The official bi-monthly publication of Bhabha Atomic Research Centre

BARC newsletter

May-June 2024 ISSN: 0976-2108

PLATINUM JUBILEE YEAR 70 Glorious Years 1954-2024

- Cancer Biology
- Mutation Breeding
- Food Processing & Preservation
- Seawater Desalination

NTD 2024 issue

Unlocking atoms for securing Water, Food, Agri & Health

BARC Newsletter
May-June 2024
ISSN: 0976-2108

50 years of Pokhran-I

An epitome of national self-reliance in advanced technologies

A Great Leap Forward - *The successful execution of Peaceful Nuclear Explosion Experiment by India on 18 May 1974 (christened 'Pokhran-I') had been a significant milestone in the country's scientific journey. The achievement singularly manifested India's complete mastery over a spectrum of scientific and technological aspects encompassing nuclear energy, materials development, precision engineering and allied disciplines. As India expands its footprints in nuclear energy research in pursuit of its national objectives, the key aspect of unlocking the potential of atoms to bring about a positive change in the quality of lives of its citizens will continue to be a permanent cornerstone.*

*From the Annals of History... *From the Annals of History... *From the Annals of History... *From the Annals of History...

Courtesy of "The Asian Age, The Financial Express, The Indian Express, The Times of India, Statesman, Pioneer and The Observer"

Atoms at the forefront of national efforts for securing Health, Food, Agri & Water

It gives me immense pleasure to write this foreword for the thematic issue of BARC Newsletter focused on multi-disciplinary R&D efforts in the vitally important areas of Water, Food, Agriculture, and Health. Understanding the significance of these key areas in our day-to-day lives and their prominence in the overall well-being of the nation, the theme of this year's edition of National Technology Day celebration in BARC was centered on them.

This carefully curated bulletin of BARC Newsletter showcases some of the important contributions of BARC to healthcare, food security and water management through unlocking the applications of radiation technologies.

In the area of healthcare, advanced nuclear technologies are being leveraged for the diagnosis and therapy of major diseases, including cancer. With the advent of cutting-edge technologies for focused targeting of radiation beam and radioisotopes to the localized disease site, nuclear technologies have now become the most indispensable and formidable tools not only for diagnosis and treatment of cancer but other life-threatening diseases (cardiovascular pathogenesis, Alzheimer etc) as well. In this regard, BARC continues to provide R&D support for production of a variety of radiopharmaceuticals for supply to hospitals and nuclear medicine centers across the country, rendering the treatment in an affordable manner.

In agriculture, an effective blend of radiation induced mutation and recombination breeding at BARC has resulted in the release of several crop varieties, which have largely benefited the farmers, nationwide. In addition, Trombay mutant lines have also extensively been used in several national breeding programs for crop improvement. In the area of food, cutting edge research programs in radiation processing ensure food security, safety and trade promotion. The SOP developed for sea-route shipment of radiation processed mangoes from India to USA has led to significant increase in the revenue obtained from export, resulting in immediate benefits to the growers.

BARC also developed several state-of-the-art technologies in treating waste water, desalination of sea water, isotopic analysis of ground water etc.

I take this opportunity to compliment the Associate Editors (Dr B. S. Patro and Dr. Anand D. Ballal) and contributing authors for their efforts in bringing out this exciting collection of articles in the field of healthcare, food, agriculture, and water. I am sure the articles will be an invaluable asset to all the researchers, especially young minds to explore the exciting fields of research in nuclear technology for societal benefits.

Dr. P. A. Hassan
Associate Director
Bio-Science Group
Bhabha Atomic Research Centre (BARC)

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ASSOCIATE EDITORS' message

Technology Development efforts in Health, Food and Agriculture

engineered by Bio-Science research at BARC

We are delighted to bring out this issue of BARC Newsletter on the momentous occasion of 'National Technology Day' (NTD). Every year, May 11 is celebrated as National Technology Day to commemorate the successful execution of "Operation Shakti", which constituted a series of nuclear explosions conducted at Pokhran in Rajasthan, on May 11 and May 13, 1998. These nuclear tests proved the country's technological and scientific prowess and had established India as a self-reliant and responsible nuclear power.

It is our honor to contribute to this special issue of BARC Newsletter as Associate Editors. Some of the important scientific and technological achievements of BARC in the field of nuclear science encompassing health, agriculture, food, and water have been curated in this issue. Supported by decades of high-quality pure and applied research in Bio-Sciences, BARC has achieved significant milestones in these areas of national importance. This has translated into a high degree of self-reliance in the development of affordable nuclear medicines, cancer therapeutics and radiation-guided diagnosis as well as therapy. Furthermore, mutation breeding for crop improvement, devising sustainable technologies for crop protection, radiation processing to extend the shelf-life of foods, and water/waste treatment are some of the diverse areas of societal applications wherein BARC has developed a unique pan-India footprint. We have attempted to cover this wide expanse of R&D work in BARC in this thematic issue. In this regard, four articles highlight the contemporary biological research and technologies evolved in the area of health, while six articles emphasize new approaches in the field of food and agriculture, whereas one article deals with seawater desalination technologies. We are hopeful that this issue will enlighten and stimulate young researchers, invigorating them to take up challenging research problems for wider societal benefit.

On behalf of the Bio-science Group (BSG), BARC we sincerely thank all the authors for their active participation and enthusiastic support. Our heartfelt, special thanks to Dr. S. Adhikari, Director, Knowledge Management Group (KMG), for extending this opportunity and guiding us throughout publication process. We also take this opportunity to thank Mr. Manoj Singh, Head, Scientific Information Research Division, KMG. We're extremely thankful to the services of Mr. Madhav N of SIRD and the Editorial Team of SIRD for their meticulous effort in curating the content, designing/formatting the articles in their final form and for providing proofs in a timely manner.

We are thankful to Associate Director, Bio-Science Group, BARC for his unstinting support in bringing out this thematic issue of newsletter.

Dr. Anand D. Ballal
Head, NA&BTD
Bio-Science Group

Dr. B. S. Patro
Head, BOD
Bio-Science Group

R&D IN HEALTH, FOOD, AGRICULTURE & WATER

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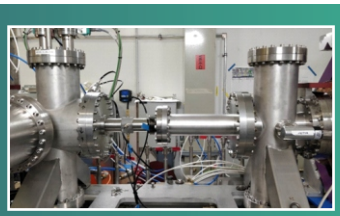
Research & Developement in Accelerator Technologies



- **High Power Radio Frequency Systems for LEHIPA.**
- **Indigenous 325 MHz Solid State Power Amplifiers.**
- **Integrated RF system for Positron Annihilation Lifetime Spectrometer.**
- **Solid-State Amplifier Systems of kW and MW level at UHF for Accelerators.**
- **Integrated RF Control System developed for accelerators under IIFC.**
- **Control and Measurement System for Superconducting Qubits.**
- **Indigenous RF systems for Hollow Cathode based Cold Atmospheric Plasma (HC-CAP) devices.**

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बीएआरसी न्यूज़लेटर, अंक: मई-जून, 2024 में सम्मिलित तकनीकी आलेखों के सारांश

पीएसएमए-617 और पीएसएमए-11 के संश्लेषण के लिए अंतर्गृह-विकसित संश्लेषण पद्धतियाँ: प्रोस्टेट कैंसर के उपचार के लिए किफायती ऑर्गेनिक लिगेण्ड

के.एस.अजिश कुमार[1,2]
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सारांश

हमारे देश में कैंसर रोगियों के लिए समुचित उपचार विधि को उपलब्ध कराने के लिए किफायती दवाओं का विकास करना आवश्यक है। कार्बनिक चेलेटर आधारित रेडियो-लिगेण्ड उपचार विधि, जिसे आमतौर पर नाभिकीय चिकित्सा की श्रेणी में रखा जाता है, विभिन्न कैंसर और संबंधित विकारों को दूर करने के लिए एक प्राथमिक उपचार विधि के रूप में उभर रही है। बायो-ऑर्गेनिक डिवीजन (बीओडी), बोर्ड ऑफ रिसर्च इन आइसोटोप टेक्नोलॉजी (ब्रिट, वाशी), और रेडियोफार्मास्युटिकल डिवीजन (आरपीएचडी) के बीच एक सहयोगी अनुसंधान कार्यक्रम के अंतर्गत, भारत में प्रोस्टेट कैंसर के रोगियों के उपचार के लिए विकिरणभेषज, [^{68}Ga] Ga-PSMA-11 और [^{177}Lu] Lu-PSMA-617 के विकास की दिशा में कार्य प्रारंभ किया गया था। इस कार्यक्रम में मुख्य रूप से, स्वदेशी संश्लेषण विधि का उपयोग करते हुए किफायती तरीके से ऑर्गेनिक लिगेंड, पीएसएमए-11 और पीएसएमए-617 का अंतर्गृह संश्लेषण करने में सफलता प्राप्त हुई थी। इस लेख में, हमने उन संश्लेषण चुनौतियों का वर्णन किया है, जिनका सामने हमें विभिन्न संश्लेषण मार्गों के उपयोग के दौरान करना पड़ा था, ताकि रेडियोलिगेण्ड विधि का उपयोग करके भारत में प्रोस्टेट कैंसर प्रबंधन को सामान्य बनाने के लक्ष्य को प्राप्त किया जा सके। **(पूरे लेख के लिए पृष्ठ संख्या 11 देखें।)**

क्लोबेटासोल प्रोपियोनेट और विकिरण के संयोंजन द्वारा उत्प्रेरित फेरोप्टोसिस उत्पेरण से कीप-1 उत्परिवर्ती मानव फेफड़ों के कैंसर कोशिकाओं के रेडियो-संवेदीकरण की संभाव्यता (i)

अर्चिता राय[1,2], राघवेंद्र एस. पटवर्धन[1], सुंदरराज जयकुमार[1], प्रदन्या पाचपाटिल[2,4], ध्रुव दास[3], गिरीश पाणिग्रही[5], विक्रम गोटा[5], सेजल पटवर्धन[2,5], संतोष के. संद्रूर[1,2]*
[1] विकिरण जीवविज्ञान एवं स्वास्थ्य विज्ञान प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई-400094, भारत
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[5] एक्ट्रेक टाटा स्मारक केंद्र (टीएमसी), खारघर, नवी मुंबई-410210, भारत

सारांश

एनआरएफ-2 (Nrf-2) अवरोधक के साथ रेडियोथेरेपी का संयोजन कीप-1 (Keap-1) उत्परिवर्ती रेडियोप्रतिरोधी फेफड़ों के कैंसर कोशिकाओं के विरूद्ध प्रभावी हो सकता है। वर्तमान अध्ययन में एनआरएफ-2 अवरोधक, क्लोबेटासोल प्रोपियोनेट (CP) के उपयोग से कैंसर से ग्रसित फेफड़ों के A549 कोशिकाओं को विकिरण द्वारा नष्ट करने के लिए, विकिरण के प्रति उनकी संवेदनशीलता को बढ़ाए जाने पर विचार किया गया है। विकिरण प्रेरित फेरोप्टोसिस के उपरांत CP उपचार करने पर, आयरन प्रेरित ऑक्सीडेटिव तनन और लिपिड पेरोक्सीडेशन के कारण कोशिका नष्ट हो जाती है। इस अध्ययन द्वारा फ्रॉपटोसिस को प्रेरित करने में माइटोकॉन्ड्रियल ROS की भूमिका को चिह्नित किया गया है। A549 कोशिकाओं में Nrf-2 की प्रमुखता के कारण CP+4 Gy प्रेरित रेडियो-संवेदीकरण का प्रतिरोध पाया गया। इस प्रकार, यूएस एफडीए द्वारा अनुमोदित एंटी-सोरायटिक दवा CP का पुन:प्रयोजता रेडियोप्रतिरोधी फेफड़ों के कैंसर कोशिकाओं के उपचार में एक आशाजनक क्रियाविधि प्रस्तुत करती है। **(पूरे लेख के लिए पृष्ठ संख्या 16 देखें।)**

एचईआर2 पॉजिटिव स्तन कैंसर से पीड़ित कैंसर रोगियों की रेडियोइम्यूनोथेरेपी के लिए [^{177}Lu]Lu-अंकित-ट्रैस्टुज़ुमैब का विकास और मूल्यांकन

मोहिनी गुलेरिया*[1, 2], जयचित्रा आमिरधानायागम[2], रोहित शर्मा[1], तपस दास[1,2]
[1]रेडियोभेषज्ञ प्रभाग ,भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई-400094, भारत

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ट्रैस्टुज़ुमैब, एक एफडीए-अनुमोदित मानवीय मोनोक्लोनल एंटीबॉडी, का उपयोग एचईआर2 पॉजिटिव स्तन कैंसर के उपचार में किया जाता है। इस अध्ययन का उद्देश्य स्तन कैंसर की रेडियोइम्यूनोथेरेपी के लिए [^{177}Lu]Lu-ट्रैस्टुज़ुमैब की रोगी खुराक की तैयारी के लिए डीओटीए-ट्रैस्टुज़ुमैब संयुग्म के प्रशीतित एवं निर्जलीकृत फॉर्मूलेशन को अनुकूलित करना है। [^{177}Lu]Lu-ट्रैस्टुज़ुमैब के लिए पारंपरिक निर्माण प्रक्रिया समय लेने वाली और अस्पताल रेडियोफार्मेसियों में नियमित तैयारी के लिए अव्यावहारिक है। इसके समाधान के लिए, सावधानीपूर्वक अनुकूलित रेडियोप्रोटेक्टेंट और क्रायोप्रोटेक्टेंट को जोड़ने के बाद प्रशीतित एवं निर्जलीकृत रूप में एक पूर्व-संश्लेषित डीओटीए-ट्रैस्टुज़ुमैब संयुग्म तैयार किया गया था। फ्रीज़-ड्राय किट का उपयोग करके तैयार किए गए [^{177}Lu]Lu-डोटा-ट्रैस्टुज़ुमैब की अंतिम रेडियोकेमिकल शुद्धता >95% पाई गई। प्रक्रिया की पुनरुत्पादकता सुनिश्चित करने के लिए, प्रशीतित एवं निर्जलीकृत फॉर्मूलेशन के लगातार छह बैच तैयार किए गए हैं और उनका मूल्यांकन किया गया है। [^{177}Lu]Lu-ट्रैस्टुज़ुमैब का प्रारंभिक नैदानिक मूल्यांकन एचईआर2-पॉजिटिव स्तन कैंसर से पीड़ित रोगियों में किया गया है। **(पूरे लेख के लिए पृष्ठ संख्या 20 देखें।)**

SARS-CoV-2 PLpro के नए संदमक की पहचान

रिमांशी आर्या,[1, 2] जननी गणेश, [1, 2] विशाल पराशर,[1, 2*] और मुकेश कुमार*[1,]
[1]प्रोटीन क्रिस्टलोग्राफी अनुभाग, जैव विज्ञान वर्ग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई-400094, भारत

सारांश

(ii)

SARS-CoV-2 वायरस का PLpro एंजाइम, वायरस प्रतिकृति (replication) और शरीर की रोग-प्रतिरोधक क्षमता की विकृति में महत्वपूर्ण भूमिका निभाता है। इसलिए, वायरस के इस एंजाइम को कोविड-19 के इलाज के लिए एक विशेष औषध-लक्ष्य (drug target) माना जाता है। वायरस के जीनोम में हो रहे लगातार परिवर्तनों के कारण, भविष्य में कोविड-19 के संभावित प्रकोपों का मुकाबला करने के लिए प्रभावी विषाणुरोधी दवाओं की तत्काल आवश्यकता है। इस संदर्भ में, हमने PLpro के संभावित अवरोधकों की खोज के लिए विविध रसायनों का परीक्षण किया। हमने पाया कि औरिन्ट्रिकॉर्बॉक्सिलिक एसिड (ATA), PLpro का एक प्रभावी संदमक है, जिसकी K_i और IC_{50} मान क्रमशः 16 μM और 30 μM हैं। ATA के PLpro के साथ बांधन के तापीय गतिशास्त्र को आइसोथर्मल टिट्रेशन कैलोरीमेट्री का उपयोग करके और अधिक विश्लेषित किया गया। पात्रे (इन विट्रो) परीक्षणों में ATA की विषाणुरोधी प्रभावकारिता 50 μM की IC_{50} के साथ प्रदर्शित हुई। इसके बाद, सीरियाई हैम्स्टर्स में भी इसके जीवे (इन विवो) विषाणुरोधी क्षमता का अध्ययन किया गया है । **(पूरे लेख के लिए पृष्ठ संख्या 23 देखें।)**

गुजरात और महाराष्ट्र में खेती के लिए गामा किरणों के उत्प्रेरण द्वारा विकसित मूंगफली की नई उन्नत किस्म

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नाभिकीय कृषि और जैव प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत

सारांश

मूंगफली के आनुवांशिक सुधार में अबतक गामा किरणों द्वारा उत्पन्न परिवर्तनशीलता (म्यूटेजेनेसिस) ने महत्वपूर्ण भूमिका निभाई है। भापअ केंद्र, मुंबई में निरंतर ऐसे स्थायी उत्परिवर्तन प्रयास करके कई उन्नत ट्रांबे मूंगफली (टीजी) किस्में विकसित की गयी हैं, जो पूरे देश में व्यावासायिक रूप से उपलब्ध हैं। इसी प्रक्रिया से, TG 38 के गामा किरण म्यूटेजेनेसिस द्वारा एक नया उत्परिवर्तीत (म्यूटेंट) किस्म TG 73 विकसित किया गया हैं। इस उत्परिवर्तीत किस्म में फली और बीज के दानों का आकार अतः तीन बीजों वाले फलियों के प्रतिशत में सुधार दिखाई दिया हैं। मोलेकुलर मार्कर्स की सहायता से TG 73 को उसके मूल, TG 38 या जांच किस्म, TAG 24 से विभिन्न बताई गई है। विभिन्न कृषि-पारिस्थितिकीय परिस्थितियों में इसकी गुणवत्ता, उपयुक्तता और अनुकूलता का परीक्षण करने के लिए, विभिन्न स्थानों में, जैसे कि जूनागढ़ कृषि विश्वविद्यालय (जेएयू), जूनागढ़, गुजरात एवं डॉ. पंजाबराव देशमुख कृषि विद्यापीठ (पीडीकेवी), अकोला, महाराष्ट्र, के सहयोग से गर्मी के मौसम के दौरान TG 73 का मूल्यांकन किया गया। इन सभी परीक्षणों में, TG 73 के फसल की औसत उपज 2541 किलोग्राम/हेक्टेयर और 3218 किलोग्राम/हेक्टेयर दर्ज की गई, जहां इस किस्म ने सबसे अच्छी जांच किस्म की औसत उपज के मुकाबले 16.6% और 14.3% उत्कृष्टता दर्ज की। नियमित फसल के उच्चतम औसत उपज के आधार पर, TG 73 को महाराष्ट्र (विदर्भ क्षेत्र) और गुजरात के गर्मी के मौसम में खेती के लिए TAG 73 और GG 37 के रूप में जारी और अधिसूचित किया गया है। **(पूरे लेख के लिए पृष्ठ संख्या 27 देखें।)**

कृषि में सतत विकास के लिए नाभिकीय विज्ञान का अनुप्रयोग: वर्तमान और भविष्य की चुनौतियों का समाधान

गौतम विश्वकर्मा[1, 2], बिक्रम किशोर दास[1, 2, *], आनंद डी बल्लाल[1, 2]
[1]नाभिकीय कृषि जैव प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई-400094, भारत

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कृषि मानव जीविका के लिए अत्यंत महत्वपूर्ण है। इसने सभ्यता के विकास को आकार दिया है। संपूर्ण विश्व जनसंख्या की खाद्य सुरक्षा काफी हद तक कृषि क्षेत्र में निरंतर नवाचार/सुधार पर निर्भर है। जलवायु परिवर्तन सहित विभिन्न जैविक और अजैविक तनावों की शुरुआत के कारण वैश्विक फसल उत्पादन को गंभीर चुनौतियों का सामना करना पड़ रहा है। निकट भविष्य में, बदलती कृषि पद्धतियों के कारण ये सभी चुनौतियाँ और अधिक बढ़ने की संभावना है। फसल उत्पादकता बढ़ाने के लिए कृषि के विभिन्न पहलुओं को बेहतर बनाने के लिए नाभिकीय विज्ञान का उपयोग कई दशकों से किया जा रहा है। यह लेख प्रेरित-उत्परिवर्तन प्रजनन दृष्टिकोण के माध्यम से बेहतर फसल किस्मों को विकसित करने के लिए नाभिकीय विकिरण के अनुप्रयोगों पर चर्चा करता है। इसके अलावा, (ए) ऐसे फॉर्मूलेशन विकसित करने के लिए नाभिकीय विकिरण के उपयोग की समीक्षा की गई है जो पौधों की वृद्धि को बढ़ावा देते हैं या फसलों को बीमारियों से बचाते हैं (बी) प्रमुख कीटों का प्रबंधन करते हैं और (सी) फसल के बाद के नुकसान को रोकते हैं। इसके अतिरिक्त, पौधों के भीतर पोषक तत्वों के ग्रहण, परिवहन और इसके वितरण का अध्ययन करने में रेडियोआइसोटोप के उपयोग पर चर्चा की गई है। यह लेख उभरती चुनौतियों से निपटने के लिए पारंपरिक प्रजनन दृष्टिकोण में आण्विक उपकरणों को एकीकृत करने की आवश्यकता पर केंद्रित है। **(पूरे लेख के लिए पृष्ठ संख्या 33 देखें।)**

कीटनाशकों के लिए बायोसेंसर: अवधारणा से प्रौद्योगिकी तक

जितेंद्र कुमार[1,2*]
[1]नाभिकीय कृषि एवं जैव प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे- 400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई- 400094, भारत

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कीटनाशक, विशेष रूप से कृमिनाशक, ऐसे रसायन हैं जिनका उपयोग कृषि, वानिकी और खाद्य उद्योग जैसे कई अलग-अलग क्षेत्रों में कीड़ों को मारने या नियंत्रित करने के लिए किया जाता है। पारिस्थितिकी तंत्र में कीटनाशकों का संचय, जो मानव और पशु स्वास्थ्य के लिए हानिकारक है, पर्यावरण के लिए हानिकारक है। इस प्रकार, इन कीटनाशकों की त्वरित और सटीक विश्लेषण के साथ निगरानी करने की आवश्यकता है। यह लेख विभिन्न जैव घटकों का उपयोग करके कीटनाशकों का बायोसेंसर-आधारित पता लगाने के लिए भाभा परमाणु अनुसंधान केंद्र में किए गए कार्य की एक संक्षिप्त समीक्षा है। हमने विभिन्न मैट्रिक्स पर माइक्रोबियल कोशिकाओं को स्थिर करके माइक्रोबियल बायोसेंसर की अवधारणा विकसित की है और प्रयोगशाला या क्षेत्र में मिथाइल पैराथियान के एकल से एकाधिक नमूनों का पता लगाने के लिए उन्हें विभिन्न ट्रांसड्यूसर के साथ जोड़ा है। बाद में, इस अवधारणा को सीधे क्षेत्र में मिथाइल पैराथियान का पता लगाने के लिए एक हैंडहेल्ड ऑप्टिकल बायोसेंसर डिवाइस में विकसित किया गया। एक ही समूह से संबंधित विभिन्न कीटनाशकों का पता लगाने के लिए एंजाइम-आधारित बायोसेंसर की एक और अवधारणा भी विकसित की गई थी, और इसे ऑर्गनोफॉस्फेट और ऑर्गेनोकार्बामेट से संबंधित कई कीटनाशकों की गुणात्मक पहचान के लिए बायोकिट की तकनीक में परिवर्तित किया गया है। **(पूरे लेख के लिए पृष्ठ संख्या 36 देखें।)**

दलहन फसलों में उत्परिवर्तन प्रजनन के सुदृढ़ीकरण हेतु अनुवांशिक संसाधनों का उपयोग

जे सौफ्रामानियन*[1], पी धनसेकर[1], वी जे ढोले[1], स्वप्नोनिल बनर्जी[2] और एल श्रीनिवास[2]
[1]नाभिकीय कृषि एवं जैव प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]नाभिकीय कृषि एवं जैव प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र सुविधाएं, विशाखापत्तनम -531011, भारत

सारांश

उच्च प्रोटीन तत्व वाली दलहन फसलें भारतीय कृषि के मूल्यवान तत्व हैं क्योंकि वे हमारी बढ़ती आबादी की पोषण संबंधी मांगों को पूरा कर सकती हैं। हालाँकि, संकीर्ण आनुवंशिक विविधता वाली इन फसलों पर तुलनात्मक रूप से कम शोध किया गया है। उत्परिवर्तन प्रजनन इन उपेक्षित, फिर भी महत्वपूर्ण दलहन फसलों जैसे कि अरहर, मूंग, उड़द और लोबिया के आनुवंशिक सुधार में सफल रहा है। इसके अलावा, प्रेरित उत्परिवर्तजनन में इन फसलों में आनुवंशिक संसाधनों को समृद्ध करने की क्षमता है, जिससे फसल सुधार कार्यक्रमों को जलवायु परिवर्तन की उभरती चुनौतियों का सामना करने के लिए सक्षम बनाया जा सकेगा। साथ ही, दलहन फसलों के त्वरित और लक्षित प्रजनन के लिए जीनोमिक संसाधनों को बढ़ाना भी उतना ही महत्वपूर्ण है।
(पूरे लेख के लिए पृष्ठ संख्या 40 देखें।)

मत्स्य-अपशिष्ट का मूल्यवर्धन

आशिका देबबर्मा[1], विवेकानंद कुमार[1,2], आरती सुधीर काकटकर[1], राज कमल गौतम[1], प्रशांत कुमार मिश्र[1], सुचंद्रा चटर्जी*[1,2]
[1]खाद्य प्रौद्योगिकी प्रभाग, भाभा परमाणु अनुसंधान केंद्र, मुंबई-400094, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्तिनगर, मुंबई-400094, भारत

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मछली और मत्स्य-अपशिष्ट पोषण की दृष्टि से पर्याप्त हैं और इसके उचित उपयोग के लिए नए तरीकों को अपनाकर इस अपशिष्ट का मूल्यवर्धन किया जा सकता है। मछली अपशिष्ट के परिनियोजन को तीन अलग-अलग तरीकों अर्थात् पालतू जानवरों का भोजन, संपुटित तेल और बायोडिग्रेडेबल फिल्म से उपयोग करके प्रदर्शित किया गया है। पशु और पालतू जानवरों के लिए किबल/टुकड़े और चूर्ण के रूप में पर्याप्त पोषण तत्व युक्त और सूक्ष्मजीव दृष्टि से सुरक्षित भोजन को गामा-विकिरण का उपयोग करके तैयार किया गया, जिसकी विस्तारित शेल्फ लाइफ 65 दिनों तक है। मत्स्य-अपशिष्ट से निकाले गए मछली के तेल का आवरण, कैल्शियम एल्जिनेट के दाने में किया गया, जिसने असंपुटित तेल की तुलना में भंडारण की गुणवत्ता को 3 गुना तक बढ़ा दिया। बायोडिग्रेडेबल फिल्मों को गामा किरणित क़ीमा के फैलाव का उपयोग करके संश्लेषित किया गया था, जहां 10 kGy उपचारित नमूने से तैयार फिल्म ने गैर-किरणित, असंपुटित की तुलना में बेहतर भौतिक गुणों का प्रदर्शन किया। इन परिणामों से संकेत मिलता है कि मत्स्य-अपशिष्ट का उपयोग मूल्यवर्धित उत्पादों के विकास के लिए किया जा सकता है, जो न केवल पर्यावरणीय खतरों को कम करता है, बल्कि सामाजिक-आर्थिक स्थितियों के उत्थान में भी योगदान देता है। **(पूरे लेख के लिए पृष्ठ संख्या 43 देखें।)**

फ्लोरोसेंट गामा डोसीमीटर का विकास

मनोज के चौधरी[1,2], बिरीजा एस. पात्रो [1,2], सौम्यदिता मुला [1,2]
[1]जैव-कार्बनिक प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा परमाणु अनुसंधान केंद्र, प्रशिक्षण विद्यालय परिसर, अणुशक्तिनगर, मुंबई-400094, भारत

(iv)

सारांश

फ्लोरीनेटेड बोरोन-डाइपिरोमेथीन (BODIPY) रंजकों पर आधारित दो फ्लोरोसेंट गामा-डोजिमीटर विकसित किए गए। एक में, 8-एनिलिनो BODIPY का उपयोग किया गया जोकि 0-100 Gy की गतिशील रेंज में गामा-उद्भासन के तहत "ऑफ-ऑन" प्रतिदीप्ति प्रदर्शित किया। जबकि दूसरे ने, 8-(एन, एन- डाइमेथायनिलिनो) BODIPY आधारित डोसीमीटर ने 0.5 Gy की संसूचन सीमा (LOD) के साथ 0-150 Gy की रेंज में लागू रतिमितीय ''ऑफ-ऑन'' प्रतिदीप्ति वृद्धि दर्शाई। ये अत्यधिक संवेदनशील फ्लोरोसेंट डोजिमीटर खाद्य विकिरण प्रक्रियाओं में अवशोषित डोज़ माप के लिए उपयोगी होंगे। **(पूरे लेख के लिए पृष्ठ संख्या 48 देखें।)**

Nuclear Medicine

1

In-house Developed Synthetic Strategies for PSMA-617 & PSMA-11: Affordable Organic Ligands for Prostate Cancer

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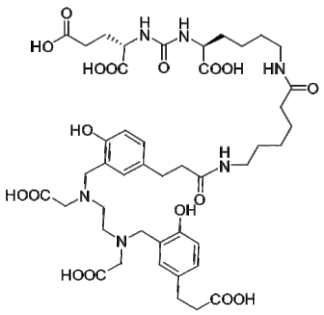

PSMA-11

ABSTRACT

Development of affordable medicines is a promising strategy to make a treatment method accessible to the cancer patients in our country. Organic chelator based radio-ligand treatment method, a branch of medicine generally called as nuclear medicine, is coming up as a primary treatment mode in addressing various cancer and related disorders. A collaborative research program between, Bio-Organic Division (BOD), Board of Research in Isotope Technology (BRIT, Vashi), and Radiopharmaceutical Division (RPhD), was directed towards the development of radiopharmaceuticals, [^{68}Ga]Ga-PSMA-11 and [^{177}Lu]Lu-PSMA-617 for prostate cancer patient treatment in India. In this program, main breakthrough was the in-house synthesis of organic ligands, PSMA-11 and PSMA-617, in cost effective manner, using indigenous synthetic method. Here in, we demonstrate the synthetic challenges that we had to surmount, while pursuing different synthetic routes, to accomplish the goal of making prostate cancer management equitable in India, using radioligand method.

KEYWORDS: *Ligands, Cancer, Prostate, PSMA, Therapy, Diagnosis*

Introduction

Prostate cancer (PC) is commonly encountered in men and it accounts for more than 15% of the total cancer cases. It is estimated that the total number of PC cases globally will be more than 2.9 million in a span of fifteen odd years [1-3], which is more than double that was observed in 2020. Hence, there is urgent need to popularize the most efficient treatment methods, currently available, as well as discovery of new treatment options. PC affects prostate gland in men and can be malignant, which makes it a perilous disease to cure. Most importantly, this disorder is the second major cause of cancer related death in men. Identification of new techniques based on tools that drive on molecular level diagnosis or therapy of PC can be rewarding. Such developments possess great relevance as there is large increase in the number of cancer incidences and related deaths in our country [4] and India is currently considered as the cancer capital of the world.

