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Unlocking the secret of soft X-ray impact on seed germination

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Abstract

- **Background**: Seed quality analysis using X-rays is increasingly explored due to its invasive and rapid nature. Yet, the current absence of reliable and standardised imaging protocols has led to contradictory effects of X-ray exposure in previous studies. Our work systematically investigated the effect of soft X-rays on a wide range of plant materials.
- **Results**: The baseline of three germination categories was established across seven species before the application of soft X-ray exposure under controlled standard germination conditions. The high inter-varietal and inter-lot variabilities, in addition to the strong interaction between X-ray exposure with variety and lot, reinforced the need to consider genetic and seed quality aspects while evaluating the impacts of X-rays. A slight stimulative effect was observed on most of the species (bean, carrot, fennel, maize, radish, and ryegrass), notably, with a repeated reduction in ungerminated seeds.

Intrinsic physical quality holds a crucial value where the minor negative impact observed in soybean originated from its degraded physical quality and not from X-ray exposure, hence, no destructive effects were detected. To understand whether seed size plays a significant role in a seed's response to exposure, linear regression models were built to predict 3D seed traits (volume) from 2D X-ray images. Yet, seed size did not explain the variation in responses to soft X-rays. However, the average density of the seven species explained both their natural germination (p < 0.01; R^2=0.82) and their germination outcomes after exposure (p < 0.01; R^2=0.88). Among all species, fennel with notably low density (0.7 g/cm^3) demonstrated the most pronounced gains in germination after exposure (4.6 ± 6.3%) due to the stimulative effect.

- **Conclusion**: Soft X-ray exposure is non-destructive with a beneficial effect on germination but can be strongly influenced by underlying genetics and the physical quality of the tested seeds. This study adopted internationally-standardised germination procedures and tested the effect of soft X-rays across diverse botanical, genetic and seed quality profiles. This work addressed important gaps in evaluating X-ray impacts and proposed a robust design and well-examined radiography protocol for a proven non-destructive seed quality analysis.
- **Keywords:** Germination, Radiography, X-ray Imaging, Seed Quality, Standard Protocols

Introduction

Seed phenotyping has been of increasing interest in recent years [1,2]. In particular, X-ray imaging has demonstrated significant value in this area. Indeed, this technology has proven useful for the phenotyping of the different stages of seed and seedling development (seed morphogenesis, characterisation of seed anatomy, seed imbibition, germination, and seedling elongation) [3–5].

Specifically, X-rays provide a solution for performing qualitative and quantitative analysis of the internal and external characteristics of seeds, which are not directly visible without breaking open the seed [6]. Its principle allows for visualising different seed components (e.g., seed coat, endosperm, embryo) based on the contrast resulting from different absorption levels of these tissues [7,8]. Currently, a few X-ray techniques exist in modern phenotyping, two of which are widely employed for seed and plant physical analysis, each with specific advantages and settings. First, 2D radiography is widely used as a rapid tool to assess seed quality [9,10] and detect mechanical or insect damage [11–13]. Second, 3D tomography is a more advanced technique that captures tiny details at higher resolution and allows the quantitative measurement of morphometric traits of seed components [14–18]. Yet, tomography image acquisition is notably slower and more costly compared to 2D radiography. Furthermore, a few studies have shown the benefit of X-rays to explore the relationship between seed morphometric characteristics and germination quality [19–21].

Once the X-ray images have been acquired, the interpretation of the images can be carried out either visually by experts or automatically using image analysis techniques [6,22,23]. Traditional visual analysis has been used for the assessment of seed quality of Brassicaceae [24] and soybean [25]. But lately, there has been a rapid rise in image analysis and artificial intelligence applications in agriculture including seed quality evaluation. Recent studies have applied machine learning techniques to X-ray imaging to facilitate and improve seed physical analysis. For instance, [26] classified the quality of *Urochloa brizantha* seeds using Linear Discriminant Analysis (LDA). While [27] introduced a high-throughput deep learning-based pipeline for X-ray data pre-processing and high-precision seed defect detection. Additionally,

[28] characterized the quality of *Jatropha curcas* seeds using 2D X-rays, combined with AI algorithms and multispectral imaging.

Unfortunately, during X-ray imaging, species are usually exposed to unequal and non-optimised radiation due to multiple reasons. First, the lack of standardized imaging protocols in seed testing may lead to satisfactory but non-optimal parameter combinations (e.g., kV, distance-to-source, and exposure time). Second, during seed physical analysis using X-ray imaging, image quality gets the highest importance in the process, while dose measurement is typically ignored. Third, both the variability in seeds' morphology and the characteristics to observe influence the analyst's choices of imaging parameters.

In most studies, X-ray imaging is largely described as physically non-destructive [22,29]. However, there are very few studies on the impact of X-ray exposure on seeds or plant physiology, unlike in the medical area [30,31]. On plants or seeds, earlier studies showed different effects of X-rays on plants at physiological and cytogenetic levels [32–35]. For example, Bless *et al.* showed an effect of X-rays on plant growth, but this impact was different, depending on the plant species [33]. More recently, further studies have been carried out on the impact of X-rays on the physiology of the plant seed [36], cytogenetic and biochemical impacts [35,37,38] and many studies have focused on the effect on germination and growth stages. Notably, the different studies have shown conflicting results, and this subject is still a matter of disagreement.

Concerning seed germination, Rudolph reported a higher germination rate for *Pinus banksiana* seeds having been exposed to X-rays at 1000R [39]. However, other studies have concluded that there is no impact of X-ray exposure on germination. For example, it was reported that exposure to X-rays did not significantly affect the germination of onion seeds [40]. Researchers implied that soft X-ray radiation did not impair the germination of sunflowers [41]. More recently, Gagliardi *et al.* have investigated the germination, and the structure of bell pepper seeds by X-rays [21] under weak X-ray exposure to avoid potential impact on seed germination, underlining the potential harmfulness of X-rays at higher doses. Finally, a recent study on *Echinacea angustifolia* showed that X-ray exposure did not significantly affect seed germination or growth [42]. On the contrary, some publications have shown that X-rays have a detrimental effect on seed germination. For example, a negative impact of X-rays on the germination of the date palm even at low doses was found by Al-Enezi *et al.* [43]. Also, germination of peanut seeds was reported stable at low exposures with an observed decline in germination at high exposure [44,45]. These contradictory findings have been reported in the literature likely due to methodological variability and the absence of standard protocols and generalised design. This research systematically addresses these gaps while focusing solely on 2D radiography for its advantages and wide use in seed physical analysis. To obtain a complete study, several variables that could interact with the effect of X-rays were taken into account. For example, species from different families were selected to cover a wide range of seed sizes. In addition, intraspecific effects were also taken into account by studying the possible influence of 'varieties', 'lots', and seed quality. Finally, given the conflicting findings in prior studies, it was hypothesized that such inconsistencies and contradictions may arise from methodological variability, lack of standard imaging and germination protocols, and insufficient control of seed physical quality. In this study, all these aspects were systematically considered to isolate and clarify the actual effect of X-rays on germination. The overall workflow of this study is illustrated in Fig. 1, which provides a graphical overview of the article's structure. The aim is to reach a clear conclusion on the potential effect of X-rays on germination under standard conditions of routine use. In addition to this conclusion, this study will also propose a safe X-ray dose to the scientific community and practical application of X-ray on seed quality analysis.

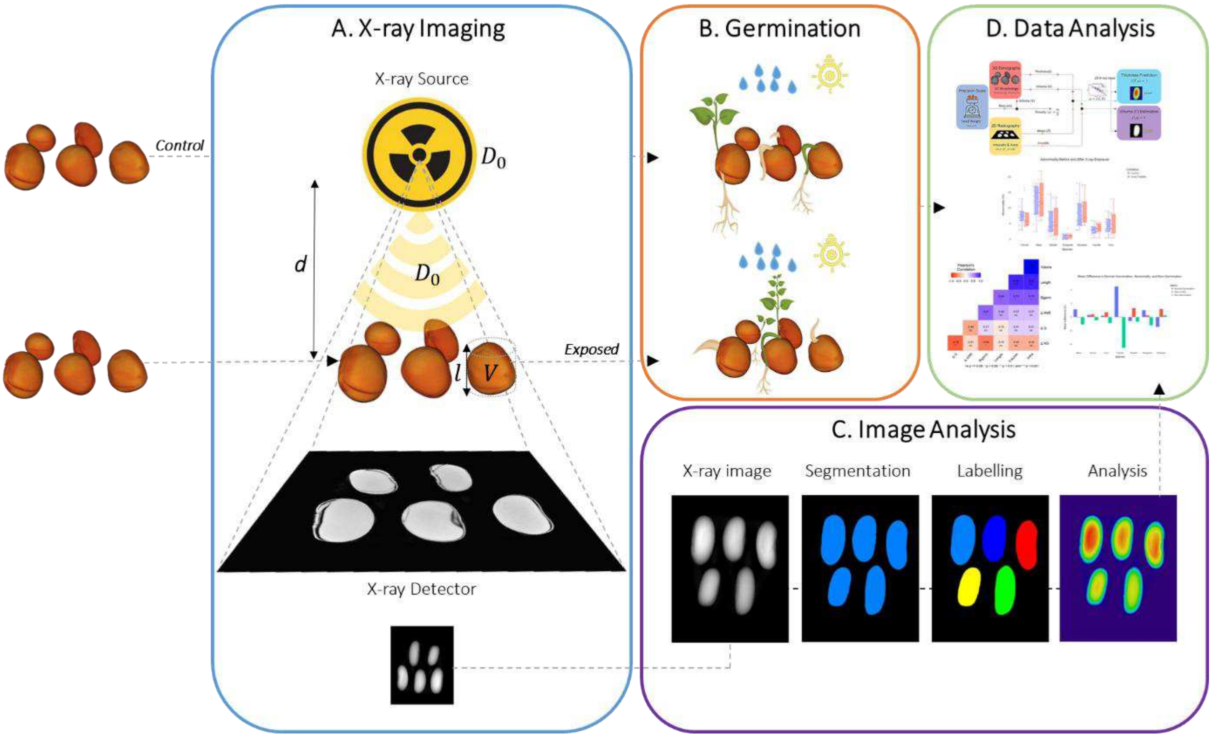

Figure 1: General overview of the evaluation of the impact of X-ray on germination.

