Molecular Tools In Conventional Breeding

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Application of Nuclear Science for Sustainable Development in Agriculture: Addressing Current and Future Challenges

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Field view of Vikram-TCR (on right hand side) along with its parent Safri-17 (on left hand side)

ABSTRACT

Agriculture, which is pivotal to human sustenance, has shaped the evolution of civilization. Food security of the entire world population is largely dependent on continuous innovations/improvements in the agriculture sector. Global crop production faces serious challenges due to the onset of various biotic and abiotic stresses, including climate change. In the near future, due to changing agricultural practices, all these challenges are likely to be aggravated further. Nuclear science has been used for several decades to improve different aspects of agriculture for enhancing crop productivity. The current article discusses applications of nuclear radiation for developing improved crop varieties via the induced-mutation breeding approach. Furthermore, the use of nuclear radiation to (a) develop formulations that boost plant growth or protect crops against diseases (b) manage major insect pests and (c) prevent post-harvest losses, is reviewed. In addition, the use of radioisotopes in studying nutrient uptake, transport and its distribution within plants is also discussed. This article concludes by emphasising the need to integrate molecular tools in conventional breeding approaches for addressing the emerging challenges.

KEYWORDS: *Agriculture, Crop-improvement, Radiation in agriculture, Mutation breeding, Sustainable agriculture*

Introduction

Agriculture has been the bedrock of human existence, allowing civilizations to flourish since ancient times. In fact, the food security afforded to millions of people by agriculture, not only provides sustenance but is also responsible for economic and political stability. Production of food has been the foremost priority of every civilization over the course of history. Agriculture began about 10,000 years ago with domestication of plants. Subsequently, harnessing of animals for various activities (e.g. plowing) led to the first agricultural revolution, boosting crop productivity in the ancient world. Presently, in more recent times, milestones such as mechanization in the 19th century, green revolution in the 20st century and the fusion with biotechnology in the 21st century, have been major events that have helped agriculture to cope up with the increasing demand for food grains [1]. However, due to burgeoning population, increasing crop productivity will continue to remain a top priority in the present as well as in the coming future. Although, India, strengthened by its robust agriculture framework, has been able to fulfill food requirement of its people, it is estimated that more than 800 million people globally do not have means to fulfill their daily dietary needs. By 2050 global population may reach 9.8 billion, which will create an estimated additional 50% requirement of food grains [2]. This escalated demand must be met not only without any significant increase in cultivable land, but also by facing challenges posed by climate change (e.g. limited water for irrigation, abnormal temperatures etc.) and multiple emerging diseases. Thus, the only sustainable and economical option to augment the current agricultural production is to develop crop varieties with enhanced yield (even when subjected to various stresses), and minimize the post-harvest losses. Ever since its discovery by Henri Becquerel in 1896, radioactivity has played an immense role in the development of different disciplines of science, including agriculture. Integrating radiation/radioisotopes into agricultural research has provided innovative solutions for crop improvement, plant environment interaction studies, insect pest management and food preservation [3]-[5]. The current articles sheds light on the application of radioisotopes in some of these areas and how they hold promise to address the future challenges.

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Radiation-induced Development of New Crop Varieties (Crop Improvement)

Since, the discovery of radiation-induced mutation in Drosophila by Hermann Muller in 1920s, radiation-induced mutation breeding has been an important tool for crop improvement over 100 years [6]. Around that same time, Lewis John Stadler pioneered mutation breeding in plants by using X-rays on maize, wheat and barley [7]. By using physical mutagens, the mutation rate can be increased by 1000 to million-fold. Also, as compared to chemical mutagens, the spectrum of mutations obtained by physical mutagensis are very different. Today, more than 70% of mutant crop varieties have been developed using radiation-induced mutagenesis.

