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Galactosyl-sucrose oligosaccharides and potential longevity of primed seeds

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Abstract

Correlative evidence indicates that sucrose and α-galactosyl-sucrose oligosaccharides (raffinose family oligosaccharides; RFOs) may be involved in seed longevity. Priming treatments (hydration in water or osmotic solutions followed by drying) can improve short-term seed performance but often result in reduced seed longevity. As RFOs are metabolized quickly following seed imbibition, loss of RFOs during priming could lead to more rapid deterioration in dry storage. This hypothesis was tested by measuring sucrose and oligosaccharide contents and potential longevity of primed seeds. Raffinose contents of whole lettuce (*Lactuca sativa* L.) seeds declined during hydration and priming and were correlated with decreased median potential viability (p_{50}). However, this relationship was less significant when only the embryonic axes were analysed. In tomato (*Lycopersicon esculentum* Mill.) and impatiens (*Impatiens balsamina* L.) seeds, planteose was the major galactosyl-sucrose oligosaccharide and only small quantities of RFOs were present. Planteose contents declined during priming in seeds of both species, while sucrose contents increased or remained constant. Post-priming treatments that restored longevity in primed impatiens and tomato seeds were not accompanied by consistent changes in RFO or planteose contents. Our data do not rule out a role for oligosaccharides in seed longevity, but they make it unlikely that changes in oligosaccharide contents alone are responsible for the reduction in longevity due to priming or its restoration by post-priming treatments.

Keywords: impatiens, *Impatiens balsamina* L., lettuce, *Lactuca sativa* L., oligosaccharides, planteose, raffinose, seed longevity, seed priming, tomato, *Lycopersicon esculentum* Mill.

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Introduction

Seed vigour is closely related to seed longevity, as seeds of low vigour generally have shorter potential longevity than high-vigour seeds (Ellis and Roberts, 1981; Sanhewe and Ellis, 1996). However, seed enhancement techniques involving hydration, such as priming or pregermination, can improve short-term germination vigour but often have detrimental effects on storage life of the subsequently dried seeds (Argerich and Bradford, 1989; Tarquis and Bradford, 1992; Bruggink *et al*., 1999). Desiccation tolerance *per se* is not lost in primed seeds, as they are capable of rehydrating and germinating rapidly following drying. Although repair mechanisms may be involved in the priming effect (McDonald, 1999), deterioration is none the less more rapid when primed seeds are stored dry compared to untreated seeds. Understanding how seed hydration and drying reduce storage life should provide insight into the relationship between desiccation tolerance and resistance to deterioration in dry seeds, as similar biochemical and biophysical mechanisms may be involved in both processes.

Recent evidence indicates that sugars and sugar derivatives may play a role in both desiccation tolerance and the longevity of dry seeds (Horbowicz and Obendorf, 1994; Brenac *et al*., 1997; Obendorf, 1997; Obendorf *et al*., 1998). Sugars, particularly disaccharides and small oligosaccharides, are involved in the maintenance of membrane integrity during dehydration (Crowe *et al*., 1988, 1989; Hoekstra *et al*., 1992; Oliver *et al*., 1998). Sugars also play a role in maintaining the proper three-dimensional structure of proteins, preventing their unfolding and denaturation due to loss of associated water (Crowe *et al*., 1992; Wolkers *et al*., 1998). Most seeds contain high concentrations of sucrose at maturity, and treatments that induce desiccation tolerance in germinated seeds result in accumulation of sucrose (Bruggink and van der Toorn, 1995). Thus, there is substantial evidence that sucrose plays a role in dehydration tolerance of seeds.

In addition to sucrose, many seeds also contain varying amounts of oligosaccharides, particularly α-D-

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galactosyl-sucrose derivatives known as the raffinose family oligosaccharides (RFOs) (Amuti and Pollard, 1977; Obendorf, 1997). Raffinose, stachyose and verbascose are formed by the sequential addition of one, two or three galactose units to sucrose via the galactosyl donor, galactinol. The presence of these (and other) oligosaccharides may enhance the ability of sucrose to protect lipid membranes during dehydration (Caffrey *et al*., 1988), although desiccation tolerance *per se* does not appear to require oligosaccharides (Ooms *et al*., 1993; Hoekstra *et al*., 1994; Lin *et al*., 1998). RFOs are also implicated in seed longevity, as seeds having ratios of sucrose to RFOs greater than 1.0 tend to possess shorter storage lives, while those having ratios less than 1.0 tend to have longer storage lives (Horbowicz and Obendorf, 1994). In addition to stabilizing membranes and proteins during dehydration, both sucrose and raffinose are correlated with the capacity of tissues to form a glassy state (Leprince *et al.,* 1993), which is a highly viscous solid state that retards molecular diffusion and slows deteriorative reactions. Dry seeds contain glasses (Burke, 1986), and among maize cultivars, both longevity in storage and raffinose content were highly correlated with the extent of glass formation in the seeds (Bernal-Lugo and Leopold, 1995). An increase in the molecular mobility of the cytoplasm in seeds is correlated with the decline in viability during accelerated ageing (Sun and Leopold, 1993; Buitink *et al*., 2000b).

Hydration and priming initiate germinative metabolism, and RFOs are among the first sugars to be metabolized following imbibition (Koster and Leopold, 1988; Dey, 1990; Hoekstra *et al*., 1994; Lin *et al*., 1998). Thus, the loss of RFOs during priming, and failure to accumulate them again during drying, could compromise glassy state formation in seeds and allow deteriorative reactions to occur more rapidly, leading to more rapid loss of viability under adverse storage conditions. To test this hypothesis, seed sucrose and oligosaccharide contents were examined before and after hydration and drying in relation to potential seed longevity. However, studies of the relationship between seed sugar contents and longevity are correlative. It is difficult to prove decisively that a given component has a critical role unless it can be selectively enhanced or removed. Thus, the methods of Bruggink *et al*. (1999) were utilized to extend longevity of primed seeds, and accompanying changes in sucrose and galactosyl-sucrose oligosaccharides in the seeds were investigated.

Materials and methods

Seed lots

Lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds produced in 1998 were provided by Seminis Vegetable Seeds Inc. (Oxnard, CA, USA) and impatiens (*Impatiens balsamina* L. cv. Accent Salmon) seeds produced in 1998 were provided by Goldsmith Seeds Inc. (Gilroy, CA, USA). Four lots of tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) seeds produced in the years 1995–1998 in the field at UC Davis (as described by Ni and Bradford, 1993) were also utilized in these studies.

Hydropriming and osmopriming

For hydropriming (imbibition in water followed by drying), lettuce, impatiens and tomato seeds were spread in a single layer on two germination blotters wetted with 125 ml water in glass dishes (25.2 × 37.5 × 3.5 cm) covered with plastic transparent wrapping. Lettuce seeds were incubated under diffuse fluorescent light and tomato seeds in the dark at 20°C, while impatiens seeds were incubated at 15°C under diffuse fluorescent light. The osmopriming procedure for lettuce seeds was identical to the method of hydropriming, except that the blotters were wetted with –1.2 MPa polyethylene glycol (PEG 8000) solution and incubated for 24 and 48 h. Osmopriming of impatiens (at 15°C) and tomato (at 20°C) seeds occurred in a cylinder (12.5 cm diameter × 40 cm high) containing twice the seed volume of an aerated –1.2 MPa solution of PEG 8000 (400 ml) for 72 or 144 h. In all cases, seeds were rinsed rapidly with water after incubation and surface water was removed by suction in a Buchner funnel.

