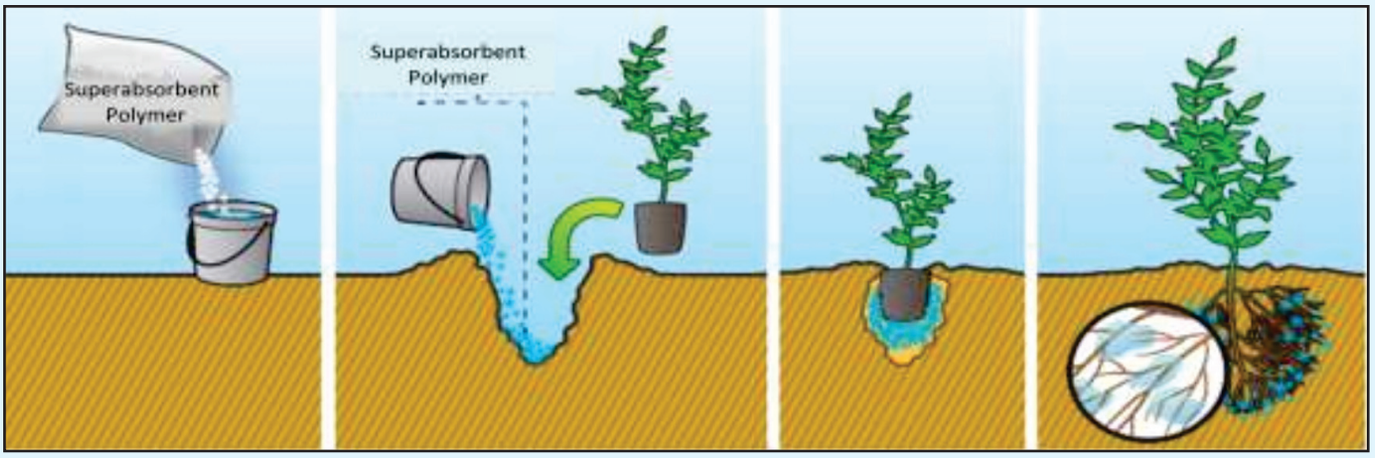

Figure 4: Application of superabsorbent polymer material for water conservation in soil for augmenting plant growth (*adopted from: www.aprotekgroup.com/case-study/*)

precursor units and are considered as soil conditioners that have the capacity to absorb and hold 300 to 500 times of water of their initial mass in the soil system. SAPs form granules upon swelling in soil to enhance soil properties by retention of more water and improved aeration for plants (Fig. 4). Due to their quick and high water retention capacity, SAPs are currently used in various agriculture and horticulture applications that include soil broadcasting, seed coating, root dipping, flower bedding, ornamental gardening, etc.).

The SAPs used in agriculture applications are usually synthesized from acrylic acid monomers and polyacrylamide co-polymer. The crosslinked polymer i.e. polyacrylate is non-toxic, non-irritating and non-corrosive in nature. It is tested to be biodegradable with an annual rate of degradation 10-15% and Absorption Capacity Index (ACI) in the scope of 30-100. However, some natural polymers like polysaccharides and polypeptides are also being used for various SAP formulations. The demand of SAPs prepared from natural precursors is increasing in global SAP market because they are the least expensive and most abundant available renewable organic materials.

The application of SAP is a well established water conservation approach in agriculture. It is widely used in different parts of the world for fulfilling the increased demand of food to growing population, which is the key factor driving the demand for the SAP market in agriculture application. Various advancements are monitored in the preparation of SAP formulations in last few decades, which involve co-polymerized composites for optimum stability and absorbency under load (AUL), control release of plant growth promoting factors, and also bio-based antifungal and antimicrobial SAP preparations. Due to their nature, SAPs can be known as mini-reservoirs of water and nutrients in soil for on-demand supply to plants to produce grain and increase biomass under limited nutrient and irrigation conditions. Their water storage capacity can last from months to years depending upon their nature of degradation. They help and improve the rate of seed germination, root growth during drought stress and also reduce transplanting stresses in plants. Authors have developed an indigenous gamma-radiation based process of SAP synthesis using novel composition and are studying its application for augmenting the plant growth by applying SAP to the soil (Fig. 5). In addition, SAP application to soil also acts as an insulating material to plant roots to reduce stress during frosty winter and can also reduce the use of fertilizers by 15 to 30 %. The agricultural industry is the fastest-growing market for super absorbent formulations. The superabsorbent polymers are presenting tremendous growth and attracting scientists and global market leaders for its advancement, low-cost production and potential utilization in agriculture to fulfil the societal requirements due to deteriorating climate conditions and global warming.

In 1990s, a Japanese company demonstrated the use of SAP in the Egyptian desert to conserve the water for agriculture application [42]. Also, Woodhouse and Jonson have shown the application of synthetic SAP as a soil conditioner in drought-prone plant growing media [43]. Most widely and efficiently used SAPs consist of polyacrylate polymer, which shows approximately 10% yearly degradation due to the mechanical and photosensitive chain scission and delamination of polyacrylate network [44]. There is also some concern

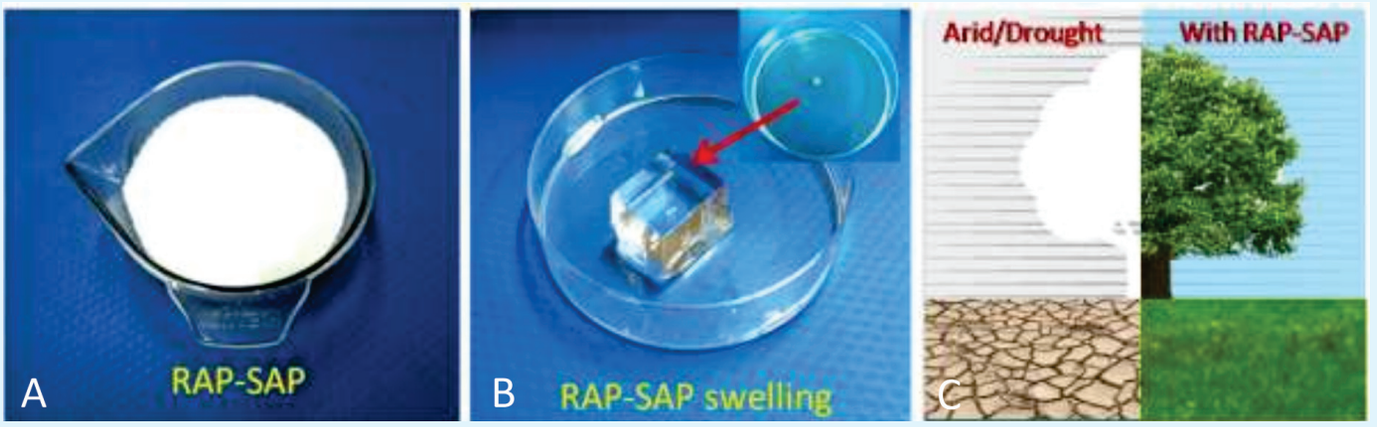

Figure 5. Radiation polymerized superabsorbent polymeric material developed at BARC-Mumbai (A), that swells >400 times of its initial dry mass (B), and reserves water during drought (C) (Author's unpublished data*)**

regarding the slow rate of degradation of polyacrylate which is anticipated to be a pollutant to the soil, therefore, several new polyacrylate bio-composites have been developed to address this environmental concern [45].

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Radiation based induced mutagenesis in Trombay groundnuts: Developments and accomplishments

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed, food and feed crop grown in India with an area of 5 million hectares with a total production of 7 million tons. With this, India contributes to 18.8% of the world's groundnut area and 15.7% of the world's groundnut production. Groundnut occupies nearly 18.7% of the cultivated area and contributes 24.2% of the production of the total oilseeds in our country. Recently, India has exported more than 5 lakh tons worth of Rs. 3300 crores of groundnut to several countries. It is widely used as principal source of cooking oil, digestible protein, minerals and vitamins in many countries. Its kernels are consumed directly after roasting, boiling or frying, used in confections or crushed for edible oil. Being a high energy food, groundnut seeds contain 48-50% edible oil, 25-34% protein, 10-20% carbohydrates and rich source of vitamins E, K and B complex, phosphorous, potassium, calcium and magnesium. About 50% of India's groundnut production is crushed for oil and the rest was used for sowing, consumption and export.

Induced mutagenesis and cross breeding in groundnut

Genetic variability is the most important requirement for success in plant breeding. Ionizing radiations and chemical mutagens enhance the mutation frequency. In most of the groundnut mutation experiments, the objectives were to develop high yielding varieties with early maturity, large seed, improved oil quality, high shelling percentage, moderate seed dormancy and tolerance to biotic and abiotic stresses. The radiation source used for groundnut breeding in the early years was X-rays, later gamma rays and recently electron beam was also employed. The effective dose of 200-350 Gy gamma rays was close to lethal dose $(LD)_{50}$ depending on the factors influencing radio-sensitivity at the time of mutagen treatment. Materials used for mutagen treatment are seeds of cultivar, mutant, selection, hybrids or advanced lines. The induced mutants are utilized directly or in cross breeding.

Trombay groundnut varieties

Mutation research in groundnut at the BARC had evolved several morphologically distinct mutants, which formed an initial gene pool for developing groundnut varieties. Sustained research using mutation and cross breeding resulted in the release of 15 Trombay groundnut (TG) varieties for commercial cultivation across the country. TG 1 was the first induced large seed mutant variety developed by X-ray irradiation of Spanish Improved variety and released in India [1]. Another direct mutant, TG 3 was released for Kerala and became popular in Odisha. Subsequently, TG 17 was released for Maharashtra. TG 17 along with TG 1 resulted in large seed variety TKG 19A for Maharashtra. Under the genomic backgrounds of Robut 33-1 and M 13, mutant and mutant derivatives of Spanish Improved generated two varieties, TG 22 and Somnath and were released for Bihar and Gujarat states, respectively. Genomic blend of four mutants of Spanish Improved under the background of M 13, TAG 24, TG 39, TLG 45 and RARS-T-1 (Bheema) were developed. TAG 24 was released for Maharashtra, Karnataka and West Bengal. TG 39 was released for Karnataka and Rajasthan, TLG 45 for Maharashtra and RARS-T-1 for Andhra Pradesh. Similarly, four mutants of Spanish Improved and one mutant of JL 24 with M 13 background resulted TG 26. TG 26 was released for Gujarat, Maharashtra and Madhya Pradesh. The genomic constitution of TG 26 was diversified with natural mutant Gujarat dwarf to evolve TG 37A; with variety Girnar 1 to evolve TG 38 and with genetic stock, Chico to evolve TG 51. Similarly, TPG 41 was resulted from four mutants of Spanish Improved and one mutant of JL 24 under M 13 and Robut 33-1 backgrounds.

In BARC, notable contribution has been in the development of early maturing confectionery grade

large seed (100-seed weight >60g) varieties (TG 39, TPG 41, TLG 45, RARS-T-1) which are suitable for export and table purpose. These varieties have benefited many farmers, traders and exporters. Semi dwarf habit, high harvest index and better partitioning in TAG 24 and TG 26 help to increase plant population per unit area and permit pegs to enter the soil early to have better uniformly developed pods. Fresh seed dormancy in TKG 19A, TG 22, TG 26 and TPG 41 prevents *in situ* seed germination due to end season rains when the crop is ready for harvest. This trait is very useful under current changing climatic conditions wherein unpredictable rains are often experienced. Early maturity in TAG 24 and TG 51 is helpful to escape end-season drought and to fit into different cropping systems. Drought tolerance in TAG 24 and TG 37A make them suitable for rainfed situations. High oleic acid (60%) in TG 39 and TPG 41 impart better oil shelf life and health benefits. Among these varieties, TG 39, TG 51 and RARS-T-1 were recently released.

TG 39

TG 39 is a confectionary large seed groundnut variety released as TBG 39 (Trombay Bikaner Groundnut 39) for arid and semi-arid regions of Rajasthan in collaboration with Rajasthan Agricultural University, Bikaner for rainy season [2]. It is also released as TDG 39 (Trombay Dharwad Groundnut 39) for northern transitional Zone 8 and northern dry Zone 2 and 3 of Karnataka in collaboration with University of Agricultural Sciences, Dharwad. It is a Virginia bunch variety developed by crossing TAG 24 and TG 19. In the evaluation trials in Rajasthan for five years, TG 39 had given a mean pod yield of 3,154 kg/ha with a superiority of 19.5% and 15.8% over check varieties, M 13 and TKG 19A, respectively. In Karnataka, TG 39 showed superiority of 12.8 to 19.7% for pod yields. It has an average 100-seed weight of 66g and maturity of 115-120 days. It has moisture stress tolerance making it suitable for rainfed situations. TG 39 is characterized by erect growth habit with semi-dwarf height, alternate flowering and dark green leaves. Seeds are rose in color and contain 49.9% oil, 26.5% protein, 12.6% carbohydrate and 4.5% sucrose. The oil contains 59.0% oleic and 23.0% linoleic fatty acid with an oleic/linoleic ratio of 2.56. Recently, TG 39 has become very popular in Gujarat and has yielded upto 5700kg/ha on farmer's fields in 100-105 days. This variety has also preferred for boiled nut consumption.

TG 51

TG 51 is released for post-rainy season (October-May) in Odisha, Bihar, and West Bengal and North-eastern states under residual moisture situation [3]. It is also being cultivated in large areas in Gujarat. It is a Spanish bunch variety derived by hybridizing TG 26 and Chico. In the All India Coordinated Research Project – Groundnut Trials conducted by Indian Council of Agricultural Research (ICAR) during post-rainy season, TG 51 recorded a mean pod yield of 2,675 kg/ha with a superiority of 12.0% and 16.3% over check varieties TAG 24 and ICGS 44, respectively. It has maturity of 90-95 days which makes farmers to harvest early and bring the produce early to the market in turn fetching the better price. This variety is also preferred for the export under Java type of groundnuts. TG 51 has an erect growth habit with sequential branching, semi-dwarf height and medium-size green leaflets. Its pods are mainly two seeded but invariably each plant contains one to two three seeded pods. Pods are with slight beak, moderate constriction and slight reticulation with average shelling out turn of 72-75%. Seeds are more spheroidal with rose colour with average size of 50-55g/100seeds. Seeds contain 49% oil with 42.9% oleic, 36.5% linoleic and 13.0% palmitic acid.

RARS-T-1

TG 47 is a Spanish bunch large seed variety released as Bheema and notified as RARS-T-1 for commercial cultivation for early rainy and post-rainy season under irrigated conditions in all agro-climatic zones of Andhra Pradesh in 2011. It is an improvement over TKG 19A with respect to higher productivity, larger seed size and greater proportion of large seeds. It is developed by crossing TAG 24 and TG 19. Its plant is erect, semi-dwarf with sequential flowering. TG 47 recorded 30% and 40% superior seed and pod yields over best check variety in evaluation trials at Tirupati during rainy and post-rainy trials. It also yielded 4000 kg/ha pods with 45% superiority over local variety in the farm trials. It matures in 115-120 days with an average 100-seed weight of 70g and 70% shelling out turn.

Trombay groundnut varieties as source of parents

Groundnut mutant/mutant derivatives developed at this centre also contributed as a parental material in the release of eight varieties by the other agricultural

universities in the country. Towards this, University of Agricultural Sciences, Dharwad has released Dh 40 from the cross Dh 3-30 X TGE 2 and GPBD 5 from the cross TG 49 X GPBD 4 for Karnataka. Similarly, University of Agricultural Sciences, Raichur has released R 9251 from the cross BARCG 1 X TG 23 for Northern Karnataka. Acharya N.G. Ranga Agricultural University, Jagatial and Tirupati have released an early maturing variety JCG 88 from the cross J 11 X TGE 1 and drought tolerant variety TPT 25 from the cross K 134 X TAG 24, respectively for Andhra Pradesh. Mahatma Phule Krishi Vidyapeeth, Jalgaon has released an early maturing variety JL 501 by the selection from TAG 24 for Gujarat and south Rajasthan. Junagadh Agricultural University has developed GG 21 by hybridizing Somnath and NCAc 2232 for Saurashtra region of Gujarat. Recently, Rajasthan Agriculture Research Institute, Durgapur has released RG 559-3 from the cross (TKG 19A X Kadiri 3) X TKG 19A for Punjab, Rajasthan and Uttar Pradesh. Besides, >300 groundnut induced mutants and breeding lines were developed and maintained at the BARC.

New Trombay groundnut breeding lines

Groundnut improvement being a continuous process, new breeding lines with high oleic acid, high protein, large seed or late leaf spot (LLS) disease resistance were developed at the BARC using mutation and recombination breeding. These studies involved gamma ray irradiation of seeds of TG 38, TG 51, TG 66 and electron beam mutagenesis of TG 26, TG 68 and recombination breeding of TG varieties with CO 3, Mutant 28-2, R 9227 and SG 99. Large seed groundnuts have greater consumer preference and export potential. Towards which, new high yielding mutants (TG 72, 73, 78, 79 and 89) and breeding line (TG 76) with large seed (70-80g/100 seeds) and 110-115 days maturity were developed. For late leaf spot resistance, two breeding lines (TG 71, 75) and 8 advanced selections with disease score of 3-5 were evolved as compared to score 9 in susceptible check. Further, consistent breeding efforts have developed 10 lines (TG 74, TG 80 to TG 88) with greater pod yield, 100-105 days maturity and ideal plant type. Of these, for the first time, 5 lines (TG 81, 82, 83, 87 and 89) were evolved with electron beam mutagenesis. A high seed protein mutant TGM 206 (31%) with 19% increment was identified from gamma ray mutagenesis of TG 66 (26%). Further, conarachin fraction was selectively increased in this mutant [4]. Groundnut seeds with high oleic acid have shown greater shelf life and their consumption has many benefits to human health. Earlier two mutants were isolated with 70% oleic acid. Further, by hybridizing these mutants with TPG 41 and TG 51, 8 high yielding advanced selections with 70-75% oleic acid were identified. Some of these lines and mutants are being evaluated in the national and state university trials.

