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Vernalization and Gibberellin Physiology of Winter Canola[1]

Endogenous Gibberellin (GA) Content and Metabolism of [^{3}H]GA_1 and [^{3}H]GA_{20}

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Winter canola (*Brassica napus* cv Crystal) is an oilseed crop that requires vernalization (chilling treatment) for the induction of stem elongation and flowering. To investigate the role of gibberellins (GAs) in vernalization-induced events, endogenous GA content and the metabolism of [^{3}H]GAs were examined in 10-week vernalized and nonvernalized plants. Shoot tips were harvested 0, 8, and 18 d postvernalization (DPV), and GAs were purified and quantified using $^2H_2$-internal standards and gas chromatography-selected ion monitoring. Concentrations of GA_1, GA_3, GA_8, GA_{19}, and GA_{20} were 3.1-, 2.3-, 7.8-, 12.0-, and 24.5-fold higher, respectively, in the vernalized plants at the end of the vernalization treatment (0 DPV) relative to the nonvernalized plants. Thermoregulation apparently occurs prior to GA_{19} biosynthesis, since vernalization elevated the concentration of all of the monitored GAs. [^{3}H]GA_{20} or [^{3}H]GA_1 was applied to the shoot tips of vernalized and nonvernalized plants, and after 24 h, plants were harvested at 6, 12, and 15 DPV. Following high-performance liquid chromatography analyses, vernalized plants showed increased conversion of [^{3}H]GA_{20} to a [^{3}H]GA_1-like metabolite and reduced conversion of [^{3}H]GA_1 or [^{3}H]GA_{20} to polar ^{3}H-metabolites, putative glucosyl conjugates. These results demonstrate that vernalization influences GA content and GA metabolism, with GAs serving as probable regulatory intermediaries between chilling treatment and subsequent stem growth.

Although plant growth and development are partially determined by genetic factors, environmental conditions including photoperiod and temperature also have major influences. Vernalization is a chilling treatment that promotes flowering in a number of plants from diverse taxa. The physiological mechanism of vernalization is interesting in that the perception and initial transduction of the environmental stimulus occur during the chilling period, but the developmental consequences are expressed after vernalization (Metzger, 1988).

Since GAs are involved in the regulation of both stem elongation and flowering in numerous plants (Zeevaart, 1983; Pharis and King, 1985; Phinney, 1985), it has repeatedly been proposed that GAs are involved in the regulation of events following vernalization (Lang, 1957, 1965; Chailakhyan and Lozhnikova, 1962; Hazebroek and Metzger, 1990; Metzger, 1990). Numerous studies have attempted to induce stem elongation and/or flowering using exogenous GA_3 treatment of non- or partially vernalized plants (Lang, 1957, 1965). Whereas exogenous GA studies investigate the physiological consequence of increased GA level, the reciprocal condition of reduced GA level has also been investigated. GA-deficient mutants are short and often have retarded flowering without supplemental GA treatment (Rood et al., 1989b; Reid, 1990). With respect to this phenotype, GA-deficient *Brassica* mutants such as *rosette* resemble nonvernalized winter annuals (Rood et al., 1989b; Zanewich et al., 1990), suggesting that the failure of nonvernalized plants to bolt and flower could be the result of reduced GA content.

In addition to manipulative experimental approaches, studies involving the quantification of endogenous GAs may be used to assess the relationships between GA content, growth, and flowering in winter annuals or biennials. Quantitative and/or qualitative changes in endogenous GA content would be expected prior to and during the transition from the vegetative to the reproductive condition (Zeevaart, 1983).

Although a few studies have investigated the endogenous GA content of vernalized compared to nonvernalized plants using either bioassays or GC-MS, results have been variable (Suge, 1970; Michniewicz et al., 1981; Joseph et al., 1983; Lin and Stafford, 1987). Bioassay results with *Brassica napus* (Margara, 1963), *Brassica rapa* (Suge and Takahashi, 1982), chicory (Joseph et al., 1983), radish (Suge, 1970; Michniewicz et al., 1981), and winter wheat (Chailakhyan and Lozhnikova, 1962; Reda et al., 1978) have indicated the presence of additional GA-like substances or increases of certain GA-like substances in vernalized tissues. In contrast, in another member of the Brassicaceae, *Thlaspi arvense*, increases in endogenous GA-like substances did not appear to be directly induced by low temperature, but rather thermoinduction may have resulted in altered GA sensitivity or GA metabolism (Metzger, 1985).

