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Research article

Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* L. seeds

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Abstract

The behaviour of the *Ginkgo biloba* L. seeds was studied during storage at 4 and 25 °C. When stored at 25 °C, all the seeds died in 6 months. Cold temperatures preserved seed tissue viability for 1 year but did not preserve their capability to germinate, since such capability decreased after 6 months. A significant increase in lipid peroxidation occurred in the seed both in the embryo and in the endosperm. During storage a progressive deterioration of the endosperm tissues was evident. The two major water soluble antioxidants, ascorbate (ASC) and glutathione (GSH), showed different behaviour in the two conditions of storage and in the two main structures of the seed, the embryo and the endosperm. The ASC content of embryos and endosperms remained quite unchanged in the first 9 months at 4 °C, then increased. At 25 °C a significant decrease in the ASC content in the embryos was evident, whereas it remained more stable in the endosperm. The GSH pool decreased at both storage temperatures in the embryos. As far as the ASC–GSH redox enzymes are concerned, their activities decreased with storage, but changes appeared to be time-dependent more than temperature-dependent, with the exception of the endosperm ascorbate free radical (AFR) reductase (EC 1.6.5.4), the activity of which rapidly decreased at 25 °C. Therefore overall the antioxidant enzymes were scarcely regulated and unable to counteract oxidative stress occurring during the long-term storage.

Keywords: Antioxidants; Ascorbate; *Ginkgo biloba*; Glutathione; Recalcitrant seeds; Seed storage

1. Introduction

A number of different plant species, both of tropical and temperate origin, produce seeds considered as recalcitrant because, differently from the orthodox seeds, they are shed from the mother plant with a high moisture content and are desiccation-sensitive. They generally directly pass from development to germination, even if in some cases a dormant phase occurs [4,9,32]. There are many types of recalcitrant seeds with different desiccation tolerance; moreover some species produce seeds with a behaviour intermediate between orthodox and recalcitrant [4]. For few recalcitrant seeds, there is consistent literature on some aspects of seed development [15], on the basic physiology and response to desiccation [11,17,28,35,38], as well as ecology and evolution [29,31,39]. However many questions are still open, for example concerning the lifespan of seeds of many species, long term-storage and the processes occurring during loss of viability [30], also because there are a wide range of differences in the post harvest responses of recalcitrant seeds. Some data report that many recalcitrant seeds, particularly those of tropical origins, are also chilling sensitive and cannot be stored at temperatures below 15 °C. Their storage lifespan is quite short varying from 2 weeks to some months [8,28]. Recalcitrant seeds of temperate origin, like *Aesculus hippocastanum* or *Quercus robur,* seem to have a storage lifespan of some months or 2–3 years, respectively [8]. During short term storage, embryonic axes of recalcitrant seeds undergo to ultrastructural changes similar to those occurring during orthodox seed germination, among which increase in cell size, extensive vacuolization, consumption of reserves and development of mitochondria.

Abbreviations: AFR, ascorbate free radical; ASC, ascorbate; DHA, dehydroascorbate; GLDH, galactono-γ-lactone dehydrogenase; GSH, glutathione; GSSG, oxidized glutathione; PAGE, polyacrylamide gel electrophoresis; ROS, reactive oxygen species.

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Such changes imply an additional water requirement and for this reason recalcitrant seeds are exposed to a progressive water depletion during storage [28]. It is well known that the antioxidant systems play a pivotal role in limiting damage during water stress in vegetative tissues [27,33] and during orthodox seed development and germination [2,3,36] by removing the reactive oxygen species (ROS) generated in these conditions. Some data relate the loss of the germination capability of *Shorea robusta* recalcitrant seeds to a drop in the antioxidant system efficiency [7]. Moreover, the decrease in specific activities of antioxidant enzymes seems to be directly associated with loss of viability in *Quercus robur* where the maintenance in the ascorbate pool alone was unable to prevent or delay peroxidative damage induced by desiccation [20]. The origin of this damage in drying recalcitrant seeds has been attributed to the formation of ROS in conjunction with a decline in protection afforded by antioxidants [6,17–20]. Some authors recently reported that the antioxidant activity, at least the lipid soluble component, varies during seed storage, but it was not related to seed viability in some Australian species [26]. The presence of antioxidant systems have been also reported in *Ginkgo biloba* L. seeds which, diversely from orthodox seeds, contain large amount of ascorbate (ASC), a certain amount of dehydroascorbate (DHA), the oxidized form of the ASC, and the enzymes of the ASC metabolism [35,37]. No information is available about glutathione (GSH) metabolism in *G. biloba* seeds and antioxidant behaviour during seed storage. The morphology of *G. biloba* seeds, such as the well developed embryo contained in an haploid endosperm from which it is easy to separate it, makes them particularly suitable as a "model system" for seed physiology studies. Consistently, *G. biloba* seeds, have already been used as a model for demonstrating that the desiccation rate is a key factor for desiccation tolerance in seeds [25]. For their dimensions, tropical origin, elevated water content, and desiccation sensitivity *G. biloba* seeds seem to be recalcitrant [25]; however, this question is still under debate because some authors consider them as orthodox ones [1]. Not much information is available about lifespan and viability in different storage conditions of these seeds.