Molecular studies in Trombay groundnuts

Conventional breeding has played an important role in delivering improved cultivars to farmers. However, integration of marker assisted breeding (MAB) with conventional breeding is expected to enhance the efficiency of developing new varieties, but MAB requires development, identification and validation of markers which are strongly associated with traits of interest. Towards development of molecular markers, a mapping population was developed from a cross between rust disease resistant parent VG 9514 and rust susceptible parent TAG 24. Rust resistance was governed by single dominant gene in this cross. Using this population, RAPD marker J7 was linked to rust resistance gene completely in repulsion phase and at 18.5cM in coupling phase [5]. Later, flanking SSR markers (pPGPseq4A05, gi56931710) for the rust resistance gene at map distances of 4.7 cM and 4.3 cM, respectively were identified in linkage group 2 [6]. Further EST-SSR markers SSR_GO340445 and SSR_HO115759 were found closely linked to a rust resistance gene at 1.9 and 3.8 cM distances, respectively in groundnut [7]. Transposable element (TE) markers offer an advantage by virtue of their simplicity, inexpensiveness and codominance nature. TE 360 and TE 498 were found associated with rust resistance gene [8]. A fine mapping approach towards the development of closely linked markers for rust resistance gene was undertaken in groundnut. Phenotyping of an RIL population at five environments for field rust score and subsequent quantitative trait loci (QTL) analysis has identified a 1.25 cM map interval that harbored a consensus major Rust_QTL in A03 chromosome [9]. This Rust_QTL is flanked by two SSR markers: FRS72 and SSR_GO340445. This 1.25 cM map interval contained 331.7 kb in the physical map of *A. duranensis* and had a TIR–NB–LRR category R gene and four glucan endo-1,3 β glucosidase genes.

Groundnut bruchid (*Caryedon serratus*) is a major storage insect pest that significantly lowers the quality and market acceptance of the produce. Screening for

resistance against groundnut bruchid in field conditions is difficult due to the variation in environmental factors and possible occurrence of biotypes. Hence, identification of tightly linked markers or QTLs is needed for selection and pyramiding of resistance genes. In groundnut, QTL analysis detected 13 main QTLs for four components of bruchid resistance in nine linkage groups and 31 epistatic QTLs for total developmental period [10].

Groundnut contains bioactive compounds like phenolics and flavonoids which determine its antioxidant activity. The groundnut recombinant inbred population contained 1.65 mg GAE/g and 240 lg CE/g of phenolics and flavonoids, respectively in its seed [11]. The DPPH radical scavenging activity of this population ranged from 1.27 to 4.40 mM of TEAC/g of seed with an average of 2.87 mM of TEAC/g and had significant positive correlation with both phenolics and flavonoids content. Genetic mapping and QTL analysis revealed five QTLs for total flavonoid content, four QTLs for DPPH radical scavenging activity and a single QTL for total phenolic content in six linkage groups. Of these 10 QTLs, six were positioned in linkage group A02 and A03 wherein rust and late leaf spot resistance genes were located.

A dark green dwarf mutant, TGM 167, was isolated from a gamma ray and sodium azide mutagenesis of TG 66 [12]. The mutant had a 45.8% reduction in height due to its shorter internodal length. Further, it was found to be insensitive towards exogenous GA3 application, although it had nearly the same level of endogenous GA3 as the parent. Genetic analysis revealed that the dwarfism is under the control of a single dominant gene. This dominant dwarfing gene was mapped with an SSR marker TC3H02 at a distance of 9.7 cM.

Realization of Yield potentials of Trombay groundnut varieties

With the release of several promising TG varieties, TAG 24, TG 37A, TG 38 and TG 51 in normal seed class and TG 39, TPG 41, TLG 45 in large seed class became popular in Gujarat, Andhra Pradesh, Maharashtra, Karnataka, Odisha, Rajasthan, West Bengal, Tamil Nadu, Madhya Pradesh and Uttar Pradesh due to incorporation of desirable traits like earliness, wider adaptability, large seeds and high harvest index through planned breeding. Several farmers have harvested significant improved productivity even upto 7,000 kg/ha as compared to national average of 1500 kg. In addition, TAG 24, TG 37A, TG 38, TG 51, TPG 41 are also used as check varieties in the respective national and state varietal groundnut improvement trials. BARC has undertaken large-scale breeder seed production as a first step to transfer the benefits of TG varieties to the farmers. Based on the allocation by the Department of Agriculture & Cooperation, Ministry of Agriculture and other seed agencies, >6000 quintals breeder seed of TG varieties is produced and supplied by the BARC to National Seed Corporations, State Farms Corporation of India, State Seed Corporations, ICAR institutes, State Agricultural Universities, State Agriculture Departments, NGOs, Seed companies and farmers for further foundation and certified seed production.

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Induced mutations for the genetic improvement of Banana and Sorghum

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Abstract

Tropical fruit crops such as banana are propagated vegetatively and creating genetic variability is highly limited due to heterozygosity, polyploidy and complex genome. Mutation induction is an important tool to create variability for fruit yield and quality traits. In the present study in vitro mutagenesis was applied to banana cv. "Giant Cavendish" to develop dwarf, early maturing mutants with increased fruit yield. Among the twelve selected gamma ray irradiated (10 Gy) plants, TBM-9 performed better than control with a bunch weight of 29 Kg with early flowering (294 days). This mutant is being evaluated for large scale yield trials.

Sorghum is extensively grown for food, fodder, fuel and other uses. India is the top producer of grain sorghum among the Asian countries. Induced mutations have played a vital role in improving earliness, yield contributing traits and seed quality parameters in sorghum. In the present study, two landraces viz., Chincholli-2 and JP 1-5 were improved for grain yield and tolerance to charcoal rot disease using gamma rays (350Gy) and Ethyl Methane Sulphonate (0.1%). In M2 generation, wide variability was also observed for other quantitative traits such as plant height (115-338 cm), stem diameter (0.8-2.2 cm), panicle length (8-34 cm) and width (7-25 cm) in these mutants. In the advanced generation (M6), TC-2 and TJP-1-5 mutants were selected for high grain yield and showed 2465-2653 Kg/ha against check 2397 Kg/ha. In addition, these mutants also showed less lodging (17.03-31.70%) due to charcoal rot disease as against 90.91 % in the susceptible check. These high yielding mutants are being evaluated in the multi-location trials of ICAR (Indian Council for Agriculture Research) for post rainy season. Our results on these aspects are summarised in this article.

1. Banana

Banana and plantains are the most important fruit crops grown in more than 130 countries in an area of 5.14 million ha producing 113.28 million tonnes [1]. India is the largest producer of banana in the world with a production of 29.12 million tonnes from an area of 0.796 million hectares and productivity of 35.7 metric tonnes per hectare. In spite of this popularity, they are one of the least genetically improved crops compared to other major food crops. The majority of production is still based on the cultivars derived from wild collections. Several diseases and pests besides abiotic stresses threaten banana cultivation and drastically reducing production. In order to improve the productivity, improved clones resistant/ tolerant to biotic and abiotic stresses are very much required. Creating genetic variability using crossbreeding and genetic recombination have limited application in banana due to their different genomic constitutions, heterozygosity, and polyploidy and parthenocarpic fruit development. This complexity needs the development of innovative approaches to support conventional breeding programs. *In vitro* mutagenesis has a great potential for the generation of useful mutants relatively rapidly in proven elite cultivars without compromising their yielding ability. Induced mutations are used to improve lodging problem in the high yielding banana cultivars, which is a severe problem during fruiting stage resulting in the significant losses to the growers. Dwarf with sturdy and strong pseudostem is a highly desirable trait, which can produce quality fruits without any mechanical damage.

Banana is propagated vegetatively by suckers, as viable seeds are not produced in most of the edible triploid bananas. The number of suckers produced in a year is limited and hence tissue culture propagation using shoot tip explants has become a common practice all over the [2, 3]. Rapid micro-propagation through shoot tip culture and somatic embryogenesis has been well established in

some of the important commercial cultivars of banana and can be exploited for *in vitro* mutagenesis for developing high yielding dwarf mutants. In the present study, multiple shoots of banana cv. 'Giant Cavendish' were gamma ray irradiated and variants were isolated and multiple copies of these variants were derived as per the procedures described earlier [4]. Twelve gamma ray irradiated (10 Gy) plants and control plants were multiplied by tissue culture and rooted plants were hardened in the Green house, BARC, Mumbai and subsequently field planted at NPCIL, Kaiga, Karnataka during 2013-14 crop season. The data were recorded for various morphological and fruit yield contributing traits (Table 1).

For morphological traits, wide range of values was observed for plant height (177.8-304.8 cm), stem diameter (76.2-86.36 cm) and number of suckers (6-12) as against the control. Two variants, TMB-2 and TMB-6 were dwarf (177.70 and 187.96 cm respectively as against control, 279.40 cm) and late flowering but attained maturity much before the control plants. These banana variants showed 9-11 hands/bunch and 135-170 fingers/bunch with a mean of 9.42 and 152.78 respectively. The main fruit yield contributing traits such as bunch weight (25-37 Kg), fruit length (17.78 – 22.86 cm) and fruit circumference (15.24-17.78 cm) have also seen wide range values as against control plants. TBM-6 variant recorded highest bunch weight (37kg) and fruit length (22.86 cm), where as TBM-8 had more number of fingers per bunch (170) and more hands/bunch (10). Although number of fingers was less in most of the variants except TBM-8, bunch weight and fruit area was substantially increased as against control. These dwarfs were initially characterized at maturity, by height, leaf shape and other agronomic characters [4, 5]. A few useful mutants such as semi-dwarf and dwarf mutants were characterized at molecular level by RAPD analysis and developed SCAR marker [4, 6]. Further field evaluation of these dwarf mutants was taken up in collaboration with National Research Centre for Banana (NRCB), Trichy, and Tamil Nadu. Based on the performance, a mutant line, TBM-9 performed better than control with a bunch weight of 29 Kg with early maturity (294 days) (Fig. 1). This mutant is being multiplied at NRCB for further large scale yield trials and registered with NBPGR, New Delhi. Thus, *in vitro* mutagenesis can be successfully applied in banana to develop dwarf mutants with comparable fruit yielding ability as compared to control.

Table: 1 Mean values for morphological and fruit yield contributing traits among the 10Gy derived banana variants

Genotypes	Plant height (cm)	Days to Flower	Fruit weight (kg)	Fruit length (cm)	Fruit circumference (cm)
TBM-1	180.34	291	28	17.78	17.78
TBM-2	177.8	244	35	20.32	16.51
TBM-3	187.96	294	28	20.32	17.78
TBM-4	187.96	284	28	22.86	15.24
TBM-5	193.04	272	29	20.32	15.24
TBM-6	187.96	255	37	22.86	16.51
TBM-7	187.96	288	34	19.05	15.24
TBM-8	180.34	251	31	21.59	15.24
TBM-9	195.58	266	30	22.86	15.24
TBM-10	187.96	266	34	20.32	15.24
TBM-11	187.96	281	25	20.32	17.78
TBM-12	195.58	276	34	20.32	15.24
Control	279.4	288	30	21.59	15.24
CV (%)	19.09	8.14	11.52	7.04	7.52

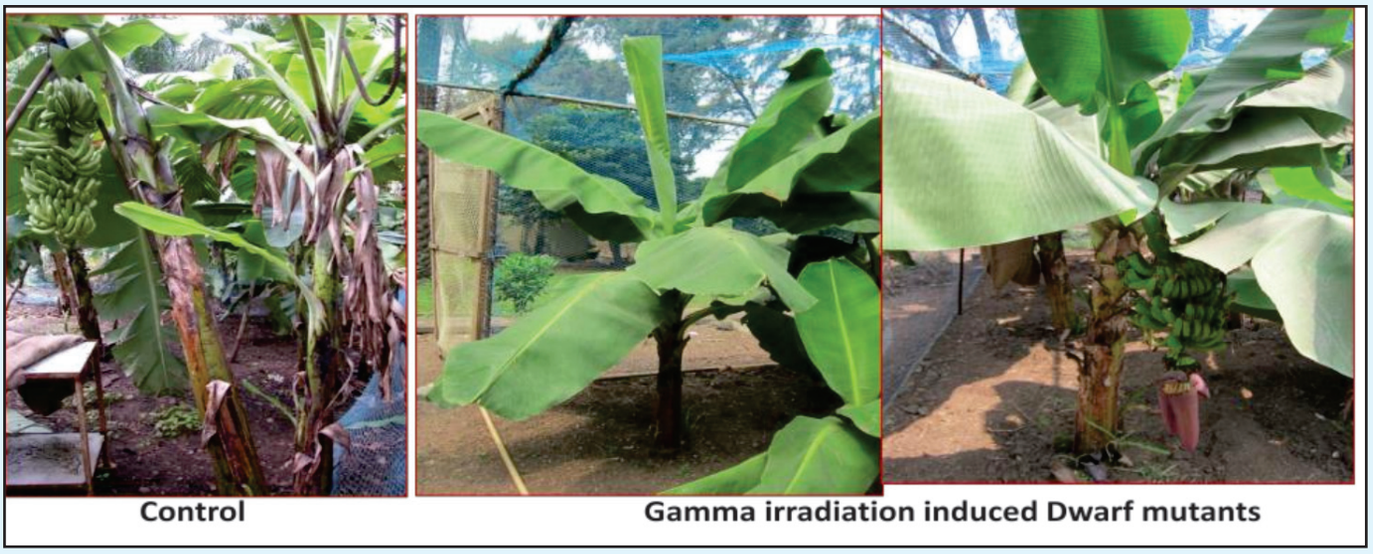

Figure 1. Field view of dwarf banana mutants and control plants of 'Giant Cavendish'

2. Sorghum

Sorghum is an important cereal crop grown across semi-arid tropics of African and South-Asian countries. Among the Asian countries, India produces about 7.29 million tonnes of grains from much reduced area of 7.69 million hectares as against 18.59 million hectares in 1970s [7]. However the productivity of kharif sorghum has gone up by 74.64%, mainly due to introduction of short duration high yielding semi-dwarf hybrids [8]. It is mainly used as staple food, livestock feed and for biofuel production. The rabi grown sorghum grain is mainly used to make roti and other value added products, such as flakes, porridge, hurda (green milky grains), pop sorghum and alcoholic beverages. Sorghum is rich in starch and contain considerable amount of protein, fat and mineral nutrients making it a cheap source of staple food for poor section of the society.

The main objective of the mutation breeding is to improve well adapted variety/landrace by altering one/two major traits. Gamma rays, Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) have been frequently used for the genetic improvement of field crops [9]. Many improved varieties have been released for commercial cultivation using ionizing radiations and chemical mutagens [10] and mutant lines were also utilized as parents in cross breeding programs. Induced mutations have played a vital role in improving biotic/abiotic stress tolerance, mineral nutrition, earliness, yield traits and seed quality parameters in cereal crops [11]. In this context, the present study was undertaken to improve grain yield and tolerance to charcoal rot disease in sorghum landraces.

Chincholli-2 and JP1-5 landraces, which have been extensively grown in north Karnataka and known for their good roti making quality, were selected for mutation breeding experiment. Dried and selfed seeds of these landraces were treated with gamma rays (300 Gy) and EMS (0.1%) and space planted at Experimental and Gamma Field Facility, Bhabha Atomic Research Centre, Trombay, Mumbai during 2012 post rainy season. The experiment was laid out on medium to deep black soil with spacing of 45 x 10cm. All the agronomic practices were followed to raise the ideal and healthy crop. In M2 and M3 generations, favourable mutants with altered morphology, earliness, panicle size and seed weight were identified. In M3 and M4 generations, 300 progenies in each of these landrace were evaluated for yield traits and screened for charcoal rot (CR) by tooth pick method. Based on percent lodging and mean node crossed, 40 tolerant mutants (TJP of JP 1-5 and TC of Chincholli-2 derived mutants) were selected as compared to checks, M-35-1, DSV-4, E-36-1 (resistant to charcoal rot) and SPV-86 (susceptible to charcoal rot) including their parents. In M5 and M6 generations, these elite mutant lines were evaluated in randomized complete block design (RCBD) with two replications at Agriculture Research Station, Gulbarga, during the post rainy seasons of 2016-17 and 2017-18 respectively. Observations on grain yield and disease reaction were recorded on five plants in each replication. The data were subjected to analysis of variance for each environment and for the combined data using PROC GLM of SAS 9.1 [12].

Popular landraces such as Chincholli-2 (TC-2) and JP 1-5 (TJP-1-5) have been grown widely for food

and fodder purpose. Although they produce marginal yields with lustrous pearl yellow seeds, but they are susceptible to charcoal rot. In 2012, mutation breeding program was initiated to improve these local landraces for yield contributing traits using gamma rays and EMS. Promising mutants having ten days early flowering were identified in TC-2 and TJP-1-5 progeny lines with high grain yield (range of 2465-2653 Kg/ha against check 2397 Kg/ha) and large seeds (4.1 g/100 seeds against check, 3.5 g/100 seeds) (Table: 2). Wide variability was also observed for other quantitative traits such as plant height (115-338 cm), stem diameter (0.8-2.2 cm), panicle length (8-34 cm) and width (7-25 cm) in these mutants (Badigannavar et al., 2017). Bold lustrous pearl yellow seeds are highly preferred by the consumers and fetch premium price in the market. Further, these elite mutant lines were also screened for charcoal rot disease using Tooth pick method. Mean Node Crossed (MNC) varied from 1.17-1.66 nodes as against 3.50 in the susceptible check (SPV-86) (Fig. 2). With respect to mean length of sclerotial growth spread (MLS), the range was 18.00-28.17 cm among mutant lines as against susceptible check (35.24 cm). The sclerotial growth has direct influence on lodging, which range from 17.03-31.70% among the mutant lines as against 90.91 % in the susceptible check. Most of the root infections were initially in the primary roots. When the temperatures were high with low water potential, extensive fungal growth was observed [13]. In addition, several anatomical and physiological characters have also been associated with CR resistance. Currently, these high yielding mutants are being evaluated in the multi-location trials of ICAR (Indian Council for Agriculture Research) for post rainy season.