Winter canola refers to genotypes of oilseed rape (*B. napus* or *B. rapa*) with nutritionally favorable oil compositions (canola) and qualitative requirements for vernaliza-

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Abbreviations: ANOVA, analysis of variance; DPV, days postvernalization; EtOAc, ethyl acetate; M^+, molecular ion; MeOH, methanol; Rt, retention time; SIM, selected ion monitoring; SiO_2, silicic acid.

tion for the induction of bolting and flowering (anthesis). Without vernalization, winter canola remains as a vegetative rosette with a phenotype partly resembling that of the GA-deficient mutants or plant growth retardant-treated plants (Zanewich et al., 1992). Consequently, studies were conducted to investigate vernalization and GA physiology in *Brassica*, a genus that has long been involved in thermoinduction studies and for which various aspects of GA physiology have been described (Rood et al., 1987, 1989a; Hedden et al., 1989; Zanewich and Rood, 1993). The endogenous GA contents of vernalized and nonvernalized winter canola plants were compared to test the hypothesis that vernalization results in elevated GA concentrations that presumably enable bolting and flowering. Furthermore, studies of the influence of vernalization on GA metabolism were conducted involving GA_1, the probable effector GA for the regulation of stem elongation in various plants (Phinney, 1985), and GA_{20}, the principal but not exclusive precursor of GA_1 in *Brassica* (Rood et al., 1990; Rood and Hedden, 1994).

MATERIALS AND METHODS

Plant Material and Growth

Certified seed of *Brassica napus* cv Crystal (from Keith Topinka, Agriculture Canada Research Station, Lethbridge, Alberta, Canada) was sown in 11.5- (diameter) × 9.5-cm pots filled with Terra-Lite 2000 Metro-Mix (W.R. Grace Co., Ajax, Ontario, Canada). Plants were watered to saturation daily and fertilized with a water-soluble 20–20–20 fertilizer with chelated trace elements (The Professional Gardener Co., Ltd., Calgary, Alberta, Canada). All plants were grown at 23 ± 4°C (day and night) in the University of Lethbridge greenhouse (latitude 49°41′ N and longitude 112°51′ W) and provided with a 16-h photoperiod provided by natural sunlight supplemented with high-pressure sodium vapor lights (Reflector PL90M [medium] N400; P.L. Light Systems Canada, Inc., Grimsby, Ontario, Canada). Lights were positioned 1.4 m above the plants and provided 280 μmol m^{-2} s^{-1} PAR.

Following 4 weeks of growth in greenhouse conditions, plants to be vernalized were transferred to an upright refrigerator maintained at 4 ± 2°C. A 10-h photoperiod was provided by cool-white fluorescent lights delivering 50 μmol m^{-2} s^{-1} PAR. After a 4°C vernalization period of 10 weeks, plants were transferred to a cold room (13 ± 2°C) for 72 h (light provided by a 150-W incandescent bulb and cool-white fluorescent tubes collectively delivering 15 μmol s^{-1} m^{-2} PAR) and then returned to the original greenhouse conditions. Plant height and developmental stage were determined at weekly intervals (Harper and Berkenkamp, 1975).

Quantitative Analyses of Endogenous GAs by GC-SIM

Samples consisting of five to seven 1.5-cm-long shoot tips containing the apical meristems and upper stem segments (dry weights 0.3–5.5 g) were collected 0, 8, and 18 DPV. Excised shoot tips were frozen in liquid N_2, lyophilized for 72 h, and stored at −20°C. Vernalized plants 18 DPV had macroscopically visible flower buds and had started to elongate.

