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Roles of Soluble Sugars in Protecting Phytochrome- and Gibberellin A_3-Mediated Germination Control in Skotodormant Lettuce Seeds

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Abstract. Skotodormant seeds of *Lactuca sativa* Grand Rapids imbibed in darkness for 10 days (10-day DS) germinated poorly upon terminal treatment with red light (R) or gibberellin A_3 (GA_3). Soluble sugars in the imbibition solutions influenced the depth of skotodormancy. Ten-day DS seeds, imbibed in 50–500 mM sucrose or 100–500 mM glucose and given terminal GA_3 germinated completely and germinated about 80% when imbibed in 100 mM galactose, mannose, lactose, or maltose. In contrast, terminal R applied to 10-day DS seeds caused only 20–50% germination. If given R at day 0 and imbibed for 10 days in darkness in 500 mM sucrose or glucose, seeds washed free of exogenous glucose or sucrose then germinated about 50% in darkness in water. These seeds responded to terminal R or GA_3 with complete germination. When seeds were given FR at day 0, germination responses following terminal R or GA_3 were significantly lower when the duration of DS was increased from 7–10 day DS to 15 days. In 10-day DS seeds given initial FR and imbibed in either solutions of 50 or 100 mM sucrose and KNO_3, either terminal R or GA_3 treatment gave complete or near complete germination. It is concluded that seed exposure to certain soluble sugars and/or nitrate during a 10-day DS protected certain substrates and thereby extended the sensitivity of the seeds to terminal R or GA_3 treatment. The study provides substantial evidence for nonhormonal factors associated with light and GA action in the control of seed skotodormancy.

Key words. Seed germination—Skotodormancy—Sucrose—Glucose—Nitrate—*Lactuca sativa*

Abbreviations: DS, dark storage; R, red light; GA_3, gibberellin A_3; FR, for red light; ANOVA, analysis of variance; GLM, General Linear Model; LSD, least squares difference; Pfr, phytochrome far-red-absorbing form.

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The exact relationship between the hormonal control of metabolic processes leading to germination and the phytochrome status within seeds is often complicated by interactions between light and other environmental factors such as temperature and nutritional status (Thomas 1992). Nitrate promotes the germination of a wide range of species, but its effect is best realized in combination with other factors such as temperature manipulations or light (see Hsiao and Quick 1996, McIntyre et al. 1996, and references cited therein). Hsiao and Quick (1996) showed that the presence of certain inorganic nitrogen salts at 25–50 mM in the dark storage (DS) imbibition solution influenced the depth of skotodormancy. The terminal red light (R) treatment was the most effective in seeds imbibed in NH_4NO_3 for 10 days in darkness and washed before the terminal treatment. Terminal GA_3 caused complete germination in 10-day DS seeds imbibed in 25 mM NH_4Cl or 50 mM NH_4NO_3, whereas complete germination followed either terminal R or GA_3 when these seeds were imbibed in higher concentrations of the four salts. We concluded that seed exposure to certain inorganic nitrogen salts, especially ammonium salts, during a 10-day DS could maintain the sensitivity of the seeds to terminal R or GA_3 treatment by reducing the depth of skotodormancy to levels at which the germination of the seeds still would be under the control of P_{fr} and/or GA_3.

The objectives in this study were to examine whether seed exposure to soluble sugars during DS imbibition produced protecting effects similar to those of seed exposure to these inorganic nitrogen salts, that is, protecting the functioning of phytochrome- and GA_3-mediated germination control mechanisms in skotodormant lettuce seeds.

