Journal of Experimental Botany, Vol. 52, No. 357, pp. 701–708, April 2001

Journal of Experimental Botany

Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality

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Received 2 May 2000; Accepted 16 September 2000

Abstract

Seeds of bean (*Phaseolus vulgaris* cv. Vernel) were collected throughout their development on the plant and dried at 15 °C and 75% relative humidity to a final moisture content of about 16% (fresh weight basis) to determine whether the onset of tolerance to this drying condition was related to changes in soluble sugars or the activities of the main antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Measurements of soluble sugars and enzyme activities were made after drying the seeds, and drying tolerance was evaluated by the ability of dried seeds to germinate and to produce normal seedlings. Seeds became tolerant to drying at 45 d after anthesis, a time marking physiological maturity. At physiological maturity, the moisture content of seeds was about 50–55% (fresh weight basis) and seed dry matter reached about 190 mg per seed. Seed vigour, evaluated by controlled deterioration and conductivity measurements, continued to increase after seed mass maturity, but decreased when seeds remained thereafter for more than 7 d on the plant. Acquisition of drying tolerance was coincident with an accumulation of raffinose and stachyose. Dried-tolerant seeds were also characterized by a high amount of sucrose, the most abundant sugar, and by a low content of monosaccharides. The (raffinose + stachyose)/sucrose ratio increased during seed filling, reaching a value close to 1 when all the seeds became tolerant to drying, and maintaining this proportion during the final stages of maturation. Acquisition of drying tolerance was also related to a reorientation of the enzymatic antioxidant defence system. Drying-tolerant dried seeds displayed high CAT and GR activities and low SOD and APX activities, while the opposite condition was observed in immature dried seeds. The shift in antioxidant enzymes corresponded to the beginning of the maturation-drying phase. These results suggest that oligosaccharide metabolism and enzymatic antioxidant defences may be involved in acquisition of drying tolerance during bean seed development, but are not related to seed vigour.

Key words: Antioxidant enzymes, bean seed, desiccation tolerance, oligosaccharides, *Phaseolus vulgaris* L., seed development.

Introduction

Desiccation of orthodox seeds on the mother plant is a part of their developmental programme which allows them to enter a dry quiescent state thereby permitting

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their storage and survival in various environmental conditions (Leprince *et al*., 1993). This terminal phase of seed development, called maturation drying, is also known to ensure the switch from a developmental mode to a germinative mode (Kermode, 1995). The ability of orthodox seeds to withstand severe desiccation generally occurs during the phase of reserve accumulation, but is dependent on the drying rate, which has been shown to affect seed survival after drying (Kermode, 1995; Pammenter and Berjak, 1999). The involvement of the maturation-drying phase in the development of seed vigour is subject to discussion. Some authors have claimed that seeds attain maximum germinability at the end of the filling phase and thereafter age during further dehydration on the plant (Harrington, 1972; Tekrony and Egli, 1997). Others have found that seed storability, which can be considered as an indicator of vigour, improves during maturation drying (Demir and Ellis, 1993; Kermode, 1995; Sanhewe and Ellis, 1996*b*).

Numerous cellular and biochemical events appear associated with acquisition of desiccation tolerance of orthodox seeds. They include modifications of ultrastructural characteristics such as vacuolation (Vertucci and Farrant, 1995), synthesis of dehydrins (Galau *et al*., 1991) or heat shock proteins (Vierling, 1991), activation of antioxidative defences (Leprince *et al*., 1993), accumulation of oligosaccharides (Obendorf, 1997) and maintenance of DNA integrity (Osborne and Boubriak, 1994).

