Pericarp, Leachate, and Carbohydrate Involvement in Thermoinhibition of Germinating Spinach Seeds

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ABSTRACT. Spinach (*Spinacia oleracea* L.) seed germination can be inhibited by high temperatures. An understanding of thermoinhibition in spinach is critical in predicting germination and emergence events. The purpose of this study was 3-fold: 1) to determine seed germination percentage and rate of spinach genotypes—‘Cascade’, ‘ACX 5044’, ‘Fall Green’, and ‘ARK 88-354’—exposed to constant and alternating temperatures; 2) to determine the nature and extent of inhibition imposed by the pericarp; and 3) to investigate leachate and oligosaccharide involvement in thermoinhibition. Germination inhibition began at >20 °C constant temperature and was totally suppressed at 35 °C. Alternating temperatures at 30/15 °C (12-hour day/12-hour night) resulted in greater germination than a constant 30 °C. The genotype sensitivity to supraoptimal temperatures was in the order of ‘ARK 88-354’ ≤ ‘Fall Green’ < ‘ACX 5044’ < ‘Cascade’, but the highly thermoinhibited ‘Cascade’ seeds retained the ability to germinate when shifted to lower incubation temperatures. The pericarp inhibited germination, since seeds deprived of the pericarp had ≈90% germination at 30 °C. ‘ACX 5044’ and ‘Cascade’ had higher ABA content in the pericarp than ‘ARK 88-354’ and ‘Fall Green’. Before imbibition at 30 °C, raffinose levels in each genotype were in the order of ‘ARK 88-354’ > ‘Fall Green’ > ‘Cascade’. After 48 hours of imbibition, sucrose and glucose levels were highest and raffinose levels were lowest in ‘ARK 88-354’ and ‘Fall Green’ seeds, while ‘Cascade’ seeds remained less active metabolically. These data suggest that the pericarp apparently acts as a physical barrier as well as a source of inhibitors during thermoinhibition.

Spinach (*Spinacia oleracea*) seed germination is inhibited by temperatures higher than 30 °C (Heydecker and Orphanos, 1968). Spinach plantings in southwest Texas occur between August and December when temperatures typically average 36/20 °C (maximum/minimum). Consequently, yields can be reduced by poor field emergence and by low and variable plant stands.

Fluctuating temperatures partially overcome primary dormancy acquired during seed development in *Sorghum halepense* (L.) Pers. (Benec Arnold et al., 1988) and *Nicotiana tabacum* L. (Bewley and Black, 1994). It is unknown whether alternating temperatures alleviate thermoinhibition in spinach genotypes, and whether seeds exposed to high temperatures lose their ability to germinate if transferred to lower temperatures. Pericarp removal promoted spinach seed germination at high temperatures (Atherton and Farooque, 1983a), but the mechanism, physical or chemical, is unclear. Removal of water soluble chemical inhibitors may be an important step to alleviate seed thermoinhibition, but it is unknown for spinach genotypes. Methods to remove seed inhibitors or to release seeds from thermodormancy include washing, scarification, and osmopriming (Atherton and Farooque, 1983b; Cantliffe et al., 1984; Hassell and Kretchman, 1997; Masuda and Konishi, 1993).

High levels of oligosaccharides may be an important mechanism to establish metabolic controls for seed germination (Vertucci

and Farrant, 1995). Changes in carbohydrates occur during seed germination, with generally the monosaccharide content increasing and the oligosaccharide content decreasing (Vertucci and Farrant, 1995; Koster and Leopold, 1988). Oligosaccharides of higher degree polymerization (DP) may protect membranes and macromolecules in seeds from desiccation damage (Bernal-Lugo and Leopold, 1992). Evidence of carbohydrate metabolism in spinach seeds imbibed at supraoptimal temperatures is lacking, as well as the role of oligosaccharides of higher DP, such as sucrose and raffinose, in preventing thermoinhibited seeds going into thermodormancy.

The purpose of this study was to determine the extent of seed thermoinhibition in four spinach genotypes, the nature and level of inhibition imposed by the pericarp and seedcoat leachates, and to identify and quantify the level of soluble carbohydrates during thermoinhibition.