Materials and Methods

Lactuca sativa cv. Grand Rapids seeds were from the same seed lot as those used in earlier studies (Hsiao and Quick 1996, Hsiao and Vidaver 1989, Hsiao et al. 1984). They had been stored at −20°C and main-

Table 1. Effects of various concentrations of glucose and sucrose and subsequent treatment with FR, FR + R, or FR + GA_3 on the germination of lettuce seeds 48 h after the onset of imbibition.[a]

Light and GA_3 treatments	Concentrations (mM) of glucose or sucrose during 48-h imbibition							
	0	10	25	50	100	200	300	500
Glucose								
FR	16e[b]	14e	13e	11ef	5g	0h	0h	0h
FR + R	96a	93a	99a	98a	94a	44c	0h	0h
FR + GA_3	97a	99a	70b	76b	31cd	5g	0h	0h
Sucrose								
FR	16de	13de	11d–f	8e–g	4f–h	0h	0h	0h
FR + R	98a	98a	96a	98a	93ab	67c	2gh	0h
FR + GA_3	96a	98a	99a	96a	90b	24d	2gh	0h

[a] Dry seeds were imbibed for 0.5 h in H_2O, glucose, or sucrose with or without GA_3, given FR or FR + R, and germinated in darkness for 48 h. Values are percent germination.

[b] An 8 × 3 factorial ANOVA was performed. Within each chemical, effects of chemical, light, or GA_3 treatment and their interaction are all significantly different at $p < 0.01$. Values within each chemical which are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

tained strong light sensitivity. Unless otherwise stated, each treatment had three replicates of 50 seeds with an initial 5 min of far red light (FR) to suppress germination 0.5 h after the onset of imbibition at 20°C. The light sources and filters for R or FR treatments were the same as in previous studies (Hsiao 1992, Hsiao and Vidaver 1989). Except for germination counts, all operations were carried out in darkness or under dim green safelights (Hsiao and Vidaver 1971, Vidaver and Hsiao 1974).

Unless otherwise stated, all light treatments were for 5 min, and the concentration of GA_3 was 0.5 mM. Dry seeds were placed in 5.0-cm-diameter Petri dishes lined with two 5.5-cm-diameter Whatman No. 1 filter paper discs. Imbibition at 20°C was carried out with 1.5 mL of distilled water or solutions of soluble sugars. In one experiment, seeds were imbibed in the dark with 50 and 100 mM sucrose or KNO_3 or combinations of the two chemicals for 10 days. In another experiment, seeds imbibed in the dark with 500 mM sucrose or glucose for 10 days were given 5 min of initial R rather than the usual FR treatment. DS refers to the 10-day period of imbibition in darkness following initial light treatment, except for one experiment involving a 35-day period of imbibition. Any germinated seeds present after DS were discarded, and subsequent germination percentages were based on the remaining skotodormant seeds. These seeds were immersed for 1 h in 125 mL of distilled water to remove residual imbibition chemicals from the seed surface and were then transferred to fresh 5.0-cm-diameter Petri dishes in the appropriate incubation medium, given light or GA_3 treatments, and incubated for 48 h in the dark at 20°C. Seeds were scored as germinated if the radicle had visibly penetrated the pericarp. Germination of 90% or better was considered complete.

In one experiment, seeds were imbibed in 100 mM sucrose for 10–35 days during a 35-day DS as follows. Dry seeds were imbibed for 0.5 h in 100 mM glucose or water, given FR, imbibed for 10 days in the dark, transferred to fresh water or 100 mM sucrose for 5 days (day 15), transferred to fresh water or 100 mM sucrose for another 20 days (day 35), and finally rinsed in H_2O for 1 h and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h.

The procedures for measuring sucrose uptake in lettuce seeds were as follows. For each sucrose treatment, three replicates of 20 dry seeds were imbibed in each 5.0-cm-diameter Petri dish lined with two 5.5-cm-diameter Whatman No. 1 filter paper discs and 2.0 mL of sucrose solutions with 5 μCi of UL-[^{14}C]sucrose (specific radioactivity of 50 mCi/mmol; Sigma Chemical Company, Mississauga, ON) for 0.5 h, given FR, imbibed for another 23.5 h, and rinsed with 2 mL of sucrose of the same concentration to remove labeled sucrose from the seed surfaces. Each seed sample was combusted with a biologic sample oxidizer (OX 500, R. J. Harvey Instrument Co., Hillsdale, NJ). The trapped $^{14}CO_2$ was quantified by a liquid scintillation analyzer (Tri-Carb 1900 TR, Canberra Packard Canada, Missisauga), and the amount of sucrose uptake/20 seeds was determined.