Staging of prostate cancer using non-invasive techniques like computed tomography (CT) or magnetic resonance imaging (MRI) are less sensitive to disclose the definite condition of the patient and hence are often less successful [5]. Therefore, the requirement for a sensitive technique like radioligand diagnosis, that could stage the disease and estimate the treatment progress after cancer therapy, at molecular level, is essential. In this approach, the identification of biomarker present in the cancer cell and targeting it with appropriate radionuclides [6] or that chelated to organic ligands is crucial [7]. This methodology is well appreciated by physicians and is being successfully utilized in the treatment of various malignant cancers, including PC. The success of this method in the treatment of PC may be credited to the identification and study of prostate specific membrane antigen (PSMA) [8], a type II transmembrane glycoprotein over-expressed on prostate cell surface. Significant expression of PSMA on the prostate cancer cells, compared to normal cells, make it an interesting molecular target. With respect to PC, few such molecules namely; PSMA-11 and PSMA 617 (collectively called as active pharmaceutical ingredients (APIs)), that target PSMA, has been identified and is providing breakthroughs in the designing of new treatment protocols [9,10].

Surgery, often a preferred treatment method for cancer, is not a desired option in PC as it is linked to a vital organ [11-13]. In such circumstances radioligand therapy (RLT) is a genuine option. But for RLT, most of the clinically approved [14] APIs are exorbitantly costly, which makes the treatment expensive. Especially, in countries with high population density and limited infrastructure, the non-availability of these medicines at an affordable cost can hamper the nations progress and most importantly, limits its availability to the needy patients. Considering these aspects, we have taken up the challenge to develop synthetic strategies to achieve these special molecules in affordable mode. Consequently, the past one decade of research resulted in the development of many established organic ligands in economical way. We were successful in achieving three important precursors (1a-c, Fig.1) for [^{18}F]-FLT **1**, [15] a positron emission tomography (PET) based brain cancer imaging agent, and more recently two highly important ligands, PSMA-617 **2** and PSMA-11 **3**, were also realized in affordable manner, using in-house developed synthetic strategies [16-19]. These developments may make the targeted treatment modality affordable to the people of our

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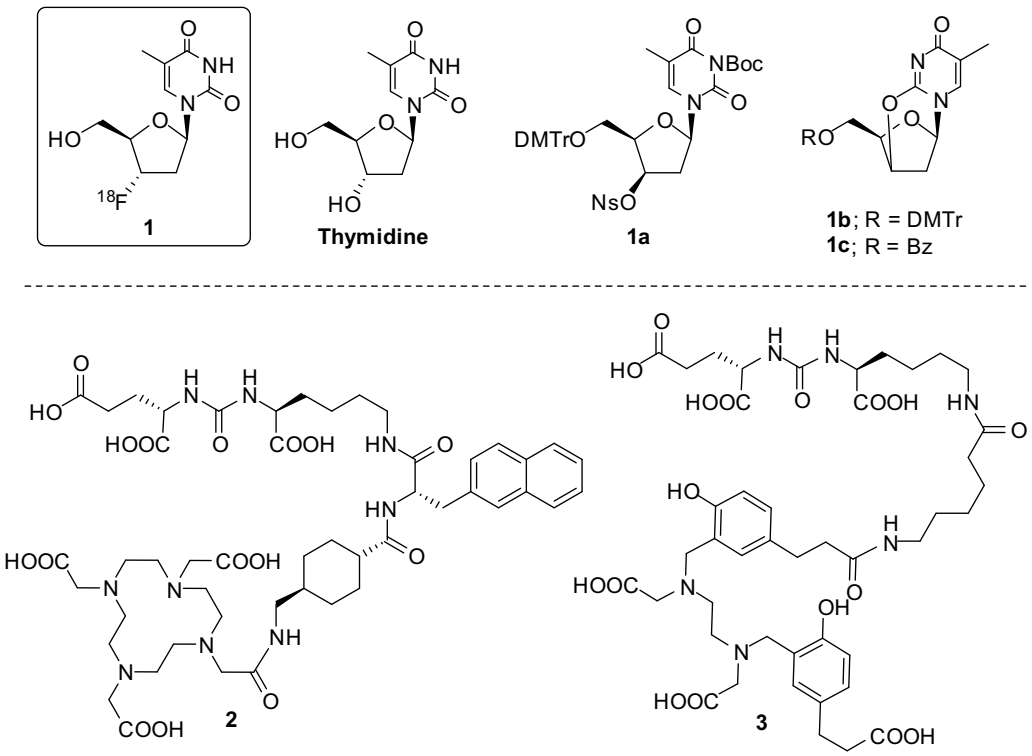


Fig.1: In-house developed organic ligands/precursors: [18F]-FLT (1), precursors for [18F]-FLT (1a-c) synthesized from Thymidine, PSMA-617 (2) and PSMA-11 (3).

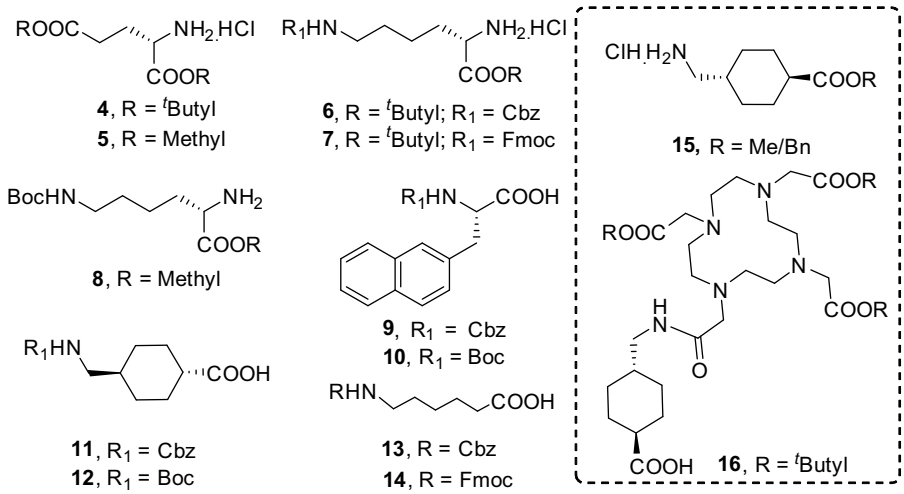


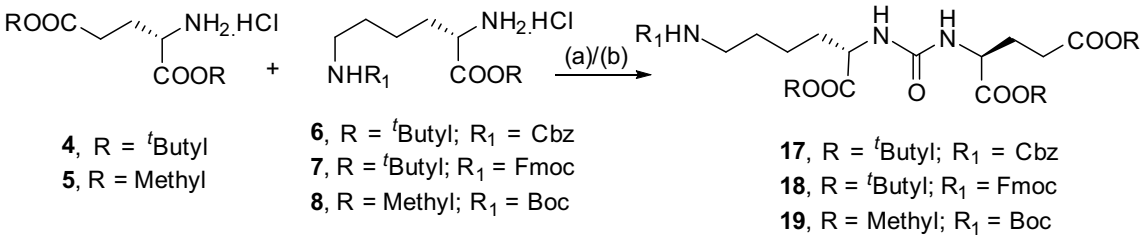
Fig.2: Amino acid templates/fragment for synthesizing PSMA-617 and PSMA-11.

country. In this account, we demonstrated the synthetic challenges that we encountered while employing different synthetic routes for achieving organic ligands, PSMA-617 and PSMA-11, in cost-effective way. After successful synthesis of these ligands, BRIT (Vashi) adopted these ligands as import substitutes, thereby significantly reducing the cost of radiopharmaceuticals, [^{68}Ga]Ga-PSMA-11 and [^{177}Lu]Lu-PSMA-617.

Results and Discussion

For the synthesis of PSMA-11 **2** and PSMA-617 **3** we opted for solution phase method which bestows the flexibility to adopt multiple approaches to the target molecule in the pursuit of a viable method. Inspection of the molecular structure of PSMA-11 **2** and PSMA-617 **3** reveals that it has one part in common, i.e. the dipeptide of glutamic acid and lysine; linked through a carbonyl moiety, which serves as the moiety that binds to the PSMA-protein. Consequently, to achieve the synthesis of this vital part of the target molecule, one can visualize it through the appropriate selection and assembly of differently protected hydrochloride salts (Fig.2) of glutamic acid **4**, and **5** and lysine **6, 7** and **8** [16-19]. Not to mention this would naturally leads towards different synthetic strategies for PSMA-617 and PSMA-11.

Among the two PSMA ligands, we first aimed for the synthesis of PSMA-617, most difficult of the two targets, primarily because of its potential to use as endotherapeutic agent. Nevertheless, due to structural similarity, parallel research efforts were in place for the synthesis of PSMA-11, using Fmoc-strategy. For the synthesis of PSMA-617, apart from the aforementioned hydrochloride salts, appropriately protected two unnatural amino acids, 3-(2-napthyl)-L-alanine derivatives, **9** and **10** and tranexamic acid derivatives, **11** and **12**, were also accomplished in efficient manner from their unprotected commercial equivalents [16-19].

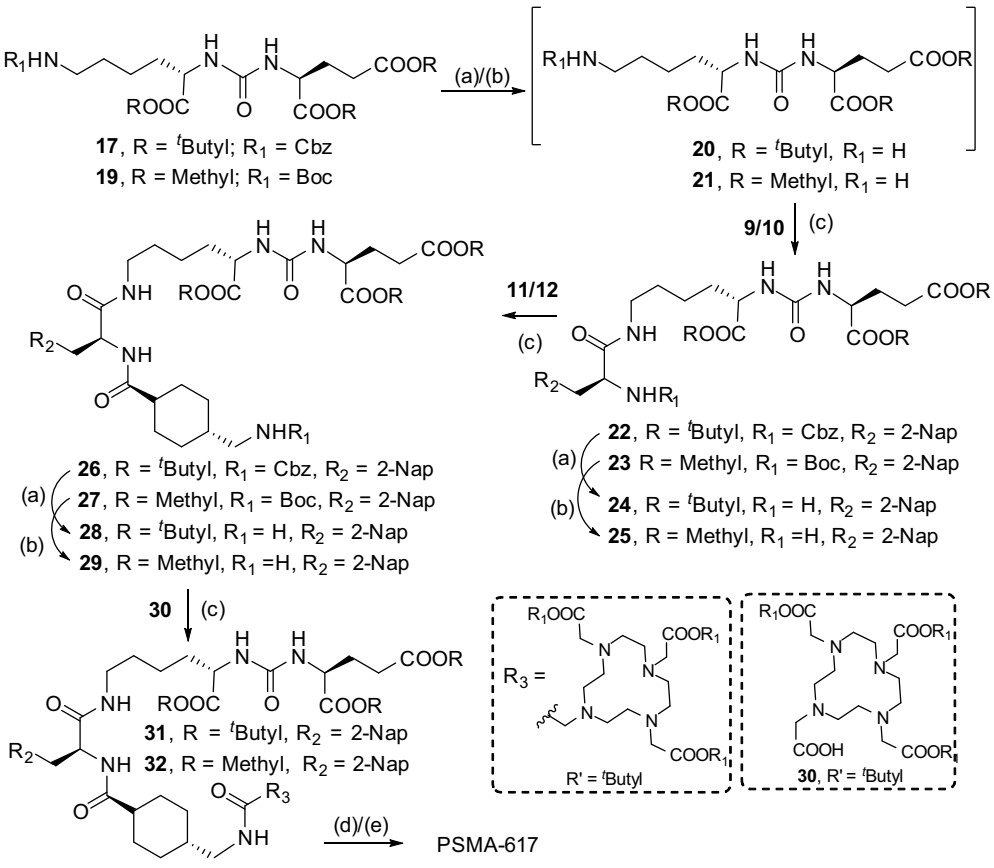


Scheme 1: Synthesis of urea templates (17/18/19): (a) Disuccinimidyl carbonate, DIEA, CH_2Cl_2; (b) $CO(OCCl_3)_2$, DIEA.

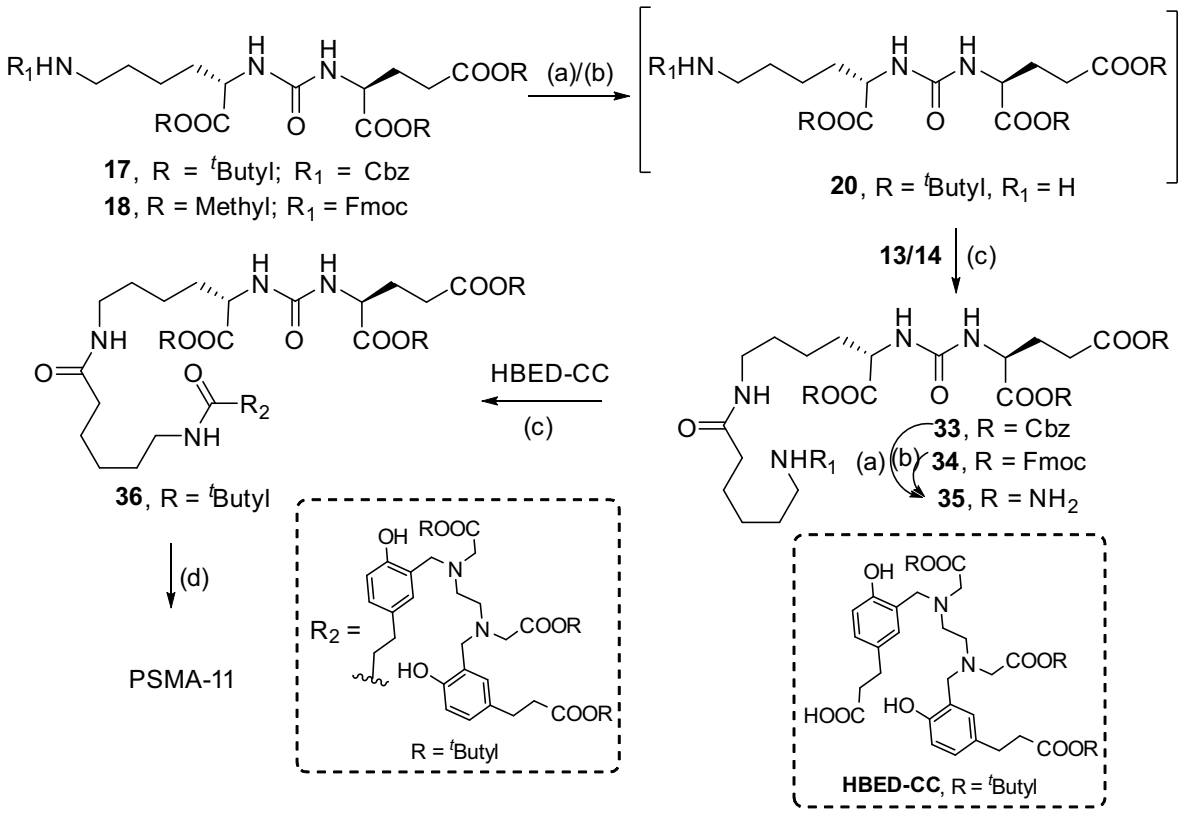
With the required amino acid precursors **4/5** and **6/7/8** in hand, we initiated the synthesis with the construction of three orthogonally protected urea templates **17-19** from the appropriately protected hydrochloride salts of glutamic acid and lysine as shown in Scheme 1. Hence, three urea templates, **17, 18** and **19** were obtained by chemical conjugation of appropriate amino acid residues using disuccinimidyl carbonate or triphosgene as the ligating reagent in the presence of an organic base. Even though the isolated yield of the products **17** and **19** were satisfactory [17,18], **18** was isolable in comparatively less yield [19]. Hence, it was decided to proceed with the synthesis of PSMA-617 using templates **17** and **19**.

Using the template **17**, the Cbz-strategy, wherein the Cbz group in the N-terminal of the synthetic sequence will be unmasked using metal catalysed nonhomogeneous reduction pathway. Therefore, in this synthetic strategy amino acids **9** and **11** would serve as the building blocks prior to the conjugation of DOTA chelator **30**. As a first step of linear Cbz strategy, protecting group in the side chain amine of **17** was deprotected using hydrogenolysis using flow reactor method in the presence of 10% Pd/C to generate corresponding amine **20** (Scheme 2). Subsequent reaction of amine with amino acid residue 9 in the presence of dicyclohexyl carbodiimide (DCC) as coupling agent furnished compound **22**. Repeating the hydrogenation step on **22** yielded amino derivative **24**. Reaction of **24** with tranexamic acid derivative **11** yielded the adduct **26**. Iteration of hydrogenation process on **26** furnished an amine **28** that on coupling with commercially available DOTA derivative **30** yielded fully protected PSMA-617 derivative **30**. Demasking of protecting group in **31** and subsequent purification yielded PSMA-617 **2** as a white foamy solid [17]. Purity and structural integrity of the synthesized compound was confirmed by HPLC, HRMS, NMR analysis and comparative studies with commercial equivalent. Alternatively, synthesis of PSMA-617 through convergent method, which theoretically provides better yield, was also exploited through the coupling of fragment **24** with **16**, (Fig.2) generated by the coupling of **15** with DOTA **30**, to yield **31**. Acidic hydrolysis of **31** furnished PSMA-617, in overall yield, almost similar to that of linear method [17].

Commercially, palladium and palladium based reagents are getting expensive. This prompted us further to search for an alternate method for making PSMA-617. In this regard, we employed Boc-strategy (Scheme 2), wherein amino protection in template **19**, prepared from amino acid constituents **5** and **8**



Scheme 2: Strategies for synthesis of PSMA-617 (2): (a) 10% Pd/C, H_2(15Psi), MeOH; (b) HCl (g), Ethyl acetate, 0 °C to rt; (c) DCC, DMF, 0 °C to rt; (d) i) LiOH, THF, ii) TFA-H_2O-PhSH; (e) TFA-H_2O-PhSH.



Scheme 3: Strategies for PSMA-11 (3): (a) 10% Pd/C, H_2(15Psi), MeOH; (b) Piperidine, CH_2Cl_2; (c) DCC, DMF; (d) (e) HBED-CC, DCC, DMF; (f) TFA-H_2O-PhSH.

(Scheme 1), was selectively made free, by reaction with HCl (g) solution in ethyl acetate, to yield **21**. This reaction was found to undergo without any side products and most importantly the resultant product after drying can be directly used for coupling with subsequent amino acid residues. Similar to Cbz strategy, subsequent coupling of **21** with amino acids **10** yielded **23**; removal of Boc-group in **23** furnished amino form **25**, which on coupling with **12** generated **27**. Deprotection of Boc-group in **27** followed by coupling with chelator **30** afforded **32**. Up to this stage the synthesis was highly efficient and there were only limited purification steps involved which made it a user-friendly process. However, the final deprotection step was not highly successful [19] due to the incomplete deprotection of three-methyl ester groups under saponification condition. Nevertheless, the strategy has the potential to further fine-tuning, employing an alternative protecting group at the carboxylic acid groups present in the binding motif.

For the synthesis of PSMA-11, we initially used Fmoc-strategy (Scheme 3) because Fmoc-template **18** could furnish **36**, precursor for PSMA-11, in few synthetic steps. Thus, deprotection of Fmoc group in **18** using piperidine, and subsequent coupling with amino acid template **14**, using DCC yielded compound **34** which was subjected to piperidine treatment to yield amino compound **35**. The conjugation of **35** with HBED-CC yielded fully protected PSMA-11 **36**. Subjecting **36** for acid hydrolysis yielded a crude mass which on purification using HPLC generated PSMA-11 **3** as a hygroscopic foamy off-white solid [19]. The structural integrity and the purity of the isolated product was confirmed by NMR, HPLC and HRMS analysis. Similarly, in Cbz-strategy, following analogous coupling sequence on **20**, obtained after the Cbz deprotection in **17** was subjected for coupling with amino acid **13** to furnish conjugate adduct **33**. The amine **35** obtained after the deprotection of Cbz group in **33** was ligated to commercially available chelator HBED-CC to furnish fully masked adduct **36** [16], which on acid hydrolysis furnished PSMA-11. Among the two approaches the isolated yield of PSMA-11 using Fmoc-strategy was understandably inferior [19] to the Cbz-strategy. From the lessons learned during the synthesis of PSMA-617, the use of Boc-strategy towards the synthesis of PSMA-11 was not explored.

After achieving the total synthesis of PSMA-617 and PSMA-11, both were tested for radiolabelling studies using radionuclides, ^{177}Lu and ^{68}Ga, respectively, at BRIT, Vashi. This study revealed the formation of radiolabelled products, [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11 in purity >98% adequate for direct human applications. Successful labelling studies were followed with a series of *in-vitro* and *in-vivo* studies (BRIT, Vashi), and subsequent clinical studies conducted at TMH, (Parel), showed that the performance of fully indigenous radiolabelled products were comparable to the corresponding products made from commercial equivalents. To validate the use of in-house synthesized ligands, PSMA-617 and PSMA-11, for prostate cancer management, BRIT and RPhD too played prominent role in developing and getting regulatory approval (DAE-Radiopharmaceutical Committee; DAE-RPC) [20,21] for [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11 and their supply to nuclear medicine centres in India. To date, using the fully indigenous [^{177}Lu]Lu-PSMA-617, >2500 Indian patients were treated.

In short, our collaboration with BRIT, RPhD, and RMC (Radiation Medicine Centre) were successful in the indigenization of three [^{18}F]-FLT precursors and two organic ligands, PSMA-617 and PSMA-11, in affordable manner, so that this treatment modality is made available to all needy patients. The work presented here demonstrates past one decade of our research efforts to decode the availability of these important API's in every nuclear medicine niches in our country.

Conclusion

Among the three solution phase strategies pursued namely; Fmoc-, Cbz-, and Boc-strategies; Cbz-strategy generated organic ligands PSMA-617 and PSMA-11 in affordable manner. Radiolabelling studies of the in-house synthesized ligands, PSMA-617 and PSMA-11, carried out at BRIT (Vashi), afforded corresponding labelled products in purity >98%. Clinical studies conducted at TMH (Parel), using the fully indigenous nuclear medicines, [^{177}Lu]Lu-PSMA-617 and [^{68}Ga]Ga-PSMA-11, showed results comparable to the commercial equivalents. Through the in-house developed strategy, we are currently capable of synthesizing these valuable import substitutes in purity >99.9%. Along with our endeavour to make these interesting ligands, complemented with the prompt supply of radionuclides by RPhD, is helping uninterrupted supply of refined radiolabelled product, [^{177}Lu]Lu-PSMA-617, by BRIT (Vashi), to nuclear medicine centres across India. BRIT (Vashi) is currently examining the possibility of local supply of [^{68}Ga]Ga-PSMA-11. Development of similar API's useful for diagnostic and therapeutic applications are in various stages of development.

Acknowledgments

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Novel Radiosensitizers

2

Ferroptosis Induction by Combining Clobetasol Propionate with Radiation Results in Radio-sensitization of Keap-1 Mutant Human Lung Cancer Cells

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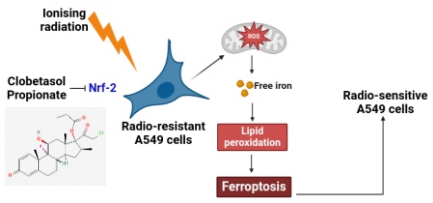

Radio-sensitization of A549 cells

ABSTRACT

Combining radiotherapy with an Nrf-2 inhibitor could be effective against Keap-1 mutant radioresistant lung cancer cells. The present study explored the use of Nrf-2 inhibitor, clobetasol propionate (CP), to enhance sensitivity of A549 lung cancer cells to radiation induced killing. CP treatment followed by radiation induced ferroptosis, a form of cell death triggered by iron induced oxidative stress and lipid peroxidation. The study identified the role of mitochondrial ROS in inducing ferroptosis. Nrf-2 overexpression in A549 cells resisted CP+4 Gy induced radio-sensitization. Thus, repurposing of US FDA approved anti-psoriatic drug, CP, presents a promising strategy for combating radioresistant lung cancer cells.

KEYWORDS: *Iron homeostasis, Glucocorticoid, Lipid peroxidation, Clobetasol propionate, Radio-sensitization, Non-small cell lung carcinoma*

Introduction

Lung cancer, responsible for roughly 18% of cancer-related fatalities worldwide, poses a significant challenge due to its resistance to radiotherapy [1]. Despite considerable efforts, the development of effective radiosensitizers that can be used in clinic remains limited, necessitating urgent exploration for safer and more efficient alternatives.

Clobetasol propionate (CP), originally approved by the US- Food and Drug Administration (FDA) for treating skin conditions like eczema and psoriasis due to its anti-inflammatory properties, has emerged as a promising candidate for repurposing in lung cancers characterized by mutations in Keap-1, a negative regulator of Nrf-2 [2]. Upregulation of Nrf-2, linked to poor prognosis in lung cancer patients, affects approximately one-third of non-small cell lung cancers (NSCLC). Further, exposure to radiation also activates Nrf-2 leading to radioresistance [3,4]. Small molecule inhibitors targeting Nrf-2 have shown promise in sensitizing cancer cells to chemotherapy, suggesting their potential as adjuvants to radiotherapy [5]. Hence, CP was combined with radiation to assess their potential to sensitize Keap-1 mutant human lung cancer cells in the current investigation. Inhibiting Nrf-2 with CP and exposure to radiation facilitated ferroptosis induction, thereby enhanced radiosensitivity in NSCLC cells [6]. Ferroptosis, an iron-dependent form of non-apoptotic cell death triggered by intracellular lipid peroxide accumulation, is hindered by Nrf-2 [7,8]. This approach underscores the potential of CP in overcoming radioresistance in lung cancer cells.

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Materials and Methods

A549 human lung adenocarcinoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS at 37^{0}C in a 5% CO_2 incubator. Cells were treated with indicated concentrations of CP and doses of γ-radiation. Cell viability was assessed using a cell titre Adenosine triphosphate (ATP) based luminescent assay. Live cell imaging was carried out in treated groups using Incucyte system. Sub-G1 population analysis was conducted using flow cytometry. Clonogenic assay was performed and colonies were counted after crystal violet staining. Spheroid formation and quantification was carried by propidium iodide (PI) staining microscopically.

Nrf-2 expression was studied by immunofluorescence microscopy and Western blotting. Gene expression studies were conducted by RNA Seq and real time PCR. Mitochondrial reactive oxygen species (ROS) levels were studied by Mitosox Red (MSR) staining, mitochondrial membrane potential (MMP) was estimated by JC-1 assay and Transmission Electron Microscope (TEM) was used for ultrastructure study.

Lipid peroxidation and iron levels were quantified using respective fluorescent probes (Liperfluo and BODIPY iron probe, respectively). Intracellular GPX4 levels were studied by fixation, permeabilization, staining with anti-GPX4 mAb

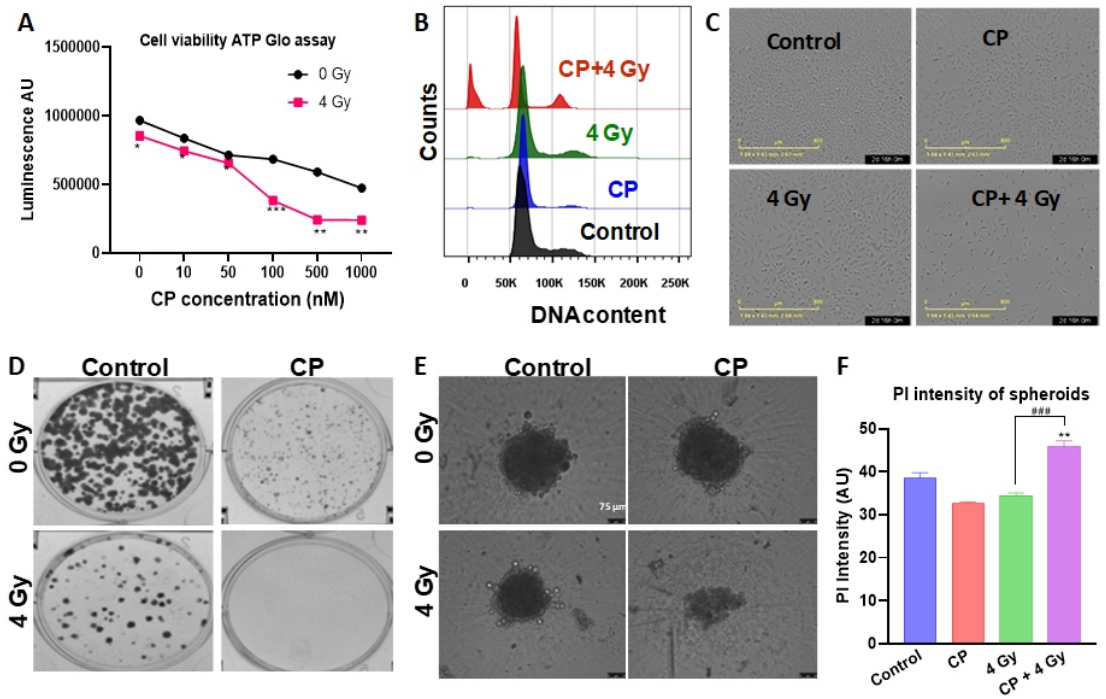


Fig.1: A) Cell viability was studied using Cell Titre ATP Glo assay in A549 cells with indicated treatments. B) Overlaid flow cytometric histograms of propidium iodide stained cells. C) Incucyte images of cells showing confluence post 64 hours of indicated treatments. D) Representative images of macroscopic colonies formed after 14 days of indicated treatments. E) Images of lung cancer spheroids 72 hours post treatment. F) Quantitation of spheroids after staining with PI is shown as bar graph. (Adapted from Rai et al., Acta Pharmacol Sin., 2024).

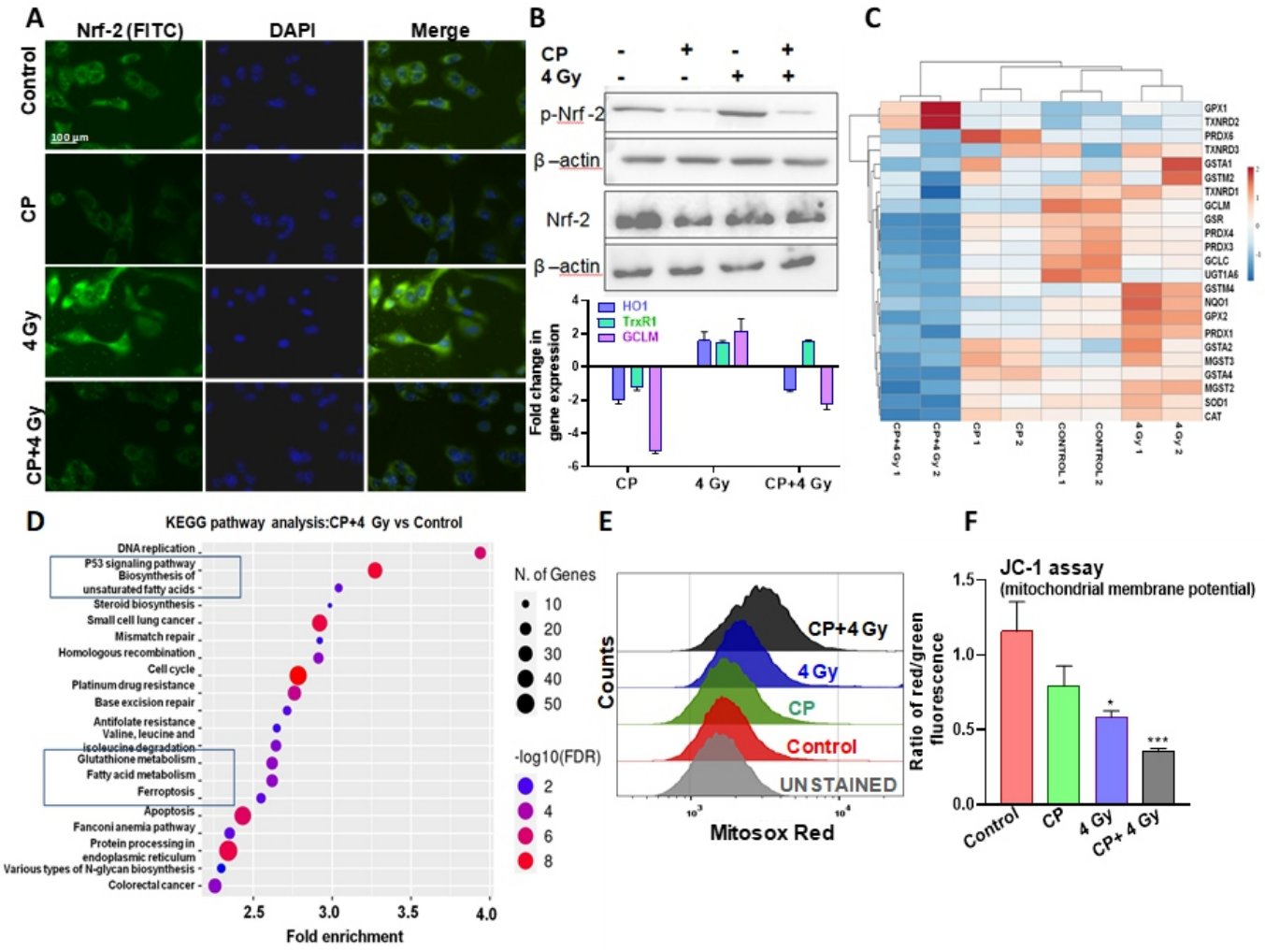


Fig.2: A) Immuno-fluorescence images showing nuclear translocation of Nrf-2 at 4 hours. B) Western blot images for Nrf-2 and phospho-Nrf-2 in cells treated with indicated groups. C) Heatmap of Nrf-2 dependent antioxidant genes studied by RNA Sequencing. D) KEGG pathway analysis of cells treated with CP+4 Gy was compared with control. E) Overlaid flow cytometric histograms showing mitochondrial ROS levels in indicated groups. F) Bar graph showing changes in MMP levels. (Adapted from Rai et al., Acta Pharmacol Sin., 2024).