Materials and Methods

GERMINATION AT CONSTANT AND ALTERNATING TEMPERATURES. Seed of ‘Cascade’ (hybrid), ‘ACX 5044’ (hybrid), and ‘Fall Green’ (open pollinated) (Alf Christianson Seed Co., Mount Vernon, Wash.) were placed on Whatman No. 1 and No. 3 filter papers in 9-cm petri dishes. Five milliliters of distilled deionized water was initially added, and followed by additions of 0.5 mL as needed. Seeds were incubated for 2 weeks on a thermogradient table at constant 10, 15, 20, 22, 23, 25, 30, and 35 ± 1 °C. To determine whether alternating temperatures could alleviate thermoinhibition, the seeds were incubated at 25/15, 30/15, 35/15, 30/20, 35/20, and 35/25 °C (12-h day/12-h night) for 4 weeks. Controls at 20, 25, and 30 °C constant temperatures were also included.

The following experiment determined whether spinach seeds held at high temperature for a prolonged period of time lose their

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Fig. 1. Shifts in the ABA standard curve due to addition of spinach seed extracts. The amount of ABA was measured by indirect ELISA in samples containing only ABA standard (open squares, $y = -0.800 + 1.168x$, $r^2 = 0.986$), ABA standard + 'Fall Green' extract (solid diamond, $y = 30.407 + 1.291x$, $r^2 = 0.984$), and ABA standard + 'Cascade' extract (solid square, $y = 50.703 + 1.264x$, $r^2 = 0.990$). Each point represents a mean ($n = 4$).

ability to germinate when returned to lower temperatures. 'Cascade' nontreated seeds, washed seeds (immersed in water at 10 °C for 4 h, followed by surface disinfection with 2% NaOCl for 10 min and rinsing three times with deionized water), and seeds only disinfected with NaOCl as described for washed seeds, were incubated at a constant 30 °C for 10 d or 35 °C for 7 d, and then transferred to 18 °C for a total incubation time of 20 d. The difference in the transfer time to 18 °C was based on preliminary observations, where seed vigor was visibly reduced when seeds were previously incubated at 35 °C for >8 d or at 30 °C for >10 d.

PERICARP ROLE ON GERMINATION. To evaluate whether thermoinhibition at 30 °C is seedcoat and/or embryo imposed, seeds were treated as follow: a) pericarp removal (coatless or naked seeds); b) pericarp removed and placed on the same medium at a distance of about ≤5 mm from the embryo (spirally coiled around the attached perisperm); c) pericarp removed, embryo reinserted back into pericarp, d) slitted-pericarp seeds, and e) intact seeds. To remove the pericarp or seedcoat, seeds were placed on Whatman No. 3 filter paper in 9-cm petri dishes, and moistened with 5 mL of distilled deionized water for 10 min. Individual seeds were then placed under a 40× magnification stereo microscope and pericarps were removed using a surgical blade and a sharp-point forceps. Slitting was carefully done with a surgical blade to a length of ≈1 to 2 mm near the radicle end point, and seeds with visible embryo damage were discarded. The open pollinated genotype 'ARK 88-354' was also included in this test. Seeds were incubated for one week in darkness at a constant 30 °C and germinated seeds were counted at 2:00 PM daily.

GERMINATION WITH EXOGENOUS ABA AND LEACHATE. To determine the high temperature sensitivity of 'Cascade' and 'ARK 88-354' to exogenous ABA, pericarpless seeds were germinated in an incubator in the dark at 30 ± 1 °C for 7 d. ABA (+/-cis, trans) (Sigma Chemical Co., St. Louis, Mo.) concentrations of 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹ M were prepared, and 5 mL of ABA solution was added to the filter paper initially, followed by additions of 0.5 mL ABA solution at 2 and 4 d.

To determine the effects of water soluble extracts on germination of spinach 'Cascade', 'ACX 5044', 'Fall Green', and 'ARK 88-354' seeds incubated at 30 °C, the following treatments were tested: a) intact seeds in distilled deionized water; b) leached seeds in distilled deionized water; c) leached seeds in leachates

extracted from whole seeds of the same genotypes, d) embryos in leachates from whole seeds of the same genotypes; and e) embryos in deionized distilled water. Leached seeds and leachates were obtained by mixing 50 g of intact seeds of ≈7% moisture content with distilled water (1:4 w/v) and stirring continuously for 1 h at 23 °C. The leachates were vacuum filtered using two No. 1 Whatman filter papers. For a bioassay, 120 mL of each extract was retained and the remainder was centrifuged at 2,500 rpm for 30 min, freeze dried, and assayed for ABA as described below.