All experiments in this study were randomized complete block in design and were repeated at least once with similar results. Germination percentages were arc sine transformed and subjected to analyses of variance (ANOVA) by the General Linear Model procedure (GLM) of Statistical Analysis System (SAS Institute Inc., Cary, NC). A Fisher's protected LSD test was used for a two-way comparison of means at 5% probability.

Results

Seeds without Dark Storage

The FR-treated seeds without DS germinated completely at 20°C if given a treatment of R or GA_3 (Table 1). The main effects of sugar, light, or GA_3 treatment, and their interaction were all significantly different at $p < 0.01$. The germination response of these seeds to R or GA_3 treatment was unaffected by concentrations of sucrose up to 100 mM. Similar concentrations of glucose did not change the germination response to R treatment, but reduced germination was present in GA_3-treated seeds imbibed in sugars at 25 mM and higher. Both sugars reduced germination significantly, with R treatment still eliciting higher germination responses than GA_3 treatment. Seeds incubated in 300 or 500 mM of either sugar for 48 h did not germinate following R or GA_3 treatments.

Seeds with Dark Storage for 10 Days

Experiment 1. Seeds imbibed in water or various concentrations of glucose or sucrose up to 500 mM during 10-day DS, transferred to water, and then held in darkness for 48 h germinated poorly (Table 2). Terminal GA_3 treatment given to the seeds imbibed in 50–500 mM sucrose and in 100 mM glucose or higher resulted in complete germination. Terminal R applied to similarly treated seeds yielded only 12–48% germination. The main effects of sugar, terminal treatment, and their interaction were all significantly different at $p < 0.01$. Sucrose was more effective than glucose in raising germination percentage of seeds imbibed at concentrations of 25–50 mM and given GA_3 treatment. Similarly, seeds

Table 2. Effects on the germination of lettuce seeds of exposure to various concentrations of glucose or sucrose during 10-day DS and subsequent treatment with R or GA_3.[a]

Terminal treatments	Concentrations (mM) of glucose or sucrose during 10-day DS					
	0	10	25	50	100	500
Glucose						
D	0h[b]	0h	0h	0h	0h	0h
R	10e	7e–g	8ef	8ef	21d	25d
GA_3	7e–g	4g	6fg	73c	91b	99a
Sucrose						
D	0e	0e	1e	0h	0h	0h
R	11d	9d	11d	12d	45c	48ab
GA_3	7d	8d	57b	96a	96a	99a

[a] Dry seeds were imbibed for 0.5 h in H_2O or in various concentrations of glucose or sucrose, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h; D, dark control. Values are percent germination.

[b] A 6 × 3 factorial ANOVA was performed. Within each chemical, effects of initial chemical, terminal light or GA_3 treatment, and their interaction are all significantly different at $p < 0.01$. Values within each chemical which are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

imbibed in 100 and 500 mM sucrose germinated better following R than did seeds imbibed in 100 and 500 mM glucose.

Experiment 2. Seeds were treated as in experiment 1 but imbibed in 100 mM of various sugars during 10-day DS and then given a terminal treatment with R or GA_3 (Table 3). The main effects of sugar, terminal treatment, concentration, and their interactions were all significantly different at $p < 0.01$. Terminal GA_3 treatment gave complete germination in seeds imbibed in 100 mM sucrose or glucose (Table 2). Terminal GA_3 treatment was able to cause about 80% germination in seeds imbibed in 100 mM galactose, lactose, maltose, or mannose (Table 3). Terminal R treatment also tended to give higher germination responses in these seeds, with sucrose and lactose most effective. The terminal treatment of R or GA_3 had no effect on germination in the 10-day DS seeds imbibed in 100 mM arabinose, fructose, or sorbose; and terminal R had no effects in the 10-day DS seeds imbibed in 100 mM maltose, trehalose, or cellobiose.