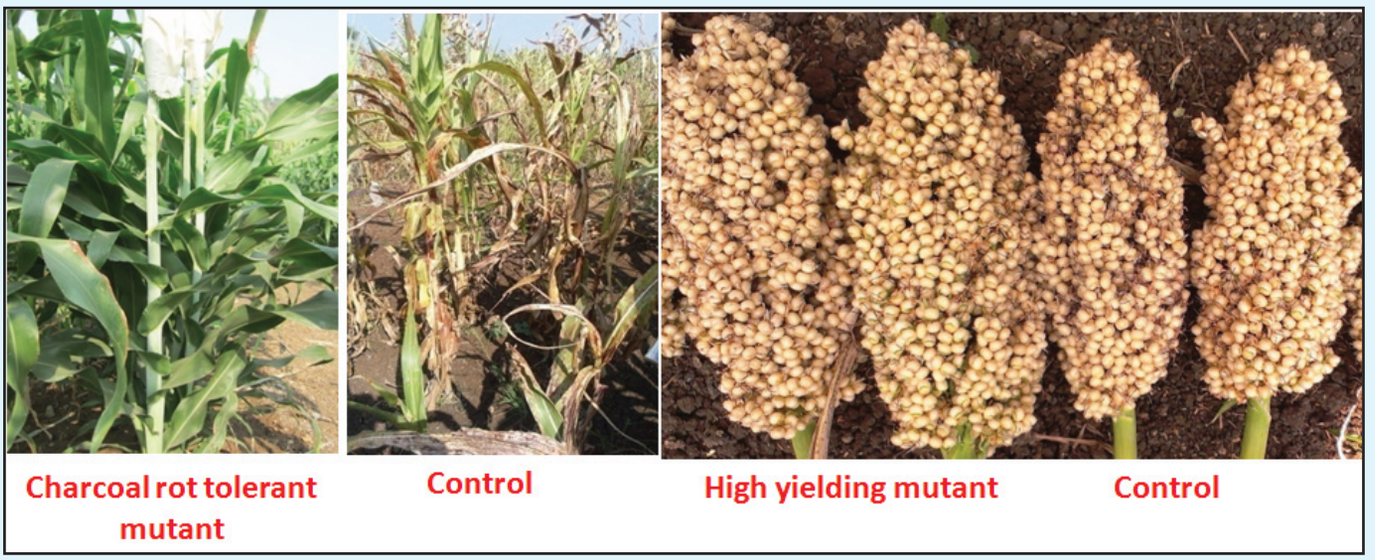

Fig: 2 Field view of high yielding and charcoal rot tolerant mutants in TC-2 landrace

Table: 2. Performance of elite sorghum mutants for grain yield and charcoal rot tolerance in M6 generation

Mutants	Seed yield (Kg/ha)	Reaction to Charcoal rot disease		
		*MNC (cm)	MLS (cm)	% Lodging
TJP-1-5	2653	1.17	20.50	26.79
TC-42	2595	1.66	26.17	31.70
TJP-11	2499	1.50	28.17	23.30
TC-109	2465	1.17	18.00	17.03
Chincholli-2 ©	2459	2.00	27.17	35.00
JP-1-5 ©	2332	2.50	21.67	41.03
M-35-1 ©	2397	2.17	28.83	52.31
SPV-86 ©	1466	3.50	22.66	90.91
Mean	2364	1.95	24.65	35.24
CV (%)	16	9.25	11.5	13.5
CD (@5%)	545			

*MNC: mean number of nodes crossed, MLS: mean length of spread

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Dr. T.R. Ganapathi joined BARC in 1991, did his Ph.D. degree in Botany from Karnatak University, Dharwad and is heading the Plant Cell Culture Technology Section in Nuclear Agriculture and Biotechnology Division. He has established protocols for tissue culture propagation and genetic transformation in banana. The technique of banana micropropagation has been transferred to user agencies. He was awarded INS medal in 2004 for his excellent contribution to the banana biotechnological research. Dr. Ganapathi has published more than 150 research publications in journals and books published by national and international publishers. He is a recognized Ph.D. guide in Biotechnology of University of Mumbai and is a Professor in Homi Bhabha National Institute, Mumbai. Four students have obtained Ph. D. degree under his supervision and three students are pursuing. Currently, he is working on *in vitro* mutagenesis in vegetatively propagated crops and genetic engineering of banana for the incorporation of useful traits. He is a Fellow of Maharashtra Academy of Sciences.

Dr. Ashok Badigannavar joined BARC as Dr. K. S. Krishnan Research Associate in 2010. He did his Ph.D. in the field of Genetics and Plant Breeding from Louisiana State University, USA. Currently he is working on development of sorghum varieties with improved grain yield and seed quality traits using conventional and biotechnological tools.

Development of eco-friendly sterile insect technique for agriculturally important insect pests

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Abstract

Sustainable agricultural production is highly influenced by various biotic and abiotic factors. Among them, insect pests can have adverse and damaging impacts on agricultural production, parasitizing livestock and health hazard to humans. It has been estimated that 25-35% of world food production is hampered by insect pests despite of extensive pesticide application. Due to the increasing awareness about the adverse effects of pesticides; need for environment friendly control methods are highly desirable. Sterile insect technique (SIT) is one of the important control method presently used for fruit fly and other insect pests control worldwide. BARC has developed sterile insect techniques for the control of different insect pests such as red palm weevil, potato tuber moth and fruit flies. This technique is an environment friendly, species-specific method of insect pest control. The successful implementation of area-wide integrated pest management (AW-IPM) programmes integrating other control strategies could help to suppress/eradicate the pest population in field.

Introduction

Insect pest management includes various control strategies such as collection and destruction of infested plant parts, suppression by using protein bait/pheromones, use of other biocontrol agents and selected insecticides. But, the success and effectiveness of these methods is greatly influenced by various environmental factors. Area Wide Integrated Pest Management (AW-IPM) including Sterile Insect Technique (SIT) programme has been implemented successfully to control several insect pests. SIT was pioneered in the 1950s by American entomologists Dr Raymond C. Bushland and Dr Edward F. Knipling. This technique has successfully been used to eradicate the Screw-worm fly (*Cochliomyia hominivorax*) in areas of North America and has been successfully implemented against a number of pest species such as Mediterranean fruit fly, *Ceratitis Capitata*; melon fly, *Bactrocera cucurbitae;* pink bollworm, *Pectinophora gossypiella*; codling moth, *Cydia pomonella* and tsetse fly, *Glossina austeni* [1,2]. Recently SIT has also been introduced for control of mosquitoes. The principle of SIT involves release of sterile males which mate with wild females after which female fails to produce any offspring. This technique is an environment friendly, sustainable and species-specific method of pest control based on mass rearing of the target pest, sterilization and release of sterile males [1]. It has been used successfully to suppress several insect pests threatening livestock, fibber crops, fruit and vegetables. SIT is compatible with other insect control methods that are used in Integrated Pest Management (IPM) programs.

Principle and requirement for SIT

The principle of SIT is the genetic effects of radiation on insect reproduction. Low dose radiation affects the reproductive cells of the insects and makes them sterile. The sterile males copulate with wild females of the target pest population and are unable to produce viable offspring. Repeated releases of sterile flies could lead to suppression of pest population or may be eradication within the release area. The theoretical model proposed by Dr Knipling predicted that the target pest population could be eradicated within five generations after introduction of SIT (Table 1).

Table 1. Theoretical model of number of generations required to eradicate the insect pest population

Gene-ration	Insects in field	Sterile insects released	Ratio of sterile : fertile	% Sterility	Fertile insects in field	Growth rate
P	1000000	9000000	9:1	90	100000	5x
F1	500000	9000000	18:1	94.7	26316	5x
F2	131580	9000000	68:1	98.6	1907	5x
F3	9535	9000000	944:1	99.9	10	5x
F4	50	9000000	180000:1	99.999	0	5x

World scenario

The successful implementation of AW-IPM integrating SIT with other control methods successfully demonstrated peaceful applications of nuclear technology. The Food and Agriculture Organization (FAO) of the United Nations and the International Atomic Energy Agency (IAEA) are engaged in supporting their member states in the development and application of these environment friendly technologies since last 50 years.

Indian scenario

BARC has developed sterile insect techniques for the control of insect pests such as red palm weevil, potato tuber moth and fruit fly species. At present, SIT is being developed to control fruit fly species, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* and tomato leaf miner, *Tuta absoluta.*

Red palm weevil (RPW), *Rhynchophorus ferrugineus*

Red palm weevil is a serious pest of coconut and other palms in India and worldwide. Efficient and economical mass rearing methods were developed and sterility dose was optimized. The optimum dose of 15-20 Gy induced 99% sterility in red palm weevil adults [3]. Field studies were carried out to demonstrate the feasibility of this technology in collaboration with three agricultural universities *viz.* Kerala Agriculture University, Thiruvanantapuram, Kerala, University of Agricultural Sciences, Dharwad, Karnataka and Dr. B.S. Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra. The release of sterile red palm weevil males in selected hot spots resulted in significant reduction in wild insect population as well as infested trees after 4 years of field studies (Table 2).

Table.2: Estimation of field population and release of sterile males of red palm weevil (2001-2004)

Red palm weevil	Estimation and release
Estimated insect population	1286
Number of sterile males released	5789
Number of insects trapped after 2004	27
Reduction of insect population (%)	>97

Potato tuber moth (PTM), *Phthorimaea operculella*

Potato tuber moth is the most destructive pest of potatoes, causing serious damage in field as well as in storage. Studies were carried out to evaluate the feasibility of using SIT for the control of potato tuber moth under storage conditions. Mass rearing technique has been developed and sterility dose was optimized [4]. The dose of 450-500 Gy induced 99% sterility in potato tuber moth. The feasibility of SIT in controlling multiplication of potato tuber moth in storage was assessed in collaboration with Directorate of Onion and Garlic Research (ICAR-DOGR) at their regional research centre in Rajgurunagar near Pune in Maharashtra. The multiplication of insect population was significantly suppressed by the release of sterile PTM males (Table 3).

Table.3: Evaluation of SIT for Potato tuber moth under field cage conditions

Potato tuber moth	First release	Second release
Tuber infestation (%)	23	2.4
Degree of infestation	6.3	6.8
Number of sterile males released	7000	20300
Reduction of insect population (%)	50	82

Development of SIT for fruit fly control

Insect species belonging to the genus *Zeugodacus* and *Bactrocera* are members of the Tephritidae family. These fruit flies are distributed worldwide exhibiting economical important agricultural pests and capable of affecting a variety of fruit and vegetable hosts. The extent of losses due to fruit fly is around 30-100%. Currently, SIT is widely used for fruit fly control worldwide.

Melon fruit fly, *Zeugodacus cucurbitae*

Melon fruit fly is a serious pest on cucurbits and solanaceous plant species (Fig. 1). We initiated IAEA-CRP to study feasibility of using SIT and improvement of sterile insect fitness using gut microbiota for the control of melon fruit fly [5,6]. Mass rearing technique has been developed and sterility dose was optimized. The dose of 50-60 Gy induced 99% sterility in melon fly. This work will help us to devise the SIT module for melon fruit fly management.

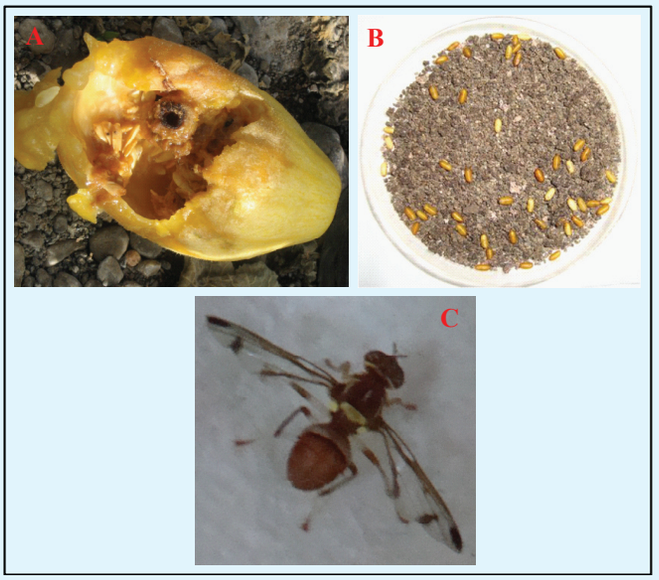

Fig. 1. SIT for melon fruit fly. A) Melon damaged due to melon fruit fly; B) Irradiated melon fruit fly and C) Sterile melon fruit fly male

Oriental fruit fly, *Bactrocera dorsalis*

The Oriental fruit fly is one of the major insect pests on economical fruits such as mango, sapota, guava etc in India. The Oriental fruit fly can damage 30-90% of fruit crop. SIT has been developed for the suppression of Oriental fruit fly population in fruit orchards for (Fig. 2) [6]. We have developed economical mass rearing method to produce large number of insects and optimised sterility dose. The dose of 70-90 Gy induced >99% sterility in Oriental fruit fly. This technology is being tested under field conditions in collaboration with Agricultural universities.

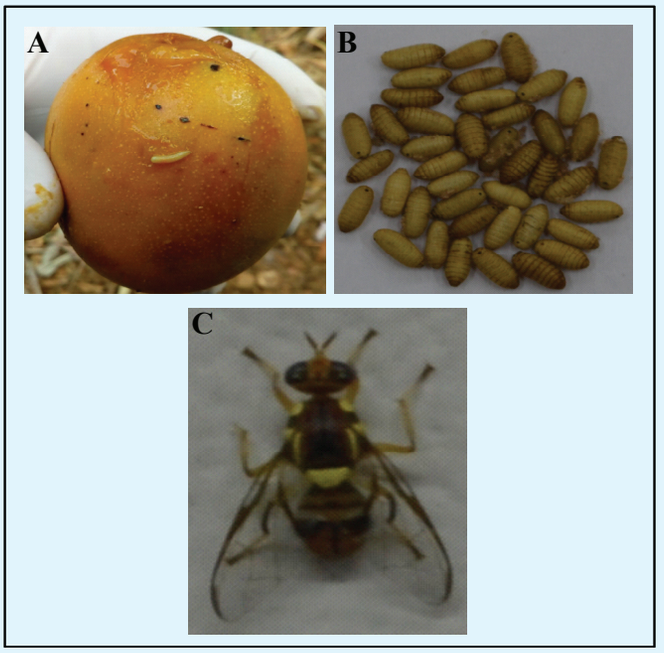

Fig. 2. SIT for Oriental fruit fly. A) Mango fruit damaged by Oriental fruit fly; B) Irradiated pupae and C) Sterile male

Conclusions and future prospects

SIT plays central role wide-area insect pests management programs to suppress devastating insect pests. These pests have been successfully controlled or being managed in different parts of the world. IAEA has initiated several Co-ordinated Research Projects (CRP) to develop SIT programs for the management of economically important insect pests like fruit flies, *Helicoverpa* sp., *Spodoptera* sp. and *T. absoluta*. BARC is actively participating in these programs to develop SIT for control of major pests of India. SIT technologies developed by BARC can be implemented over a wide area for suppression of these pests after clearance from Government agencies. This will help in upliftment of farming community by improving export potential of fruits and other crops.

Acknowledgements

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Dr. A. B. Hadapad joined BARC in 2008 through 15th batch of KSKRA. His area of interest includes molecular entomology, insect pathology, sterile insect technique and integrated pest management. At present, he is working development of SIT for fruit flies and tomato leaf miner and also involved in IAEA-CRP on development of SIT. He is also working on development of biopesticide based on *Bacillus sphaericus, B. thuringiensis* subsp. *kenyae,* and *B. thuringiensis* subsp. *israelensis* and use of other entomopathogens for various insect pest control. He is working on optimization of sterility dose for insect pests, profiling the insect gut microbiota and their attractant potential. Detection of insect associated endosymbionts like *Wolbachia* and potential to use in insect control.

Dr. R. S. Hire joined BARC in 1999 and since then working in the area of integrated pest management. He is heading the group on Pest Biocontrol Research in NA&BTD. His area of interest includes biological control of insect pests by biopesticides, sterile insect technique and molecular characterization of insecticidal proteins. He is involved in development of biopesticides based on bacteria such as *Bacillus thuringiensis* subsp. *kenyae, B. thuringiensis* subsp. *israelensis* and *B. sphaericus*. He has successfully characterized insecticidal proteins from these bacteria. He is also involved in IAEA-CRP on development of SIT for fruit flies and tomato leaf miner and also working on fruit fly associated endosymbionts using molecular techniques.

Methyl parathion Biosensor: From lab to field

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Abstract

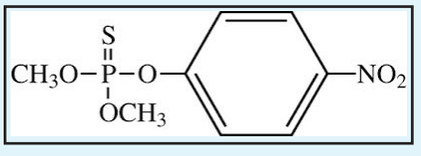
Methyl parathion (MP) is an organophosphate (OP) pesticide, which is being used as an insecticide in agriculture to protect the crops from insects. It acts as a nerve poison and causes overstimulation of muscle and nerve fibers, uncontrollable twitching, convulsions, difficulty in breathing or death. Thus, it has been classified by the WHO under 'Category Ia' (extremely toxic) and by the USEPA under 'Toxicity Category I' (most toxic) insecticide. Therefore, economically feasible, rapid, sensitive, selective and reliable methods for detection of MP are necessary. For this purpose, we have developed microbial biosensors by immobilizing microbial cells on different matrices and associated with different transducers for detection of single to multiple samples of MP in the laboratory in a very short period of time. The above biosensors use high end costly detection system and can be used in the laboratory only because of its size. Therefore, a handheld optical biosensor device was also designed and developed using the above colorimetric concept. This handheld biosensor is small in size, battery (DC) operated and directly displays the concentration of MP in ppm (detection range 1-10ppm). This can be utilized for detection of MP directly in the field.

