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Research article

Deterioration mechanisms in air-dry pea seeds during early aging☆

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ABSTRACT

The deteriorative reactions underlying seed aging, namely, lipid peroxidation and non-enzymatic carbohydrate hydrolysis, were studied in pea seeds differing in quality. Aging air-dry seeds were subdivided to three fractions using the application to individual seeds of room temperature phosphorescence. These fractions were strong seeds (fraction I) producing normal seedlings, weak seeds (fraction II) producing mainly abnormal seedlings, and dead seeds (fraction III). Enzymatic processes cannot operate in dry seeds due to the absence of free water, and thus an analytical method was needed that does not require the addition of water. The content of lipid peroxidation products was similar in both strong and weak seeds; this excluded the possibility that lipid peroxidation induced the transition of strong to weak seeds during early aging. Lipid peroxidation was activated only in dying seeds. However, glucose content in weak seeds was much higher than in strong seeds, suggestive of non-enzymatic hydrolysis of carbohydrates, probably of oligosaccharides, which utilized bound water in air-dry seeds. This process resulted in lowered water content in weak seeds. Therefore, associated with deterioration of air-dry seeds during early aging is the non-enzymatic hydrolysis of carbohydrates, whereas lipid peroxidation is not the decisive event.

1. Introduction

Seed aging is usually estimated by a decrease in germination percentage measured as an average value for a seed lot. However, the aging of individual seeds occurs at various rates that results in the discrimination of aged seeds by their quality. The criteria for assessing seed quality standardized by the International Seed Testing Association (ISTA rules, 1996) are based on seed germinability. Strong seeds, i.e. seeds of high quality, produce normal seedlings, and they account for the germination percentage of a seed lot. Seed deterioration manifests itself in the appearance of weak seeds producing seedlings with some morphological abnormalities. Long-term storage of seeds results in the loss of germination ability; these seeds remain alive for some time, but do not germinate. During further aging, such seeds die.

A reliable method suitable for estimating seed quality without conducting germination tests was devised. Its advantage is that it can be applied to individual air-dry seeds and predict what will be the quality of subsequent seedling growth. The method involves the recording of room temperature phosphorescence (RTP) of individual air-dry seeds (Veselova et al., 1999; Veselova, 2002) and can discriminate between strong, weak and dead seeds. Here, strong and weak seeds are compared in order to study some key processes leading to the seed deterioration during aging.

Because these processes occur in air-dry seeds, they proceed without participation of free water, and thus are not enzymatic. Three non-enzymatic processes that are relevant to air-dry seed deterioration are lipid peroxidation, hydrolysis of carbohydrates and amino-carbonyl reactions (Murthy et al., 2003).

A common opinion is that seed aging and loss of germination capacity during artificial aging are due to accumulation of the products of free-radical lipid peroxidation (Stewart and Bewley, 1980; Smith and Berjak, 1995; McDonald, 1999; Murthy et al., 2003; Bewley et al., 2013). However, lipid peroxidation (POL) needs the participation of oxygen, which is present in mature seeds in low amounts (Borisjuk and Rolletshek, 2009). A low concentration of oxygen inside air-dry seeds is evident from a significant RTP emission because phosphorescence can be recorded only when O_2 is very low (Veselova et al., 2003), oxygen being a strong quencher of phosphorescence (Frank and Pringsheim, 1943; Terenin, 1967).

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For this reason, active lipid peroxidation as a primary deterioration mechanism in aging dry seeds is questionable.

The next candidate offered for the role of primary aging-induced process is non-enzymatic glycosylation of proteins and amino acids (Feeney and Whitaker, 1982; Smith and Berjak, 1995; Sun and Leopold, 1995; Murthy et al., 2003). This reaction known also as the Amadori–Maillard reaction represents the interaction of the carbonyl group of glucose with the free amino group of amino acids in protein to form glycosylamine. Its subsequent rearrangement to a so-called "advanced glycosylamine end product" leads to membrane injury (Smith and Berjak, 1995; Sun and Leopold, 1995). This reaction requires the presence of reducing sugar, glucose in particular. However, the content of free glucose in air-dry pea seeds is low. Pea and soybean seeds contain about 0.1 mg glucose per g fresh weight or less (Koster and Leopold, 1988; Locher and Bucheli, 1998).