In terms of seed germinability, carbohydrate metabolism and capacity to scavenge Active Oxygen Species (AOS) seem to be of a particular interest. Accumulation of non-reducing sugars have often been shown to be associated with acquisition of dehydration tolerance of orthodox seeds (Leprince *et al*., 1990; Blackman *et al*., 1992; Black *et al*., 1996; Brenac *et al*., 1997; Corbineau *et al*., 2000). Oligosaccharides might facilitate the formation of aqueous glasses (Koster and Leopold, 1988) or substitute for water thereby preventing phase transitions in the lipid bilayer (Crowe *et al*., 1992). Moreover, some authors (Bernal-Lugo and Leopold, 1992; Horbowicz and Obendorf, 1994; Steadman *et al*., 1996; Obendorf, 1997) have suggested that seed sugar content, particularly the ratio of oligosaccharides to sucrose, might be used as an indicator of seed vigour and storability. However, few other data indicate that accumulation of the raffinose family of oligosaccharides is not clearly correlated with the degree of desiccation tolerance (Bochicchio *et al*., 1997; Black *et al*., 1999) or longevity.

There is a lack of data on the role of antioxidant systems during seed development and therefore in acquisition of seed desiccation tolerance. However, AOS generation is known to occur during dehydration of various plant tissues (Smirnoff, 1993) and recalcitrant seeds (Hendry *et al*., 1992). The ability of seeds to withstand desiccation might be related to their ability to scavenge AOS in order to avoid deleterious events such as lipid peroxidation caused by these compounds (Hendry *et al*., 1992; Leprince *et al*., 1993; Vertucci and Farrant, 1995). These mechanisms would involve enzymes such as superoxide dismutase (SOD), catalase (CAT) and enzymes of the ascorbate-glutathione cycle, or antioxidant compounds such as reduced glutathione or ascorbate. However, it has been shown that seed germinability might be related to the efficiency of free radical scavenging because this scavenging may affect merely seed storability and vigour (Priestley, 1986; Bailly *et al*., 1998, 2000).

In the present study, drying tolerance of bean seeds has been investigated throughout their development on the mother plant. Tolerance to drying has been evaluated by the capability of seeds to germinate and to produce normal seedlings after drying. It is shown that a high (raffinose + stachyose)/sucrose ratio in the dried embryonic axis is related to the onset of drying tolerance in developing seeds, and that the acquisition of drying tolerance is concomitant with a reorientation of the enzymatic antioxidant defence system.

Materials and methods

Plant material and drying conditions

Seeds of bean (*Phaseolus vulgaris* L., cv. Vernel) were sown in the experimental fields of FNAMS (Fédération Nationale des Agriculteurs Multiplicateurs de Semences) at Brain-sur-l'Authion (near Angers), France, on 10 June 1996 with a density ranging from 25–30 seeds m^{-2}. Irrigation, fertilization and crop protection were designed to ensure optimal crop growth. Flowering started on 22 July and pods were hand collected from 32–57 d after anthesis (dpa, days post-anthesis) at the level of the first node of the raceme in order to get pods of similar age (Sage and Webster, 1987). Seeds were threshed from pods after harvest and placed at 15 °C and 75% relative humidity (RH) to a final moisture content of 16% (fresh weight basis). They were then stored at 4 °C in hermetically sealed polyethylene bags until their use for experiments.

Germination tests and seed moisture content determination

Germination tests were performed by placing seeds (400 seeds in four replicates) in boxes containing sand moistened with water to 9% (w/w) at an alternate temperature of 20/30 °C (16/8 h) with a photoperiod of 8 h. After 7 d, seedlings were characterized according to ISTA (ISTA, 1993). A seed was considered tolerant to drying when it germinated and gave a normal seedling, or intolerant when it did not germinate or did not give a normal seedling.

Seed moisture contents were determined using the ISTA method (ISTA, 1993). Fifty seeds per sample were ground and dry matter was evaluated by oven drying at 130 °C for 1 h. Results correspond to the means of the moisture content values obtained with three measurements ± SD.

Controlled deterioration

Seeds, equilibrated at a moisture content of 16% (fresh weight basis) as according to the method described above, were placed for 11 d at 40 °C in tightly closed boxes. Subsequent germination ability was evaluated according to ISTA recommendations (ISTA, 1993).

Conductivity measurements

Electrolyte leakage measurements were carried out using an automatic seed conductivity meter (ASAC 1000, Neogen Corporation, USA) with 50 seeds placed individually in 3.5 ml deionized water at 20 °C for 24 h. Results are expressed as mA g^{-1} of seeds (fresh weight basis) since the conductivity meter ASAC 1000 is in fact an ammeter. They are expressed on grams of fresh matter of dried seeds (16% moisture content) in order to take into account possible changes in the total electrolyte pool during seed development. It has been verified with another batch of seeds that changes in total electrolyte leakage measured after boiling the seeds were similar to those in seed weight.

