## फसल कटाई के बाद के नुकसान का न्यूनीकरण एक एकीकृत दृष्टिकोण के माध्यम से प्याज़ की फसल कटाई के बाद के नुकसान के न्यूनीकरण हेत् वृहद पैमाने पर वाणिज्यिक परीक्षण

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5.47 41.1 28.27 1.71 3.76 58.9 94.53 12.83

सारांश

(A) Untreated (B) Radiation treated M ble bulb (%) Total loss (%) Weight loss (%) Loss due to rotti

भंडारण के दौरान गैर-विकिरणित और विकिरण उपचारित प्याज में वजन घटाने की रूपरेखा

प्याज़ का दीर्घकालिक विस्तारित संरक्षण फसल कटाई के बाद के प्रमुख मुद्दों के कारण सीमित है, जिसमें वजन कम होना, सूक्ष्मजीव खराब होना, सड़ना और अंक्रूरित होना शामिल है, जिससे 35-40% से लेकर समग्र नुकसान होता है। नियमित और पारंपरिक भंडारण सुविधाओं में रखी जाने वाली प्याज़ इन खराब होने की समस्याओं के प्रति अत्यधिक संवेदनशील होती है। इस संदर्भ में, अत्यधिक चिंता के इस मुद्दे को हल करने के लिए तकनीकी हस्तक्षेप एक पूर्व-आवश्यकता है। खाद्य प्रौद्योगिकी प्रभाग में अनुसंधान और विकास प्रयासों के परिणामस्वरूप, एक ऐसी प्रौद्योगिकी विकसित की गई है जिसमें विकिरण प्रसंस्करण (डोज़ Dmin 60 Gy.) और विशिष्ट भंडारण स्थितियों Dmin 60 Gy) से युक्त एक एकी कृत दृष्टिकोण शामिल है। यह सुनिश्चित करता है कि विकिरण प्रसंस्कृत प्याज को व्यावहारिक रूप से 7.5 महीने तक न्यूनतम वजन घटाने ( 5 प्रतिशत) और गुणवत्ता विशेषताओं के प्रतिधारण के साथ संग्रहीत किया जा सकता है। इस दृष्टिकोण के साथ, विकसित दृष्टिकोण के महत्व का पता लगाने के लिए हाल ही में 1200 टन के बड़े पैमाने पर सफल वाणिज्यिक परीक्षण भी किए गए। किरणित प्याज़ के मामले में विस्तारित भंडारण के दौरान प्याज़ की गुणवत्ता विशेषताओं को बनाए रखा गया। गैर-किरणित और विकिरण प्रसंस्कृत प्याज़ के प्रतिलेखीय विश्लेषण से पता चला है कि प्रमुख डाउन-रेगुलेटेड जीन में कोशिका विभाजन, कोशिका चक्र, कोशिकीय विकास, विकास, कोशिका दीवार मॉड्यूलेशन, डीएनए प्रतिकृति और गैर-विकिरणित प्याज़ की तुलना में विकिरण उपचारित प्याज़ के बल्बों में मरम्मत शामिल हैं। वर्तमान अन्वेषण प्याज संरक्षण के लिए एक प्रौद्योगिकीय समाधान के रूप में कार्य करती है और साथ ही विकिरण मध्यस्थ अंकुरण अवरोध में योगदान करने वाला एक तंत्र प्रदान करती है।

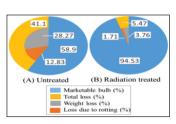
# **Mitigation of Post-harvest Spoilage**

## Large Scale Commercial Trials for the Mitigation of Post-harvest Spoilage in Onion Through an Integrated Approach

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## ABSTRACT



Weight loss profile in non-irradiated and radiation treated onions during storage

The long-term extended preservation of onions is limited owing to key post-harvest issues including weight loss, microbial spoilage, rotting and sprouting leading to overall losses ranging from 35-40%. Onions that are kept in routine and traditional storage facilities are highly vulnerable to these spoilage issues. In this context, technological intervention is a pre-requisite to address this issue of immense concern. As an outcome of the R&D efforts at the Food Technology Division, a technology has been developed which involves an integrated approach comprising of radiation processing (Dose: Dmin 60 Gy) and specific storage conditions (0.2-0.5℃; RH: 65-68%; CO₂: 8000-9000 ppm). This ensures that radiation processed onion can be practically stored for 7.5 months with minimal weight loss ( $\leq$  5%) and retention of quality attributes. With this approach, large scale successful commercial trials to the tune of 1200 Ton were also recently conducted ascertaining the significance of the developed approach. The quality attributes of onion were retained during extended storage in the case of irradiated onions. Transcriptome analysis of non-irradiated and radiation processed onions showed that major down-regulated genes included the ones involved in cell division, cell cycle, cellular growth, development, cell wall modulation, DNA replication, and repair in radiation treated onion bulbs compared to non-irradiated onions. The current investigation serves as a technological solution for onion preservation as well as provides a mechanism contributing to radiation mediated sprouting inhibition.