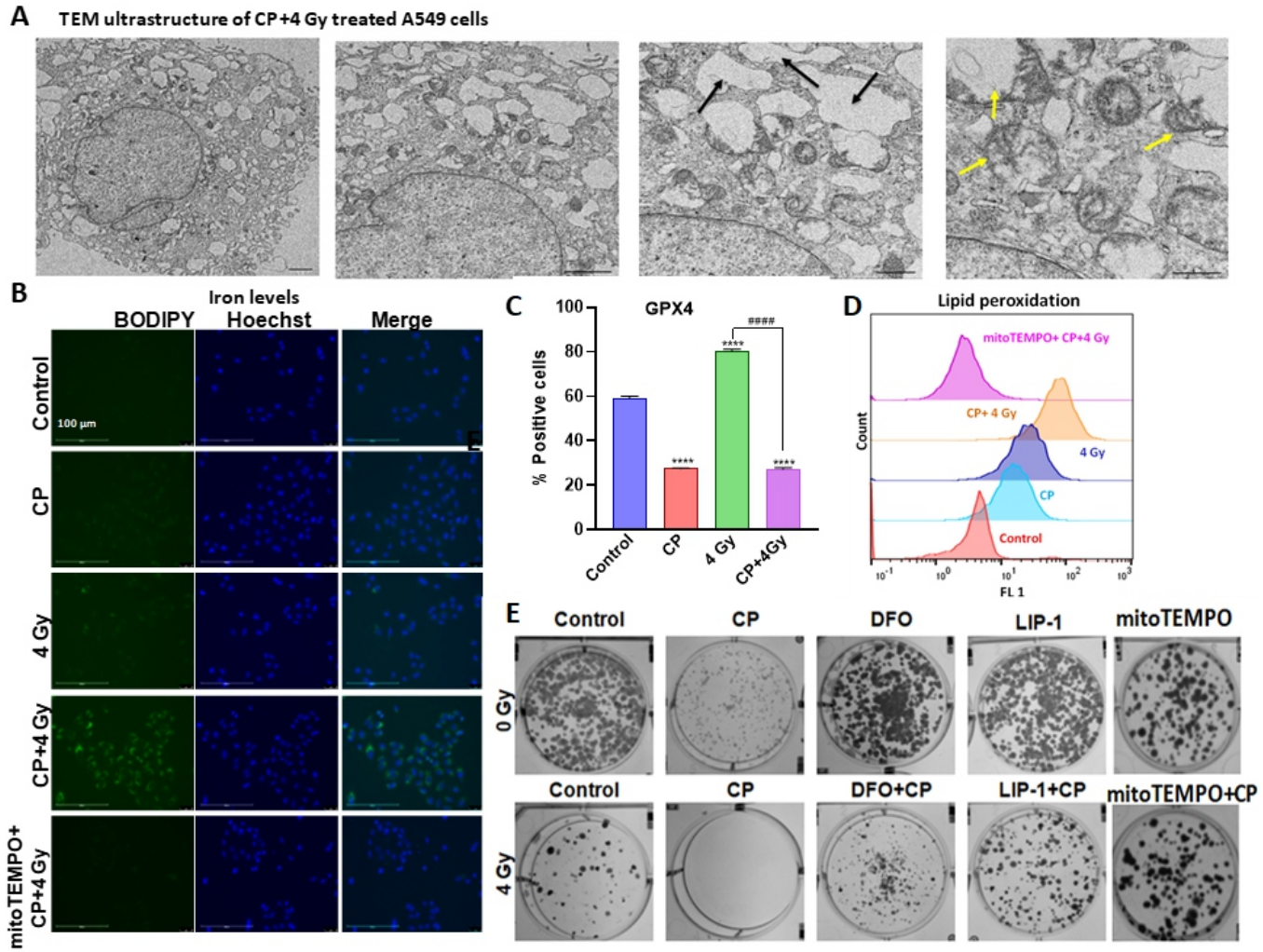


Fig.3: A) TEM images of cells treated with CP+4 Gy for 24 hours indicating increased vacuolisation (black arrows) and loss of cristae (yellow arrows). B) Images representing intracellular free iron levels in indicated groups. C) Bar graph showing GPX4 levels as studied by flow cytometry in cells treated with indicated groups. D) Overlaid flow cytometric histogram representing lipid peroxidation. E) Representative images of colonies of lung cancer cells treated with indicated groups. (Adapted from Rai et al., Acta Pharmacol Sin., 2024).

followed by PE-conjugated secondary antibody and acquired using a flow cytometer. Nrf-2 overexpression was achieved via lipofectamine based transfection. Animal experiments were performed using NOD/SCID BALB/c mice. Statistical analysis was conducted using Graphpad Prism 9.0 software.

Results and Discussion

CP sensitized radiation induced killing of lung cancer cells: Combination of CP and radiation resulted in significant loss of viability of A549 cells along with increase in cell death, as compared to individual CP or 4 Gy alone treated group (Fig.1 A, B). CP+4 Gy treatment resulted in loss of confluence due to cell death and severely diminished the clonogenic potential of A549 cells as compared CP or 4 Gy alone (Fig. 1 C, D). A549 tumor spheroid size and integrity were reduced along with higher PI uptake in the presence of CP+4 Gy treatment (Fig.1 E, F), as compared to CP or 4 Gy group. These results strongly suggested that CP enhanced radiation induced killing of Keap-1 mutant A549 cells.

CP inhibited Nrf-2 and upregulated genes involved in ferroptosis: Treatment of A549 lung cancer cells with CP downregulated the constitutive as well as radiation induced expression and nuclear translocation of Nrf-2 (Fig.2A, B). Due to inhibition of Nrf-2, several Nrf-2 anti-oxidant and cytoprotective genes were also downregulated by CP as studied by RT-PCR (Fig.2B). Transcriptomics data also suggested upregulation of genes involved in ferroptosis in CP+4 Gy treated cells (Fig.2C, D). To validate the role of ferroptosis, mitochondrial parameters were studied. Mitochondrial ROS was elevated and the mitochondrial membrane potential was compromised in CP+4 Gy treated cells as compared to CP or 4 Gy group (Fig. 2E, F).

Validation of ferroptosis by CP+4 Gy treatment: TEM studies indicated severe damage to mitochondrial ultrastructure like loss of cristae and increased vacuolisation strongly associated with ferroptosis in CP+4 Gy treated cells (Fig.3A). The combination treatment resulted in increase in intracellular free iron levels which was abrogated by using mitochondrial ROS scavenger mitoTEMPO (Fig.3B). CP and CP+4 Gy treated cells had downregulation of GPX4 which is reported to inhibit ferroptosis (Fig.3C). Lipid peroxidation caused by increase in iron levels was high in CP+4 Gy treated cells as compared to CP or 4 Gy group. This effect was abrogated by mitoTEMPO (Fig.3D). Scavenging mitochondrial ROS (mito-TEMPO) or pre-treatment with iron chelator (DFO) or ferroptosis inhibitor (Liproxstatin) resulted in abrogation of ferroptosis induced by CP+4 Gy treatment (Fig.3E).

Role of Nrf-2 in CP+4 Gy mediated radio-sensitization: Overexpression of Nrf-2 resulted in abrogation of lipid peroxidation and eventually inhibited CP mediated radiosensitization (Fig.4A,B). This substantiated the role of Nrf-2 in ferroptosis induced by combination treatment of CP

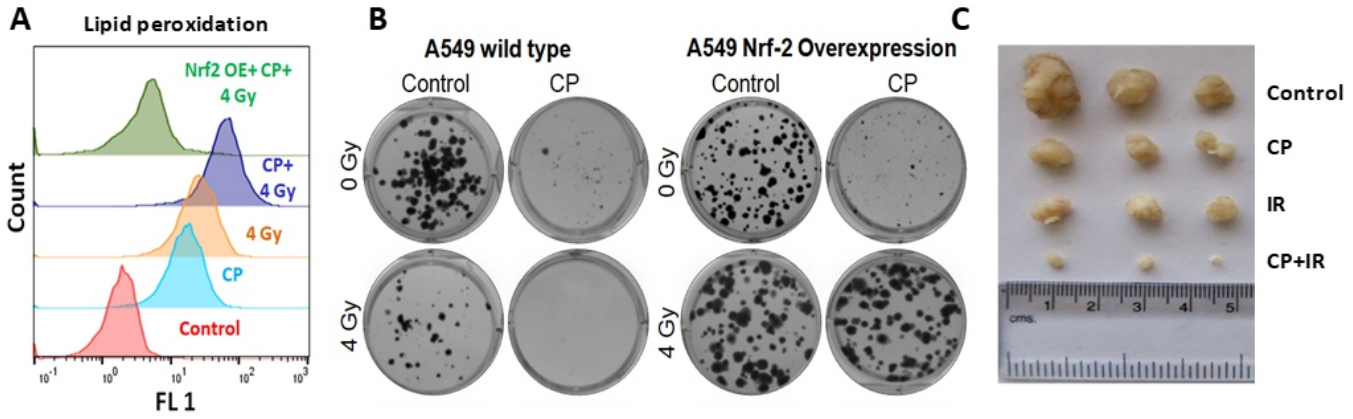
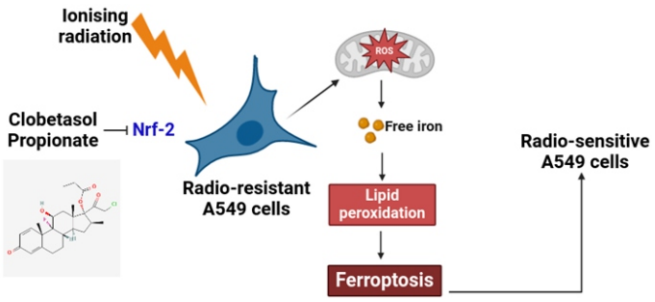


Fig.4: A) Overlaid flow cytometric histograms showing lipid peroxidation in indicated groups. B) Representative images of colonies formed by cells treated with indicated groups. C) Representative images of tumors excised from SCID mice transplanted with A549 cells from indicated groups. (Adapted from Rai et al., Acta Pharmacol Sin., 2024).



Scheme 1: Nrf-2 inhibition by CP prior to radiation exposure increases mitochondrial ROS mediated ferroptosis which results in radio-sensitization of A549 cells.

and 4 Gy. The in vivo studies clearly showed reduction in tumor burden in A549 xenograft tumor bearing SCID mice administered with CP and 3 x 2 Gy radiation (Fig.4C). The results strongly suggested that Nrf-2 inhibition by CP can exhibit radiosensitization in Keap-1 mutant Nrf-2 overexpressing lung cancer cells.

Conclusion

Multiple studies have suggested the potential of ferroptosis in overcoming therapy resistance and achieving better clinical outcome [9,10]. This underscores the promise of novel radiosensitizers that induce ferroptosis. The current study has identified clobetasol propionate, an FDA-approved agent, as a novel inducer of ferroptosis through Nrf-2 inhibition when combined with radiation (Scheme 1).

Acknowledgments

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Breast Cancer Treatment

3

Development and evaluation of [^{177}Lu]Lu-labeled-Trastuzumab for Radioimmunotherapy of Cancer Patients Suffering with HER2 Positive Breast Cancer

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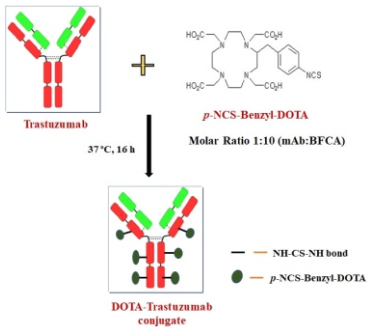

Pictorial depiction of conjugation reaction between Trastuzumab and p-NCS-benzyl-DOTA

ABSTRACT

Trastuzumab, an FDA-approved humanized monoclonal antibody, is utilized in the treatment of HER2-positive breast cancer. This study aims to optimize a freeze-dried formulation of DOTA-Trastuzumab conjugate for the preparation of patient dose of [^{177}Lu]Lu-Trastuzumab, intended for radioimmunotherapy of breast cancer. The conventional formulation process for [^{177}Lu]Lu-Trastuzumab is time-consuming and impractical for routine preparation in hospital radiopharmacies. To address this, a pre-synthesized DOTA-Trastuzumab conjugate in freeze-dried form was prepared after the addition of carefully optimized radioprotectant and cryoprotectant. The final radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab, prepared using the freeze-dried kit, was found to be >95%. To ensure the reproducibility of the process, six consecutive batches of the freeze-dried formulation are prepared and evaluated. Preliminary clinical evaluation of [^{177}Lu]Lu-Trastuzumab was performed in patients diagnosed with HER2-positive breast cancer.

KEYWORDS: *[^{177}Lu]Lu, [^{177}Lu]Lu-Trastuzumab, DOTA-Trastuzumab, Freeze-dried kit, Radioimmunotherapy*

Introduction

Trastuzumab, a monoclonal antibody (IgG) has been approved by US-FDA for immunotherapy of the HER2 positive breast cancer in 1998. However, in many cases, conventional immunotherapy involving Trastuzumab suffers from various issues, such as cardiotoxicity and other related complications [1,2]. Receptor heterogeneity and receptor down-regulation during the course of immunotherapy are additional factors resulting in low response of the breast cancer patients during Trastuzumab therapy [1,2]. To circumvent these issues, instead of using 'cold' (non-radioactive) antibody, the use of Trastuzumab in radiolabeled form has been envisaged, wherein the antibody is radiolabeled with a suitable radionuclide for exerting therapeutic effects. The aforementioned technique is termed as 'radioimmunotherapy' (RIT), where unlike immunotherapy, the associated radionuclide is responsible for therapeutic efficacy whereas the monoclonal antibody acts as the targeting vector only [3-5]. The application of [^{177}Lu]Lu-DOTA-Trastuzumab is currently being explored clinically for diagnosis and radionuclidic therapy of patients suffering from HER2 positive breast cancer [3-5]. Lutetium-177 [$E_{\beta max}$= 0.497 MeV] is a therapeutic radionuclide suitable for development of radiolabeled antibodies especially due to its long half-life ($T_{1/2}$ = 6.73 d) which matches well with that of the biological half-life of monoclonal antibodies. Given recent interest in the application of radiolabeled antibodies in cancer care, convenient and simple formulation of radiolabeled antibodies at hospital radiopharmacies is highly desirable. Therefore, an attempt has been made to formulate a robust 'freeze-dried' Trastuzumab-kit which will enable the preparation of patient doses of ^{177}Lu-labeled Trastuzumab at hospital radiopharmacy in a simple and convenient manner. In the present work, systematic optimization of the kit constituents was carried out to arrive at the formulation which consistently give high and reproducible radiolabeling yields for [^{177}Lu]Lu-DOTA-Trastuzumab using medium specific activity ^{177}Lu [555-740 MBq/µg].

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Materials and Methods

Conjugation of Trastuzumab with *p*-NCS-benzyl-DOTA (subsequently referred as DOTA) was performed by following the reported method [5]. The average number of DOTA-units attached per Trastuzumab molecule was analysed by two different methods viz. UV-Vis spectrophotometry as well as MALDI-TOF mass spectrometry. The purified DOTA-Trastuzumab conjugate was also analysed for protein content by employing Bradford protein assay. The formulation of freeze-dried kit of DOTA-Trastuzumab was carried out by following a procedure mentioned as: A stock solution 4.0 mL containing 32 mg of DOTA-Trastuzumab in 0.2 M NaOAc buffer (pH 5.0) was used. The other stock solutions (aqueous) utilized were of sucrose (50.0 mg/mL), ascorbic acid (50.0 mg/0.5 mL) and 0.2 N NaOH (10 mL). Aliquots of 375 µL (3.0 mg) of DOTA-Trastuzumab conjugate, 100 µL of sucrose (5.0 mg), 50 µL of ascorbic acid (5.0 mg), 0.2 N NaOH (1.0 mL) and 0.2 N NaOAc buffer (1.0 mL) were withdrawn and added to each sterile glass vial. The vials were lyophilized and stored at 4°C till further use or till shelf-life of kit (6 months).

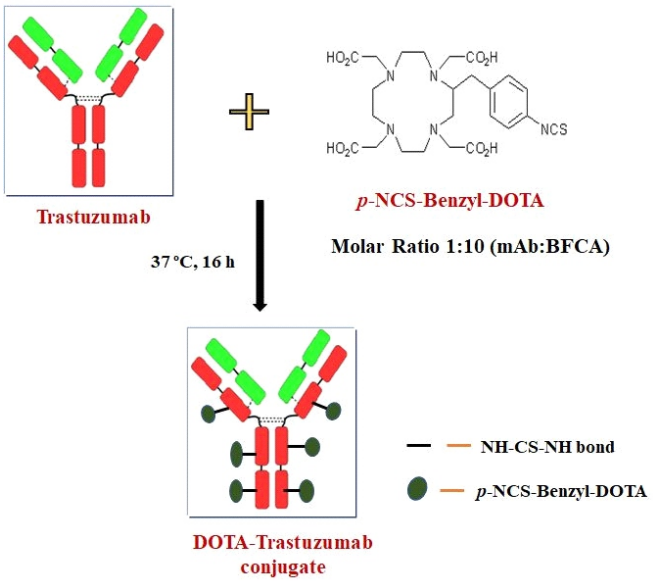

Fig.1: Pictorial depiction of conjugation reaction between Trastuzumab and p-NCS-benzyl-DOTA.

For radiolabeling, the freeze-dried DOTA-Trastuzumab formulation was reconstituted with 0.5 mL of deionized water followed by addition of 100-200 µL (1.94-2.40 GBq) of [^{177}Lu]$LuCl_3$ (in 0.01 M HCl). The reaction mixture was incubated at 37^{0}C for 30 minutes. Radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab was confirmed using paper chromatography (PC) and High Performance Liquid Chromatography (HPLC). PC was performed with 0.01 M sodium citrate buffer (pH 5.0) as mobile phase, while HPLC utilized a size-exclusion column (TSK Gel G3000SWXL) with isocratic elution using 0.05 M phosphate buffer (pH 7.0) containing 0.05% NaN_3 as the mobile phase.

Post-radiolabeling, [^{177}Lu]Lu-DOTA-Trastuzumab was evaluated in three different cancer cell lines (SK-OV-3, SK-BR-3 and MDA-MB-231) for determination of binding and specificity towards HER2 receptors. Cells were treated with [^{177}Lu]Lu-DOTA-Trastuzumab [5 MBq, 50 nM] for 2 h followed by washing with ice-cold 0.05 M PBS (pH 7.0) and solubilization with of 1N NaOH. The extracted cells were centrifuged, supernatant was removed and the activity associated with the cells was counted. Inhibition studies were carried out under same conditions by incubating the cancer cells with [^{177}Lu]Lu-DOTA-Trastuzumab and cold/non-radioactive Trastuzumab (3.3 µM).

Several physico-chemical studies were performed before [^{177}Lu]Lu-DOTA-Trastuzumab, formulated using DOTA-Trastuzumab kit, was released for further experimentation/use. Bio-evaluation of [^{177}Lu]Lu-DOTA-Trastuzumab was performed in healthy Swiss mice (n=3) at different post-administration (p.i.) time points namely 1, 2, 5 and 7 d as well as in SK-OV-3 xenografted SCID mice at 48 h p.i. Post-administration, the animals were sacrificed, dissected and activity associated with various organs was determined as percentage injected activity per gram of organ (%IA/g of organ).

[^{177}Lu]Lu-DOTA-Trastuzumab prepared using in-house optimized freeze-dried kit of DOTA-Trastuzumab was utilized for limited clinical evaluation in patients suffering with HER2 positive breast cancer.

Results and Discussion

DOTA-Trastuzumab conjugate was synthesized by conjugating -NH_2 group of lysine amino acid in Trastuzumab with isothiocyanate group of DOTA derivative employing 1:10 molar ratio (Fig.1). Mass analyses revealed the average number of BFCA molecules attached per Trastuzumab as 6.0±1.1, whereas using the UV-Vis spectrophotometric assay the same was determined to be 6.2±0.8 (Fig.1).

Optimized freeze-dried Trastuzumab kit comprised 3.0 mg DOTA-Trastuzumab, 5.0 mg sucrose, 5.0 mg ascorbic acid, 8 mg NaOH and 16.5 mg NaOAc. Considering that minimum radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab should be ~95% for clinical application, it was observed that maximum 2.22 GBq of ^{177}Lu could be added in the kit vial, when ^{177}Lu having specific activity of 555 MBq/µg was used for the formulation of the radiolabeled agent.

PC and HPLC were used for the determining of RCP of [^{177}Lu]Lu-DOTA-Trastuzumab formulated using kit. In PC, [^{177}Lu]Lu-DOTA-Trastuzumab remained at the origin (R_f = 0.0-0.1) whereas free ^{177}Lu moved to the solvent front R_f = 1.0). In HPLC, [^{177}Lu]Lu-DOTA-Trastuzumab exhibited a retention time (R_t) of 14.5±0.5 min whereas uncomplexed ^{177}Lu eluted from the column at 21.2±0.9 min. The percentage binding [^{177}Lu]Lu-DOTA-Trastuzumab in SK-OV-3 and SK-BR-3 cells ranged from 14.6±2.1 to 23.0±1.4 and 19.5±0.9 to 32.0±7.2, respectively; whereas the same in MDA-MB-231 cells was observed to vary from 3.2±1.4 to 4.6±1.0. In addition to a lower binding in negative control, a significant decrease in the % binding of [^{177}Lu]Lu-DOTA-Trastuzumab in HER2 positive cancer cells in presence of excess of unmodified Trastuzumab was also observed. As a part of additional quality control studies, visual examination of [^{177}Lu]Lu-DOTA-Trastuzumab showed the formulation as a clear, colorless and transparent solution. The pH of the final preparation was observed to be between 5.0 and 6.0.

Bio-evaluation studies revealed considerable uptake and prolonged retention of the radiolabeled agent in blood (21.47±4.81, 17.06±0.79, 15.55±2.84 and 12.32±3.94%IA/g at 1, 2, 5 and 7 d p.i., respectively). Low accumulation and retention of the radiotracer was observed in majority of the organs except in liver and intestine. Bio-distribution studies performed in SCID mice having SK-OV-3 xenografted tumor showed tumor uptake of 9.07±2.60 % IA/g at 48 h p.i. which indicated the *ex-vivo* tumor targeting potential of [^{177}Lu]Lu-DOTA-Trastuzumab formulated by using kit.

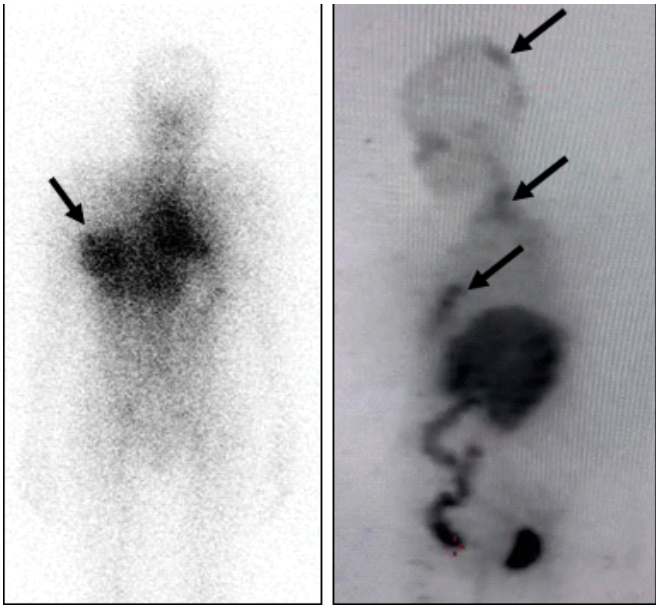

Fig.2: SPECT scans of two patients depicting the accumulation of [^{177}Lu]Lu-DOTA-Trastuzumab (shown with arrows) in cancerous lesions (Image courtesy: Dr. Venkatesh Rangarajan, TMH Parel and Dr. Nandini Pandit, JIPMER, Puducherry).

During clinical evaluation, [^{177}Lu]Lu-DOTA-Trastuzumab exhibited accumulation in cancerous lesions thereby showcasing the retention of its targeting efficacy post-functional modification and radiolabeling procedures (Fig.2).

Conclusion

The formulation of DOTA-Trastuzumab as a lyophilized kit, suitable for the formulation of patient dose of [^{177}Lu]Lu-DOTA-Trastuzumab, has been standardized. The kit was used for the formulation of patient dose of [^{177}Lu]Lu-DOTA-Trastuzumab with high RCP in a reproducible manner. Availability of DOTA-Trastuzumab freeze-dried kits will help in easy, convenient and consistent formulation of [^{177}Lu]Lu-DOTA-Trastuzumab patient doses in the hospital radiopharmacies.

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Development of Effective Antivirals

4

Identification of Novel Inhibitor of SARS-CoV-2 Plpro

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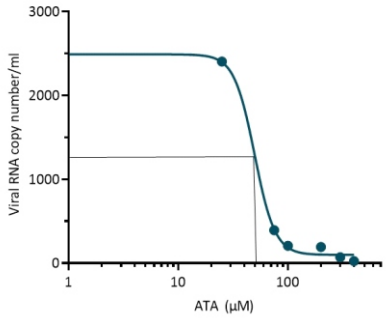

In vitro IC_{50} determination of ATA

ABSTRACT

The papain-like protease (PLpro) of SARS-CoV-2 is an important drug target against COVID-19 due to its essential role in viral replication and modulation of host immune responses. With the virus undergoing mutations across its genome, there's a pressing need for effective antivirals to combat the disease and its potential future outbreaks. In this study, we conducted screening of small molecule libraries to identify potential PLpro inhibitors. We found that aurintricarboxylic acid (ATA) inhibits PLpro, with K_i and IC_{50} values of 16 μM and 30 μM, respectively. The thermodynamics of ATA binding to PLpro were further characterized using isothermal titration calorimetry. *In vitro* assays demonstrated the antiviral efficacy of ATA with IC_{50} of 50 μM. Subsequently, its *in vivo* antiviral potential was also studied in Syrian hamsters.

KEYWORDS: *SARS-CoV-2, COVID, Aurintricarboxylic acid, PLpro, Papain-like protease, Antiviral*

Introduction

Developing effective antivirals against SARS-CoV-2 is crucial for managing the COVID-19 disease and preparing for potential future coronavirus outbreaks. Targeting essential steps in the virus's life cycle, such as blocking the interaction between the viral spike protein and the host ACE2 receptor, inhibiting viral genome replication by targeting viral RNA-dependent RNA polymerase (RdRp), and inhibiting viral proteases like the main protease and the papain-like protease (PLpro), are key strategies for drug development [1-3].

Papain like protease (PLpro) of SARS-CoV-2 cleaves viral polyproteins (pp1a/1ab) at three sites to yield mature non-structural proteins, Nsp1, Nsp2, and Nsp3, essential for virus replication. Besides, PLpro is also implicated in the evasion of host antiviral immune responses through deubiquitination and deISG15lation of several host/viral proteins hampering the signalling cascades of the innate immune system [4-7]. Therefore, inhibition of PLpro will not only suppress the viral multiplication but also help in overcoming the dysregulation of host innate immune response caused by viral infection. While several potential PLpro inhibitors have been identified previously, their clinical efficacy remains limited. Therefore, there is a need to find novel PLpro inhibitors.

In this study, employing our previously established optimized assay conditions [8], we undertook a high-throughput biochemical screening with small molecule libraries to identify novel inhibitors of SARS-CoV-2 PLpro. Among the compounds screened, aurintricarboxylic acid (ATA) emerged as a potent inhibitor of the PLpro enzyme with inhibition constant in the low micromolar range.

Subsequently, we explored the potential of ATA in inhibiting PLpro both *in vitro* and *in vivo*.

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Materials and Methods

Expression and purification of PLpro: The recombinant protein of SARS-CoV-2 PLpro was expressed and purified as previously described [8]. Briefly, the protein was expressed in BL21(DE3) strain of *E. coli* and purified from the soluble fraction of the cell lysate using Nickel-affinity chromatography followed by size exclusion chromatography. The purified protein, concentrated to 10 mg/ml, was stored at 4°C in a storage buffer (50 mM HEPES pH 7.5, 100 mM NaCl, and 5 mM DTT) for further studies.

High throughput screening: The experiments were conducted using 96-well black non-treated plates (Thermo Scientific, Denmark) in a CLARIOstar Plus multi-mode microplate reader (BMG Labtech, Germany), with excitation and emission filters set at 355 nm and 430 nm, respectively. The assay buffer consisted of 50 mM MES (pH 6.5), 100 mM NaCl, 0.5 mM EDTA, 5 mM DTT, and 0.1 mg/ml BSA. Initial screening assays were performed in a reaction volume of 50 μl, comprising 200 nM enzyme, 300 μM test compound, and 20 μM fluorogenic tetrapeptide substrate (Z-Leu-Arg-Gly-Gly-AMC). IC_{50} values were determined through three/four parameters non-linear regression fitting of velocity data for different compound concentrations (0-300 μM) at a fixed concentration of the substrate (20 μM) using GraphPad Prism software (www.graphpad.com). The plotted data in Fig.1 (a,b) represent mean values derived from three independent experiments.