Sugar measurements

Excised embryonic axes were used for soluble sugar determinations instead of whole embryos because they are the most important organs in desiccation tolerance. Soluble sugars were extracted according to Black *et al*. (Black *et al*., 1996). Ten to 15 axes (*c*. 0.03 g FW) were ground at room temperature in a mortar in 1 ml 80% aqueous ethanol containing melezitose (2.5 mg ml^{-1}) as internal standard, and the extract was heated for 15 min at 80 °C. After centrifugation for 15 min at 14 000 *g* and removal of the supernatant, the pellet was resuspended and reextracted in 0.5 ml and then 0.3 ml 80% aqueous ethanol at room temperature and centrifuged again. The supernatants were combined and reduced to dryness in a centrifugal evaporator (RC 10–22, Jouan, France). The dry extracts were dissolved in 100 µl of ultra-pure water and then filtered through acetate filter (0.45 µm pore size, Nalgene) before being analysed by HPLC. Ten µl samples were injected onto a Spherisorb-NH_2 column (Thermo Separation Products, France) and eluted with 80/20 (v/v) acetonitrile/H_2O at a flow rate of 1 ml min^{-1} using a Spectra Physics 8700 pump. The eluents were analysed with a differential refractometer (Spectra Physics 8430) and the peak areas were integrated by a Spectra Physics 4290 integrator. Fructose, glucose, sucrose, raffinose, and stachyose were identified by co-elution with standards (Sigma).

The results were expressed as µg of sugars mg^{-1} of dry weight of embryonic axes and correspond to the means of three measurements ± SD. Expression of results as µg of sugars per embryonic axis gave similar results.

Enzyme extraction and assays

Isolated embryos (about 1.5 g FW) were ground in liquid nitrogen and then homogenized in 20 ml of potassium phosphate buffer (0.1 M, pH 7.8) containing 2 mM α-dithiothreitol, 0.1 mM EDTA and 1.25 mM polyethylene glycol 4000, and mixed for 15 min. The homogenate was centrifuged at 16 000 *g* for 15 min, and the supernatant was filtered through Miracloth and desalted on a PD 10 column (Pharmacia).

Superoxide dismutase (SOD, EC 1.15.11) and catalase (CAT, EC 1.11.1.6) activities were determined as previously described (Bailly *et al*., 1996). Results were expressed as units (SOD) and nmol H_2O_2 decomposed min^{-1} mg^{-1} protein or mg^{-1} dry weight (CAT).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured according to Nakano and Asada (Nakano and Asada, 1981). The enzyme assay contained 0.5 mM ascorbate, 0.4 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7.0), and 50 µl of enzyme extract for a total volume of 1 ml. APX activity was determined by following the decrease of ascorbate absorbance at 290 nm and 30 °C, and was expressed as nmol ascorbate decomposed mg^{-1} of protein, or mg^{-1} of dry weight, min^{-1}.

Glutathione reductase (GR, EC 1.6.4.2) activity was determined at 35 °C as described previously (Esterbauer and Grill, 1978). The assay mixture contained 0.5 mM NADPH, 8 µM GSSG and 5 µM $MgCl_2$ in 0.1 mM potassium phosphate buffer (pH 7.8), in a total volume of 500 µl. GR activity was estimated by following the rate of NADPH oxidation at 340 nm and was expressed as nmole of NADPH oxidized mg^{-1} of protein, or mg^{-1} dry weight, min^{-1}.

The results presented correspond to the means ± SD of the values obtained with nine measurements carried out on three different extracts (three measurements per extract).

All the results were expressed on the basis of mg protein or mg dry weight, but the shapes of the curves obtained were similar when they were referred to one embryo.

In order to have enough material for extraction, enzyme extracts were performed with whole embryos but it has been checked for some points that the values of enzyme activities were similar in excised embryonic axes and in whole embryos when results are referred to mg protein or mg dry weight.