The radiation-induced mutation breeding programs for improving crops have spread to over 70 countries, and more than 3500 mutant varieties of ~210 plant species have been released so far [8]. With the first mutant variety of Tobacco "Chlorina" developed using X rays in 1934, many economically beneficial radiation induced mutants have been released over the years. Some of the key mutant varieties include, the semi dwarf and good-malting varieties of barley "Golden Promise" and "Diamant", derived using gamma and X rays, respectively. These were released in Europe with estimated economic value of 417 million US$ during 1977-2001 [9]. In USA, the semi-dwarf rice variety "Calrose 76", generated employing gamma irradiation, provided more than 15% yield advantage. In India the TG series of groundnut varieties developed by BARC using radiation-induced mutagenesis has shown immense success and are preferred for cultivation by farmers in many areas of the country. Similar success stories of the radiation-induced mutant breeding programs have resulted in the development of salinity-tolerant rice with high yield and short duration in Vietnam, a high-altitude Barley variety in Peru and many other such examples can be cited. Other indirect use of radiation methods in plant breeding include the seminal work carried out by ER Sears in 1956, which involved the radiation-induced translocation of *Aegilops* species genome into the common bread wheat [10]. Many of the rust disease resistance genes used in the presently-cultivated wheat varieties have been transferred from these radiation-derived translocation lines, are used extensively in wheat breeding all over the world. Recognizing the importance of radioisotopes in crop improvement, in 1964, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture was established. In India, at BARC, Mumbai, with collaboration of Indian Council of Agricultural Research (ICAR), New Delhi, as well as various state agricultural universities, has established an extensive radiation-induced mutation breeding program for various crops. This research program has resulted in the release of 62 varieties, including cereals, pulses, oilseeds etc. and many more improved germplasms currently in pipeline for release [11]. To address the global future challenges posed by climate change and the increasing food demand, some of the priority traits that can be addressed by using radiation-induced mutagenesis are elaborated below.

Growing crops with lesser water requirement

It is predicted that due to higher temperatures brought on by climate change, the evapo-transpiration loss of water from farms and plants would be much higher, creating an estimated 10-30% higher demand of irrigated water. Several, models also predict aberrations in precipitation pattern and reduction in annual rainfall, which will further limit the current resources available for irrigation [12]. Thus, it is important to breed varieties with lesser water requirement, which can be economically useful in drought-like conditions. Radiation-induced mutagenesis has been employed to develop drought tolerant mutants in rice, wheat, maize and sorghum, which are major cereals (and also have a high irrigation demand), thus producing more crop per drop [8].

Plants with resistance to diseases

Plant diseases caused by pathogenic viruses, bacteria and fungi, are estimated to cause 30-40% loss in yield annually. Due to changing agro-climatic conditions, threat from emergence of new pathotypes and spread of diseases to new areas has increased. Further, resistance sources in the existing 'crop genepool' for these emerging diseases are limited and too cumbersome to incorporated using the traditional recombination-based breeding methods. Mutation breeding using radiation has been extensively used for developing disease resistance in various crops with an added advantage of conserving the desired genetic background. Resistance to stem and stripe rust disease of wheat, powdery mildew of barley and yellow mosaic virus in mungbean has been induced using the radiation-mutagenesis approach. In future, this approach holds promise to combat emerging diseases in other major crops [13].

Plants with tolerance to salt stress

It is estimated that approximately 30% of the global farm area is affected by salt stress, either in form of salinity or sodicity of soil, thus making it one of the most important abiotic stress. Climate change is expected to not only aggravate the severity of salt stress, but also increase the total area affected by this stress. Mutation breeding for developing salt-tolerant varieties holds promise, as most of the genetic resource for salinity tolerance are in local landraces with poor agronomic values. Success in obtaining salt-tolerant mutants has been reported in China, Korea, and many other participating countries of FAO/IAEA mutation breeding program, and may be extended in salinity affected area in India too [14], [15].

Crop varieties to combat "hidden hunger"

A large swathes of population, especially in marginal countries, consume only one variety of cereal as staple. Thus, their diet lacks diversity in terms of pulses, vegetables, dairy as well as other animal products. Although they full fill their calorific requirement, such diets often lack in the daily dietary requirements of essential micronutrients, leading to "hidden hunger". The currently cultivated high-yielding varieties of these cereals lack adequate amounts of micronutrients, primarily iron and zinc. Many countries including India, are trying to overcome this deficiency by fortification of grains with micronutrient using various approaches. Biofortification or enhancing micronutrient content 'biologically' within the plant is one of the most economical and effective strategy. Local landraces and traditional farmers' varieties of these cereals have been identified with high micronutrient contents, but their agronomic traits are poor, which limits their use in widespread cultivation. Using radiation-induced mutagenesis, yield attributes and other agronomic traits of these landraces can be improved, leading to development of biofortified mutants with acceptable yield performance. In BARC, R&D work is being carried out to validate the nutritional and medicinal properties of traditional rice varieties and simultaneous improvement of their agronomic traits using radiation-induced mutation breeding. Similarly, local landraces with premium qualities such as aroma, special product-making capability (such as *poha, kheer, murmura* etc) are also being undertaken, which will potentially help in increasing farmers income.