Isothermal Titration calorimetry: Isothermal titration calorimetry (ITC) experiments were performed to study thermodynamics of PLpro-ATA interaction using MicroCal iTC200 instrument (Malvern Panalytical Ltd., UK). Protein and ATA were used at concentrations of 25 μM and 0.7 mM, respectively in Tris (pH 7.5), 100 mM NaCl, and 5 mM DTT. Sample cell contained the protein with temperature and reference power set at 25°C and 5 μcal/sec, respectively. Each titration with ATA comprised an initial injection of 0.4 μl

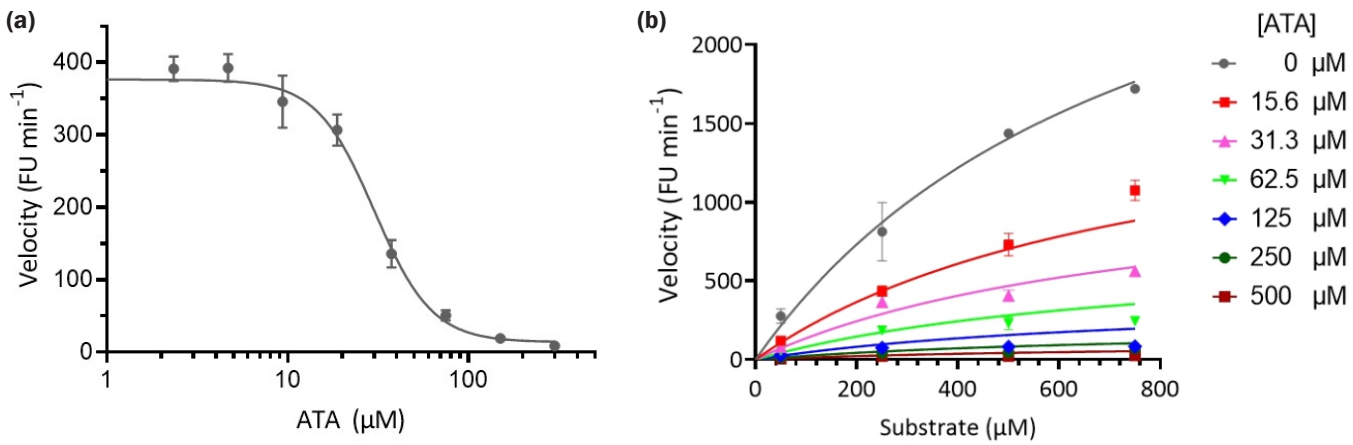

Fig.1: Biochemical characterization of PLpro inhibition by ATA (a) Concentration-dependent inhibition of PLpro enzymatic activity by ATA; [PLpro]=200nM, [Substrate]=70µM; (b) Enzymatic activity of PLpro at different concentrations of Z-LRGG-AMC substrate (50, 250, 500, 750 µM) and ATA (0-500 µM) to estimate K_i. FU: Fluorescence units.

followed by 18 identical injections of 2 µl, lasting 4 seconds per injection, and spaced 120 seconds apart. An initial delay of 120 seconds was applied, with stirring at 800 rpm. The heat of dilution derived from ATA titration in buffer was averaged and subtracted from each injection. Interaction parameters were computed using MicroCal iTC200 analysis software.

In vitro toxicity and antiviral assay of ATA: Before proceeding for in vitro antiviral assay, cytotoxicity assessment of ATA against Vero E6 cells was carried out. After seeding cells in a 96-well plate at 80% confluency, various concentrations of ATA (up to 1 mM) were added. MTT assay was performed after 48 hours to gauge cell viability. For the antiviral assay, Vero E6 cells, similarly seeded in a 96-well plate at 80% confluency, were infected with SARS-CoV-2 at 0.1 m.o.i. for two hours. Post-infection, different concentrations of ATA (ranging from 25 to 400 µM) in fresh media were added to the cells. Supernatants were collected at 24 hours post-infection, and RNA isolation followed by RT-qPCR analysis using specific primers for viral spike, nucleocapsid, and ORF1a was conducted. Mean Ct values from the RT-qPCR were utilized to estimate viral RNA copy numbers, and IC_{50} for ATA was determined using GraphPad Prism with four parameters variable slope non-linear regression fitting.

Evaluation of antiviral potential of ATA in Syrian hamsters: The antiviral efficacy of ATA was evaluated *in vivo* in 24 Syrian hamsters, divided into four groups of six animals each: one control and three treatment. All animals were intra-nasally infected with SARS-CoV-2 ($TCID_{50}$ = 10^5) on day zero. While the control group received no treatment, the treatment groups (A, B, and C) were administered ATA at doses of 15, 30, and 45 mg/kg body weight, respectively, starting four hours post-infection via oral route in a 100 µl solution. Dosages were repeated daily for four days (day 1-4), and animals were euthanized on day 5. Daily monitoring and recording of body weights were conducted throughout the study. Throat swabs and lung tissues were collected from all the animals for further analysis. RT-qPCR was employed to estimate the viral loads in throat swabs and left lung tissues, while histopathological evaluations were performed on the right lung tissues. Statistical analysis to determine differences among groups was conducted using Welch's t-test (one-tailed).

Results

High throughput screening

A screening of 350 drug-like compounds, including natural product compounds, was carried out against PLpro under optimized assay conditions as previously reported [8]. Significant inhibition of the enzyme was observed only with ATA. Concentration-dependent inhibition of PLpro enzymatic activity revealed an IC_{50} value of 30 µM for ATA (Fig.1(a)). To ascertain the type of inhibition and the inhibitory constant (*Ki*), enzymatic assays were performed at four substrate concentrations (50, 250, 500, 750 µM) with varying ATA concentrations (0 µM – 500 µM) (Fig.1(b)). The kinetic data were analysed using SigmaPlot software, fitting them into four models of enzyme inhibition (competitive, non-competitive, uncompetitive, and mixed). The Akaike Information Criterion corrected (AICc) values were employed to determine the most plausible mode of inhibition. The kinetic data demonstrated the best fit with the non-competitive model of enzyme inhibition, yielding a *Ki* value of 16 µM.

Isothermal titration calorimetry

The binding energetics of ATA to PLpro were investigated using isothermal titration calorimetry (ITC) (Fig.2). Analysis of thermodynamic data, fitted into a one-site binding model, revealed a dissociation constant (K_D) of 11.3 µM with enthalpy (ΔH) and entropy (ΔS) changes of -2.47 kcal/mol and 14.3 cal/mol/deg, respectively. These thermodynamic findings indicate favourable contributions from both enthalpy and entropy to the overall binding energy (ΔG), suggesting that the interaction between ATA and PLpro involves a combination of hydrophobic and hydrogen bonding interactions.

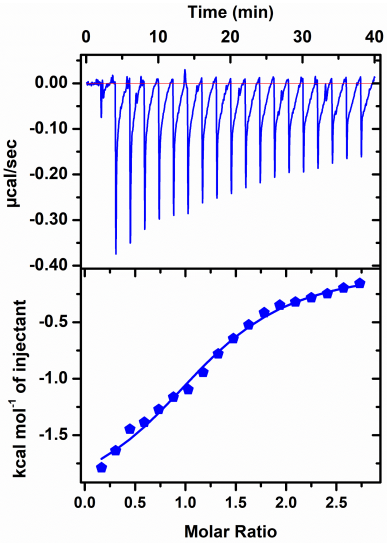

Fig.2: Thermodynamic and kinetic parameters of ATA binding to PLpro. Top panel: Raw data of calorimetric titration showing exothermic heat changes with successive injections; Bottom panel: Integrated binding isotherm plotted against molar ratio (ATA: Plpro).

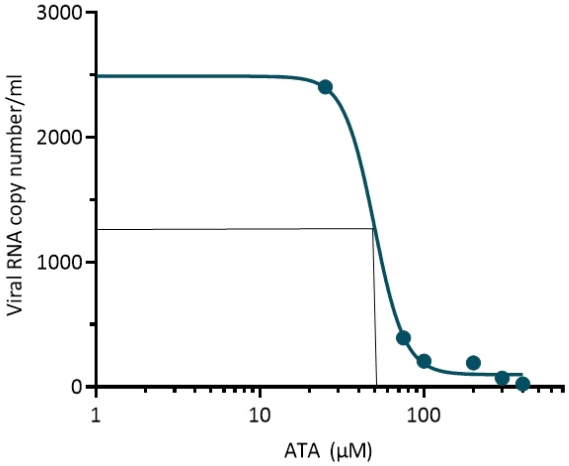

Fig.3: In vitro IC_{50} determination of ATA.

In vitro toxicity and antiviral potential of ATA

The *in vitro* antiviral potential of ATA was explored by first assessing its cytotoxicity in Vero E6 cells. Treatment with 1.0 mM ATA showed 95% cell viability after 48 hours, indicating its non-toxic nature at this concentration. Subsequently, antiviral efficacy of ATA was evaluated at concentrations below 1.0 mM. Vero E6 cells infected with SARS-CoV-2 were treated with different ATA concentrations (ranging from 0 to 400 μM). The viral load, estimated in terms of viral RNA copy numbers via RT-qPCR, exhibited a notable reduction as ATA concentration increased from 25 μM to 75 μM, indicating an IC_{50} value between these concentrations. Using four-parameter variable slope non-linear regression fitting, the IC_{50} was determined as 50 μM (Fig.3).

Evaluation of in vivo antiviral potential of ATA in Syrian hamsters

The *in vivo* antiviral efficacy of ATA was assessed in Syrian hamsters using a previously established protocol [9]. ATA was orally administered to SARS-CoV-2-infected animals in three treatment groups, designated as (a), (b), and (c), at doses of 15, 30, and 45 mg/kg body weight, respectively. The treatment schedule for ATA is illustrated in Fig.4(a).

Throughout the five-day observation period, no notable weight loss, complications, or mortality occurred in any groups (treatment or untreated controls). RT-qPCR analysis revealed a decrease in virus RNA copy numbers in throat swab samples from ATA-treated groups compared to the untreated control (Fig.4(b)). Significance of this reduction was determined using one-tailed Welch's t-test, showing a 0.4-log reduction in groups A and B (*p*-values: 0.0592 and 0.0580, respectively) and a 1-log reduction in group C (*p*-value: 0.0439) compared to the untreated control.

However, in lung tissues, no significant reduction in virus RNA levels was observed in the treatment groups compared to the untreated control (Fig.4(c)). Additionally, there were no noticeable differences in lung pathology between the untreated and ATA-treated groups.

Conclusion

In this study, we have screened a library of compounds using high-throughput biochemical assay to find potential inhibitors of SARS-CoV-2 PLpro. ATA emerged as a promising inhibitor of PLpro. Further, its binding parameters were assessed through biochemical and biophysical methods. We also evaluated its in vitro antiviral efficacy against SARS-CoV-2 using viral culture in VeroE6 cells. Subsequent *in vivo*

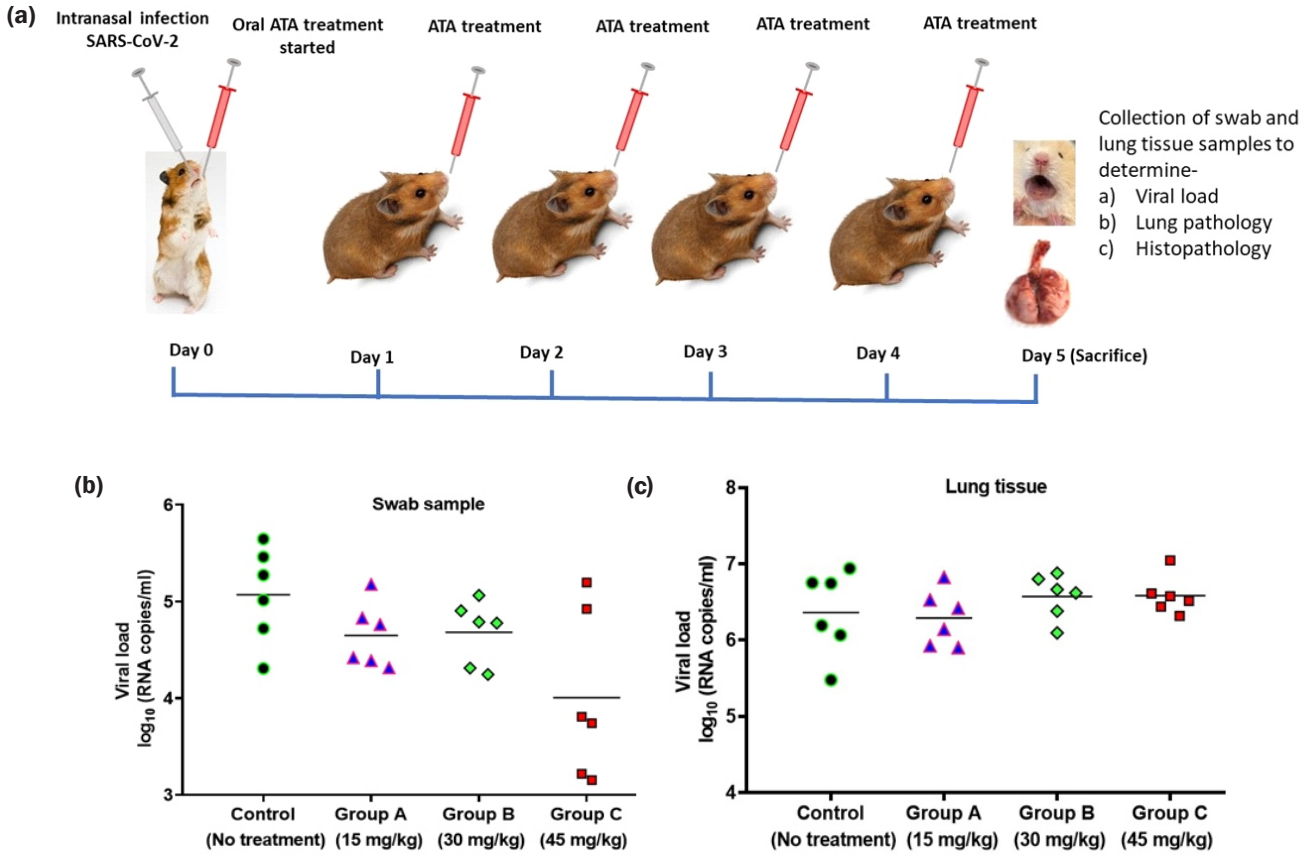

Fig.4: In vivo antiviral assay of ATA. (a) Treatment design in Syrian hamsters. (b) Throat swabs and (c) Lung tissues of SARS-CoV-2-infected animals from the untreated control (six animals) and three treatment groups (six animals each). Horizontal bars represent mean values.

investigations in SARS-CoV-2-infected Syrian hamsters demonstrated that oral administration of ATA led to a reduction in viral load in the throat. However, no discernible improvement in lung pathology was observed, possibly due to limited cell permeability of ATA. However, ATA's low toxicity suggests its potential as an oral gargle or nasal cleaning solution to reduce viral transmission. Alternative administration routes, like nebulization or nasal inhalation, may enhance bioavailability of ATA in lung tissues.

Acknowledgments

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New Approaches to Value Addition

5

Valorization of Fish Waste

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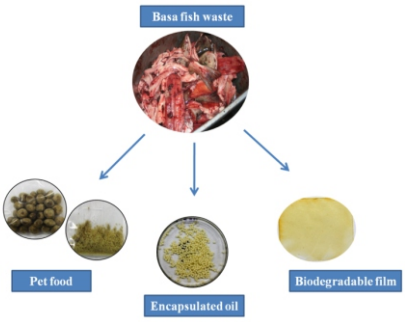

ABSTRACT

Fish and fishery waste are nutritionally adequate and valorization of this waste can be done by adapting new approaches for proper utilization. Deployment of fish waste has been demonstrated by using three different approaches viz. pet food, encapsulated oil and biodegradable film. Nutritionally adequate and microbiologically safe pet food in kibble and powder form was prepared with an extended shelf life of 65 days using gamma irradiation. Encapsulation of fish oil extracted from fish waste was carried out in calcium alginate beads, which increased the shelf life and storage quality up to 3 times as compared to the non-encapsulated oil. Biodegradable films were synthesised using gamma irradiated mince dispersion where, film prepared from 10 kGy-treated sample exhibited enhanced physical properties compared to the non-irradiated one. These results indicate that fish waste can be utilized for development of value-added products, which not only reduces environmental hazards, but also results in the upliftment of the socio-economic conditions.

KEYWORDS: *Fish waste, Circular economy, Pet Food, Fish oil, Encapsulation, Biodegradable film.*

Introduction

Fish and other types of seafoods are highly nutritious, and can be an important part of healthy and well-balanced diet. They are also considered as unique sources of natural drugs for deadly diseases like cancer, AIDS, coronary disease etc. It is known to be rich in protein, fat, omega-3 fatty acids, vitamins and minerals. Fisheries and its allied industries are the major economic sectors in the world. Consumption of fishery products has seen a huge increase in the last few years. In 2020, the world's total fisheries and aquaculture production reached a record 214 million tonnes. India is the 3rd largest fish producer (16.25 million tonnes), and this contributes to 1.1% of the country's GDP [1].

Unfortunately, commercial processing (beheading, de-shelling, degutting, removal of fin and scales, filleting) of fish results in a huge amount of solid waste, offal or by-products, which are discarded either at sea or in landfills. Waste disposal in the open environment creates pollution and hazards, which has potential to become the source of many infectious diseases [2].

However, fishery waste is an enriched source of nutrients and bioactive compounds that can be utilized in production of several value-added products. The head, bone, and intestine are rich in lipids with high polyunsaturated fatty acids (PUFAs) content and the skin is a rich source of protein [3]. These wastes can be gainfully utilized for several purposes such as preparation of biodegradable/edible films, fish oil, low-cost nutraceuticals, pet food/animal feeds, silage & organic fertilizers and industrial products such as bio-active compounds (gelatin, chitin, chitosan, carotenoids, glucosamine hydrochloride, squalene) etc. Fish oil from waste contains beneficial bioactive compounds (eicosapentaenoic acid, EPA, C20:5 n-3 and docosahexaenoic acid, DHA, C22:6 n-3) that can be encapsulated, thus providing a specific and controlled release of these bioactives. Encapsulation of bioactives and fish oil is of great pharmaceutical significance due to its growing demand in the market [4].

Fish and shellfish proteins from waste offer a wide variety of substrate material for the extraction of new peptides with specialized or multi-functional bioactivity because of their considerable structural diversity. The skin of the larger fish provides leather that could be utilized in the manufacture of handbags, belts, clothing, wallets, belts, etc [5]. Basa fish offal is a good source of potential lipids and can be utilized for preparation of aquatic feed for commercially important fishes [6]. Utilization of fish waste will provide an important tool for lowering the problem of malnutrition and food shortage in developing countries. In addition, it will help in generation of employment opportunities and as a result, turn out to be advantageous both for the environment and the economy.

Basa (*Pangasius bocourti*), an Asian freshwater catfish, is one of the most popular fish worldwide due to its desirable qualities such as white flesh, high PUFA content and digestible protein. It contains high quality balanced essential amino acids and fatty acids with adequate ω-6/ω-3 ratio [7]. During processing of Basa into fish fillet, fingers, steaks etc., 50-70% of the biomass ends up as solid or liquid wastes. The waste tissues/organs such as viscera, head, bones, skins and fins, generated in the filleting industry, are a boon in direction of fish-waste utilization.

Circular economy is the need of the hour for efficient and sustainable utilization of fish and fishery wastes to recourse, reallocate and reduce the negative environmental effects. An improved and efficient technique will help in utilization of fish-processing discards to generate superior quality co-products.

The present article shows three different ways by which seafood processing waste can be utilized for production of valuable commodities i.e. (i) pet food (ii) encapsulated fish oil and (iii) biodegradable films, which could find various applications in food and feed industries.

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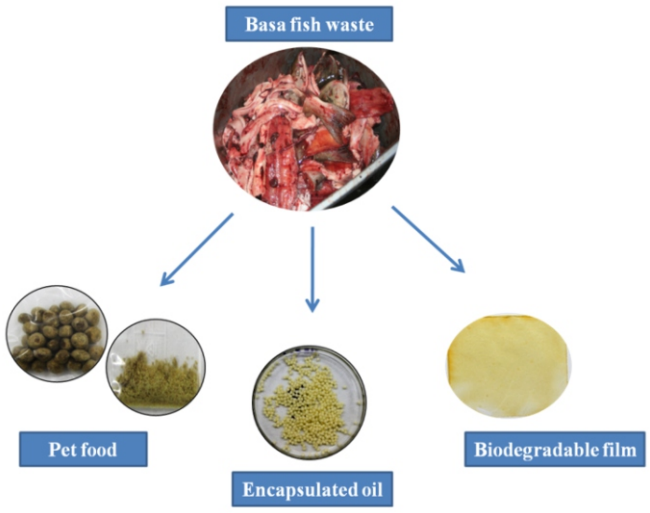


Materials and Methods

Oil and mince extraction

The oil from head, bone and viscera was extracted separately by solvent extraction method as described earlier using chloroform: methanol; ratio of 2:1 [8]. Mince was extracted from the bones and spines using deboning machine. The protein content in the mince was analysed by the Kjehldahl method.

GC-MS analysis of oil

This fish oil was esterified with methanol and 2N potassium hydroxide [8]. These esterified methyl esters of fatty acid were analysed using the GC-MS technique.

Preparation of pet food

Initially fish waste (100 g) was mixed with 50 ml of potable water and homogenised to make a thick paste; rice and wheat flour (1:1 w/w), 1% turmeric powder and salt 3% were then mixed properly. For powder preparation, this thick paste was dried directly in Infra-red dryer at 50^{0}C for 3 h to a moisture content of 5 - 8%. For kibble preparation, 20 ml water was slowly added to the mixture and round kibbles were made from the prepared dough. Kibbles were dried to a moisture content of 5 - 8 %. These pet foods were packed and irradiated at 2.5 and 5 kGy and quality parameters such as proximate, microbial load and lipid peroxidation (in terms of thiobarbituric reactive substances, TBARS) was studied during the storage.

Encapsulation of oil

Oil was mixed with various concentrations of sodium alginate to prepare loading concentrations of 10%, 20%, 30%, 40% and 50%. Stability of emulsion was determined by keeping 50 ml of alginate-oil emulsion undisturbed for 1 h at room temperature. To prepare beads, simple extrusion process was used for the encapsulation of fish oil as per the previous report [9]. The size, surface and cross-sectional view of beads was observed with the scanning electron microscope at 20 kV. The quality parameter of beads such proximate analysis, acid value (AV), peroxide value (PV) and free fatty acid (FFA) was done as per the methodology already published [10].

Preparation of biodegradable films

The washed and minced meat was homogenized with one volume of chilled distilled water using a homogenizer. Later, 0.5% glacial acetic acid was added slowly to reduce pH with stirring to induce gelation. The gel was homogenized with three volumes of a chilled solution of 3% glycerol and 0.75% glacial acetic acid to obtain film-forming dispersion. The obtained film-forming dispersion was divided into three parts, irradiated at 0, 10 and 25 kGy and change in viscosity as well as protein content was studied. 100 ml of each sample was poured and spread evenly on 21 cm × 21 cm polystyrene plate and kept at 50^{0}C in a dry air oven for 12 h and further conditioning of films was done at 25^{0}C at 50% relative humidity for 5 days. After conditioning, films were peeled and analysed for different properties [11].

Results and Discussion

Fish waste is a source of various nutraceuticals and bioactive compounds such as polyunsaturated fatty acids (PUFAs), peptides, vitamins and minerals. The fish waste can be effectively utilized for development of value-added products, thus, reducing environmental pollution and generating economic benefits. This waste can be utilised for the extraction of these bioactives for preparation of different value-added products for e.g. nutrient supplements, packaging materials, animal feeds etc. In the present study, fish oil was extracted from total waste (bones, head and viscera) whereas, fish mince was extracted only from bone waste of Basa (*Pangasius bocourti*). The Basa fish waste contains about 12% oil and 49% mince with 15% protein. Total fatty acid composition of the extracted oil (Table 1) showed that most monounsaturated fatty acid (48.27 ± 1) predominated, followed by saturated fatty acid (35.93 ± 1.13%) and polyunsaturated fatty acid (15.88 ± 0.26%). These results suggest that Basa fish waste is nutritionally adequate with substantial amount of essential fatty acids and protein [12]. Hence, this waste was utilized to develop three value-added products namely (i) pet-food (ii) encapsulated oil and (iii) biodegradable film.

Table 1: Total fatty acids (%) of fish waste.

Sr. No.	Fatty Acid Composition	Total fatty acid (%)
1.	Myristic Acid (14:0)	2.27 ± 0.04
2.	Palmitic Acid (16:0)	22.47 ± 1.02
3.	Stearic Acid (18:0)	11.19 ± 0.07
4.	Oleic Acid (18:1, n-9)	48.27 ± 1
5.	Alpha Linolenic Acid (18:3, n-3)	0.55 ± 0.09
6.	EPA (20:5, n-3)	0.29 ± 0.1
7.	DHA (22:6, n-3)	2.1 ± 0.01
8.	Linoleic Acid (18:2, n-6)	12.1 ± 0.02
9.	Gamma-Linolenic Acid (20:3, n-6)	0.45 ± 0.02
10.	Arachidonic Acid (20:4, n-6)	0.39 ± 0.02
11.	n-3/n-6	0.23 ± 0.014
12.	Σ SFA	35.93 ± 1.13
13.	Σ MUFA	48.27 ± 1
14.	Σ PUFA	15.88 ± 0.26

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9).

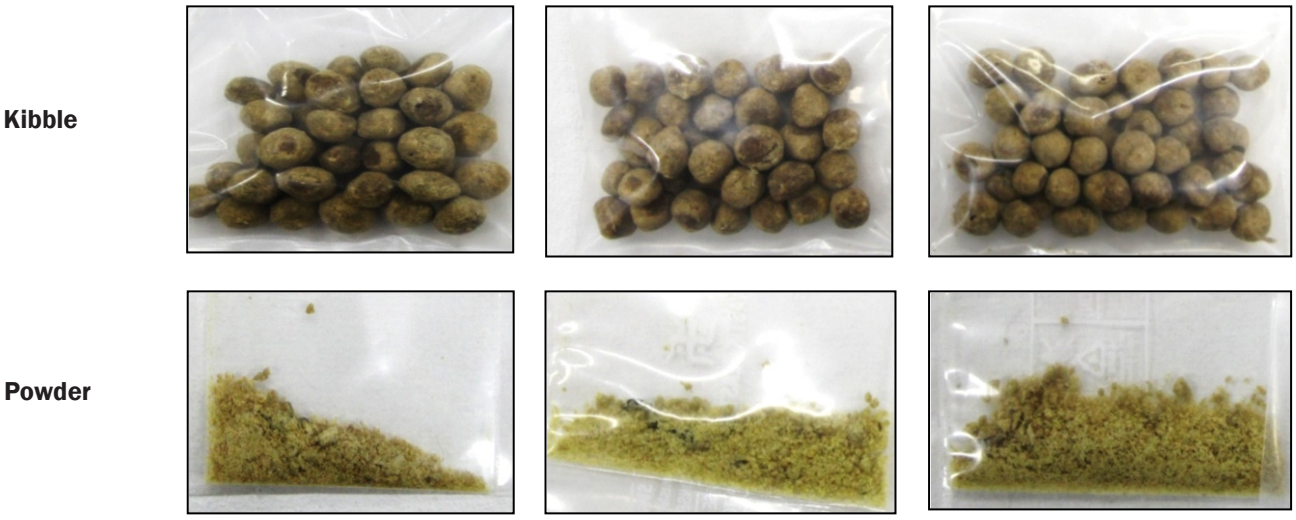


Fig.1: Different forms of pet foods before and after irradiation.

Pet food preparation

Valorization of Basa fish to prepare safe pet foods i.e. kibble or in powder form (Fig.1) using gamma irradiation (for extending its shelf life) was attempted. Microbiological (Fig.2) and lipid peroxidation analyses (Fig.3), showed an extended shelf life of 65 days for both the pet foods when irradiated at 2.5 and 5 kGy whereas, non-irradiated kibble and powder samples were spoilt within 28 and 35 days respectively. No significant changes in the proximate composition (Table 2) of both these pet foods were observed on irradiation, indicating that both were nutritionally adequate and microbiologically safe [13-15].

Encapsulated fish oil

Fish oil was extracted from total waste and encapsulated with alginate as a coating material using simple extrusion process. The total fatty acid composition of encapsulated and uncapsulated oil was analysed where no significant difference was observed. Alginate concentration (2.5%) with oil loading of 30% showed higher emulsion stability (Fig.4) with least pore space, hence, was considered optimum for encapsulation of oil [16]. The encapsulated beads were 2.39 ± 0.10 mm in size, with smooth surface (Fig.5) and had 91% encapsulation efficiency. The proximate analysis depicted, ash, moisture, fat, protein and carbohydrate content of oil beads to be 2.95 ± 0.07, 2.26 ± 0.06, 91.07 ± 0.02, 0.08 ± 0.01 and 3.64 ± 0.01 respectively (Table 3). Beads were stable up to 12 days as determined by peroxide value (18.67 ± 1.15 meq/Kg), acid value (14.13 ± 0.35 mg KOH/g) and free fatty acid value (8.42 ± 0.51%) where as the un-encapsulated oil got rancid on day 4

Table 2: Proximate analysis of prepared pet foods before and after irradiation.

Sr. No		Kibble			Powder		
		Control	2.5 kGy	5.0 kGy	Control	2.5 kGy	5.0 kGy
1	Moisture	6.60 ± 0.14^{a}	6.92 ± 0.87^{a}	6.98 ± 0.81^{a}	6.12 ± 0.04^{a}	5.90 ± 0 .29^{a}	5.84 ± 0.01^{a}
2	Protein	20.69 ± 1.92^{a}	16.16 ± 0.53^{a}	19.66 ± 4.36^{a}	13.59 ± 0.38^{b}	12.87 ± 0.35^{a}	14.37 ± 0.32^{c}
3	Fat	14.99 ± 2.35^{a}	16.48 ± 2.59^{a}	14.6 5±1.91^{a}	15.36 ± 0.06^{a}	16.57 ± 2.79^{a}	15.3 ± 1.06^{a}
4	Ash	7.0 ± 0.07^{b}	6.8 ± 0.42^{b}	4.48 ± 0.01^{a}	5.05 ± 0.21^{a}	4.95 ± 0.49^{a}	4.95 ± 0.64^{a}
5	Carbohydrate	50.72 ± 0.02^{a}	53.64 ± 0.55^{ab}	54.23 ± 0.07^{b}	59.88 ± 0.15^{a}	59.71 ± 0.05^{a}	59.54 ± 0.08^{a}

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences ($p < 0.05$).

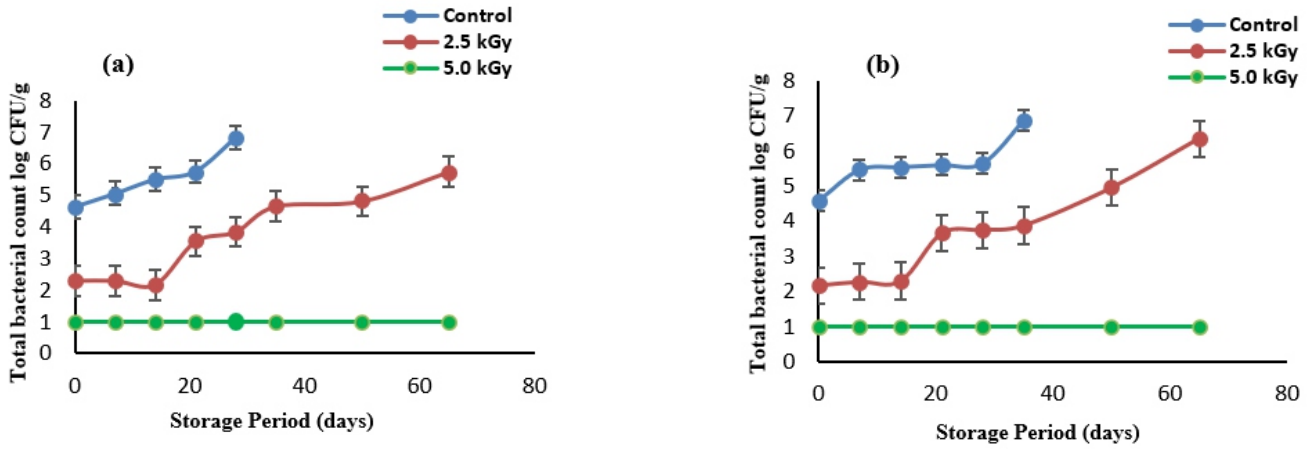


Fig.2: Total bacterial load of developed pet foods on irradiation and storage (a) Kibble form and (b) Powder form.