Experiment 3. Seed samples were imbibed in 500 mM glucose or sucrose, given initial R or FR 0.5 h after the onset of imbibition, and held for 7, 10, or 15 days DS, followed by terminal treatment of R or GA_3 (Table 4). The main effects of initial and terminal treatment and their interactions were all significantly different at $p < 0.01$. Given R at day 0, 10-day DS seeds germinated about 50% in darkness in water after removal of exogenous glucose or sucrose. These seeds responded to terminal R or GA_3 with complete germination. When given FR at day 0, terminal R or GA_3 gave similar germination responses in 10-day DS seeds imbibed in 500 mM of both sugars as in experiment 1 (compare Table 4 with Table 2). When given FR at day 0, germination responses by terminal R or GA_3 were significantly lower when the duration of DS was increased from 7–10-day DS to 15 days (Table 4).

Experiment 4. Seed samples were imbibed in 50 or 100 mM sucrose or KNO_3 or combinations of the two chemicals for 10 days, followed by terminal treatment of R or GA_3 (Table 5). The main effects of initial and terminal treatment and their interactions were all significantly different at $p < 0.01$. When seeds were given FR at day 0, terminal R or GA_3 produced similar germination responses in 10-day DS seeds imbibed in 50 or 100 mM sucrose as in experiment 1 (compare Table 5 with Table 2), with complete germination in seeds given terminal GA_3 treatment. Confirming our previous study (Hsiao and Quick 1996), terminal R gave complete germination in 10-day DS seeds imbibed in 50 or 100 mM KNO_3 (Table 5). When seeds were imbibed during 10-day DS in mixed solutions of 50 or 100 mM sucrose and KNO_3, either terminal R or GA_3 treatment gave complete or near complete germination.

Seeds with Dark Storage for 35 Days

For seed imbibed in 100 mM sucrose for varying periods during the 35-day DS, the main effects of initial and terminal treatment and their interactions were all significantly different at $p < 0.01$ (Table 6). Terminal GA_3 gave about 50% germination responses in seeds imbibed in 100 mM sucrose for the last 5 days of the 35-day DS. Seeds imbibed in 100 mM sucrose for the first 10 days and then in water for the next 25 days during the 35-day DS did not germinate with terminal GA_3. Terminal R gave reduced germination responses in these 35-day DS seeds compared with the 10-day DS seeds imbibed in 100 mM sucrose regardless of sucrose pretreatment (compare Table 6 with Tables 2–5).

Sucrose Uptake in Seeds without Dark Storage

The effect of sucrose concentration on uptake (Table 7) was significantly different at $p < 0.01$. The concentration of sucrose in the imbibition solution was significantly correlated with the sucrose uptake by the seeds with positive correlation coefficient $r = +0.907$ ($df = 4$, $r =$

Table 3. Effects on the germination of lettuce seeds of exposure to various sugar analogs (100 mM) during 10-day DS and subsequent treatment with R or GA_3.[a]

Chemical during DS	Formula	Molecular weight	Terminal R or GA_3 treatments		
			D	R	GA_3
Water	H_2O	18.02	0l[b]	8hi	6hi
Ribose	$C_5H_{10}O_5$	150.13	0l	23f	62d
Arabinose	$C_5H_{10}O_5$	150.13	1l	7hi	3hi–k
Glucose	$C_6H_{12}O_6$	180.16	0l	25f	94a
Galactose	$C_6H_{12}O_6$	180.16	0l	18fg	87b
Mannose	$C_6H_{12}O_6$	180.16	0l	22f	79bc
Fructose	$C_6H_{12}O_6$	180.16	0l	8hi	11hi
Sorbose	$C_6H_{12}O_6$	180.16	0l	5hi	4h–i
Sucrose	$C_{12}H_{22}O_{11}$	342.30	0l	48e	96a
Lactose	$C_{12}H_{22}O_{11}$	342.30	0l	42e	83b
Maltose	$C_{12}H_{22}O_{11} \cdot H_2O$	342.30	1l	11e	86b
Trehalose	$C_{12}H_{22}O_{11} \cdot 2\ H_2O$	342.30	0l	3i–k	71cd
Cellobiose	$C_{12}H_{22}O_{11}$	342.30	0l	6hi	23f

[a] Dry seeds were imbibed for 0.5 h in HO_2 or in a 100 mM concentration of various sugar analogs, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h; D, dark control. Values are percent germination.