Crop varieties with tolerance to herbicides

Increasing global temperatures and changing weather patterns, have led to the rampant growth of weeds, which has also resulted in their spread into newer areas. Weeds compete with the crop for resources and are estimated to cause 10–80% loss in yield. The traditional methods of control involve manual intervention, which has become difficult due to reduction of manpower in the agriculture sector. Chemical control remains the only viable option with acceptable results, and currently this is the most widely used strategy to control weeds. The major challenges in the use of selective-weedicides (i.e. these do not harm the crop but kill the weeds), is their incomplete effectiveness to all types of weeds, and the threat of herbicide tolerance arising in weeds. In case of broad-spectrum weedicides, the weed control is complete, but the crops are also affected, resuling in lower yield. Using chemical mutagenesis, many herbicide-tolerant mutants have been developed in various crops (e.g. wheat, rice, soybean and maize). Radiation-induced mutagenesis is also being explored for developing resistance to various selective and broad-spectrum herbicides [13], [15].

Other agronomically important traits

Population growth and increased human activities has led to other challenges in agriculture. Some of the important priorities in the future plant breeding objectives include, developing crops with enhanced nutrient use efficiency (NuUE) (for e.g. Nitrogen, Potassium and Phosphorus) so that lower (fertilizer) application of these nutrients is required. Some other important traits include resistance to major insects and pests that cause substantial damage to production (e.g. fall armyworm in maize), or tolerance to heavy metals, which also affecting crop yields (e.g. cadmium, copper and arsenic toxicity in rice fields). In future, more focus on conservation agriculture (CA) is expected, which would require developing crops with better rooting system (making them amenable for CA practices like zero tillage) and reduced length (suitable for vertical farming) will also assume importance. Radiation-induced mutagenesis holds promise in delivering these improved genotypes with desired traits for protecting the future food security of our world [16].

Radiation-enhanced Crop Production and Protection

In addition to genetic improvement of crops, radiation techniques have also been used in enhancing crop productivity and protection. In this context, some of the key achievements of BARC are discussed in brief. A radiation-induced hydrogel was developed with ability to absorb and retain high amount of water. This water is subsequently released when externally-available water becomes limited. This prolonged release of water is helpful in protecting plants from drought stress at all stages, particularly in arid areas with severe irrigated water shortage. Another example includes development of a gamma ray-induced plant growth stimulator, Anu-Chaitanya, which was derived from irradiation of chitosan using gamma rays. Due to reduced particle size and other improved physical properties, this formulation helps in increasing yield as well as vigour in both agronomic and horticultural crops. Similarly, a radiation-induced mutant strain of *Trichoderma virens* was developed as a purely organic biocontrol formulation for seed treatment [4].

Plant Environment Interaction

Radiotracers for studying nutrient uptake

Employing radiotracers, study of the movement of various solutes has played a considerable role in understanding plant physiology and helped to unravel complex phenomenon such as photosynthesis, nutrient cycling, specific metabolic pathways and plant – microbe interactions. Radioisotopes (C^{14}, N^{15}, P^{32}, K^{40}), used extensively as tracers in plant physiology studies, have given rise to remarkable discoveries. In one of the most seminal studies, Samuel Ruben and Martin Kamen (1930) used C^{14} to trace the path of carbon in photosynthesis, carbon fixation, and synthesis of organic molecules. The N^{15} radiotracer was used to study nitrogen fixation by bacteria and to subsequently follow the movement of nitrogen to plants.

Radiotracers have also been utilized in studying the primary and secondary metabolic process. Similarly, studies employing various other radiotracers have helped in understanding the spatial distribution of nutrients in plant organs at cellular and subcellular levels. Radiotracers have been useful in optimizing the nutrient amount be applied, which is crucial for enhancing crop productivity with limited input application. Sustainable increase in crop production involves mitigating the environmental pollution associated with excessive fertilizer runoff, and radiotracers have played a critical role in this aspect. Radiotracers have also helped in understanding soil-plant interactions, particularly in rhizospheres, where interaction of microorganisms in the vicinity of plant roots plays a major role in affecting yield.