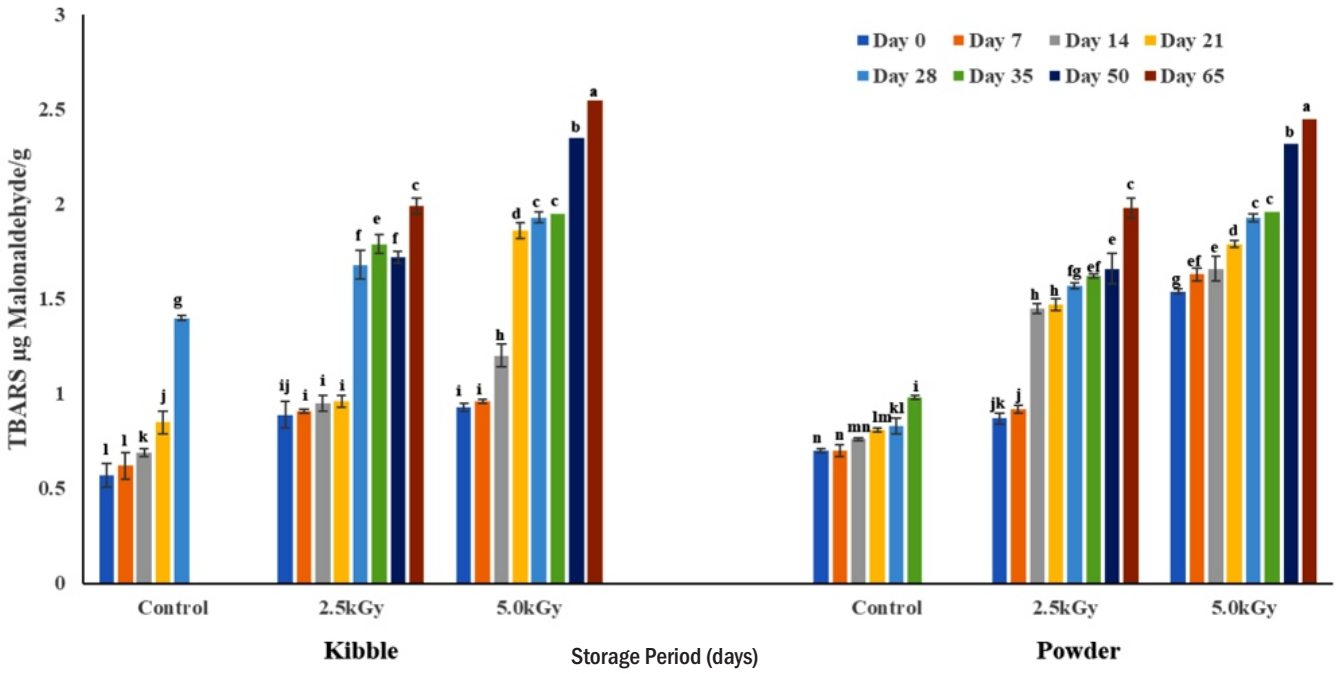


Fig.3: Lipid peroxidation in terms of TBARS for different pet foods on irradiation and storage.

The values are mean ± standard deviation of three independent experiments in triplicates (n=9). Different letters on the error bar indicate significant differences ($p < 0.05$).

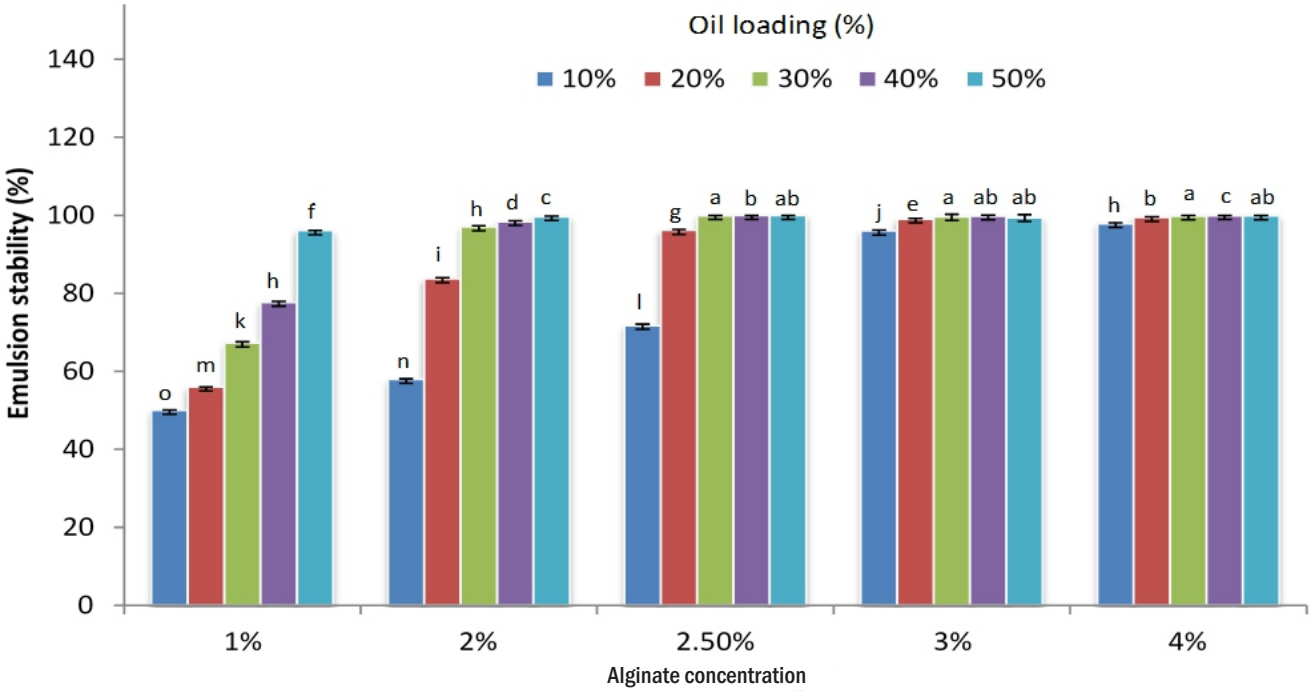


Fig.4: Emulsion stability (ES) at different alginate concentration and oil loading %.

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in error bars indicate significant differences ($p < 0.05$).

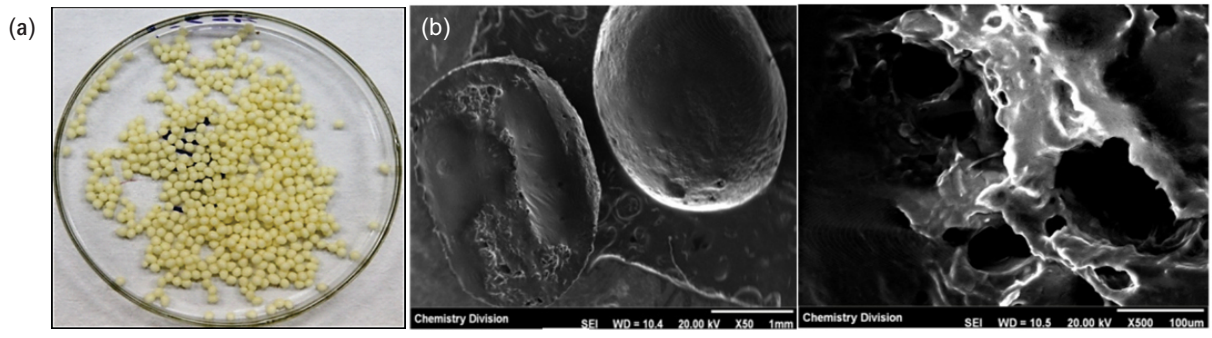


Fig.5: Characteristics of oil beads of 2.5% alginate concentration at 30% oil loading: (a) Physical appearance (b) SEM image of Surface and Cross-sectional view.

Table 3: Proximate analysis of per 100 g of encapsulated oil beads.

Sr. No.	Proximate analysis	Content (%)
1.	Ash	2.95 ± 0.07
2.	Moisture	2.26 ± 0.06
3.	Fat	91.07 ± 0.02
4.	Protein	0.08 ± 0.01
5.	Carbohydrate	3.64 ± 0.01

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9).

Table 5: Viscosity and protein content of non-irradiated and irradiated film forming dispersions

Sr. No.	Sample	Viscosity (cp)	Protein content (%)
1.	Control	506 ± 2.3^{a}	1.06 ± 0.02^{A}
2.	Dispersion 10 kGy	365 ± 2.2^{b}	1.19 ± 0.02^{A}
3.	Dispersion 25 kGy	20 ± 0.6^{c}	1.23 ± 0.03^{A}

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences ($p < 0.05$).

Table 4: Peroxide value (PV), Acid value (AV) and Free fatty acid (FFA) values of oil beads.

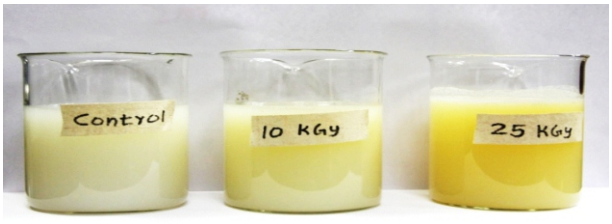
Sr. No	Storage period (days)	PV (meq/kg)		AV (mg KOH/g)		FFA (% wt)	
		Fish oil	Encapsulated	Fish oil	Encapsulated	Fish oil	Encapsulated
1	0	2.33 ± 0.32^{f}	2.67 ± 0.15^{f}	4.7 ± 0.3^{g}	5.46 ± 0.41^{f}	1.86 ± 0.08^{g}	2.73 ± 0.45^{f}
2	4	28 ± 2.65^{b}	5 ± 0.26^{e}	17.20 ± 0.7^{b}	10.02 ± 0.56^{e}	7.87 ± 0.65^{c}	5.01 ± 0.28^{e}
3	8	ND	6.67 ± 0.15^{d}	ND	12.84 ± 0.69^{d}	ND	6.92 ± 0.34^{d}
4	12	ND	18.67 ± 1.15^{c}	ND	14.13 ± 0.35^{c}	ND	8.42 ± 0.51^{b}
5	16	ND	43.33 ± 2.89^{a}	ND	34.03 ± 0.47^{a}	ND	17.02 ± 0.23^{a}

The results shown are mean ± standard deviation of three independent experiments in triplicate (n= 9). Different letters in the row/column indicate significant differences ($p < 0.05$). ND- Not determined

when stored in amber coloured glass bottle in dark at room temperature (25°C) with relative humidity of 65% (Table 4). Encapsulation of fish oil increases the shelf life and storage quality for longer period by reducing oxidation and bio dehydrogenation [17].

Biodegradable film

Biodegradable packaging material from mince dispersion of fish waste was synthesized to serve as "Wealth from Waste"- an eco-friendly initiative. Film dispersions were gamma-irradiated at a dose of 10 and 25 kGy before casting. A significant decrease in viscosity (Table 5) and increase in yellowness (Fig.6) was observed with irradiation which can be linked to higher oxidative fragmentation of proteins at 25 kGy as compared to 10 kGy. The appearance of different films is shown in Fig.7. The physical properties of prepared films were tested (Table 6), where film prepared after irradiation at 10 kGy showed better Tensile strength, Young's Modulus with lower water solubility and elongation at break as compared to the control, which had minimum tensile strength and maximum elongation at break. The 25 kGy irradiated film had highest opacity with yellowness and water vapour permeability. Gamma-irradiation affects the physical properties of the film, hence, irradiation of protein dispersion from waste is an effective tool to develop films with improved quality [18,19].



(a) (b) (c)

Fig.6: Dispersions treated with different doses of γ- irradiation (a) Control (b) 10 kGy (c) 25 kGy .

(a) (b) (c)

Fig.7: Film prepared from control and irradiated dispersion (a) Control (b) 10 kGy (c) 25 kGy .

Conclusion

Basa fish waste is nutritionally adequate and valorization of this waste can be done by adapting new approaches for value addition. In the present study, three different approaches have been demonstrated for sustainable management of waste and resource reallocation by developing commercially important commodities such as pet food, encapsulated oil and biodegradable films. The fish waste can be effectively utilized for development of value-added products, thus, reducing environmental hazards and generating economic benefits.

Acknowledgment

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Table 6: Physical properties of films obtained from control and irradiated dispersion.

Sr. No	Sample (film)	Control	10 kGy	25 kGy
1	Moisture (%) #	14.07 ± 0.29^{a}	14.06 ± 0.03^{a}	13.76 ± 0.07^{a}
2	Protein (%) #	30.93 ± 0.5^{a}	29.88± 0.71^{a}	29.97 ± 1.01^{a}
3	Water solubility (%) #	61.9 ± 4.76^{a}	15.47 ± 1.19^{b}	61.04 ± 5.7^{a}
4	Water vapour permeability (g mm h^{-1} cm^{-2} Pa^{-1})x 10^{-8} ##	7.08 ± 1.02^{a}	7.74 ± 1.27^{a}	11.42 ± 0.09^{b}
5	Thickness (mm) ##	0.16 ± 0.009^{a}	0.17 ± 0.0^{a}	0.29 ± 0.037^{b}
6	Tensile strength (N/mm^2) ###	0.76 ± 0.13^{a}	1.27 ± 0.26^{b}	1.11 ± 0.22^{b}
7	Young's Modulus (N/mm^2) ###	3.82 ± 1.28^{a}	8.39 ± 2.32^{b}	9.77 ± 1.98^{b}
8	Elongation at break (%) ###	162.94± 19.64^{a}	87.64 ± 15.23^{b}	65.43 ± 6.96^{b}
9	L* (black to white) ##	38.37 ± 1.0^{a}	35.63 ± 0.28^{b}	33.32 ± 0.78^{c}
10	a* (green to red) ##	7.86 ± 0.74^{a}	9.38 ± 0.48^{b}	12.77 ± 1.36^{c}
11	b* (blue to yellow) ##	3.84 ± 0.78^{a}	10.38 ± 1.02^{b}	14.11 ± 0.43^{c}
12	ΔE* (total colour change) ##	59.19 ± 1.03^{a}	62.92 ± 0.43^{b}	66.26 ± 0.96^{c}
13	Opacity (%) ##	13.59 ± 0.38^{a}	14.75 ± 0.58^{b}	17.91± 1.25^{c}

 # (n=3), ## (n=6), ### (n=4), Different letters in the same row indicate significant differences ($p < 0.05$).

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Resilient Crop Varieties

6

A New Gamma Ray-induced Mutant Variety of Groundnut for Cultivation in Gujarat and Maharashtra

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Plant of TG 73

ABSTRACT

Gamma ray based-induced mutagenesis has played a significant role in genetic improvement of groundnut. Sustained mutation breeding efforts at BARC, Mumbai, have evolved several improved Trombay groundnut (TG) varieties that have been commercialized across the country. In continuation, a new mutant was developed by gamma ray mutagenesis of cultivar TG 38. This mutant showed an improvement in pod, kernel size and percentage of three-seeded pods. Molecular markers distinguished TG 73 from its parent TG 38 or the check variety, TAG 24. In order to test its suitability and adaptability in different agro-ecological situations, TG 73 was evaluated in multi-location trials over the years in collaboration with Junagadh Agricultural University (JAU), Junagadh, Gujarat, and Dr. Panjabrao Deshamukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, during summer. In these trials, TG 73 recorded a mean pod yield of 2541 kg/ha and 3218 kg/ha with a superiority of 16.6% and 14.3% over the best check variety, respectively. Based on consistent greater pod yields, TG 73 has been released and notified as TAG 73 and GG 37 for summer cultivation for Maharashtra (Vidharbha region) and Gujarat, respectively.

KEYWORDS: *Groundnut, Gamma rays, Mutant, Pod yield, Seed size.*

Introduction

Groundnut (*Arachis hypogaea* L.), an important edible food, feed and oilseed crop, covers 19% of the country's oilseed area, contributing 26.3% to the total oilseed production. It is distinct from other legume species by having aerial flower and subterranean fruit. It is widely used as source of cooking oil, digestible protein, minerals, vitamins and contributes considerably to food security as well as in alleviating poverty. Groundnuts have recently attracted attention as a functional food. Several studies have revealed groundnut consumption to reduce the risk of heart disease, cancer, total cholesterol, bad cholesterol and triglycerides without affecting the beneficial cholesterol. This is due to the presence of mono-unsaturated fatty acid, resveratrol, beta-sitosterol, vitamin E, folic acid and fibre [1]. In Gujarat and Maharashtra, groundnut is cultivated on 19,87,000 ha and 2,95,500 ha, with a production of 44,94,800 tonnes and 3,76,100 tonnes, respectively. Groundnut cultivation in *rabi* and summer (post-rainy) season is gaining prominence and its area is expanding with better productivity. There are very few summer-adapted, high input-responsive groundnut varieties in these states.

Groundnut exhibits narrow genetic base because of its monophyletic origin, limited gene flow due to ploidy barrier, and self-pollination. Breeding for high yield has been and will remain as one of the most important objective of any groundnut improvement programs. Induced mutagenesis using ionizing radiations has been the convenient and desirable approach for broadening genetic variability to overcome the limitations associated with a narrow genetic basis. This method is suitable for bringing specific improvement without significantly affecting other traits in groundnut [2]. Effective application of induced mutagenesis along with cross breeding has resulted in generation of a wide spectrum of mutants and mutant varieties [2]. Towards breeding of groundnut varieties with high yield potential, better heat tolerance and summer suitability, a new gamma ray groundnut mutant was developed and released for summer cultivation in Gujarat and Maharashtra.

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Materials and Methods

Seeds of groundnut cultivar, TG 38 were irradiated with 200 Gy of gamma rays (M_1 generation) from the Cobalt-60 source at the Bhabha Atomic Research Centre (BARC), Mumbai. TG 38, a gamma ray mutant, was released for cultivation for *rabi*/summer season in Odisha, West Bengal, Assam and North-Eastern states in 2006 [3]. Irradiated seeds were sown in the field along with untreated seeds in rainy season, 2008. In the M_2 generation, plants were examined carefully for various economic traits and 39 variants were selected and harvested individually. In the M_3, one progeny (TG 38-38) having more number of three-seeded, larger pod and seed compared to its parent, bred true (Fig.1). This mutant was ensured for its true breeding nature for pod and other traits in subsequent generations by growing alternately in rainy and summer seasons from M_4 to M_9 generations and designated as TG 73 (Fig.2). To test its suitability and adaptability, TG 73, was evaluated at Dr. Panjabrao Deshamukh Krishi Vidyapeeth

Fig.1: Pods of parent, TG 38 (left) and mutant, TG 73 loading %.

Fig.2: Plant of TG 73.

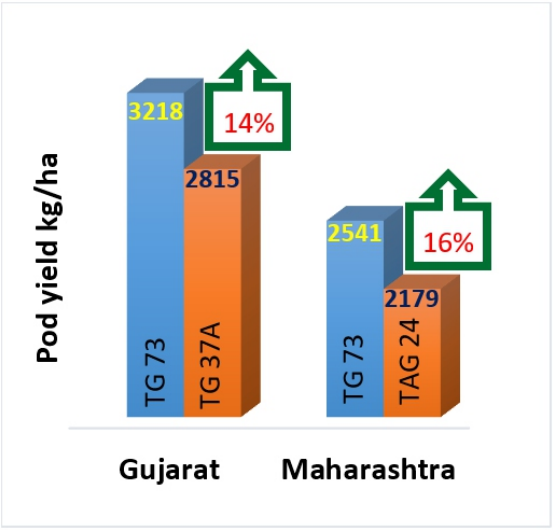

Fig.3: Pooled mean pod yield of TG 73 and best check variety in Gujarat and Maharashtra.

(PDKV), Akola, Maharashtra, during summer 2014 to 2019; and Junagadh Agricultural University (JAU), Junagadh, Gujarat, during summer 2016 to 2020. The evaluation trials included Station trials, Multi-location trials and Adaptive (On Farm) trials. Simultaneously, TG 73 was evaluated in initial varietal trials (IVT-I and II) of ICAR-All India Coordinated Research Project on Groundnut (AICRP-G) during summer 2017-18 and 2018-19.

Results and Discussion

In Maharashtra, evaluation of TG 73 was initiated in the station trial at PDKV, Akola during summer 2014 for the Vidharbha region. In this trial, TG 73 recorded pod yield of 4900 kg/ha with 14.4% advantage over check variety, TAG 24 (4282 kg/ha). Based on its superiority, TG 73 was subsequently yield-tested in the multi-location trials at four locations during summer 2015 to 2019 wherein, it had pooled mean pod yield of 2541 kg/ha, which was 16.6% more thanr TAG 24 (Fig.3). Further to study its adaptability, TG 73 was demonstrated on farmers' fields in adaptive trials wherein, it recorded 19% higher yield over TAG 24 during summer 2019. It has also greater fodder yield (which is a key contributor for livestock feed) improving the overall farm income. Considering the yield superiority, better pod or seed size and summer adaptability, TG 73 was released as TAG 73 (Trombay Akola Groundnut 73) by State Varietal Release Committee, Maharashtra. This variety was subsequently notified for commercial cultivation in Maharashtra in 2021.

For evaluation of TG 73 in Gujarat, the station trial was started at JAU, Junagadh, in summer 2016, wherein it recorded pod yield of 2355 kg/ha having 19.3% advantage over check variety, TG 37A (1973 kg/ha). Subsequently, TG 73 was evaluated in multi-location trials at four locations during summer 2016 to 2020. In these trials, it has pooled mean pod yield of 3218 kg/ha with an advantage of 14.3% over TG 37A (Fig.3). In parallel national ICAR evaluation trials, mutant TG 73 has also registered a mean pod yield of 1662 kg/ha and 3175 kg/ha with 24.2% and 6.0% superiority over the national check, TAG 24 and with 15.3% and 14.5% increase over the

Fig.4: Field view of TG 73 in Maharashtra.

Fig.5: Farmers from Andhra Pradesh, Karnataka, Gujarat and Maharashtra are displaying the better pod yield of TG 73.

zonal check, TG 37A, at Akola and Junagadh, respectively during the summer of 2017-18 and 2018-19. With consistent superior performance and desirable pod and seed features during summer, TG 73 was released as GG 37 (Gujarat Groundnut 37; Sorath Gaurav) by State Varietal Release Committee, Gujarat, followed by central notification in 2023.

TG 73 has semi-dwarf height, medium-size dark green leaflets, erect growth habit with sequential branching and maturity of 110-115 days (Fig.2). In TG 73, mutation was for the increased pod and seed size and higher number of three-seeded pods. TG 73 has greater mean hundred pod and kernel weight, shelling-out turn in comparison to the check varieties. The proportion of three seeded pods was more in TG 73 (40 %) than in its parent TG 38 (1 %) or TAG 24 (9%). Pods of TG 73 are with slight constriction and without beak and reticulation, while its seeds are more spheroidal with rose colour. Nutritionally, TG 73 has 24.5% protein, 49.1% oil content, 49% oleic acid and 32% linoleic acid. DNA profiling was carried out for TG 73 along with check variety, TAG 24 and its parent TG 38 using simple sequence repeat (SSR) and transposable element (TE) markers. Of the several markers screened, two SSR (IPAHM 23, GM 1996) and two TE (TE 113, TE 457) markers showed allelic variation among these varieties. To translate the benefits of new mutant variety, breeder seed of TG 73 were supplied to farmers of different states and farmers have cultivated and obtained encouraging returns (Fig.4, 5).

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Molecular Tools In Conventional Breeding

7

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.

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Biosensors

8

Biosensors for Pesticides: From Concept to Technology

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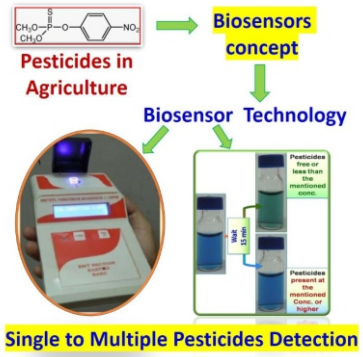

BARC technology for detection of single to multiple pesticides

ABSTRACT

Pesticides, especially insecticides, are chemicals used in many different sectors, such as agriculture, forestry, and the and the food industry, to kill or control insects. The accumulation of pesticides in the ecosystem, which are harmful to human and animal health, is detrimental to the environment. Thus, there is a need to monitor these pesticides with prompt and accurate analysis. The present article is a brief review of the work carried out at BARC for biosensor-based detection of pesticides using different biocomponents. We have developed the concept of microbial biosensors by immobilizing microbial cells on different matrices and associated them with different transducers for the detection of single to multiple samples of methyl parathion in the laboratory or field. Later on, this concept was translated into a handheld optical biosensor device for the detection of methyl parathion directly in the field. Further, a concept of enzyme-based biosensors was also developed for the detection of various pesticides belonging to the same group, and the same was translated into the technology of Biokit for the qualitative detection of multiple pesticides belonging to the organophosphate and organocarbamate groups.

KEYWORDS: *Biosensors, Pesticides, Insecticides, Microbial biosensors, Transducers, Methyl parathion*

Introduction

Due to the extensive use of pesticides in agriculture and other allied sectors, the presence of pesticides and their residues in food chains or food commodities has become a major cause of concern all over the world. Food safety has become crucial for all, and consumers have to be assured that they are not exposed to an unacceptable level of pesticide residues. Many newspapers, including The Hindu (10 June 2015 title: Chemical contaminants in household spices), The Deccan Chronicle (8 June 2015 title: Washing vegetables does not reduce pesticides), have reported the presence of pesticides in vegetables and spices [1-2]. On May 7, 2017, The Hindu and The Deccan Chronicle, reported the presence of pesticides in dried ginger powder [3–4]. In 2018, the Government of India banned 18 pesticides, which cover many pesticides belonging to the organophosphate (OP) and organocarbamate (OC) groups [5–6]. Keeping in view the dietary exposure and risk assessment, the Food Safety and Standard Authority of India (FSSAI) under the Ministry of Health and Family Welfare uses the Good Agricultural Practice (GAP) data for the fixation of the Maximal Residual Level (MRL) of each pesticide. A central scheme, "Monitoring of Pesticide Residues at National Level," was set up, and NABL-accredited laboratories located in different parts of India under the Department of Agriculture, Cooperation, and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, participated in collecting and analysing the food samples for the possible presence of pesticide residues [7].

Many traditional analytical methods like HPLC and GC/LC MS-MS have been widely used for pesticide analysis, but they require not only expensive equipment but also highly-trained technicians and are time consuming and laborious. Over a course of time, researchers have put efforts to develop biosensors, which can be used for easy, online and prompt detection of pesticides with accuracy and sensitivity comparable to that of other techniques. A biosensor is an analytical device that integrates an immobilized biological element with a transducer to recognize the analyte. The signal generated by the interaction of analyte and the biological element is proportional to the concentration of analyte. Contrast to the traditional analytical methods, biosensor facilitates onsite detection of a large number of samples with almost no or very little preparation, in less time and without the requirement of expensive apparatus or trained [7-8]. In this direction, NABTD, BARC, has also developed the concept of microbial and enzyme-based biosensor technology for the detection of pesticides.

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Concept of Microbial Biosensors for Detection of Methyl Parathion Pesticide in Laboratory

Methyl parathion (MP), an extremely toxic organophosphate pesticide that was used as a non-systemic insecticide in agriculture to protect crops, is currently banned

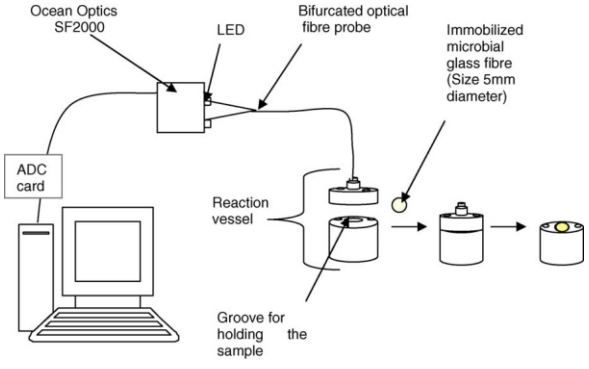


Fig.1: Schematic diagram of optical biosensor using disposable microbial biocomponent .

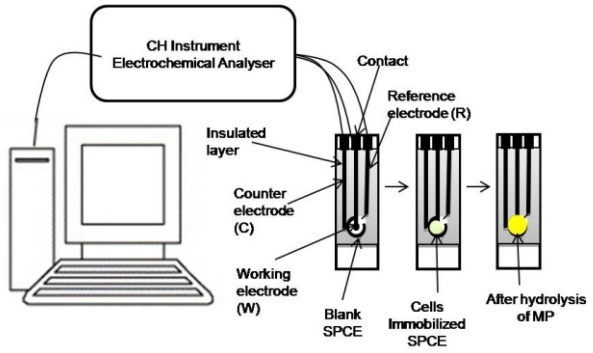


Fig.2: Schematic diagram of electrochemical biosensor using cells immobilized SPCE.

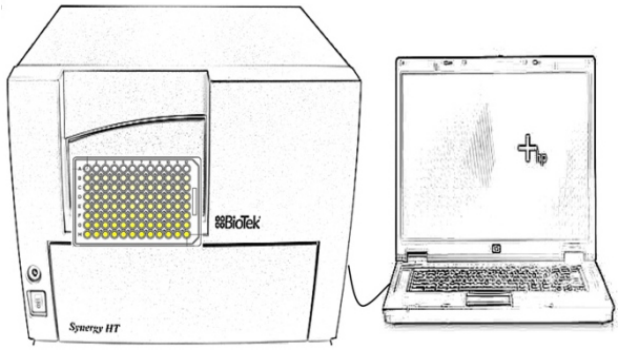


Fig.3: Schematic diagram of microplate based optical biosensor for multiple samples.

[9–11]. Our laboratory has established the concept of microbial biosensors for the detection of this pesticide. The characteristic feature of the developed microbial biosensors was their ability to detect MP in the laboratory from single or multiple samples. The first optical microbial biosensor was based on the immobilisation of whole cells of *Flavobacterium sp.* (containing organophosphorus hydrolase enzyme) on glass fibre filters. This biosensor dealt with a disposable biocomponent [12] and consequently, these biocomponents could be used for single-sample analysis only. In the second study, an electrochemical microbial biosensor was developed by immobilising recombinant *E. coli* on a screen-printed carbon electrode (SPCE) and integrating this system with an electrochemical analyzer [13]. Here, the biocomponent was reusable and required a low amount of sample. In the third study, a microplate-based optical biosensor was developed by immobilising *Sphingomonas sp.* (a) directly onto the surface of the 96-well microplate or (b) indirectly on an onion membrane fixed inside the wells of the microplate. These two types were linked with the optical transducer of a multi-detection microplate reader (MDMR). The microplate technique enables the acquisition of the whole array of data simultaneously and provides an innovative concept where multiple samples can be detected in a very short period of time [14–15]. Further integrating *Sphingomonas sp.* cells with fSi nano-particles increased the sensitivity and stability of the biocomponent [16].

All these microbial biosensor techniques require high-end, costly detection transducer systems and can only be used in laboratories due to their large size and high cost. Therefore, later on, the concept of microbial biosensors was exploited and translated into a technology for the simplified detection of MP.