[b] A 13 × 3 factorial ANOVA was performed. Effects of initial chemical, terminal light, or GA_3 treatment, and their interaction are all significantly different at $p < 0.01$. Values that are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Table 4. Effects on the germination of lettuce seeds of exposure to 500 mM glucose or sucrose during 10-day DS and subsequent treatment with R or GA_3.[a]

Initial light treatment at day 0	Dark storage		Terminal light or GA_3 treatment		
	Chemical	Days	D	R	GA_3
R	Glucose	10	45d[b]	97a	100a
FR	Glucose	10	0f	25e	98a
R	Sucrose	10	52cd	98a	100a
FR	Sucrose	10	0f	48cd	100a
FR	Sucrose	7	0f	57c	100a
FR	Sucrose	15	0f	18e	73b

[a] Dry seeds were imbibed for 0.5 h in 500 mM glucose or sucrose, given R or FR, imbibed for 7–15 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3 or given R or FR, and germinated in darkness for 48 h; D, dark control. Values are percent germination.

[b] A 6 × 3 factorial ANOVA was performed. Effects of initial and terminal treatment and their interaction are all significantly different at $p < 0.01$. Values that are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

0.811 at 5%). However, the proportional increase in the rate of sucrose uptake by seeds was greatly reduced as the concentration of sucrose in the imbibition medium increased.

Table 5. Effects on the germination of lettuce seeds of exposure to 50 or 100 mM sucrose or KNO_3 and some of their mixtures during 10-day DS and subsequent treatment with R or GA_3.[a]

Incubation medium during 10-day DS	Terminal R or GA_3 treatment	
	R	GA_3
50 mM sucrose	18de[b]	90bc
100 mM sucrose	37d	97ab
50 mM KNO_3	100a	7e
100 mM KNO_3	100a	38d
50 mM sucrose + 50 mM KNO_3	84bc	87c
100 mM sucrose + 100 mM KNO_3	91bc	88bc

[a] Dry seeds were imbibed for 0.5 h in incubation medium, given FR, imbibed for 10 days during DS, rinsed in H_2O for 1 h, and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h. Values are percent germination.

[b] A 6 × 2 factorial ANOVA was performed. Effects of initial and terminal treatment and their interaction are all significantly different at $p < 0.01$. Values that are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Discussion

Hsiao et al. (1984) suggested that the deeper dormancy of skotodormant over primarily dormant lettuce seeds might be due to reduced levels of P_{fr} and endogenous GA in skotodormant seeds. Skotodormant seeds normally require both systems to induce complete germination (Hsiao and Quick 1996, Hsiao and Vidaver 1989, Hsiao et al. 1984, Speer et al. 1974, Vidaver and Hsiao 1974, 1975), whereas either one of the phytochrome or GA promotion systems is sufficient to terminate primary dormancy. In our previous study (Hsiao and Quick 1996), we showed that the skotodormancy of 10-day DS seeds imbibed in certain inorganic nitrogen salts can be overcome by either terminal R or GA_3 treatment. More specifically, the terminal treatment of R caused complete germination in the 10-day DS seeds imbibed in 50 and 100 mM KNO_3, NH_4NO_3, or NH_4Cl, but at these concentrations terminal GA_3 treatment gave complete germination only in seeds imbibed in the two ammonium compounds. In the present study (Table 2), complete germination resulted if terminal GA_3 treatment was given to the seeds imbibed in 50–500 mM sucrose or in 100–500 mM glucose, whereas terminal R in those seeds yielded only 12–48% germination. Also, when seeds were imbibed during 10-day DS in mixed solutions of 50 or 100 mM sucrose and KNO_3, either terminal R or GA_3 treatment gave complete or near complete germination (Table 5). Thus, we can conclude that the depth of seed skotodormancy is related to levels of P_{fr} (with or without initial R treatment) or to endogenous GA and that the presence of certain soluble sugars and inorganic nitrogen salts during DS protects or maintains the sensitivity of the seeds to either terminal R or GA_3 treatment. Whether