Following hydropriming or osmopriming, seeds were dried rapidly in a ventilated oven for 2 d at 29°C to final moisture contents (MC) of 6–8% (fresh weight basis). Seed moisture contents approached the final values within 2 h for lettuce and impatiens seeds, and within 6 h for tomato seeds. In some experiments, a proportion of osmoprimed seeds was dried slowly at 20°C by spreading on a mesh for 2 d in a sealed chamber over 25% w/v glycerol (93% RH; –10.2 MPa). Initial drying was still relatively rapid, with loss of approximately half of the initial water content within the first 2 h of drying. Subsequently, the rate of drying declined exponentially and the seeds reached a final MC of 13–14%. The seeds were then dried to 6–8% MC using the rapid method. Following dehydration, all seeds were stored in airtight vials at –80°C.

While hydropriming is often not as effective as osmopriming in improving subsequent germination rates, the objective of these studies was not to optimize priming treatments, but rather to study the loss of seed longevity following priming. As hydropriming is simple, rapid and highly effective in reducing seed storage longevity (e.g. Tarquis and Bradford, 1992), this procedure was used in these studies.

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Post-priming treatments of tomato and impatiens seeds

Surface moisture of hydroprimed or osmoprimed seeds was removed by suction and blotting, and seed MC was determined before and after short-term drying in a ventilated oven without heat (29°C) to lower the total fresh weight by approximately 10% (e.g. 10 g of wet seeds were dried until the total weight reached 9 g). Following this reduction in MC, seeds were incubated in sealed boxes at 32, 37 or 40°C for different periods of time before rapid drying as described above. To determine the effects of different MC and temperatures during the post-priming treatment, the wet weight of hydroprimed tomato seeds was lowered by 1, 5 or 7%, and the seeds were then incubated for 3 h at 25, 30, 33, 37 or 40°C followed by rapid drying.

Determination of oligosaccharide contents

For lettuce, three replicates of 30 embryonic axes or five whole seeds were analysed for sucrose and oligosaccharide contents. For impatiens and tomato, three replicates of 30 micropylar regions (radicle tips and surrounding micropylar endosperm) or 30 whole seeds were analysed. After weighing, the samples were ground in liquid nitrogen in a mortar and pestle. Sugars were extracted with four successive 250 µl aliquots of 80% (v/v) ice-cold ethanol and further grinding. An internal standard (30 µl of 10 mM melezitose) was added with the initial aliquot of 80% ethanol. Pooled extracts were boiled for 5 min, centrifuged at 16,000 *g* for 10 min, and the supernatant immediately evaporated to dryness using a Speedvac concentrator (Savant Instruments Inc., Farmingdale, NY, USA). The resulting dry residue containing the ethanol-soluble sugars was dissolved in 0.5 ml distilled water and stored in sealed vials at –80°C until analysis.

Sugars were separated and quantified by high-performance anion-exchange chromatography (Dionex Bio-LC gradient pump and a 4 × 250 mm Carbopac PA-1 column; Dionex Corporation, Sunnyvale, CA, USA) attached to a pulsed amperometric detection system (HPAE-PAD) (Townsend, 1988a, b). The protocol was optimized for disaccharides and oligosaccharides, which were eluted with a solution of 168 mM NaOH and 6 mM sodium acetate. Pulse potentials used for detection were E1 = 0.06 V, E2 = 0.05 V, and E3 = –0.80 V with 540, 120 and 60 ms pulse durations. The detection output of the PAD was set to 300 mA. Amounts of sucrose and oligosaccharides were quantified utilizing the amperometric detector response factors (RF) of authentic standards relative to melezitose, setting the RF of melezitose as 1.0. All sugar contents are reported as µmol of sugar or oligosaccharide per g of tissue dry weight (i.e. at 6–8% MC).

A fraction containing an unidentified oligosaccharide was collected, but was partially hydrolysed when concentrated following elution, using a solution of 168 mM NaOH and 6 mM sodium acetate as eluate. Hence, NaOH (180 mM) was used to elute the unknown oligosaccharide peak, and the sodium was subsequently removed using a cation exchange resin (Marathon C, Sigma-Aldrich Laboratory Products, Milwaukee, USA) prior to the concentration step, resulting in a neutral pH aqueous solution containing the purified unknown sugar. Stepwise enzymatic analyses were performed to elucidate its identity as planteose, utilizing invertase (β-fructofuranosidase, EC 3.2.1.26, Sigma Chemicals, St. Louis, MO, USA), β-glucosidase (EC 3.2.1.21, Sigma) and α-galactosidase (EC 3.2.1.22, Calbiochem-Navabiochem Corporation, La Jolla, CA, USA) (Dey, 1990; Duke, 1992). We were unable to obtain an authentic sample of planteose, so its RF was derived by comparing the area of the planteose peak before hydrolysis with α-galactosidase with the peak areas of the products (sucrose and galactose) after complete hydrolysis (see Fig. 4B). Since planteose is hydrolysed to equimolar amounts of galactose and sucrose, having known RFs, the initial amount of planteose could be determined, and an RF of 0.678 was calculated for planteose relative to melezitose.

Controlled deterioration

Seeds for controlled deterioration tests were adjusted to 10–11% MC by incubation over saturated NaCl solution (75% RH) for 24 h at 2.5°C in a closed glass cylindrical vessel (20 cm diameter by 25 cm tall) containing a fan for air circulation. The seeds with elevated MC were sealed in microfuge tubes and placed inside a jar that was submerged in a heated water bath (40°C for lettuce and impatiens and 50°C for tomato). Samples were removed after different ageing periods and stored at –80°C until tested for viability.

Germination tests and seedling evaluations

Seeds were incubated in 4.7-cm diameter Petri dishes on single circular germination blotters wetted with distilled water (4–4.5 ml). Each dish contained 25 seeds and there were three replicates per treatment. Lettuce seeds were incubated under fluorescent light and tomato seeds in the dark at 20°C. Impatiens seeds were incubated at 15°C in the dark. Germination was scored as radicle protrusion of 1 mm. When a treatment was tested for germination for the first time, germinated seeds at each scoring interval were transferred to germination papers (double thickness) supported by Plexiglass plates. Plates were slanted and held in a tray of water incubated at 25°C, and seedlings were scored as normal or abnormal

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(International Seed Testing Association, 1999). Radicle emergence percentages were always highly correlated with normal seedling percentages, so the former was used routinely for determining potential viability.

Median germination rates (t_{50}) and median potential viability periods (p_{50}) were determined by probit analysis of germination or survival time courses using the SAS statistical package (SAS Institute Inc., Cary, NC, USA). Each protocol was replicated with different seed lots.