Microbial Biosensors Based Technology for Detection of MP

BARC has exploited the concept of microbial optical biosensors for detection of MP and translated the research work into technology for developing a prototype handheld colorimetric biosensor, which can be used in the field for monitoring of MP pesticides (Fig.4). It has two components: The first component is the biocomponent, consisting of immobilised microbial cells of *Sphingomonas sp.* with the organophosphorus hydrolase (OPH) enzyme. The second component is the handheld optical colorimeter with an ultraviolet 3W LED light source, a small cuvette, and a microcontroller circuit. This handheld biosensor is small in size, battery-operated, and directly displays the concentration of MP in ppm (detection range 1–10 ppm) and can be utilised for the detection of MP directly in the field [17]. A microplate-based optical biosensor was able to detect MP pesticides in the range of 0.1–1.0 ppm (the FSSAI set MRL) and can detect many or multiple samples (No. 96) in a very short period of time (10 min). This was subsequently translated into technology (AB35NABTD, 18).

Biosensor Kit (Biokit) For Detection of Organophosphate and Organocarbamate Pesticides

Using the above technology, one could detect only one pesticide, methyl parathion. However, under the real field conditions, multiple pesticides are used in large amounts on crops, resulting in the accumulation of many pesticides in fruits and vegetables as well as in water bodies. Organophosphate (OP) and organocarbamate (OC) pesticides are the most commonly used pesticides for agriculture and domestic use to control insects. BARC has developed a colorimetric visual biosensor kit (BioKit: AB37NABTD) for the detection of safe levels of OP (Methyl Parathion, Parathion, Monocrotophos, Chlorpyrifos, Phorate, Profenfos, Quinalphos, and Dichlorvos) and OC (Aldicarb, Carbaryl, Carbofuran, and Carbosulfan) pesticides (Fig.5). This qualitative type of detection indicates the presence of either a single or all the 12 pesticides (as mentioned in Table included in Fig.5) that belong to the OP and OC groups. In this Biokit, a colour code of blue and green was optimised and calibrated with their respective concentrations of pesticide (Fig.5). If pesticides are either absent or present at a concentration lower than the mentioned concentration (Fig.5), then the colour will change from blue to green, and if pesticides are present at concentrations higher than the mentioned concentration, then there will be no change in colour and the blue colour will persist. This biokit is able to detect six pesticides among the 18 banned pesticides. This technology was recognised as a rapid food testing kit by

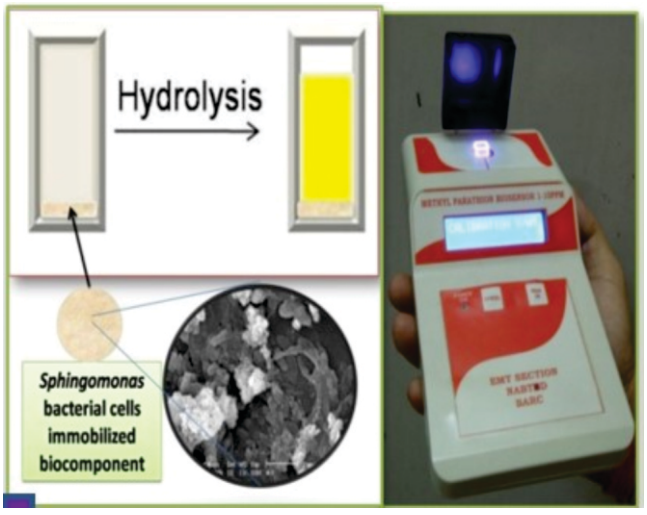
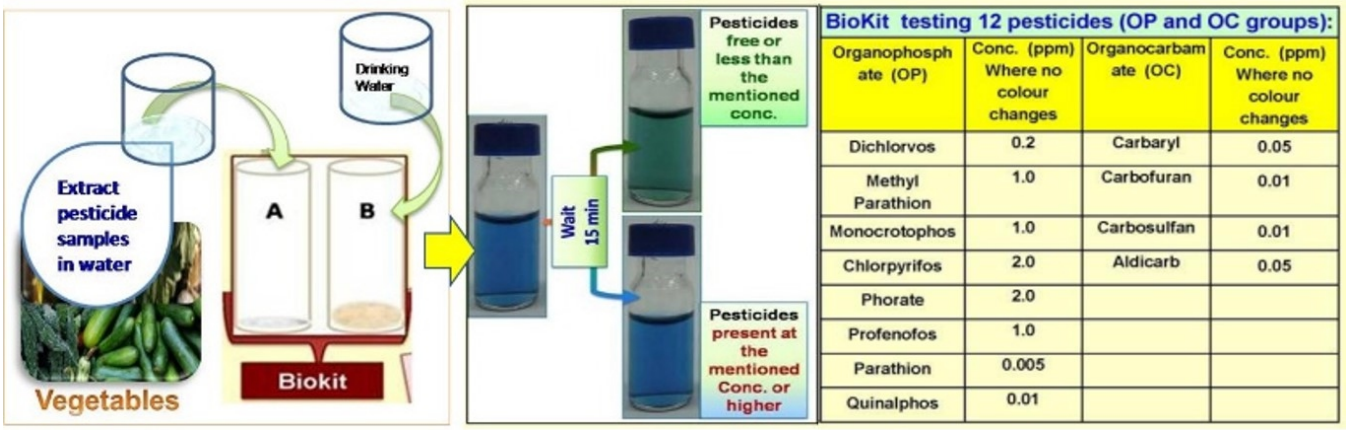


Fig.4: Handheld colorimetric microbial biosensor for detecting methyl parathion
(AB28NABTD: http://barc.gov.in/technologies/biosensor/index.html)



BioKit testing 12 pesticides (OP and OC groups):

Organophosphate (OP)	Conc. (ppm) Where no colour changes	Organocarbamate (OC)	Conc. (ppm) Where no colour changes
Dichlorvos	0.2	Carbaryl	0.05
Methyl Parathion	1.0	Carbofuran	0.01
Monocrotophos	1.0	Carbosulfan	0.01
Chlorpyrifos	2.0	Aldicarb	0.05
Phorate	2.0		
Profenofos	1.0		
Parathion	0.005		
Quinalphos	0.01		

Fig.5: Biokit for detection of OP and OC pesticides (AB37NABTD:https://www.barc.gov.in/technologies/biokit/index.html)

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Ref.

Date .....

"TO WHOM IT MAY CONCERN"

No. FSC 20/2021/609

This is to certify that the "Blokit for detection of Pesticides" developed and demonstrated by Dr. Jitendra Kumar, Scientist-G, Bhabha Atomic Research Centre (BARC), Mumbai on 1st May, 2023 at Conference Hall of Directorate of Horticulture & FP, Khanapara, Guwahati-22 was found to be functioning satisfactorily and qualitatively response to 12(twelve) numbers of Organophosphate (8 nos.) and Organocarbonate (4 nos.) pesticides as per its detection protocol. The kit was found to be easy to handle and use.

Dated Guwahati
the 3rd May, 2023

(ANUPAM GOGOI)
Food Analyst to the Govt. of Assam,
State Public Health Laboratory, Assam,
Bamunimaidam, Guwahati-21

Food Analyst to the Govt. of Assam, Guwahati-21

Fig.6: Validation certificate from Food Analyst, Governemnt of Assam, for satisfactory performance of Biokit detecting pesticides.

FSSAI in their press release (December 31, 2019) (20). Recently, the technology was validated by the State Food Analyst, Govt. of Assam (Fig.6) and transferred to many entrepreneurs, including Patanjali.

Conclusion

In this article, we have summarised the research work carried out at BARC, which has led to the development of biosensors for the detection of pesticides. Initial research work was carried out to develop the concept of biosensors. After a thorough validation, these were later translated into technologies such as handheld biosensors for the detection of methyl parathion pesticides for field application and biokits for the detection of multiple pesticides belonging to organophosphate and organocarbamate groups.

Acknowledgement

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Mutation Breeding

9

Reinforcing Mutation Breeding by Augmenting Genomic Resources in Pulse Crops

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Maruca pod borer tolerant cowpea mutant Mr2

ABSTRACT

Pulse crops, with high protein content, are valuable components of Indian agriculture as they can satisfy the nutritional demands of our growing population. However, these crops with narrow genetic diversities, have received comparatively less research attention. Mutation breeding has been successful in the genetic improvement of these orphan, yet important pulse crops such as pigeonpea, mungbean, black gram and cowpea. Also, induced mutagenesis has the potential to enrich the genetic resources in these crops, equipping the different crop improvement programs with arsenal to meet the challenges of climate change. Concurrently, augmenting the genomic resources is equally significant for accelerated and targeted breeding of pulse crops.

KEYWORDS: *Genetics, Genomics, Mutation breeding, Next-generation sequencing, Pulses, SSRs, SNPs, Whole genome*

Introduction

Pulse crops, the cornerstone of Indian agriculture, are the primary source of dietary proteins for the predominantly vegetarian population of our country. These crops play an immense role in enhancing and ensuring nutritional security by complementing carbohydrate-rich cereals-based diets[1]. India predominantly cultivates chickpeas, pigeonpeas, mungbeans, black gram, cowpeas, lentils, and various beans. Despite being the leading global producer, consumer, and processor of pulses, India's reliance on net imports is concerning [2]. Mutation breeding is a GMO-free [3] complementary method to the narrow genetic base limited conventional breeding for inducing novel variations and improving yields of pulse crops.

Traditionally, ionizing radiations such as X-rays, gamma rays, beta particles, and fast and thermal neutrons were employed to induce mutations in these crops. However, new energy sources like electron-, proton-, and heavy-ion beams are increasingly adding new perspectives to mutation breeding.Though induced mutation alone or in combination with conventional breeding has the potential to create variations, the availability of genomic resources profoundly influences the pace of accelerated genetic crop improvement. The advent of next-generation sequencing (NGS) technologies has resulted in the development of extensive molecular resources, including transcriptome sequence data, genetic and physical maps, and molecular markers, enabling trait mapping and marker-assisted breeding faster and more reliable. For fast-tracking pulse crop improvement, it is imperative to use radiation to broaden the variation and concurrently develop exhaustive genomic resources.

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Materials and Methods

Induced mutagenesis

Thousands of genetically pure, uniform, dry seeds (12% moisture content) of pulse crops after exposure to predetermined doses of ^{60}Co gamma rays were used to raise M_1 and M_2 generations. Variants with putative mutations for desirable traits, identified in the M_2 generation, were ascertained for their stability and uniformity (homozygosity) in the later generations M_3–M_5. If found to be agronomically superior, these were directly evaluated for their potential release as varieties. Conversely, the poor mutants were improved through recombination breeding before entering into varietal trials. In either case, the promising mutants or their derivatives underwent a battery of rigorous testing with other competitors and check varieties (in station to multi-location and adaptive varietal trials) for different parameters such as yield, nutrition, disease, insect pest and nematodes resistance, in coordination with various State Agricultural Universities and or All India Coordinated Research Project on Pulses of the Indian Council of Agricultural Research. Subsequently, potential candidates that successfully emerged from these meticulous testing were released and notified through the Gazette for commercial cultivation by the Ministry of Agriculture and Farmers Welfare.

Developing genomic resources

Next-generation sequencing-based Illumina short reads and the third generation Oxford Nanopore sequencing-based long reads of the whole genome sequences of the black gram cultivar 'Pu31' and that of cowpea cultivar 'CPD103' were individually hybrid assembled de novo using the MaSuRCA (Maryland Super Read Cabog Assembler) genome assembler. The completeness of the assembled genomes was determined through BUSCO analysis. These assemblies were subsequently

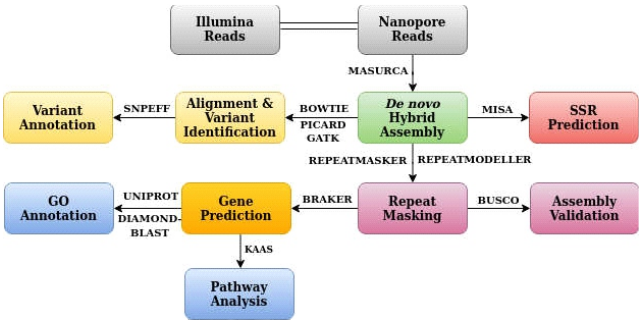

Fig.1: Whole genome sequencing bioinformatics workflow.

used for downstream analysis and also as reference genomes for resequencing of some induced mutants in these crops. While the entire set of genes and the encoded proteins across the genomes were predicted using BRAKER tool, the SSR motifs were deciphered using the MISA tool. The genome analysis tool kits, BOWTIE and PICARD, were employed to identify single base substitutions and small insertion or deletion variants (Indels) in the mutants as against the parental genomes. The gene products were subjected to gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for assigning gene ontology terms to describe their biological functions, processes, and cellular components and to map genes to complex interaction networks as per the KEGG database, respectively. The entire bioinformatic workflow is depicted in Fig.1. The whole genome sequences were also mined in silico for R-genes, transcriptionally active proteins and protein kinases involved in regulating gene expressions under a myriad of biotic and abiotic stresses. In addition, RNA-seq based transcriptome profiling enabled development of genic-SSRs and SNPs and treatment context-based identification of differentially expressed genes.

Results and Discussion

Induced mutagenesis

Of the 24 mutant varieties of pulse crops developed through mutation breeding at this Centre, five of them (three in black gram and one each in mungbean and cowpea) have been released in the last five years for commercial cultivation

Fig.2: Maruca pod borer tolerant cowpea mutant Mr2.

Fig.2: Recent varieties of Trombay pulse crops suitable for summer season. (a) Mungbean: TRCRM-147, (b) Cowpea: TC-901.

(Table 1, Fig.2). The cowpea variety, TC-901, is a gamma ray-induced direct mutant [4], while the rest of the four varieties are mutant derivatives involving mutant x cultivar or cultivar x mutant crosses (Table 1). The success of any mutation breeding program is largely dependent on the selection of appropriate parental genotype for mutation induction, appropriate dose for maximizing mutation recovery, size of the mutagenized population in M_1 and subsequent generations, and proper screening techniques for rapid and cost-effective identification of mutants [1]. A series of radio-sensitivity assays are conducted for determining the genotype-specific optimal dose prior to embarking on mutation breeding experiments. Optimal doses causing less than 50% lethality eg. LD_{20} or LD_{30}, or less than 50% growth reduction (GR_{50}) are understandably more desirable in autogamous plants like pulses that ensure maximal recovery of useful mutations with least mortality-like unintended damages. Successful outcomes of mutation breeding programs in pulse crops suggest that doses in the range of 100–200 Gy, 200–300 Gy and 300–400 Gy, are effective in pigeonpeas, cowpeas, and in mungbeans/urdbeans, respectively. Effective doses of electron beams were also in close proximity to that of gamma rays in different pulse crops (mungbean:500 Gy; urdbean:400 Gy; and cowpea:270 Gy) [5]. New mutant varieties have been carefully compared to competitors and check varieties, and have undergone extensive multi-location and adaptive varietal trials lasting almost a decade to ensure their success in the field. Thanks to this process, the farmers now have access to a range of high-quality, disease-resistant mutant pulse varieties that have improved yields and supported sustainable agriculture practices. As perceived in the present research outcome, the induced mutants often by themselves are not appropriate for varietal release, but have the potential to be more productive when used in cross breeding [6]. The contribution of induced-mutagenesis in enhancing the genetic resources can also be appreciated from the wide range of novel mutants identified in these pulse crops. For instance, the unavailability of useful resistance sources against *Maruca* pod borer in the cowpea gene pool was partially overcome by identifying tolerant mutants (Fig.3) (major accomplishment of BARC under the FAO/IAEA ongoing CRP project D22006) [7]. The induced mutants also serve as genetic treasures for identifying candidate genes and for exploring the intricacies of developmental biology in pulse crops.

Development of Genomic Resources

Draft whole genome sequence

A draft whole genome sequence of blackgram cultivar Pant U-31 was constructed for the first time by employing hybrid genome assembly based on Illumina and Nanopore reads with a total sequencing depth of ~148X. The final de novo whole genome spanned 475 Mb with a maximum scaffold

Table 1: Trombay mutant varieties notified in the last five years in different pulse crops.

Sr. No.	Mutant variety	Mutant/Mutant derivative	Year of release	Released for	Important attributes	Collaborating Institute
	Blackgram					
1	TJU-130	Cultivar x mutant	2023	MP	YMD resistant, Summer suitable	JNKVV, Jabalpur
2	TJU-339	Mutant x cultivar	2023	MP	YMD resistant, Summer suitable	JNKVV, Jabalpur
3	TRCRU-22	Mutant x cultivar	2023	Karnataka	Medium large seeds, YMD resistant, summer suitable	UAS, Raichur
	Mungbean					
4	TRCRM-147	Mutant x cultivar	2023	Karnataka	Large seed size, YMD resistant, Summer suitable	UAS, Raichur
	Cowpea					
5	TC-901	Direct mutant	2018	North Zone	Mosaic and root-rot resistant, early, summer suitable	IIPR-Kanpur

length of 6.3 Mb and scaffold N50 of 1.42 Mb[8]. Along similar lines, whole genome assembly of cowpea cultivar CPD103 resulted in an estimated genome size of ~377 Mb with a maximum scaffold length of 1.6 Mb and scaffold N50 of 26.7 Kb at a sequencing depth of ~120X [9]. Next generation sequencing reads are too short to resolve plant genomes abundant with repetitive elements resulting in assembly ambiguity [10]. Therefore, in the present study, a hybrid assembly approach involving the third generation Nanopore sequencing technique that is capable of delivering several kilobases of long reads was integrated with more accurate Illumina short reads to construct scaffold level reference genome assembly. The completeness of the genome assembly was gauged using the BUSCO analysis wherein 96.8% and 90.0% of complete genes in respect of black gram and cowpea, respectively, were recovered suggesting robustness of the assembly. About 42,115 genes were predicted with a mean coding length of 1131 bp in black gram as against 65,708 genes with a mean coding length of 915.7 bp in case of cowpea. The cowpea coding sequences varied between 42 bp and 15.2 Kb. Roughly, 80.9% and 85.4% of the predicted genes in black gram and cowpea, respectively, could be annotated (Fig.4).

Transposable elements (TEs)

In black gram, almost half of the assembled genome comprised of repetitive elements, majorly retrotransposons (RNA, 47.3% of genome) and minority of DNA transposons (2.29% of genome). But unlike the other related *Vigna* species, black gram housed more members of *Copia* super family

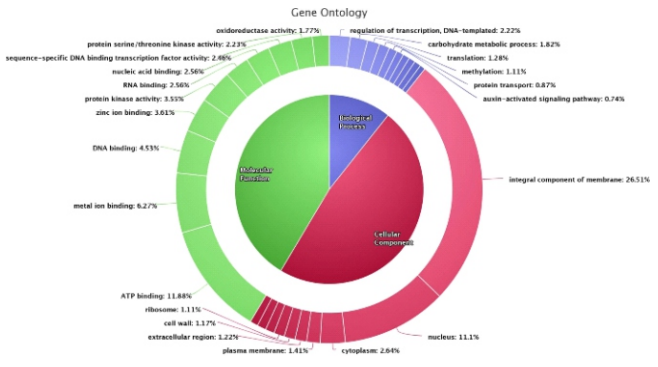

Fig.4: Gene ontology chart of Vigna mungo [8].

(34.5%) of LTR retrotransposons as against the Gypsy elements (13.4%). In cowpea also the retrotransposons (91.1%) were the major TEs with DNA transposons forming a minor component (4.4%). But among the LTR retrotransposons, the Gypsy superfamily predominated compared to the *Copia* elements. TEs, the potential resources of phenotypic variation and plasticity, are the major drivers of genome expansion [8]. They aid crop improvement programs by serving as molecular markers within or are closely linked to various QTLs as well as defense-related genes. They have been widely used for diversity studies and trait mapping [11].

Identification of genomic SSR motifs

From the whole genome sequencing of black gram, a total of 166,014 SSRs, including 65,180 compound SSRs, were identified and primer pairs for 34,816 SSRs were designed. Among 1,66,014 SSRs (excluding mono nucleotide repeats) identified, the proportions of dinucleotide repeats were higher (38.1%) than the other repeats in *V. mungo* [8]. Similarly, in cowpea, SSRs numbering 1,73,866 were identified from 16,063 sequences including 23,082 compound SSRs. Among these SSRs, barring the mono nucleotide repeats, tetra nucleotide repeats predominated (19.5%) in contrast to the dinucleotide repeats (15.6%). Pulses exhibited variation with respect to the predominance of particular nucleotide repeats. Mungbean showed prevalence of dinucleotide repeats, while in cluster beans tetra nucleotide repeats prevailed (19.9%), whereas common beans exhibited preponderance of trinucleotide repeats. Proportion of tri-, tetra-, penta-, and hexa-nucleotide SSRs in black gram were more or less same in comparison to *V. radiata* (24.6%, 2.5%, 1.2%, 0.2%) but lower than *V. marina* (49%, 3%, 7%, 5%), except for the tetra-nucleotide repeats. In cowpea, the corresponding proportions were 5.9%, 19.5%, 0.12% and 0.001%. These SSR markers are valuable for genomic mapping, DNA fingerprinting, and marker-assisted selection in plant species and pulses in particular [12].

Discovery of R-gene domains

In black gram, of the 33,959 annotated gene products, 1659 proteins showed the presence of R-gene (resistance gene) related domains, among which the predominant KIN class (905) was followed by RLK (239) and RLP classes (138) [8]. In contrast, a total of 2188 R-gene domain containing proteins were identified in cowpea, which also exhibited

predominance of KIN class (855) genes followed by the transmembrane receptors, RLKs (258) and RLPs (238). The classic nucleotide binding sites (NBS)-harbouring genes numbered 142 in black gram, which was in sharp contrast to 392 in cowpea. Only twenty-seven proteins were found to represent the class of cytoplasmic proteins (CNL and TNL) in black gram that was distinctly differing from 183 proteins in cowpea. These R-genes could be exploited in pyramiding resistance genes in new cultivars favouring effective and environment friendly approach of plant disease control [8].

KEGG pathway analysis

KEGG analysis identified 16,404 unique pathways in black gram of which majority of the genes were grouped into protein families (genetic information processing, signalling and cellular processes, and metabolism) followed by carbohydrate metabolism and transcription-related activities. In cowpea, 17,283 unique KEGG pathways were identified. Like in black gram, majority of them were grouped into protein families, followed by carbohydrate metabolism and transcription activities. KEGG pathway analysis provides a comprehensive overview of biological pathways enabling scientists to identify key molecules and pathways involved in specific biological and cellular processes. They could be applied to different organisms that share orthologous genes and help uncover hidden features in large-scale biological data, such as cellular and organism-level functions [13].

Orthologous gene comparison

Orthologous gene comparison study involving genes from black gram, mungbean, cowpea and adzuki bean showed 19,095 gene clusters to be shared by all four species, while 1970 clusters were specific to black gram. Cowpea exhibited 789 unique clusters among the four species, while it shared 20,091 orthologous genes with black gram (Fig.5). Such orthologous gene study aids in comparative genomics, phylogenetics, protein function annotation, understanding genome rearrangements, providing hypotheses about gene function across different species. In, it also provides insights into evolutionary relationship and gene family dynamics [14, 15].

Identification of genic-SSRs and SNPs

Transcriptome sequencing using NGS technologies allows quick and inexpensive identification of SNPs/SSR within the coding regions. These are more likely to be related with various biological functions. In cultivated blackgram (TU94-2), towards developing genic-SSR and SNP markers, a

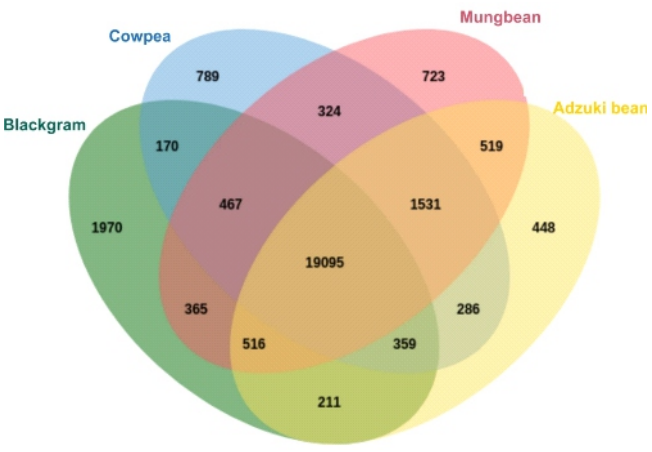

Fig.5: Venn diagram showing shared orthologous gene clusters among black gram, cowpea, mungbean and adzuki bean [8].

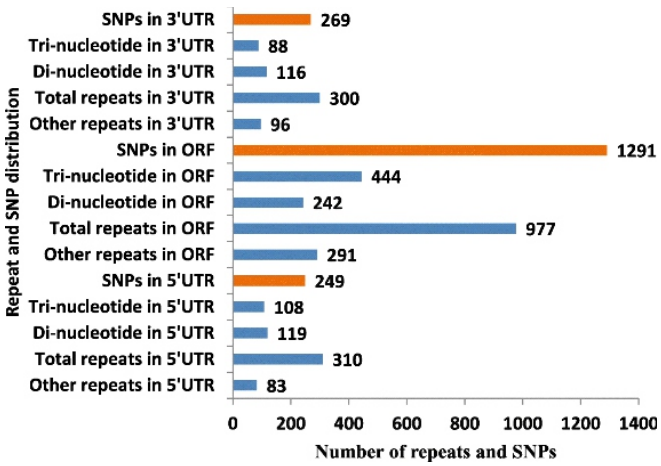

Fig.6: Frequency and distribution of SSRs and SNPs in coding sequence and untranslated region (UTRs) of blackgram TCS [17].

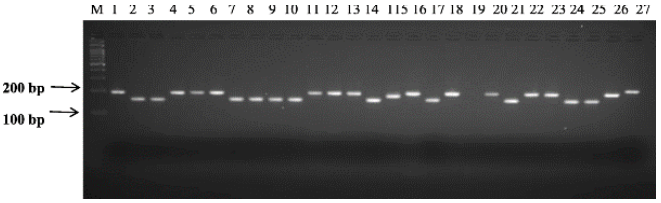

Fig.7: PCR amplification of a genic-SSR marker in 27 blackgram genotypes [17].

total of 48,291 transcript contigs (TCS) were searched for SSRs and 1,840 SSRs were identified in 1,572 TCS with an average frequency of one SSR per 11.9 kb. The tri-nucleotide repeats were most abundant (35%), followed by di-nucleotide repeats (32%). PCR primer pairs were successfully designed for 933 SSR loci. Sequences analyses indicate that about 64.4% and 35.6% of the SSR motifs were present in the coding sequences (CDS) and untranslated regions (UTRs), respectively (Fig.6) [16,17]. Similarly in the wild blackgram accession (*Vigna mungo* var. *silvestris*), out of 40,178 TCS, 1621 SSRs were identified in 1339 (3.3%) TCS, with an average frequency of one SSR per 11.1 kb. Tri-nucleotide repeats were found to be most abundant (646,39.9%) followed by di-nucleotide (490,30.2%), which together constituted 70.1% of the identified SSRs. Of the 1621 SSR motifs that were identified, PCR primer pairs were successfully designed for 1171 SSR loci. Of the total of 1844 SNPs that were identified in TW samples, 17 SNPs were heterozygous and 1828 SNPs were homozygous. Out of 1844 SNPs identified, PCR primer pairs were successfully designed for 1749 SNP loci and validated in a set of black gram genotypes (Fig.7) [17]. In cowpea, a total of 41,506 transcript contigs (TCS) were searched for SSRs and 3878 SSRs were identified in 3315 TCS with an average frequency of one SSR per 9.34 kb in cultivar CPD103. The tri-nucleotide repeats were most abundant (54.3%), followed by tetra-nucleotide repeats (21.5%). The most frequent motif type was A/T, which was followed by GAA/CTT. The availability of such a large number of sequence-based markers allows genetic diversity analysis, linkage mapping, comparative genomics, and association studies [17].

Nature of Gamma Rays-induced Stable Mutations at Whole Genome Level

Gamma rays induced single base substitutions and indels in three cowpea mutants (M_6 generation) (disease resistant, large seed size and small seed size mutants) as deduced through Illumina based next-generation

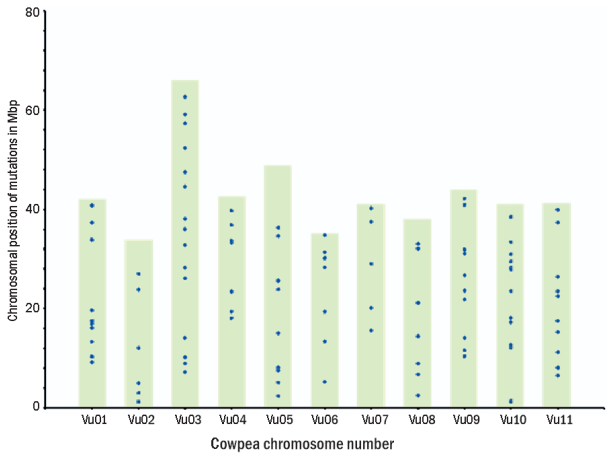

Fig.8: Chromosomal distribution of induced mutations (SBSs and indels) in gamma ray-induced cowpea mutants (M_6) [19] .

resequencing. A relatively higher frequency (88.9%) of single base substitutions (SBSs) with an average transition to transversion ratio (Ti/Tv) of 3.51 was observed [9]. A>G transitions, including its complementary T>C transitions, predominated the transition mutations and all four types of transversion mutations were detected. Among the indels (11%), small insertions (6.3%) were relatively more prominent than small deletions (4.8%). Single-base indels (involving A/T bases) showed preponderance, but indels of up to three bases were also detected in low proportions. Scattered across all the 11 chromosomes, 5.61±0.67 SBSs and 0.7±0.15 indels were induced per chromosome on an average (Fig.8). Only a fraction of SBSs (19.45%) and indels (20.2%) potentially altered the encoded amino acids/peptides, which concurs with previous reports [18]. The mutation rate (1.4×10^{-7} per bp) induced by gamma rays in the present study is about 0.6× of that in rice ($2.31 \pm 1.5 \times 10^{-7}$) [19], probably due to genomic differences.

Impact of Mutant Pulse Crop Varieties in India

Indian farmers are increasingly adopting Trombay mutant pulse crop varieties due to their superior yields, which are leading to higher on-farm incomes. One of the earliest black gram mutant varieties, TAU-1, released in 1985, is still highly coveted among farmers in Maharashtra and remains the mainstay of black gram cultivation in the state. TU-40 black gram variety is gaining popularity in the southern states, especially in the rice fallow niches. Pigeonpea varieties, TJT-501 and TT-401, are also incredibly popular, with the former being grown in almost half of the pigeonpea growing areas in Madhya Pradesh, while the latter, released for the central zone, is making inroads into the southern states with yields as high as 2500 kg/ha. The Maharashtra farmers cultivating PKV-TARA are reaping record pigeonpea yields under drip irrigation. The mungbean variety TMB-37 released for North East Plain Zone is garnering huge demands in the country for its earliness and summer suitability, and is re-released in the Punjab state. Likewise, the powdery mildew and *Corynespora* leaf spot disease-resistant varieties, TM-96-2 and TM-2000-2, are bringing rice fallow areas under mungbean cultivation in Andhra Pradesh and Chhattisgarh, respectively [5]. The recently released pulse varieties, TC-901 (cowpea), TRCRM-147 (mungbean) and TRCRU22, TJU339 and TJU-130 (blackgram), with resistance to yellow mosaic disease are contributing to area expansion under summer cultivation. These Trombay mutant pulse varieties, enjoying the sustained patronage of the Indian farmers, are instrumental in boosting the productivity and nutritional security of the country and now fulfill more than 3% of the national breeder seed demand of pulse crops in the country.