To measure ABA content in pericarps, the pericarps were manually separated from intact dry seeds of 'Cascade', 'ACX 5044', 'Fall Green', and 'ARK 88-354' genotypes as described previously. Pericarp samples were ≈80 to 160 mg with four replications per cultivar. ABA was extracted from the pericarps in methanol containing butylated hydroxytoluene and assayed by an indirect ELISA (Belefant and Fong, 1989). Seed leachates from each genotype were also assayed for ABA content. When extracts were tested for interference by some substances in the binding of ABA to the monoclonal antibody (Crozier et al., 1985), the linearity or slope of the standard curves of ABA was not altered (Fig. 1). A similar procedure was used to determine ABA content in whole seeds, but due to high interference from substances in the perisperm, ABA was not measured.

EFFECT OF 'CASCADE' PERICARPS ON 'ARK 88-354' EMBRYOS. To confirm the inhibition of 'Cascade' pericarps on 'Cascade' and 'ARK 88-354' embryos incubated at 30 °C in deionized distilled water, the following embryo treatment manipulations were tested: a) 'Cascade' embryo; b) 'Cascade' pericarp removed, embryo reinserted back into pericarp; c) 'Cascade' pericarp removed, 'ARK 88-354' embryo reinserted into 'Cascade' pericarp; d) 'ARK 88-354' embryo; e) 'ARK 88-354' pericarp removed, embryo reinserted back into pericarp; and f) 'ARK 88-354' pericarp removed, 'Cascade' embryo reinserted into 'ARK 88-354' pericarp.

SOLUBLE CARBOHYDRATES. Carbohydrates were determined for 'Cascade', 'Fall Green' and 'ARK88-354' imbibed seeds at 30 and 36 °C. Seeds were sampled at various times over 96 h, flash frozen with liquid N, freeze-dried, ground to powder, trimethylsilyl (TMS) derivatized, and analyzed for oligosaccharide content by gas chromatography using a DB-1 capillary column with a glass insert (HP 5181-3316) used as a splitless liner. Runs were started at 160 °C oven temperature with the injector and detector at 350 °C. The oven was programmed to hold for 4 min at 160 °C, and then increased to 325 °C at 8 °C per min and held for 25 min. Peaks were identified by using the internal standard 1-O-methyl-D-

Fig. 2. Germination of spinach seeds incubated for 14 d at constant temperatures. Each point represents a mean ($n = 4$) ± SE (where larger than data point).

Table 1. Germination of spinach seeds at alternating temperatures (12-h day/12-h night) for 14 d.

Genotype	Temp (°C)					
	25/15	30/15	35/15	30/20	35/20	35/25
	Germination (%)					
Fall Green	94	90	58	90	90	70
ACX 5044	98	94	44	78	85	68
Cascade	69	64	40	54	59	32
LSD _(0.05)	16	18	---	18	17	22
	T_{25%} (d)^z					
Fall Green	2.3	2.6	2.8	2.2	2.8	3.2
ACX 5044	3.6	3.8	7.9	3.9	6.0	7.2
Cascade	6.9	7.9	9.1	8.9	8.9	10.8
LSD _(0.05)	1.7	0.3	2.3	1.6	1.5	2.7

^zT_{25%} = Time to 25% germination.

Fig. 3. Germination of 'Cascade' untreated control and washed seeds incubated at a constant 30 °C for 10 d or 35 °C for 7 d, and then transferred to 18 °C for a total incubation time of 20 d.

glucopyranoside and by comparing the retention times with those of known standards of each saccharide of interest.

STATISTICAL PROCEDURES. Each temperature test was arranged in randomized complete block design, and each genotype was replicated four times. Fifty seeds were used in each replication. Seeds were considered germinated when the radicle protruded (≥ 2 mm) from the pericarp. The number of germinated seeds was counted daily and total germination, time to 25% germination ($T_{25\%}$), and mean days of germination (MDG) were calculated. Because final percentage germination varied widely in the alternating temperature tests, $T_{25\%}$ was used instead of $T_{50\%}$. Percentage of germination was transformed to square root arcsine prior to performing an analysis of variance. Data were analyzed using PROC GLM and PROC REG of SAS (SAS Institute, Inc., Cary, N.C.). Means were separated by LSD (0.05). The main effect of exogenous ABA

concentration was partitioned into linear or quadratic orthogonal contrasts. In those experiments where significant interactions ($P \leq 0.05$) were detected for germination, they were partitioned for each genotype.

