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OLIGOSACCHARIDE METABOLISM IN WHEAT EMBRYO AS A POSSIBLE INDICATOR OF SPROUTING INJURY

A. LEHNER, D. CÔME and F. CORBINEAU

Physiologie Végétale Appliquée, EA 2388 « Physiologie des semences », Université Pierre et Marie Curie, 3, rue Galilée, 94200 Ivry-sur-Seine, France. Email : corbi@ccr.jussieu.fr, http://lpva.snv.jussieu.fr



INTRODUCTION

Dormancy of cereal seeds, which corresponds to their inability to germinate at relatively high temperatures, generally above 20°C, prevents germination during seed development on the mother-plant. Lack of dormancy results in preharvest sprouting during adverse weather conditions (cold temperature and high rainfall), and induces loss in grain and flour quality due to synthesis of enzymes during germination, notably α -amylase (Meredith and Pomeranz, 1985).

The aims of the present work were to investigate whether soluble sugar metabolism in the embryo is an early indicator of sprouting injury, and whether it is correlated with dormancy in wheat (*Triticum aestivum* L., cv Charger).

Soluble sugar contents of the embryo were measured by HPLC in dry freshly harvested (dormant, D) seeds and seeds stored dry for 5-6 months at 20°C (non dormant, ND), and in both types of seeds incubated in water or in the presence of polyethylene glycol (PEG) (-2.5 MPa) for various durations at 20°C and 30°C.

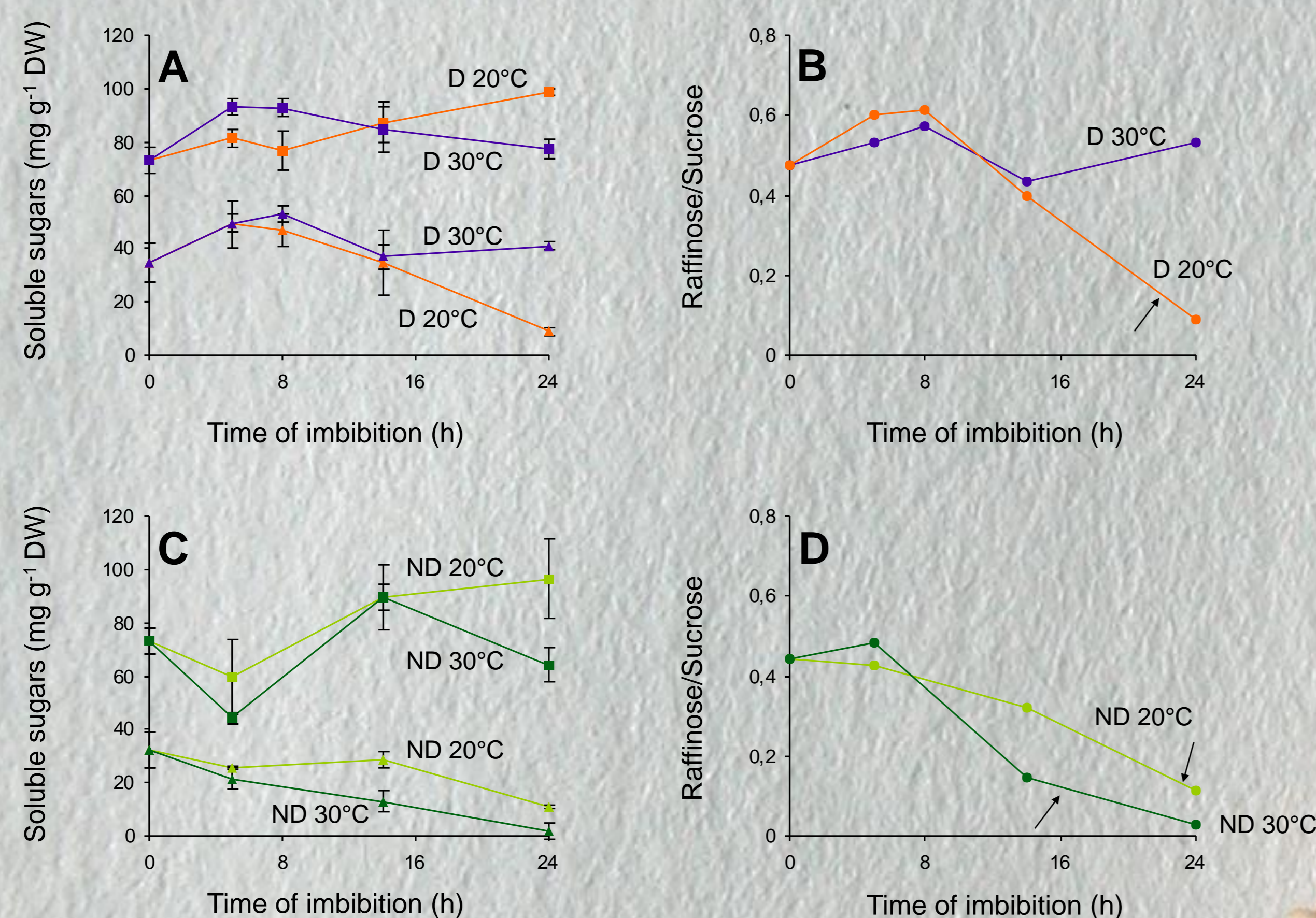


Figure 1 - Changes in sucrose (■) and raffinose (▲) contents (A, C) and in the raffinose/sucrose ratio (B, D) in embryos isolated from dormant (D) and non dormant (ND) seeds imbibed on distilled water at 20 and 30°C. Arrows indicate the beginning of germination. Means of 3 measurements \pm SD. Where no bars are shown, the spread of SD is less than the size of the symbols.