Results

Sucrose and RFO contents and p_{50} *following priming of lettuce seeds*

The effects of two types of hydration treatments on germination rates, potential seed longevity and sucrose and RFO contents of lettuce seeds were determined. Hydropriming for up to 7 h accelerated germination of lettuce seeds, but longer times were detrimental (Fig. 1A). By 16 h, some seeds had initiated radicle emergence, and germination of most seeds was delayed upon rehydration, indicating that some damage had occurred during drying or imbibition. All hydropriming treatments significantly reduced p_{50} (days of ageing required to reduce viability to 50%) (Fig. 1B). By the time the maximum reduction in t_{50} had been attained, p_{50} had been reduced by half, and thereafter, continued to decrease as t_{50} increased (Fig. 1C).

To test whether sucrose and/or RFOs are related to the reduction in p_{50}, soluble sugar and oligosaccharide contents of the hydroprimed seeds were determined. In whole seeds of lettuce, sucrose content decreased by 30%, while raffinose content declined by 75% during hydropriming (Fig. 2A). Consequently, the sucrose to raffinose ratio increased from 2.5 to 6.8. When p_{50} (from Fig. 1B) was plotted as a function of sucrose or raffinose contents, significant correlations were obtained for both sugars, but the relationship was more significant for raffinose than for sucrose (Fig. 2B). A decrease in soluble sugars might be more pronounced in embryonic axes, where germinative metabolism is initiated first and damage due to ageing is first evident (Tarquis and Bradford, 1992). Hence, sugar contents in embryonic axis tissues were also measured. In the axes, the sucrose content remained constant or rose slightly during 16 h of hydropriming, while total RFO content remained relatively constant (Fig. 3A). With respect to the individual RFOs, a

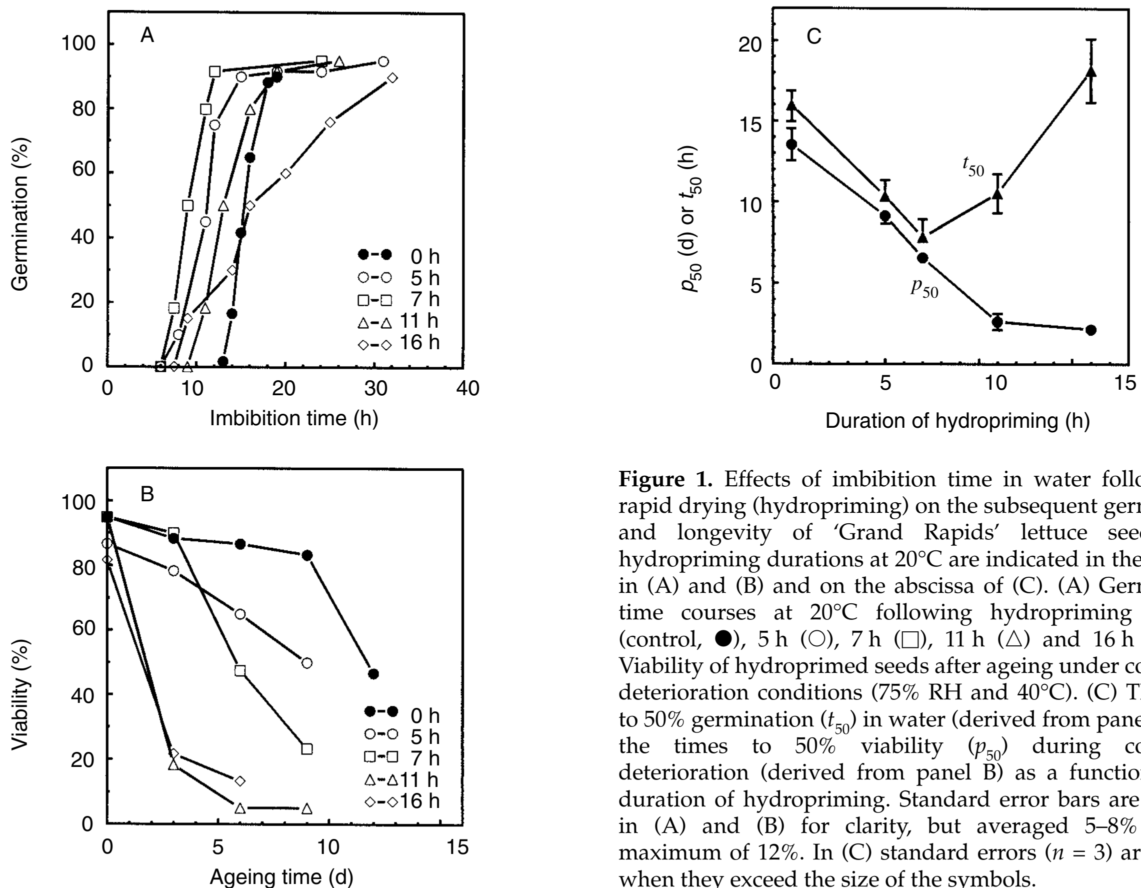

Figure 1. Effects of imbibition time in water followed by rapid drying (hydropriming) on the subsequent germination and longevity of 'Grand Rapids' lettuce seeds. The hydropriming durations at 20°C are indicated in the legends in (A) and (B) and on the abscissa of (C). (A) Germination time courses at 20°C following hydropriming for 0 h (control, ●), 5 h (○), 7 h (□), 11 h (△) and 16 h (◇). (B) Viability of hydroprimed seeds after ageing under controlled deterioration conditions (75% RH and 40°C). (C) The times to 50% germination (t_{50}) in water (derived from panel A) and the times to 50% viability (p_{50}) during controlled deterioration (derived from panel B) as a function of the duration of hydropriming. Standard error bars are omitted in (A) and (B) for clarity, but averaged 5–8% with a maximum of 12%. In (C) standard errors (n = 3) are shown when they exceed the size of the symbols.

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decline in raffinose content was largely compensated by the appearance of small amounts of verbascose and stachyose after 7 h of imbibition (Fig. 3B). As p_{50} declined linearly between 0 and 11 h of hydropriming (Fig. 3A), there was no evident relationship between longevity and sugar content of the axes.