Materials and Methods

Experimental design

For a direct evaluation of the possible impacts of X-ray exposure on germination, our experimental design involved a controlled setup where seeds from two different lots of five varieties of seven species (2 lots × 5 varieties × 7 species) were analysed. A total of 200 seeds per lot were exposed to X-rays at a specified X-ray dose used in our everyday analysis, with an equivalent number of unexposed seeds serving as a control group (Fig. 2).

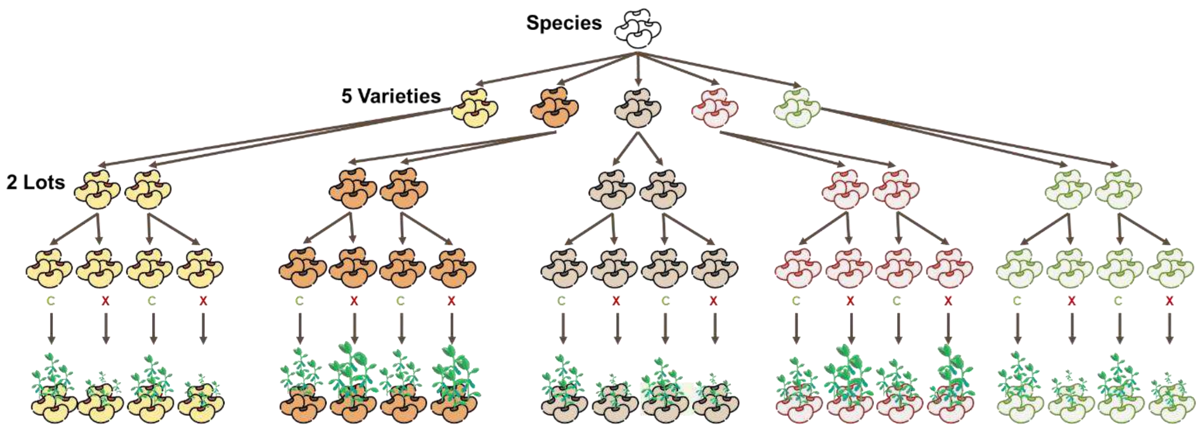

Figure 2: Experimental design for evaluating the effect of X-ray exposure on a species. Letter (C) denotes the control group while letter (X) denotes the group exposed to X-rays.

To avoid any environmental variability or any source of bias, all the project's samples were prepared under the same conditions in the following stages: (1) Preparation of the samples on the supports in the morning, (2) Radiography in the afternoon at room temperature, (3) Storage in an air-conditioned room (10°C) overnight, (4) Germination test the following morning. The control samples were kept in the same room as the samples X-rayed during the first 2 stages.

Biological materials

The species selection was based on their agricultural importance of culture, frequent physical analysis using X-rays at the laboratory, and morphological and botanical family diversity. Four botanical families are represented in this study *Fabaceae, Apiaceae, Poaceae* and *Brassicaceae* corresponding to the 7 species (Table 1). For each species, 5 varieties were analysed and each variety underwent X-ray exposure by exposing two different lots to capture potential variations and assess the impact of X-rays on seed germination.

Table 1: List of the species used in this study.

Botanical family	Latin name	Common names

Fabaceae	*Phaseolus vulgaris*	Bean
Apiaceae	*Daucus carota*	Carrot
Apiaceae	*Foeniculum vulgare*	Fennel
Poaceae	*Zea mays*	Maize
Brassicaceae	*Raphanus sativus*	Radish
Poaceae	*Lolium* sp.	Ryegrass
Fabaceae	*Glycine max*	Soybean

X-ray imaging

Seeds were exposed to soft X-ray using digital X-ray equipment at GEVES, France. 200 seeds were taken at random from each lot and placed on one of two sample holders, polyamide plates or floral foam, in groups as indicated in Table 2. A cabinet X-ray system for specimen radiography (Faxitron X-ray, model MX-20 DC12, Tucson, USA), operating from 10 to 35 kV, 300 μA and with a maximum pixel resolution of 7 μm was used to obtain the X-ray images (Fig. 3). The parameters used for each of the 7 species are presented in Table 2.

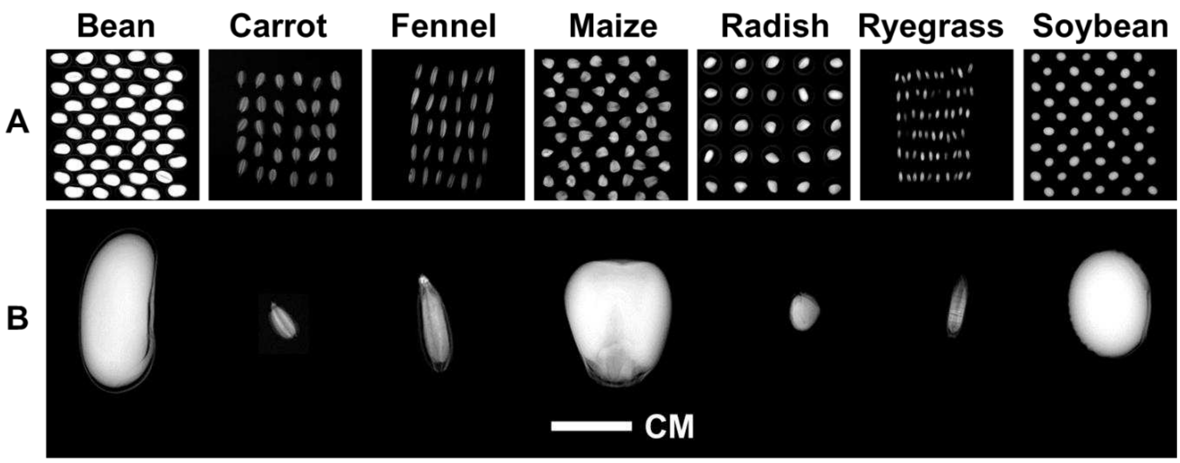

Figure 3: (A) Examples of X-ray images of 25 or 50 seeds per species. (B) zoom on 1 seed of each species to demonstrate the morphological variation of the selected species.

To measure the entrance dose D_0 (mGy) during the X-ray image acquisition, a radiation dosimeter was placed at the same distance as the exposed seeds.

Table 2: Biological material and imaging parameters used to acquire radiographic images.

Species	**Distance (cm)**	**kV**	**Time (s)**	**mA**	**Entrance dose (D_0)**	**Sample holder**	**#seeds/ image**
Bean	57.2	23	12		0.27	Polyamide plates	50
Carrot	14.3	18	16		1.82	Floral foam	25
Fennel	19.1	19	12		1.1	Floral foam	30
Maize	57.2	19	12	0.3	0.1	Polyamide plates	50
Radish	19.1	19	12		1.1	Polyamide plates	25
Ryegrass	28.6	25	19		2.34	Floral foam	50
Soybean	57.2	20	12		0.14	Polyamide plates	50

Germination procedure

To address the lack of consistency and eliminate methodological variability that may explain the contradictory effects of X-ray in literature, standard germination protocols were strictly applied to the control and exposed groups of the 7 species as defined by the ISTA (International Seed Testing Association) [46], which is responsible for developing standard seed testing methods. Various parameters are defined, such as temperature, substrate and test duration, to analyse the germination capacity of seed lot under optimal conditions [46]. The methods used in the laboratory to assess the germination quality of each species are illustrated in Table 3. During the tests, the samples were exposed to 8 hours of light per day.

Table 3: Detailed methods for germination test per species.

Species	**Substrate**	**Temperature (°C)**	**Duration (day)**
Bean	Organic growing medium	20-30	7
Carrot	Top of paper	20-30	14
Fennel	Pleated paper	20-30	14
Maize	Between paper	20-30	7
Radish	Pleated paper	20-30	6
Ryegrass	Top of paper	20-30	10
Soybean	Organic growing medium	25	7

The results of this test were expressed as normal seedlings, abnormal seedlings and ungerminated seeds as described by the ISTA rules (Table 3). Normal seedlings are seedlings that develop well with all their organs without any major defect when grown in favourable conditions of moisture, temperature and light. This category included: intact seedlings, seedlings with slight defects, and seedlings with secondary infection. Abnormal seedlings are seedlings that do not show the potential to develop into normal plants in favourable conditions. This category includes all types of seedling growth anomalies: root defects, shoot system (hypocotyl, epicotyl and mesocotyl), cotyledons and primary leaves, buds (Fig. 4). The ungerminated seeds corresponding to seeds which have not germinated by the end of the test several types of seed are included in this category: hard seeds, fresh seeds, dead seeds, empty seeds, embryoless seeds, and insect-damaged seeds.

