



## ***In vivo* Metabolic Investigation of Oxygen, Light, and Temperature Effects on Dormancy Alleviation of Sesame (*Sesamum indicum* L.) Seeds**

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### ABSTRACT

As an edible seed, sesame seeds require careful storage to maintain their quality. Dormancy helps seeds extend their lifespan by slowing down metabolic processes, reducing energy consumption and natural aging. However, seeds may exit dormancy and begin germination during storage due to variations in temperature, light, and oxygen conditions. This transition is not easily visible, but nutritional components within the seeds can start to deplete. In this study, non-invasive magnetic resonance spectroscopy and imaging were used to monitor sesame seeds stored under different temperature, light, and oxygen conditions for over 120 hours. Results showed that seeds remained dormant at 15 °C under oxygen deprivation and in the absence of light. When exposed to continuous light at 15 °C, under anaerobic or aerobic conditions, changes in metabolic resonances were observed through spectroscopy, indicating

moisture and fatty acid transfer between seed structures. Despite these changes, magnetic resonance imaging showed that the embryo did not develop. At 24 °C with continuous light and aerobic conditions, both spectroscopy and imaging analyses revealed significant metabolic changes, and all internal seed structures developed normally, with visible signs of germination. This study highlights that although sesame seeds are non-photoblastic, light can still trigger metabolic activity within the seeds, while suitable temperature is essential for complete seed development. These findings provide valuable insights into the dynamic molecular-level metabolic changes from dormancy to early seed germination using magnetic resonance technology and offer guidance for maintaining seed dormancy during storage.

Keywords: NMR, MRI, <sup>1</sup>H NMR spectroscopy, Chemical Shift Selective Imaging, Germination, Storage

## 1. Introduction

Seed storage conditions, including temperature, oxygen availability, and light exposure, significantly impact their viability, germination rate, and overall quality (Gebeyehu 2020). Low temperatures help to slow down seed metabolism and reduce moisture loss, which aids in maintaining seed viability. However, temperatures that are either too low or too high may have detrimental effects on the seeds (Simon et al. 1976; Egli & Wardlaw 1980; Geneve 2003). Oxygen is a critical gas for seed metabolism, promoting water absorption and thereby promoting seed germination (Gay et al. 1991; Corbineau 2022). Certain seeds are light-sensitive, and exposure to light can trigger photosynthesis and produce excessive free radicals which may compromise seed quality (An et al. 2020; Cheng et al. 2023). As a widely consumed edible seed, sesame seeds require meticulous attention to their storage conditions to ensure their consumable quality and safety. Currently, the primary technical methods for investigating the effects of storage and cultivation conditions on seeds include: the measurement of phenotypic date (Cao et al. 2020; Chen et al. 2023; Elboghdady et al. 2023; Pasternak et al. 2024), gas chromatography mass spectrometry (Taylor et al. 1999; Yan et al. 2018), liquid chromatography-mass spectrometry (Chiwocha et al. 2003), high-performance liquid chromatography mass spectrometry (Jander et al. 2004; Kołodziejczyk et al. 2015; Wu et al. 2017), infrared spectroscopy and imaging (Tigabu et al. 2004; Song et al. 2015; Zhang et al. 2020). However, during the transition from dormancy to the early stage of germination, seeds do not exhibit observable morphological changes. As a result, phenotypic data collection is more applicable to the mid-to-late stage of germination, where visible changes occur. During germination, even in the absence of external changes, various metabolites may still be produced within the seeds. Therefore, understanding the physiological changes, as well as the composition and distribution of metabolites, in seeds during the early stage of germination is crucial. Gas chromatography mass spectrometry (Mota et al. 2021), liquid chromatography-mass spectrometry (Li et al. 2020) can obtain detailed, high-resolution metabolic profiles. However, these methods are destructive, and cannot be used for dynamic monitoring of seeds. Infrared spectroscopy and imaging, while enabling non-destructive detection of seeds (Amanah et al. 2020; Ma et al.

2020; Xiao et al. 2024), suffer from relatively low resolution and decreased sensitivity when applied to seeds with low moisture content. In addition, a variety of data preprocessing steps is required before analyzing infrared spectral data, such as the baseline correction, smoothing, and standard normal variate.