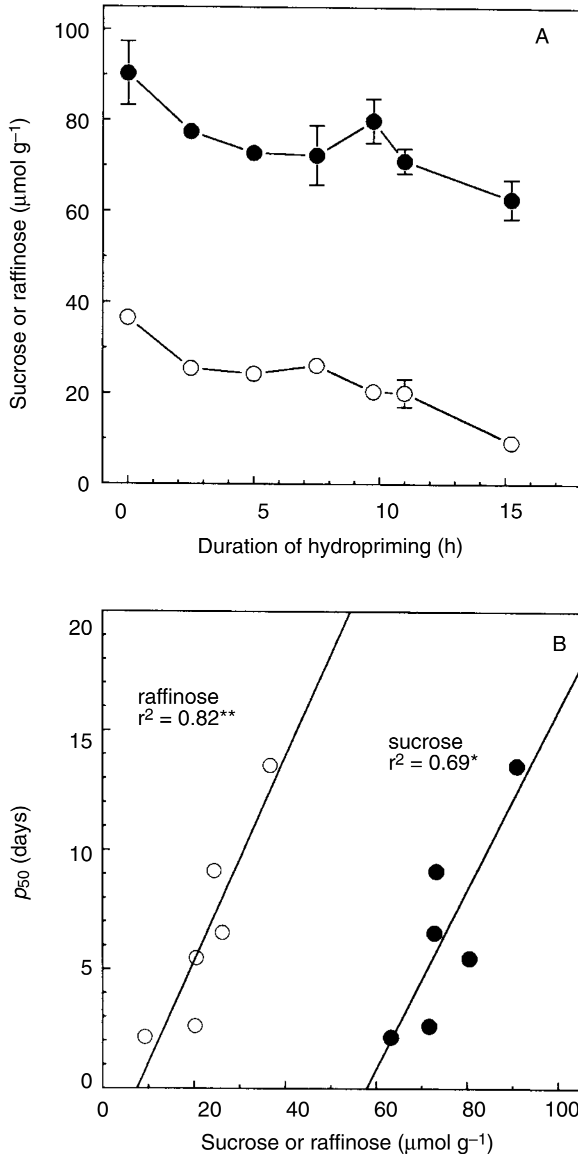

Figure 2. (A) Sucrose (●) and raffinose (○) contents of whole 'Grand Rapids' lettuce seeds as a function of hydropriming time. Error bars indicate ± SE (n = 3) when they exceed the size of the symbols. (B) Relationships between sucrose or raffinose contents of whole lettuce seeds and median potential longevity (p_{50}; data from Fig. 1) for hydroprimed seeds. Correlations were statistically significant at the 1% (**) or 5% (*) levels. Stachyose and verbascose contents in whole seeds were very low compared to raffinose (data not shown). All seeds were dried rapidly before sugar analyses.

In similar experiments using lettuce seeds osmoprimed for 24 or 48 h in –1.0 MPa PEG solutions, both priming durations reduced p_{50}, and raffinose contents declined in whole seeds and in axes (Table 1). Stachyose contents were low and did not vary consistently with treatment, while verbascose content increased during priming. The sucrose to RFO ratio tended to increase in osmoprimed seeds and axes (Table 1).

Sucrose and oligosaccharide contents and p_{50} following priming of tomato and impatiens seeds

Impatiens and tomato seeds contained relatively little raffinose or RFOs (Table 2). However, among the

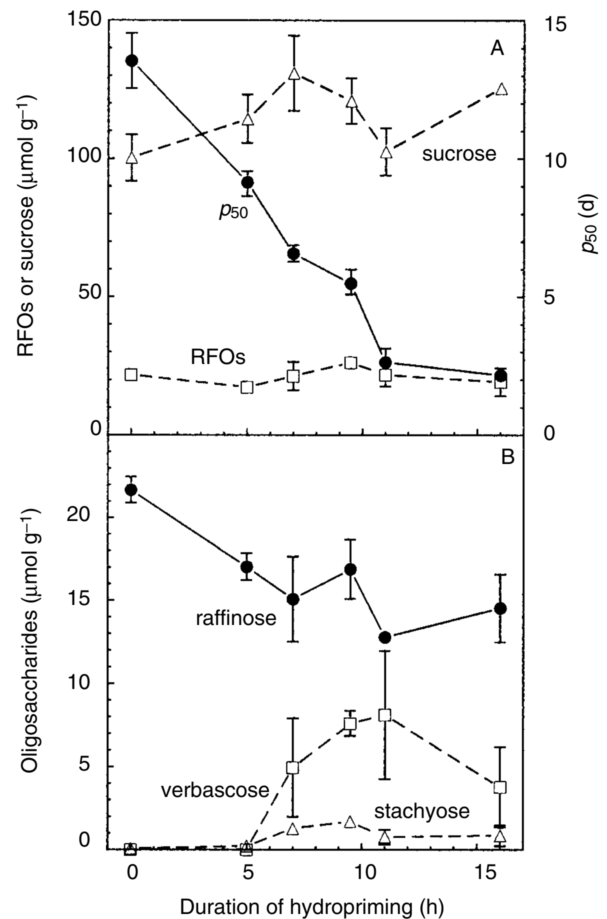

Figure 3. (A) Changes in p_{50} (median potential longevity measured by controlled deterioration) and in sucrose and total RFO contents of 'Grand Rapids' lettuce embryonic axes after hydropriming (imbibition in water at 20°C followed by rapid drying). (B) Changes in individual RFOs (raffinose, stachyose and verbascose) in lettuce embryonic axes during hydropriming. Note the differences in the y-axis scale of oligosaccharides in panels A and B. Error bars indicate ± SE (n = 3) when larger than the symbols.

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Table 1. Seed quality parameters (t_{50} and p_{50}) and sucrose and RFO contents of whole 'Grand Rapids' lettuce seeds and embryonic axes before and after osmopriming. Seeds were primed for 24 and 48 h in –1.0 MPa PEG at 20°C, followed by rapid drying before sugar analysis. Potential longevity (p_{50}) was determined by controlled deterioration at 75% RH and 40°C. Means ± SE (n = 3) are shown

Tissue	Treatment	t_{50} (h)	p_{50} (d)	Sucrose (μmol g^{-1})	Raffinose (μmol g^{-1})	Stachyose (μmol g^{-1})	Verbascose (μmol g^{-1})	Sucrose: RFO ratio
Seed	Control	15 ± 0.9	14 ± 2.0	83 ± 2.1	27 ± 1.9	4.0 ± 1	0 ± 0	2.7
	Primed (24 h)	13 ± 1.9	4 ± 0.2	82 ± 3.3	15 ± 1.9	1.1 ± 1	2.8 ± 1.3	5.1
	Primed (48 h)	13 ± 2.0	6 ± 0.1	102 ± 3.0	12 ± 1.5	2.1 ± 1	2.9 ± 1.3	6.0
Axis	Control			127 ± 3.6	40 ± 1.2	0.5 ± 1	0.6 ± 0	3.1
	Primed (24 h)			89 ± 2.5	24 ± 1.6	1.0 ± 1	2.0 ± 1	3.3
	Primed (48 h)			80 ± 2.8	15 ± 0.4	1.0 ± 1	1.0 ± 1	4.7

oligosaccharides present in tomato and impatiens seeds were significant quantities of one that did not belong to the raffinose family, based on its retention time in HPAE-PAD (Fig. 4A) and thin-layer chromatography (data not shown). Incubation of the oligosaccharide with invertase (Fig. 4A) or β-glucosidase (data not shown) alone did not liberate any products, even after 24 h of incubation. Incubation with α-galactosidase at pH 7 liberated equimolar amounts of D-galactose and sucrose (Fig. 4B). When these products were further hydrolysed with invertase, the sucrose was converted to D-glucose and D-fructose (Fig. 4C). D-Glucose and D-galactose resolved at the same retention time in this chromatographic system, but it was evident that the specific enzymes used in the stepwise digestions liberated galactose first and then glucose plus fructose.