KEYWORDS: Onion, Ionizing radiation, Sprouting inhibition, Gene expression, Transcriptome

## Introduction

Onion (*Allium cepa*) is a highly consumed agri-produce known for its culinary as well as industrial applications [1]. As per the recent data (2020-2021), the annual onion production in India is around 26.91 million MT and Maharashtra is the leading producer followed by Madhya Pradesh and other states (NHB, 2019). Onion crop harvested during the Rabi season accounts for ~65% of the onion production (Tripathy et al., 2014). Its proper storage thus becomes extremely important and relevant to ensure the crop's sustainability as well as fluctuations in onion market price.

However, approx. 35-40% of the onion crop undergoes losses during storage due to weight loss, microbial spoilage, rotting, and sprouting (Laferriere et al., 1988). As per the recent data by the Ministry of Consumer Affairs, Government of India, every year onion crop to the tune of Rs. 11,000 Crore is lost due to improper storage. Therefore, technological interventions specifically in the onion sector are very much essential not only in ensuring a sustained supply of quality produce during the lean period but also in effectively controlling the price stabilization to a greater extent.

To address the issues of sustained availability of quality onions and stabilized market price as well as to establish economic viability, a meticulously designed large-scale study of the most commonly consumed 'Rabi' cultivar was conducted in association with various agencies. Additionally, to unravel the underlying molecular mechanism behind gamma radiation-induced inhibition of sprouting and to provide further molecular insights, transcriptome analysis of onion bulbs was also comprehensively undertaken.

#### **Materials and Methods**

## Radiation processing and storage

Commercial storage trials were done in multiple batches such as 15 tons (2021), 30 tons (2022), 30 tons (2023), and 1200 tons (2023). Properly cured Rabi onion was procured, packed in mesh bags and radiation processed ( $D_{min}$  60 Gy) at KRUSHAK facility, Maharashtra. Dosimetry was done using a standard Fricke dosimeter. Irradiated onions were transported to multiple cold storage facilities including the newly commissioned cold storage facility at KRUSHAK, Lasalgaon as well as cold storage facility located at Shahapur, Maharashtra (0.2-0.5°C,  $65\pm1\%$  RH) in customized wooden bins with air gaps for proper ventilation.

#### Quality assessment

Periodically during storage, weight loss, texture (using a Texture Analyzer), and color (using a colorimeter) of onion samples was evaluated. The nutritional profiling of the onion samples was also done as per the standard procedures (BIS, 1984; AOAC, 2001). The onions were also evaluated by the trained panellist for the organoleptic quality attributes on a 9-point hedonic scale.

#### Transcriptome analysis

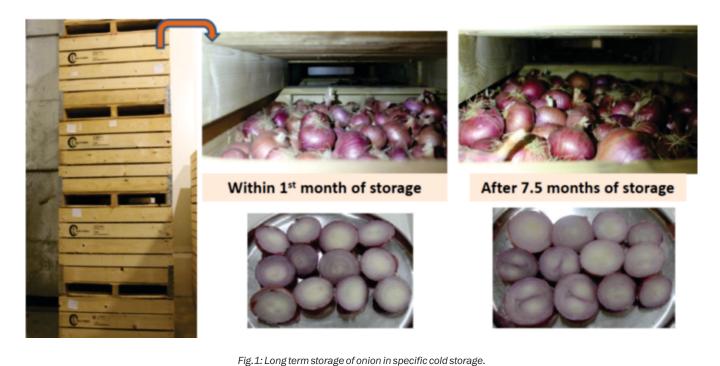
To understand the underlying mechanism of gamma radiation induced sprout inhibition in onion bulbs, transcriptome analysis was performed. This involved RNA isolation, library preparation (concentration assessed through qPCR), sequencing (Illumina NovaSeq 6000 was utilized for Paired End Sequencing ) and bioinformatics analysis. Alignment of filtered reads was done using Hisat 2 (version - 2.1) genome assembler onto the *Allium sepa* reference genome GCA\_000226075.1 Functional annotation clustering was done using DAVID bioinformatics tool (Database for Annotation, Visualization and Integrated Discovery, LHRI). GO enrichment analysis was based on Uniport database and GO terms were placed under molecular function, biological process and cellular compartmentalization.

