Changes in antioxidant enzymes during sunflower seed development

Arnaud Lehner, Christophe Bailly, Sandra Rousseau, Françoise Corbineau, Daniel Côme

CHANGES IN ANTIOXIDANT ENZYMES DURING SUNFLOWER SEED DEVELOPMENT

A. LEHNER, C. BAILLY, S. ROUSSEAU, F. CORBINEAU et D. CÔME

Physiologie Végétale Appliquée, Université Pierre et Marie Curie, Tour 53, 1er étage, 4, place Jussieu, 75252,Paris, cedex 05.

INTRODUCTION

Seed germinability and vigour are closely related to the conditions of seed development and maturation on the mother plant. The development of orthodox seeds is associated with reserve accumulation, which provides the energy source for seedling growth, and with a pronounced desiccation phase, which allows the seeds to enter a dry quiescent state.

Desiccation of plant tissues has been shown to be related to production of active oxygen species (AOS), the accumulation of which may generate various cellular damage (Leprince *et al*, 1993; Smirnoff, 1993). Therefore, the ability of seeds to escape oxidative injuries during their programmed desiccation on the mother plant, through AOS scavenging, might be related to their subsequent germinability. The aims of this work were (I) to study the changes in the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) during sunflower seed development and after artificial drying, and (II) to investigate whether there was a relationship between acquisition of seed vigour and expression of these enzymes.

RESULTS

SEED DEVELOPMENT

Seed dry mass accumulated from 24 to 42 days after pollination (Fig. 1). At the end of reserve accumulation, so called physiological maturity, thousand seed weight (TSW) was *ca* 55 g and moisture content was around 30 % fresh weight (FW). Seed water content regularly decreased from 60 % FW at 24 DAP to 10 % FW at 58 DAP, thus showing no marked desiccation phase.

Table I : Germination ability and seed vigour of dried seeds collected at various stages of their development. MTG, mean time to germinate. T50, duration of controlled deterioration which reduced seed germination by 50 %.

DAP	Normal seedlings (%)	MTG at 15¡C (h)	T50 (h)
24	89,7	80	122,4
34	97,7	64	132,0
42	95,3	57	144,0
50	90,7	47	156,0

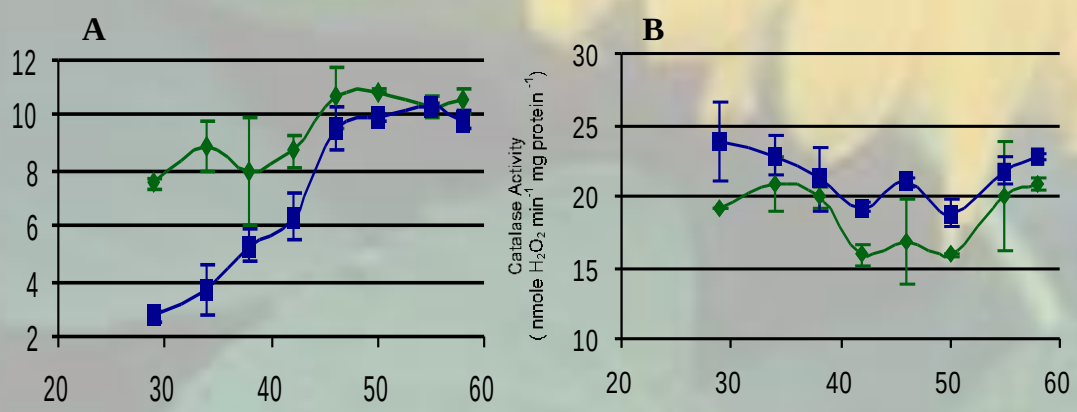

Figure 2 : Changes in glutathione reductase (A) and catalase (B) activities in seeds collected at various stages of development. Measurements were carried out in fresh seeds (■) and in dried seeds (◆). Means of 3 measurements. Verticals bars denote standard deviations.