Invertase hydrolyses only terminal non-reducing β-D-fructofuranoside residues (Dey, 1990), so the inactivity of invertase on the unknown trisaccharide revealed that it does not contain a terminal β-D-fructofuranoside. However, α-galactosidase released galactose and sucrose, indicating that the fructofuranoside unit was present between α-D-

Table 2. Seed potential viability (p_{50}) and soluble sugars in micropylar tissues (endosperm caps and radicle tips) of 'Accent Salmon' impatiens seeds and two lots (1996 and 1997) of 'Moneymaker' tomato seeds before and after osmopriming followed by different methods of drying. Seeds were primed in –1.2 MPa PEG for 3 or 6 d, followed by either rapid or slow drying. For rapid drying, seeds were held in a ventilated oven at 29°C until the moisture content reached 6–8%. For slow drying, seeds were held for 2 d at 93% RH and 20°C until the moisture content reached 13% (fresh weight basis) followed by drying as above to 6–8% moisture content. Only trace amounts of stachyose and verbascose were detected (data not shown). Median potential longevity (p_{50}) was determined by controlled deterioration at 75% RH and either 40°C (impatiens) or 50°C (tomato). Means ± SE (n = 3) are shown

Treatment	Drying rate	p_{50} (d)	Sucrose (μmol g^{-1})	Planteose (μmol g^{-1})	Raffinose (μmol g^{-1})
Impatiens					
Control		8.2 ± 0.1	6 ± 1	58 ± 12	0.2 ± 0.2
Primed (3 d)	Rapid	6.5 ± 0.3	39 ± 9	60 ± 2	0.5 ± 0.0
	Slow	8.5 ± 1.0	21 ± 8	42 ± 2	1.0 ± 0.2
Primed (6 d)	Rapid	4.7 ± 0.2	29 ± 4	29 ± 2	0.2 ± 0.1
	Slow	7.3 ± 0.3	44 ± 2	28 ± 2	0.1 ± 0.1
Moneymaker 1996					
Control		>40	51 ± 3	25 ± 2	0.4 ± 0.9
Primed (2 d)	Rapid	8.3 ± 0.2	66 ± 11	29 ± 5	4.1 ± 0.3
	Slow	8.4 ± 0.3	66 ± 3	28 ± 2	3.8 ± 0.3
Primed (4 d)	Rapid	9.2 ± 0.2	66 ± 8	22 ± 1	1.2 ± 0.2
	Slow	11.0 ± 0.5	69 ± 2	25 ± 2	3.4 ± 0.4
Moneymaker 1997					
Control		18 ± 3.3	45 ± 3	25 ± 2	2.8 ± 0.9
Primed (3 d)	Rapid	6.6 ± 3.3	57 ± 11	24 ± 1	2.1 ± 0.3
	Slow	7.6 ± 0.3	54 ± 3	23 ± 2	1.7 ± 0.3
Primed (6 d)	Rapid	6.7 ± 0.6	50 ± 8	17 ± 2	0.8 ± 0.2
	Slow	6.3 ± 0.6	58 ± 2	22 ± 1	1.9 ± 0.4

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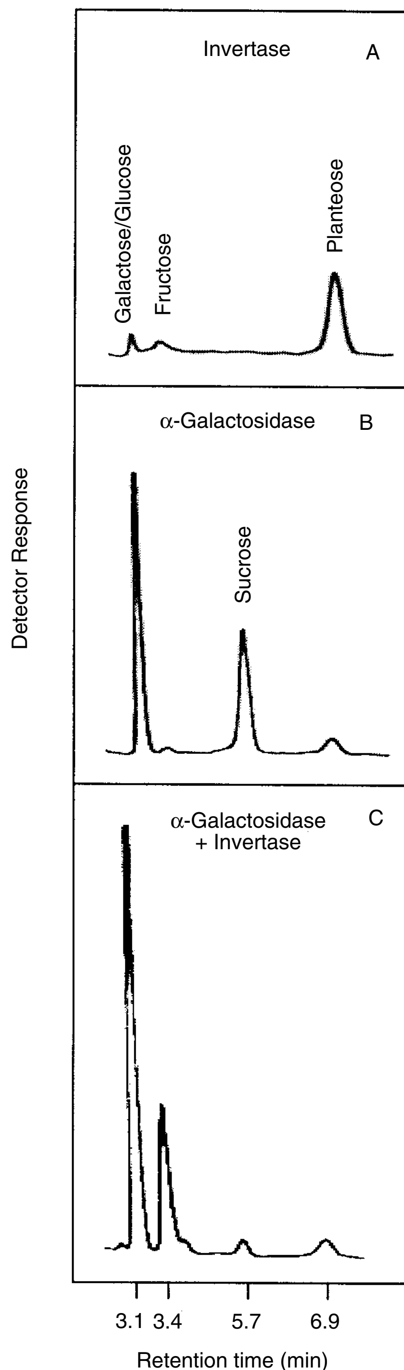

Figure 4. Chromatograms showing stepwise enzymatic hydrolysis of an unidentified sugar isolated from impatiens and tomato seeds. (A) The intact trisaccharide (labelled planteose) was not hydrolysed by overnight incubation with invertase. (B) Hydrolysis with α-galactosidase released D-galactose and sucrose with a corresponding reduction in the unknown (planteose) peak. (C) Hydrolysis with α-galactosidase followed by invertase released D-glucose (co-eluted with D-galactose) and D-fructose. This pattern of hydrolysis indicates that the unknown trisaccharide is planteose.

galactopyranosyl and α-D-glucopyranoside units. Therefore, the unidentified sugar has the characteristics of planteose ([*O*-α-D-galactopyranosyl-(1–6)-β-D-fructofuranosyl-α-D-glucopyranoside]; Wattiez and Hans, 1943; French *et al*., 1959), which has previously been reported to be present in seeds of the *Solanaceae* (Duke, 1992) and *Balsaminaceae* (Youcef *et al*., 1977).

Impatiens and tomato seeds were osmoprimed, followed by either rapid or slow drying. Osmopriming reduced p_{50} of seeds of both species (Table 2). Drying rate had little effect on p_{50} of tomato seeds, but slow drying did have a small beneficial effect on p_{50} of impatiens seeds (Table 2). Sucrose contents increased markedly in impatiens seeds and less so in tomato seeds due to priming, while planteose contents decreased with extended priming. Raffinose contents were low in both types of seeds and did not change consistently with priming or drying treatments (Table 2).

There was a sharp decline in p_{50} after hydropriming impatiens seeds for 36 h and tomato seeds for 24 h (Fig. 5). RFO contents of the micropylar tissues were low and changed little during hydropriming in both impatiens and tomato. Micropylar tissues of impatiens seeds contained large amounts of planteose, which declined somewhat with longer durations of hydropriming, as sucrose content increased (Fig. 5A). Similarly, the amount of planteose in tomato micropylar tissues declined concomitantly with the decrease in p_{50}, while sucrose content increased somewhat (Fig. 5B). The decreases in p_{50} and in planteose content were significantly correlated for impatiens seeds ($r^2 = 0.59$, $P < 0.07$) and for the 1996 lot of tomato seeds ($r^2 = 0.77$, $P < 0.009$), but not for the 1997 tomato seed lot in a second experiment (data not shown). No significant relationship was found between seed RFO content and p_{50} in any experiment with impatiens or tomato.