Prior to extraction, all tissue was relyophilized for 24 h. Tissue was ground in cold (4°C) 80% aqueous MeOH and extracted for 12 h at 4°C. Extracts were vacuum filtered, buffered with sodium phosphate (0.1 M, pH 8.0), and 20 ng each of [17-$^2H_2$]GA_1, [17-$^2H_2$]GA_3, [17-$^2H_2$]GA_4, [17-$^2H_2$]GA_8, [17-$^2H_2$]GA_9, [17-$^2H_2$]GA_{19}, and [17-$^2H_2$]GA_{20} (all greater than 99% enrichment) were added as quantitative internal standards. The MeOH was removed in vacuo at 35°C, and the pH of the resulting aqueous extract was increased to 9 with 1.5 N KOH. Aqueous extracts were partitioned twice against water-saturated diethyl ether, the aqueous phase was then acidified to pH 7 using 1.5 N HCl, and polyvinylpolypyrrolidone was added. After 30 min, the aqueous polyvinylpolypyrrolidone slurry was vacuum filtered, and the filtrate was acidified to pH 3 with 1.5 N HCl and partitioned three times against water-saturated EtOAc. The EtOAc extracts were frozen and cold filtered to remove water. EtOAc was then evaporated in vacuo to leave a solid residue. Residues were dissolved in a small volume of 1:1 (v/v) MeOH:EtOAc and transferred to Whatman GF/D glass microfiber discs. Chromatographic standards consisting of 250 Bq each of [1,2-^{3}H]GA_1 and [1,2-^{3}H]GA_4 (Amersham) were added to the discs.

The residues were further purified using stepwise elution silica gel (SiO_2) partition chromatography (Durley et al., 1972; Rood et al., 1983). A 5-g silica column was poured, and following low-pressure compaction, the glass microfiber discs were placed on top of the column's stationary phase. The column was eluted with 30 mL of a 60:40 (v/v) *n*-hexane:EtOAc solution, followed by 35 mL of a 5:95 *n*-hexane:EtOAc solution. Fractions of 5 mL were collected, and ^{3}H distribution was determined by liquid scintillation counting of aliquots.

Based on radiocounting results, appropriate SiO_2 fractions were dried, dissolved in MeOH, filtered through 0.45-μm filters, passed through Waters C_{18} Sep Pak cartridges, and dried under N_2 at room temperature. The GAs were further purified and fractionated by gradient-eluted reversed-phase HPLC using a μBondapak C_{18} column (3.9 × 300 mm; Millipore-Waters) (Koshioka et al., 1983).

HPLC fractions were air dried and then grouped according to the Rts of authentic standards (Koshioka et al., 1983), methylated in 100 μL of ethereal diazomethane at room temperature for 60 min, dried under N_2, and remethylated. Methylated samples were dried under N_2 and silylated with 50 μL of pyridine and 50 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane at 90°C for 30 min. GA_8 samples were silylated twice.

After drying under a stream of N_2, methylated and trimethylsilylated samples were dissolved in hexane, and 1-μL aliquots were introduced by cool on-column injection into a precolumn fitted to a J&W Scientific DB-5 ms 15-m × 0.25-mm silica column with a 0.25-μm film of polymethyl (5% phenyl) siloxane (Chromatographic Specialties, Brockville, Ontario, Canada). GAs were resolved using a Hewlett-Packard 5890 series II gas chromatograph (Zanewich and Rood, 1993) and analyzed by a Hewlett-

Packard 5980 mass-selective detector in the SIM mode. The M^+ and five other characteristic ions were monitored for each GA.

The concentrations of endogenous GA_1, GA_3, GA_4, GA_5, GA_8, GA_9, GA_{19}, and GA_{20} were calculated from the M^+ peak area ratios of 506/508, 504/506, 418/420, 416/418, 594/596, 298/300, 434/436, and 418/420, respectively, using a modified version of the equation for isotopic dilution analysis described by Fujioka et al. (1988). Two separate experiments were performed to assess endogenous GA content. Similar results were observed in both experiments, and only results from the second experiment are presented. That experiment included four replicates that were independently ground, extracted, purified, and analyzed.

Metabolism of [^{3}H]GA_1 and [^{3}H]GA_{20} in Vernalized and Nonvernalized Plants

The metabolism of [^{3}H]GAs was examined in two separate experiments. In one experiment, 2.1 kBq of either [1,2-^{3}H]GA_1 (from Amersham; specific activity about 1.2 TBq mmol^{-1}) or [1,2,3-^{3}H]GA_{20} (from Professor R.P. Pharis, University of Calgary [Murofushi et al., 1977]; specific activity about 62 GBq mmol^{-1}) in 95% aqueous ethanol were applied by syringe to the shoot tip of vernalized and nonvernalized plants at 5 DPV (no stem elongation) or at 11 DPV (some stem elongation). In a second experiment, 2.1 kBq of either [^{3}H]GA_1 or [^{3}H]GA_{20} were applied to the shoot tips of plants 14 DPV (stem elongation). Following an incubation period of 24 h, shoot tips were harvested, frozen in liquid N_2, and lyophilized. All tissue was stored with desiccant at −20°C until analysis.