Figure 4: Illustrations of germination outcomes of fennel characterised into 3 groups. (A) normal seedling, (B) and (C) abnormal seedlings, and (D) ungerminated seeds (according to current ISTA rules).

The variables measured are as follows, all expressed in percentages (%):

- Normal germination/seedlings: percentage of germinated seeds with no or slight seedling defect out of the total seeds.
- Abnormal germination/seedlings: percentage of germinated seeds with a major seedling defect.
- Ungerminated seeds: percentage of ungerminated seeds (empty, dead, or fresh).

Morphological parameters of seeds obtained by X-ray image analysis

For studying the potential link between seed morphology and the response to X-ray exposure, a comprehensive image analysis pipeline was employed to extract 8 morphological characteristics from X-ray images. The pipeline (Fig. 1. C) processed multiple batches of 2D greyscale images to analyse seeds individually. This resulted in a set of morphological features for each seed lot, variety and species, stored in a data structure for further analysis. These traits were measured in pixel units. Then, the measurements were calibrated using the corresponding equivalent in millimetres (mm or mm²) in Table 4.

Table 1: Seed morphological features measured by the image analysis pipeline.

Parameter	**Description**	**Unit**
	2D Radiography	
Perimeter	Total circumference of the seed, providing an overall measure of its shape	mm
Surface Area	The area enclosed by the seed's contours allows the assessment of the overall size	mm²
Length	Maximum distance between opposite ends, representing the longitudinal dimension	mm
Width	Measurement of maximum width, indicating the transverse dimension	mm
Mean Intensity	The mean intensity value of a seed in the radiographic image	0-255
Intensity Standard Deviation	Measure of dispersion of intensity values relative to the mean	0-255
Intensity Median	Central value of intensity in the distribution of values	0-255
Intensity Majority	The most frequent intensity value in the seed	0-255

Predictive modelling of seed 3D morphology from 2D features

To effectively evaluate our hypothesis on whether the soft X-ray exposure elicits a significant or contradictory response depending on seed size and physical characteristics (e.g., density), accurate estimation of key structural parameters such as seed volume (V), thickness (l), and density (ρ) is crucial. However, these parameters remain inaccessible from 2D X-ray images. Thus, an alternative approach (Fig. 5) was designed to estimate these characteristics.

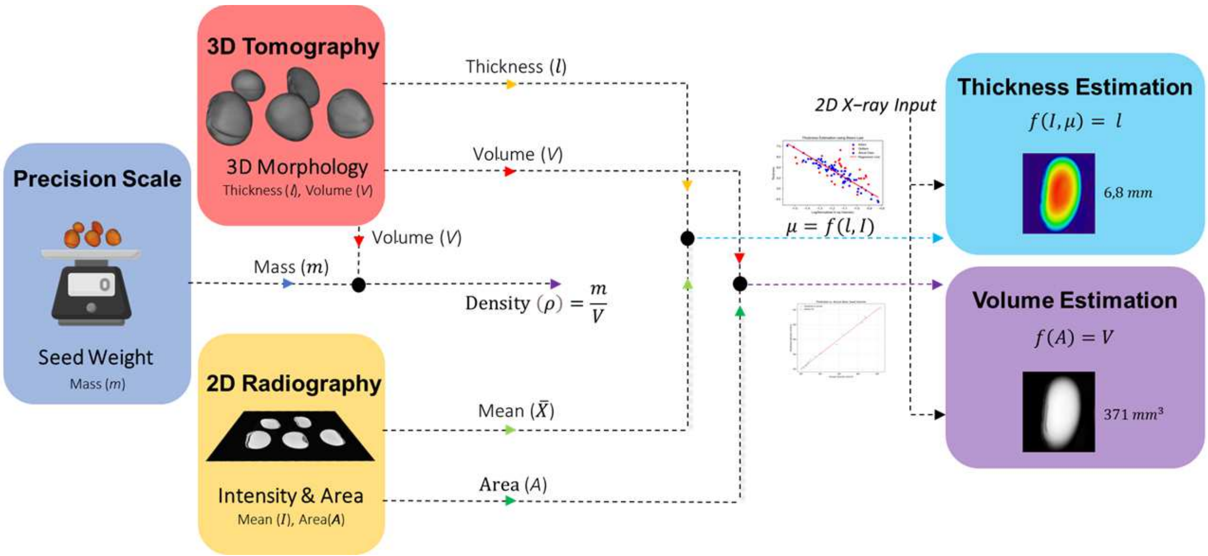

Figure 5: Species' average density estimation, and Volume and thickness estimation from 2D X-ray images.

To achieve this, a calibration dataset was necessary to build the predictive models. To construct our calibration dataset, 100 additional seeds from the same lots of each species were individually measured for thickness (l) and volume (V) using 3D tomography (NSI X-50 X-ray imaging system, North Star Imaging, Inc., Minnesota, USA), surface area (A) and pixel intensity using 2D radiography at the same initial imaging parameters (Table 2), and seed mass (m) using a 4-digit precision scale.

Table 5: Estimated seed morphological and physical features.

Parameter	**Description**	**Unit**
	3D Tomography	
Thickness	The length travelled by the X-rays through the seed	mm

Volume	The three-dimensional space occupied by a seed	mm^3
Physical Property		
Density	The estimated average ratio of the seed's mass to its volume	g/cm^3
Mass	The average weight of a seed	g

Beer's law (1) governs the attenuation of X-rays through an object (e.g., seed):

$$I = I_0 e^{-\mu l}, \qquad (1)$$

where linear attenuation (μ) quantifies the rate at which the initial intensity (I_0) of X-rays travelling through a seed declines per unit thickness (l) which is an indicator of the ability of a species to absorb or scatter X-rays linearly regarding its thickness (l), supposing that I_0 is the pixel intensity if no absorbing or scattering seed tissues between the X-ray source and the detector. The calibration data were split into training (70%), validation (15%), and testing (15%). Then, the linear regression models were trained on the train-validation set to fit a regression line between pixel log-transformed intensity (I/I_0) extracted from 2D radiography, versus the corresponding seed thickness (l) measured using 3D tomography. This linear regression model allowed the recovery of irradiated seeds' thickness (l) through only their 2D X-ray images and the slope that corresponds to the linear attenuation (μ) of a species at specific imaging settings. The model with the highest validation score (R^2) was then chosen. Later, our best model was tested on the unseen test set. Similar to the previous method, the linear relationship between the 2D Area of a seed measured in 2D radiography and the corresponding 3D Volume of the same seed measured in 3D tomography allowed recovering missing morphological information from 2D images, by the estimation of seed volume (V). Finally, species average density (ρ) was estimated using the measured seed mass (m) using precision scale and volume (V) data measured using 3D Tomography. All measured or estimated characteristics are detailed in Table 5. All measured or estimated characteristics are detailed in Table 5.

Statistical analysis

Within each species, the effects of X-ray exposure (Modality), variety (Variety), lot (Lot) and their interactions, on germination categories were tested using a Binomial Generalized Linear Model (GLM) with a logit link function [47]. Binomial GLM was proven efficient for non-normally distributed germination and categorical data [48,49]. To prevent the model from convergence a small constant (c = 0.01) was added or subtracted from extreme germination metrics with values of (0% and 100%). Multiple binomial GLMs (2) were fitted for each germination category by modelling the proportion of seeds in each category relative to the total number of seeds tested, as follows:

$$\operatorname{logit}(p_{ijk}) = \log\left(\frac{p_{ijk}}{1-p_{ijk}}\right) = \beta_0 + \beta_1 \cdot \mathrm{Mod}_i + \beta_2 \cdot \mathrm{Var}_j + \beta_3 \cdot (\mathrm{Var}_j \times \mathrm{Lot}_k)$$

$$+\beta_4 \cdot (\mathrm{Mod}_i \times \mathrm{Var}_j) + \beta_5 \cdot (\mathrm{Mod}_i \times \mathrm{Var}_j \times \mathrm{Lot}_k) + \varepsilon_{ijk}\ , \qquad (2)$$

where p_{ijkl} is the probability of success (for instance, a seed lot germinating normally) for the observation corresponding to modality Mod_i, variety Var_j, and seed lot Lot_k; β_0 is the intercept; β_1 to β_5 are the regression coefficients associated with the main factors and their interactions; and ε_{ijk} is the error. The analysis was conducted using RStudio [50]. The mean difference of any germination category was also calculated based on the control (C) and exposed (X) germination data to highlight the practical impacts of our findings.

Results

This section presents our findings on the effect of X-ray exposure on several germination aspects. These results include the direct and indirect impacts of X-rays, followed by investigating the underlying reasons behind these observations.

Determination of baseline germination prior to X-ray exposure

The germination outcomes of the control groups of each species were analysed to establish individual baselines (Fig. 6). The results showed noteworthy variations in the three germination categories (normal germination, ungerminated seeds, and abnormality) in the seven species. Regarding normal germination (Fig. 6. A), the fennel control group recorded the lowest normal germination with the second highest variability (73.6 ± 9.3%) after the bean control group which recorded the highest variability of normal germination (83.8 ± 10%). Baseline normal germination was high (>90%) in radish, ryegrass, soybean, and maize, with maize demonstrating the highest normal germination and lowest variability (93.8 ± 2.9%). For ungerminated seeds (Fig. 6. B), Fennel exhibited the highest ungerminated seeds with the highest variability of (18.8 ± 8.5%), followed by carrot (9.2 ± 4.5%). On the contrary, soybean and maize recorded the lowest ungerminated seeds and the lowest variability (0.6 ± 0.6% and 1.7 ± 1.8%, respectively). For abnormality (Fig. 6. C), the bean control group showed the highest seedling abnormality with the highest variability (12.6 ± 5.5%). Fennel and soybean exhibited relatively elevated seedling abnormality (7.7 ±2.9% and 8 ± 5.1%, respectively) while ryegrass recorded the lowest abnormal germination with a notable low variability (1 ± 0.8 %).