③ Changes in sugar contents during imbibition on PEG

Raffinose content decreased in the embryo of non dormant seeds incubated at 30°C for 24 h on a PEG solution at -2.5 MPa (Fig. 2C), even though no germination occurred during this time (data not shown). In contrast, raffinose did not significantly change within 24 h of incubation of dormant seeds at 20 and 30°C (Fig. 2A), and of non dormant ones at 20°C (Fig. 2B).

Sucrose content of the embryo remained constant in dormant seeds incubated at 30°C (Fig. 2A), whereas it slightly increased in the embryo of dormant seeds placed at 20°C (Fig. 2A) and in that of non dormant ones placed at 20 and 30°C (Fig. 2B).

The raffinose/sucrose ratio value remained then high in the embryo of dormant seeds at 20 and 30°C (Fig. 2C) and in that of non dormant ones at 20°C (Fig. 2D), but it strongly declined after 8 h of incubation in the case of non dormant seeds at 30°C, reaching 0.1 at 24 h (Fig. 2D).

CONCLUSION

➔ Germination was associated with a strong decrease in raffinose, but not in sucrose, thus leading to a sharp reduction in the raffinose/sucrose ratio value in the embryo of both dormant and non dormant seeds.

➔ Dormant seeds did not germinate at 30°C, and their embryo did not show any change in raffinose content nor in the raffinose/sucrose ratio.

➔ Our results demonstrate that the raffinose/sucrose ratio in the embryo might be a good criterion for detecting that sprouting occurred, even though any visible symptom is observed.

RESULTS

① Germination of dormant and non dormant seeds

Table 1 shows the germination percentages obtained with dormant and non dormant seeds imbibed for various durations on water at 20 and 30°C.

Non dormant seeds germinated rapidly at both temperatures; all the seed population germinated within 48 h. In contrast, freshly harvested seeds (dormant seeds) germinated at very low percentages at 30°C, whereas they were able to germinate at 20°C, but at a lower rate than non dormant seeds.

Dormant seeds did not germinate at all at 20 and 30°C on a PEG solution at -2.5 MPa, and only few non dormant seeds (8%) germinated within 48 h at 30°C whereas they were unable to do so within the same period of time at 20°C (data not shown).

Table 1- Germination percentages obtained after 8, 24 and 48 h with dormant (D) and non dormant (ND) seeds placed on water at 20 and 30°C. Means of 4 replicates \pm SD.

Imbibition time (h)	Germination (%) at			
	20°C		30°C	
	D	ND	D	ND
8	0	0	0	6 \pm 1
24	35 \pm 5	62 \pm 3	1 \pm 1	89 \pm 1
48	59 \pm 2	99 \pm 1	7 \pm 4	99 \pm 1

② Changes in sugar contents during imbibition on water

In the embryos of dry dormant and non dormant seeds, soluble sugars were mainly sucrose (73.0 mg g⁻¹ DW) and raffinose (32.2 mg g⁻¹ DW) (Fig. 1). Germination of dormant seeds at 20°C and non dormant ones at 20 and 30°C resulted in a decrease in raffinose as early as 8 h of incubation, and in sucrose after 24 h, i.e. after radicle protrusion (Figs 1A and 1C). Consequently, the raffinose/sucrose ratio declined rapidly (Figs 1B and 1D). In dormant seeds, which did not germinate at 30°C, soluble sugars remained constant at their initial levels (Fig. 1A), and the raffinose/sucrose ratio value was at least 0.45 within 24 h of imbibition (Fig. 1B).