Conclusion

The accomplishments in mutation breeding of pulse crops at this Centre underscores the significance of the physical mutagens such as gamma rays in broadening the genetic variability of pulse crops and their contribution in sustained enhancement of the productivity to meet the growing demands and emerging challenges of climate change. The impact of mutant varieties demonstrates the immense potential of induced mutagenesis in rescuing the conventional breeding and contributing towards an environmentally sustainable agriculture. The rich genomic data generated through the molecular studies provide valuable resources for accelerated genetic enhancement of pulse crops through marker assisted breeding and enables adoption of newer genome editing tools like CRISPR-Cas in these important 'orphan' crops.

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Dose Measurement in Food Irradiation

10

Development of Fluorescence Gamma Dosimeters

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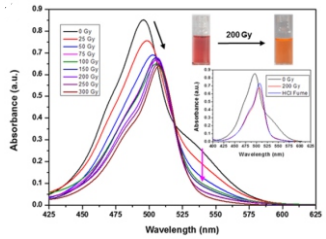

Change in absorption of anilino-Boron Dipyrromethene (BODIPY) dye in $CHCl_3$ under γ-irradiation exposure of increasing doses

ABSTRACT

We have developed two fluorescent γ-dosimeters based on BODIPY dyes. In one case, 8-anilino BODIPY was used which showed "off-on" fluorescence under g-exposure with dynamic range of 0-100 Gy. On the other hand, 8-(N,N-dimethyanilino) BODIPY based dosimeter showed ratiometric "off-on" fluorescence enhancement applicable in the range of 0–150 Gy with limit of detection (LOD) of 0.5 Gy. These highly sensitive fluorescence dosimeters will be useful for absorbed dose measurement in food irradiation processes.

KEYWORDS: *Dosimeter, Gamma radiation, Low dose, Bodipy dyes, Sensitivity, Colourimetric and Fluorometric measurement*

Introduction

Gamma (γ) is highly ionizing radiation, thus a very low dose γ-radiation can cause serious harm to the living biological systems. Thus, detection of gamma radiation is highly important to protect the lives [1]. Also, various safe uses of the gamma radiation are known like food, blood and sludge irradiation, radio-imaging and therapy, medical equipment sterilizations etc [2]. For all these applications, different doses of gamma radiation are being used which required to be precise to protect the materials and lives. Thus, accurate dosimeters are in high demands.

Conventional gamma-ray detectors like ionization chambers, scintillators and semiconductor detectors suffer from various limitations such as bulkiness, lack of sensitivity, high costs, inability to provide real-time three-dimensional data etc. [1,3]. Different dosimeters such as Fricke, clear poly(methyl methacrylate) (PMMA), dyed PMMA and radiochromic film dosimetry along with various luminescent dosimeters like TLD, RPLD, OSLD are also known for gamma dose measurements [1,4]. But they also have various limitations like low sensitivity and accuracy, dose range, anomalous fading, change in sample properties in repeated uses etc. Thus, detection of γ-radiations especially low dose gamma radiation is a real challenge. As a result, there is an urgent need for the development of simpler, highly sensitive and cost-effective gamma-ray detection systems.

Uses of fluorescence techniques are highly popular in chemical and biological sensors due to its high sensitivity, low cost, easy operation etc. But its use in γ-radiation detection is not very known so far [5-8]. We envisaged that use of fluorescence technique could be highly useful for the development of the low dose gamma dosimeters. For the current studies, we have chosen two anilino-Boron Dipyrromethene (BODIPY) dyes (1, 2) (Fig.1(a)). Their synthesis, photophysical and gamma dosimetry studies are discussed below.

Materials and Methods

BODIPY dyes 1-2 were synthesized by using the previously reported methods [8]. Dye 1-2 in chloroform

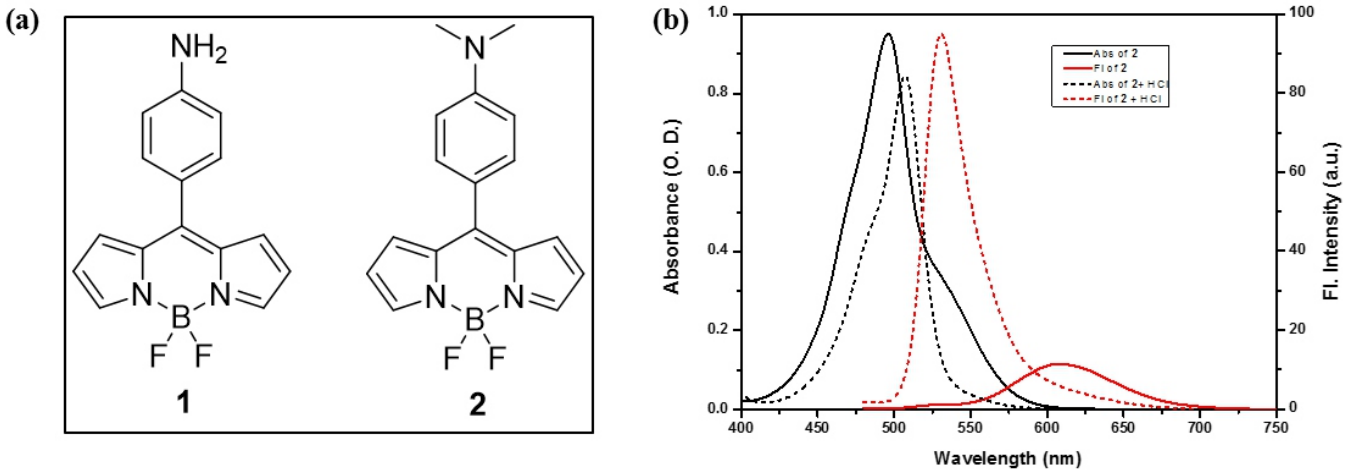

Fig.1: (a) Chemical structures of the anilino-BODIPY dyes (1,2). (b) Absorption and fluorescence spectra of dye 2 in absence and presence of acid.

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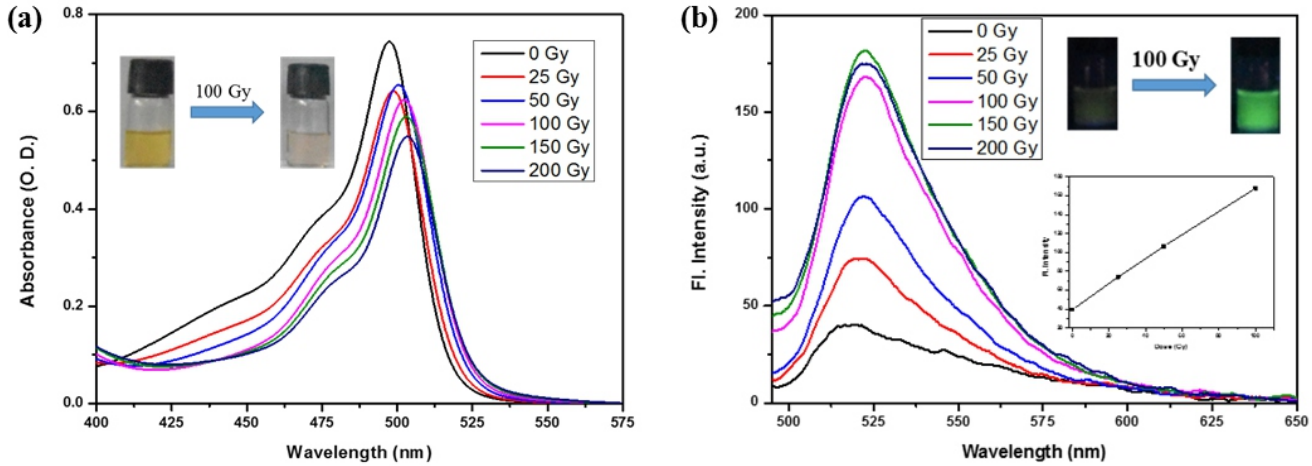

Fig.2: (a) Change in absorption of dye 1 in $CHCl_3$ under γ-irradiation exposure of increasing doses. Inset: Change in colour of the solution after 100 Gy γ-exposure. (b) Change in fluorescence spectra of dye 1 in $CHCl_3$ under γ-irradiation exposure of increasing doses. Inset: Plot of fluorescence intensity changes with γ-irradiation doses and colour change of the solution after 100 Gy γ-exposure.

(~10^{-6} M, 2 ml) were exposed with specified gamma radiation doses using the Blood Irradiator 2000 (Sr. No.14; Dose rate: 0.386 Gy/min; Source: ^{60}Co) and Blood Irradiator 2000 (Sr. No.48; Dose rate: 5.794 Gy/min; Source: ^{60}Co) (Make: Board of Radiation and Isotope Technology (BRIT), DAE, Mumbai, India). Then the absorbance and fluorescence of the solutions were recorded after 10 min.

Results and Discussion

Photophysical properties of the dyes 1-2 were evaluated in chloroform. Dye 1 showed narrow absorption with λ_{abs} at 497 nm and low fluorescence with λ_{fl} at 522 nm. While dye 2 showed absorption band with λ_{abs} at 496 nm and very week fluorescence (λ_{fl} = 609 nm). Both the dyes are low-fluorescent in organic solvent due to the photo-induced electron transfers of the nitrogen lone pairs to the BODIPY core. In presence of acids, this nitrogen lone pairs are blocked by the H^+ ion, thus both the anilino-BODIPY dyes became fluorescent [9].For example, in presence of acid, dye 1 showed slightly red shifted absorption spectra (λ_{abs} = 503 nm) with enhanced greenish fluorescence. Similarly, for dye 2, λ_{abs} is red shifted by 11 nm with highly enhanced greenish yellow fluorescence (Fig.1(b)). Thus, both the dyes are capable of sensing acids. Under γ-exposure, chloroform produced hydride and chloride radicals which generate HCl after recombination [8]. Thus, detection of HCl in γ-exposed chloroform can be used for sensing of γ-radiation. This prompted us to check the gamma sensing abilities of the dyes 1-2 in chloroform.

Upon γ-exposure with increasing doses, absorption of dye 1 started decreasing with slight red shift in λ_{abs} (5 nm). Yellow coloured solution changed to faint yellow after 100 Gy of γ-exposure. The fluorescence peak at 522 nm increases gradually upto 100 Gy and the then saturated. Thus, due to this "off-on" fluorescence sensing, very low yellowish fluorescence converted to intense greenish-yellow. Intensity of λ_{fl} (522 nm) enhanced by 5 folds under 100 Gy of γ-irradiation. Therefore, this dosimeter is useful for γ-radiation detection in the range of 0-100 Gy

Then, γ-radiation sensing ability of dye 2 was checked. Under γ-exposure, the λ_{abs} of dye 2 was red shifted (11 nm) and the colour of the solution changes from purple to orange colour. The fluorescence peak at 530 nm enhances sharply (100 folds) with decrease in 609 nm peak showing change in the low reddish fluorescence to intense greenish-yellow upon 200 Gy of γ-radiation exposure. Thus, this "off-on" ratiometric change in fluorescence can be used for the measurement of the gamma doses. The fluorescence intensity ratio, I_{530}/I_{609} is

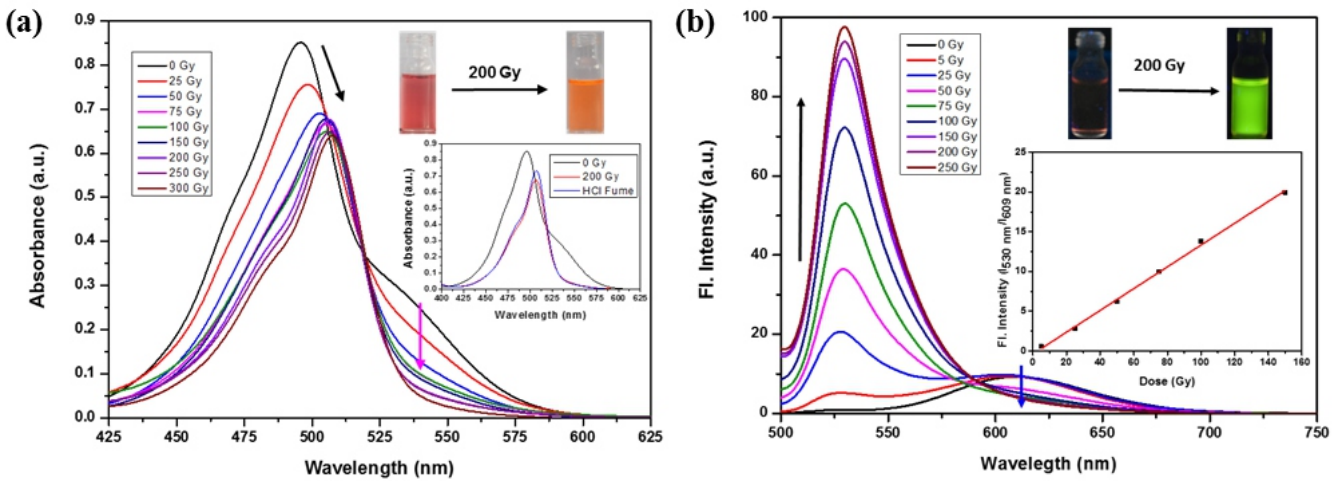

Fig.3: (a) Change in absorption of dye 2 in $CHCl_3$ under γ-irradiation exposure of increasing doses. Inset: Change in colour of the solution after 200 Gy γ-exposure and and HCl fumes exposure. (b) Change in fluorescence spectra of dye 2 in $CHCl_3$ under γ-irradiation exposure of increasing doses. Inset: Fluorescence intensity ratio (I_{530} nm/I_{609} nm) changes with γ-irradiation doses and colour change of the solution after 200 Gy γ-exposure. Reproduced with permission from ref. [9]. Copyright: Royal Society of Chemistry.

linearly increases upto 150 Gy of gamma doses before it reaches saturation. Thus, the dosimeter can be used in the range of 0-150 Gy with a limit of detection (LOD) of 0.5 Gy. Thus, the new dosimeter is highly sensitive as compared to commonly used absorption based Fricke dosimeter (LOD: 20 Gy). Also, unlike the Fricke dosimeter, response of the newly developed dosimeters does not depend on non-fluorescent impurities [10]. Thus, this anilino-BODIPY based dosimeter will be useful for the absorbed dose measurement in food irradiation processes.

Conclusions

Thus, two "off-on" fluorescent dosimeters were developed with dynamic range of 0-100 Gy and 0-150 Gy. These dyes are highly sensitive and easy to use. Thus, these newly developed dosimeters can be used for absorbed dose measurement in γ-irradiation of food materials (sprouting inhibition).

Acknowledgements

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Affordable Healthcare

11

Panoramic Photo of RMRC, Kolkata

Radiation Medicine Research Centre at Kolkata

A brief narrative of newly started centre of BARC

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Radiation Medicine Research Centre (RMRC), a state-of-the-art Nuclear Medicine Facility has been established at Kolkata under BARC. This Facility is setup and commissioned by RMC, BARC, Mumbai, with an aim to provide affordable state-of-the-art diagnostic and therapeutic Nuclear Medicine services to patients of Eastern and North Eastern states of India. In addition, this facility will provide teaching & training for medical and science post-graduates to produce Nuclear Medicine professionals and will also carry out research and development work in Clinical Nuclear Medicine, Nuclear Medicine imaging technology and in development of diagnostic & therapeutic radiopharmaceuticals.

Introduction

The Radiation Medicine Center (RMC), Mumbai was started on September 3, 1963 by Dr. Homi Jehangir Bhabha with objectives to provide Nuclear Medicine services and start research in Nuclear Medicine using radioisotopes to make the country self-reliant in the field of Nuclear Medicine diagnosis and therapeutic applications for welfare of mankind. RMC started initially with few instruments donated by Nobel Laureate Ernest Lawrance and expanded to a full-fledged NM facility.

In continuation with the same noble vision to provide low-cost affordable Nuclear Medicine services, Dr. Sekhar Basu, former Chairman, AEC incepted the dream of establishing an advanced Nuclear Medicine facility in Kolkata in eastern zone of India. The RMRC project was formulated and sanctioned under MTA in 2016 (OM No.3/1/2016/BARC/R&D-I/3519 dated 14.03.2016; XII-N-R&D-068). RMRC work started in new DAE campus at Rajarhat in August, 2017 and achieved various milestones during development stage (Fig.1) and the facility was commissioned to start patient services since Jan 3, 2024.

Activities at RMRC

RMRC is aimed to provide services for more than 10000 new patients annually and more than 25000 patients for follow ups which includes: Diagnostic services using imaging modalities for PET/SPECT scans, radionuclide therapy & Thyroid disorders management. Currently, these services are available at the centers where necessary clinical services are provided to the patients.

Diagnostic & Therapeutic Services: RMRC will offer Nuclear Medicine diagnostic services on PET/CT, SPECT/CT & SPECT systems using various radioisotopes like ^{99m}Tc, ^{18}F, ^{68}Ga etc. Total four imaging systems (SPECT- 2 Nos, PET/CT-1 No and SPECT/CT-1 No.) have been procured, installed and commissioned at RMRC in first phase for this purpose. The Centre will also offer therapeutic services utilizing various radioisotopes like ^{131}I, ^{177}Lu, ^{90}Y, ^{153}Sm, ^{225}Ac etc. A dedicated radionuclide therapy ward is established with 25 nos. beds for this purpose.

Hospital Radiopharmacy: A clean room facility is developed as radiopharmaceutical (RP) laboratory for preparation of various diagnostic and therapeutic radiopharmaceuticals used in the Centre. The clean room of class 10000 and class

Clockwise from top: Patients undergoing OPD services at RMRC, SPECT 830 unit in routine operations, Group photograph of DAE officials.

Fig.1: Project Milestones.

100000 has been established. RP lab will be mainly involved in labelling of ^{99}mTc, ^{68}Ga, ^{177}Lu, ^{90}Y, ^{225}Ac with pharmceuticals and to carry out Bio QC before adminisetering to patients.

Engineering Services: RMRC is a standalone facility and therefore various support services are necessary for operation and maintenance of infrastructure development. All engineering services (Mechanical, Electrical, Civil and I&C system) and associated systems like delay tank facility & Clean room etc. have been established. Major systems installed at campus are main power supply system, HVAC system, Fire protection system, I&C systems etc.

Health Physics Unit: RMRC has a radiation hazard unit to provide radiation surveillance and required radiation safety support to the facility. HPU also supports in radiation management system and routine radiation surveillance.

Research and Development Work: Research and training activities will be initiated in the Centre after diagnostic and therapeutic service are established. The research activities are in the field of Nuclear Medicine imaging and development of diagnostic & therapeutic radiopharmaceuticals. Other major research area will include clinical translation of radiopharmaceuticals, thyroid cancer & thyroid disorder-metabolomics, diagnosis (cell free), mutation studies and personalized treatment, Anti-cancer drug and nanobodies development, development of radionuclide therapy protocols in association with cancer research institutes & hospitals.

The Centre will provide teaching/training for medical and science graduates in Nuclear Medicine. The training courses are MD (NM), Msc (NMMIT, HRP), RSO etc.

Defect Powered Zinc Gallo Germanates for Tunable Light Emitting Diodes

▪ Rare Earth Free Phosphors

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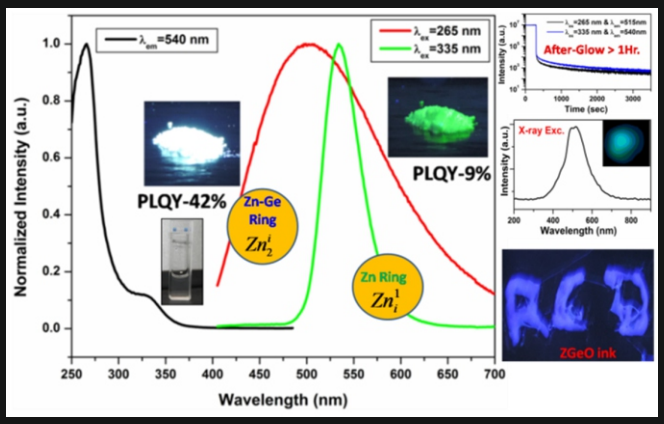
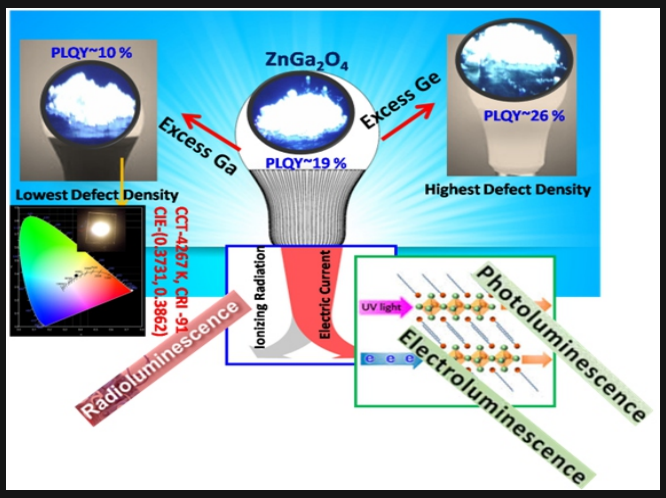
The entire world is going through a severe energy crunch owing to increased industrialization, ever increasing population and depleting fossil fuel resources. It is believed the maximum damage is triggered by electricity sector as of the total global power consumption around 15% is required for electric lighting and replacing the existing light source with phosphor converted light emitting diodes (pc-LEDs) can be a game changer in resolving this issue. However, light-emitting diodes (LED) and other advanced lighting technologies can reduce lighting power consumption by half due to their high efficiency. This can potentially reduce CO_2 emissions by 800 million tons per year, which is equivalent to 684 coal-fired power plants. But most of the existing pc-LEDs are driven by rare earth which is neither benign from safety aspects nor conducive from cost of production.

To reduce the burden of solid state lighting and phosphor converted light emitting diode (pc-LEDs) technologies on rare earths (RE) and avoid their potential supply risks we have developed RE free Zn_2GeO_4 (ZGeO) [Gupta et al. *ACS Appl. Electron. Mater*. 2023, 5, 12862] and $ZnGa_2O_4$ (ZGeO) phosphor. ZGeO demonstrated color tunable emission from bluish white to green on changing the excitation wavelength from 265 to 335 nm linked to zinc interstitials in zinc (Zn_i^2) and zinc-germanium rings (Zn_i^1) respectively with photoluminescence quantum yield (PLQY) of 42 and 9 % respectively. We have further synthesized, defect-induced long and brightly emitting rare-earth free $ZnGa_2O_4$ (ZGaO) with PLQY~19%. Defects generated in large numbers via high temperature annealing along with antisites boosted the generation of trapping centres leading long persistent luminescence in both the samples.

The defect was further engineered in $ZnGa_2O_4$ by reacting it with different percentages of Ga_2O_3/GeO_2 yielding stoichiometric $Zn3Ga_2GeO_8$ (S-ZGG), gallium excess $Zn_3Ga_4GeO_{11}$ (Ga-ZGG) and germanium excess $Zn_3Ga_2Ge_2O_{10}$ (Ge-ZGG) of zinc gallo germanate samples [Gupta et al. *Chem. Eng. Journal*, 2023, 74, 145595]. The defect density follows the trend Ge-ZZG>S-ZGG>ZGaO>Ga-ZGG which is directly culminated in achieving a high PLQY of ~ 26% in Ge-ZGG. But a lower defect in Ga-ZGG aided in achieving the finest white light emission with correlated color temperature (CCT) of 4267 K, color rendering index (CRI) of 91, and CIE of (0.3731, 0.3862). We have further proposed a flexible and rare pathway to engineer such defects that tuned the broadband emission from cold white to the energy efficient warm white lighting. We believe this work is an excellent contribution to resolve the issues of expensive RE doping, doping induced strain, complex organic synthesis, safety concern, and serve as a strategic pathway to design thermally stable and cost effective on demand rare earth free tunable LED with suitable CCT and high CRI.



▲ Scheme 1: Excitation Tunable emission, afterglow decay, radioluminescence and anticounterfeting application of Zn_2GeO_4.



▲ Scheme 2: All inorganic high PLQY (~19%) and persistent bluish-white light emitting $ZnGa_2O_4$ exhibiting radio and electroluminescence and designing rare earth free compositionally modulated defect powered Zinc gallo germanate Tunable LEDs.

Dr. Santosh K. Gupta, is currently working as a Scientific Officer/F at Radiochemistry Division, BARC, Mumbai. His research area focuses on designing light emitting materials for health, energy and environment etc. Dr. Gupta is the recipient of several awards notable among them are Fulbright and JSPS Fellowship, DAE and Scientific India Young Scientist award, IANCAS Tarun Datta Medal, SMC Bronze and CRS silver medal. He has also been bestowed with membership of Indian National Young Academy of Sciences (INSA-INYAS) & National Academy of Sciences (NASc) and young associate of Maharashtra Academy of Sciences (MASc). Dr. Gupta has more than 225 peer reviewed international journal articles to his credit.

Development of Deuterated Titanium Target on Chemically Etched Copper Substrate

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Metal hydride target is one of the important parts of the compact accelerator based neutron generators. Concentration of the deuterium/tritium in the target is also important for the neutron yield. Deuterated titanium target are under development in our laboratory. A comparative study between smooth and rough target were carried out to estimate deuterium concentration. To create roughness, chemical etching was performed.

Two types of copper substrate were used; i.e. mechanically polished and chemically etched. Both the substrates were coated with titanium by thermal evaporation method. Thickness of the titanium film was 2 µm.

The coated substrate was shifted to deuteration chamber and activated for one hour at a temperature of 500°C at a vacuum of the order of 10^{-7} mbar and left for cool down to room temperature. At room temperature the substrate was charged with deuterium. Deuterium charging was carried out for different charging pressure and duration. Higher charging pressure and longer duration is effective for higher deuterium concentration in the film [1].

For comparative study both polished as well as chemically etched samples were charged at 3 bar deuterium pressure and for 120 hours. The deuterium concentration is higher in the case of chemically etched sample. Higher deuterium concentration in chemically etched sample is due to the higher porosity, reduced grain size and different grain boundaries. The D/Ti ratio found out from weight measurement is 1.03 for polished sample whereas in case of chemically etched sample, it is 1.54 [2].

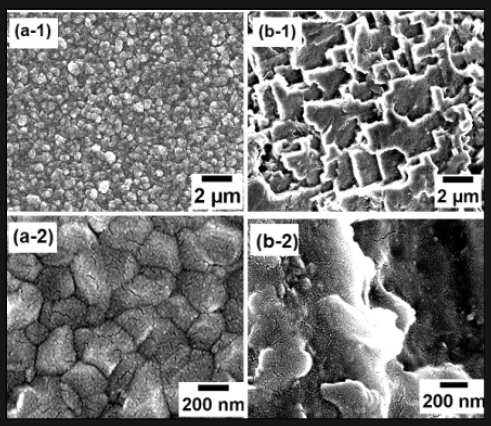

Fig.1: SEM images of Ti film on (a) Polished and (b) Chemically etched copper substrate.

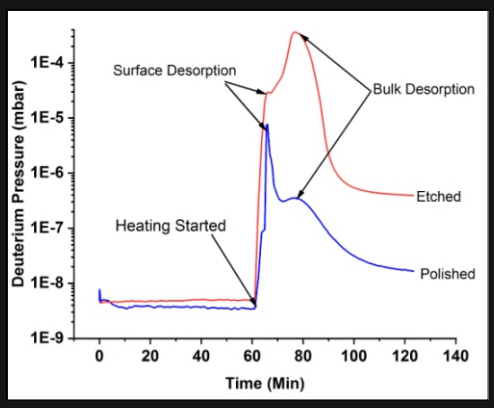

Fig.2: RGA spectra for deuterium emission from polished and chemically etched sample.

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BARC

CHINTAN BAITHAK

brainstorming science & technology for cutting edge in-house R&D

@High Intensity Proton Accelerators

Roadmap

High Intensity Proton Accelerators (HIPA)

- Normal Conducting Low Energy Front End upto 10-20 MeV: LEHIPA
- Superconducting Medium Energy Section: upto 200 MeV: MEHIPA
- Superconducting High Energy Section: ~1 GeV: HEHIPA

High Intensity Proton Accelerators delivering proton beam of around 1GeV energy would play a key role in nuclear energy sector and specifically in the realization of Accelerator Driven Sub Critical System, nuclear waste incineration and advancing utilisation of thorium in nuclear reactors. The first step on the path of High Energy High Intensity Proton Accelerator (HEHIPA) has been successful demonstration of 20 MeV energy in indigenously developed Low Energy High Intensity Proton Accelerators (LEHIPA). BARC is also significantly contributing towards construction of 800 MeV Proton accelerator at Fermilab National Laboratory. Both these endeavors have boosted the confidence in moving ahead with constructing 200 MeV Medium Energy High Intensity Proton Accelerator (MEHIPA) at BARC, Visakhapatnam. Subsequently, Super Conducting Elliptical type cavities will be developed to reach 1 GeV. The fabrication and design of these cavities would be on similar concepts adopted for Fermilab Proton Improvement Plan (PIP)-II project.

Applications

- Advanced physics & material science research.
- Nuclear physics studies
- Radioactive Ion Beams
- Rare Radio Isotopes
- Medical research
- Demo ADSS
- Accelerator Driven Systems (ADSS)
- Spallation Neutron Sources (SNS)

Technologies Developed

- Normal Conducting Accelerating Structures: RFQ, DTL
- High Current Ion Source
- Regulated High Voltage Power Supply
- LLRF, RFPI, Control & Interlock System
- Beam Diagnostics
- Magnets
- Liquid Helium Plant
- RF Amplifiers
- RF Couplers
- Super Conducting Cavities

Speaker
Jose V. Mathew

Key resource persons
U. D. Malshe, Associate Director, MRG
Rajesh Kumar, Head, IADD

Contributors
Multi-disciplinary Research Group, Beam Technology Development Group, Electronics & Instrumentation Group, Engineering Services Group, Physics Group, Reactor Projects Group, Reactor Design & Development Group, Health Safety & Environment Group, BARC (F) Visakhapatnam, BARC Safety Council, Raja Ramanna Centre for Advanced Technology & Variable Energy Cyclotron Centre.

‘Indians had a very imaginative past in their DNA; revving it up holds good promise’

Emphasized **General Anil Chauhan** in his keynote address at National Technology Day-2024 event.

‘Operation Shakti’ and its overall significance...

Operation Shakti signified more than technical prowess of the country. It actually demonstrated two important things. Firstly, it had demonstrated team work. A team of scientists, from BARC, DRDO and Army Engineers, worked in unison and in complete secrecy to achieve this difficult mission. Secondly, it signified our national resolve and the will of our political leadership. India took this historic decision knowing fully well its consequences. We were confident that the country would be able to overcome subsequent hurdles. We firmly believed that 'Hum Honge Kaamyaab (We shall emerge successful)'.

Innovation in Technology...

Technology affects education, communication, economy of the country and many other areas. It ultimately goes on to change the socio-cultural behavior of a particular society. Modernization of a society is equated to technological advancement. Developed nations will more reluctantly share advanced technologies to other countries, particularly with technologically-backward nations.

A (nuclear) deterrence is important because India prophesies a No-First-Use (NFU) doctrine. It mandates us to maintain a credible minimum deterrent. This particular aspect will always remain important for India.

We have heard of technology denial regimes in the past and these were of extreme forms. BARC had been subjected to this and it was under embargo for most of the times. India had experienced this in the past and the development of a nation cannot be made hostage to geopolitical desires and policies of other nations. There's a saying that 'Today's Science is Tomorrow's Technology'. Today's science fiction is tomorrow's reality. Today's dreams are tomorrow's missions.