Protein content of the extracts was determined using the BioRad assay kit with bovine serum albumin as the calibration standard.

Results

Seed development

Figure 1 shows the changes in dry weight and moisture content of bean seeds during their development *in planta*. Seeds were small (about 105 mg DW) at 32 dpa and their mean moisture content was 69.4% (fresh weight basis). Dry mass increased approximately linearly from 32–43 dpa. At the end of reserve accumulation, the mean

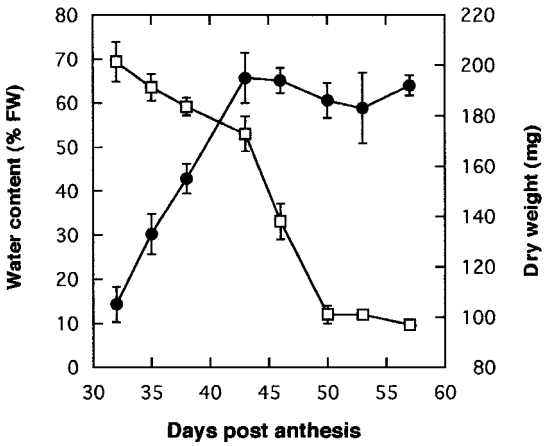

Fig. 1. Changes in moisture content (□) and in dry weight (●) of bean seeds during their development. Means of three measurements ± SD.

seed dry weight was about 190 mg and the moisture content decreased to *c*. 53% (fresh weight basis). Further maturation of seeds corresponded to a sharp increase of water loss, with final moisture content of about 12% and 9.6% at 53 and 57 dpa, respectively.

Changes in seed germination ability and vigour during seed development

Seeds younger than 46 dpa, the moisture content of which was higher than 33% (fresh weight basis) (cf. Fig. 1), did not survive drying at 15 °C and 75% RH (Fig. 2A). Tolerance to drying developed fully between about 46 and 50 dpa, i.e. a few days after the end of seed filling (cf. Fig. 1).

Seed vigour was assessed using controlled deterioration and electrolyte leakage measurements. Ageing of seeds for 11 d at 40 °C markedly altered their germination at 20 °C (Fig. 2A). However, this deleterious effect of controlled deterioration varied depending on the developmental stage of the seeds. It was a little less intense at 50 dpa than at 46 dpa, but it clearly increased thereafter.

Drying of very young seeds (33–38 dpa), which were not at all tolerant (Fig. 2A), induced strong deterioration of cell membranes as indicated by high electrolyte leakage (Fig. 2B). This damaging effect of drying decreased during seed development and was minimal between 46 and 50 dpa, i.e. when the seed moisture content dropped down to 33% fresh weight basis (cf. Fig. 1). Electrolyte leakage tended to increase slightly when seeds remained on the mother plant for longer periods (insert in Fig. 2B), which corresponded to an increase in seed sensitivity to controlled deterioration (Fig. 2A).