1. Introduction

India is world's second largest populous nation with a population of 1.3 billion which is approximately 18% of the global population. The Global population is expected to cross 9 billion by 2050 from 7.5 billion today. A UN study on global population trends predicts that India will surpass China to become the most populous nation in the world by 2022. With a present size of 1.32 billion, India currently supports nearly 17.84% of the world population, with 2.4% land resources and 4 % of water resources. Keeping pace with these growing numbers, the country will not only have to raise its agricultural production but also the productivity to ensure food and nutrition security of the nation. Rising population has led to increasing the food demand. It is also mentioned that about 15-25% of produce in agriculture is lost due to insect pests, weeds and diseases. Pesticides have played an important key role for crop production to control the crop pests such as insects, fungal, weeds and rat etc., and thereby increase the productivity to meet the food demands and security by decreasing the crop loss from pests (1).

Methyl parathion (MP) is an organophosphate pesticide which is used as non-systemic insecticide in agriculture to protect the crops. Organophosphates pesticides are a class of insecticides, several of which are highly toxic. Earlier they were among the most widely used insecticides however, in the past decade, several notable organophosphates pesticides have been discontinued for use, including parathion, which is no longer registered for any use. Among organophosphate pesticides some widely used pesticides were methyl parathion, malathion, chlorpyrifos, diazinon, phorate, dichlorvos, dimethoate, monocrtophos and profenfos [2-4].

MP is used as insecticide in agriculture to protect the crops from insects. The IUPAC chemical name of MP is *O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate. Its chemical structure is shown in schematic 1:



Schematic 1

MP kills pests by acting as a potent irreversible acetylcholinesterase inhibitor. It was used to control a variety of insects and mites, including thrips, weevils, aphids and leafhoppers, in a very wide range of crops including cereals, fruit, nuts, vines, vegetables, ornamentals, cotton, and field crops [2-6].

MP was initially registered in 1954 in the United States for application as insecticide but its uses was restricted in 1978 as a result of detrimental effects to humans. MP causes inhibition of acetylcholinesterase which lead to excess accumulation of acetylcholine and causes overstimulation of muscles and nerve fibers, uncontrollable twitching, convulsions, difficulty in breathing and death. Environmental Protection Agency (EPA) has classified MP as a restricted-use pesticide and has given approval for outdoor use only. It was classified by the World Health Organization (WHO) as a Category Ia (extremely toxic) and by the United States EPA (U.S. EPA) as a Toxicity Category I (most toxic) insecticide [2-9]. Although banned in developed countries, it is used in developing countries like India as a restricted insecticide. As per statistical report by Directorate of PPQS, India and Centre of Science and Environment (CSE), consumption of MP in India was 5286 MT during 2010-2016 [10]. In India, Central Insecticide Board and Registration Committee (CIBRC) has recommended MP in two different concentrations either in 2% DP or 50% EC for controlling the pests from the cotton, paddy, wheat, pulses such as green gram and black gram and oilseeds such as ground nut and mustard crops [11]. However many newspapers, Hindu (10 June 2015 title: Chemical contaminants in household spices) and Deccan Chronicle (8 June 2015 Title: Washing vegetables does not reduce pesticides) have reported the presence of this pesticide in vegetables and spices [12-13]. In 2017, many news papers such as Hindu and Deccan chronicle on 7th May 2017 reported the presence of MP in concentration range 1.1 to 4.85 ppm in dried ginger powder [14-15]. Very recently some of the pesticides including MP were completely banned by Government of India with effect from August 2018 because of the high toxicity concern [16-17].

Although extensive use of pesticides has improved in securing enough crops, these pesticides are equally toxic or harmful to non-target organisms like mammals, birds etc and thus their presence even in small amounts can cause serious health and environmental problems. Pesticides have thus become environmental pollutants and they are often found in soil, water, atmosphere products. Thus monitoring of these pesticides and its residues become extremely important.

2. Research Highlight

2.1. Concept of biosensors for monitoring of Methyl parathion

Many traditional analytical methods like GC and HPLC have been widely used for pesticide analysis, but they require not only expensive equipments but also highly-trained technicians. Also these traditional techniques are time consuming and laborious because it requires pre sample preparation before analysis. Over a course of time, researchers have put efforts to develop promising alternatives for the detection of pesticides which can be used for easy, online and prompt detection with comparable accuracy and sensitivity. Also approach is such that the sample preparation can be avoided and minimized.

A biosensor is an analytical device that integrates an immobilized biological element with a transducer to recognize the analyte and the signal due to interaction between analyte and biological element is proportional to the concentration of analyte (Figure 1). Biosensor facilitate onsite detection of large number of sample with no or low preparation, less time requirement and no requirement of expensive apparatus and trained personnel which are generally limitation in traditional analytical methods [18-21].

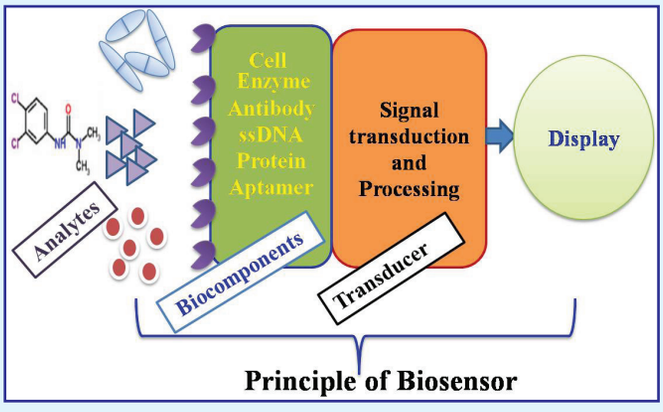


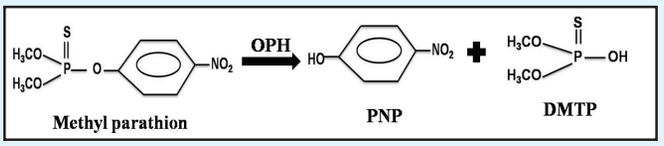
Figure 1. Schematic diagram of principle of biosensor

On the basis of biocomponent, if enzymes are used, it is known as enzymatic biosensor and if microbial cells are used, it is called microbial biosensor. Both biocomponent (enzyme/microbial cells) have certain limitations and advantages. Purified enzymes have very high specificity for their substrates or inhibitors, their

application in biosensor construction may be limited by the tedious, time-consuming and costly enzyme purification steps and requirement of cofactor/coenzyme to generate the measurable product. Microbes provide an ideal alternative to these bottle-necks. The enzymes and co-factors that co-exist in the microbes give the ability to consume and hence detect large number of analytes. Microbial cells can be easily manipulated and adapted to consume and degrade new substrates under certain cultivating condition. The above makes microbial cells an excellent biosensing element for developing biosensors. Thus, using microorganisms as biorecognition element provides an ideal alternative to purified enzyme. [2,4]

Biosensors require different types of transducer for sensing the signal generated because of interactions of biocomponent with analytes. Electrochemical transducers are widely used in the development of biosensors. According to the detection principle, electrochemical techniques can be divided into potentiometry, amperometry, conductometry and voltammetry. Amperometry is conducted at a given applied potential between the working electrode and the reference electrode and the current signal is recorded between working and counter electrode and correlated to the concentration of MP. Cyclic voltammetry is a very versatile electrochemical technique which allows probing the mechanism of redox and transport properties of a system in solution. This is accomplished with a three electrode arrangement whereby the potential relative to some *reference* electrode is scanned at a *working* electrode while the resulting current flowing through a *counter* (or *auxiliary*) electrode is monitored in a quiescent solution. The technique is ideally suited for a quick search of redox couples present in a system; once located, it may be characterized by more careful analysis of the cyclic voltammogram. In one of our study, a cyclic voltammetry based microbial biosensor for MP was described. Optical transducer is also commonly used system in biosensors. Optical detection is usually based on the measurement of absorbance, color, luminescence, fluorescence, or any other optical signal produced by the interaction of microorganism with the analyte and correlates the observed optical signal with the concentration of target compound. Optical sensing techniques are especially attractive in high throughput screening since they enable for simultaneous analysis of multiple analytes. The colorimetric sensing technique in microbial biosensors involves the conversion of a chromogen substrate into a colored compound by the metabolic activity of the sensing element. The colored product can be distinguished by the naked eye or a spectrophotometer. Because of its simple and inexpensive measurement setup, colorimetric technique has been widely applied in the fabrication of cost-effective microbial biosensors. Colorimetric biosensors involve the generation of colored compound which can be measured and correlated with the concentration of analyte [2-4, 18-21].

Among the various biosensors for MP determination and analysis, systems based on acetylcholinesterase (AChE), organophosphorus hydrolase (OPH) and MP hydrolase (MPH) contribute major share. The basic principle and mechanism of AchE based biosensor is the ability of pesticide to inhibit acetylcholinesterase. On the other hand, OPH and MPH are used based on the enzymatic hydrolysis of MP to generate an acid and alcohol. Below is the structural presentation of MP hydrolysis with OPH into p-nitro phenol (PNP) and dimethyl thiophosphate (DMPT) [2].



PNP is an optically detectable product which can be detected by electrochemical and colorimetric methods. Thus, this hydrolytic step has been extensively exploited to develop the biosensor for detection of methyl parathion. MPH also a member of OPH family acts specifically on MP in a similar way. This makes OPH and MPH a suitable recognition element for the detection of MP pesticide. In this article we will be discussing about the research work carried out in our laboratory for developing the microbial biosensor for monitoring of MP pesticides.

2.2. Biosensors for MP detection in laboratory

Our first study involved an optical microbial biosensor for the detection of MP (Fig. 2). In this study, whole cells of *Flavobacterium sp.* expressing OPH enzyme, were immobilized on glass fibber filters and were used as biocomponent along with an optical fibber system. Detection was based on the relationship between the amount MP hydrolyzed and the amount of chromophoric product PNP formed which was quantified by measuring the absorbance at the λ_{max} of 410 nm. A lower detection limit of 0.3 µM and linear detection range of 4 - 80 µM of MP was established. The immobilized microbial

biocomponent was disposable, cost-effective and showed high reproducibility and uniformity. Applicability of biosensor was also demonstrated with synthetic MP spiked samples [2, 22].

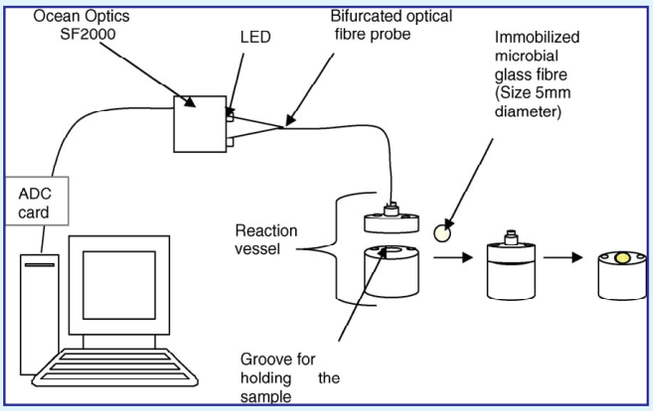


Figure 2. Schematic diagram of optical biosensor using disposable microbial biocomponent

In the second study, recombinant *E. coli* cells with high periplasmic expression of OPH was immobilized on screen printed carbon electrode (SPCE), associated with cyclic voltammetry system and cyclic voltammogram were recorded before and after hydrolysis of MP (Fig. 3).

It was calibrated based on the relationship between the changes in the current observed at +0.1 V potential. As the concentration of MP was increased the oxidation current also increased. Detection range of biosensor was reported between 2-80 µM of MP. A single SPCE with immobilized cells could be reused for 32 reactions and showed storage stability for 22 days [2, 23].

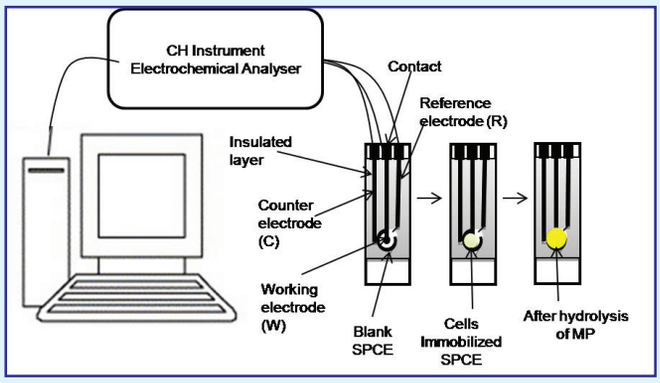


Figure 3. Schematic diagram of electrochemical biosensor using cells immobilized SPCE

In the third study, a microplate-based biosensor was described where isolated cells of Sphingomonas *sp.,* were immobilized directly onto the surface of the wells of a polystyrene make 96 wells microplate using glutaraldehyde as the cross-linker (Fig. 4). MP was hydrolyzed to a chromophoric product PNP. Microplate with immobilized bacteria was directly associated with the optical transducer of a microplate reader and PNP was quantified by measuring the absorbance at a λmax of 410 nm. In this case linear detection range of the biosensor was also between 4 - 80 µM MP but the cells-immobilized microplate showed reusability up to 75 reactions and storage stability of 18 days [2, 24]. In another study, inner epidermis of onion bulb scale was used as a natural support for immobilization of microbial cells of *Sphingomonas sp.* In this study, cells immobilized on onion membrane were placed inside the wells of the microplate and same mechanism as mentioned above was used for detection. Detection range was similar because microbial cells and transducer were same but there was a difference in reusability and storage stability which was 52 reaction and 32 days respectively [2, 25].

In another study we described the synthesis of a functional biohybrid component by integrating *Sphingomonas* sp. cells with functionalized silica nano particles (^{f}Si NP). Biohybrid was further immobilized onto 96 well microplate for biosensor application. The detection range of this optical biosensor for the detection of MP is 0.1–1 ppm and the storage stability of the biocomponent is 180 days [26].

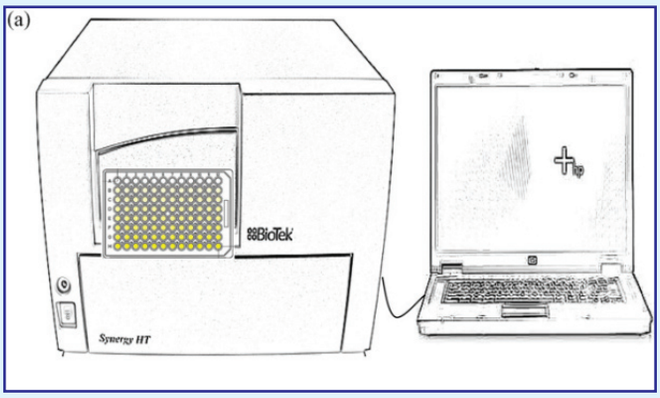


Figure 4. Schematic diagram of microplate based optical biosensor for multiple samples

In summary a characteristic feature of the developed microbial biosensors was the ability to detect MP pesticides in laboratory from single to multiple samples. The first optical microbial biosensor which was developed based on immobilization of whole cells of *Flavobacterium sp.* containing organophosphorus hydrolase enzyme on glass fibre filters dealt with a disposable biocomponent. Biocomponent could be used for single sample analysis only. In the second study, an electrochemical microbial

biosensor was developed by immobilizing *recombinant E.coli* on Screen Printed Carbon Electrode (SPCE) and associated with electrochemical analyser. Here the biocomponent was reusable and required low amount of samples. In the third study, a microplate based optical biosensor was developed by immobilizing *Sphingomonas sp.* directly onto the surface of the 96 wells microplate and indirectly on onion membrane fixed inside the wells of microplate and associated with optical transducer of multi detection microplate reader (MDMR). Microplate technique enables to acquire the whole array of data simultaneously and provides an innovative concept where multiple samples could be detected in very short period of time. Further integrating *Sphingomonas* sp. cells with ^{f}Si NP increased the sensitivity and stability of biocomponent.

These promising concepts have been published in high reputed journal Biosensors Bioelectronics (Impact Factor 8.17). All these microbial biosensors techniques required high end costly detection transducer systems and can be used in laboratory only due to its voluminous size and high cost. Recently this concept of microbial optical biosensor for detection of MP was exploited and translated into a technology for developing a prototype of handheld colorimetric biosensor.

2.3. Handheld Biosensors for field detection of MP

Concept of microbial optical biosensor for detection of MP was exploited and translated into technology for developing a prototype of handheld colorimetric biosensor which can be used in the field for monitoring of MP pesticide (Fig. 5). It has two components: First component is the biocomponent consisting of immobilized microbial cells of *Sphingomonas* sp. with organophosphorus hydrolase (OPH) enzyme. The second component is the handheld optical colorimeter with an ultraviolet 3W LED light source, a small cuvette and microcontroller circuit. Monochromatic light (410nm) coming from LED passes through glass cuvette (sample in solution) and photodiode for determining the light intensity. This handheld biosensor was initially calibrated with PNP and MP in association with immobilized biocomponent. This handheld biosensor is small in size, battery (DC) operated and directly displays the concentration of MP in ppm (detection range 1-10ppm). This handheld biosensor can be utilized for detection of MP directly in field. This technology is available on our BARC (AB28NABTD: http://barc.gov.in/technologies/biosensor/index.html) webpage and has been transferred to industry in 2018 [27].

