



## Effect of $\gamma$ -irradiation on rice seed vigor assessed by near-infrared spectroscopy



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### ABSTRACT

High-energy radiation such as  $\gamma$ -ray can kill pests, insects and bacteria with high efficiency and low-cost, and so it has been utilized as one of the most promising tools for food storage. On the other hand, high-energy radiation may also induce physiological, biochemical and genetic changes of crops. In this study, near-infrared (NIR) spectroscopy as a non-destructive, fast, low-cost analytical tool was employed to investigate the effect of  $\gamma$ -ray irradiation on the irradiated rice seeds. The alteration of seed vigor was assessed after the rice seeds were irradiated with different irradiation doses and at different storage ages. The relationship between seedling height, seed vigor and irradiation dosage was established based on the analysis of the NIR spectral data. The accuracy of the PLS (partial least squares) prediction model was checked by parameters such as the root mean square error of prediction (RESEP) and the residual prediction deviation (RPD). Our results showed that with increase of irradiation dosage and storage age, seed injury increased resulting in reduced seed vigor and seedling height. This work thus demonstrates the successful application of NIR for the rapid and non-invasive evaluation of rice seeds after irradiation and storage.

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## 1. Introduction

Every year microorganisms and pests cause severe damage in rice seeds and food crops and lead to huge economic losses. For example, insect pests bring about 5–10% or even higher percentage losses of the total grain harvest in tropical countries. To extend storage time and maintain food quality traits, researchers have applied irradiation treatment to kill the associated molds, insects and bacteria (Si et al., 2013; Lankmayr et al., 2004). In particular, the irradiation by  $\gamma$ -rays has become a widely applied method because of the advantages in high effectiveness, low cost and easy operation (Wang, 2002). Being a kind of electromagnetic radiation,  $\gamma$ -rays possess high energy and penetrating power, inducing production of

free radicals in pathogens and pests which affect cellular metabolic processes, eventually causing death when at sufficiently high dose (Maity et al., 2008; Liu et al., 2013).

While a proper dosage of  $\gamma$ -ray irradiation can inhibit insects and bacteria and so be beneficial for rice seed storage, a dosage that is too high can change the physiological, biochemical and genetic traits of the rice seeds, resulting in mutation or complete loss of the vitality of the seeds. It has been reported that the irradiation processing dose for insect disinfestations is 0.3–0.5 kGy, and this exposure is not harmful to grain edible quality and function characteristics (Li et al., 2007). It has also been reported that the range of irradiation dose is 100–1500 Gy for cereal storage treatment to reduce the dangers of insect and mold deterioration caused by fungal activity (National Standards of P.R. China, 2001). It is therefore very critical to optimize the irradiation dosage for the best treatment of seeds. To achieve this, it is necessary to look for an effective tool and method which can quickly and non-destructively assess the effect of  $\gamma$ -ray irradiation.

In this context, we initiated the study of applying near-infrared (NIR) spectroscopy for the assessment of the radiation-induced

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effects on rice seeds. Near-infrared light is an electromagnetic wave with wavelength within the range of 780–2500 nm, which covers the region for the combination and octave frequency vibration absorption of the molecular hydrogen functional groups in X–H (X = C, N, O, S, etc.), so NIR spectroscopy can be utilized to analyze agricultural products and ingredients such as protein, fat, moisture, amino acids, starch, sugar etc. (Xue et al., 2011; Herrero Latorre et al., 2013; Xu et al., 2013; Escuredo et al., 2013). For example, Kays and Barton (2002) assayed soluble and insoluble dietary fiber fractions of cereal food products through NIR spectroscopy, Giacomo and Stefania (2013) established a multivariate regression model for detection of fumonisins content in maize from based on NIR measurements and András and Szilveszter (2012) analyzed wheat grain development using NIR spectroscopy. However, to the best of our knowledge, the application of NIR spectroscopy in the evaluation of  $\gamma$ -ray irradiation effects on rice seeds has never been reported. It was thus intriguing for us to explore if NIR spectroscopy could also be useful for effective evaluation of the vigor or vitality of irradiated rice seeds. This work therefore aimed to apply NIR spectroscopy in the assessment of irradiated rice seeds, to establish the possible relationship between irradiation dosage, storage age and vitality of rice seeds.

## 2. Material and methods

### 2.1. Seed sample preparation

Rice seeds of variety 9311 were used in the experiments, which were harvested in 2013 with 99% germination rate. Preliminary screening of seeds was performed and the seeds were placed in an incubator with temperature at 42 °C and relative humidity (r.h.) at 80% for continuous aging.