Management of Insect Pests

Insect pests cause a serious threat to crop production. It is estimated that 20-40% of the global production is lost annually due to pests, leading to a monetary loss of ~ 220 billion dollars. Traditionally, insect pests are controlled using chemical insecticides, which have deleterious effects on human health. These also negatively affect the survival of the beneficial pests present in the environment. Nuclear science via the ‘Sterile Insect Technique (SIT)’ provides an innovative and sustainable solution to manage insect pests. It involves sterilization of insect pests (specifically male) using radiation. These insects are not able to reproduce (i.e. produce offspring), which restricts their numbers, consequently minimizing crop damage. SIT can also be integrated with other management practices, including biological control, modification of habitat, cultural practices etc., to obtain a more effective, durable, and sustainable pest suppression in a large area. In India at BARC, SIT has been developed for Red Palm Weevil (*Rhynchophorus ferrugineus*), a serious pest of coconut and other palms [4]. With release of sterile insects, a significant decrease in insect population as well number of trees infested with it has been observed. Similar success has been achieved in the case of fruit fly in Chile, Medfly in Mexico and Tsetse in Zanzibar.

Preventing Post-harvest Losses

It is estimated that a 10-15 % of agricultural produce is lost after harvest, causing a loss of billions of dollars. Preventing these losses by extending the shelf-life of agricultural produce, and making them available for consumption for a longer period of time, can contribute significantly in ensuring food security for millions of people. Traditional approaches include the use of chemical preservatives, which are now known to be hazardous to health. Nuclear science offers a unique solution to extend the shelf life of agricultural produce without the usage of hazardous chemical preservatives, maintaining the sensory as well as nutritional attributes. Radiation-induced shelf-life extension inhibits sprouting in foods under normal storage conditions. In addition, irradiation of food helps in eliminating harmful organisms that include bacteria, fungi and insects, thus preventing spoilage by them. In India, extensive work is being carried out at Food Technology Division of BARC, and shelf-life of many agriculture-related products (e.g. onions and potatoes) has been extended by preventing sprouting. Post-harvest losses due to insect infestation in cereals and spices has also been mitigated by radiation treatment. Radiation processing has also enabled international trade of mangoes from India, which where earlier restricted due to regulatory and sanitary requirements [5].

Future Directions

Nuclear science and radiation technologies hold promise to address many of the noteworthy challenges to agriculture in current and future scenarios. Gamma-rays and X-rays have been the most widely used mutagens over several decades in the past. However, presently, the use of other physical mutagens with higher relative biological effectiveness and distinct mutation spectrum (as compared to gamma rays), for developing mutants with desired traits is also being explored. Japan and China are at the forefront of using accelerated heavy ion, proton and neutron beams for mutagenesis in many crops species (e.g. wheat, rice, soybean and several vegetables). In, India at BARC, mutation breeding using proton ion and neutrons has been initiated in the case of cereals and holds potential for developing new mutants with agronomically superior traits.

One of the limitations in conventional breeding involves the long time required for the mutant trait to stabilize before direct or indirect applications of the mutant may be explored. Thus, it is necessary to integrate mutation breeding with rapid generation advancement (also known as speed breeding) as well as double-haploid methods to fix the mutant trait in less time. Also, with advancement in reverse genetics approaches, it is now possible to integrate molecular biology tools with mutation breeding. TILLING (Targeting Induced Local Lesions in Genomes) may be utilized for directed identification of mutations in a specific gene. Conventional mutant resources can be coupled with NGS-based bioinformatic pipeline (e.g. MutMap, MutMap+, MutantHunter, TENSeq, MutRenSeq, AgRenSeq, k-mer GWAS, and MutChromSeq) for rapid identification of mutant gene(s) and understanding the molecular basis of the mutation(s). Plant phenotyping has been boosted by the development of high-throughput phenotyping platforms and its data analysis has been assisted by artificial intelligence models. These phenotyping methodologies need to be integrated for screening large mutagenized populations to identify mutants, which are otherwise difficult to recognise by visual screening. Although, marker-assisted genome editing techniques are currently under regulatory restrictions for plant breeding, however, DNA-free editing approaches can be explored for crop improvement. Success in the case of wheat, rice and other important crops has been reported with this approach, and hence in future, this method may be used for targeted manipulation of desired traits.