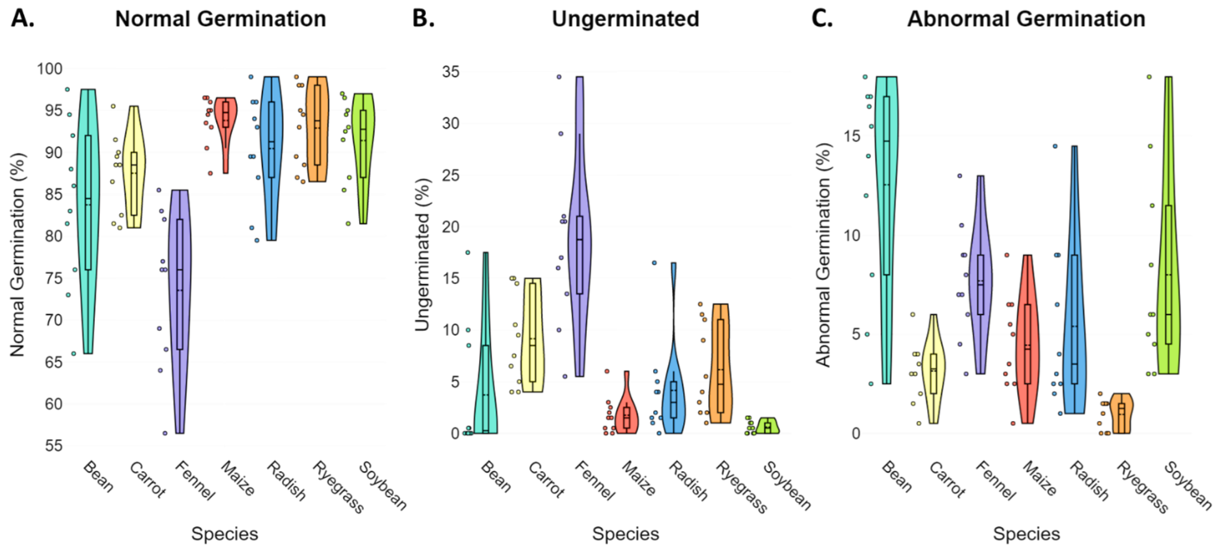

Figure 6: Germination results of control seed lots across the seven species.

Effect of X-ray exposure on germination

Table 6 provides the results of the statistical analysis on the overall effects of X-ray exposure ‘Modality’, different varieties ‘Variety’, seed lots ‘Lot’, and their interactions, on normal germination, ungerminated seed, and abnormal germination across the seven species.

*Table 6: Generalized linear model (GLM) testing the effect of main factors and their interactions on the three germination categories. * p < 0.05, ** p < 0.01, and *** p < 0.001.*

Species	Source	Df	Normal Germination			Ungerminated			Abnormality		
			LR Chisq	P-Value	Sig	LR Chisq	P-Value	Sig	LR Chisq	P-Value	Sig
Beans	Modality	1	1,5	0,22		14,1	0,00	***	0,4	0,50	
	Variety	4	38,4	0,00	***	106,8	0,00	***	2,6	0,62	
	Variety × Lot	5	58,4	0,00	***	31,8	0,00	***	34,4	0,00	***
	Modality × Variety	4	5,3	0,26		13,5	0,01	**	6,6	0,16	
	Modality × Variety × Lot	5	14,7	0,01	*	1,1	0,95		13,2	0,02	*
Carrot	Modality	1	0,0	0,87		0,0	0,84		0,2	0,63	
	Variety	4	21,9	0,00	***	23,0	0,00	***	14,0	0,01	**
	Variety × Lot	5	18,1	0,00	**	24,6	0,00	***	5,1	0,40	
	Modality × Variety	4	4,7	0,32		1,7	0,79		4,8	0,31	

	Modality × Variety × Lot	5	8,0	0,16		3,7	0,60		3,6	0,61	
Fennel	Modality	1	7,0	0,01	**	3,5	0,06		3,3	0,07	
	Variety	4	53,4	0,00	***	69,7	0,00	***	8,7	0,07	
	Variety × Lot	5	36,4	0,00	***	53,0	0,00	***	17,1	0,00	**
	Modality × Variety	4	5,0	0,29		6,7	0,15		8,8	0,07	
	Modality × Variety × Lot	5	3,4	0,63		3,6	0,62		7,8	0,17	
Maize	Modality	1	0,0	0,84		2,8	0,10		0,0	0,84	
	Variety	4	8,2	0,08		8,9	0,06		14,5	0,01	**
	Variety × Lot	5	12,1	0,03	*	14,1	0,02	*	11,6	0,04	*
	Modality × Variety	4	5,5	0,24		7,9	0,09		4,7	0,32	
	Modality × Variety × Lot	5	12,3	0,03	*	5,4	0,37		14,0	0,02	*
Radish	Modality	1	1,4	0,24		0,0	1,00		2,1	0,14	
	Variety	4	51,6	0,00	***	52,2	0,00	***	33,7	0,00	***
	Variety × Lot	5	30,4	0,00	***	19,5	0,00	**	14,7	0,01	*
	Modality × Variety	4	32,6	0,00	***	10,3	0,04	*	25,6	0,00	***
	Modality × Variety × Lot	5	17,2	0,00	**	8,6	0,13		16,3	0,01	**
Ryegrass	Modality	1	0,0	0,83		0,5	0,48		1,1	0,30	
	Variety	4	42,2	0,00	***	38,3	0,00	***	2,6	0,62	
	Variety × Lot	5	3,9	0,56		2,1	0,83		3,2	0,67	
	Modality × Variety	4	0,8	0,94		0,8	0,94		2,4	0,66	
	Modality × Variety × Lot	5	6,3	0,28		4,9	0,42		1,4	0,92	
Soybean	Modality	1	2,3	0,13		0,2	0,65		2,9	0,09	
	Variety	4	26,7	0,00	***	7,2	0,13		27,7	0,00	***
	Variety × Lot	5	7,9	0,16		6,6	0,25		5,5	0,35	
	Modality × Variety	4	2,6	0,63		8,6	0,07		2,0	0,73	
	Modality × Variety × Lot	5	5,7	0,34		6,4	0,27		4,5	0,49	

Genetic and lot-specific factors

The results (Table 6) revealed strong impacts of both ‘Variety’ and ‘Lot’ on the germination outcomes of the tested species regardless of being exposed to X-rays. For normal germination, a significant effect of Variety on normal germination was observed in the majority of species ($p < 0.001$), except for Maize where it was marginal ($p = 0.08$). In bean, carrot, fennel, maize, and radish Variety × Lot interaction had a significant ($p < 0.05$) impact on normal germination. Regarding ungerminated seeds, it was observed that the effect of Variety on ungerminated seeds was significant ($p < 0.001$) in all species except for maize, and soybean. Furthermore, the interaction Variety × Lot was significant ($p < 0.05$) in beans, carrots, fennel, maize and radish. While in abnormal germination, the impact of Variety on abnormal germination was highly significant ($p < 0.001$) in carrot, maize, radish, and soybean. Furthermore, the interaction Variety × Lot was significant ($p < 0.05$) in beans, fennel, maize, and radish. This analysis revealed that Variety and Lot highly affected the three categories of germination of the tested species. Thus, 'Variety' and 'Lot' received careful attention while investigating the impact of X-ray exposure on germination outcomes.

X-ray impact on normal germination

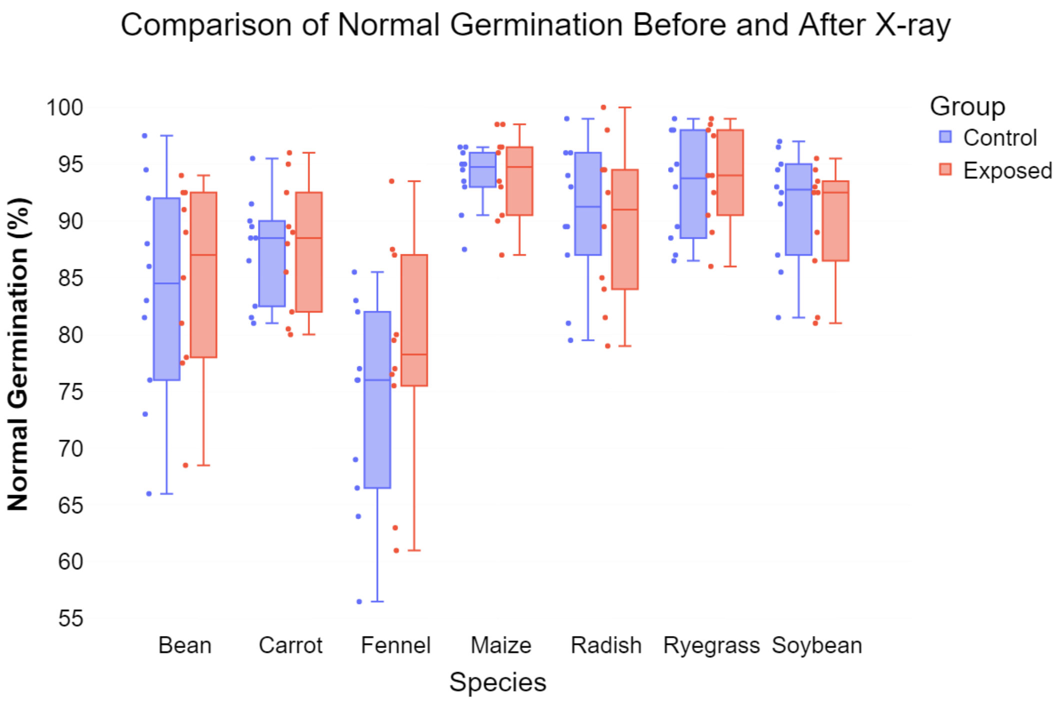

Figure 7: Comparison of normal germination (G) before and after the application of soft X-ray. Data points represent the tested seed lots.

