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Changes in respiration and structure of non-heading Chinese cabbage seeds during gradual artificial aging

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ABSTRACT

The aging of seeds is a major problem in the agricultural sector. It is also a complex biological question that remains unclear for many crop seeds. In this experiment, we investigated how different ageing levels influence seeds germination, storage components, structure, respiration metabolism, and whether respiration is a promising indicator that can non-destructively evaluate seed aging. First, we obtained non-heading Chinese cabbage seeds with varying ageing levels by applying artificial aging treatments over a period of six days (0 d–6 d). Subsequently, respiration related indexes and ultrastructural observations were conducted. Our results showed that the respiratory rate remains low when seeds have a low water content. With the increased water content, the activities of respiratory-related enzymes, phosphohexose isomerase (PGI) and malate dehydrogenase (MDH), reached maximum levels when seeds were aged for two days. Respiration rates peaked one day later, followed by a rapid decline with increasing age. The ageing process consumed a large amount of storage components (e.g. soluble sugar, sucrose, and starch), leading to the degradation of mitochondria, proteasomes, fat bodies and cell nuclei in the aged seeds, which resulted in the reduction of respiratory rates. This, in turn, negatively affected seed germination. When seeds germinated (1 h–6 h, 1 d–6 d) under standard germination condition, the germination rate and respiratory rate showed significant correlation. Especially for 5 h and 6 h, the correlation coefficients reached 0.954 and 0.976 respectively. Measured at those times, respiratory rate should be a promising indicator for seed aging.

1. Introduction

Seeds is important materials in agriculture. It will subject to deterioration with prolonged storage time or inappropriate storage conditions, which is one of the major problems faced by the agricultural sector and germplasm preservation research units. Seed aging will lead to the decrease of the seed's germination ability and subsequent seedling establishment, which in turn have great impact on yields, economic benefits, and germplasm preservation, thus resulting in enormous commercial and genetic losses (Jacoba et al., 2016; Blauer et al., 2013; Bewley et al., 2013). Seed aging is a complex biological process, and understanding this process has great potential for predicting seed aging or providing new methods for seed conservation and improving longevity.

Even under optimal storage conditions, however, seeds suffer a variety of biochemical and physiological alterations (Rajjou et al., 2008; Sveinsdóttir et al., 2009). The predominant reserves in seeds, such as carbohydrate and protein, play an important role in normal metabolism and are closely related to seed vigour. The soluble sugar is the important substrate for respiration, which is reduced with the ageing time (Woodstock et al., 1985). Stored protein in the seeds is the main nitrogen resource during seed germination and seedling growth. The ageing process of seeds is progressive generally. In normal conditions, biochemistry changes (such as the change of enzyme activities, the membrane lipid peroxidation, and accumulation of deterioration products) come first, followed by physiological changes (such as the disorder of respiratory metabolism and the changes of reserve substance), and are finally reflected in morphological and structural changes in the cells. Furthermore, the changes of morphology and structure in turn will affect the biochemistry and physiology (Heydecker, 1972; Tao and Zheng, 1991).

Free-radical theory has widely been accepted as one of the foremost explanations for seed aging. The reactive oxygen species, ROS, can influence cell structure and the function of seeds (Gao et al., 2016; Diaz-Vivancos et al., 2013; Chen et al., 2013; Hsua et al., 2003; Miquel et al., 1980). Respiration, the center of substance and energy metabolism, is also closely related to the vigour of seeds (Mendes et al., 2009; Wang et al., 2015). Seed respiration, due to its non-destructive characteristic,

Abbreviations: MDH, malate dehydrogenase; PGI, phosphohexose isomerase; ROS, reactive oxygen species

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might be a new promising detection technique for seeds aging. Until now, respiration has been regarded as the indicator of general metabolic rate (Makarieva et al., 2008). Mendes et al. (2009) suggested that the measurement of respiratory activity was a promising approach to identify the degree of vigour of soybean seeds.

Seed respiration, generally requiring oxygen, initiates as dry seeds absorb water. Water is needed to restart seed metabolism through activation of food reserves (Xia et al., 2015; Bewley and Black, 1994). The initial materials for seed respiration are soluble sugars, followed by lipids or starches (Botha et al., 1992). Respiration is also one of the important processes that influence cell division and seed growth (Smiri et al., 2009; Bewley, 1997; Morohashi, 1980; Nawa and Asahi, 1971). Yang et al. (2014) adopted digital gene expression tag profiling to investigate the differentially expressed genes in the embryos of aged maize seeds and non-aged seeds. They reported that in the aged seeds, respiration-related genes were changed, i.e., 59 genes in glycolysis and gluconeogenesis, 50 genes in pyruvate metabolism respectively. The dysfunction of mitochondria in artificially aged seeds was a result of poorly developed mitochondria and ASC-GSH cycle activity and increased reactive oxygen species (ROS) accumulation. These studies indicated that respiration, which is involved in many important metabolic processes, is also closely associated with seed vigour, ROS, storage components, and water content.