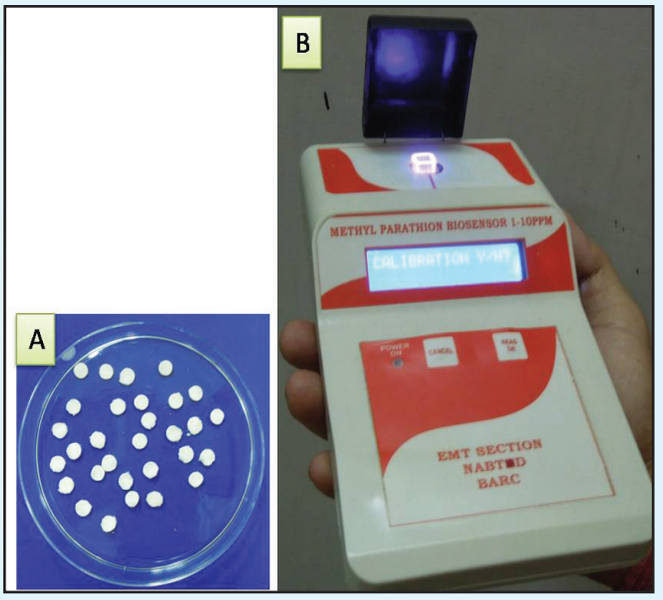


Figure 5. (A) Biocomponent disc (Microbial cells immobilized) for selectivity (B) Handheld colorimetric biosensor for detecting methyl parathion

3. Conclusion

In this article we have summarized the research work carried out in our laboratory which led the development of biosensor for detection of MP, an organophosphate pesticide. Initial research work was carried out for developing concept using OPH based microbial biosensor using optical and electrochemical biosensor. Biocomponent was developed by immobilizing microbial cells on various matrices in such a way that it can be used as disposable to reusable components and also transducers was selected so that it can be exploited for single to multiple sample analysis in laboratory. Later on concept was translated into technology (handheld biosensor) for field application in detection of MP pesticide. In agriculture field many pesticides from organophosphate and organocarbamate group are used while our developed biosensor is specific for methyl parathion pesticide, therefore there is requirement to monitor multiple pesticides in samples. Currently we are focusing to develop a biokit to detect multiple pesticides that belong to organophosphate and organocarbamate pesticides.

4. Acknowledgement

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Dr J. S. Melo career spans over three decades in the field of research and development. He obtained his Masters degree in Biochemistry from Mumbai University in 1984 and then joined Bhabha Atomic Research Centre where he is currently Head of the 'Enzyme and Microbial Technology Section'. In the year 1990 he received his doctoral degree in Biochemistry from Mumbai University. He is a Ph.D. guide at Mumbai University, Pune University and also a Professor at Homi Bhabha National Institute. He has to his credit over hundred publications in International Journals, Symposiums and Workshops with an overall citation of 2500 and h-index of 29. In the field of bioprocessing, he has developed a number of novel techniques for stabilizing enzymes and cells. His other areas of interest are in bioremediation of inorganic and organic pollutants, nanobiotechnology and biosensors. Due to his excellent contributions to science he has been honored as a Fellow of the Maharashtra Academy of Sciences and Society of Applied Biotechnology.

Dr. Jitendra Kumar is currently Scientific Officer F, Nuclear Agriculture and Biotechnology Division, Bioscience Group, BARC is working in the field of biosensor for pesticides, glucose, urea and mycotoxin producing organism. He has significantly contributed in the area of biosensor and published papers in high impact journal like Biosensor and Bioelectronics. He translated his research work "Biosensor for Methyl parathion" into technology and transferred to industry. He received best thesis award for high impact publication during his Ph.D. He is the recipient of DAE-Young Scientist Award 2011. He is a Fellow member of the Society of Applied Biotechnology, India and member of Indian Society for ElectroAnalytical Chemistry (ISEAC), Association of Microbiologist of India (AMI), Biosensor Society of India (BSI) and Association of Environmental Analytical Chemistry of India.

Improvement of Soybean and Linseed through Mutation Breeding

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Introduction

India is the fourth leading oilseeds producing country in the world, next only to the USA, China, and Brazil [1]. Soybean, groundnut, rapeseed-mustard, sesame, sunflower, castor, safflower, linseed and niger are the main oilseed crops grown in India. Highest average area contribution to total oilseed area is of soybean (39%) followed by rapeseed-mustard (24%) and groundnut (24%) [2]. Soybean which is mainly grown as a *kharif* crop has emerged as one of the major oilseeds crop and has revolutionized rural economy and lifted the socio-economic status of farmers in India. As a result of high protein and fat soybean has a multifold use and can be utilized domestically for meeting the acute protein deficiency in our country. Linseed is one of the important *rabi* oilseed crops and it is used as oil, feed and in industries for the manufacture of paints, varnish, linoleum and printing ink. The productivity of soybean and linseed in India is low as compared to the world average. Along with other reasons narrow genetic base of cultivated varieties in soybean and linseed is one of the reasons for low productivity. At BARC, genetic variability is generated in soybean and linseed through induced mutations. Mutants for morphological traits, high oil, low Trypsin inhibitor content (TI), low phytic acid content (*lpa)*, altered protein profile, altered fatty acid content, high harvest index and high yield have been identified and characterized. Mutants with desirable characters were used in hybridization with varieties and several high yielding recombinant lines developed which are being evaluated in various state and national trials. High yielding lines having low linolenic acid content has been developed in linseed and are in advanced trials of Indian Council of Agricultural Research (ICAR). Two soybean varieties TAMS-38 and TAMS 98-21 has been developed and released for commercial cultivation. In this article, the success story of genetic improvement for productivity and quality of soybean and linseed using conventional, molecular and mutation breeding approaches at BARC will be discussed.

Soybean (*Glycine max* L. Merr)

Soybean is ranked number one in world oil production and is widely cultivated in the United States, Brazil, Argentina, China and India. In India, soybean is mainly grown as a *kharif* crop under rainfed condition and it occupies an area of about 11.50 million hectares with and production of over 10.50 million tonnes. In India, it contributes about 25 per cent to the domestic edible oil pool and the country earns substantial foreign exchange to the tune of Rs. 3731 Crores through export of soy meal [3]. The major soybean growing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Rajasthan. The average yield of soybean in India is 1 tonne per hectare as compared to a world average of 2.5 tonnes per hectare. The major constraints for low productivity of soybean are poor seed viability, non-availability of early maturing, photoperiod insensitive high yielding cultivars carrying resistance to biotic and abiotic stresses. Soybean seed contains 18-23% oil and about 38-40% protein and together, they account for 56% of dry soybeans by weight. As a result of high protein and fat soybean has a multifold use. It is mainly grown for seeds, which are used for fresh, fermented and dried fruit products. A large quantity of seed is crushed to extract oil for food and industrial purposes. The oil is converted to margarine, mayonnaise, shortening, salad oils and salad dressing. The soybean meal remaining after oil extraction is used primarily as a source of high protein for animal and poultry feeds. It is also being used in the production of fermented foods like soy sauce, miso, natto, tempeh and sufu and non-fermented foods like soymilk and tofu. The soybean protein is also used in the form of concentrates, isolates and textured protein for human consumption. Thus, the role of soybean as a protein rich food crop and oil crop is well known and can be utilized domestically for meeting the acute protein deficiency in our country. Despite its rich nutritional profile, use of soybean in food has been limited because soybean proteins are often associated with compounds, which could be considered toxic or harmful to the animal body. These are called the anti-nutritional factors. Some of these anti-nutritional factors can be destroyed by heat (protease inhibitors, lectins, goitrogens, antivitamins)

while others (saponins, tannins, estrogens, flatulence factors, lysinoalanine, allergens, phytate) cannot be destroyed [4]. Hence, development of cultivars with low or null anti-nutritional factors will help to improve nutritional quality of soybean for export and domestic use.

RADIATION INDUCED GENETIC VARIABILITY

Morphological mutations

Soybean genotypes NRC-37, JS 93-05, Bragg, JS 71-05, PUSA-5, EC 241780, JS 80-21 and VLS-2 were irradiated with 250 Gy gamma rays for improving yield and biochemical characters. A large number of mutants affecting the morphological characters were identified and characterized. The viable chlorophyll mutants include chlorine, viridis and virescens. Morphological mutants with altered leaf characters, flower colour, branching pattern, plant architecture and maturity were identified. Early maturing mutants were identified in the genotypes NRC-37, EC-241780 and PUSA-5. More than sixty mutants for different traits are maintained at BARC.

Mutants for low phytic acid and trypsin inhibitor content

Soybean cultivar JS 93-05 and NRC-37 were irradiated with 250 Gy gamma rays to induce mutation for various morphological and biochemical characters. Ninety true breeding mutant lines in M_6 generation were screened for TI and phytic acid (PA) content. The PA content in the mutants varied from 7.59 to 24.14 mg/g. Two mutants lines TSG-62 (7.59 mg/g) and TSG-66 (9.62 mg/g) showed significant low phytic acid (*lpa)* content as compared to the parent JS 93-05 (20.19 mg/g). The TI concentration in the mutants varied from 19.92 to 53.64 Trypsin inhibitor unit (TIU)/mg and one mutant line (TSG-14) was found with the lowest TI inhibitor concentration of 19.92 TIU/mg compared to parent JS 93-05 (50.90 TIU/mg). Five mutant lines TLPM-14, TLPM-20, TLPM-34, TLPM-47 and TLPM-52 were identified in the M_2 generation and were advanced to M_3 generation. Five *lpa* mutant lines TLPM-14, TLPM-20, TLPM-34, TLPM-47 and TLPM-52 were also identified in the genotype NRC-37. All the *lpa* mutant lines showed good germination and plant vigour in also gave higher yield than the parent NRC-37 indicating that seed emergence and plant growth is not affected by *lpa* mutations. Multilocation analysis carried out at two locations showed no significant effect of environment and genotype × environment (G X E) interaction on PA concentration. The correlation studies showed no significant correlation of PA with maturity, plant height, pods per plant, yield per plant, oil, protein and fatty acids. The preliminary study indicates that *lpa* trait in the mutant lines may be controlled by recessive alleles at two independent loci [5, 6].

Mutants for high oleic acid and low linolenic acid

A typical soybean cultivar contains about 11% palmitic (16:0), 3% stearic (18:0), 22% oleic (18:1), 56% linoleic (18:2) and 8% linolenic acid (18:3). The 18:3 is considered as an unstable component and is prone to oxidation and spoilage, rancidity and off-flavors and decreases shelf life of oil. Hydrogenation is carried out to overcome the above problems but it leads to formation of trans fats responsible for heart diseases. Development of soybean lines with high 18:1 and low 18:3 content will improve the oxidative stability of soybean oil. Four hundred soybean genotypes including mutants, Indian germplasm, exotic germplasm and released varieties were analyzed for fatty acid composition for identification of high 18:1 and low 18:3 lines. Genotypic variation was observed for all the 5 major fatty acids. Twelve lines were identified with high 18:1 (more than 35%) and five lines low 18:3 (range of 3 to 5%). Molecular assays were developed based on polymerase chain reaction (PCR) amplification of the region of interest from earlier reports (FAD3 gene) and validation of these markers were carried out in high 18:1 and low 18:3 lines [7].

Mutants in cross breeding

Single, double and three-way crosses were made to develop high yielding recombinant lines resistance to diseases and pests. Yellow mosaic virus resistant variety PK-564 and SL-742, rust resistant variety DSb-12, multiple disease and pest resistant BARC mutant selections and high yielding varieties were used in the crossing programme. Based on high yield and good agronomic characters 122 lines were identified and are maintained in F_8 generation. All the lines gave 10-20 % higher yield over the best checks at Trombay. Recombinant inbred lines (RILs) were developed for mapping yellow mosaic virus (YMV) and rust disease resistance in soybean

Molecular Studies

Genetic diversity studies

The average yield of soybean in India is about 1353 kg/hectare which is significantly lower in comparison to the world average 2500 kg/ha). Narrow genetic diversity in

Indian soybean is considered as one of the main reason for low average yields of the Indian soybean cultivars. Inbreeding and evolutionary events such as domestication can results in reduction of genetic diversity and modify the allele frequencies in the populations. The study of genetic variation is a prerequisite and vital step in the improvement of any crop plant and is important in identifying suitable parents to develop segregating populations with maximum genetic diversity. Genetic diversity in 90 Indian soybean cultivars was assessed using 45 SSR markers distributed on 20 soybean chromosomes. In cluster and structure analyses, soybean cultivars DS228, MACS-13, LSb-1, Hardee, Improved Pelican, and Pusa-24 were six most genetically distinct cultivars identified. The study reported a moderate genetic diversity in Indian soybean cultivars and findings would be useful to the soybean breeders in selecting genetically distinct parents for soybean improvement program [8].

Soybean mosaic virus (SMV)

Soybean mosaic disease caused by soybean mosaic virus (SMV) is one of the major viral diseases prevalent in India and causes significant yield loss and also affects seed quality. In India, soybean cultivars resistant to SMV have been identified but source of resistance has not been characterized. Therefore, the study was carried out to study the genetic variation at three SMV resistance loci in a set of SMV resistant and susceptible Indian soybean genotypes using the mapped 13 SSR markers. Cluster analysis grouped the 23 soybean genotypes into three major clusters and results of the Principal Coordinate Analysis also congruent well with the cluster analysis. This study showed that Indian soybean genotypes have sufficient genetic variability at the three SMV resistance loci and it would be helpful in the selection of suitable parents in breeding for SMV resistance [9].

Yellow Mosaic Virus (YMV)

Yellow Mosaic Virus (YMV) disease caused by mungbean yellow mosaic virus is a serious disease of soybean and adversely affects soybean production in India. Growing YMV resistant cultivar is the most economical and environment friendly approach to prevent yield losses due to YMV. The YMV resistance gene in soybean is reported to be present on two different chromosomes (chromosome 17 and chromosome 18) in two independent studies. A total of 22 soybean genotypes were used in the study, which included 8 YMV resistant and 14 YMV susceptible genotypes. A total of 52 SSR markers (26 markers from chromosome 17 and 26 markers from chromosome 18) were screened on all 22 soybean genotypes. SSR marker GMHSP179 present on chromosome 17 was able to distinguish successfully between YMV susceptible and resistant soybean genotypes. The identified marker will be helpful in breeding YMV resistant soybean cultivars through marker assisted selection [10].

PCR based characterization of soybean mutant and germplasm lines having reduced phytic acid content

Soybean seed contains about 4.3 g kg^{-1} phytic acid P (PA-P) and 0.6 g kg^{-1} inorganic P (Pi). PA is considered as an anti-nutritional factor as it can chelate with important mineral micronutrients, e.g., Zn, Fe, Ca rendering them virtually indigestible by humans and non-ruminant livestock. Four mutants of soybean cultivar NRC-37 developed by γ-irradiation with PA content ranging from 0.07 to 0.10 mg/g and four soybean germplasm lines having *lpa* (0.87 to 1.07 mg/g) were used in the study for PCR based molecular characterization. To identify any large deletion/addition mutation in the four MIPS genes (MIPS1, MIPS2, MIPS3 and MIPS4), PCR primers were designed to amplify the predicted exons of the MIPS genes. Exons of all four MIPS genes are highly similar and based on the gene sequence of the MIPS genes, 12 degenerate primer pairs were designed to amplify all the 10 exons of four MIPS genes. These primer pairs were screened on low and high phytate mutant and germplasm lines. PCR products of expected size were obtained with all exon specific primers. However, no DNA variation was detected for any of the exons indicating that no large deletions are present in the MIPS genes of soybean genotypes having reduced phytic acid content. PCR primers identified as linked to four MIPS genes were also used to screen the low phytate mutant and germplasm lines of soybean. The primers linked to gene MIPS1, MIPS2 and MIPS3 did not show any variation among the low phytate and high phytate soybean genotypes. However, primer linked to MIPS4 gene showed length variation among the low phytate and high phytate genotypes. Association of this variation with low phytate trait in soybean will be confirmed through linkage analysis [11].

Linseed *(Linum usitatissimum* Linn)

Linseed is one of the important *rabi* oilseed crops of India and is cultivated over an estimated area of 292.1 thousand hectares with a production of around 141.2 thousand

tonnes [12]. The major linseed growing states of India are Madhya Pradesh, Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Orissa, Andhra Pradesh and West Bengal. It is used as oil, feed and in industries for the manufacture of paints, varnish, oilcloth, linoleum and printing ink. The productivity of linseed in India is 525kg/ha which is very low as compared to the world average. The reasons for low productivity are lack of high yielding varieties, cultivation of linseed under no input condition and utera system of cropping wherein majority of linseed crop is grown under moisture stress situation. Piara or utera cropping system has been in practice for efficient use of residual moisture in rice fields, where tillage is a problem. About 25% of the linseed area (0.5 million ha) is under utera cropping. Development of early maturing cultivars with high yield will be best suited for utera cropping to overcome the poor yield levels by maximising utilisation of residual moisture and nutrients present in the soil. . Linseed oil is a rich source of unsaturated fatty acids like oleic acid (16–24 %), linoleic acid (18–24 %) and linolenic acid (36–50 %). The high level of linolenic acid makes it useful for industrial purposes because of high drying quality but makes it unsuitable for use as edible oil because of its susceptibility to oxidation by developing off-flavours during storage. Development of varieties with low linolenic acid content will help to make linseed oil edible oil.