Tissue was ground in cold 80% aqueous MeOH and extracted for 12 h at 4°C. Following vacuum filtering, extracts were dried in vacuo at 35°C, redissolved in a total of 1.5 mL of 1:1 MeOH:EtOAc, and loaded onto glass microfiber discs. Subsequently, a minimum volume of 50% aqueous MeOH was also used to transfer remaining substances to the discs. Samples were purified using stepwise elution silica gel (SiO_2) partition chromatography (Durley et al., 1972; Rood et al., 1983). The column was eluted with 50 mL of 60:40 (v/v) *n*-hexane:EtOAc, followed by 50 mL of 5:95 *n*-hexane:EtOAc (EtOAc-eluted GAs), and finally 40 mL of MeOH (MeOH-eluted GAs). Fractions of 5 mL were collected, and aliquots were removed to determine the distribution and ratio of EtOAc-eluted or "free" acidic [^{3}H]GAs to the MeOH-eluted or putative conjugated [^{3}H]GAs. Recovery of GAs in either the EtOAc or MeOH fractions was expressed as a percentage of the total recovered radioactivity.

Radioactive fractions were combined into two groups representing either the EtOAc-eluted (free) or MeOH-eluted (conjugated) fractions, and aliquots were chromatographed on reverse-phase C_{18} HPLC (Koshioka et al., 1983). Tentative identification of ^{3}H-metabolites was based on comparison of chromatographic Rts of authentic [^{3}H]GA standards and Rts described by Koshioka et al. (1983) for GAs that had previously been identified as metabolites following [$^2H_2$]GA feeds to *Brassica* (Rood et al., 1990). Putative GA metabolite recovery was expressed as a percentage of total recovered ^{3}H and used to assess substrate turnover.

RESULTS AND DISCUSSION

Influence of Vernalization on Endogenous GA Concentration in Winter Canola

The winter canola cv Crystal has an absolute vernalization requirement of at least 8 weeks that subsequently induced stem elongation and flowering (Zanewich et al., 1992). After 10 weeks of vernalization at 4°C, vernalized and nonvernalized plants had distinctive phenotypes. Significant differences (e.g. at 16.5 weeks following vernalization; ANOVA, $F = 15.5$, $P < 0.003$) in height were apparent following vernalization and were maintained until the conclusion of the study (Fig. 1). By 18 DPV, nonvernalized plants remained as slow-growing vegetative rosettes, whereas the stems of all vernalized plants had started to elongate and macroscopic flower buds were evident.

During extraction, $^2H_2$-labeled 13-OH and non-13-OH GAs that had previously been identified from *Brassica* (Rood et al., 1987; Hedden et al., 1989; Zanewich and Rood, 1993) were added as quantitative internal standards. Whereas all [$^2H_2$]GA standards were detected by GC-SIM, only endogenous 13-OH GAs were sufficiently abundant for quantitation. Trace quantities of GA_4 were present but could not be confidently quantified. The ranking of endogenous GAs in both the vernalized and nonvernalized canola plants in decreasing abundances were $GA_3 > GA_{19} > GA_8 > GA_1 = GA_{20} > GA_4$ (Fig. 2). There were no apparent qualitative changes in the endogenous GA profiles between vernalized and nonvernalized winter canola shoot tips. Furthermore, the relative proportions of endogenous GAs were generally similar to those found in shoots of annual *Brassica* plants (Rood et al., 1987, 1989a; Zanewich and Rood, 1993).