Effects of post-priming treatments on seed longevity and oligosaccharide contents

Post-priming treatments, involving a slight reduction of seed MC and incubation at elevated temperatures before drying, have been reported to extend seed longevity (Bruggink *et al*., 1999). The effects of these treatments on longevity and oligosaccharide contents were tested following hydration of tomato and impatiens seeds. Seeds were either osmo- or hydroprimed sufficiently to result in a reduction in p_{50} following rapid drying, but after the hydration, seeds were allowed to dry until they had lost 10–13% of their wet weight. They were then held at 32°C for 2 d or at 40°C for 3 h before rapid drying. In both species, these treatments largely prevented the loss of viability exhibited by primed seeds during controlled

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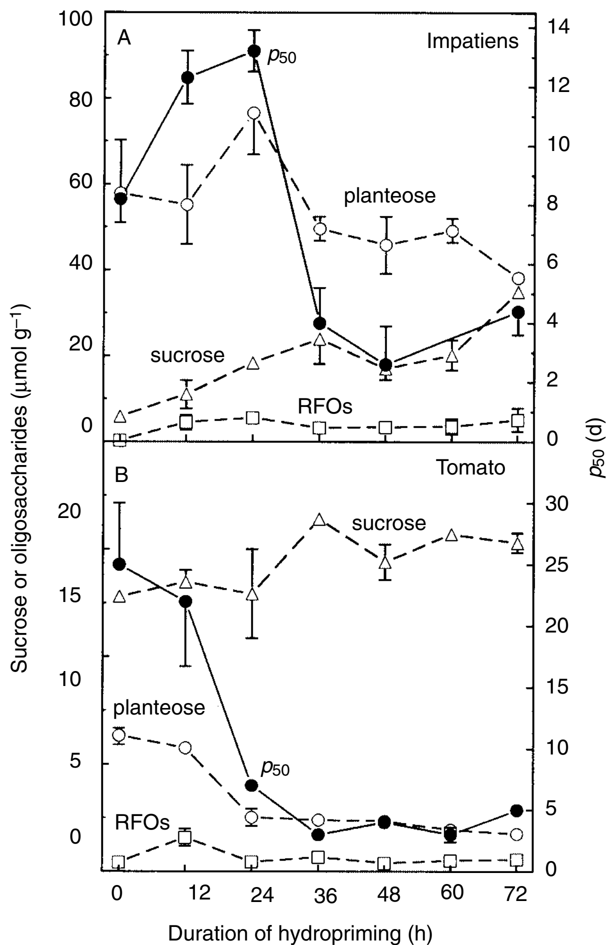

Figure 5. Changes in median potential longevity (p_{50}) and sucrose and oligosaccharide contents of micropylar regions (radicle tips and surrounding endosperm tissue) of (A) 'Accent Salmon' impatiens seeds and (B) 'Moneymaker' tomato seeds (1996 lot) with increasing duration of hydropriming (imbibition in water followed by rapid drying). Standard errors (n = 3) are shown when they exceed the size of the symbols.

deterioration (Table 3). Remarkably, the tolerance to ageing of impatiens seeds treated in this way was even greater than that of the control seeds. Two days at 32°C, or only 3 h at 40°C, had similar effects on tomato seed longevity, although the former treatment had the additional effect of improving germination rate, probably by extending the priming duration (Table 3).

To determine whether both the initial reduction in moisture content and the high-temperature incubation were required for extended longevity, tomato seeds were imbibed in water for 24 h at 25°C. If the seeds were dried rapidly immediately following hydropriming, p_{50} was reduced from 19 to 5.1 d during controlled deterioration. Other seeds were dried by 1, 5 or 7% of their initial wet weight and then incubated at 25, 30, 33, 37 or 40°C for 3 h before rapid drying. When the post-priming reduction in wet weight of the seeds was less than 10%, little or no increase in longevity occurred at any temperature (Table 4). Neither the smaller wet weight reductions nor the higher temperatures were effective in this test in extending longevity of the hydroprimed seeds. A slight improvement of p_{50} was evident only at 40°C, throughout the range of wet weight reductions (Table 4). Additional treatments where wet weight was reduced by 10% and seeds were incubated at a lower temperature (25°C) were also ineffective in extending longevity (data not shown), while incubation of the same seed lot at 40°C for 3 h after 10% wet weight reduction resulted in improvement of p_{50} (Table 5). Thus, it appears that a combination of both an initial reduction in wet weight of at least 10% and incubation at elevated temperature were required to extend longevity of primed tomato seeds.

As these post-priming treatments had a beneficial effect on seed longevity, it was then determined whether this was associated with changes in seed sucrose and oligosaccharide contents. Hydropriming reduced p_{50} of two tomato seed lots, and post-priming treatments at 32, 37 or 40°C were effective in substantially restoring seed longevity (Table 5). However, there was no obvious relationship between soluble oligosaccharide contents of micropylar tissues and seed longevity (Table 5). Changes in sucrose and oligosaccharide contents were generally relatively small and were not consistently related to variation in p_{50}.

Further observations were made by withdrawing seed samples at hourly intervals during the high-temperature incubation at 37°C and 40°C and then determining p_{50} and whole-seed sucrose and oligosaccharide contents (Table 6). In experiment 1, the p_{50} decreased from 24 to 6 d due to hydropriming and rapid drying, but this increased to 13–17 d following the best post-priming treatments (Table 6). The benefits of incubation at 40°C decreased after 2 h exposure to the high temperature. This reduction in effectiveness of the heat treatment with increasing time at 40°C was confirmed in a second experiment with a different seed lot (Table 6). In this experiment, p_{50} was reduced from 26 to 12 d by the hydropriming treatment, but was restored to 28 d by incubation for 1 h at 40°C after a 10% wet weight reduction. However, 2 or 3 h at 40°C resulted in p_{50} values of only 17 d, which none the less was still better than the hydroprimed and rapidly dried seeds (Table 6). There was no clear relationship among whole-seed sucrose and RFO contents and p_{50} (Table 6). Raffinose and verbascose contents were low and did not change consistently with the treatments. Planteose, the major oligosaccharide found in tomato seeds, consistently

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Table 3. Effects of post-priming treatments on viability of 'Accent Salmon' impatiens and 'Moneymaker' tomato seeds. Impatiens seeds were primed in –1.2 MPa PEG for 24 h at 15°C, followed by either rapid drying or loss of 10% wet weight and incubation at 32°C for 2 d before rapid drying. Tomato seeds (1995 lot) were hydroprimed for 18 h and then were treated as above. Sugar contents, mean germination rate (t_{50}) and viability were determined, and seeds were subjected to controlled deterioration at 75% RH and either 40°C (impatiens) or 50°C (tomato) for 12 d. Means ± SE (n = 3) are shown

Species	Priming/post-priming treatment	t_{50} (h)	Viability before ageing (%)	Viability after 12 d of ageing (%)
Impatiens	Control	175 ± 4.5	99 ± 1	44 ± 7
	Osmoprimed	160 ± 2.1	99 ± 1	9 ± 2
	Osmoprimed, then held at 32°C for 2 d	164 ± 0.8	99 ± 1	96 ± 3
Tomato	Control	121 ± 4.8	98 ± 1	93 ± 2
	Hydroprimed	89 ± 9.5	99 ± 0	26 ± 8
	Hydroprimed, then held at 32°C for 2 d	66 ± 9.5	99 ± 0	96 ± 1

decreased by approximately 50% due to hydropriming, but did not return to original levels during the heat treatments (Table 6).