RADIATION INDUCED GENETIC VARIABILITY

Linseed genotypes NL-97 and NL-264 were irradiated with 600 Gy gamma rays for improving yield and biochemical characters. In the M_2 generation, chlorophyll and viable mutants affecting morphological and physiological characters were identified. The morphological mutants included those affecting plant height, flower colour, sterility, leaf shape, number of pods per plant, seed colour and days to maturity. Breeding behaviour and salient features of the true breeding mutants were studied up to $M_3 – M_7$ generations. Ninety six mutant lines for earliness, seed colour and size, quality and high yield were evaluated in M_7 generation for studying their quantitative and qualitative characters. One of the mutants TL-145 flowered in 39 days and matured in 107 days as in comparison to the parent NL-97 (125 days). The early maturing mutant TL-145 (7.6 gm) showed significantly higher seed yield per plant as against the parent NL-97 (4.5 gm). TL-145 was evaluated in ICAR trials and it gave yield of 1422 kg/ha as compared to best check Divya (1289 kg/ha) and is promoted to Advanced varietal trials. High yielding mutant lines TL-13, TL-16, TL-24, TL-30, TL-66, TL-89, TL-7, TL-187 and TL-253 are being were evaluated in University trial at College of Agriculture, Nagpur [13].

Development of low linolenic acid lines in Linseed

A simple, reliable method of screening for large number of plants for identifying biochemical mutants is a pre-requisite for an efficient breeding programme. At BARC, A modified rapid and simple spot test technique was developed for determining linolenic acid content (McGregor 1974). To improve the oil quality of linseed, a low linolenic acid content genotype Solin was used hybridisation with high yielding genotypes RLC-6, GS-234, Y-28 and mutants of NL-97. Ten lines were developed with good agronomic characters and low linolenic acid content. The linolenic content in the selected lines ranged from 2% to 34.5 % as compared to the parent the parents (56.8%) [14]. One of the lines TL-99 having low linolenic acid (2%) was evaluated in university and ICAR trials for yield and other characters. In Advanced varietal Trial -1 of ICAR it gave yield at par with zonal check Shekhar and National check T-397. Based on yield and quality trait TL-99 is promoted for one more year testing in ICAR trials.

Conclusion and Future Prospects

The degree of genetic variability available for selection can play an important role in overcoming yield barriers. Improvement in yield is normally attained through exploitation of the genetically diverse genotypes in breeding programmes. Mutations, spontaneous or induced, are an important source for inducing genetic variability. Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time. These identified mutants and mutant derivatives can be utilised in the breeding programme for developing elite varieties of soybean and linseed. High yielding mutant lines will be evaluated in state and ICAR trials.

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Genetic improvement of pulse crops through induced mutation and biotechnological approaches

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Introduction

Pulses form the major sources of proteins among the predominantly vegetarian population of the country. They form a unique and essential component of the diet by complementing the staple cereals with proteins, essential amino acids, vitamins and minerals. On an average, pulses contain 22-24% protein which is almost two to three times that of wheat and rice. Pulses are known to contribute immensely to the sustainability of the farming systems as they can be grown on range of soil and climatic conditions, play a determinative role in crop rotation, mixed and inter-cropping, enrich soil fertility through in situ nitrogen fixation and help liberate soil-bound phosphorus.

Area, production and yields of pulses in India

Globally, India ranks first as the largest producer and consumer of pulses. The major pulses grown in the order of predominancy include chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), lentil (*Lens culinaris*), urdbean (*Vigna mungo*), mungbean (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum*), grass pea or khesari (*Lathyrus sativus*), cowpea (*Vigna unguiculata*), and faba bean (*Vicia faba*). Pigeonpea, urbean and mungbean constitute the major kharif pulses, while chickpea, lentils and peas represent rabi pulses. The sturdier pulses like moth bean, horse gram and cowpea are classified as arid legumes. India contributes 35% and 25 % of the global acreage and production, respectively. In spite of annual pulses production of 22.95 Mt during 2016-17, India imports pulses to the tune of 6.6 Mt to meet the domestic demand (DGCI&S, Ministry of Commerce, Kolkata). By 2030, the projected pulse requirement is 32 million tons with an anticipated growth rate of 4.2% (IIPR Vision 2030). Though India has made considerable progress in improving the productivity to 738kg/ha (2016-17), it is far behind the global average (Source: Agricultural Statistics at a glance 2016).

Genetic constraints in improving the productivity

The scope for genetic improvement of pulses by conventional means is limited owing to the very narrow genetic base. In addition, the repeated use of limited cultivars in hybridization for evolving improved varieties has further accelerated genetic erosion. The breeders are entrusted with the mammoth task of creating large genetic variability to broaden the genetic base so as to break the yield plateau. Induced mutation is one of the important tools for generating genetic variability that can be suitably made use of to the advantage of the breeders. A large number of varieties have been developed across the world through mutation breeding, many of which have brought out economic revolution to the farmers. Apart from this, production of major pulses is constrained by both biotic / abiotic stresses and socio-political problems. Pulses being rich in N and P make them attractive for insect pests and diseases. Most of the pulses in India are grown in low fertility, problematic soils and unpredictable environmental conditions. More than 87% of the area under pulses is rainfed and are subjected to drought and heat stress, which brings down its yield. Further, the arid and semi arid areas of the country face problem of alkaline and acidic soils. Pulses in our country continue to be grown on poor soils with low inputs. The high rate of ovule abortion and flower drop adds to the poor productivity. The non-availability of quality seeds also dents the pulses productivity.

Radiation for induced mutations

Success of a crop improvement programme depends on the availability of large genetic variability, which a plant breeder can combine to generate new varieties. This variability is the outcome of spontaneous and induced mutations. Induced mutations have become the most common method for breeding plants through sexual reproduction. Induction of mutations using physical and chemical mutagens has been exploited by breeders to develop new crop varieties. Induced mutagenesis through physical mutagens is well proclaimed in comparison to

the chemical mutagenesis as the latter is known to cause point mutations. A wide range of physical mutagens including UV rays, beta rays, gamma rays, x-rays, fast neutrons, thermal neutrons, electron beams, ion beams etc are available for use in mutagenesis. The frequency with which the desired mutants appear depends on the efficiency of the mutagen. Therefore, application of more powerful mutagens with different mutation spectra is of great significance in induction of variability.

Different kinds of radiation have different energy transfer patterns. The mutation effect of radiation is known to be a function of its linear energy transfer (LET), which is defined as the energy deposited to the target material when ionizing radiations pass through it. Once an accelerated particle encounters any substance, it gradually loses its own energy (i.e., the same amount of energy is transferred to the substance causing damage.) and eventually stops at the point where the maximum energy loss is observed. LET is usually expressed in kilo electron volt per micrometer (KeV/μm) which represents the average amount of energy lost per unit distance. Biological effects induced by high LET radiation are greater than those induced by low LET radiation (gamma ray, X-ray) [1]. Mutation frequency and spectrum induced by high LET radiation such as ion beams were reported to be higher compared with those induced by low LET radiations [2].

In general, a variety of ion species, from protons to uranium ions, can be utilized for ion beam applications. Several kinds of energies and ions, such as helium (He), carbon (C), neon (Ne) and argon (Ar) can be used, with 220MeV carbon ions being the most commonly used in mutagenesis. In ion beam mutagenesis, positively charged ions are accelerated at a high speed (around 20–80% of the speed of light) and used to irradiate target plant material. As a physical mutagen, ion beams are similar to other forms of radiation such as X-rays, gamma-rays, and electrons, but they are different from them in that ion beams have much higher LET. This characteristic is important to understand the high biological effectiveness of ion beams [1]. Ion beams have a relatively high LET (around 10–1000 keV/mm or higher), while X-rays, gamma-rays and electrons have low LETs (around 0.2 keV/mm). Therefore, ion beams are able to cause more severe damage to living cells than other forms of radiation, resulting in the high relative biological effectiveness. Ion beams can cause large DNA alterations (large deletions, inversions, and translocations).

Electron beam has been demonstrated to induce mutation with increased mutation frequency as compared to gamma rays. Both γ-rays and electron beams have low LETs of around 0.2KeV/ μm. However, electron beam has a higher dose rate compared to gamma-rays and is administered as short pulses, while gamma irradiation is continuous. Absorbed dose-rate exhibits its strong influence on relative biological effectiveness (RBE), which was called dose-rate effect relationship [3]. The higher the dose-rate, greater is the RBE. The absorbed dose rate of electron beam on biomaterials may reach 10^{10} $Gy.s^{-1}$, which is much higher than that of γ-rays (usually under 60 $Gy.s^{-1}$) and those of other radiation methods [4]. With such high dose-rate, electron beam can produce high density free radicals in a very short time, resulting in a large number of DNA double strand breaks, bringing mutation effects with high mutation efficiency and wide variety [4]. Compared with γ-rays or X-rays, the electron beam is limited to treating relatively thin packages because of the low penetrating power (< 2 inches) of electrons.

Mutation breeding in pulses

The induced mutants with desirable agronomic traits can either be directly released as varieties or could be used in hybridization with cultivars or other mutants to develop mutant varieties. As per the latest enumeration by Food and Agriculture Organization of the United Nations (FAO/IAEA), as many as 3281 varieties have been released so far in 232 different crop and plant species. More than 70% of these mutant varieties have been developed using physical mutagens especially ionizing radiations [5]. Despite mutation being random, the success of obtaining desired mutant traits depend on three factors viz., the efficiency of mutagenesis, the starting plant material and efficient screening technique [6]. Since 1950, India has developed about 343 mutant varieties in 57 crops through direct mutagenesis of which major varieties have been developed for rice, wheat, barley, pearl millet, jute, groundnut, soybean, chickpea, mungbean, cowpea, black gram, sugarcane, chrysanthemum, portulaca, tobacco and Dahlia. Out of these 343 mutant varieties, about 50 varieties have been developed through use of mutant lines in breeding programmes. In India, mutation breeding has contributed some of the best varieties in pulses. In total, 56 mutant varieties have been released in pulses. Induced mutagenesis either alone or in combination with hybridization has resulted in development of 56 crop varieties in pulses. Almost 50% of these mutant varieties have been bred from Bhabha Atomic Research Centre, Mumbai. Apart from the BARC bred varieties

like TAU-1 in blackgram, some other mutant varieties of economic significance released in India are Pusa 408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) of chickpea, Co-4 of blackgram, and MaruMoth-1 of mothbean [7].

Induced mutagenesis at Bhabha Atomic Research Centre (BARC)

Bhabha Atomic Research Centre is one of the leading institutions in India wherein mutation breeding is actively being practiced under the peaceful application of atomic energy. A total of 42 mutant crop varieties have been released and notified for commercial cultivation across the country in 10 different crops. The Pulses Improvement Section under Nuclear Agriculture and Biotechnology Division of BARC has been actively engaged in the genetic improvement of pulse crops namely urdbean, mungbean, pigeonpea and cowpea. Twenty varieties belonging to these four crops have been released and notified for commercial cultivation across the country (Table 1).

Table 1. Trombay pulse crop varieties released and notified for commercial cultivation by Ministry of Agriculture, Government of India

Variety	Year of release	States	Special features
Mungbean (Green gram)			
TM 2000-2	2010	Chhattisgarh	Suitable for rice fallows and resistant to powdery mildew
TM-96-2 (Trombay Pesara)	2007	Andhra Pradesh (*rabi* and summer) and rice fallows	Resistant to powdery mildew and *Corynespora* leaf spot
TJM-3	2007	Madhya Pradesh	Resistant to powdery mildew, Yellow mosaic virus and *Rhizoctonia* root –rot diseases.
TMB-37	2005	Eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam	Tolerant to yellow mosaic virus
TARM-18	1995	Maharashtra	Resistant to powdery mildew
TARM-1	1995	Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Kerala, Karnataka, Tamil Nadu, Odisha	Resistant to powdery mildew
TARM-2	1992	Maharashtra	Resistant to powdery mildew
TAP-7	1983	Maharashtra, Karnataka	Tolerant to powdery mildew
Pigeonpea (Tur)			
PKVTARA	2014	Maharashtra	Resistance to *Fusarium* wilt disease
TJT- 501	2009	Madhya Pradesh, Gujarat, Maharashtra, Chhattisgarh	High yielding, Early maturing, Tolerant to *Phytophthora* blight
TT-401	2007	Madhya Pradesh, Gujarat, Maharashtra, Chhattisgarh	High yielding, tolerant to pod borer and pod fly damage
TAT-10	1985	Maharashtra	Early maturing
TT-6	1983	Madhya Pradesh, Gujarat, Maharashtra, Karnataka, Kerala, Andhra Pradesh	Large seed
Uridbean (Black gram)			
TU40	2013	Andhra Pradesh, Karnataka, Kerala, Tamil Nadu	Powdery mildew resistance
TU 94-2	1999	Andhra Pradesh, Karnataka, Kerala, Tamil Nadu	Resistant to yellow mosaic virus
TAU-2	1992	Maharashtra	High yielding
TPU-4	1992	Maharashtra, Madhya Pradesh	Large seed
TAU-1	1985	Maharashtra	Large seed
Cowpea			
TRC-77-4 (Khalleshwari)	2007	Chhattisgarh *(rabi)*	Suitable for rice based cropping system
TRC-901	2018	Northern zone (Gujarat, Rajasthan, Madhya Pradesh, West Bengal, Maharashtra and Uttarakhand)	Suitable for summer season and resistant to cowpea mosaic virus

Many of these varieties have been developed in collaboration with different State Agricultural Universities with whom BARC has entered into Memorandum of Understanding (MoU). BARC's stronghold has been in the development of early maturing varieties with disease and pest resistance. Apart from varietal development a large spectrum of mutants in these pulse crops have been generated and are being maintained for use in breeding and basic studies. Of late, work has also been initiated and good progress has been made in chickpea and cluster bean. The work related to induced mutation in different pulse crops have been discussed under various sections.

Effective doses for mutation induction

The effective dose for mutation induction depends on the nature of mutagen, the crop species and as well on the genotype within a crop species. Hence, a series of experiments need to be conducted to determine the effective dose for a particular crop and for a particular variety. Generally, a dose which is slightly below the lethal dose 50 (LD_{50}) or growth reduction 50 (GR_{50}) is found to be effective in inducing useful mutations. Based on the previous experiments, the doses for mutation breeding in different pulse crops have been standardized for gamma rays at our Centre. A dose range of 300-400 Gy is effective in case of mungbean and urdbean, whereas in pigeonpea a dose range of 100-200 Gy is helpful. In cowpea 200-300 Gy has been found to be efficient, while in chickpea 300-400 Gy has been effective in inducing useful mutations. A number of mutant varieties (either direct or mutant derivatives) have been released in mungbean, urdbean, pigeonpea and cowpea from our centre by employing the above mentioned effective doses. In recent times, radiosensitive assays of different crops for electron beam have been done and LD_{50} for different crops have also been ascertained (mungbean: 500 Gy; urdbean: 400 Gy; chickpea: 300 Gy and cowpea: 270 Gy).

Chlorophyll mutations

In any mutation breeding programme, the effectiveness of mutagen is gauged by its ability to induce chlorophyll mutations. A large spectrum of mutations affecting chlorophyll development is observed in M_2 generation. As in other crops, albinos (completely devoid of chlorophyll and resultantly dies after few days), xanthas (yellow coloured), chlorinas (yellow with tinge of greenness) and viridis (viruscent) have been widely observed in these pulse crops [8]. Xanthas have been found to be the most frequent followed by chlorina and albinos. Also, the overall frequency of chlorophyll mutations has been found to be higher in electron beam treated population in comparison to gamma rays treated population [9,10]. In addition, variegated chlorophyll mutations affecting different parts of plant have also been isolated.

Morphological mutations

A wide spectrum of morphological macro mutants in pulse crops have been isolated over the years and are being maintained at our centre. Mutations affecting leaf size, leaf shape, leaf margin, leaflet number, phyllotaxy, plant stature, plant type, growth habit, flowering time, maturity time, internodal length, flower colour, seed size, seed shape, testa colour, pod colour, pod placement, pod wall proportion, root length and sterility have been identified in different pulse crops at our centre [11, 12,13,14]. Many of these mutants are valuable genetic resources for use in mutation breeding or in basic studies for studying developmental biology. The mutants with pods above canopy [15] could be used for developing mechanical harvest compliant varieties. Similarly, the determinate mutants in cowpea [16] could be used to restructure plant ideotype for developing synchronous maturing genotypes. The early maturing mutants identified in chickpea, mungbean and cowpea are valuable donors for reducing the growth duration with potential for escaping terminal water stress. The long root mutants identified in mungbean, urdbean, cowpea and chickpea could be promising under receding water stress situations. The seed size and seed colour mutants identified in these pulse crops could be used to develop market ideotypes based on regional consumer preferences. It has also been observed in cowpea that a single mutation could lead to simultaneous changes in a number of characters due to epistatic interactions [17].

Mutations for disease resistance

The major trust of BARC in induced mutations has been in the field of disease resistance [18]. The first powdery mildew (PM) resistant mutant mungbean variety (TARM-2) in the whole of Asia was developed from BARC through induced mutagenesis [19] and subsequently, a series of PM resistant mungbean varieties were evolved by utilizing PM resistant mutant [20]. BARC is also acclaimed for developing yellow mosaic virus (YMV) resistant mutants in urdbean and mungbean [18,21]. These mutants have been successfully used in hybridization leading to development of YMV resistant varieties [21]. Mutants with resistance to cowpea aphid borne mosaic virus and leaf crinkle diseases have also

been identified in cowpea [22] and are currently being utilized in the crossing programme towards varietal development.

Mutations for pest resistance

BARC has received accolades for doing pioneering research towards development of urdbean genotypes resistant to the serious storage pest namely bruchids (*Callosobruchus maculatus*) [23]. Trombay wild urdbean (*Vigna mungo* var *silvestris)* collected from the Trombay hills was identified to possess resistance to bruchids and has been registered (INGR10133) as potential genetic donor for bruchid resistance. By involving these genotypes in the mutation breeding programme at our centre, a number of mutant derivatives have been developed and are being tested for bruchid resistance under the All India Coordinated Research Programme (AICRP) of ICAR [24]. Similarly, in mungbean, a unique genotype 'Thokalwadi wild' belonging to *Vigna radiata* var *sublobata* group has been deciphered to possess bruchid resistance [25] and is currently being utilized in hybridization with our high yielding mutants to develop bruchid resistant varieties.