#### **Results and Discussion**

Storage of onion was done in customized cold storage having standardized storage conditions including temperature: 0.2-0.5°C; RH: 65-68%;  $CO_2$ : 8000-9000 ppm. Inside the chamber, onion were placed in wooden bins in stacked manner with a provision for proper ventilation (Fig. 1).

## Retention of nutrients & sensory attributes during the storage of irradiated onion bulbs

The initial energy content of the irradiated and nonirradiated bulbs varied from 49 to 58 kCal/100 g. In irradiated samples, this did not appear to change significantly throughout



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the course of storage. Changes in non-irradiated tubers were negligible for the first ninety days; however, because of substantial sprouting, these onion bulbs were unfit for both consumption and nutritional quality evaluation.

The color and texture of radiation treated onion samples remained better as compared to the untreated onion samples. After storage for 7.5 months also, the taste and overall acceptability of radiation treated samples remained unaffected ascertaining the efficacy of radiation processing. Besides, radiation treated bulbs retained their natural internal and external colour over long term cold storage.

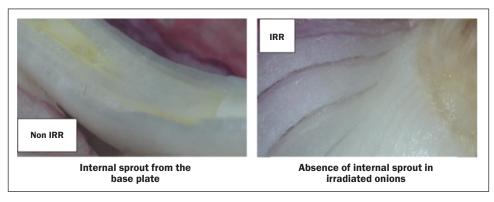
## Radiation processing mediated sprouting inhibition in onion

Onion has a diploid genome (2n=16), with a genome size of ~16.4 GB. Radiation processing led to sprouting inhibition in onion during storage (Fig. 2). Transcriptome analysis of gamma irradiated and non-irradiated onion bulbs showed total 1194 differentially expressed genes, out of which 862 genes were annotated. Among these, 515 genes showed down-regulation, while 347 genes showed up-regulation of transcript levels in irradiated onion bulbs (depicted as Fig. 3).

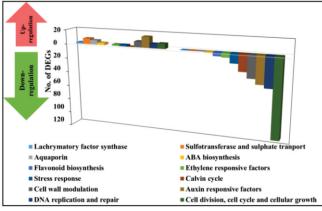
The major down-regulated genes were found to be associated with cell division, cell cycle, cellular growth as well as development, cell wall modulation, DNA replication and repair in radiation treated onion bulbs. In the group of cell wall modulation, expansin was maximally down-regulated and pectinesterase was found to have 14 exons down-regulated. Decrease in the expression of these enzymes indicated possible compactness of the cell wall being manifested as unchanged texture parameters in irradiated onions. Auxin

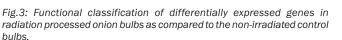
responsive family proteins were also found to be downregulated which included 37 genes with log2 FC of -1.5 to -2.8, while ethylene responsive transcription factors were also found to be down-regulated showing log2 FC of -1.6 to -2.8. Dormancy break in onion bulbs is triggered through modifications in cell wall structure, cellular division and growth which occur through the action of these phytohormones (Puccio et al., 2022). Therefore, down-regulation of their responsive factors will result in dormancy progression, as was observed in the case of irradiated onion bulbs. Interestingly, abscisic acid (ABA) biosynthesis gene, zeaxanthin epoxidases showed up-regulation (log2 FC: 3.4 to 4.7). It has been reported earlier that abscisic acid may serve as a plant growth promoter or inhibitor which depends upon the circumstances. In general, it is known to inhibit growth prior to plant establishment (Chope et al., 2012).

Transcriptome analysis shows that five chalcone synthase exons have been up-regulated in the radiated bulb [Chalcone synthase A: log2 FC 1.8 and Chalcone synthase B: log2 FC 2.0]. Anthocyanin 5-aromatic acyltransferase was found to be up-regulated (log2 FC:1.9) as compared to the non-irradiated onions, indicating the retention of the color pigment in the scales of onion, which was also observed in the color analysis of the irradiated bulb samples. Another very important enzyme lachrymatory factor synthase (LFS) was found to be up-regulated in irradiated bulbs (log2 FC: 6.0). Since LFS is the enzyme responsible for pungency of onions (Imai, 2002; Eady et al., 2008; Silvaroli et al., 2017), as it is known to catalyse the production of lachrymatory factor, propanethial S-oxide, from 1-propenyl sulphenic acid. Up-regulated LFS will provide pungency to onions and their characteristic flavour.