In comparison to the baseline, positive changes were observed in normal germination after the exposure (Fig. 7) in bean (+1.2 ± 4.7 %), carrot (+0.3 ± 4.8 %), maize (+0.2 ± 4%), and ryegrass

(+1 ± 2.2 %). In addition, negative changes were also observed in radish (-0.6 ± 6.3 %) and soybeans (-1.5 ± 3.3%). However, these changes were a trend and did not stem from the independent effect of X-ray exposure on normal germination (Table 6) of these six species and were not significant ($p > 0.05$). An exception was observed for fennel, which experienced significant ($p < 0.01$) positive changes after the exposure by a notable increase of normal seedlings (+4.5 ± 7.4%). Furthermore, Variety and Lot effects were important where the X-ray had a significant interaction with Lot (Modality × Variety × Lot) in bean, and maize ($p < 0.05$), and the interaction was more pronounced in radish ($p < 0.01$). By comparing the exposed and the control groups of all species, the results of the statistical analysis revealed that X-ray exposure (Modality) did not directly/independently alter the development of normal seedlings of the tested species except for fennel at the used doses. However, X-ray exposure (Modality) had clear interactions with 'Variety' and 'Lot'.

X-ray impact on ungerminated seeds

A non-significant decreasing trend was observed in the ungerminated seeds of six species after X-ray exposure compared to the baseline (Fig. 8): bean (-1.1 ± 3%), carrot (-0.5 ± 3.1%), fennel (-4.6 ± 6.3%), maize (-0.9 ± 1.9%), radish (-0.7 ± 2.7%), and ryegrass (-1.3 ± 2.4%) (Figure 8). As observed in (Table 6), a near-significant ($p=0.06$) direct effect of X-ray on fennel, where the average of ungerminated seeds declined from 18.8 % to 14.2 %. Unlike the rest of the species, the negative impact observed in soybean was not significant ($p > 0.05$), where ungerminated seeds increased by (+0.25 ± 1.1%). However, there was a near-significant ($p = 0.07$) interaction between X-ray and Variety (Modality × Variety) in soybean. In addition, this interaction (Modality × Variety) was significant in beans ($p < 0.01$) and radish ($p < 0.05$). The overall decline in ungerminated seeds observed across the species after the exposure suggested a positive influence of soft X-rays, while the variability in response suggested the influence of varietal properties.

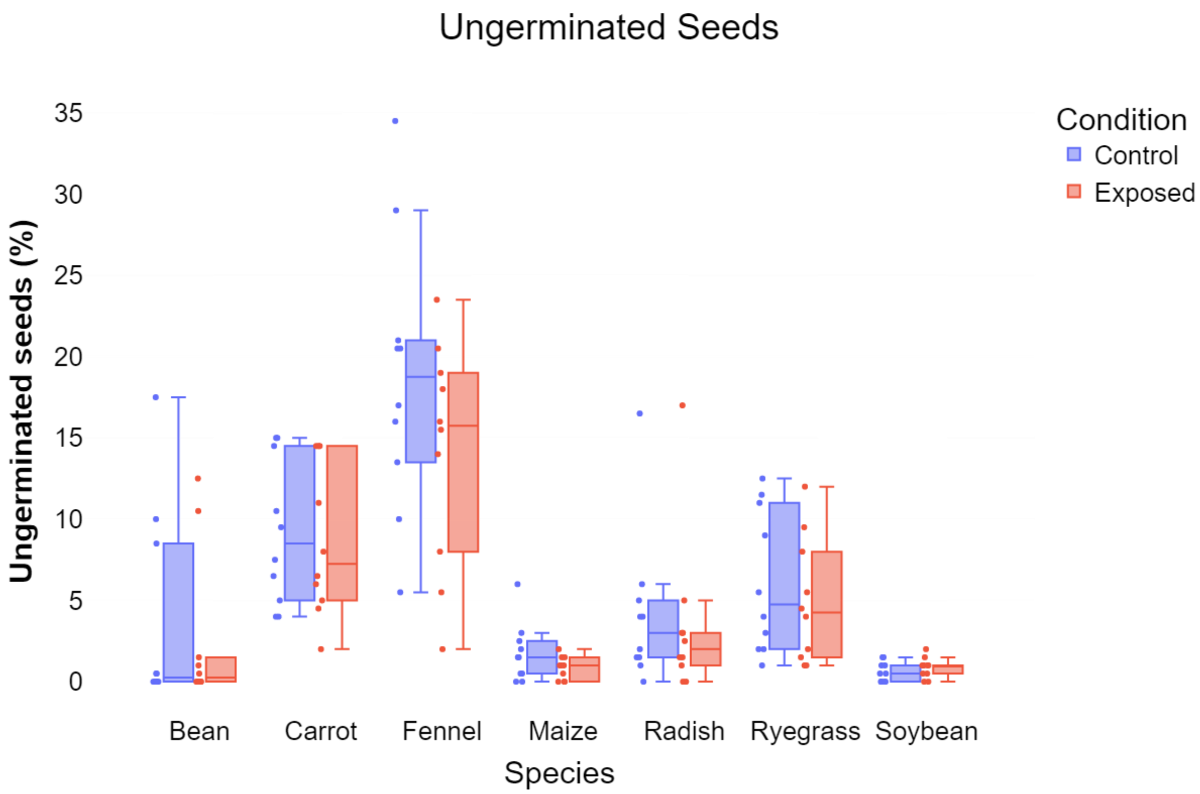

Figure 8: Comparison of ungerminated seeds (NG) before and after the application of soft X-ray. Data points represent the tested seed lots.

X-ray impact on abnormality

By comparing the abnormal germination after the X-ray exposure to the abnormality baseline, variable differences were observed across the seven species (Fig. 9). Bean (-0.1 ± 5%), carrot (+0.4 ± 3%), fennel (+0.1 ± 5.3%), maize (+0.7 ± 3.8%), and ryegrass (+0.3 ± 1.4%) recorded slight changes in abnormal germination, while radish (+1.3 ± 4.7%) and soybean (+1.2 ± 3.4%) exhibited elevated changes. However, the results (Table 6) exhibited that there was no significant direct/independent effect of X-ray exposure on the development of abnormal seedlings in any species. Moreover, the interaction (Modality × Variety) was highly significant in radish ($p < 0.001$). Furthermore, the interaction (Modality × Variety × Lot) was significant ($p < 0.05$) in beans and maize, and stronger in radish ($p < 0.01$), highlighting the influence of Variety and Lot.

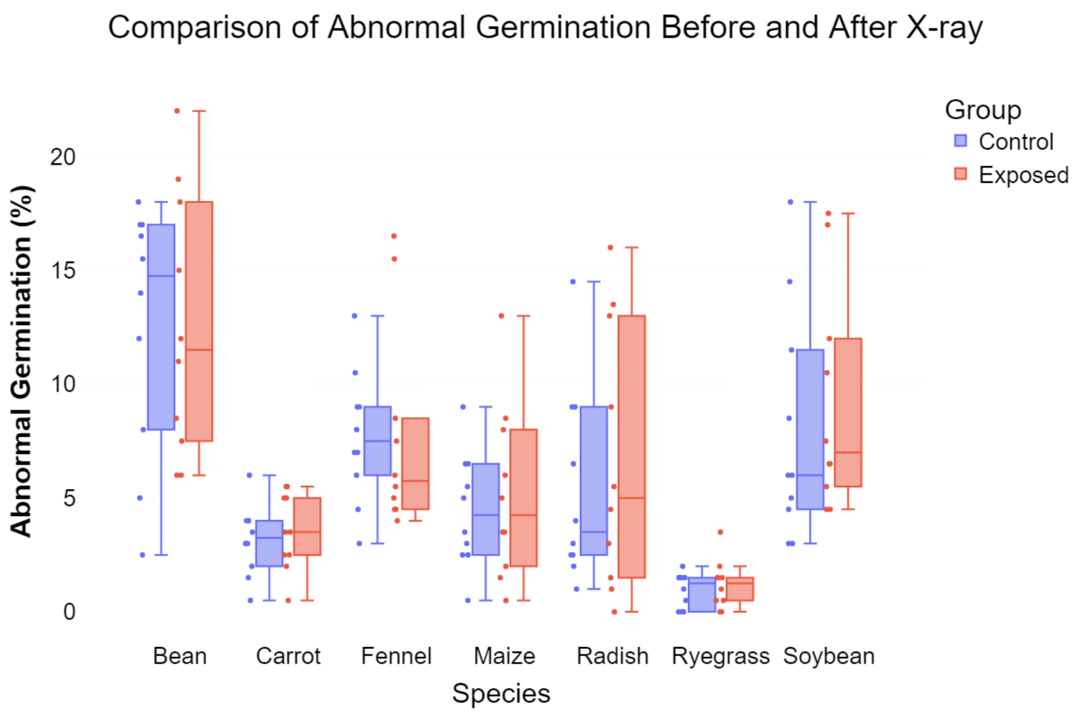

Figure 9: Comparison of Abnormal germination (ANRX) before and after the application of soft X-ray. Data points represent the tested seed lots.

