Effect of Radiation Processing on Water Absorption and Germination of Kodo and Kutki Millets

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ABSTRACT

Background: In pursuit of exploring gamma irradiation as a technology-intervention for de-husking of Kodo and Kutki millets grown widely, authors of this paper observed that irradiation leads to easy de-husking of hardest Kodo grains. Calibration for appropriate dose of irradiation is needed and hence, this study was planned. This technology, if established, may check millet farmers shifting to other crops.

Methods: Kodo and Kutki millets were irradiated by gamma radiations for doses of 0 kGy to 10 kGy. Water absorption capacity and germination potential of irradiated grains were measured to determine the dose for desired results.

Result: Water absorption and germination potential of Kodo and Kutki grains improved on irradiation; dose of 2.5 kGy and above resulted in an increase in the quantity of water absorbed and rate of water absorption. The germination also increased but registering maxima at 7.5 kGy for Kodo and 5.0 kGy for Kutki. Irradiation at doses higher than maxima caused decline. Thus, appropriate dose of irradiation was found to be 7.5 kGy for Kodo and 5 kGy for Kutki.

Key words: Gamma irradiation, Germination potential, Kodo, Kutki, Water absorption capacity.

INTRODUCTION

Irradiation means exposure of materials to radiations of short wavelength, e.g. gamma rays, accelerated electrons and X-rays for desired changes. Novel materials like highly cross-linked polymers could be produced by radiation processing (Technical Document, Department of Atomic Energy, Government of India, 2014). Radiation processing needs neither any toxic catalyst nor thermal energy (Diehl, 1990; Josephson and Peterson, 1983) and hence, this is termed as a green process. Irradiation is the most potent tool for ensuring safety of the biomedical devices sterilised by irradiation.

Ionising radiations like gamma radiations (Co-60 as a radioactive source) are known to be useful and more effective for food processing (Josephson and Peterson, 1983; WHO, 1994). Low doses (0.2 kGy to 1.0 kGy) are sufficient to: a) reduce insect infestation (El-Naggar and Mikhail, 2011) as well as microbial load (AL-Bachir, 2004), b) delay ripening of fruits and c) inhibit sprouting in perishable vegetables (Khandal, 2004). Leafy vegetables, meat etc. are irradiated (at 1 kGy to 10 kGy dose) for making them pathogen-free (Khandal, 2004). Higher doses (10 kGy to 50 kGy), are used to produce gene-mutated seeds with unique characteristics (Ambavane et al., 2015; Subramanian et al., 2011; Nirmalakumari et al., 2007; Ahmad and Qureshi, 1992). Here, it must be mentioned that countries like the USA allows gamma irradiation of foods but EU countries have banned it for foods in fact, consumers in USA are ready to pay extra for food (leafy vegetables, meat etc.) that is gamma-irradiated. But for getting regulatory approvals for gamma irradiation, research studies are a must to establish appropriate dose, safe for consumers (WHO, 1994). The irradiation of food by gamma radiations is neither banned nor popularised, in India. Several food products are allowed, by Indian regulators, for gamma irradiation, based on studies that established safe irradiation doses (Diehl, 1990; WHO, 1994; Sreenivasan, 1974; Hassan et al., 2009; Pankaj et al., 2013; Rachie, 1975). WHO has also issued a list of products approved for gamma irradiation, specifying safe doses for a given purpose (WHO, 1994). The present study deals with effect of gamma irradiation (doses from 0 kGy to 10 kGy) of Kodo millet and Kutki millet on their water absorption capacity and germination potential (Aparna et al., 2013; Borzouei et al., 2010; Garcia et al., 1982; Harding et al., 2012; Melki et al., 2009; Santosh, 2017). Earlier studies on Kodo and Kutki by the authors of this paper had shown that irradiated grains could be de-husked easily (Unpublished results). Present study is to validate the irradiation dose that is beneficial also for germination, besides helping in the de-husking.
**MATERIALS AND METHODS**

**Materials**
Kodo and Kutki grains, sourced from Krishi Vigyan Kendra, Madhya Pradesh were packed (2000 g in each packet) and thermal-sealed in food grade laminated LDPE packets (procured from registered supplier). This study was conducted during 2019-2020 at NIFTEM, Haryana, India.