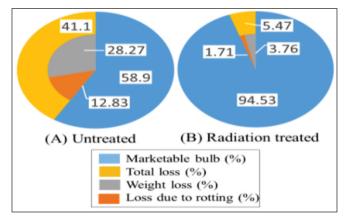


Fig.4: Weight loss profile in non-irradiated and radiation treated onions during storage.

## Significant reduction in post-harvest storage loss was noted in radiation treated onion

A substantial 28% loss was noticed in non-irradiated onions, the radiation processed onions suffered a meagre ~4% weight loss. Reduction in bulb weight is a direct consequence of sprouting where, the growing sapling metabolizes the nutrition stored in the scales and thereby resulting in shrinkage. At the end of the storage, 41% of the untreated onion was lost while 95% radiation treated onion was marketable (Fig. 4). These findings ascertain the potential of application of gamma radiation at commercial scale as a sprout suppressant in onion during extended storage.

## Loss of firmness of onion bulbs during long term cold storage was improved upon radiation treatment

No significant variation in texture was noted between the untreated control and the radiation treated bulbs immediately after gamma radiation. However, after ninety days of storage, the firmness of non-irradiated control onion decreased noticeably as the control bulbs became 32.9% softer. On the contrary, the loss of firm texture of the radiation treated bulbs over the storage period was not pronounced, as 1.3 and 5.8% reduction in maximum force were recorded at ninety days and two hundred and twenty-five days respectively with respect to its initial day's value.

## Un-irradiated control onion suffered significant moisture loss due to actively transpiring sprouts compared to irradiated bulbs

As discussed above, loss of moisture together with sprouting are the major causes of weight loss and texture deterioration in the stored onion. The loss of moisture became pronounced with progression of storage in both the nonirradiated and irradiated samples. The RH at 65  $(\pm 2)$  % exerts an inhibitory effect on the mold growth, especially Aspergillus growth in the cold storage and, thus higher RH is not conducive for bulk storage at industrial scale. Hence, the difference in moisture content between the control and the radiated samples arises gradually during storage due to indirect effect caused by the differential physiological changes. Suppression of sprouting being the most prominent effect of radiation, it seems that the non-irradiated bulbs underwent dormancy break at an earlier stage and the enhancement in growth could be associated with higher rate of transpiration in the control bulb.

## Industrial relevance of integrated approach for long term extended preservation

Overall, it appears that success of long-term storage of onion bulb at large scale depends upon careful regulation of interplay among humidity, temperature and radiation treatment as each of these factors could affect weight loss, dehydration, sprouting, rotting and fungal contamination in a different way. Cold temperature is an essential storage condition for prevention of rotting of bulb and reducing physical water loss by evaporation. Low moisture is a prerequisite for restricting fungal growth while it compromises with evaporation. Additionally, during gradual warming of the coldstored non-irradiated onion prior to market released resulted in profuse sprouting.

This problem was circumvented by introducing gamma radiation at low dose, which was capable of complete sprout inhibition during long-term low temperature storage as well as during gradual warming of the stored commodity for selling in the market at ambient temperature. Thus, the current largescale trials indicated that properly cured onions, if treated with gamma radiation at  $D_{min}$ : 60 Gy and storage at specific condition (RH 65-68%, temp: 0.2-0.5°C) retain the quality attributes with reasonably low post harvest loss ~ 6 % i.e., 2% rotting & 4% weight loss to thrive over the lean period, leading to price stabilization and its market availability throughout the year.

## Conclusion

Post-harvest storage spoilage in onions is quite significant and technological interventions are a pre-requisite to provide sustainable solutions to this challenging concern. The current study provides credible evidence ascertaining the efficacy of gamma radiation processing in ensuring extended preservation of onions based upon various commercial trials with multiple agencies. The outcome of this study provides a substantial basis for the further widespread adoption and implementation of an integrated approach involving radiation technology for the extended preservation of onion with quality retention leading to sustained supply and better commercial gains during the lean period.

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