Although proportions of the different GAs were similar in shoot tips from vernalized and nonvernalized plants, GA concentrations were much higher in the vernalized plant shoot tips (Fig. 2). Elevated GA concentrations were observed for almost all GAs for all three harvests, with the exception of GA_{19} at 18 DPV, which was similar in vernal-

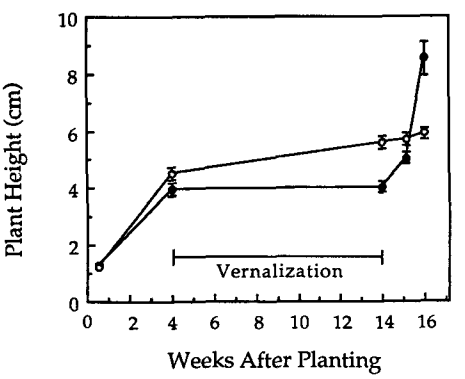

Figure 1. Heights of vernalized (●) and nonvernalized (○) plants of *B. napus* cv Crystal. Plants were vernalized for 10 weeks (from week 4 until week 14). Values represent the means ± SE of 20 plants.

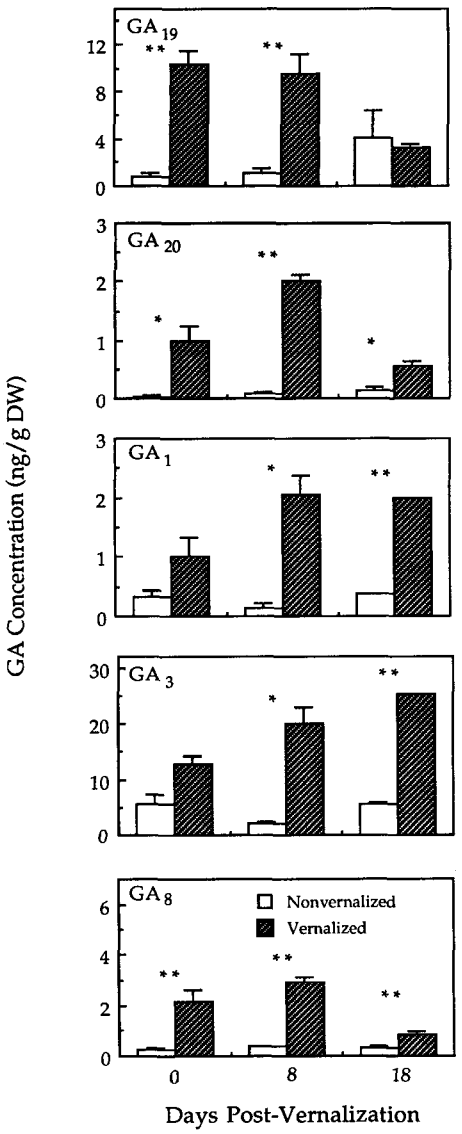

Figure 2. Contents of GA_{19}, GA_{20}, GA_1, GA_3, and GA_8 in shoot tips from vernalized and nonvernalized *B. napus* cv Crystal plants at 0, 8, and 18 DPV. Values plotted are the means ± SE of four separate samples. The asterisks indicate that values are significantly different between the vernalized and nonvernalized treatments (ANOVA; *P < 0.05; **P < 0.01). DW, Dry weight.

ized and nonvernalized plants. Concentrations of the dihydroxylated GAs that are likely to be particularly important, at least for the regulation of shoot elongation, GA_1 and GA_3, were significantly higher in vernalized stem tissue of plants, with increases being 3.1- and 2.3-fold at 0 DPV, 15.8- and 9.6-fold at 8 DPV, and 5.2- and 4.6-fold higher at 18 DPV for GA_1 and GA_3, respectively. Concentrations of GA_8 were also greater (e.g. 8 DPV, by ANOVA, $F = 114.16$, $P < 0.01$) in vernalized plants throughout the entire sampling period. GA_{19} and GA_{20}, the precursors of the putative effector of elongation, GA_1, were significantly increased (e.g. GA_{19} 8 DPV, by ANOVA, $F = 24.55$, $P < 0.01$) in vernalized shoots and tended to decline by the third harvest (Fig. 2). This may reflect the metabolism or conversion of these precursor GAs to GA_1, whose concentration apparently increased and then remained high.

Although GAs from both the early 13-OH and non-13-OH pathway have previously been detected in *Brassica* (Rood et al., 1987; Hedden et al., 1989; Zanewich and Rood, 1993), based on the detection of GAs in the present study, the GAs from the early 13-OH biosynthetic pathway were most abundant and were substantially influenced by vernalization in winter canola. Qualitative comparisons of GAs were similar in the shoot tips of vernalized and nonvernalized winter canola, but quantitative changes in endogenous GAs were detected and the differences were positively correlated with stem elongation following vernalization.