Results and Discussion

GERMINATION AT CONSTANT AND ALTERNATING TEMPERATURES. Germination percentage was highest between 10 and 20 °C, and inhibition occurred at temperatures higher than 20 °C for 'Cascade' and 'ACX 5044' and at higher than 23 °C in 'Fall Green' (Fig. 2). After 14 d of incubation at 30 °C, total germination ranged from less than 10% for 'Cascade' to between 40% and 55% for 'ACX 5044' and 'Fall Green' respectively, and was totally suppressed at 35 °C in 'Cascade' and 'ACX 5044' (Fig. 2). Similarly, rates of germination (data not shown) were reduced at temperatures higher than 20 °C.

Thermoinhibition was less pronounced at alternating temperatures compared to constant 30 °C (compare Table 1 with Fig. 2). Germination was higher at 25/15 °C, 30/15 °C, 30/20 °C and 35/20 °C (12/12 h) than at 35/15 °C or 35/25 °C (Table 1). Therefore, 20 °C, rather than 25 or 15 °C, improved germination when alternating with 35 °C for 12 h. The $T_{25\%}$ was generally faster for 'Fall Green', than 'ACX 5044' and 'Cascade' at almost all alternating temperatures.

When seeds of 'Cascade', the spinach genotype most sensitive to high temperature, were incubated at 30 °C for 10 d, germination was only 23%, but after being transferred to 18 °C for an additional 10 d, germination proceeded and reached 88% (Fig. 3, control 30 °C). Similarly, germination of Cascade seeds was reduced to 3% at 35 °C after 7 d, but increased to 75% after being transferred to 18 °C for an additional 13 d (Fig. 3, control 35 °C). This indicates that 'Cascade' spinach seeds held at supraoptimal temperatures can maintain the ability to germinate when returned to lower

Table 2. Germination of spinach genotypes at 30 °C when subjected to various pericarp (P) modifications.

Genotype	P removed	P removed embryo ^z	P removed + embryo reinserted	P slitted to 1–2 mm	Intact seed (control)
			Germination (%)		
ARK 88-354	92	92	87	71	65
Fall Green	99	95	81	32	9
ACX 5044	96	83	69	18	1
Cascade	96	87	66	9	1
LSD _(0.05)	---	5	13	29	18

^zP removed embryo = pericarp removed and placed on the same medium at a distance of ≤ 5 mm, but not touching the embryo.

Table 3. Germination of pericarpless spinach seeds 'ARK 88-354' and 'Cascade' in contact with ABA solutions. Germination test was performed in petri dishes at 30 °C in darkness for 1 week.

ABA (M)	ARK 88-354		Cascade	
	Germination (%)	MDG ^z (d)	Germination (%)	MDG (d)
0 (Control)	92	1.5	91	2.7
10 ⁻⁶	90	1.7	87	2.4
10 ⁻⁵	88	1.6	90	2.9
10 ⁻⁴	91	2.3	34	3.4
10 ⁻³	59	2.6	16	3.1
Orthogonal contrasts				
Control vs. others	NS	**	**	NS
ABA	Q*	Q**	Q**	L**

^zMDG = mean days to germination.

NS, *, ** Nonsignificant or significant F test at $P = 0.05$ or 0.01 , respectively. ABA concentration effect was linear (L) or quadratic (Q).

temperatures. Germination of washed 'Cascade' seeds increased to 38% at 30 °C for 11 d and to 12% at 35 °C after 7 d, and this improvement was carried over when seeds were transferred to 18 °C (Fig. 3, washed).

PERICARP ROLE ON GERMINATION. The germination of intact seeds held at 30 °C for 1 week was very low in 'Cascade', 'ACX 5044', and 'Fall Green', although germination of 'ARK 88-354' reached 65% (Table 2). However, germination of embryos with the pericarp removed was similar to that for intact seeds at ≤ 20 °C temperatures (compare Table 2 with Fig. 2).

When seeds were incubated with the pericarp near the embryo, germination was slightly reduced in 'ACX 5044' and 'Cascade' compared with 'ARK 88-354' and 'Fall Green'. This decrease was more evident when embryos were reinserted back into pericarp (Table 2). Apparently, both structural and chemical factors in the pericarp prevented spinach germination at high temperature. Although O₂ supply was reported to be critical for spinach embryos to germinate at high temperatures (Heydecker and Orphanos, 1968), in this study, slitting the pericarp had little effect on improving germination (Table 2).

GERMINATION WITH EXOGENOUS ABA AND LEACHATE. 'Cascade' embryos were sensitive to ABA concentration; germination was significantly reduced to 16% at 10⁻³ M ABA and mean days of germination increased linearly from 2.4 to 3.1 d as ABA concentrations increased from 10⁻⁶ to 10⁻³ M (Table 3). Germination of 'ARK 88-354' pericarpless seeds was reduced to 59% at 10⁻³ M ABA, and mean days of germination increased by 0.9 d from 10⁻⁶ to 10⁻³ M ABA. In rape (*Brassica napus* L.), 10⁻³ M ABA completely inhibited germination (Schopfer and Plachy, 1984).