ROS, an accepted indicator for seed vigour, can be produced through many ways. However, the largest portion of cellular ROS can be attributed to the mitochondria. The resumption of respiration in the mitochondria of imbibed seeds might result in electron leakage and the raise of ROS production (El-Maarouf-Bouteau and Bailly, 2008; Balaban et al., 2005), which indicated a close correlation between ROS and chondriosome especially at the imbibition period. The activated free radicals may damage the integrity of the seed membrane, leading to the degradation of DNA and RNA (Paparella et al., 2015; Kranner et al., 2011; Davies, 2005; Berlett and Stadtman, 1997; Levine et al., 1990). The mitochondrial DNA was among those that have the closest proximity to the source of oxidants in the chondriosome (Balaban et al., 2005). With the degradation of DNA and RNA, some proteins required for seed germination could not be synthesized, and the ability to repair faulty DNA in cells decreased, resulting in a reduction of energy metabolism, i.e., respiration (Rajjou et al., 2008; Bailly, 2004; McDonald, 1999; Kalpana and Madhava Rao, 1997; El-Maarouf-Bouteau et al., 2011).

We may speculate that storage components, structure, respiration, and ROS all correlate with seed vigour, and seed respiration rate might be a promising non-destructive testing technique for seed quality. However, a more profound and integrated study should be carried out to further verify the correlation between them, and to determine the appropriate time to adopt respiration as an indicator to evaluate seed aging. In this experiment, non-heading Chinese cabbage seeds were used as the plant materials. Controlled aging was conducted, putting seeds in a high temperature and high humidity environment over a period of six days (0 d–6 d), which can be used to provide seeds with different ageing levels (Pandita et al., 2014). We investigated the effects of seed aging on seed respiration (during the ageing processes, and germination for 1 h–6 h and 1 d–6 d under a standard germination environment) in regard to developmental indicators of seed physiological age (seeds germination, storage components, microstructure and ultrastructure).

2. Materials and methods

2.1. Plant material

Seeds of non-heading Chinese cabbage (*Brassica campestris* ssp. *Chinensis* Makino), cultivar 'Hanxiao', obtained from the non-heading Chinese cabbage research group at Nanjing Agricultural University were used in this experiment.

2.2. Experimental design and treatments

Artificial seed aging followed the protocol of high temperature and high humidity treatment according to Yan (2001). Initial seed moisture was 6.21%. Before germination, seeds were equilibrated at natural conditions for 24 h. Accelerated ageing treatments were then carried out at 100% relative humidity and 42 °C for various durations (0, 1, 2, 3, 4, 5, 6 d).

The seeds were divided into five groups for artificial ageing treatments.

The first group was used to measure the germination indexes, specifically, the aged seeds were dried to initial relative humidity, and these seeds were then kept under standard germination conditions (25 °C in the dark under humid condition) for several days, finally, germination indexes were measured.

The second group was used to detect changes in moisture content and respiratory-related indexes during seed aging, specifically, moisture content, respiratory rate, activities of phosphohexose isomerase (PGI), malate dehydrogenase (MDH), and the contents of reserve components on day 0–6 after seed aging.

The third group was used to measure the respiratory rate of aged seeds at 0, 1, 2, 3, 4, 5, 6 h under standard germination conditions. Specifically, after 1 d–6 d of artificial aging, seeds were dried back to their initial relative humidity. The seeds of different ageing levels were then imbibed at standard germination conditions and respiratory rates were evaluated at various times.

The fourth group was used to evaluate the respiratory rate of aged seeds at 0, 1, 2, 3, 4, 5, 6 d under standard germination conditions. Specifically, the respiratory rates were measured immediately after ageing treatment as initial values. Then, the seeds of different ageing levels were germinated at standard germination conditions and respiratory rates were measured every day.

The fifth group was used to determine the microstructure and ultrastructure of the seeds. Seeds were artificially aged for 0, 2, and 4 d and then dried back to their initial relative humidity. Subsequently, the seeds of different ageing levels were imbibed at standard germination conditions for 12 h in the dark and then used in the experiment.

2.3. The measurement indexes

2.3.1. Germination indexes

Seeds were kept at standard germination conditions (25 °C in the dark under humid conditions) with 50 seeds in each dish and three replications for each treatment. Germinated seeds were counted every day; subsequently, germination potential, germination rate, germination index, vigour index, and abnormal seedling rate were calculated according to the method described in the ISTA manual of determinative seedlings (Association IST International rules for seed testing, 2010). The calculation formulas of the indexes were as follows.

Seed germination potential (%) = [number of germinated seeds at the initial stage (the fifth day)/number of total samples] × 100%;

Seed germination rate (%) = [number of germinated seeds in the end stage (the seventh day)/number of total samples] × 100%;

Abnormal seedling rate (%) = (number of abnormal seedlings/number of germinated seeds) × 100%;

$$\text{Germination index(GI)} = \sum \frac{Gt}{Dt};\quad \text{Vigour index(VI)} \sum \frac{Gt}{Dt} = \times S;$$

Note: Gt stands for the number of germinated seeds on day t; Dt stands for the corresponding germination days; S stands for the root length of seedlings.

2.3.2. Storage components

Starch and total soluble sugar assays were performed using the