Mutations for biochemical parameters

Phytic acid (PA), an anti-nutritional factor is known to conjugate phosphorus and other essential elements like iron and make it unavailable to organisms that feed on seeds rich in PA. Mutations affecting PA content have been identified and low PA mutants have been isolated in mungbean [26], chickpea [27], blackgram and cowpea [28]. These mutants are being exploited in varietal development programme so as to reduce the PA content to reasonable levels without affecting the physiological balance. PA has also been found to impart basal resistance against biotic stresses like diseases and pest in mungbean [25] and is also established to have implications in imparting drought tolerance in chickpea [29]. In a recent study undertaken to detect molecular diversity in a collection of chickpea germplasm and to use this information for developing genotypes suitable for drought conditions, a distinct clustering of a group of drought tolerant accessions of Indian origin indicated the presence of a common genetic base for drought tolerance in these accessions [30]. PA content has also been found to be positively correlated with canopy temperature in cowpea and therefore, may impart drought tolerance [28].

Raffinose family oligosaccharides (RFOs) are known to cause flatulence in organisms ingesting seeds rich in RFOs. Therefore, it becomes imperative to identify genotypes with low RFOs content. Mutants with low RFOs have been identified in urdbean [31] and cowpea [32] that could be potential donors for developing varieties with low RFOs. The RFOs content in blackgram ranged from from 26.64 to 61.57 mg/g with an average of 43.6 mg/g.. Low content of total RFOs was observed in blackgram mutant TU43-1 (26.64 mg/g) as compared to its parent TU94-2 (58.3 mg/g). TU43-1 which recorded low content of total RFOs also showed lowest verbascose and stachyose content respectively. TU1-820- 1-5 recorded high verbascose (31.02 mg/g) with low stachyose (11.13 mg/g) and raffinose (0.18 mg/g) content (31).The RFOs content in cowpea ranged from 4.27 to 9.67 mmoles/100gm with a mean value of 7.77 mmoles/100gm of seed flour. The lowest total RFO content was obtained in the cowpea mutant TC501 (4.27 mmoles/100gm)[32]. Contrarily, mutants with high RFOs in cowpea have been found to possess long roots and could play a prospective role in better performance potential under water stress conditions [28].

Tannins (or tannoids) are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. The breeding programme is aimed at reducing these anti-nutritional factors without disturbing their protective role. The low tannin containing mutants identified in cowpea [33] could be used to develop pro nutritious varieties.

Mutation for genetic biofortification

Genetic biofortification is a cost effective strategy to address the iron (Fe) and zinc (Zn) mineral micronutrient deficiencies that are most prevalent worldwide. Chickpea, a food legume grown and consumed around the globe is a good target for biofortification as it constitutes a rich and cheap source of proteins, particularly for the developing world and the populations that are vegetarian by choice or non-affordibility of animal protein. India is the largest producer and consumer of chickpeas. Genetic biofortification for improved Fe and Zn with moderate PA can be a better breeding target in chickpea. Genotypes with consistently high iron (>70 ppm) and/ or Zn content (>40 ppm) with low/moderate PA were identified [25,32]. In addition, 17 mutants ($M_{2:3}$ seeds) isolated for their large seed size (100 seed weight>25 g,

compared to 20g in control) were also screened for Fe and Zn content using atomic absorption spectroscopy. One mutant from electron beam (EB200/M36-1) and one from gamma ray irradiation (G400/36-1) were found to contain significantly higher Fe content (156 and 12 ppm respectively) than the control (range 81-102 ppm). Advanced progenies ($M_{3:4}$ seeds) of these two mutants also showed significantly higher Fe content (110.9 and 109 ppm respectively) than the control (70.8 ppm) [34].

Mutations for varietal development

Varieties developed through mutation breeding could be direct mutants with good agro-morphological traits or the resultants of hybridization with mutants for mutant trait introgression in more adaptable and elite genetic backgrounds. Positive mutations affecting yield attributing traits such as plant height, number of branches per plant, clusters per plant, pods per plant, seeds per pod, seed yield per plant, seed test weight have been identified in urdbean [11], mungbean [14], pigeonpea [35], cowpea [16] and chickpea [37]. In urdbean, large seed mutants, UM-196 (dark green leaf mutant) and UM-201 were hybridized with an elite cultivar T-9 resulting in development of high yielding varieties TAU-1, TAU-2 and TPU-4. Likewise, in pigeonpea a fast neutron induced large seed size mutant variety TT-6 was hybridized with ICPL 84008 and three early maturing and high yielding varieties (TT-401, TJT-501 and PKV-TARA) have been developed in pigeonpea. In mungbean, crosses involving Kopergaon and TARM-2 have resulted in the development of early maturing variety TMB-37 for North East Plain zone. This variety has recently been readopted by State of Punjab owing to its earliness and yield superiority. The cowpea variety TRC-77-4 is a classical example wherein a dwarf determinate mutant has been directly released as a variety in the state of Chhattisgarh. Some of the mutant varieties like TM-96-2, TM2000-2 (mungbean), TU-40 (urdbean) and TRC-77-4 (cowpea) are also suitable for special niches like rice fallows. BARC also has the distinction of developing the first summer suitable cowpea variety TC-901 (gamma ray induced direct mutant of EC394763), identified for North Zone under the National Network Project on Arid Legumes of ICAR [38]. Many of the mutants and mutant derivatives in these pulse crops are at various stages of trials under ICAR and State Agricultural Universities.

Novel mutations

Three gamma ray induced determinate mutants were identified in cowpea that carried novel SNP mutation in the widely conserved Arabidopsis Terminal Flower 1 (TFL1) gene homolog. The non-synonymous exonic point mutation resulting from transversion of cytosine (C) to adenine (A) (Pro-136 to His) in determinate mutants had a deterimental effect on TFL protein function and stability leading to loss of function [39]. In addition, agronomical mutants like early maturity, tall and erect plant type, salt tolerant mutant and slow transpiring mutant have also been identified in different cultivars of chickpea [36]. In mungbean, mutants with early and synchronous maturity, large seed size, top pod bearing, long root and drought tolerant were also identified. The drought tolerant mutant showed significantly greater root length (40 cm), leaf thickness (0.704 to 0.803 mm) and lower leaf canopy temperature (32.4°C) than control (20.5 cm root length, 0.573 mm leaf thickness and 40.9 °C leaf canopy temperature) [14]. In cluster bean terminal flowering mutant was identified in the electron beam treated population. This mutant will be helpful in developing determinate type cultivars in cluster bean.

Biotechnological tools in crop improvement

The advent of molecular markers has effectively complemented conventional approaches in accelerating the selection and breeding programmes. Successful breeding depends on the existence of large genetic diversity and identification of diverse genotypes for developing suitable recombinants. The pulse crops exhibit great morphological diversity but are found to be very similar at the genetic level. Molecular markers have the potential to identify mutants / genotypes that are genetically distinct and enable selection of parents that has high probability of generating potential recombinants.

Molecular markers based on RAPD, SSR, ISSR, REMAP and SNPs have been used to evaluate the genetic diversity among various mutants and genotypes in urdbean [40,41], mungbean [42], pigeonpea [43], cowpea [44] and chickpea [30]. Molecular markers have also been used to tag important traits such as disease resistance, pest resistance, growth habit etc in different pulse crops (Table 2).

Table 2. List of traits/gene mapped /tagged with molecular markers in pulses

Crop	Trait/gene	Markers	References
Blackgram	Yellow mosaic disease resistance	ISSR ISSR811$_{1357}$ and SCAR (YMV1F-1R) markers	[52]
	Bruchid resistance	QTLs (Cmrae1.1,Cmrae1.2, Cmrdp1.1, Cmrdp1.2. Cmrdp1.3, Cmrdp1.4, Cmrdp1.5 and Cmrdp1.6	[50]
Mungbean	Yellow mosaic disease resistance	RAPD marker (OPB-07$_{600}$ and SCAR (MYMVR0583) marker	[53]
Pigeonpea	Cytoplasmic male sterility	OPC11$_{600}$	[54]
	Plant type	OPF04$_{700}$ and OPA09$_{1378}$	[43]

Association studies in chickpea has showed that microsatellite variations present in the 5' UTR of inositol monophosphatase gene differentially regulates its expression, associates with phytic acid content and contributes to drought tolerance in chickpea [29]. Transcriptome sequencing of the immature seeds of black gram cv. TU94-2, by Illumina paired end sequencing technology has generated transcriptome sequences for gene discovery and genic-SSR and SNP marker development in urdbean. PCR primer pairs were successfully designed for 933 SSR loci from the transcriptome sequence of blackgram variety TU94-2 [45]. For identifying SNPs, high quality reads of *Vigna. mungo* var. *silvestris* were aligned with the assembled transcripts of TU94-2 publicly available in NCBI short read archive (submission ID. SRR1616991, SRX710526). Out of 1845 SNPs identified, 1291 SNPs fall in ORF regions of TU94-2 transcripts. A total of 50 primer pairs flanking single SNP were designed and genotyped by HRM (High Resolution Melting) analysis in two black gram genotypes [46]. These valuable genomic resources could be exploited for studying genetic diversity, evolution, linkage mapping, comparative genomics and marker-assisted selection in black gram.

Molecular tools also help in the generation of DNA fingerprints that can uniquely identify specific mutants or genotypes or cultivars and are pre-requisites for release of varieties. RAPD based DNA fingerprints of important cowpea mutants have been developed wherein each mutant could be identified using single or combination of primers [44]. Reverse transcription-PCR have also been used to identify specific pathogens such as cucumber mosaic viruses which are hitherto difficult to diagnose based on symptoms [22]. Real time-PCR has also been employed to study differential expression analysis in urdbean and chickpea. High resolution melting (HRM) analysis has been used in conjunction with RT-PCR for SNP genotyping in urdbean [47]. Molecular markers provide valuable tools for rapid and accurate selection of genotypes carrying the required allele through marker assisted selection/breeding. The genomics tools available for mungbean and urdbean genetic improvement have been reviewed in detail [48].

Developing linkage map and identifying QTLs associated with bruchid resistance in blackgram

Linkage maps provide the platform for mapping and tagging of useful traits using the information of tightly linked markers. An inter-subspecific mapping population was generated by crossing cv. TU 94-2 (bruchid susceptible) and *V. mungo var. silvestris* (bruchid resistant). About 37.8% of the bruchid completed their lifecycle on seeds of *V. mungo var. silvestris* compared with 100% on the susceptible variety TU 94-2. The total developmental period of *Callosobruchus maculatus* on *Vigna mungo var. silvestris* was considerably extended (88 days as compared with 34 days on TU 94-2). A genetic linkage map of black gram was constructed with 428 molecular markers using an F_9 recombinant inbred population of 104 individuals [49]. The linkage analysis at a LOD score of 5.0 distributed all 428 markers (254 AFLP, 47 SSR, 86 RAPD, and 41 ISSR) into 11 linkage groups. The map spanned a total distance of 865.1 cM with an average marker density of 2 cM. The largest linkage group spanned 115 cM and the smallest linkage group was of 44.9 cM. One hundred four individuals were used for detection of QTLs associated with bruchid resistance. The RILs exhibited a high level of variation in percentage adult emergence (0–100%) and developmental period (0–105 days). Two QTLs, Cmrae1.1 and Cmrae1.2, were identified for percentage adult emergence, on linkage group (LG) 3 and 4,

respectively. For developmental period, six QTLs were identified, with two QTLs (Cmrdp1.1 and Cmrdp1.2) on LG 1, three QTLs (Cmrdp1.3, Cmrdp1.4, and Cmrdp1.5) on LG 2, and one QTL (Cmrdp1.6) on LG 10 [50].

Impact of mutant varieties

Many of the mutant varieties developed in pulses are very popular among the farming community owing to their superior yield attributes. The mutant variety TAU-1 (developed in collaboration with Dr. PDKV, Akola) in urdbean released way back in 1985 is still very popular among the Maharashtra farmers. This variety is extensively grown throughout Maharashtra and occupies more than 50% of the area under urdbean. The variety TJT-501 (developed in collaboration with JNKVV, Raipur) occupies almost 60% of the area under pigeonpea in Madhya Pradesh and is one of the topmost varieties receiving 12% of national breeder seed indent [51]. In mungbean, the variety TMB-37 is widely preferred by farmers throughout the country for its earliness and suitability for summer cultivation though it was originally released for North East Plain Zone. This variety has been readopted and re-released in the state of Punjab. Likewise, the pigeonpea variety TT-401 initially released for central zone, has been found promising in southern states like Tamil Nadu, Andhra Pradesh and Karnataka where it has given record yield of 2500kg/ha. The mungbean varieties TM96-2 (in collaboration with ANGRAU, Lam, AP) and TM 2000-2 (in collaboration with IGKVV, Raipur) with resistance against powdery mildew and *Corynespora* leaf spot occupy large areas under rice fallows in Andhra Pradesh and Chhattisgarh, respectively. The recently released high yielding urdbean variety TU-40 is gaining popularity in the southern states. The farmers of Maharashtra cultivating PVK-TARA pigeonpea are reaping high yields especially under drip irrigation.

Conclusion

Mutation breeding in conjunction with conventional breeding has immense potential to enhance the genetic variability in pulses with narrow genetic base, paving way for developing elite varieties suitable for different agro-climatic zones and improving the pulses production and nutritional security of our country. Over the years, pulse crop varieties developed through mutation breeding has greatly benefitted the farming community by enhancing their livelihood. During the last decade, induced mutations have also been gaining increasing importance in plant molecular biology as a tool to identify and isolate genes and to study their structure and function. Knowledge of genes controlling important agronomic and quality traits is critical for plant breeders to develop proper strategies and efficiently implement breeding programmes. Multiple mutant alleles are the sources of genetic diversity for crop breeding as well as functional analysis of the targeted gene in many cases. Therefore, induced mutations can contribute further to increasing global food production both directly and indirectly by increasing yield potential and stability.

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Mutation breeding technology in rice for improvement of plant architecture and better grain quality

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Abstract

With the inevitable risk posed by global climate change affecting crop yield and ever increasing demands of agricultural produce, today crop improvement techniques need to be more precise in developing smart crop varieties. Rice, a staple food for a majority of population across the world, holds a significant role to play in alleviating the global hunger problem. With the burgeoning size of world population at an unprecedented rate, limited fertile land resources, climate resilience, emerging new races of pests and diseases and consumer preferences for quality attributes, it is imperative to increase crop diversity with better selection efficiency addressing the challenges of future rice production. Mutation breeding is a fundamental and very successful tool helping in increasing crop diversity and allowing plant breeders to exercise their skill in developing desirable crop varieties. The induction of mutations has been used to enhance the yield, better nutritional quality and wider adaptability of world's most important crops such as wheat, rice, pulses, millets and oilseeds. India is considered to be one of the primary centres of origin with very high genetic diversity in traditional landraces for different agronomic traits of economic importance. Plant architecture including plant height, branching habit (tiller number), leaf shape and patterns, floral and grain traits etc and quality traits including aroma, amylose content, cooking quality, etc are of tremendous importance for rice improvement programme. Traditional landraces in rice have premium grain quality fetching premium price but the cultivation is being marginalized due to tall stature resulting crop lodging, late maturity and poor yield. Mutation breeding technology has been successfully implied in rice improvement programme which has resulted in improvement of aromatic rice varieties viz., Pusa Basmati 1, Dubraj, Jawaphool etc. Two high yielding mutants rice varieties i.e., TCDM-1 (Trombay Chhattisgarh Dubraj Mutant-1) and TKR Kolam (Trombay Karjat Rice Kolam) has been released for cultivation in Chhattishgarh and Konkan region of Maharashtra state respectively. Both these varieties possess dwarf plant stature (~110 cm), medium maturity (~130 days), premium grain quality, resistance to major pest and diseases. Improvement of other traditional rice varieties are underway which will bring these varieties back into cultivation and help in improving the tribal and marginal farmer's economy.

Key words: Mutation, Rice, Plant Architecture, Grain quality, Landraces.

Introduction

Rice (*Oryza sativa* L.) is most important cereal crop which is grown under a wide range of climatic and geographical conditions on all five major continents. More than 60% of global population and more than 75% of Asian population are dependent on rice based cropping system for food and economy. Global rice production statistics reveal production of 486.2 million metric tonnes milled rice in 2016-17 (https://www.statista.com/statistics/271972) with estimated world acreage of 161.1mha (https://www.statista.com/statistics/271969). India, with a rice production volume of approximately 111.3 mtons, ranked second in global rice production in 2016-17, with estimated harvest area to be about 44.5 mha of rice (https://www.statista.com/statistics/255937). With increasing world population at an unprecedented rate, limited land resources, climate change, emerging new races of pests and diseases and consumer preferences for quality attributes, it is necessary to increase crop diversity with better selection efficiency addressing the challenges of future rice production. In order to meet the dietary requirements of increasing population and challenges emerging due to the adverse

impacts of climatic changes, genetic gains in the grain yield per hectare of major crops like rice, need to rise faster than the current rate (1,2).

Traditional landraces of rice in India

India has a rich and diverse genetic wealth of rice and it has been estimated from various surveys that nearly the country had been endowed with more than 2 lakh (200,000) rice varieties, a rich biodiversity that no other country on earth (3-6). The switch over to high yielding varieties with the spread of modern agriculture, has posed a great threat to the security of the age old practice of growing traditional varieties and landraces which may have immense potential for different important traits (7-9). These diversity hotspots have been found in North-eastern region, central India and Southern India accompanying unique scented and aromatic, medicinal and quality rice. Some important rice landraces are enlisted in Table 1. Traditional and aromatic rice varieties are very unique in terms of its pleasant aroma, fine grain size, better cooking quality and consumer preference for special culinary preparations. Farmers get premium price due to its excellent cooking quality and low production owing to its poor yield, lodging losses, cultivation by tribal and marginal farmers etc. Improvement of these agronomic attributes will help in increasing the production potential of these varieties and bring them back into cultivation.