The ABA content of the pericarp (fresh weight basis) was ≈ 2.4 -fold higher in 'Cascade' and 'ACX 5044', 29 and 31 ng·g⁻¹ fresh weight, respectively ($\approx 1.2 \times 10^{-7}$ M) than in 'Fall Green' and 'ARK 88-354' (Fig. 4). However, the content of ABA in the leachate was not consistently higher in the first two cultivars. Leaching removed up to 90% of ABA in 'Fall Green' and 'ARK 88-354' and between 20% to 50% in 'Cascade' and 'ACX 5044', respectively (Fig. 4). This may indicate that ABA was present in the apoplasm in 'Fall Green' and 'ARK 88-354'.

Washing significantly improved germination at 30 °C only in 'Fall Green', whereas leachates inhibited germination significantly only in 'ACX 5044' (Fig. 5). The genotype 'ARK 88-354' appeared least sensitive to either the presence or absence of other possible inhibitors that might be present in the leachates. Germination of embryos was not affected when seeds were incubated with the leachate coming from intact seeds (Fig. 5), evidence that the pericarp is necessary for thermoinhibition or that the concentration

was not high enough (as shown in Table 3) to inhibit germination.

EFFECT OF 'CASCADE' PERICARPS ON 'ARK 88-354' EMBRYOS. Germination of 'ARK 88-354' embryos in 'Cascade' pericarp was 17% lower than 'ARK 88-354' embryos in 'ARK 88-354' pericarps. Conversely, germination of 'Cascade' embryos in 'ARK 88-354' pericarps was 13% higher and proceeded at a lower speed (2.4 vs.

Fig. 4. ABA concentration (fresh weight basis) in pericarps and leachates from intact seeds of four spinach genotypes. Each bar represents a mean ($n = 4$) $\pm$ SE.

Fig. 5. Germination of spinach seeds at 30 °C in the presence of seed leachates. Intact (intact seeds in deionized distilled water), W = washed or leached seeds in deionized distilled water, W + Leachate = washed or leached seeds in leachates extracted from whole seeds of the same genotypes, E + Leachate = embryo with perisperm in leachates from whole seeds, and E = embryo with perisperm. Each bar represents a mean ($n = 4$) $\pm$ SE.

Table 4. Germination of spinach embryos incubated at 30 °C when reinserted to similar or different pericarps.

Treatment ²	MDG ³ (d)	Germination (%)
Ae + Cp	2.3	76
Ae + Ap	2.1	93
Ce + Ap	2.4	76
Ce + Cp	3.3	63
LSD _(0.05)	0.5	10

²Ae + Cp = 'ARK 88-354' embryo reinserted into 'Cascade' pericarp; Ae + Ap = 'ARK 88-354' embryo reinserted into 'ARK 88-354' pericarp; Ce + Ap = 'Cascade' embryo reinserted into 'ARK 88-354' pericarp; Ce + Cp = 'Cascade' embryo reinserted into 'Cascade' pericarp.

³MDG = mean days to germination.

3.3 d) compared to 'Cascade' embryos in 'Cascade' pericarps (Table 4). Therefore, this result confirms the greater inhibitor capacity of 'Cascade' pericarps. Increased germination as a result of pericarp removal were reported in 'Prickly New Giant' spinach; however, the germination level of that genotype did not reach those found at optimum temperatures (Atherton and Farooque, 1983a), such as reported in our study.

SOLUBLE CARBOHYDRATES. The major sugars present in spinach seeds imbibed at 30 °C for 96 h were the monosaccharide glucose (Glu), and the di- and tri-oligosaccharides sucrose (Suc) and raffinose (Raf), respectively. In addition, *myo*-inositol was also detected. Glu content of 'Fall Green' seeds remained unchanged until 24 h; thereafter, Glu amounts increased more rapidly than in 'ARK 88-354', while Glu content of 'Cascade' seeds remained low throughout the 96 h incubation (Fig. 6). Suc amounts were slightly higher in 'Fall Green' and 'ARK 88-354' compared to 'Cascade' seeds at 72 h. At 96 h, however, Suc levels in 'Fall Green' were 4-fold greater than the other two genotypes. Prior to imbibition (0 h) Raf content was significantly different among the three genotypes and was in the order of 'ARK 88-354' > 'Fall Green' > 'Cascade'. After 48 h, Raf levels declined in 'Fall Green' compared to 'ARK 88-354'. In 'Cascade', Raf levels remained about constant after 24 h and higher than the other two genotypes after 36 h. *Myo*-inositol was highest for 'ARK 88-354' only at noticeable peaks at 16 and 72 h imbibition. At 36 °C, Glu, Suc, and *myo*-inositol levels were negligible and remained fairly unchanged in the three genotypes (Fig. 6). Raf declined immediately after imbibition, increasing 2-fold at 96 h imbibition only for 'Cascade' seeds.