Nuclear magnetic resonance (NMR) is a suitable non-invasive technique for studying plants. Time-domain nuclear magnetic resonance relaxometry offers a reliable approach for assessing both oil seed temperature within soils and soil thermal diffusivity (Carosio et al. 2018).  $^{31}\text{P}$  NMR spectroscopy helps to enhance the comprehension of herbicide-induced metabolic changes detected in live plants (d'Avignon et al. 2018). Quantitative and high-resolution fingerprints of marine microalgae have been established using  $^1\text{H}$  High-resolution magic angle spinning (MAS) NMR (Caprara et al. 2023). The field of magnetic resonance imaging (MRI) is also rapidly expanding and offers significant advantages in the *in vivo* plant sciences (Blystone et al. 2024). Magnetic resonance imaging technology can measure the water content of leaves and study its variation throughout the day, such as detecting changes in the cellular and tissue water status and distribution in *Brassica napus* leaves during blade development and dehydration processes (Boulc'h et al. 2024). It also plays a beneficial role in detecting plant diseases. For instance, it facilitates the early detection of cotton *Verticillium wilt* (Tang et al. 2023), as well as the characterization of changes induced by *Phytophthora cactorum* infection in strawberry seedlings (Tuomainen et al. 2024).

Presently, research using NMR and MRI technologies to observe metabolic changes in seeds during the germination process remains limited in scope (Sarkar et al. 2009; Cai et al. 2018a; Xiao et al. 2024; Liao et al. 2024). Sesame, as an oil seed, primarily derives its economic and nutritional value from its rich oil content, which includes a range of fatty acids beneficial to human health, including linoleic acid, oleic acid, stearic acid, and palmitic acid (Dossa et al. 2017). However, during the germination process, sesame seeds use their stored oils as a source of nutrients and energy required for growth (Xiao et al. 2024). Therefore, the effective management of seed dormancy and prevention of the transition to the germination stage is of significant importance. As far as we know, there is limited research (Cai et al. 2018a) on the metabolism of seeds from dormancy to the early germination stages under different storage conditions.

One-dimensional  $^1\text{H}$  NMR spectroscopy (Bharti & Roy 2012) is among the most prevalent techniques in NMR. It requires only a single pulse sequence to acquire spectra, with relatively fewer parameter adjustments. In contrast to certain other NMR methodologies like MAS NMR (Cai et al. 2016a; Cai et al. 2018a) and intermolecular multi-quantum coherence technique (Cai et al. 2016b; Cai et al. 2018b),  $^1\text{H}$  NMR does not necessitate the use of specialized rotors, complex sequences and parameter configurations. Chemical Shift Selective (CHESS) imaging is a specific MRI technique (Haase et al. 1985). It selectively detects specific metabolites through the chemical shift effect, enabling accurate quantification of their concentration and distribution within complex biological tissues (Fujii et al. 2022). Given the distinctive features and advantages of these two techniques, this study proposed to dynamically monitor the biophysical properties of sesame seeds using one-dimensional  $^1\text{H}$  NMR spectroscopy and CHESS. These approaches would elucidate how temperature, light, and oxygen influence metabolite variations and distributions. By investigating the impact of these factors on sesame seed metabolites, the aim is to identify key factors that induce sesame seeds to transition from dormancy to early germination, providing scientific insights for sesame seed storage.

## 2. Material and Methods

### 2.1. Sample preparation

Black sesame seeds (*Sesamum indicum* var. *radiatum*) were purchased from a local seed retailer one week after their harvest. A germination test of 50 seeds was performed to determine their viability, and the germination rate reached 96%, meeting the requirement for research purposes. For preparing acidic liquid culture medium (pH=5.4), the  $\text{HNO}_3$  was purchased from Sigma-Aldrich Co (St. Louis, MO, USA). The procedure for preparing the medium is as follows: Add deionized water to a diluted 10 ml 1%  $\text{HNO}_3$  solution and use a pH meter to monitor the pH until the target pH is reached. After preparation, sterilize the medium using an autoclave. Once cooled, store the medium in sterile containers to avoid contamination. The reason for preparing an acidic liquid culture medium was that this study investigated the impact of temperature, light, and oxygen on sesame seeds from dormancy to early germination. To enable rapid germination under controlled conditions, sesame seeds were cultivated in an acidic liquid culture medium. Oxygen conditions were varied by the extent to which the seeds were submerged in the acidic liquid. Complete submersion simulates hypoxic conditions, while partial coverage mimics aerobic conditions. Light for the sesame seeds was provided by a 50-watt full-spectrum LED lamp (Oppl Lighting Co., China).

### 2.2. Experiment 1

Sesame seeds were not exposed to light, cultivated at 15 °C, and fully covered with acidic liquid culture medium. There measurements were taken at 35 time points: 1-110, 2-510, 3-835, 4-1120, 5-1560, 6-1915, 7-2160, 8-2630, 9-2880, 10-3300, 11-3615, 12-3960, 13-4320, 14-4680, 14-5080, 15-5474, 16-5820, 17-6140, 18-6505, 19-6890, 20-7260, and 21-7660 min.