Discussion

It is a reasonable hypothesis that loss of sucrose or (particularly) oligosaccharides during priming, and failure to reaccumulate them upon drying, might contribute to the reduced longevity of primed seeds (Fig. 1). There were significant correlations between the decline in sucrose and raffinose contents of whole hydroprimed lettuce seeds and the reduction in p_{50} (Fig. 2B). Since the axes are the site of initial growth associated with germination, and are the tissue most sensitive to ageing (Tarquis and Bradford, 1992), sugar contents were also measured in this tissue only. While p_{50} declined linearly between 0 and 11 h of hydropriming, neither sucrose nor total RFO contents showed a consistent decrease in lettuce embryonic axes (Fig. 3A), although raffinose content did exhibit a declining trend (Fig. 3B), as was observed in whole seeds (Fig. 2). In lettuce embryonic axes, the r^2 of the regression of p_{50} on raffinose content was only 0.64 ($P < 0.05$), compared to 0.87 ($P < 0.006$) for whole seeds. The ratio of sucrose to total RFOs increased following osmopriming, but all values were greater than 1.0 (Table 1), which has been suggested to be associated with shorter storage life (Horbowicz and Obendorf, 1994; Brenac *et al*., 1997). While a decrease in lettuce seed raffinose content was correlated with reduced longevity, it is not possible from these experiments to test whether the relationship is causal. The interpretation is further complicated by the fact that considerable amounts of sucrose and raffinose were still present even in seeds having reduced longevity (Figs 2 and 3; Table 1).

An unknown oligosaccharide was present in large amounts in seeds of both impatiens and tomato. This oligosaccharide was identified as planteose via a series of enzymatic analyses (Fig. 4). Planteose is known to be resistant to hydrolysis by invertase (Dey, 1990; Fig. 4A), which ruled out the possibility that the unknown trisaccharide was raffinose or its isomer umbelliferose (found only in the order *Umbellales*; Cronquist, 1968). Invertase readily releases melibiose

Table 4. Effect of different reductions in seed wet weight following hydropriming and temperatures of post-priming incubation on potential viability (p_{50}). 'Moneymaker' tomato seeds (1997 lot) were hydroprimed for 24 h at 25°C, then allowed to lose 1, 5 or 7% of their wet weight before incubation at 25, 30, 33, 37 and 40°C for 3 h before rapid drying. The p_{50} value of non-primed (control) seeds was 19 d, while that of hydroprimed seeds incubated at 27°C without an initial reduction in wet weight was 5.1 d. In a separate experiment with the same seed lot, the p_{50} value after 10% reduction in wet weight and incubation at 40°C for 3 h was 18 d, as shown in Table 5

Temperature of incubation	p_{50} (days)		
(°C)	1% reduction in wet weight	5% reduction in wet weight	7% reduction in wet weight
25	4.8 ± 0.2	5.6 ± 0.6	7.5 ± 0.2
30	6.6 ± 0.6	5.7 ± 1.0	6.4 ± 0.1
33	5.8 ± 0.3	5.1 ± 0.2	6.1 ± 0.1
37	4.7 ± 0.3	6.2 ± 0.6	5.5 ± 0.5
40	9.2 ± 0.5	8.4 ± 0.7	8.7 ± 0.3

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Table 5. Effects of post-priming treatments on median germination rate (t_{50}), median potential viability (p_{50}) and soluble sugar contents of tomato seeds. 'Moneymaker' tomato seeds (1995 and1997 lots) were hydroprimed for 18 or 24 h at 20°C, then either rapidly dried or allowed to lose 10% fresh weight before incubation at 32°C or 37°C for 2 d or 40°C for 3 h before rapid drying. Contents of sucrose, planteose, raffinose, stachyose and verbascose were quantified in dry micropylar tissues. Means ± SE (n = 3) are shown

Treatment	t_{50} (h)	p_{50} (d)	Sucrose (μmol g^{-1})	Planteose (μmol g^{-1})	Raffinose (μmol g^{-1})	Stachyose (μmol g^{-1})	Verbascose (μmol g^{-1})
1995 seed lot							
Control	150 ± 1.2	>40	25 ± 1	18 ± 1	1.6 ± 0.1	1.2 ± 0.2	0.4 ± 0.2
Hydroprimed (18 h)	135 ± 1.2	9 ± 0.5	22 ± 4	12 ± 2	1.7 ± 0.6	0.6 ± 0.2	1.1 ± 0.3
32°C, 2 d	80 ± 4.1	17 ± 0.3	32 ± 4	10 ± 2	2.2 ± 0.5	0.6 ± 0.4	0.4 ± 0.4
40°C, 3 h	130 ± 4.3	39 ± 1.2	21 ± 2	9 ± 2	1.9 ± 0.7	0.7 ± 0.5	0.1 ± 0.3
1997 seed lot							
Control	106 ± 1.4	17 ± 0.3	24 ± 1	18 ± 1	1.6 ± 0.2	1.1 ± 0.2	0.4 ± 0.4
Hydroprimed (24 h)	104 ± 1.7	11 ± 0.1	27 ± 5	21 ± 2	0.7 ± 0.5	0.7 ± 0.6	0.7 ± 0.2
37°C, 2 d	123 ± 3.2	24 ± 5.0	23 ± 3	14 ± 2	1.9 ± 0.4	0.7 ± 0.3	1.1 ± 1.9
40°C, 3 h	111 ± 0.9	18 ± 0.3	22 ± 2	14 ± 2	1.0 ± 0.5	0.8 ± 0.3	0.0 ± 0.0

or isomelibiose, respectively, by cleaving the exposed terminal non-reducing β-D-fructofuranoside residue from these trisaccharides (Dey, 1990). Cleavage of the trisaccharide by α-galactosidase to release galactose and sucrose (Fig. 4B) is characteristic of planteose (Dey, 1990). Planteose was present in all parts of the tomato seed, including the radicle tip, endosperm and the rest of the embryo, and in seeds of other species of the *Solanaceae*, including potato and tobacco (data not shown; Amuti and Pollard, 1977; Duke, 1992). Planteose has also been found in impatiens seeds (Youcef *et al.*, 1977), and it is likely that the unidentified oligosaccharide reported by Buitink *et al.* (2000a) in impatiens and pepper (*Capsicum annuum* L.) seeds is also planteose. No information is available on the effectiveness of planteose as a desiccation or