It is likely that for seeds imbibed past 36 h at 30 °C the hydrolysis of Raf into Suc and D-galactose contributed to a relative greater content of Suc and Glu in 'ARK 88-354' and 'Fall Green'. Since galactose was not detected, it is possible that it was metabolized for energy or transformed into sucrose. The decline of Raf in 'ARK 88-354' and 'Fall Green' could be associated with the onset of germination. The biosynthesis of Raf involves the successive transfer of a D-galactosyl residue from galactinol, which is formed from UDP-galactose and *myo*-inositol (Goodwin and Mercer, 1983). Whether increases in *myo*-inositol in 'ARK 88-354' are important for the synthesis of additional Raf beyond 96 h is unknown. In 'Cascade' seeds, Raf content decreased less rapidly than in 'ARK 88-354' and 'Fall Green'.

Endo- β -mannanase and α -galactosidases are two important enzymes capable of mobilizing soluble sugars, decreasing the mechanical restraint imposed on the tomato embryos (Groot et al., 1988; Hilhorst and Downie, 1996). Mannose activity and synthesis can be suppressed by ABA (Malek and Bewley, 1991). Whether

the activity of these enzymes is greater in 'Fall Green' and 'ARK 88-354' than in 'Cascade' and 'ACX 5044' seeds is unknown. ABA content in the seedcoat was leached easily in 'Fall Green' and 'ARK 88-354', but not in the other two genotypes. We could speculate that if levels of ABA were measured and found to be high either in the perisperm or the embryonic axis in the sensitive genotypes, ABA could be another component of the inhibitory system, altering the seed metabolism, e.g., by suppressing the activity or synthesis of endo- β -mannanase or delaying seed germination until seeds encounter lower temperatures such as observed for 'Cascade' (Fig. 3).

In a recent overview of primary dormancy in tomato, Hilhorst (1997) described that all seed parts, including the thin testa may play a major role in seed dormancy. During seed germination inhibition in *Chenopodium album* L., a species that, like spinach, belongs to the Chenopodiaceae, one site of secondary hormonal action was located inside the covering structures where ABA blocks GA-induced hydrolysis of the endosperm (Bewley and Black, 1994; Karssen 1976). Based on our studies in spinach we do not have enough evidence to explain the inhibitory effect on the basis of ABA alone since the concentration of ABA in the seedcoat was too low (30 ng·g⁻¹) to inhibit germination. Karssen (1976) concluded that ABA had no function in the regulation of dormancy in *C. album* seeds, containing 8 to 10 pg ABA/seed, a much lower concentration than measured in the seedcoats in our study.

The differential responses of seed germination to high temperatures among the genotypes used in this study could also be related to the different genetic background of each cultivar. For

Fig. 6. Glucose, sucrose, raffinose, and *myo*-inositol concentrations (dry weight basis) in 'Fall Green', 'ARK 88-354', and 'Cascade' spinach seeds imbibed at 30 and 36 °C for 96 h.

example, 'ARK 88-354' is an open-pollinated smooth leaf cultivar developed by selecting seedlings of segregating material from 'Fall Green', an open-pollinated savoy leaf type cultivar, followed by intercrossing with superior flat leaf plants (T. Morelock, University of Arkansas, personal communication). Both cultivars exhibited similar levels of ABA in the pericarp and leachate fraction (Fig. 5). The hybrids 'Cascade' and 'ACX 5044', genetically dissimilar to 'Fall Green', share the same male but not the female parent. The pericarp that surrounds the embryo and perisperm is part of the diploid mother plant and therefore genetically different from the diploid embryo. In addition to genetic factors, further investigations are needed to elucidate the effect of environmental conditions during spinach seed development and maturation on pericarp structure, ABA, non hormonal inhibitor compounds, and the presence of enzymes capable of mobilizing soluble sugars during seed germination.

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