**Gamma Irradiation**
Kodo and Kutki grains packed in LDPE packets were irradiated (in triplicates) at doses: 0.5, 2.5, 5.0, 7.5 and 10 kGy at room temperature using cobalt 60, as a source of radiations, at irradiation facilities of Shriram Institute for Industrial Research, Delhi, using standard procedure (AERB, 2015) of dosimetry, for estimating irradiation doses. Un-irradiated samples were used as control.

**Effect of Irradiation on water absorption capacity (WAC)**
WAC of unirradiated and irradiated grains of Kodo and Kutki was measured using a method described by Yadav and Jindal, 2007; Shafaei et al., 2014; Dedeh et al., 2006; Agbo et al., 1987. Accurately weighed (~100 g) grains soaked in distilled water (500 ml) for 6 hrs to 96 hrs was placed in a 1000 ml stoppered flask at room temperature. The soaked grains were taken out and extra water on grain surface was removed by blotting paper and weighed to determine increase in weight of grains and expressed as WAC (g of water absorbed g⁻¹ of grains).

**Effect of irradiation on germination potential (GP)**
GP of water soaked seeds of Kodo and Kutki both irradiated and unirradiated were determined by placing 200 seeds of each treatment in germination potential at room temperature. Number of seeds germinated was observed daily, up to 14 days, ensuring moisture of seeds. Seeds with visible radicle were considered germinated (Association of Official Seed Analysis, 1983; ISTA, 2006). As per standard method (ISTA, 2006), GP (% of grains germinated) was calculated by the formula:

\[
GP = \frac{\text{Number of grains germinated} \times 100}{200 \times \text{Number of grains kept for germination}}
\]

All data are average of triplicate observations.

**Statistical analysis**
Statistical analysis of the data was done, using ANOVA with post-hoc Turkey HSD test (Snedecor and Cochran, 1987). Differences between means were compared for their significance (p ≤ 0.05) to assess the effect of gamma irradiation on the GP of irradiated and unirradiated grains.

**RESULTS AND DISCUSSION**

**Water Absorption Capacity**
Result (Fig 1 and 2) show that absorption of water increased with increase in radiation dose. Soaking time also played a role: for a given dose, water absorption increased with soaking time until it reached a plateau after reaching maxima. Time interval when absorption reached maxima, varied with radiation dose. For un-irradiated grains of Kodo and Kutki, the plateau reached after 96 hours with maximum water absorption of 51.3 and 54.4 percent respectively. When irradiated at 0.5 kGy, grains of both Kodo and Kutki registered almost same amount of absorption of water, reaching plateau in same time (96 hrs) as observed for un-irradiated grains. Studies (Yadav and Jindal, 2007; Shafaei et al., 2014; Dedeh et al., 2006; Agbo et al., 1987) on various grains like sorghum, rice, soybean, barley etc. also showed that initially water absorption rate increased fast but reached maxima after a certain time beyond which there was no further absorption. At doses from 0.5 kGy to 5 kGy, the plateau reached in 72 hours for all Kodo and Kutki. Irradiation at doses beyond 7.5 kGy there was a decrease in the time period for reaching water absorption maxima from 48% in case of 0.5 kGy to 59.2% in case of 10 kGy; a gradual increase in maximum amount of water absorbed, with the increase in irradiation dose.

Kutki grains (Fig 3 and 4), registered similar trends as observed for Kodo. Plateau reached after 96 hours for:

![Fig 1: Effect of irradiation dose on WAC of Kodo millet (dose vs time).](image-url)
unirradiated and irradiated at 0.5 kGy. Grains irradiated at 5 kGy, reached plateau in ~ 48 hours. The amount of water absorbed increased from 54.4% for unirradiated to 62% for grains irradiated at 10 kGy. Like Kodo, WAC of Kutki grains also increased with the increase in irradiation doses.

Water absorption or imbibition is the first and foremost step for germination process to set in. As water enters into seeds, it rehydrates cellular and molecular components of seeds, which drive water uptake, ascribed to changes by irradiation (Agbo et al., 1987; Association of Official Seed Analysis, 1983; ISTA, 2006). Irradiation causing seeds to absorb maximum possible amount of water within the least time is an achievement. By rehydration of internal parts, seeds swelled putting pressure on the outer layers followed
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by processes leading to germination; reactivation of metabolism followed by protrusion of a radicle which ultimately gets out of seeds as a root! Increase in WAC, thus, must impact positively on germination.