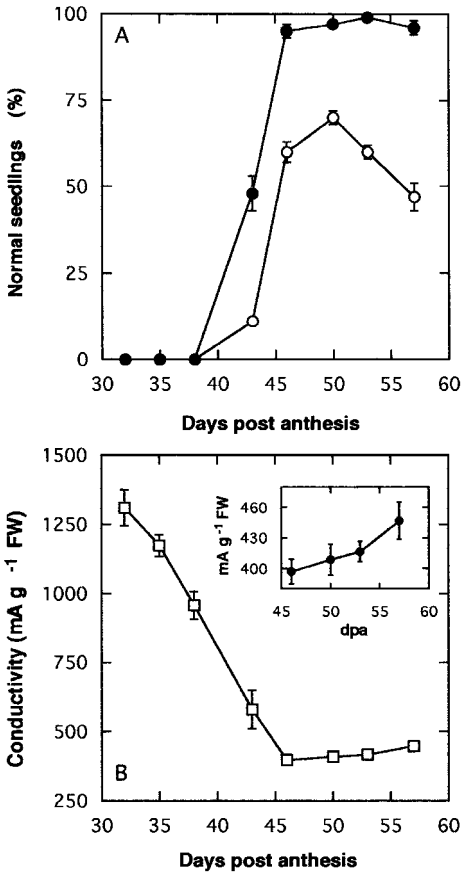

Fig. 2. Germination ability and seed vigour of seeds collected at various stages of their development and dried at 15 °C and 75% RH. (A) Seed germination ability and vigour are expressed as percentages of normal seedlings obtained after 7 d at 20 °C before (●) and after (○) controlled deterioration at 40 °C for 11 d. Means of four replicates ± SD. (B) Seed vigour is characterized by electrolyte leakage measured at 20 °C after 24 h of soaking. Means of 50 measurements ± SD. Insert shows the increase in electrolyte leakage from 46–57 dpa at a larger scale.

Changes in soluble carbohydrate contents in the embryonic axis

Figure 3A shows the changes in soluble carbohydrate contents in the axis of bean seeds collected at various developmental stages and dried at 15 °C and 75% RH. Hexose (glucose + fructose) contents remained low (around 10 μg mg^{-1} DW) throughout seed development. Sucrose content increased from 32–38 dpa, reaching 65 μg mg^{-1} DW, then it decreased slowly and remained

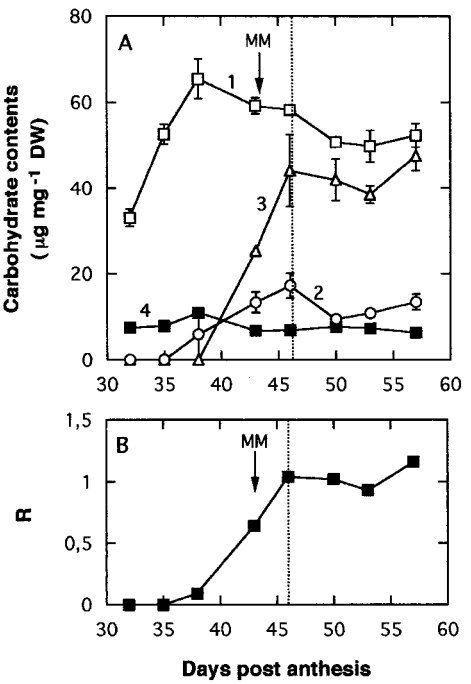

Fig. 3. Changes in soluble carbohydrate contents (A) and in the (raffinose + stachyose)/sucrose ratio (R) (B) in axes of seeds collected at various developmental stages and dried at 15 °C and 75% RH. 1, sucrose; 2, raffinose; 3, stachyose; 4, glucose + fructose. MM, the arrow indicates the end of mass accumulation (mass maturity). Vertical dotted lines indicate the onset of tolerance to drying at 15 °C and 75% RH. Means of three measurements. Vertical bars denote standard deviations.

at around 50–60 μg mg^{-1} DW during the maturation-drying phase. Acquisition of drying tolerance during seed development on the mother plant was concomitant with the synthesis of raffinose and stachyose. These oligosaccharides were not detected in the axis of dried immature seeds (younger than 38 dpa), but their content increased until the onset of drying tolerance, reaching 18 and 42 μg mg^{-1} DW, respectively. The (raffinose + stachyose)/sucrose ratio increased during seed filling (Fig. 3B). It reached 1.04 at the onset of drying tolerance, i.e. at 47 dpa, and then its value remained around 1 during seed dehydration.

Changes in antioxidant enzyme activities

Figure 4 shows the changes in SOD, CAT, APX, and GR activities, expressed as a function of protein content or of dry weight, in seeds collected at various developmental stages and dried at 15 °C and 75% RH. Patterns of the curves were similar whatever the way of expression of results, which indicated that protein and mass accumulations were similar.

SOD activity (Fig. 4A) was high at 32 dpa (7.2 units SOD mg^{-1} protein), and decreased markedly until 43 dpa (i.e. until the end of reserve accumulation), reaching only 50% of its initial value (Fig. 4A). It remained then constant at approximately 4.7 units SOD mg^{-1} protein during the maturation-drying phase. Like SOD activity, that of APX decreased until the onset of drying tolerance (Fig. 4C). After 46 dpa, it corresponded to about 50% of that measured in immature dried seeds. It remained at around 30 nmol ascorbate reduced mg^{-1} protein min^{-1} until 53 dpa and decreased to 24 nmol ascorbate reduced mg^{-1} protein min^{-1} at 57 dpa.