Table 1: Important rice landraces, their quality attributes and their undesirable traits

S. No.	Name of variety	Special character (HRR)	Undesirable character (negative trait)
1	TulsiManjari	ASG, Good grain quality, suitable for *Kheer,* Head rice recover (>65%)	Late maturity, lodging, poor grain yield
2	Gopal Bhog	ASG, Good grain quality, strong aroma, famous among the farmers, Head rice recover (>65%)	Late maturity, lodging, poor grain yield
3	Tilkasturi	ASG, Good grain quality, famous among the farmers, Head rice recover (>65%)	Late maturity, lodging, poor grain yield
4	Govardhan Kali Kamod	ASG, Nutritious, Good grain quality, Head rice recover (>65%)	Late maturity, lodging, poor grain yield
5	KumhdaPhool	Bold, Good for scented Poha/ Chiwda, Good grain quality, HRR (46 %)	Late maturity, lodging, poor grain yield
6	Alsakar	Bold, Good for aromatic Poha/ Chiwda, Good grain quality, HRR (45 %)	Late maturity, lodging, poor grain yield
7	Danighoda	Bold, Good for Poha/ Chiwda, Good grain quality, HRR (45 %)	Late maturity, lodging, poor grain yield
8	AdangaDhan	High biomass, awned, Bold, Good for Poha, HRR (48 %)	Late maturity, lodging, poor grain yield
9	RudraDhan	Good grain quality, short bold, good Idali making quality, HRR (49 %)	Late maturity, lodging, poor grain yield
10	Roti Dhan	Suitable for roti and Poha making, coarse grain, HRR (42 %)	Late maturity, lodging, poor grain yield, chalky grain
11	MakdoDhan	Bold grain, Good for Poha, and Good grain quality, HRR (45 %)	Late maturity, lodging, poor grain yield
12	Dubraj	Aromatic, better grain quality, very fine grain	Late maturity, lodging, poor grain yield
13	Jawaphool	Short slender and aromatic grain, better grain quality	Late maturity, lodging, poor grain yield
14	Ilayachi	Good for mouth ulcer, Improve digestion, short grain, good grain quality, (58 %)	Late maturity (140-150 days), tall (140-145 cm), poor yield
15	Bangalaya	Aromatic Short grain rice	Late maturity and Tall stature
16	Kalanamak	Aromatic Short grain rice	Very late maturity and tall stature

Mutation breeding

Genetic improvement work without a wide variability in the population is not possible. Intraspecific variations are pre-requisite for initiating any plant improvement programme, which forms the basis for selection and improvement (10). The number of genes expressed during the lifetime of a particular plant is estimated to be between 16,000 and 33,000 (11). The genetic architecture of living organisms is influenced by mutations, in a confounding manner. Mutations form the foundation for selection, which ultimately is the driving force for progressive breeding among crop plants. This driving force may have a natural (spontaneous) origin or it may be artificially induced. Spontaneous mutations, the naturally occurring heritable changes to genetic material are rather rare and random events and take millions of years to evolve. Not only this gradual process can be greatly accelerated through artificial induction of mutation, but it also supports the maintenance of biodiversity. Germplasm resources act as a reservoir having tolerance to various emerging stresses which can be used in breeding programme to help introgression of traits in elite crop varieties. Conventional breeding is limited by tight linkages (genetic load) of undesirable traits and genetic differentiation at the expense of genetic diversity (12). For rice breeders, it is a continuous task to find the new sources of genomic variation that can be directly used in rice breeding programs (13). Mutation breeding is an efficient technology in the hands of plant breeder which helps in breaking undesirable linkages and also results in genetic changes desirable for breeders in the background of an elite variety. In rice, more than 820 mutant varieties have been developed all over the world through mutation breeding (http://mvd.iaea.org/).

Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms. The forward genetic approach enables the identification of improved or novel phenotypes that can be exploited in conventional breeding programmes. Powerful reverse genetic strategies that allow the detection of induced point mutations in individuals of the mutagenized populations can address the major challenge of linking sequence information to the biological function of genes and can also identify novel variation for plant breeding (14).

Role of mutation breeding in rice improvement programme

The impact of induced rice mutants in applied research is best exemplified by the development of improved rice varieties through mutation breeding. During the past five decades, more than 800 varieties of rice have been developed across the globe, either directly from induced mutations or as a result of crossing such mutants with other breeding lines (15). The first rice varieties KT 20-74 and SH 30-21, developed through induced mutation, were released in China in 1957 and the first variety Yenhsing-1, developed by a cross- breeding programme with a mutant (16). Soon afterwards, the semi dwarf mutant Reimei was released in Japan (17) which have significantly increased yield because of their lodging resistance. Calrose 76 and Basmati 370, semi dwarf varieties of rice with short and stiff straw has revolutionised the rice production in USA and Pakistan respectively. In Pakistan, a new variety 'Kashmir Basmati' which matures early and has cold tolerance, and retains the aroma and cooking quality of the parent, was derived from induced mutation in Basmati 370 (18). Several high yielding rice mutants were released in India under the series PNR and some of these were early in maturity and had short height (19). Among these, two early ripening and aromatic mutation-derived rice varieties, 'PNR-381' and 'PNR- 102', are popular for cultivation in Haryana and Utter Pradesh. A Rice mutant, 'Zhefu 802' was cultivated on more than 10.6 million ha in China in a span of ten years. In Thailand, gamma ray irradiations expedite the release of an aromatic indica variety of rice 'RD6' in 1977. It was extensively grown on 2.4m ha during the year 1994-95. Similarly mutant 'RD15', released in 1978, was grown over 0.2 million ha, equivalent to 3.2% of the area under rice (20). In Australia nine rice mutant varieties Amaroo; (1987), 'Bogan'(1987), 'Echua'(1989), 'Harra'(1991), 'Illabong' (1993), 'Jarrah' (1993), 'Langi' (1994), 'Millin' (1995) and 'Namaga' (1997) have been developed. The induction of thermo sensitive genic male-sterile (TGMS) mutant in Japonica rice mutant PL-12, which is controlled by a single recessive gene has an immense contribution in designing the strategies for the production of hybrid rice varieties (21). In China '26 Zhaizao' was developed by gamma ray irradiation of indica rice (22). These mutants play an important role in two line heterosis breeding. In India, out of 335 mutant varieties released, out of which 59 are in rice.

Improvement of aromatic and traditional landraces in rice in India

India is presently facing a rice/ food crisis mainly due to the erosion of its biodiversity and increase of mono-cropping in agriculture. As climate change in the environment has led to frequent floods and prolonged droughts, the practice of monoculture primarily due to cultivation of popular high yielding and hybrid rice varieties render them vulnerable to these natural calamities leading to partial or total loss of crops. The major reason for the disappearance of thousands of local rice varieties is their steady replacement with the high-yielding varieties (HYVs) introduced in 1960's coinciding with Green Revolution (GR). This has slowly led to the gradual extinction of tradition landraces and aromatic rice varieties due to their poor yield, undesirable plant architecture, lodging problems resulting yield losses and late to very late maturity resulting not fitting into cropping pattern. In India, every state has some landmark rice landraces and premium aromatic varieties which are being cultivated by the marginal farmers only. Improvement of plant architecture in these traditional landraces will help in improving the area under these varieties.

Development of TDCM-1 (Trombay Chhattisgarh Dubraj Mutant-1) rice variety

'Trombay Chhattisgarh Dubraj Mutant -1' (TCDM-1) is an improved mutant of highly priced and aromatic local rice variety 'Dubraj' which is known for its premium grain quality and aroma. This variety was released by state variety release committee of Chhattisgarh state in 2018 under joint collaboration of BARC, Trombay and IGKV, Raipur. The salient features of TCDM-1 include dwarf plant type (90-95cm), longer and denser panicles (235-240 grains/ panicle), more numbers of tillers/ plant (10-12 tillers/plant), mid-late maturity (135-140 days), better aroma, better grain quality, resistant to major pest and diseases and higher yield (4.7t/ ha). This variety has become very popular among the farmers of Chhattisgarh state during adaptive trials because of its lodging tolerance resulting no yield losses during late monsoon rains, improved aroma and better post cooking grain quality (Fig. 1).

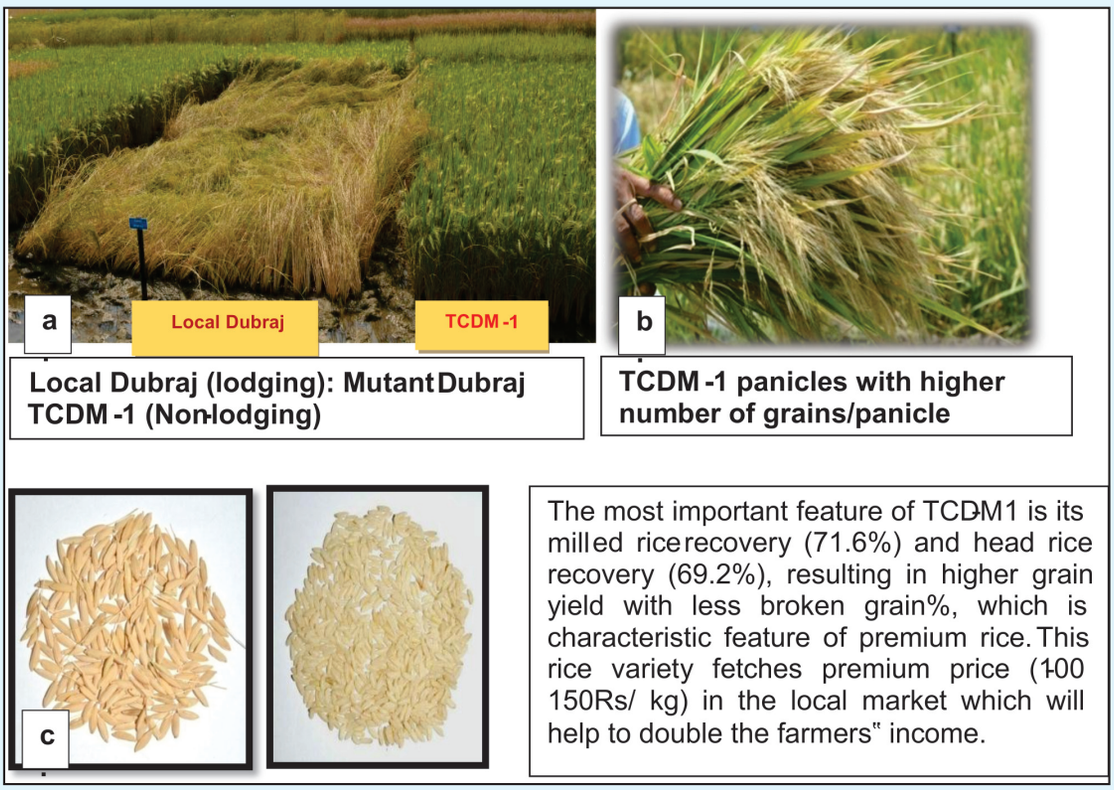

Fig 1. (a) Field view of Local Dubrajand Mutant Dubraj TCDM-1 (b) TCDM-1 Panicles (c) TCDM-1 paddy and grains

Development of TKR Kolam rice (Trombay Karjat Rice Kolam) variety

Konkan region of Maharashtra is the major rice producing area of Maharashtra state. Nearly 3.79 lakh ha area of Konkan is under rice crop with production of 16.10 lakh tones rice. Consumers in the Central and South-West India prefer small and fine grain (short slender) rice varieties, colloquially named as Kolam type rice. Traditionally Kolam rice varieties have specific ecological niche which is required for their specific grain quality. Wada Kolam, Surti Kolam, Silk Kolam, Lachkari Kolam, HMT Kolam, are popular Kolam rice predominant in western and southern India. These rice are traditionally very tall (>150cm) and very late (>150 days) in maturity. Their yield potential is also very poor. These varieties are highly susceptible to lodging during late monsoon rains resulting complete loss to the farmers. BARC has embarked upon a strong mutation breeding programme in rice. BARC in collaboration with RARS, Karjat of DBSKKV, Dapoli has developed an improved version of Kolam rice which is dwarf (110cm), mid late in maturity (130-135 days), lodging resistant and high grain yielder (4.5 – 5.0t/ha) as compared to traditional Kolam rice which yields <2.5t/ha. This variety has been developed through mutation breeding of rice variety PB1 for over 6 years. This varieties underwent yield trials under Maharashtra state and national all India co-ordinated rice improvement (AICRIP) yield trail testing, followed by farmers adaptive and agronomic trails for over 4 years. This variety has surpassed the high yielding Kolam type rice variety Karjat 4 with more than 20% yield advantage. TKR Kolam rice (Trombay Karjat Rice Kolam) variety was released for cultivation for the farmers in the Konkan region of Maharashtra in the year 2018 (Fig. 2).

TKR Kolam rice is super fine small slender grain type rice with 1000 grain weight (test weight) <11g as compared to traditional rice varieties with 22-24g test weight (Fig. 3).In spite of very low grain weight, the grain yield of this variety is very high due to its compact panicle, more number of grains per panicle, more panicle/m^2 and no lodging losses along with high milling and head rice recovery.

Fig. 2. Field view of Trombay Karjat Kolam rice

Salient features of TKR Kolam rice variety:
Dwarf stature (100cm) and non-lodging.
130 to 135 days to maturity (Mid-late duration).
Short Slender (SS) grain type.
High yielding (4.5-5.0 t/ha) with superfine grain
High milling (73.7 %)
High head rice recovery (67.98 %)
Translucent kernel type
Amylose content (23.09%)
Moderate Gel consistency indicating superior grain quality and highly palatable rice grains
Moderately resistant to prevalent pest.
Moderately resistant to Bacterial leaf blight, rice blast, Gall midge, leaf spot, bacterial leaf streak etc.

Fig. 3. (a) Paddy and grains of TKR Kolam rice (b) Major agronomic features of rice variety TKR Kolam

TKR Kolan rice may replace the traditional Kolam type rice varieties in Konkan region which are highly susceptible to lodging and diseases and pests resulting economic losses to the farmers.

Improvement of other traditional landraces of rice

India is known for its richness in diversity for rice especially for aromatic and quality rice. BARC has strong mutation breeding programme for improvement of traditional landraces of Chhattisgarh and Maharashtra state in collaboration with Indira Gandhi Krishi Viswa Vidyalaya and Dr. BalaSahab Konkan Krishi Vidyapeeth, Dapoli. Gamma ray and proton beam induced mutagenesis have been employed to improve traditional landraces of rice such as Luchai, Hundar, LoktiMachi, Jawaphool, Sonagathi, Badsahbhog, Safri, Tilkormel, Swarna, Chinoor, Ambe Mohar etc (Fig. 4). We have been able to develop dwarf and mid-early high yielding mutants of these varieties which are in state and national yield trials. JhilliDhan,

Wada Kolam, Zinia, Bangalaya, Palghar series of fine quality rice are very popular in different pockets of Maharashtra state. These traditional landraces are also being improved by Radiation induced mutation breeding.

Conclusion

At present, the genetic variability present in rice is narrowed using conventional breeding approaches and use of more popular and hybrid rice varieties in India for a longperiod. Induced mutagenesis is one of the most important approaches for broadening the genetic diversity in rice to circumvent the bottleneck conditions. This become more evident as the future rice production will have immense pressure from consumers' preferences, changing climatic conditions, resurgence of new races of pests and diseases, demand for bio-fortified rice etc. Induced mutagenesis has demonstrated the potential in broadening the plant genetic base and there by avail plant breeders the raw materials required to address different problems related to rice production and consumer preferences. Crop varieties generated through the exploitations of mutation breeding are significantly contributing to global food and nutritional security and improved livelihoods.

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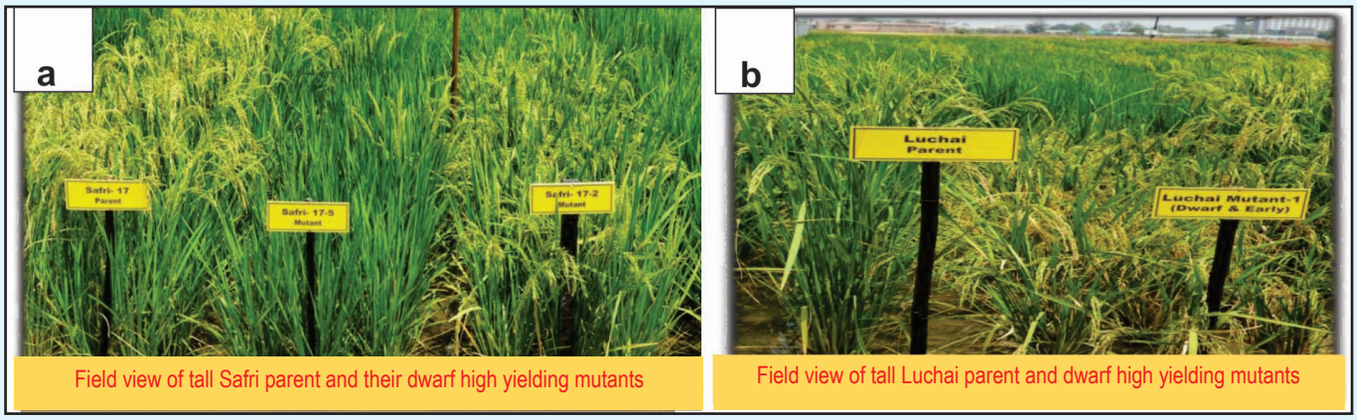

Fig 4. (a) Improvement of Safri rice variety (b) Improvement of Luchai rice variety

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