The observation that all of the 13-OH GAs studied were elevated following vernalization suggests that a thermoinduced biosynthetic step occurs prior to GA_{19} in *Brassica*. Lin and Stafford (1987) observed an abundance of C_{20}-GAs, such as GA_{53}, GA_{44}, and GA_{19}, and a reduction in levels of C_{19}-GAs, such as GA_1 and GA_3, in vernalized shoots of wheat seedlings, suggesting that turnover or the conversion of C_{20}- to C_{19}-GAs might be influenced by low-temperature treatment. The present study with *Brassica* does not support regulation at the point of GA_{19} oxidation, since levels of GA_{19} were elevated rather than reduced in vernalized plants (Fig. 2). In thermoinduced *Thlaspi* tissue, early metabolic precursors of all GAs (such as *ent*-kaurenoic acid) were elevated and had increased turnover rates, suggesting that kaurenoid metabolism is involved in the thermoinductive response in this crucifer (Hazebroek and Metzger, 1990). An influence of vernalization on the biosynthesis or metabolism of GA precursors would be consistent with the results of the present study.

In a spring canola cultivar (Westar), the concentrations of endogenous GAs and GA-like substances were relatively low during the vegetative phases of growth but increased during floral initiation and again during silique filling (Rood et al., 1989a; Zanewich and Rood, 1993). However, since stem elongation and floral initiation generally occur relatively synchronously, it is difficult to determine whether increases in endogenous GAs are specifically associated with stem elongation and/or flower induction. In some previous studies involving *Brassica* and *Lunaria*, increases in endogenous GA-like substances were observed in thermoinduced plants but were proposed to be associated with stem elongation rather than flower induction (Margara, 1963; Zeevaart, 1983).

In the present study, GA concentrations were elevated at the end of the vernalization treatment, demonstrating that GAs had accumulated during the chilling treatment. Low temperature might be expected to reduce the rate of GA biosynthesis, but net accumulation could still occur if GA metabolism or utilization were reduced by low temperature proportionally more than GA biosynthesis. This hypothesis remains speculative without metabolic studies but is consistent with the observed growth response. During the vernalization treatment, no shoot growth occurred despite the GA accumulation (Fig. 2). This might reflect the absence of GA utilization, and consequently, even

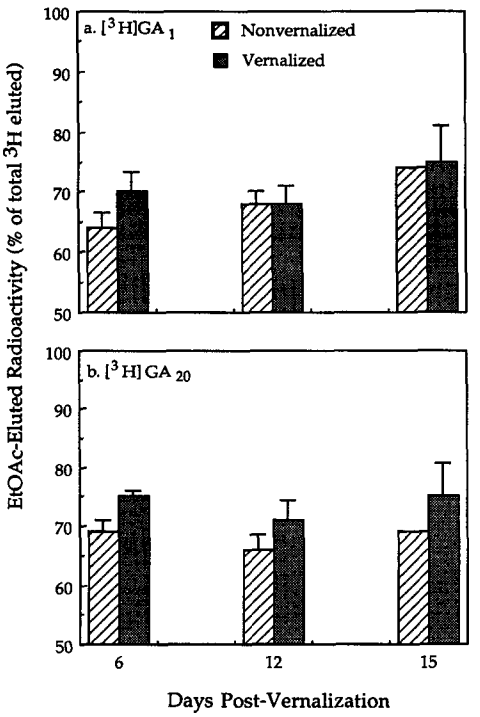

Figure 3. Percentage of extracted radioactivity that eluted with EtOAc from step-elution SiO_2 partition column loaded with extracts from vernalized and nonvernalized shoot tips of plants of *B. napus* cv Crystal fed $[^3H]GA_1$ (a) and $[^3H]GA_{20}$ (b) at 6, 12, and 15 DPV. Values are means ± SE of five (6, 12 DPV) or two (15 DPV) replicates. SE values for nonvernalized plants on d 15 are smaller than the width of the bar.

a slow rate of GA biosynthesis could still result in GA accumulation.