The fruits of being Imaginative...

The ability of being imaginative holds the most important key towards developing new technologies. A society that is imaginative will innovate and by virtue of this the development cycle and transformation will be much faster. Albert Einstein had once said: “True measure of intelligence of a human being is not knowledge, but it is imagination”. “Logic will get you from A to Z but imagination will get you everywhere”.

The imaginative India of the past and its scientific excellence...

India gave to the world the concept of zero, it

evolved the decimal system, it also gave astrology and astronomy. It was far ahead of the time as compared to other nations of that era. In the field of medicine India propounded the concept of Ayurveda. Texts like Charaka Samhita and Sushruta Samhita provided comprehensive knowledge of anatomy, surgery, pharmacology, and disease management. The extraction of metals like iron, copper, bronze etc., were well documented in our ancient texts, reflecting advanced knowledge of chemical processes as well as metallurgy. The pioneering research by Aryabhatta and Brahmagupta in the field of mathematics and astrophysics laid the foundations for algebra, trigonometry and the helio-centric model of our solar system.

India of the past and characteristics of an evolved society…

Architecturally, India was much advanced and much ahead of those times. The cities of Mohenjo-Daro and Harappa showcase the civilizational advancement in terms of urban planning and drainage systems. The temples of Khajuraho or the rock cut architecture of Ellora are enduring examples of India's architectural brilliance of those times. Societies which are technologically advanced also invest most of their leisure time in development of art and culture. This feature was also there in the past with us. I think there is no civilization other than that of India where ancient dances and music forms are still practiced today. It was true to us being imaginative, innovative, informative and inventive. This we did it as individuals, and as a complete society.

India of the past occupied centerstage in knowledge sharing networks…

India shared its ancient knowledge with the rest of the world. We didn't keep it to ourselves the way the West is doing today by putting sanctions and restricting the flow of technologies. Merchants, traders and students from all parts of the world came to India to gain knowledge. The universities of Takshashila and Nalanda were probably the MITs, Harvards and Oxfords of today. So, India was not just a Vishwaguru but it was also a Vishwabandhu and a Vishwamitra.

Knowledge enshrined in ancient scriptures, a reflection of India's imaginative past…

Ancient Indian literature spoke of imaginative technologies and a variety of astras (weapons) that were deployed in the battlefields. Mahabharata talked about situational awareness of distant battlefield. Robert J. Oppenheimer of Project Manhattan had reminded us the knowledge enshrined in ancient Sanskrit texts and had quoted the lines from the Gita when he saw the tremendous power of the atom. “If the brilliance of thousand suns were to shine together in the sky that would not match the brilliance of the mighty one (bomb),” which is a manifestation of the concept of Brahmastra envisioned in our ancient times. All of this is a reflection of our imaginative past, which we have inherited in our DNA. Somehow, because of history, it has got obscured. This imaginative Indian and the thought process underscoring the fact that we can do it and we could be better than the rest exists as part of our DNA. We only need to revive it and we would be able to occupy the centerstage in both science and technology.

Revolution in Technology and its impact on Armed Forces…

Historically, technology has had a revolutionary impact on the conduct of warfare. This has been true from ancient times. Technology has been a strategic enabler and has been responsible for what is called as revolution in military affairs. Now, we have the advent of emerging and disruptive technology like AI, autonomous weapons, stealth, quantum, bio-technology, and human advancements in hypersonics, space, novel materials, energy propulsion, next generation of communication networks, which are impacting warfare. Our defense forces, including our strategic assets therefore remain ahead of this technological curve. For embracing this, I believe that the troika of soldiers, scientists and scholars would prove to be the most important enabler. All three should work cohesively to provide a decisive technological edge over the adversaries over the entire spectrum of conflict, including nuclear.

Nuclear Technology key to maintaining peace…

A (nuclear) deterrence is important because India prophesies a No-First-Use (NFU) doctrine. It mandates us to maintain a credible minimum deterrent. This particular aspect will always remain important for India.

Securing the needs of the country through development of multi-faceted technologies…

Technology is not about developing hard power, alone. It has many facets to it and reflects many dimensions and domains of our individual and collective well-being. Tremendous efforts have been put in by scientific community of BARC in areas of water desalination, water purification and waste water treatment technologies. Some of these technologies have been deployed across strategic locations along the Indo-Pak border. Radiation technologies that enhance food safety and increase the shelf life of perishable food items and reduce post-harvest loss contribute to overall national food security. Application of radioisotopes in diagnosis of tumors, pathogens and treatment of cancers secures the well-being of the people.

BARC commissioned Seawater Desalination Plant dedicated to the nation

The state-of-the-art indigenous technology caters to the sustained requirement of process water at IREL OSCOM in Chatrapur, Odisha by processing seawater.

By Desalination & Membrane Technology Division
Chemical Engineering Group, BARC

Taking a leaf out of Government of India's renewed push for high-end technology innovation and increased deployment of home-grown capabilities (spirit of 'Atmanirbhar Bharat') in diversified areas of science and technology applications, Bhabha Atomic Research Centre has setup a seawater desalination facility within the operations of Odissa Sand Complex (OSCOM) at Chatrapur. The newly commissioned seawater desalination plant was dedicated to the nation by Prime Minister of India on March 5, 2024. The desalination facility comprises 4.5 million litres per day (MLD) Seawater Reverse Osmosis (SWRO) plant and 0.5 MLD Multi Effect Distillation Thermo Vapor Compression (MED-TVC) desalination plant. Importantly, total 5 MLD high quality water generated from this desalination facility adequately meets full-scale requirement of process water in the day-to-day operations of OSCOM complex and drinking water supply of nearby areas.

Process Description

Multi-Effect Distillation with Thermo Vapor Compression (MED-TVC)

Two units of 250 m^3/d MED-TVC plant (0.5 MLD total product capacity) were designed, installed and commissioned to meet the demand of high-quality process water at OSCOM, IREL. The units are designed to produce an average 11 m^3/hr distilled water of conductivity <5 µS/cm directly from sea water without any pre-treatment, using 1.1 TPH steam (@ 10 bar) from the IREL boiler. This plant uses multiple evaporators in series and thermo vapor compressor (TVC) to achieve the steam economy (10 kg distilled water per kg of steam). The high-quality distilled water produced from the plant will be used for boiler make-up water of OSCOM. Each evaporator consists of a horizontal tube bundle and the feed seawater is sprayed on top of the tube bundle, which then drips from an array of tubes until it is collected at the bottom of the effect.

Feed water forms a thin film on the outer side of the tube surface and gets heated due to the condensing steam inside the tube. Sub-atmospheric pressure is maintained inside the

The newly commissioned seawater desalination plant by BARC was dedicated to the nation by Prime Minister of India on March 5, 2024. The desalination facility comprises 4.5 million litres per day (MLD) Seawater Reverse Osmosis (SWRO) plant and 0.5 MLD Multi Effect Distillation Thermo Vapor Compression (MED-TVC) desalination plant. Importantly, total 5 MLD high quality water generated from this desalination facility adequately meets full-scale requirement of process water in the day-to-day operations of OSCOM complex and drinking water supply of nearby areas.

effect to reduce the boiling temperature, thereby minimising the scaling threat to evaporator tubes. A fraction of the heated seawater boils to form water vapours. After separating the entrained seawater droplets, the vapour flows into the tube bundle of the next effect, heating and evaporating more seawater. The vapour condenses to produce fresh water while transferring the latent heat to evaporate a portion of feed seawater in the next effect and this process continues for 6 effects in the MED plant. TVC is used to improve the overall steam economy. The vapour produced in the last effect is compressed in the TVC using medium-pressure steam (10 bar) and reused in the first effect.

This plant offers several advantages such as high steam economy; compact evaporator due to high heat transfer coefficient in thin film; reduced scaling potential due to low temperature operation. The plant is equipped with a PLC-SCADA system that enables precise control over the various process parameters. The system automation minimizes the need for manual intervention, resulting in optimum workforce requirement for plant operation. The plant utilizes high-quality, corrosion-resistant materials to ensure long-lasting operation in a marine environment (SS 316L shell and Ti grade 2 tubes). The plant requires 40 kW electrical power. VFD controlled pumps are used for smooth operation. With proper operation and maintenance, the MED-TVC plant is expected to have a design life of 40 years. The scaled-up version (1 MLD) MED TVC Technology is available for technology transfer.

Seawater Reverse Osmosis (SWRO)

The SWRO plant is designed to produce water at the rate of about 4500 m^3/day with salinity (TDS) of less than 500 ppm from seawater with an overall electrical power consumption of Total 940 kW. 950 m^3/hr of seawater is drawn from deep sea 900 m away from shore location through sea water pump house station located at Gopalpur Ports Ltd (GPL) adjoining OSCOM, IREL. Seawater is first pre-treated by passing it through clarifier, multigrade filter and then sent through ultrafiltration membranes for making it free from suspended solids. The treated water is then pressurised by high pressure pumps & fed to RO membrane modules for removal of dissolved salts from seawater. These membranes are manufactured by BARC technology licensees based on the BARC's know-how for the preparation of sea water reverse osmosis (SWRO) membrane. The performance of these membranes is at par with the commercially available membranes. The RO product (187.5 m^3/hr) having total dissolved solids less than 500 ppm, conforming to WHO guidelines and Indian drinking water standard IS-10500 after suitable post-treatment is supplied to reservoir of OSCOM. The concentrated seawater from desalination plant is discharged back to deep sea through diffuser.

Successful deployment of indigenous desalination technologies at IREL OSCOM is a major step towards ensuring high quality water availability in DAE units as a part of 'Atmanirbhar Bharat'. Through indigenisation of SWRO

technology, BARC aims to provide a viable import substitute to industry and municipalities. The MED-TVC technology, with its high efficiency, low maintenance requirements, and ability to produce high-purity water, holds immense potential to meet the water demand in various industries facing severe challenges. As the country strives for greater water sustainability, BARC-developed seawater desalination technologies are poised to play a significant role in capacity building to bridge the gap between demand and supply in the coming years for ensuring water secure India.

Benefits of BARC commissioned Seawater Desalination Plant

The successful commissioning of the seawater desalination plant offers significant benefits. These are as follows:

Enhanced Process Water Security & Operational Availability: The desalination plant ensures a reliable and consistent source of high-quality water for various process water requirements of IREL OSCOM facility, minimizing disruptions due to water scarcity at IREL OSCOM

High-Purity Distilled Water: The MED-TVC plant produces distilled water with a conductivity less than 5 µS/cm, ideal for applications requiring high-quality water, such as boiler makeup water and various process water needs within the facility.

Societal benefits: As a part of CSR activities of IREL, the potable water from the desalination plant can be supplied to nearby village.

The article has been compiled and edited by Saurabh, K. P. Bhattacharyya, A. K. Adak, K. T. Shenoy and Madhav N., of BARC.

Inside view of Seawater Desalination Plant equipped with BARC Technology commissioned at OSCOM in Odisha.

Mr. Raj Chengappa visits BARC

Humanizing scientific research...

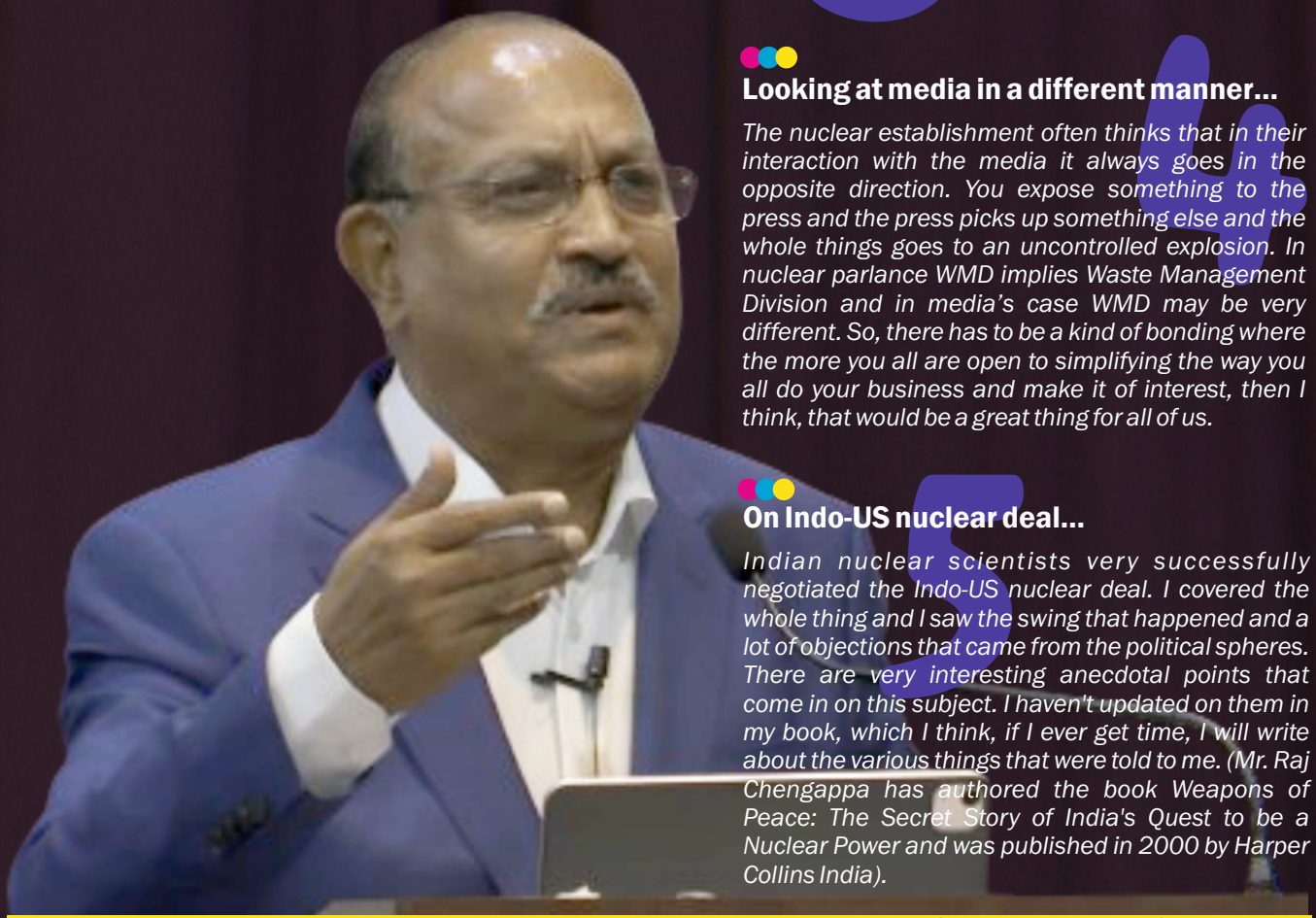
Science is not innate. Science is all about people and those who do it. Science is also about conflicts and the collaboration it has with the society. If you have to communicate science better, you have to bring the human dimension of it into all your expansion and in the media interactions you do. The human dimension is very critical to understanding science. You have to do science which directly serves the public, and that public must begin to appreciate the work you all do; for you all to not only get the recognition that you deserve but also for the funding that you need to do better and better research.

Showcasing scientific work to the public...

It is very important that society and the public are sensitized to the work you do; for the kind of support that they can give you. We have seen that happen in Space (sector) where we have seen that the Moon Mission and others have captured the imagination of the public. And it is time that the work that's being done here (in BARC and DAE), whatever the constraints are, the magnificent work that you all do should start getting showcased. Not so much to do with publicity but because to inspire a nation and a whole generation of youngsters to take this very noble profession that you all are doing.

Translating landmark developments in science into moments of excitement...

Space captured the imagination of public. Nuclear science is in the same trajectory. You have to increasingly market your talent and your capability to convince people. You all need to catch the public eye in a manner that excites them.



Looking at media in a different manner...

The nuclear establishment often thinks that in their interaction with the media it always goes in the opposite direction. You expose something to the press and the press picks up something else and the whole things goes to an uncontrolled explosion. In nuclear parlance WMD implies Waste Management Division and in media's case WMD may be very different. So, there has to be a kind of bonding where the more you all are open to simplifying the way you all do your business and make it of interest, then I think, that would be a great thing for all of us.

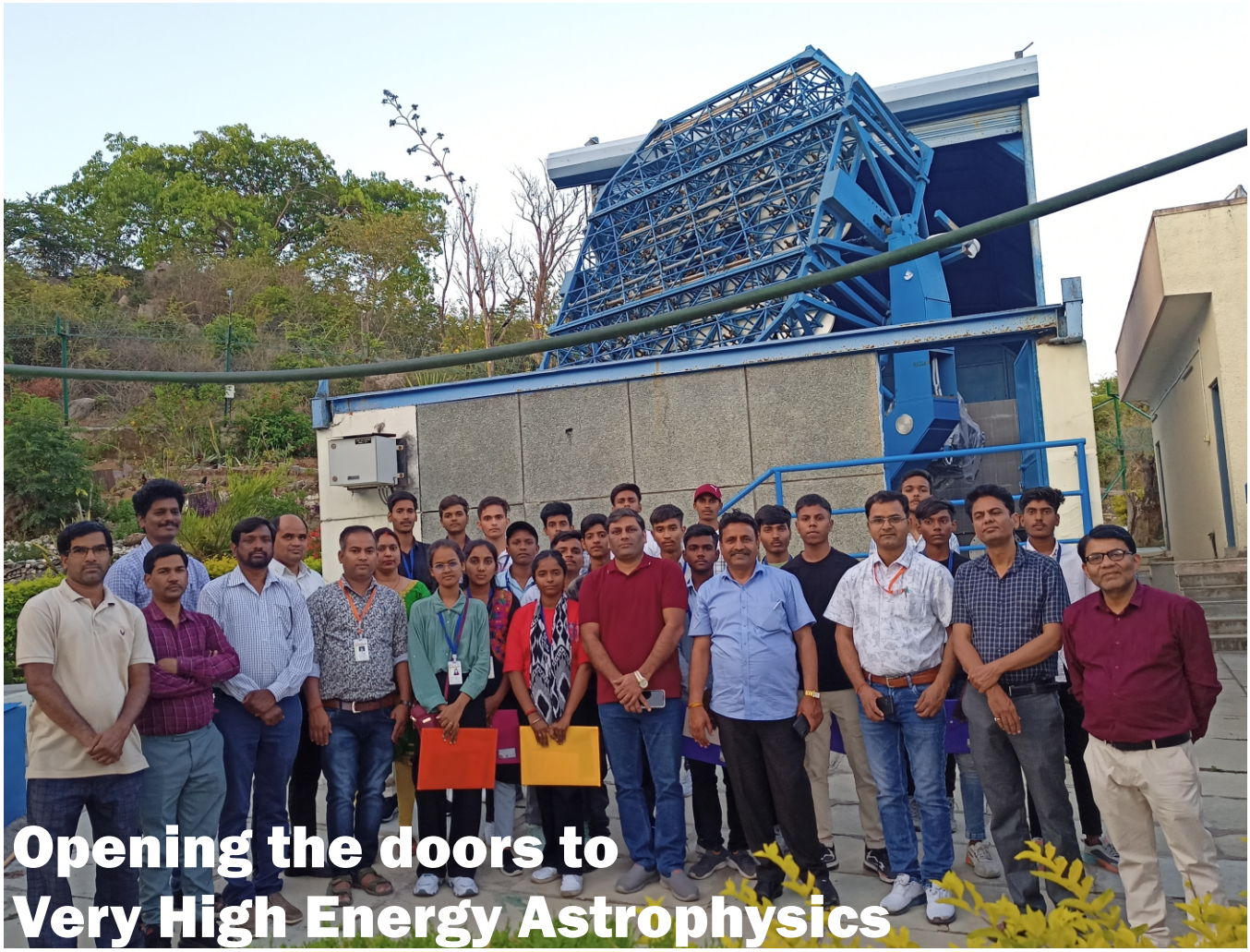
On Indo-US nuclear deal...

Indian nuclear scientists very successfully negotiated the Indo-US nuclear deal. I covered the whole thing and I saw the swing that happened and a lot of objections that came from the political spheres. There are very interesting anecdotal points that come in on this subject. I haven't updated on them in my book, which I think, if I ever get time, I will write about the various things that were told to me. (Mr. Raj Chengappa has authored the book Weapons of Peace: The Secret Story of India's Quest to be a Nuclear Power and was published in 2000 by Harper Collins India).

'Nuclear should catch public eye in a manner that excites them'

Selected excerpts of the talk by Mr. Raj Chengappa of India Today Group at Special Trombay Colloquium on the topic Science and Media on 31st of May, 2024

Reports from conferences, theme meetings, workshops, and outreach



Opening the doors to Very High Energy Astrophysics

Young minds converge at BARC's GOALS Observatory in Mount Abu, Rajasthan

A premier scientific R&D organization of the Department of Atomic Energy, Bhabha Atomic Research Centre (BARC), organizes an array of scientific outreach programs regularly with an aim to reinvigorate and infuse the spirit of scientific inquiry in the minds of younger generation. As part of this, it has recently conducted two-day outreach program during April 23-24, 2024 at its Very High Energy (VHE) Gamma Ray Astronomy facility–GOALS (Gurushikhar Observatory for AstrophysicaL Sciences) – situated in Mount Abu for the benefit of students of Uttar Pradesh based Hiralal Ramniwas Intermediate College.

The program focused on popularizing research and development activities of BARC through lectures, particularly in the field of VHE, gamma-ray Astronomy and Astrophysics. It may be noted that BARC has a robust R&D program in these niche areas of physics and had recently commissioned state-of-the-art MACE telescope - a national facility for high energy astrophysics research at Hanle in Ladakh.

During the two-day outreach program, scientific officers of Astrophysical Sciences Division (ApSD), part of BARC Physics Group, briefed the visiting students on the gamut of scientific research activities at its GOALS facility as well as the MACE facility situated in Hanle. Students were arranged a site visit to GOALS wherein ApSD officials demonstrated the key functional and operational aspects of the facility, including source localization, tracking of astronomical sources, control room features of the TACTIC (TeV Atmospheric Cherenkov Telescope with Imaging Camera) telescope deployed at GOALS. A quiz session was also organized as part of the program, which saw enthusiastic participation from the students. Certificates of merit and prizes were awarded to meritorious students in the quiz competition by the officer-in-charge of GOALS. In addition to this, students were briefed on career opportunities in BARC and DAE.

Few glimpses of student outreach program held recently at GOALS Observatory in Mount Abu, Rajasthan.

Reports from conferences, theme meetings, workshops, and outreach

Release of 'Handbook on Radiation Environment (Vol 1 & 2) during inaugural function of the theme meeting (NECE-2024). Inset photograph: Special Issue of Mapan (a Springer Journal) during the meeting.

Theme meeting on Nuclear Energy and Clean Environment

The Health, Safety and Environment Group, BARC, in collaboration with the Indian Association for Radiation Protection (IARP) and the Association of Medical Physicists of India (AMPI) organized a two-day (June 5-6) theme meeting - *Nuclear Energy and Clean Environment (NECE-2024)* - on the occasion of World Environment Day. The topics covered at the meeting across various sessions include *Nuclear Power for Sustainable Development in India; Ionizing Radiation for Inclusive Growth; Nuclear and Radiation Medicine in Healthcare; Nuclear and Radiation Technology in Food and Agriculture, and Nuclear and Radiation Technology Driven Industry.*

Notably, at the meeting two volumes of the book *Handbook on Radiation Environment* were released. Published by Springer Nature the twin volumes were edited by Dr. D. K. Aswal, Director, Health, Safety and Environment Group, BARC. In addition to this, a special issue of *Mapan* (a Springer Journal), dedicated to advanced radiation metrology techniques and related applications was also released during the meeting.

Activities across pan-India AKRUTI Kendras.

A multi-decadal glimpse of AKRUTI program

By Technology Transfer & Collaboration Division
Bhabha Atomic Research Centre, Trombay, Mumbai-400085

Department of Atomic Energy (DAE) in the year 2007 has launched DAE-Societal Initiative for utilization of non-power applications and spin-off technologies in the area of water, agriculture, food processing and agricultural-land improvement through urban and rural waste management. Within this framework, a structured program called "AKRUTI" has been formulated by BARC. Initially, it was implemented through technically-oriented non-governmental organizations for techno-economic growth of the rural sector.

AKRUTI Program demonstrates the usefulness of BARC technologies for rural and urban sector leading to societal benefit. Technically oriented human resource in rural and urban sector can deploy technologies for their use under the guidance of BARC scientists and engineers. This program has potential to encourage techno-preneurship based on DAE-BARC technologies and provides livelihoods for villages in village itself.

AKRUTI Program implementation was being carried through 'AKRUTI Kendra' to create structured and scalable network of DAE-BARC based RUrban Technology and to provide easy access to modern technologies to all rural and urban sectors.

AKRUTI - At a Glance

Awareness Camps/Workshops conducted in villages nearby AKRUTI Kendras	> 250
Meetings/Orientation Programs conducted	> 600
Awareness on AKRUTI technologies among village households	> 120,000
Soil Sample Collection Exercise	1200
Installation of Water Purifiers	250
Installation of Community Water Purifiers	05
Installation of Rapid Bio-composting units in Farms	12
AKRUTI Food Technology demonstrations	152

An AKRUTI Kendra can be setup by the academic institutes/ Govt. Organization/ PSU/ Science Park/ Mega Food parks to encourage entrepreneurship among its students/ visitors/ farmers/ educated, skilled youth and, also, to nearby rural areas to create awareness about the facilities available in the Kendra and help technically oriented youth to have first-hand experience in observing, evaluating and try-out making BARC technology products in the Kendra. These Kendras will be the first point of contact with DAE-BARC for budding entrepreneurs for registering their firm and applying for commercialization of license for BARC technologies.

Under its corporate responsibility program, NPCIL has made functional two AKRUTI Kendras, one at Unchamala, District Tapi in Gujarat in the vicinity of its Kakrapar Atomic Power Station and the other one established at Tarapur.

Recently, MoUs were inked with four academic bodies for establishment of AKRUTI Kendras and these include: Pandit Ravishankar Shukla University, Raipur, Chhattisgarh; Raipur Institute of Technology (RIT), Raipur, Chhattisgarh; Brahmadevdada Mane Institute of Technology (BMIT), Solapur, Maharashtra; Shri Vithhal Education and Research Institute (SVERI), Pandharpur, Maharashtra.

Respite in pain

A new lease of life for pelvic cancer patients

With the launch of regenerative nutraceutical AKTOCYTE tablets.

As part of sustained efforts for ensuring affordable cancer care availability to a wider cross section of the society, the constituent units of Department of Atomic Energy have joined hands to address the requirements of pelvic cancer patients. BARC, ACTREC and TMH came out with a new and highly effective drug formulation to provide pelvic cancer patients a new lease of life.

The newly launched AKTOCYTE tablets - a joint effort of DAE bodies - can now address radiotherapy-induced side effects in pelvic cancer patients, thereby ensuring better quality of life to patients in the long run. The tablets are designed to function as an adjuvant to cancer radiotherapy, and are poised to play the role of a regenerative nutraceutical, immunomodulator, and as an antioxidant.

Prior to its commercial launch, AKTOCYTE has proven its worth during rigorous clinical trials, and was accorded approval by the Food Safety and Standards Authority of India.

Speaking on the launch of AKTOCYTE, Shri Vivek Bhasin, Director, BARC appreciated the efforts of DAE scientists and the private industry for the successful commercialization of AKTOCYTE. Dr. Ajit Kumar Mohanty, Secretary, DAE and Chairman, AEC hailed it as a continuation of DAE's efforts in ensuring the health security of the country and expressed satisfaction that it was happening in the Platinum Jubilee Year of the department.

Bengaluru based M/s. IDRS Labs Pvt. Ltd., collaborated with DAE in the release of AKTOCYTE tablets.

The newly launched AKTOCYTE tablets - a joint effort of BARC, ACTREC and TMH - can now address radiotherapy-induced side effects in pelvic cancer patients, thereby ensuring better quality of life to patients in the long run. The tablets are designed to function as an adjuvant to cancer radiotherapy, and are poised to play the role of a regenerative nutraceutical, immunomodulator, and as an antioxidant.

Industry

BARC's Nuclear

Bhabha Atomic Research Centre (BARC) continues to make a positive impact on the country's overall technology landscape through sustained transfer of new and advanced technologies, for the benefit of Indian industry. In the previous year itself, BARC inked a total of 188 agreements with the industry players for transfer of more than 105 nuclear spin-off technologies. Besides, it has introduced around 20 new technologies encompassing chemical, radiation, agriculture and bio-science domains for targeted commercial production.

Tapping Hydrogen Energy

BARC transfers its workhorse Alkaline Water Electrolyser Technology to PSU major BHEL

The technology for production of hydrogen energy from alkaline water through electrolysis route, developed by BARC, has been picked up by PSU major Bharat Heavy Electricals Limited (BHEL) for deployment in industry. The official agreement towards this end was formally inked at an event organized at BARC Mumbai campus recently. The agreement entails transfer of BARC's 50 kW Alkaline Water Electrolyser (AWE) technology to BHEL, which has concrete plans to up-scale the technology with an aim to explore potential opportunities for the technology's ultimate deployment in sectors such as refineries, fertiliser, steel, transportation, etc.

The BARC developed AWE technology is the only 100% indigenous technology for AWE as on today and intends to support BHEL in its long-term plans for the development of sustainable hydrogen production methods, thereby supporting India's transition towards cleaner energy sources.

The Prototype 0.5MW Alkaline Water Electrolyser cell stack undergoing testing in BARC.

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beckons

Spin-off technologies

Photograph taken during the event organized at BARC to formalize BARC-BHEL agreement for transfer of AWE technology for hydrogen production. The agreement was signed in the presence of (seated from left to right): Jai Prakash Srivastava, Director (Engineering, Research & Development), BHEL, Dr. S. Adhikari, Director (Knowledge Management Group), BARC, K. Ravishankar, Executive Director (Corporate Technology Management and Corp R&D), BHEL, and K.T. Shenoy, Director (Chemical Engineering Group), BARC. Others present during signing ceremony include Daniel Babu P., Head (Technology Transfer & Collaboration Division), BARC and senior officials from the ranks of BARC Chemical Engineering Group.

Advanced instruments for Applied Scientific Research

Scanning Electron Microscope equipped with Thermionic Electron Emitter transferred to industry

A tungsten filament-based SEM which facilitates microscopy & microanalysis of specimen with spatial resolution down to 20nm was developed in BARC. The technological know-how key to manufacturing of this specialized high-end sophisticated instrument has been transferred to a Roorkee-based private firm M/s. Mars Design & Automation Services (MDAS) by BARC. SEM is a popular microscopy & microanalysis instrument that uses a finely focused electron beam probe to simultaneously image morphology and carry out compositional analysis of bulk specimen surface. It finds wide applications in all disciplines of science & engineering and is an indispensable tool for material and alloy characterization, mineral characterization & geology, membrane and powder metallurgy, pharmaceutical research, semiconductor development and bioscience to name a few. Despite tremendous demand in India, the instrument currently has no indigenous manufacturers, which hampers cost-effective and customized availability of the instrument in the country. BARC developed indigenous SEM technology is envisaged to become a potential cost-effective import-substitution for Indian institutions of higher education, research laboratories and industries.

Tungsten filament-based SEM developed in BARC

Photograph taken during an event organized in BARC to formalize the agreement for transfer of SEM know-how to Roorkee-based firm MDAS. The agreement was signed in the presence of (seated from left to right): Dr. Raghvendra Tewari, Director (Materials Group), Shrikant Vidwans, Representative, M/s. MDAS, Dr. S. Adhikari, Director (KMG), Dr. S. Mukhopadhyay, Director (E&IG), BARC.

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