### 2.3 Experiment 2

Sesame seeds were exposed to continuous light, cultivated at 15 °C, and fully covered with acidic liquid culture medium. The

measurements were taken at 35 time points: 1-360, 2-480, 3-720, 4-1050, 5-1410, 6-1680, 7-1800, 8-1920, 9-2160, 10-2760, 11-3240, 12-3360, 13-3600, 14-3840, 15-4380, 16-5280, 17-5490, 18-5580, 19-5740, 20-6120, 21-6720, 22-7440, 23-7600, 24-7825, 25-8390, 26-9060, 27-9760, 28-9900, 29-10080, 30-10440, 31-10800, 32-11320, 33-12360, 34-13800, and 35-15180 min.

#### 2.4 Experiment 3

Sesame seeds were exposed to continuous light, cultivated at 15 °C, and two-thirds covered with acidic liquid culture medium. The measurements were taken at 35 time points: 1-100, 2-510, 3-540, 4-1050, 5-1260, 6-1920, 7-2310, 8-2790, 9-3300, 10-3840, 11-4170, 12-4590, 13-5070, 14-5670, 15-5970, and 16-9950 min.

#### 2.5 Experiment 4

Sesame seeds were exposed to continuous light, cultivated at 20 °C, and two-thirds covered with acidic liquid culture medium. The measurements were taken at 35 time points: 1-90, 2-720, 3-1080, 4-1440, 5-1620, 6-2460, 7-2730, 8-3240, 9-4159.8, 10-5740.2, 11-6439.8, 12-6840, and 13-8650.2 min.

#### 2.6 $^1\text{H}$ NMR spectroscopy

The  $^1\text{H}$  NMR spectra were acquired on a Varian UNITY INOVA 500 MHz NMR spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) at 20 °C. A single 90° pulse excitation was used with the following parameters: a 90° pulse width of 2  $\mu\text{s}$ , a recycle delay of 3 s, an acquisition time of 0.02 s, and 16 transients. The spectra were processed using the Vnmrj 4.0 software (Agilent Technologies Inc., Santa Clara, CA, USA), which involved Fourier transformation, phase correction, baseline correction, zero filling. To ensure reproducibility and reliable error control, the experiments were conducted on five sets of samples, and the results were averaged.

#### 2.7 Chemical shift selective imaging

CHESS experiments were conducted using a Varian UNITY INOVA 500 MHz NMR spectrometer with a  $\mu\text{-MRI}$  System, employing the CHESS sequence (Haase *et al.*, 1985) at 20 °C. The TR and TE used for CHESS were 1000 ms and 15 ms respectively, and the contrast of the CHESS images was T1-weighted. As the sesame seeds grew, some imaging parameters of CSSI were adjusted accordingly.

#### 2.8 Experimental flowchart

The experimental flowchart is shown in Figure 1. The process can be summarized as follows: Sesame seeds were cultivated under four different conditions.  $^1\text{H}$  NMR and CHESS were used to analyze seeds at various time points. The obtained NMR data were combined with phenotypic data for analysis to study and compare the metabolic and structural changes in sesame under different cultivation environments.

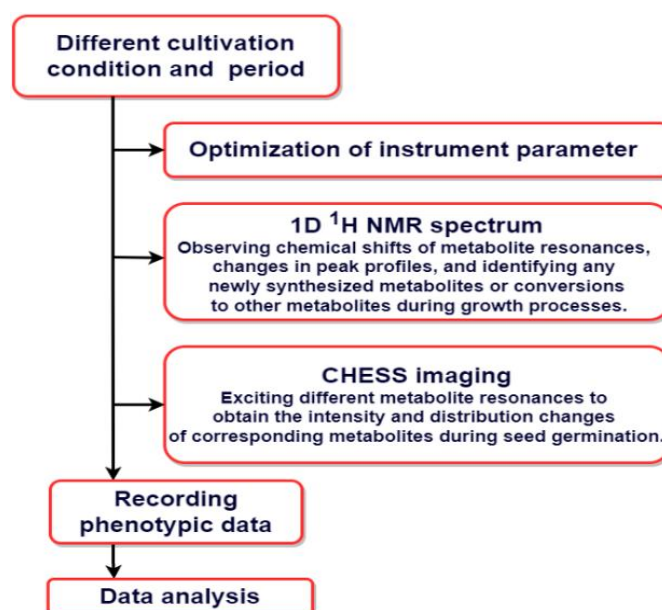
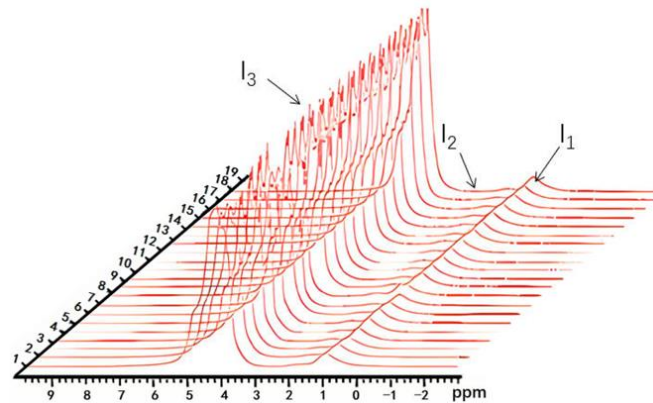