Understanding the X-ray effects through physical quality analysis

Species-specific physical quality analysis was performed to understand the observed effects of X-ray exposure. This analysis focused on the physical damage or injury due to the mechanical, environmental or biological factors that may impact seed viability and germination. Among the 7 analysed species, soybean and fennel exhibited the highest level of physical damage (Fig. 10) with 12.3 ± 6% and 8.7 ± 5.2% of the seeds where most of the physical damage that the fennel and soybean seeds suffered was insect damage on both endosperm and embryo of the seeds. Moreover, high variability was detected in the physical conditions of the lots of the beans, carrots, fennel, maize, and soybeans. In contrast, radish and ryegrass exhibited the lowest physical damage.

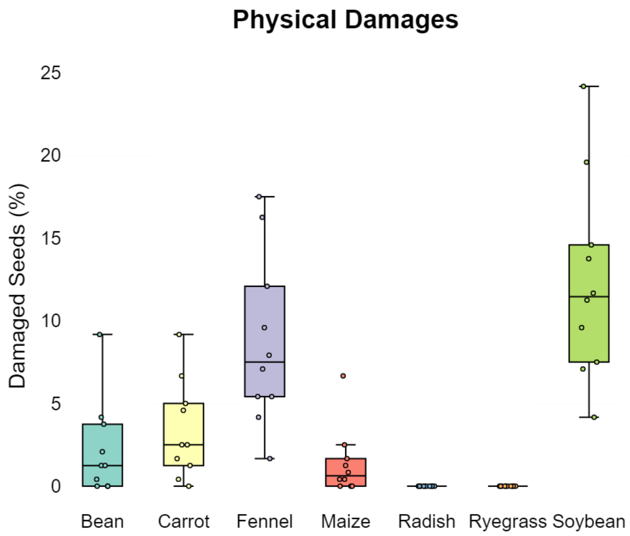

Figure 10: Physical analysis results of the seven species showing the percentage of damaged seeds.

Of all species analysed, fennel showed the strongest and only significant reaction to X-ray exposure. Thus, further analysis was carried out on fennel to better understand this effect (Fig. 11). The change in fennel normal germination Δ G exhibited a significant strong negative correlation (-0.78; $p < 0.01$) with the change in ungerminated seeds Δ NG. The tested fennel lots contained bigerm seeds which is a natural developmental defect characterised by the presence of two embryos within the seed leading to twin or deformed seedlings. Bigerm seed count showed a significant strong positive correlation (0.74; $p < 0.05$) with the standard deviation of ungerminated seeds of the control group. In addition, it exhibited a significant moderate negative correlation with the change in ungerminated seeds Δ NG (-0.66; $p < 0.05$) and a significant moderate positive correlation (0.67; $p < 0.05$) with the change in abnormality Δ ANR. As expected, bigerm had a significant strong positive correlation with morphometric traits e.g., Volume (0.74; $p < 0.05$). The mean pixel intensity (Mean) had a significantly strong negative correlation with fennel morphometric traits Length, Area, and Volume (-0.84; $p < 0.01$). Moreover, the mean pixel intensity (Mean) had a significantly strong negative correlation with Damages% (-0.7; $p < 0.05$) and Empty seeds count (-0.74; $p < 0.05$), suggesting a useful use of pixel intensity to measure fennel seed quality. The natural defects (e.g., bigerm) were a determinant of the variability in germination and the seed's response to the exposure (normal or abnormal seedling).

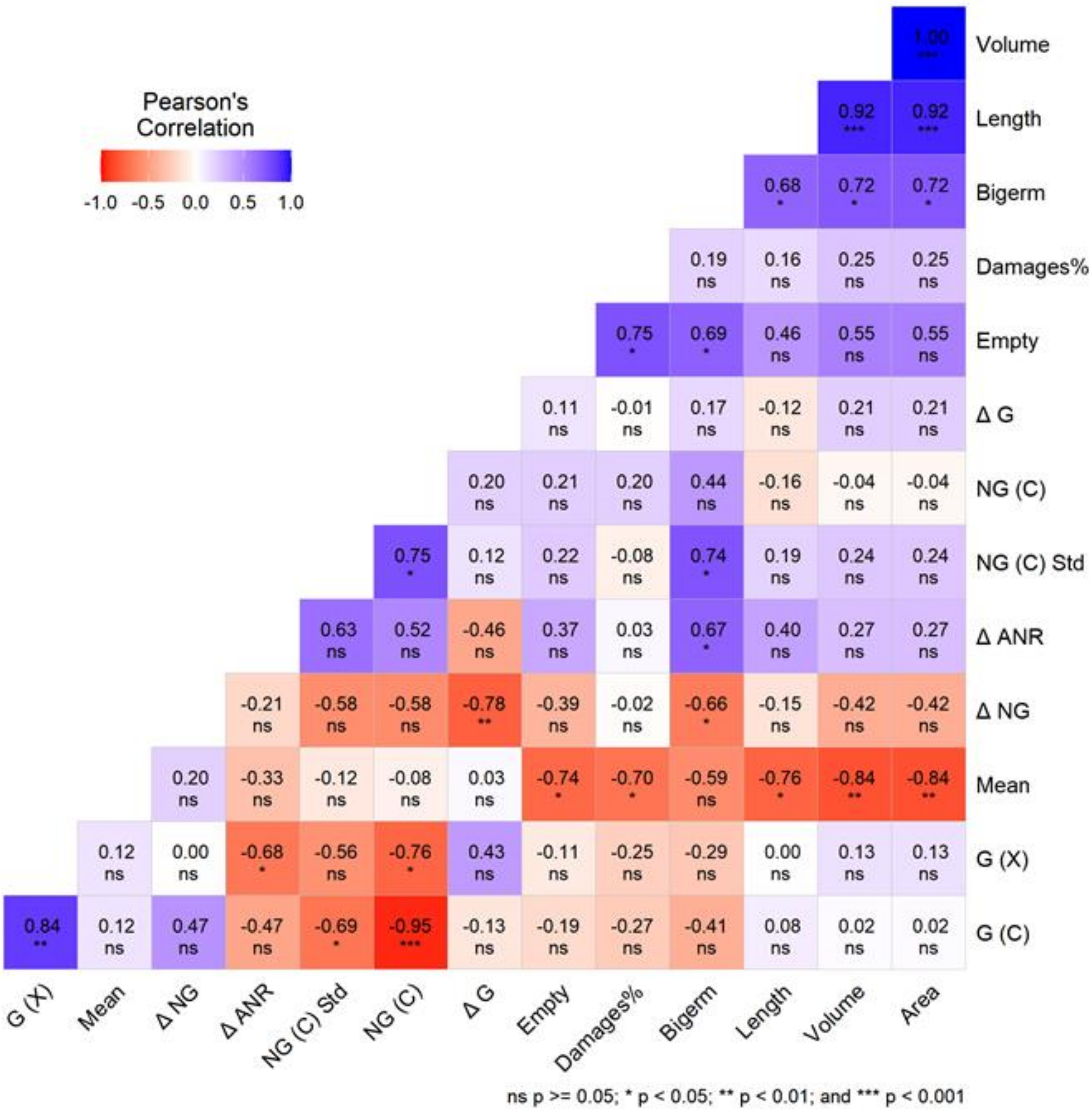

Figure 11: Pairwise correlation matrix between fennel germination, morphology, and seed quality parameters.

To address the negative effect of X-ray exposure observed on soybeans and the high damage level found in soybeans during the physical analysis, an in-depth analysis was carried out on this species (Fig. 12). The morphometric traits of soybeans exhibited multiple noteworthy correlations between one another. The change in ungerminated soybean seeds before and after the exposure (Δ NG) exhibited a significant strong positive correlation with seed morphometric traits Volume, Length, Area, and Perimeter: for instance, Seed Volume (0.78; $p < 0.01$). Moreover, significant strong positive correlation between Defects% in soybean seed lots and morphometric traits e.g., Length (0.73; $p < 0.05$). On a different note, embryo damages had a significant moderate negative correlation with normal germination of control group NG (C) (-0.69; $p < 0.05$) and the correlation was stronger with normal germination of exposed group NG (X) (-0.74; $p < 0.05$).