CHANGES IN CATALASE EXPRESSION DURING SEED DEVELOPMENT

Expression of one of the four genes coding for CAT, CATA1, has been investigated in seeds collected from capitula 29, 42 and 58 DAP (Fig. 3). In fresh seeds, CATA1 expression increased continuously during desiccation, and reached values close to 80 arbitrary units (AU) in fully mature seeds (58 DAP). CATA1 expression was stongly stimulated by artificial drying of seeds at 29 and 42 DAP, reaching values close to that found in fully mature dry seeds (Fig. 3). Drying of seeds at 58 DAP did not change CATA1 expression.

CONCLUSION

The results obtained give new insights on acquisition of germinability and on changes in antioxidant enzymes during sunflower seed development :

- Desiccation tolerance is acquired very early in the seed developmental program (at least when drying is mild);
- Seed vigour increases after physiological maturity;
- Although SOD and GR are active, they do not appear to be involved in acquisition of desiccation tolerance or elaboration of seed vigour;
- Catalase is associated with seed desiccation through a transcriptional regulation of its expression. CAT activity is correlated with the onset of physiological maturity but probably not with seed germinability.

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MATERIAL AND METHODS

Plant material : Experiments were carried out with seeds of sunflower (*Helianthus annuus* L., cv Fructidor) issued from cut capitula that were hand collected 24 to 58 DAP (days after pollination) in 1999 in experimental fields of Limagrain (Dronne, France). Seeds were either frozen in liquid nitrogen immediately after threshold and stored at -35¡C (fresh seeds) or dried on the capitula at ambient temperature for 3 days and stored at 20¡C and 75 % relative humidity (RH) (dry seeds).

Germination tests : Germination was tested according to International seed Testing Association (ISTA) rules by estimating the percentage of normal seedlings. Mean time to germination (MTG) was determined at 15¡C using the following formula : $MTG = [n_1 \times 1 + (n_2 - n_1) \times 2 + \ldots + (n_j - n_{j-1}) \times n_j] / r$ (where n_j represents the number of germinated seeds at day ja after sowing, and r represents the total number of germinated seeds).

Controlled deterioration : Seeds, equilibrated at a moisture content of 8 % (fresh weight basis) by placing them at 20¡C in 75 % RH for 7 days, were placed for 3 to 7 days at 45¡C. Subsequent germination was evaluated according to ISTA rules.

Enzyme extraction and assay : Extraction of proteins and measurements of the activities of catalase (CAT, EC 1.16.1.6) and glutathione reductase (GR, EC 1.6.4.2) were carried out as previously described (Bailly *et al.,* 1996). Results are expressed as specific activity (per mg of protein).

RNA isolation and northern blot analysis : RNA extraction was carried out according to the method described by Verwoerd *et al.* (1989). Ten µg of total RNA, for each sample, were loaded on gel, transferred on to blot and then hybridizated with a partial cDNA of CATA1 gene (clone L28740 purchased by Pr Heinze, MÜnster University).

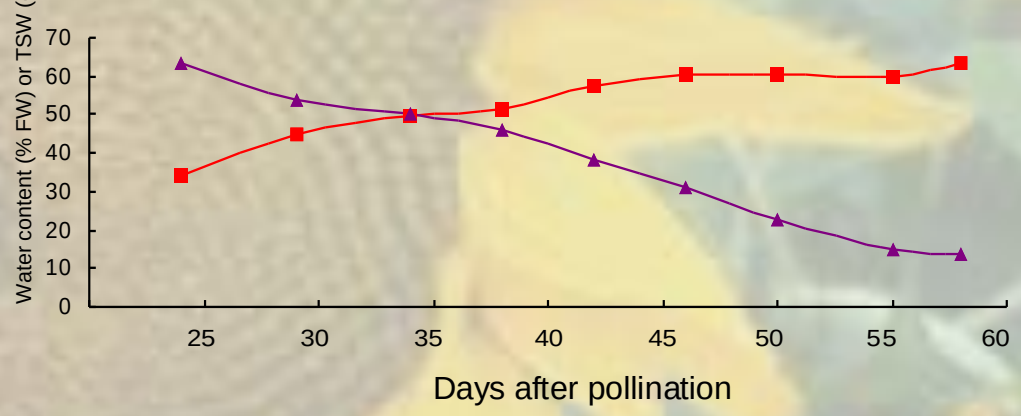

Figure 1 : changes in moisture content (▲) and in thousand seed weight (TSW, ■) of sunflower seeds during their development.