Table 6. Effects of post-priming treatments on median germination rate (t_{50}), median potential viability (p_{50}) and soluble sugar contents of tomato seeds. In two experiments, 'Moneymaker' tomato seeds (from two separate harvests in 1998) were hydroprimed for 24 h at 25°C and either rapidly dried or allowed to dry until they lost 10% of their wet weight. Following the 10% reduction in wet weight, some seeds were rapidly dried while others were incubated at either 37°C or 40°C for 1, 2, 3 or 4 h before rapid drying. Median potential longevity (p_{50}) was determined by controlled deterioration at 75% RH and 50°C. Contents of sucrose, planteose, raffinose and verbascose were quantified in whole dry seeds. Means ± SE (n = 3) are shown

Treatment	p_{50} (d)	Sucrose (μmol g^{-1})	Planteose (μmol g^{-1})	Raffinose (μmol g^{-1})	Verbascose (μmol g^{-1})
Experiment 1					
Control	24 ± 3.5	8.9 ± 0.5	10.0 ± 0.7	1.2 ± 1.0	0.7 ± 0.3
Hydroprimed (24 h)	6 ± 0.2	12.0 ± 1.8	4.7 ± 0.8	0.5 ± 1.0	0.6 ± 0.1
10% wet wt reduction	7 ± 0.2	9.8 ± 0.1	4.5 ± 0.7	0.7 ± 0.7	0.7 ± 0.2
37°C, 1 h	7 ± 0.1	6.5 ± 1.0	5.2 ± 0.1	0.9 ± 0.5	0.8 ± 0.2
37°C, 2 h	13 ± 0.5	7.9 ± 1.0	6.7 ± 0.3	0.8 ± 0.2	0.8 ± 0.1
37°C, 3 h	13 ± 1.2	7.4 ± 0.7	6.1 ± 0.1	0.6 ± 0.2	1.0 ± 0.5
37°C, 4 h	17 ± 0.1	10.0 ± 0.3	3.5 ± 0.4	0.9 ± 0.4	0.3 ± 0.5
40°C, 1 h	7 ± 0.5	8.1 ± 1.0	6.5 ± 0.1	0.9 ± 0.3	0.7 ± 0.2
40°C, 2 h	16 ± 0.5	8.5 ± 0.8	5.3 ± 0.7	1.5 ± 0.3	2.1 ± 0.2
40°C, 3 h	9 ± 0.3	11.0 ± 3.2	5.4 ± 0.1	1.0 ± 0.1	0.9 ± 0.3
40°C, 4 h	9 ± 0.3	6.9 ± 0.5	3.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.3
Experiment 2					
Control	>26	8.8 ± 0.6	13.5 ± 1.0	0.72 ± 0.5	0.49 ± 0.0
Hydroprimed (24 h)	12 ± 0.3	8.8 ± 2.0	8.1 ± 1.7	0.61 ± 0.1	0.75 ± 0.3
40°C, 1 h	28 ± 4.0	7.1 ± 1.0	9.4 ± 0.5	0.76 ± 0.0	0.95 ± 0.2
40°C, 2 h	17 ± 1.2	5.9 ± 0.0	7.5 ± 0.6	0.86 ± 0.2	0.74 ± 0.2
40°C, 3 h	17 ± 1.3	5.7 ± 4.9	7.2 ± 0.9	1.1 ± 0.3	0.50 ± 0.1

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deterioration tolerance factor, but it was abundant in both tomato and impatiens seeds (Fig. 5; Tables 2, 5, 6), which are considered to be good- and poor-storing seeds, respectively.

In both impatiens and tomato seeds, planteose content decreased during hydropriming while sucrose content increased (Fig. 5). There was a significant negative correlation ($r^2 = 0.83$, $P < 0.0001$) between sucrose and planteose contents in tomato (1996 and 1997 lots) and impatiens seeds when all data were combined, suggesting metabolic breakdown of planteose to release sucrose during imbibition. Total RFO contents were low and changed little during hydropriming in both species, and were not correlated with changes in p_{50} (Fig. 5). The reduction in p_{50} following hydropriming was significantly correlated with the decline in planteose content in impatiens seeds and one lot of tomato seeds, but not in the other tomato seed lot. The results confirm that planteose is rapidly metabolized following seed imbibition as p_{50} also declines, but do not establish a causal relationship between these two events.

As some reports indicated that slow drying would improve longevity following priming (Bruggink *et al.*, 1997), osmoprimed impatiens and tomato seeds were dried for 2 d at 93% RH until the MC was 13%, then dried to 6–8% MC as for the rapid drying treatment. Osmopriming decreased p_{50} of both impatiens and tomato seeds, and while slow drying was slightly beneficial for impatiens seeds, drying rate had little effect on p_{50} of tomato seeds (Table 2). Overall, the drying method did not affect sugar content of micropylar tissues in either tomato or impatiens seeds (Table 2). Sucrose content increased during osmo-priming in both tomato and impatiens, but planteose content decreased only in impatiens micropylar tissues.

Post-priming treatments involving a slight reduction of seed MC and incubation at elevated temperatures before drying increase the longevity of primed seeds (Bruggink *et al.*, 1999). As an additional approach to test the role of oligosaccharides in seed longevity, the sugar contents of treatments successful in restoring seed longevity were analysed. Those treatments included an initial 10% reduction in seed wet weight after priming, followed by incubation at 32, 37 or 40°C (Tables 3, 5, 6). Both an initial reduction in seed wet weight of at least 10% and incubation at elevated temperature were required for extension of longevity (Table 4). However, regardless of the effect of these treatments on p_{50}, no unique relationship was found between longevity and sucrose or oligosaccharide content (Tables 5, 6).

While there are a number of reports suggesting a role for sucrose and oligosaccharides in seed longevity, conclusive non-correlative evidence is difficult to find. Studies in *Arabidopsis* using recombinant inbred lines differing in RFO content failed to find any significant genetic relationship between seed oligosaccharide content and tolerance to controlled deterioration (Groot *et al.*, 2000). Furthermore, Buitink *et al.* (2000a) examined the intracellular glass stability of impatiens and pepper seeds before and after priming and could not detect significant changes in the mobility of spin probes that were introduced into the cytoplasm, despite a reduction in seed oligosaccharide content and p_{50} due to priming. Since improving the stability of intracellular glasses is one of the mechanisms by which oligosaccharides are proposed to increase storage life (e.g. Bernal-Lugo and Leopold, 1995), these biophysical data do not support the hypothesis.

In summary, since RFOs and other oligosaccharides are often metabolized during seed imbibition and priming, a reduction in oligosaccharide content of primed seeds could lead to more rapid deterioration in storage. In lettuce seeds, both sucrose and raffinose contents decreased as p_{50} declined during hydration or priming. However, this relationship was less convincing in the axis tissues, where deterioration first becomes evident. In impatiens and tomato seeds, planteose was the major oligosaccharide present. Planteose content decreased and sucrose content increased during hydration or priming, with these changes being somewhat correlated with the decrease in p_{50}. Restoration of tolerance to controlled deterioration by post-priming treatments, however, was not associated with concomitant changes in tissue sucrose or oligosaccharide content. These results do not rule out a role for oligosaccharides in seed longevity, as the restoration of tolerance to controlled deterioration by post-priming treatments may involve different mechanisms than are present initially in mature seeds. None the less, these data do not support the hypothesis that oligosaccharide loss is responsible for the reduced longevity of primed seeds.

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