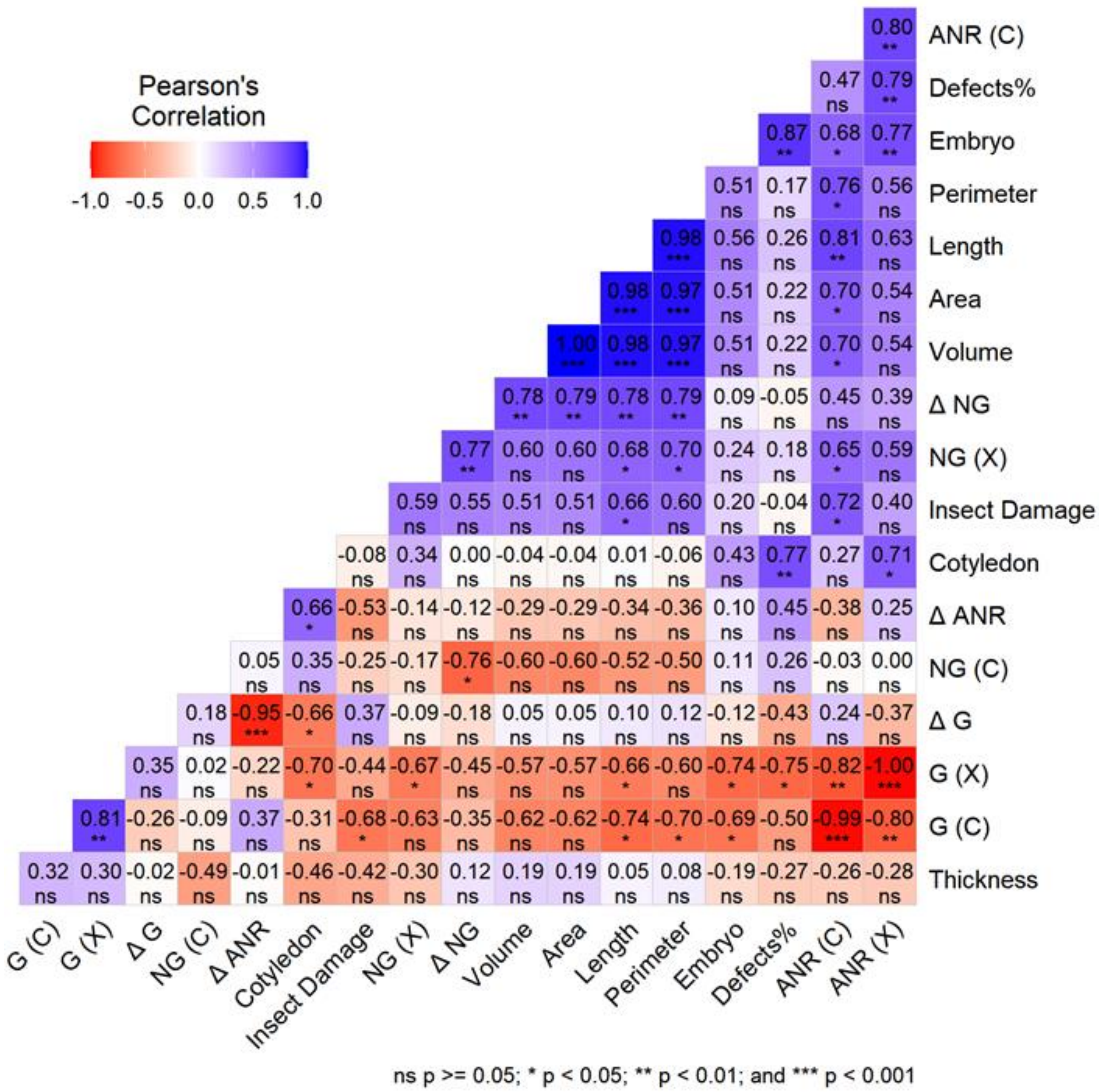

Figure 12: Pairwise correlation matrix between soybean germination, morphology, and seed quality parameters.

In addition, insect damages had a significant moderate negative correlation with normal germination G (C) (-0.68; $p < 0.05$) and a significant strong positive correlation with abnormal germination ANR (C) (0.72; $p < 0.05$) of the control group. Furthermore, cotyledon damages had a significant strong negative correlation (-0.70; $p < 0.05$) with normal germination ANR (X) and a significant strong positive correlation (0.71; $p < 0.05$) with abnormal germination ANR (X) after exposure. Overall, seed physical quality was found to play a crucial role in understanding the observed changes and contradictions, and may be considered an underlying factor in explaining and potentially predicting the effects of X-ray on germination.

Influence of density on germination

To investigate the effect of physical properties on germination outcomes, the estimated density of the seven species was analysed. The seven species exhibited unequal densities where the average density of maize (1.4 g/cm³) and bean (1.31 g/cm³) were the highest among the seven species, followed by soybean (1.2 g/cm³), ryegrass (1.12 g/cm³), radish (1.07 g/cm³), and carrot (0.94 g/cm³), respectively. Meanwhile, fennel recorded the lowest average density (0.7 g/cm³). Further analysis of the control groups of these species revealed that the relationship between the average density of the species and the percentage of ungerminated control seeds NG (C) or germinated control seeds NG+ANR (C) regardless of the normality of the seedling (Fig. 13. C and Fig. 13. D) was linear ($p < 0.01$; R^2 =0.82) and ($p < 0.01$; R^2=0.82), respectively. In contrast, the average density had weaker relationships with both normal germination G (C) and abnormal germination ANR (C) (R^2=0.5 and R^2=0.02, respectively) of the control groups.

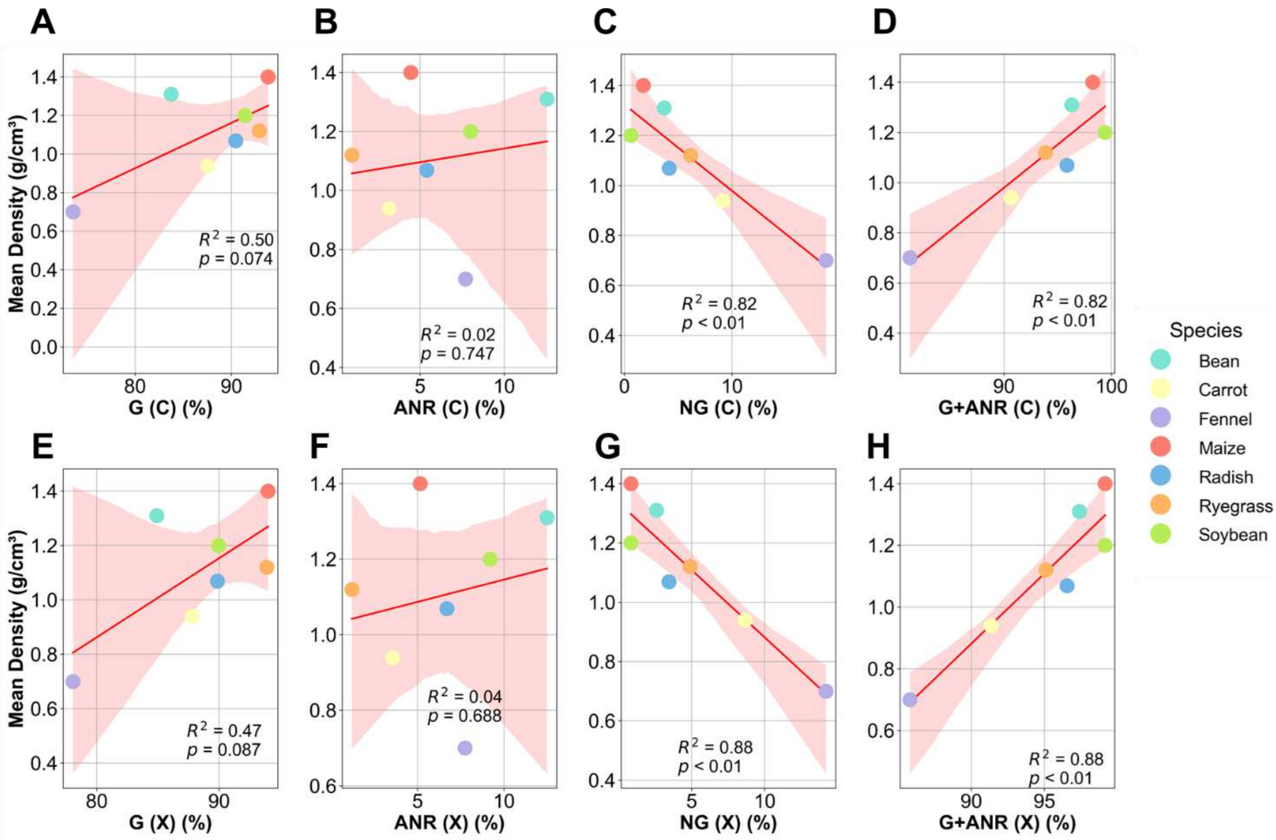

Figure 13: The relationship between the average density and the average germination outcomes of the control (first row) and exposed (second row) groups of the seven species. Germination outcomes are labelled as follows: C= control, X= exposed, G= normal germination, ANR= abnormal germination, NG= ungerminated, G+ANR= germinated seeds regardless of their normality. A linear regression was shown with the 95% confidence interval of the fit.

Following the soft X-ray exposure, the linear relationships between species average density and ungerminated seeds NG (X) or normal/abnormal germination G+ANR (X) (Fig. 13. G and Fig. 13. H) were further strengthened (p < 0.01; R^2=0.88). On the other hand, species density remained uncorrelated with abnormal germination ANR (X) and normal germination G (X) of exposed groups. Overall, species average density emerges as a significant explanatory factor in the germination variability observed between the seven species. In particular, it highly contributes to the species' probability of succeeding or failing to produce a seedling under standard germination conditions.

4. Discussion

This study examined the impact of soft X-ray exposure on germination using control and exposed groups of 70 different seed lots from 35 unique varieties representing 7 species across 4 botanical families while following a standard germination procedure (ISTA) for accurate, standardized, and reproducible comparison. Earlier studies varied considerably in methodological aspects while evaluating the effect of X-ray on germination, including a limited number of tested seeds, a single species tested, different germination procedures on the same species, absence of control groups to quantify the effect, unexamined physical quality, and partially reported imaging parameters with unknown doses in standard units [32,33,38,40–45,51,52]. These limitations complicated the direct comparisons and highly contributed to the contradictory interpretations of the effect of X-rays on germination. In our work, this gap was addressed by a systematic assessment of the exposure's impact and its interaction with varietal and lot-specific factors (e.g., seed physical quality) across a wide diversity of plant material to minimise variability, limit other additional effects that may be produced due to protocol differences, and to clarify the contradictory and species-specific effects of X-rays, which have not been addressed before to the best of our knowledge.