Conclusion

Agriculture, which ensures food security, is a key priority area in the development of any nation. Radiation techniques have played a major role in addressing many of the challenges specific to the agriculture sector. These include, crop improvement by radiation-induced mutation breeding, boosting crop productivity by application of radiation-developed stimulants, crop protection by radiation-induced improved biocontrol agents, study and management of nutrients using radiotracers and managing pests with help of radiation-induced sterility. By utilizing nuclear science approaches, it is possible to increase the income of farmers by reducing their input cost and/or by getting higher value for their crop produce. Radiation methods in agriculture, apart from enhancing biodiversity, are both economical and sustainable. Integrating molecular biology with mutation breeding helps in the targeted improvement of the desired trait, which in turn holds promise to overcome future challenges that threaten global agriculture.

References

[1] "The Nobel Peace Prize 1970," NobelPrize.org, Apr. 10, 2024. https://www.nobelprize.org/prizes/peace/1970/borlaug/biographical/ (accessed Apr. 10, 2024).

[2] M. van Dijk, T. Morley, M. L. Rau, and Y. Saghai, "A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050," Nat Food, vol. 2, no. 7, Art. no. 7, Jul. 2021, doi: 10.1038/s43016-021-00322-9.

[3] S. Penna, S. Shirani Bidabadi, and S.M. Jain, "Mutation Breeding to Promote Sustainable Agriculture and Food Security in the Era of Climate Change," in Mutation Breeding for Sustainable Food Production and Climate Resilience, S. Penna and S. M. Jain, Eds. Singapore: Springer Nature, 2023, pp. 1–23. doi: 10.1007/978-981-16-9720-3_1.

[4] A. Tripathi, A. Shrivastava, A. Hadapad, R. S. Hire, and P. K. Mukherjee, "Application of radiation technology for improving crop productivity," in Non-Power Applications of Nuclear Technologies, pp. 51–67.

[5] G. Sumit and P. S. Variyar, "Radiation in food processing," in Non-Power Applications of Nuclear Technologies, pp. 69–89.

[6] "The Nobel Prize in Physiology or Medicine 1946," NobelPrize.org, Apr. 10, 2024. https://www.nobelprize.org/prizes/medicine/1946/muller/facts/ (accessed Apr. 10, 2024).

[7] M.C. Kharkwal, "History of Plant Mutation Breeding and Global Impact of Mutant Varieties," in Mutation Breeding for Sustainable Food Production and Climate Resilience, S. Penna and S. M. Jain, Eds. Singapore: Springer Nature, 2023, pp. 25–55. doi: 10.1007/978-981-16-9720-3_2.

[8] "Mutant Variety Database - Home," Apr. 10, 2024. https://nucleus.iaea.org/sites/mvd/SitePages/Home.aspx (accessed Apr. 10, 2024).

[9] "HvDep1 Is a Positive Regulator of Culm Elongation and Grain Size in Barley and Impacts Yield in an Environment-Dependent Manner | PLOS ONE," Apr. 10, 2024. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0168924 (accessed Apr. 10, 2024).

[10] "Use of Radiation to Transfer Alien Chromosome Segments to Wheat-Sears-1993-Crop Science-Wiley Online Library," Apr. 10, 2024. https://acsess.onlinelibrary.wiley.com/doi/abs/10.2135/cropsci1993.0011183X003300050004x (accessed Apr. 10, 2024).

[11] A.M. Badigannavar et al., "Radiation technology for genetic enhancement of crop plants," in Non-Power Applications of Nuclear Technologies, pp. 33–49.

[12] W. Elnashar and A. Elyamany, "Managing Risks of Climate Change on Irrigation Water in Arid Regions," Water Resour Manage, vol. 37, no. 6, Art. no. 6, May 2023, doi: 10.1007/s11269-022-03267-1.

[13] Q.Y. Shu, "Induced plant mutations in the genomics era," 2009.

[14] M.A. Haque et al., "Advanced Breeding Strategies and Future Perspectives of Salinity Tolerance in Rice," Agronomy, vol. 11, no. 8, Art. no. 8, Aug. 2021, doi: 10.3390/agronomy11081631.

[15] F.-I. S. Medina III, S. Tano, and E. Amano, "Mutation breeding manual," 2005.

[16] J.Ali, Z. A. Jewel, A. Mahender, A. Anandan, J. Hernandez, and Z. Li, "Molecular Genetics and Breeding for Nutrient Use Efficiency in Rice," Int J Mol Sci, vol. 19, no. 6, Art. no. 6, Jun. 2018, doi: 10.3390/ijms19061762.