Influence of Vernalization on the Metabolism of $[^3H]GA_{20}$ or $[^3H]GA_1$

To further investigate the relationship between vernalization and GA physiology, $[^3H]GA_1$ or $[^3H]GA_{20}$ was applied to vernalized and nonvernalized winter canola plants. Subsequently, metabolites were extracted, and the distributions of ^3H in extracts from the $[^3H]GA_1$ and $[^3H]GA_{20}$ feeds are shown in Figure 3, a and b, respectively. These data represent the proportion of radioactivity eluting from SiO_2 columns with EtOAc, fractions that generally represent free GAs. The remaining radioactivity was eluted with MeOH and that would include GA glucosyl conjugates, if present (Rood et al., 1983; Schneider, 1983; Schneider et al., 1992). There were significant differences (by three-factor ANOVA, vernalized versus nonvernalized effect, $F = 5.02$, $P = 0.03$) in the proportion and in the amounts of recovered EtOAc-eluted $[^3H]GAs$ between vernalized and nonvernalized winter canola shoots (Fig. 3). At all three harvests following the $[^3H]GA_{20}$ application and following the $[^3H]GA_1$ application 6 DPV, the vernalized plants had higher proportions of EtOAc-eluted radioactivity (free $[^3H]GAs$) (Fig. 3). Conversely, nonvernalized plants had higher proportions of MeOH-eluted metabolites, suggesting more rapid conversion to more polar forms, presumably consisting partially of glucosyl conjugates.

In vernalized and nonvernalized plants, the principal ^3H-metabolite following $[^3H]GA_{20}$ feeds eluted at the Rt of $[^3H]GA_1$. The conversion of $[^2H_2]GA_{20}$ to $[^2H_2]GA_1$ has previously been demonstrated in *Brassica* (Rood et al., 1990). Apparently, based on the amount of radioactivity recovered, the conversion of $[^3H]GA_{20}$ to $[^3H]GA_1$ was more rapid in vernalized winter canola plants by 12 DPV (by ANOVA, $F = 9.42$, $P = 0.015$) and also tended to be more rapid at 15 DPV (Table I). Thus, the rate of 3β-hydroxylation was apparently increased by between 6 and 29% in vernalized winter canola plants. Smaller peaks accounting for approximately 6% of the total recovered EtOAc-eluted $[^3H]GA$ in either the vernalized or nonvernalized plant tissue corresponded to the Rt of authentic $[^3H]GA_{29}$ (Table I). There were no significant differences in the apparent rate of formation of $[^3H]GA_{29}$ in vernalized compared to nonvernalized plants (Table I).

Table I. *Distribution of putative metabolites from $[^3H]GA_{20}$ feeds to vernalized and nonvernalized plants of B. napus cv Crystal at 6, 12, and 15 DPV, as determined by HPLC Rt*

Following silica column purification, free GAs would normally be contained in the EtOAc-eluted fractions, whereas more polar substances, including GA glucosyl conjugates, would be contained in the MeOH-eluted fractions. Values are means ± SE from five (6, 12 DPV) or two (15 DPV) replicates.

Days after Vernalization	Percentage of Total Recovered Radioactivity					
	EtOAc-eluted GAs from SiO_2			MeOH-eluted GAs from SiO_2		
	GA_{20} (30–31)[a]	GA_1 (24–25)	GA_{29} (18–19)	GA_{20} (29–30)	GA_1 (24–25)	GA_{29} (18–19)
Nonvernalized						
6	37.1 ± 0.8[b]	27.0 ± 1.2	5.0 ± 0.5	12.4 ± 0.7[b]	14.5 ± 0.8[b]	4.0 ± 0.5
12	40.1 ± 1.1	19.8 ± 0.9[b]	4.2 ± 0.3	17.1 ± 1.3	15.2 ± 0.7	3.6 ± 0.3
15	57.4 ± 4.1	11.4 ± 5.1	2.2 ± 0.7	18.6 ± 2.4	11.1 ± 3.7	2.0 ± 0.3
Vernalized						
6	42.2 ± 0.6[b]	28.8 ± 0.8	4.7 ± 0.3	9.6 ± 0.4[b]	11.7 ± 0.8[b]	3.3 ± 0.6
12	41.1 ± 1.2	26.0 ± 1.3[b]	5.3 ± 0.5	13.2 ± 1.3	11.6 ± 1.8	2.9 ± 0.3
15	55.9 ± 0.4	15.9 ± 1.4	2.8 ± 0.4	14.0 ± 4.3	10.3 ± 4.4	1.1 ± 0.6

[a] HPLC fraction is shown in parentheses. [b] Significant difference between nonvernalized and vernalized treatments (ANOVA and/or *t* tests).