Figure 1- Experimental flowchart

### 3. Results and Discussion

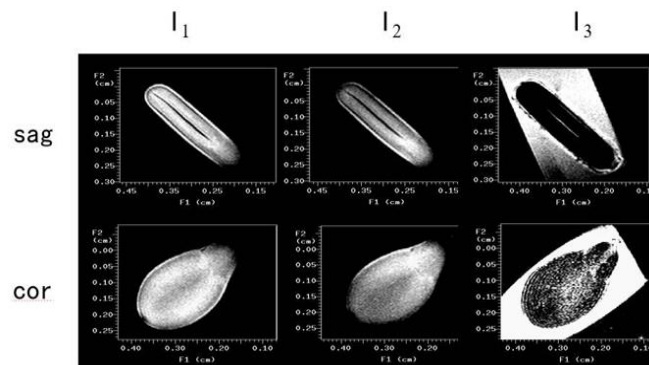
#### 3.1 Experiment 1

The 1D  $^1\text{H}$  NMR spectra collected at various time points during Experiment 1 are shown in Figure 1. The primary identifiable resonances in the spectra are  $I_1$ ,  $I_2$ , and  $I_3$ . Peaks  $I_1$  and  $I_2$  are associated with fatty acid features, specifically  $-(\text{CH}_2)_n-$  (methylene protons) and  $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2$  (allylic methylene protons), respectively. Peak  $I_3$  corresponds to the water signal,  $\text{H}-\text{O}-\text{H}$  (water protons). Over time, there were no significant changes in the chemical shifts of these characteristic peaks, nor were any new or transformed metabolites detected. This suggested that the sesame seeds remained in a dormant state throughout the duration of the experiment (Terskikh et al. 2011).



**Figure 2- The 1D  $^1\text{H}$  spectra of sesame seeds at different time points (Experiment 1)**

Figure 3 shows the images obtained using CHES after exciting the resonances of sesame seeds labelled as  $I_1$ ,  $I_2$ , and  $I_3$  at 7260 min. From the images, it was evident that the sesame seeds exhibited no apparent signs of germination. Lipids were evenly distributed within the sesame seeds, while moisture predominantly accumulated in the underdeveloped cotyledons, embryo shoot, axis, and root. The metabolic profiles indicated no spatial interactions or transformations, reflecting the seed dormancy in physiological structure (such as seed coat, endosperm and embryo) and metabolism, which was consistent with the findings from the  $^1\text{H}$  NMR spectroscopy.



**Figure 3- The CHES sagittal (sag) and coronal (cor) images of sesame seeds at 7250 min, excited resonances at  $I_1$ ,  $I_2$ , and  $I_3$ . Imaging parameters: FOV =  $0.5 \times 0.5$  cm, matrix size =  $260 \times 130$ , slice thickness = 0.3 mm**

#### 3.2 Experiment 2

The  $^1\text{H}$  NMR spectra at various time points for Experiment 2 are presented in Figure 4. From the spectra, a notable difference compared to Experiment 1 was observed: due to continuous light exposure, a marked increase in the water signal at 720 min indicated that the sesame seeds absorbed moisture through the micropyle, a key indicator of seed readiness for germination. However, from 720 to 3600 min, the spectra of Experiment 2 closely resembled those of Experiment 1, showing relative stability in water and lipid signals without apparent additions or transformations in metabolites. Starting at 3840 min, the initially sharp water signal transformed into a broader, lower peak. This change stems from two factors: a portion of the free water is oil seed d for cellular expansion and metabolic activities, while another portion binds with biomolecules within cells (such as proteins, polysaccharides), forming bound water. The signal associated with bound water appears as a broader and lower peak in the  $^1\text{H}$  NMR spectra, primarily due to restricted molecular movement and shorter relaxation time (Xiao et al. 2024).