Contrary to SOD and APX activities, CAT activity was low in dried immature seeds (Fig. 4B), but it increased markedly from 35 to 46 dpa, i.e. at the onset of acquisition of drying tolerance. It remained constant until 57 dpa. GR activity increased continuously during seed development (Fig. 4D).

Discussion and conclusion

As in other leguminous species, such as soybean (Blackman *et al.*, 1992), lupin (Gorecki *et al.*, 1997) and pea (Corbineau *et al.*, 2000), bean seeds became tolerant to dehydration at the end of, or a few days after, dry matter accumulation (Figs 1, 2A). As previously shown with the same species (Sanhewe and Ellis, 1996*a*), acquisition of drying tolerance was associated with the normal decrease in seed water content described as maturation drying. However, it must be noted that the tolerance of seeds to desiccation depends on the rate of water loss (Blackman *et al.*, 1992; Sanhewe and Ellis, 1996*a*; Bochicchio *et al.*, 1997; Black *et al.*, 1999; Corbineau *et al.*, 2000). In pea (Corbineau *et al.*, 2000) and bean (Sanhewe and Ellis, 1996*a*), slow drying of

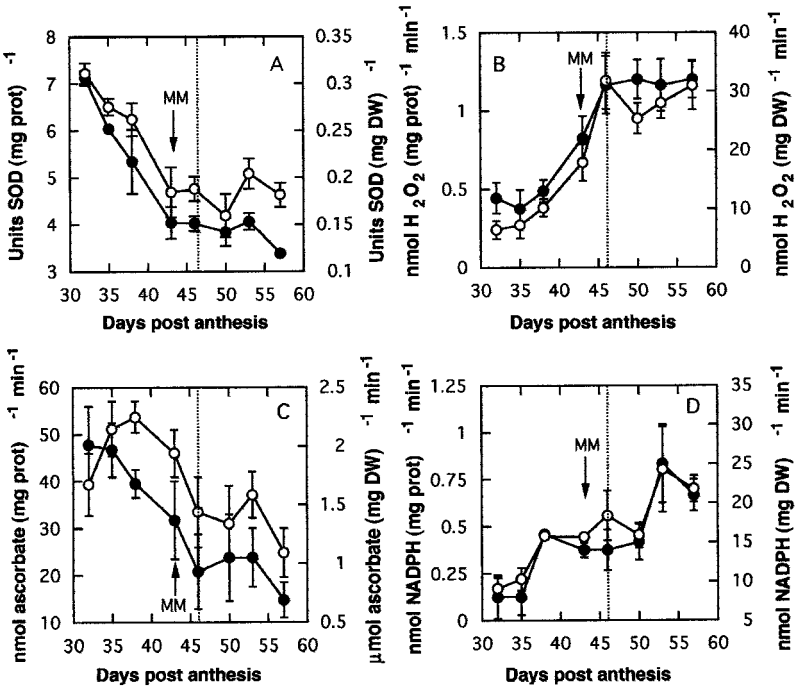

Fig. 4. Changes in superoxide dismutase (A), catalase (B), ascorbate peroxidase (C), and glutathione reductase (D) activities in seeds collected at various developmental stages and dried at 15 °C and 75% RH. Results are expressed as a function of seed protein content (○) or of seed dry weight (●). MM, the arrow indicates the end of mass accumulation (mass maturity). Vertical dotted lines indicate the onset of tolerance to drying at 15 °C and 75% RH. Means of three measurements. Vertical bars denote standard deviations.

detached seeds or drying of seeds within the pods improved desiccation tolerance of immature seeds.