Table II. *Distribution of putative metabolites from [^{3}H]GA_1 feeds to vernalized and nonvernalized plants of B. napus cv Crystal at 6, 12, and 15 DPV, as determined by HPLC Rt*

Following silica column purification, free GAs would normally be contained in the EtOAc-eluted fractions, whereas more polar substances, including GA glucosyl conjugates, would be contained in the MeOH-eluted fractions. Values are means ± SE from five (6, 12 DPV) or two (15 DPV) replicates.

Days after Vernalization	Percentage of Total Recovered Radioactivity			
	EtOAc-eluted GAs from SiO_2		MeOH-eluted GAs from SiO_2	
	GA_1 (24–25)[a]	GA_8 (13–14)	GA_1 (24–25)	GA_8 (13–14)
Nonvernalized				
6	47.5 ± 3.6[b]	14.0 ± 2.4	23.1 ± 1.0	13.4 ± 2.2
12	45.4 ± 6.4	6.4 ± 2.7	19.4 ± 1.7	10.4 ± 1.2
15	67.5 ± 0.7[b]	3.2 ± 1.6	22.3 ± 0.6	1.8 ± 0.7
Vernalized				
6	61.7 ± 2.9[b]	8.3 ± 1.1	19.6 ± 4.4	6.5 ± 0.6
12	52.3 ± 2.6	15.1 ± 1.2	19.0 ± 3.2	10.4 ± 1.2
15	59.1 ± 0.3[b]	12.8 ± 9.9	19.5 ± 7.1	3.4 ± 0.4

[a] HPLC fraction is shown in parentheses. [b] Significant difference between nonvernalized and vernalized treatments (ANOVA).

Accompanying the apparently increased rate of GA_{20} metabolism in the vernalized plants were reduced rates of production of ^{3}H-labeled MeOH-eluted substances, putative GA glucosyl conjugates (Table I). The most abundant MeOH-eluted ^{3}H-metabolites detected using HPLC chromatographed near the Rt of authentic [^{3}H]GA_{20}, with lower amounts of radioactivity corresponding to the Rts of authentic [^{3}H]GA_1 and [^{3}H]GA_{29}. These three peaks probably represent GA glucosyl conjugates, which elute from HPLC slightly before or coincidentally with their corresponding free GAs (Koshioka et al., 1983). The putative conjugates of both [^{3}H]GA_{20} and [^{3}H]GA_1 were significantly reduced in the vernalized plants at 6 DPV, and a similar pattern was observed for all nine comparisons for the three GA fractions at three harvests (Table I).

Results of the [^{3}H]GA_1 application were more variable but generally consistent with those of the [^{3}H]GA_{20} application (Table II). Proportions of [^{3}H]GA_1 were higher in the vernalized plants at 6 DPV, and proportions of putative conjugates tended to be reduced. However, an opposite pattern for [^{3}H]GA_1 was observed following the 14-DPV application, complicating interpretation.

Collectively, these metabolic studies indicate that vernalized plants generally had higher proportions of putative free GA metabolites and reduced proportions of putative conjugates. The physiological role of GA conjugates is not well understood, although they could represent either temporary or permanent removal of GAs from the biologically active form (Schneider et al., 1992). The present results suggest that conjugation might play a role in the control of free GA level and subsequent induction of elongation following vernalization. Vernalization apparently reduced the rates of GA-conjugate formation, a process that removes GAs or precursors through chemical inactivation. The metabolic differences observed in the present study were generally slight. In other cold-requiring annuals, enhanced metabolism early in the GA biosynthetic pathway has been observed (Hazebroek and Metzger, 1990; Moore and Moore, 1991; Hazebroek et al., 1993).

Differential GA metabolism between vernalized and nonvernalized plants supports the influence of vernalization on GA physiology. Combined with the observation that GA concentration is elevated following vernalization, these studies indicate that GAs probably serve as phytohormonal intermediaries between the perception of low temperature (vernalization) and at least some components of the subsequent growth and developmental responses.

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