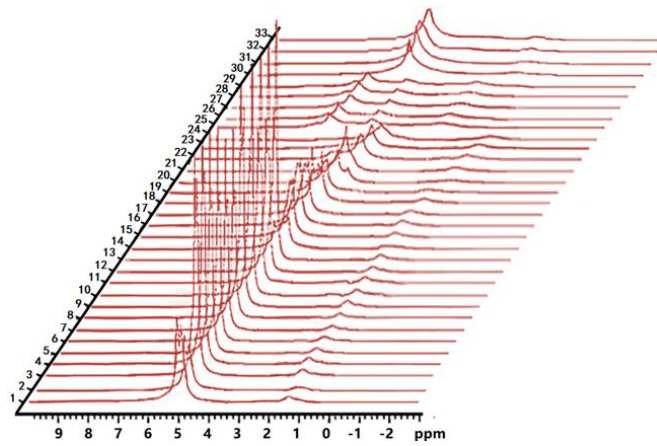


Figure 4- The 1D  $^1\text{H}$  spectra of sesame seeds at different time points (Experiment 2)

To gain a clearer understanding of changes in lipid signals, we selected the spectra at key time points as shown in Figure 5. From the figure, at 8390 min, the prominent lipid characteristic peak  $I_1$  near 1.35 ppm dramatically decreased and became less detectable, while the  $I_2$  peak around 2.50 ppm notably increased. Moreover, both signals exhibited chemical shift variations simultaneously. During the time interval from 9760 to 10800 min, a new signal  $I_{\text{new}}$  appeared near 6.20 ppm, which subsequently disappeared after 11320 min. This suggested that various metabolic processes occurred within sesame seed cells, potentially yielding intermediate products such as metabolic transformations of fatty acids. As sesame germination progressed, these metabolites might have undergone further transformation or consumption, leading to signal disappearance.

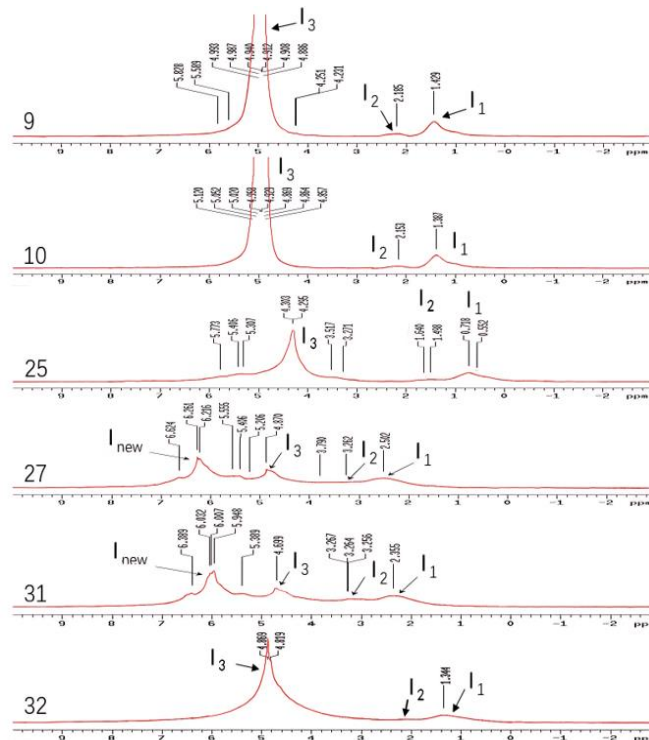
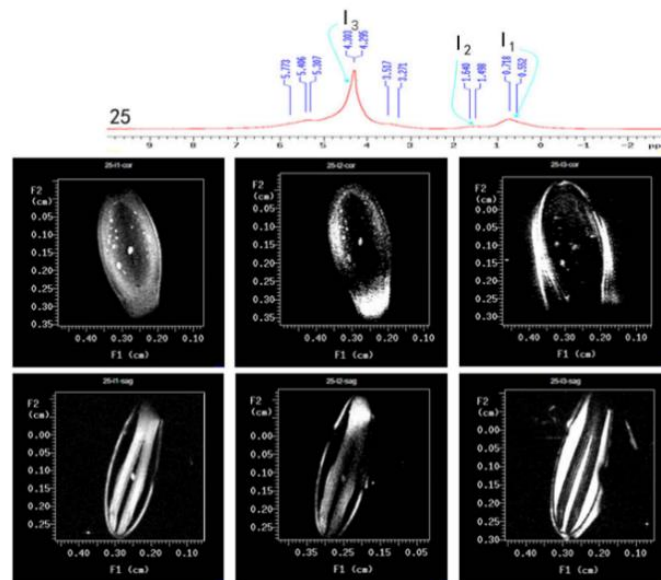


Figure 5- The 1D  $^1\text{H}$  spectra of sesame seeds at key time points (Experiment 2)