Table 6. Effects on the germination of lettuce seeds of exposure to 100 mM sucrose during 35-day DS and subsequent treatment with R or GA_3.[a]

Incubation medium during dark storage			Terminal light or GA_3 treatment at day 35		
Day 0	Day 10	Day 15	D	R	GA_3
Water	Water	Water	0d[b]	0d	0d
Water	Water	Sucrose	0d	14b	46a
Sucrose	Water	Water	0d	8c	0d
Sucrose	Water	Sucrose	0d	15b	51a
Sucrose	Sucrose	Sucrose	0d	18b	56a

[a] Dry seeds were imbibed for 0.5 h in 100 mM glucose or water, given FR, imbibed for 10 days during DS, transferred to fresh water or 100 mM sucrose for 5 days (day 15), transferred to fresh water or 100 mM sucrose for another 20 days (day 35), and finally rinsed in H_2O for 1 h and then transferred to fresh dishes with H_2O, GA_3, or given R, and germinated in darkness for 48 h; D, dark control. Values are percent germination.

[b] A 5 × 3 factorial ANOVA was performed. Effects of initial and terminal treatment and their interaction are all significantly different at $p < 0.01$. Values that are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test, using two-way comparison of means.

Table 7. Effects of various concentrations of sucrose on sucrose uptake of lettuce seeds.[a]

Concentrations of sucrose 24-h inbibition (mM)	Amount of sucrose uptake/20 seeds (μM)
0.25 (control)	5d[b]
10	181cd
25	347bc
50	395bc
100	476b
500	901a

[a] Dry seeds were imbibed for 0.5 h in solutions of sucrose with 5 μCi of [^{14}C]sucrose/20 seeds/2.0 mL, given FR, imbibed for another 23.5 h, rinsed with 2 mL of sucrose of the same concentration, and then combusted, and the amount of sucrose uptake/20 seeds was determined.

[b] An ANOVA was performed. Effect of sucrose concentration is significantly different at $p < 0.01$. Values that are followed by the same letter are not significantly different at $p < 0.05$ as determined by Fisher's protected LSD test.

the K moiety of KNO_3 plays a significant role in this relationship is not established. However, the importance of P levels to the establishment of dormancy suggests that N:P:K ratios may be significant (Quick et al. 1997).

Dry lettuce seeds contained about 2.5% sugars (Halmer et al. 1978) with sucrose the major free sugar found (Drews and van Staden 1991). Before radicle emergence, sucrose and raffinose levels of the lettuce embryos declined, and glucose and fructose levels increased. Halmer et al. (1978) suggested that sucrose, an intermediate product of gluconeogenesis in the cotyledons, was hydrolyzed to glucose and fructose, which served as the main osmotica supporting growth. However, in embryos of skotodormant lettuce seeds, no changes in glucose, fructose, mannose, and galactose were found during up to 15 months storage (Powell et al. 1983). Embryo sucrose declined rapidly over the 1st month, and endosperm sucrose also declined over the first 2 weeks. Foley (1992) and Foley et al. (1992) reported that fructose, maltose, glucose, and sucrose all broke dormancy in excised wild oat (*Avena fatua*) embryos. Application of GA_3 to dormant wild oat caryopses significantly increased the level of glucose and decreased the level of sucrose in the embryo. Foley and his coworkers (Foley 1992, Foley et al. 1992, 1993) suggested that GA_3 may promote the synthesis or activation of enzymes involved in embryo and/or endosperm carbohydrate metabolism, thereby providing sugar for breaking primary dormancy. Our studies are the first to extend these findings to seeds that have become secondarily dormant and clearly demonstrate that for terminal GA_3 to be effective in enhancing germination of intact 10-day skotodormant lettuce seeds, there must be an adequate endogenous level of soluble sugars retained by the skotodormant seeds (Table 6). Externally supplied sucrose and glucose were more effective (Tables 2–5) than maltose and lactose, whereas fructose, arabinose, and sorbose had no effect (Table 3). This contrasts with studies on excised wild oat embryos (Foley 1992, Foley et al. 1992) where fructose was most and sucrose least effective. The differences are probably due to the different structures of wild oat and lettuce seeds as well as to the use of excised wild oat embryos.