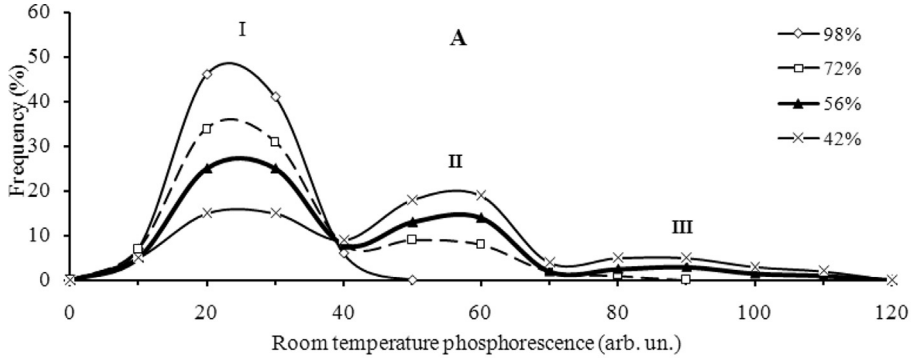
Oligosaccharides can be considered as a source of glucose, as in many aging legume seeds (Locher and Bucheli, 1998; Zalewski and Lahuta, 1998). Apparently, glucose can be formed during non-enzymatic oligosaccharide hydrolysis. This precedes glycosylation (the first step of the Amadori–Maillard reaction) because this reaction required glucose in a linear form, with a free carbonyl group (H–C=O), and this is produced during non-enzymatic oligosaccharide hydrolysis (Stepanenko, 1977). Therefore, non-enzymatic hydrolytic oligosaccharide degradation resulting in the accumulation of glucose can be considered a likely candidate in the early deterioration of aging seeds.

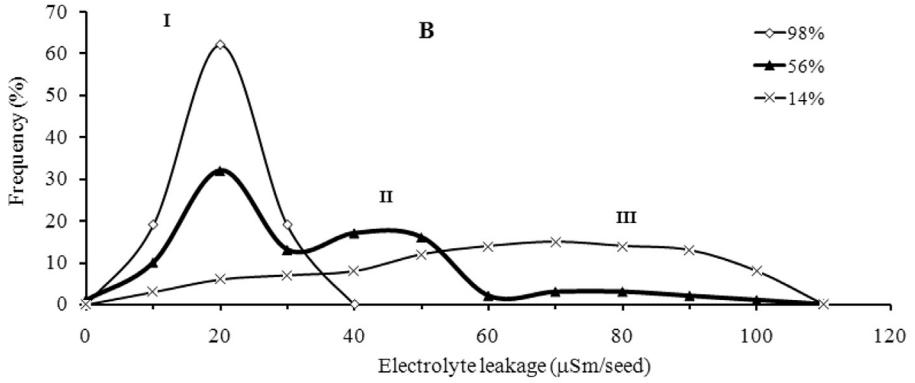
The aim of this work was to study the accumulation of the products of lipid peroxidation and non-enzymatic carbohydrate hydrolysis as related to the transition of strong seeds to weak seeds at early aging.

2. Materials and methods

2.1. Plant material

Air-dry pea seeds (*Pisum sativum* L., cv. Nemchinovskii-85) were received from the Institute of Grain Farming in non-chernozem soil regions (Nemchinovka, Moscow region, Russia). Seeds were stored at room temperature (20–23 °C) in open boxes for 5 years. Visually undamaged seeds of uniform size and weight of 225 ± 25 mg were selected for experiments.





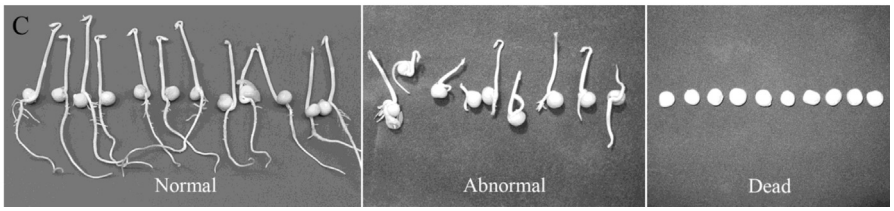


Fig. 1. (A) Distribution of individual aging air-dry pea seeds by the levels of room temperature phosphorescence (RTP, in arbitrary units) in seed lots of 98, 72, 56 and 42% germination. I, II and III indicate seed fraction numbers. (B) Distribution of individual aging pea seeds by the extent of electrolyte leakage in seed lots of 98, 56 and 14% germination. (C) Photograph of normal seedlings, abnormal seedlings and dead seeds after 5 days from the start of imbibition.