Figure 6 depicts the images obtained using CHES after exciting after exciting the resonances of sesame seeds labelled as  $I_1$ ,  $I_2$ , and  $I_3$  at 7440 min. Compared to Figure 3, the lipid distribution was no longer uniform but was concentrated around the radicle and micropylar endosperm near the micropyle, with lesser presence in the cotyledon portion of the embryo. From the sagittal image of  $I_3$ , it was observed that moisture, after permeating through the micropyle, primarily resided in the endosperm and internal cavities of the seed, gradually absorbed by the internal embryo. Moisture remained primarily concentrated in the underdeveloped cotyledons, embryo shoot, axis, and root, but compared to Figure 3, the extent of water signals had expanded. At the end of the experiment at 10800 min, the sesame seeds showed no visible signs of germination. However, observations from the spectra and CHES imaging indicated that the sesame seeds had exited dormancy.

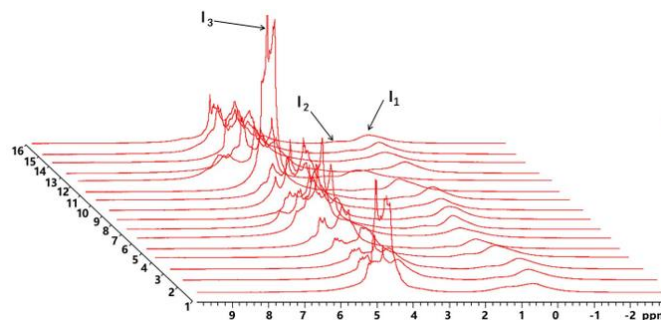


**Figure 6-** The CHES sagittal (sag) and coronal (cor) images of sesame seeds at 7440 min, excited resonances at  $I_1$ ,  $I_2$ , and  $I_3$  (The images are arranged from right to left). Imaging parameters: FOV =  $0.5 \times 0.5$  cm, matrix size =  $260 \times 130$ , nt = 16, slice thickness = 0.3 mm

### 3.3 Experiment 3

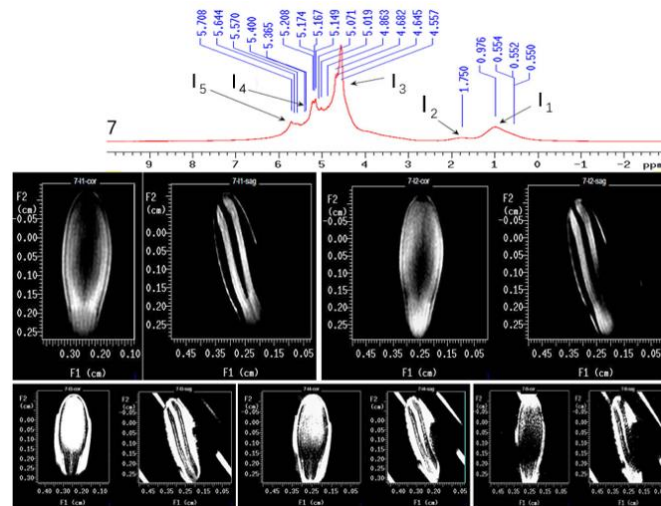
The  $^1\text{H}$  NMR spectra at various time points for Experiment 3 are shown in Figure 7. Compared to Experiment 2, the spectra from Experiment 3 reveal continuous metabolic transformations beginning from the second (2-510) time point, leading to consistent fluctuations in chemical shifts. Notably, the signals at 5.2 ppm and 5.7 ppm were significantly enhanced, corresponding to the metabolic products of aliphatic protons and phenolic compounds. This indicated that metabolic activity in the sesame seeds in Experiment 3 had become markedly more active. At the twelfth (12-4590) time point, a new characteristic peak around 6 ppm appeared, likely corresponding to glycoside compounds. This signal subsequently diminished as these compounds were metabolized into energy and carbon sources.

In Experiment 2, with the seeds completely submerged in the culture medium and under anoxic conditions, the metabolic processes of the seeds were likely restricted. The seeds may have shifted towards anaerobic metabolic pathways, such as lactic acid fermentation or alcoholic fermentation, to generate a small amount of energy to sustain basic survival needs, thereby limiting seed growth and development. In Experiment 3, where the seeds were only partially submerged in the culture medium, the seeds were able to undergo respiration, using carbohydrates to release energy. This process generated substantial amounts of energy, which supported seed growth and germination. Moreover, aerobic conditions promote the oxidation of fatty acids, providing additional energy.