The irradiation must have affected seed structure raising its porosity and capillary forces drawing in water molecules at a faster rate. This may have ensured penetration of moisture deep inside the seed structure leading to increase in WAC with irradiation doses. Unirradiated seeds have with its unaltered compact structure and poor porosity may inhibited water uptake.

Thus, it is evident from the data that a minimum of 5 kGy of dose in case of Kodo and 2.5 kGy dose in case of Kutki are required to achieve a significant increase in imbibition behaviour. Although, imbibition was better for grains irradiated at 10 kGy, but cannot be recommended as appropriate dose until there is no adverse impact on germination of the seed. As stated by Dedeh et al. (2006) that hydration rate was mainly affected by the seed coat and cotyledons, an increase in the rate of absorption of water could thus, be ascribed to changes in the seed coat and cotyledon due to irradiation. The same effect is evident here: increase in irradiation dose impacted in an increase in WAC. Irradiation of Kodo and Kutki therefore, can be a useful technology-intervention for millet farmers. However, it is imminent to determine an appropriate dose for this.

Germination potential

The data (Fig 5 and 6) reveal that the GP of seeds increased with increase in irradiation dose from 2.5 kGy to 7.5 kGy for Kodo and 2.5 to 5.0 kGy for Kutki. Length of shoots of unirradiated seed was smaller and of light green colour as compared to that of seeds irradiated at 0.5 kGy. Kutki seeds, irradiated at doses beyond 5 kGy, registered a decrease in GP. Kodo seeds registered a decrease when irradiated at doses beyond 7.5 kGy. Both Kodo and Kutki, exhibited significant rise in GP, when irradiated for doses beyond 2.5 kGy; doses of <2.5 kGy affected insignificant change.

Kutki seeds irradiated from 2.5 kGy to 5 kGy had the best germination whereas the similar behaviour for Kodo seeds was observed at doses from 5.0 kGy to 7.5 kGy. Although GP of seeds irradiated at 0.5 kGy and unirradiated were comparable, shoots were darker green for 0.5 kGy dose for both millets.
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The irradiation at doses from 2.5 kGy to 7.5 kGy could cause following changes: a) weakening adhesion between coat and inner surface of grains and b) constituents of grains getting more porous driving water in to penetrate faster resulting and deeper. However, higher irradiation doses are known to have a role in breaking of protein structure, altering the constitution of grains, Though both Kodo and Kutki showed similar trend but Kodo grains proved to be harder as evident from their highest GP at 7.5 kGy compared with 5 kGy for Kutki. These results are on similar lines to the earlier studies of Gupta and Yashvir, 1975 and Ambavane et al., 2015 who reported that chlorophyll concentrations decreased with irradiation dose. Ambavane et al., 2015, reported improved germination when finger millet grains were irradiated. It must be mentioned that doses > 7.5 kGy for and > 5.0 kGy for Kutki caused decrease in WAC and GP.

The effect of irradiation on the easying of de-husking of Kodo and Kutki was established (unpublished data) earlier by the authors. Irradiation made the dehusking of grains much easier; could be done without much effort when grains were irradiated at high doses. The question that remains to be answered: “what is the appropriate irradiation dose?” The present study provides answer to that question: dose range of 5.0 kGy to 7.5 kGy for Kodo and 2.5 kGy to 5.0 kGy for Kutki was found to be the best range. Thus, findings of this paper could help decide the right dose of irradiation for Kodo and Kutki. Incidentally, dehusking was also found to be significantly easier, at these doses.

CONCLUSION
From this study, following conclusions can be drawn: a) gamma irradiation helps in imbibition process of grains; improvement in rate and the quantity of water uptake, b) GP increased on irradiation; reached maxima at 7.5 kGy for Kodo and at 5.0 kGy for Kutki but at higher doses, GP decreased. These findings were seen in tandem with results (under publication elsewhere) of the effect of irradiation on de-husking. It established an appropriate dose of gamma irradiation as a technology-intervention for millets.

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Declarion of interest
The authors declare no conflict of interest.

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