Sensitivity of seeds to controlled deterioration and electrolyte leakage have been considered as good indicators of seed vigour in various species among which is snap bean (McDonald, 1999). As previously demonstrated in snap bean (Siddique *et al*., 1987; Sanhewe and Ellis, 1996*b*) and soybean (Zanakis *et al*., 1994), seed vigour increased after the end of the seed filling phase, i.e. until about 46 dpa in these experiments, as shown by a decrease in electrolyte leakage (Fig. 2B). An increase in sensitivity to the controlled deterioration of seeds harvested at 53 and 57 d after anthesis (Fig. 2A), and, to a lesser extent, an increase in solute leakage by these seeds (insert in Fig. 2B) suggest that loss of seed vigour occurred if seeds were not harvested immediately after physiological maturity (Fig. 2), probably because the ageing processes might be induced precociously (Harrington, 1972).

Acquisition of drying tolerance in bean seeds was coincident with an accumulation of raffinose and stachyose in the embryonic axis (Fig. 3A). Such a relationship between an increase in oligosaccharides and the acquisition of desiccation tolerance has been previously shown in wheat (Black *et al*., 1996), maize (Bochicchio *et al*., 1997; Brenac *et al*., 1997), *Brassica campestris* (Leprince *et al*., 1990), and leguminous species such as soybean (Blackman *et al*., 1992) and pea (Corbineau *et al*., 2000). The results obtained do not allow an evaluation of whether the changes in oligosaccharides were closely correlated to dehydration tolerance *per se* or were a consequence of water loss during drying at 15 °C and 75% RH, since experiments were performed only with dried seeds. Data obtained with immature embryos of wheat (Black *et al*., 1999) and with embryonic axes of pea (Corbineau *et al*., 2000) suggest that oligosaccharide biosynthesis is regulated by the rate of water loss; the slower the dehydration is, the higher the oligosaccharide content is. However, in pea seeds, Corbineau *et al*. have demonstrated that only cotyledons, not the embryonic axis, were concerned by such an effect of drying rate (Corbineau *et al*., 2000). Sucrose and the RFO (raffinose family oligosaccharides) might be involved in stabilization of membranes during dehydration or in improving glass formation (Vertucci and Farrant, 1995). Biosynthesis of raffinose and stachyose is catalysed by raffinose synthase and stachyose synthase in the cytosol of seed tissues during maturation (Obendorf, 1997). The small decrease in sucrose and stability of the hexose pool observed concomitantly with the increase in both oligosaccharides suggest that phloem unloading of carbon, mainly as sucrose (Thorne, 1985), is not restrictive during reserve accumulation. Accumulation of RFO could also result from the conversion of monosaccharides in order to decrease respiratory substrates availability, and therefore metabolic activity, when seeds undergo desiccation (Leprince *et al*., 1992; Pammenter and Berjak, 1999). In these experiments, the time-course of acquisition of drying tolerance was well correlated with the increase in the oligosaccharides to sucrose ratio (R) (Fig. 3B). At the onset of acquisition of drying tolerance, i.e. by 46 dpa (Fig. 2A), the oligosaccharides/sucrose ratio was maximum at 1.04 (Fig. 3B), a value higher than has been reported for wheat (0.6) (Black *et al*., 1996) and similar to the one calculated in pea seeds (1.0) (Corbineau *et al*., 2000). However, contrary to the suggestion of some authors (Bernal-Lugo and Leopold, 1992; Horbowicz and Obendorf, 1994), this ratio cannot be considered as a good indicator of seed vigour in bean (Figs 2, 3B), at least for the latest stages of development, since its value remained close to 1 (Fig. 3B) while seeds became more sensitive to controlled deterioration (Fig. 2A).