This study is also the first one using soluble sugars or a mixture of sucrose and KNO_3 as a pretreatment during 10-day DS, rather than as an incubation medium, to study the mechanisms involved in the induction and breakage of skotodormancy in lettuce seeds. Our study suggests a reciprocal relationship between endogenous GA and sugars in determining the depth of skotodormancy in lettuce seeds. Seeds supplied with sufficient exogenous sugars during DS remain more sensitive to terminal GA_3, exhibiting shallow skotodormancy. A similar, but less marked relationship appears to exist between endogenous P_{fr} and sugars. The results of this study (Table 5) indicate that soluble sugars play a role similar to that found for certain inorganic nitrogen salts during DS imbibition (Hsiao and Quick 1996). Seeds supplied with nitrate during DS remain more responsive to phytochrome system (Table 5; Hsiao and Quick 1996). Both soluble sugars and nitrate may exert a nutritional effect as well as an osmotic effect on membrane functioning of phytochrome- and GA-mediated control systems in breaking skotodormancy in lettuce seeds (Hsiao and Quick 1996, Hsiao et al. 1984, Taylorson and Hendricks 1977).

The significant positive correlation between sucrose

uptake and various concentrations of sucrose (Table 7) suggests a relationship between membrane integrity and seed sensitivity to terminal GA_3 in skotodormant seeds. The decline of soluble sugars during DS in skotodormant lettuce seeds (Halmer et al. 1978, Powell et al. 1983) might result in limited availability of respiratory substrates for germination (Edje and Burris 1970) or in a reduced protective effect of sugars on structural integrity of membranes (Crowe et al. 1987).

The R pretreatment, given at day 0 to seeds imbibed in 500 mM sucrose or glucose, reduced the degree of skotodormancy and also dark reversion of P_{fr}. Terminal R applied to such seeds yielded complete germination instead of the 25–50% germination found in seeds with FR pretreatment at day 0 (compare Table 4 with Table 2). Our results also showed that the seeds imbibed in 500 mM sucrose had about 180 times higher sucrose uptake than the seeds imbibing in 0.25 mM sucrose (Table 7), which corresponded to their sensitivities to terminal R or GA_3 treatment. GA_3 can induce complete germination in 10-day DS FR-treated skotodormant seeds imbibed in 100–500 mM sucrose and glucose, and either terminal R or GA_3 caused complete or near complete germination in seeds imbibed in mixed solutions of sucrose and KNO_3, indicating that the sugars prevented leaching of certain substrates that are required to maintain sensitivities of these 10-day DS seeds to terminal R or GA_3 treatment.

Hsiao and Vidaver (1989) formulated a model to interpret the effects of R and growth promoters in breaking skotodormancy in light-sensitive Grand Rapids lettuce seeds. Hsiao and Quick (1996) suggested that certain inorganic nitrogen salts present during DS block the conversion of $X' \rightarrow X$ and $Y' \rightarrow Y$ in the model, so that either terminal R or GA_3 can still cause complete germination in seeds so incubated. A similar relationship appears to exist with respect to sugars available during DS. Thus, the germination response is determined among other factors by the product of the concentrations of P_{fr} and GA, the effectiveness of which is in turn dependent on soluble sugars and/or inorganic nitrogen salts. An intricate interplay of action and reaction is thus involved in the breaking of dormancy. This study provides substantial evidence for nonhormonal factors associated with light and GA action in the control of seed skotodormancy and may represent different aspects of a general survival mechanism in skotodormant seeds of numerous species sensitive to such environmental stimuli.

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