The goal of this work was to obtain information about the lifespan of the *G. biloba* seeds, to verify the possibility of their conservation in middle and long term and to investigate the putative relation between seed viability, germinability and antioxidant metabolism. The content and the redox state of ASC and GSH as well as the enzymes involved in the metabolism of these redox pairs were studied during storage of *G. biloba* seeds at different temperatures.

2. Results

2.1. Seed viability and germinability

Among the freshly collected *G. biloba* seeds, only 80% contained an embryo. The viability of the seeds during storage at 4 and 25 °C was expressed as a percentage of the embryos showing respiratory capability (Table 1). At the beginning of storage 100% of the embryos were viable (i.e. positive to formazans test, see Section 4); after 3 months the percentage of viable embryos delete had decreased both at 4 and 25 °C. After 6 months the number of viable embryos had decreased up to 53% at 25 °C, while at 4 °C only up to 90%. All the embryos from the seeds stored at 25 °C were no longer viable after 9 months, whereas at 4 °C embryos viability of 87% was maintained for 12 months.

Table 1
Ginkgo biloba seed viability during storage at 4 and 25 °C
Seed viability was measured with TTC test on isolated embryos. The results are given as the mean value of 10 experiments ± S.D. Values with different letters indicate differences statistically significant according to the Student's *t*-test ($P < 0.05$); nd = not determined

Storage (months)	Viable embryos (%)	
	4 °C	25°C
0	100 a	100 a
3	97 ± 3 a/b	93 ± 4 b
6	92 ± 2 b	53 ± 10 d
9	90 ± 5 b/c	0
12	87 ± 4 c	nd

Table 2 reports data on germinability, and on water content of the embryos and the endosperms from the *G. biloba* seeds stored at the two different temperatures. At the beginning of storage, 100% of the seeds equipped with embryos were able to germinate. The germinability of the seeds lowered to 99%, 86%, 13% and 7% after 3, 6, 9 and 12 months of storage at 4 °C, respectively (Table 2). When the seeds were stored at 25 °C their germinability decreased much more rapidly; after 6 months more than 50% of the seeds did not germinate, and after 9 months none of the seeds germinated. In the freshly harvested seeds the moisture content of the embryos was 67%; whereas that of the endosperms was significantly lower (36%). The water content decreased to a value of 52% in the embryos and of 28% in the endosperm after 12 months of storage at 4 °C. In the seeds stored at 25 °C for 6 months, the water content reached values similar to those of seeds maintained at 4 °C for 12 months (Table 2).

Fig. 1 shows cell viability in embryos and endosperm from freshly collected seeds (Fig. 1A, E), after 6 months at 4 °C (Fig. 1B, F) and 25 °C (Fig. 1C, G) and after 1 year at 4 °C (Fig. 1D, H). The embryo tissues did not show visible damage symptoms due to storage (Fig. 1D), even if the cotyledons became thinner at 25 °C (Fig. 1C) than at 4 °C (Fig. 1B). On the other hand, the endosperm tissues showed a progressive deterioration; dead cells appeared after 6 months of cold sto-

Table 2
Germinability and water content of seeds of *Ginkgo biloba* during storage at 4 and 25 °C. The results are given as the mean value of 10 experiments ± S.D.; nd = not determined

Storage (months)	Germinability (%)		Water content (%)			
	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
			Embryos		Endosperms	
0	100	100	67 ± 0.7	67 ± 0.7	36 ± 1.6	36 ± 1.6
3	99 ± 1	80 ± 2	66 ± 0.6	60 ± 1.1	35 ± 1.2	33 ± 2.1
6	86 ± 2	46 ± 5	64 ± 0.6	51 ± 0.8	30 ± 1.1	27 ± 1.2
9	13 ± 3	0	63 ± 1.3	nd	28 ± 1.3	nd
12	7 ± 2	0	52 ± 0.9	nd	28 ± 0.8	nd