Soft X-ray is non-destructive with potential benefit

Our results exhibited that soft X-ray exposure (< 3 mGy) is non-destructive and rather has a beneficial impact. First, no statistically significant reduction in normal germination was detected in any of the seven species after the soft X-ray exposure. Second, soft X-ray exposure did not directly induce any seedling abnormality. Finally, no significant increase was observed in ungerminated seeds in any of the seven species. Particularly, soft X-rays had a positive effect on overall germination observed through a declining trend in ungerminated seeds in six species (bean, fennel, carrot, maize, radish, and ryegrass), suggesting that X-rays stimulated the initial germination of seeds that might not otherwise germinate at the standard germination timeframe described by ISTA. This stimulative pattern resulted in a transition from ungerminated seeds towards an increase in seedlings regardless of their normality, as observed in the significant increase in normal germination of Fennel (+4.6%). Except for fennel, these observed improvements were minor, thus, considerable attention must be paid not to misjudgment or overestimate the germination quality of the exposed seeds relative to the regulatory standards. Our findings agreed with previously published studies on the positive impact of X-rays on germination [39,51] and pointed to a potential Hormesis effect. In contrast, soybean broke the detected positive pattern when an increase in abnormal seedlings and ungerminated was observed. This observation partially agreed with previous studies that reported a decrease in the germination of the exposed palm seeds [43] and peanuts [44,45]. However, these studies failed to report if any other effect (e.g., seed quality or genetics) interacted with the exposure. Thus, further analysis was required to clarify the underlying reasons for these observations.

Revealing contradictory responses to X-ray exposure using physical quality

Inter-lot variability results demonstrated that seed lots hold a considerable value in determining germination outcomes. This is consistent with [53] who exhibited that germination rates among different corn lots varied between 83% and 98%, and lot-specific aspects led to yield losses of up to 25%. The physical quality of the seed sheds light on the so-called negative effect of soft X-rays. As put forward by previous studies on seed quality, seed physical damage impaired germination and seedling development. For instance, [54] exhibited that mechanical damage reduced seedling length by 24% and biomass by 65% in maize. Particularly, insect damage is a rising issue that compromised the germination of many species such as soybeans [25], and maize [51], and reduced the seedling growth of chestnut by 50% [55]. Our study confirmed these findings through the significant interactions of X-ray exposure and seed lot, and furthermore, it proved that physical damage such as insect infestation made the seeds more vulnerable and sensitive to additional stress, even if it was mild (e.g., soft X-rays), which led to germination failure, or abnormal seedlings at best, as observed in soybean. This highlights that the so-called negative influence of soft X-ray is only a co-factor, and it only amplified the already existing physical damage which supports [25]. Thus, it's highly recommended to evaluate the effect of X-ray exposure in conjunction with the quality of the tested seeds as this interaction plays a decisive role in determining whether a seed responds positively or negatively to the X-ray exposure at a specified dose.

Revealing contradictory responses to X-ray exposure using genetics

Beyond physical quality influences, the high inter-varietal variability in germination within the same species proved that the genetic factors determine the germination outcomes. This is consistent with previous studies that reported a noteworthy variability in germination of the 14 *Cannabis sativa L.* varieties ranging from 17 to 70% was reported in [56]. In addition, [57] reported a significant difference in germination between two varieties of *Glycine max L.* of 10% at 25°. Similarly, genetics play a crucial role in determining the response to the X-ray exposure as observed in the significant interactions of soft X-ray with the varieties within the same species. This indicated the crucial importance of studying a variety's sensitivity to X-rays while performing variety testing.

Does size really matter?

Morphometric traits are extracted from seeds and widely used in phenotyping to predict the potential germination outcomes [58]. [59] showed that medium to large Soybean seeds have better growth effects, and resistance levels, and can better adapt to the environment. [60] reported that large Wheat seeds had higher seed germination (91.9%) compared to small seeds

(82.8%). [61] indicated that Soybean seeds with small diameters had shorter seedlings. In our study, seed traits such as seed volume and thickness were recovered from 2D images using linear regression and Beer's law to investigate if seed volume or thickness plays any significant role in the seed's response to soft X-ray exposure. Our results diverged from theirs in that superior soybean seed volume was linked to lower normal germination. However, considering morphometric traits only can be misleading, as it was observed that seed lots with superior volume had a degraded physical quality, particularly, insect damage. Thus, morphometric traits such as seed size should not be considered in isolation but rather in combination with seed physical quality for more robust interpretation.

Species density as an explanatory factor of its germination outcomes

Our results point to the likelihood that the direct significant impacts of X-rays were observed in Fennel only due to its low baseline, which was found highly correlated with its low average density. The rest of the species with notably elevated densities had a higher germinative performance close to their maximum potential, where improvements were less detectable. Previous works explored the effect of seed density on germination intra-species. For instance, *Gossypium hirsutum L.* seeds with relatively high density (1.04 to 1.06 g/cm^3) had the highest germination (> 96%) and vigour due to compositional parameters oil and protein [62]. While high-density *Triticum aestivum* seeds produced more vigorous seedlings than those of low-density [63], linking the high density to carbohydrates and proteins (gliadin and glutenin). Our findings provided strong initial evidence on the importance of density in understanding the natural inter-species variability in germination, rather than focusing only on seed size or mass. In correlation with these previous results, our study adopted a broad perspective by investigating the relationship between a species' average density and its germination outcomes. Species with elevated average density (> 1 g/cm^3) had a higher probability (> 90%) of succeeding in producing a seedling regardless of its normality. In addition, species average density also holds an additional value such as the prediction of the species response to X-ray dose. However, to ensure broader significance, further investigations should be conducted across more species, botanical families, genotypes, ageing, storage conditions, and maturity.

Standard and safe X-ray protocol for seed quality analysis

Unlike previous studies with limited details about the imaging acquisition protocol, our imaging protocol is provided (Table 2). In addition, our method was explicitly designed to support reproducibility in terms of imaging parameters, the expected dose, genetic, and seed quality aspects, with no influence on germination for physically and physiologically non-destructive quality analysis. Our approach proposes this work as a baseline that future research can adopt or build upon.

Limitations and future directions

Radiography is a rapid and powerful technique for seed quality analysis with relatively limited capacity compared to tomography machines equipped with significantly higher capacities that produce extremely larger X-ray doses. Higher doses may probably have a greater impact on germination [44]. Our study was carried out using radiography only with soft X-ray exposure. Therefore, the use of tomography at higher doses for a similar study could potentially reveal additional impacts of X-ray on germination. A single time interval (16-20 hours) between X-ray exposure and sowing was tested in our study. It is recommended to test shorter and longer time intervals to assess the influence of this criterion on the impact of X-rays on germination. In our study, ISTA rules were followed to evaluate the germination capacity at 7 to 14 days depending on the species [46]. Due to data constraints, our study focused on the short-term effects of X-ray exposure but did not explore long-term impacts on post-germination (e.g., seedling development, plant growth, growth rate of organs, length of aerial part, root system) of these seven species, which remains an open area for future contributions. Shull *et al.* showed the potential impact of X-ray exposure of seeds on plant growth at low-dose X-ray exposure [64]. This was subsequently confirmed by similar results on cotton [65] and wheat [66]. Recently, X-rays were found to significantly accelerate bean plant development [67]. However, the growth and characteristics of seedlings of irradiated tomato seeds were not significantly impacted [68]. Thus, further investigations are required to understand the molecular mechanisms behind our and previous observations. This mild stress may induce specific changes at physiological and molecular levels, for instance, influencing transgenerational memory. Acquired stress can last over the long term, at later stages of development, and even in subsequent generations [69]. In addition, the impact of long-term storage of seeds after exposure to X-rays was not studied, as this could have another effect on water content, the germination potential of seeds, and the development of seedlings. In other matters, Rousseau *et al.* [70] demonstrated the possibility of optimising the acquisition time of Raman micro-imaging. A similar concept could be applied in the future to assist seed quality analysts in adapting their X-ray imaging parameters. This could be coupled with a dose recommendation system based on artificial intelligence.

Conclusion

Our work evaluated the impact of soft X-ray exposure on seed germination across a wide range of plant materials while applying standard germination protocols and analysing the influence of genetics and seed quality on the response to the X-rays. This approach clarified the contradictory effects of X-ray exposure reported in the literature and established a reproducible X-ray imaging protocol for non-destructive seed quality assessment.

List of abbreviations

2D: Two-dimensional space

3D: Three-dimensional space

ANR: Abnormal germination/seedling

C: Control group

G: Normal germination/ seedling

GEVES: the French Variety and Seed Study and Control Group

NG: Ungerminated seeds

X: Exposed group

Δ: Difference between control and exposed group

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Declarations

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Competing interest

The authors declare that they have no competing interests.

Authors' contributions

LST, AD, SD, AC, SH and DR conceived the study and contributed to the methodological design. LST and AD carried out X-ray and germination tests. LST and AD acquired and managed the data. SH developed image analysis algorithms and machine learning models. SH, LST, and AD analysed and interpreted the data. SH, LST, and AD wrote the manuscript, with revisions from AC, SD, and DR. AC, SD, PR, and DR supervised the study. All authors read and approved the final manuscript.

Consent for publication

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Ethics approval and consent to participate

Not applicable