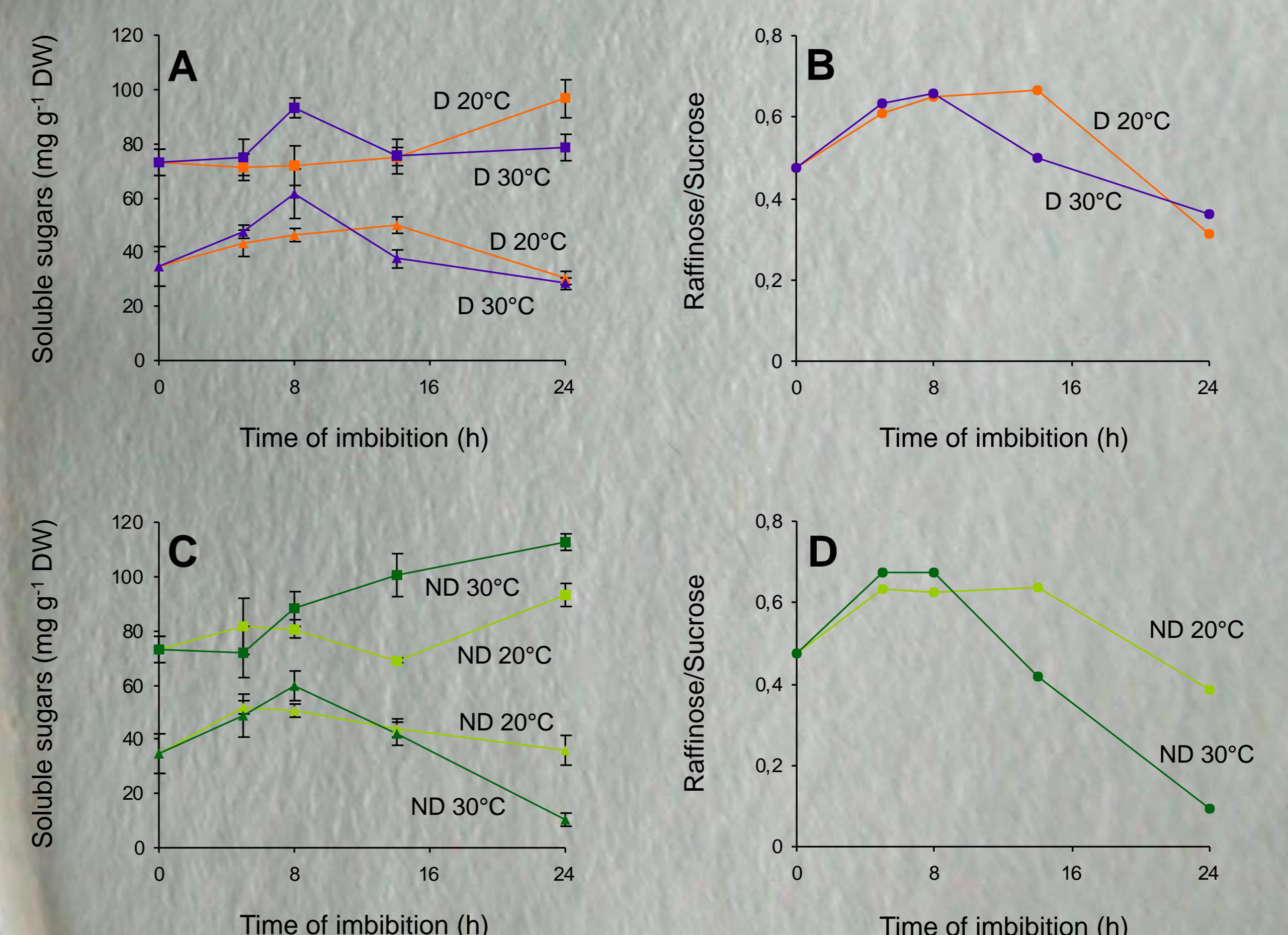


Figure 2 - Changes in sucrose (■) and raffinose (▲) contents (A, C) and in the raffinose/sucrose ratio (B, D) in embryos isolated from dormant (D) and non dormant (ND) seeds imbibed on a PEG solution at -2.5 MPa at 20 and 30°C. Arrows indicate the beginning of germination. Means of 3 measurements \pm SD. Where no bars are shown, the spread of SD is less than the size of the symbols.

PROCOLES

Seed imbibition : Seeds were placed on a layer of cotton wool moistened with distilled water or a PEG solution (-2.5 MPa) for 5, 8, 14 and 24 h at 20 and 30°C. At the end of the imbibition periods, embryos were immediately isolated and fixed in liquid nitrogen before sugar extraction.

Sugar extraction : Soluble sugars were extracted according to Black *et al.* (1996). Ten isolated embryos were ground at room temperature in a mortar in 1 ml 80% aqueous ethanol containing melezitose (1.5 mg ml⁻¹) as internal standard, and the extract was heated for 30 min at 80°C. After centrifugation for 15 min at 14 000 g and removal of the supernatant, the pellets were resuspended and reextracted twice, in 0.5 ml and then 0.35 ml 80% aqueous ethanol, at room temperature and centrifuged again. The three supernatants were combined and reduced to dryness in a centrifugal evaporator (RC 10-22, Jouan, France). The dry extracts were dissolved in 100 μ l of ultra-pure water and then filtered through acetate filter (0.45 μ m pore size, Naigene, USA) before being analysed by HPLC.

Sugar determination : Ten μ l samples of sugar extracts were injected onto a Spherisorb-NH2 column (Thermo Separation Products, France) and eluted with 70/30 (v/v) acetonitrile/H₂O 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, France), and the peak areas were integrated by a Spectra Physics 4290 integrator. Sucrose and raffinose were identified by co-elution with standards (Sigma, USA). The results are expressed as mg of sugars g⁻¹ DW of embryos and correspond to the means of 3 measurements \pm SD.

References : Black, M., Corbineau, F., Grzesik, M., Guy, P., Côme, D., 1996. Carbohydrate metabolism in the developing and maturing wheat embryo in relation to its desiccation tolerance. *Journal of Experimental Botany* 47, 161-169.

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