### 2.2. Irradiation treatment

The 9311 rice seeds were divided into 8 groups and treated by  $^{60}\text{Co}$ - $\gamma$ -ray irradiation, with the irradiation dose being 0, 60, 100, 200, 300, 400, 500, 600 Gy, respectively. The germination rate and survival rate were then measured for each group. After irradiation, the seeds were stored at –20 °C in a refrigerator.

### 2.3. Acquisition of NIR spectra

A Fourier transform near-infrared spectrometer (Bruker MPA) with PbS detector was employed to collect the spectra. The NIR reflection spectra were recorded in the range of 4000–12,500  $\text{cm}^{-1}$  with the spectral resolution of 16  $\text{cm}^{-1}$ . For each irradiation treatment, 20 grains were measured for the NIR spectra.

### 2.4. Germination test and seedling height measurement

At each dosage of irradiation, 10 seeds were gathered for NIR measurements. The seeds were treated with 1% sodium hypochlorite for 15 min, and then arranged in sequence in a 96-well plate with open bottom placed in a big dish lined with filter paper. The filter papers were sterilized without any toxic chemicals. Each irradiated seed was cultivated with light intensity at 420–450  $\mu\text{mol}/(\text{m}^2\text{s})$  for 12 h each day, with temperature at 30 °C, and the seedling height was measured from the 3rd to the 15th day (International Seed Testing Association, 1995).

### 2.5. Definition and measurement of single seed vigor index

According to the national standard vigor index (seed vigor index VI), the definition of VI is  $\text{VI} = \text{S} \times \text{GI}$ , which is suitable for

germination after  $t$  days when the total length of hypocotyls and radical is  $\text{S}$ .  $\text{GI} = \sum \text{Gt}/\text{Dt}$ , where  $\text{Gt}$  is seed germination rate at day  $\text{Dt}$ . Similarly, here we define the “single rice seed vigor index” (SSVI) as  $\text{SSVI} = \sum \text{St}/\text{Dt}$ , where  $\text{St}$  is rice seedling height measured at day  $\text{Dt}$  (National Standards of P.R. China, 1995).

## 2.6. Chemometrics evaluation

The original NIR spectra were processed by software OPUS7.0 provided by Bruker. After data pretreatment, Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression was used to establish the prediction model. PCA is a statistical procedure that uses an orthogonal transformation to convert a set of correlated variables into a set of values of linearly uncorrelated variables called principal components (PCs). The number of PCs is less than or equal to the number of original variables. The first PC has the largest possible variance, and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components (Jolliffe, 2002). The PLS regression is a statistical method that finds a linear regression model by projecting the predicted variables and the observable variables to a new space.

The model performance was determined by  $R^2$  (The Coefficient of Determination), RMSECV (Root Mean Square Error of Cross Validation), RPD (Residual Prediction Deviation, which is defined as the ratio of standard deviation to SEP) and Bias (system bias error). The model prediction accuracy was determined by RMSEP (Root Mean Square Error of Prediction),  $r$  (Correlation Coefficient), RPD (Residual Prediction Deviation) and Bias (Li et al., 2008).

## 3. Results and discussion

### 3.1. Measurement of germination rate

Fig. 1 shows the initial germination rate and survival rate for the seeds. The germination test began 3 h after irradiation, and the germination period lasted for 15 days. The germination and survival rate curves help us to determine the appropriate irradiation dosage for the rice seed vigor test. The germination rate measurement showed that when the dosage range exceeded 400 Gy, the germination rate fell to below 87% and the survival rate fell to below 43%, indicating that the irradiation treatment adversely influenced the rice seed vigor (Fig. 1).

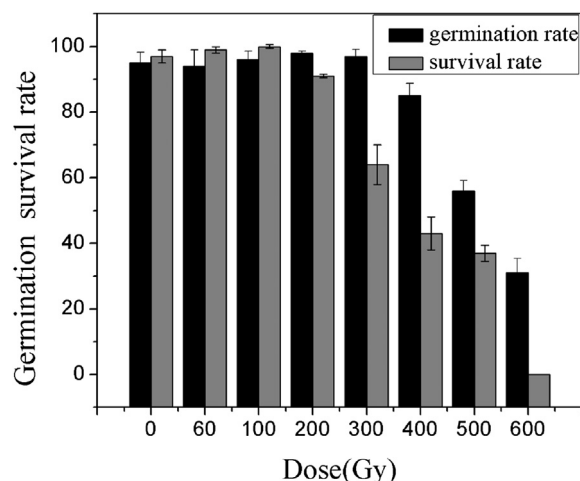


Fig. 1. Measurement of initial germination rate and survival rate.

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