Plant tissue dehydration is known to be associated with an increased production of active oxygen species (AOS) which can react together and trigger numerous deleterious oxidative processes (Leprince *et al*., 1993; Smirnoff, 1993). Antioxidative defences are widely suspected to play an important role in acquisition of desiccation tolerance (Hendry *et al*., 1992). However, there is little information regarding AOS production during orthodox seed drying, whereas it is well documented for recalcitrant seeds (Leprince *et al*., 1999). In non-photosynthetic organs like seeds, AOS are generally generated through mitochondrial activity by electron leakage to oxygen (Puntarulo *et al*., 1988). AOS production may thus decrease when respiration drops down, which is the case during maturation drying of orthodox seeds (Leprince *et al*., 2000). Vertucci and Farrant have reported that AOS production could occur until 0.25 g H_2O g^{-1} DW during seed dehydration which fits well with mitochondrial activity (Vertucci and Farrant, 1995). Nevertheless, existence of other sources of free radicals during further drying cannot be ruled out. The lack of data concerning oxidative processes during orthodox seed development therefore addresses the question of the role of the antioxidant enzymes. In bean seeds, acquisition of drying tolerance clearly seems to be associated with a reorientation of the enzymatic antioxidant defence systems. Dried mature desiccation-tolerant seeds displayed high CAT and GR activities and low SOD and APX activities, whereas it was the opposite in immature desiccation-intolerant seeds (Fig. 4). The shift between these two contrasting situations correlated with the beginning of the maturation-drying phase (Fig. 4). These results show that antioxidant enzyme activities are probably related to acquisition of seed desiccation tolerance, even though it is difficult to ascertain their physiological role. Loss of SOD activity during seed filling might lead to an accumulation of superoxide anion, whereas impairment of APX activity might be

compensated by the increase in CAT activity which also scavenges hydrogen peroxide. These enzymes could co-operate to avoid oxidative damage that might occur as a consequence of AOS accumulation during desiccation (Smirnoff, 1993). Few reports document the changes in SOD, CAT, APX, and GR activities during seed development. Catalase isoform pattern has been shown to vary during development of cotton and maize seeds (Ni and Trelease, 1991; Scandalios, 1997*a*), and SOD genes are differentially expressed throughout seed development (Inzé and Van Montagu, 1995; Scandalios, 1997*b*), however, their role in relation to seed germinability has not been elucidated. In recalcitrant cocoa embryonic axes, Li and Sun have recently demonstrated that desiccation sensitivity is related to a decrease of antioxidant enzyme activities (Li and Sun, 1999).

The changes observed in antioxidant enzyme activities might also be related to the modulation of the antioxidant or the AOS levels. Ascorbate is known to be involved in cell division and growth, and it decreases during seed maturation (Arrigoni *et al*., 1992), which might explain the loss in APX activity (Fig. 4C). Beside its antioxidant properties (Foyer *et al*., 1997), glutathione, and therefore GR, have been implicated in various metabolic processes such as regulation of enzyme activities or regulation of gene expression (Mullineaux and Creissen, 1997). Recent studies have demonstrated that $O_2^{\cdot -}$ and H_2O_2 are involved in molecular signalling (Inzé and Van Montagu, 1995; Foyer *et al*., 1997). Even though marked changes in antioxidant enzyme activities occurred during bean seed development, by the time there was a switch from developmental to germination mode, one might be questioned whether antioxidants or AOS could play a role in this process.

As an alternative hypothesis, the decrease in SOD and APX activities could also reflect a decrease in AOS production which could be associated with the decline of mitochondrial activity occurring during maturation drying. However, these experiments were carried out with dried seeds (16% moisture content). It is therefore difficult to postulate for such a hypothesis, since all seeds had experienced a drying period. Measurements on undried seeds during their development would allow this question to be answered.

Finally, it must be kept in mind that these enzymes belong to various cellular compartments such as mitochondria (SOD, CAT, GR), cytosol (APX, GR, SOD), plastids (SOD, GR) or peroxisomes (CAT, SOD to a lesser extent) (Scandalios, 1997*b*). Drying might affect these compartments differently, since different critical moisture levels have been postulated for triggering or inhibiting various metabolic and cellular events (Vertucci and Farrant, 1995). The changes in enzyme activities might therefore be related to changes in organelle activities or to their dedifferentiation during desiccation (Vertucci and Farrant, 1995).

The results of this study indicate a marked trend for a role of carbohydrate accumulation and antioxidant enzyme activities in the acquisition of bean seed drying tolerance. However, the generation and the putative role of AOS remain to be determined as does the evolution of the biochemical processes studied during physiological drying of seeds on the mother plant. This will be the subject of further investigations.

Acknowledgements

This work was supported by the 'Région des Pays de la Loire' in the frame of the 'Contrat de plan Etat-Région 1994/1999'.

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