ELABORATION OF SEED GERMINABILITY

Dry seeds were able to germinate, and then to tolerate desiccation, as soon as 24 DAP, *ie* when they were still immature (Table I). Germination percentages increased during seed development until seeds reached physiological maturity. Further decrease of percentages of normal seedlings was related to fungus contamination.

Seed vigour, as assessed by germination rate at 15¡ C and tolerance to controlled deterioration, improved during seed development and after physiological maturity (Table I). Indeed, MTG decreased and duration of controlled deterioration necessary to reduce seed germination by 50 % (T50) increased during development.

CHANGES IN ENZYME ACTIVITIES DURING SEED DEVELOPMENT

GR activity did not evolved significantly during seed development on the mother plant and was very similar in fresh and dry seeds (Fig. 2A). SOD activity displayed the same profile of activity and therefore did not show any significant change during seed development (data not shown).

CAT activity increased markedly at the end of seed filling, *ie* after 42 DAP, and remained thereafter at a high level, close to 10 nmole H_2O_2 min^{-1} mg $protein^{-1}$ (Fig. 2B). Drying of immature seeds was associated with a stimulation of this enzyme activity, which reached a value close to that found in mature seeds. The effect of dehydration on CAT activity was not observed after physiological maturity, when seed moisture content was low.

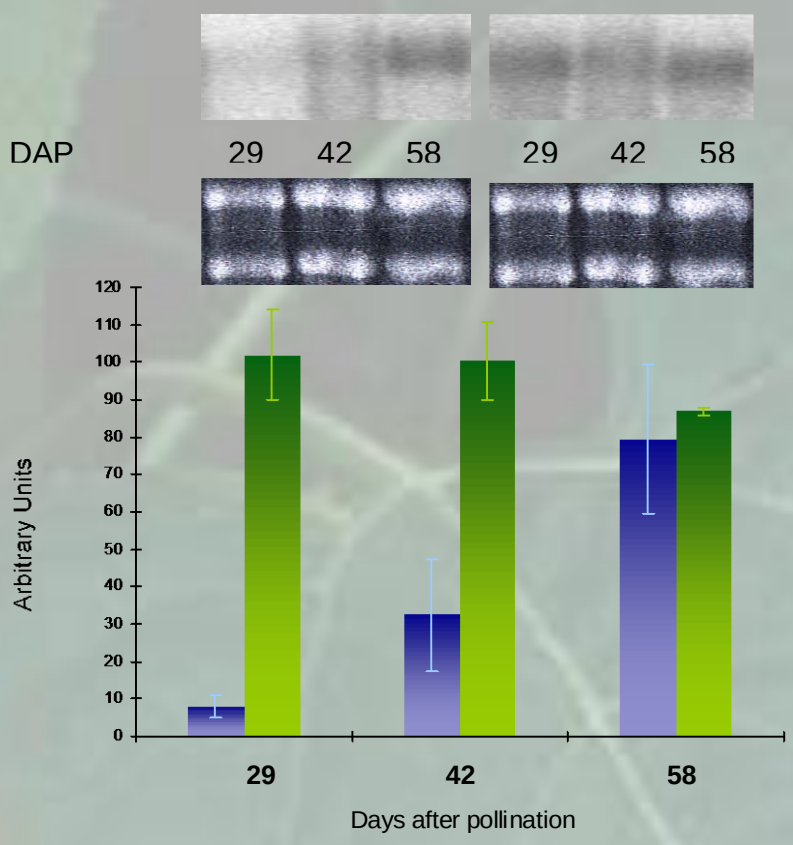

Figure 3 : A, Northern hybridization of CATA1 mRNA prepared from fresh and dried seeds collected at 29, 42, 58 days after pollination (DAP). B, electrophoresis gel of the membrane shown in A. C, Hybridization signal intensity data from northern hybridization of CATA1 gene in the fresh (■) and dried (■) seeds during their development. collected at 29, 42 and 58 DAP.