**Figure 7-** The 1D  $^1\text{H}$  spectra of sesame seeds at different time points (Experiment 3)

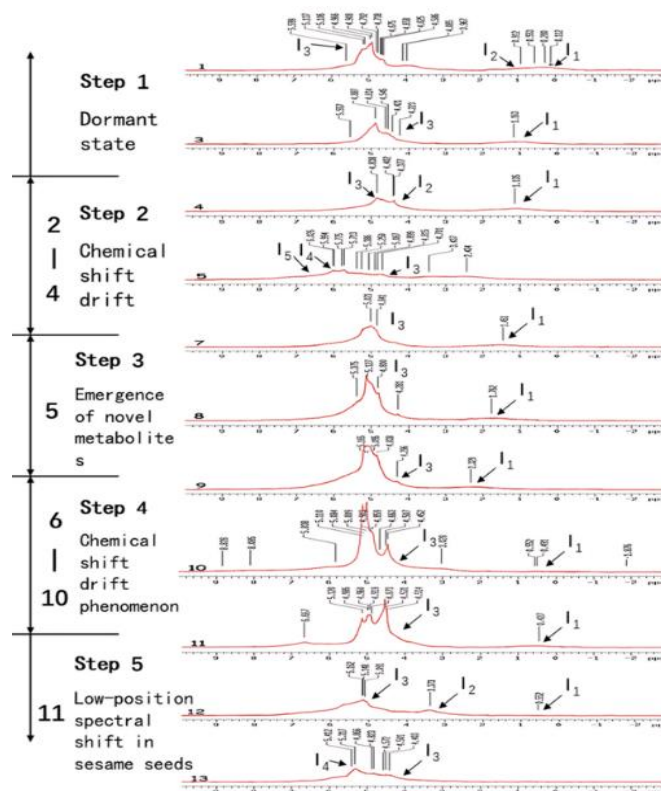
Thus, Experiment 3 yielded a higher production of metabolites in sesame seeds. We excited these metabolites by using CHES, and the results are depicted in Figure 8. From the CHES images, we observed a broader distribution and stronger signals of metabolites, indicating enhanced seed growth and metabolism under aerobic conditions. However, similar to Experiments 1 and 2, no visible signs of germination were observed until the final time point of 9950 min.



**Figure 8- The CHES sagittal (sag) and coronal (cor) images of sesame seeds at 7250 min, excited resonances at I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>, and I<sub>5</sub> (Clockwise arrangement of the images). Imaging parameters: FOV = 0.5 × 0.5 cm, matrix size = 160 × 80, nt = 16, slice thickness = 0.3 mm**

### 3.4 Experiment 4

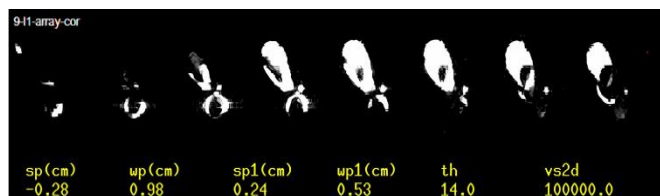
The <sup>1</sup>H NMR spectra at various time points for Experiment 4 are depicted in Figure 9, showing four distinct growth phases. From the figure, it is evident that seed growth changes rapidly. At the first (1-90) and second (2-720) time points, a conversion phenomenon was observed (characteristic peaks of sesame metabolites shift to higher frequencies), followed by a movement towards lower frequencies by the fourth (4-1440) time point. At the fifth (5-1620) time point, a new characteristic peak appeared at a high-frequency position, marking the onset of the third phase. Subsequent chemical shifts near lower frequencies indicated transformations of sesame characteristic peaks towards higher frequencies. By the eleventh (11-6439.8) time point, these peaks were completely engulfed by water peaks, yet a new peak emerged around 6.67 ppm, identified as phenolic compounds, gradually diminished thereafter. Ultimately, the overall waveform broadened and flattened, indicating that the sesame seeds were maturing, as the reduction or transformation of lipid metabolic pathways impacted the intensity of lipid components reflected in the NMR spectra.



**Figure 9- The 1D <sup>1</sup>H spectra of sesame seeds at different time points (Experiment 4)**

A series of array images of sesame seed  $I_1$  at the time point 4610 min obtained using CHES are shown in Figure 9. From the figure, it was observed that the radicle of the sesame seed had completely penetrated the seed coat and endosperm, initiating germination. Metabolic signals were present in the future first pair of cotyledons. Seed metabolic activity is typically more active during this stage, including respiration, enzyme activity, and synthesis of metabolic products. Plant hormone synthesis and signalling are typically optimized, facilitating regulation of seed germination and growth processes (Castroverde & Dina 2021).

At the final time point of Experiment 4, 8650 min, sesame seeds exhibited clear signs of germination, such as visibly softened seed coats and the emergence of the embryo shoot extending from the seed coat. This phenomenon was distinct from Experiments 1, 2, and 3.



**Figure10- The CHES array images of sesame of seeds at 4160 min excited resonances at  $I_1$ . Imaging parameters were FOV =  $1 \times 1$  cm, matrix size =  $128 \times 64$ , nt = 16, slice thickness = 0.3 mm**

Based on the above experimental results, we found that sesame seeds only remain dormancy, both physiologically and metabolically, under conditions of low temperature, darkness, and low oxygen. In continuous light and aerobic environments, although metabolic reactions occur inside the seeds, they cannot germinate normally due to the lack of suitable temperature. Seeds can only develop properly when exposed to the right temperature. Yin et al. conducted a similar study on *Ottelia alismoides* seeds (Yin et al. 2013). Their study also found that an optimal temperature (25 to 30 °C) was favorable for seed germination. However, they observed that germination occurred only in light conditions, which is inconsistent with our findings. Sesame seeds are not photoblastic; they can germinate in both light and dark conditions. Additionally, our results are more comprehensive, as we found that even in light conditions, the seeds only escape metabolic dormancy but still do not germinate normally without suitable temperature. Although studies on the effects of oxygen, light, and temperature on seed germination are already well-established (Simon et al. 1976; Chahtane et al. 2017; An et al. 2020), these studies primarily rely on phenotypic data observed through naked eye or microscope. Such research aims to detect significant signs of germination in seeds. However, at the point when the seeds have recently exited dormancy and are approaching the germination stage, no apparent signs of germination are evident. Therefore, previous research on seed germination primarily focused on the later stages of observable germination. The findings indicated that temperature and oxygen were essential conditions for seed germination (Simon et al. 1976; Corbineau 2022), whereas light was not a prerequisite (Zhang et al. 2012). Our study provides molecular-level metabolic information of seeds transitioning from dormancy to early germination, a phase in which sesame growth changes are not visible to the naked eye. Through our method, a more precise analysis of sesame seed germination can be achieved. For example, we found that light exposure alone triggers sesame seeds to exit dormancy. However, previous studies (Kim 1983) indicates that sesame seeds are shown not to be photoreceptive, but this is not contradictory. In Experiment 2, although light triggered sesame seeds to exit dormancy and initiate metabolic activity, normal development did not occur.

Biological biochemical methods (Yan et al. 2020) such as enzyme activity analysis, proteomics, and gene expression profiling, are destructive and cannot provide continuous monitoring of seeds. Regarding NMR studies (Allen et al. 2009; Munz et al. 2017), these research efforts have used either NMR spectroscopy or MRI alone. In contrast, our study combines both methods. As a result, our approach provides a more comprehensive analysis and reveals both metabolic and physiological dynamics during seed germination.

This study has several limitations. First, it did not quantitatively discuss the effects of light, oxygen, and temperature on seed germination. Secondly, humidity is also an important factor influencing seed germination (Kauth et al. 2015; Li et al. 2020). Due to equipment limitations, we did not investigate the influence of humidity on seed dormancy release. Finally, applying the machine learning and computer vision models that we developed in other fields (Gao et al. 2024a; Gao et al. 2024b; Qiu et al. 2024; Xiao et al. 2024) to analyze the NMR and MRI data of seeds significantly enhances the practical applicability of our research.

#### 4. Conclusions

This study used  $^1\text{H-NMR}$  and CHES to examine the variations in metabolite intensity and distribution during the transition of sesame seeds from dormancy to early germination under different light, oxygen, and temperature conditions. Under conditions of hypoxia (with seeds fully submerged in the culture medium), low temperature (15 °C in this study), and absence of light, NMR spectra and CHES analysis revealed no changes in the intensity and distribution of metabolites, indicating that the seeds remained in a dormant state. Exposure to light alone or in combination with oxygen triggered dormancy release in sesame seeds, yet normal development did not occur; instead, metabolic activity occurred internally within the seeds, the embryo cannot



penetrate the seed coat. This indicates that although sesame seeds are non-photoblastic, light can still trigger metabolic activity within the seeds. Normal germination and metabolic activity were observed only when the appropriate temperature was applied (20 °C in this study). Therefore, to enhance the germination, temperature is a decisive factor. Conversely, for sesame seed storage, to prevent metabolic activity from depleting nutritional reserves, seeds should be maintained under low temperature, darkness, and hypoxic conditions. Future work will focus on analyzing and studying metabolites in sesame seeds under various temperatures, oxygen concentrations, light intensities, and humidity levels to enable precise control of sesame seed storage conditions, and establish mathematical models, thereby maximizing seed quality and germination rates.

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#### Data availability:

The data that support the findings of this study are available upon request.

#### Conflict of interest statement:

The authors declare no conflict of interest.

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