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# Effects of Abscisic Acid (ABA) and ABA Analogs on Freezing Tolerance, Low-Temperature Growth, and Flowering in Rapeseed

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**Abstract.** *Brassica napus* and *B. campestris* are grown in Western Canada in areas subject to unseasonable frosts. At the seedling stage, cultivars of *Brassica* are very sensitive to frosts of −2° to −5°C, which are either lethal or delay the development of the plant. Seedlings of *B. napus* and *B. campestris*, germinated and grown at 10°C (16-h photoperiod), were treated with a foliar spray of either 100 μM racemic abscisic acid (ABA), 100 μM of various ABA analogs, 0.1% acetone, or were untreated. Freeze tests indicated 2°C of frost tolerance could be gained in *B. napus* following an application of three ABA analogs. In *B. campestris*, three analogs also increased freezing tolerance approximately 1.5°C. The analogs 2′,3′ dihydro ABA and acetylenic divinyl methyl-ABA were effective in both species. Plant fresh weight and dry weight increased in treated plants relative to control or acetone-treated plants after 3 weeks at 10°C. The effect of frost and/or analog treatment on flowering was determined in both species. In *B. campestris* and *B. napus*, a mild frost advanced flowering by approximately 2 days compared with nonfrozen control plants. The promotive effect of frost on flowering decreased with increasing severity of the frost. Several of the analog treatments, particularly 2′,3′ dihydro ABA and acetylenic divinyl ABA, advanced flowering by 2–3 days in both species. The benefit of these ABA analog treatments on flowering was enhanced additionally by a mild frost. Plants treated with either ABA, 2′,3′ dihydro ABA, 2′,3′ acetylenic dihydro ABA, or acetylenic divinyl ABA flowered up to 5 days earlier than control plants.

*Brassica campestris* and *B. napus* are grown in Western Canada and Northern Europe, in regions that are subject to late summer or early fall frosts. These frosts, although usually not lethal, can severely reduce seed quality, primarily by increasing the percentage of green seeds. The increased green seed number results in higher processing costs and a lower price for the producer. Therefore, decreasing or eliminating the damaging effects of frost on seed quality is of considerable economic interest.

Improved oil processing of green seed and investigations into the molecular mechanisms controlling the degreening process are two areas of interest. The possibility of frost avoidance, through earlier germination, earlier flowering, or faster seed maturation, are also being investigated. Plant breeders have introduced numerous new cultivars of canola-quality rapeseed; however, most of the past and current research in this area has focused on disease resistance and oil quality. Other efforts have investigated the promotion of low-temperature seed germination, and rapid and even seedling emergence (Abrams and Gusta 1989). If this latter approach is to be effective, the problems of spring frosts and decreased seedling growth rate during cool weather must be addressed. One possible solution is the use of applied frost protectants and growth promoters on newly emerged seedlings. The plant hormone abscisic acid (ABA) has long been implicated in the process of cold acclimation in plants. Racemic (±) ABA confers frost tolerance when applied to whole plants (Chen et al. 1983, Lang et al. 1989) or cell cultures (Chen and Gusta 1983, Churchill et al.

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1992) and has been shown to increase frost tolerance in *Brassica* species (Johnson-Flanagan et al. 1991, Orr et al. 1986). Endogenous ABA also increases in response to cold temperature (Chen et al. 1983, Daie et al. 1981, Dorffling et al. 1990).

Although ABA may be involved in frost protection, there are only a few reports of increased frost resistance using ABA as a foliar spray (Flores et al. 1988), which is the only practical method of field application for canola. In addition, whereas (±) ABA does promote some developmental processes, it has been shown to inhibit above-ground growth in many studies (Biddington and Dearman 1982, Creelman et al. 1989, Rehm and Cline 1973, Watts et al. 1981) and thus may compound the problem of low-temperature growth inhibition.

Recent work has suggested that the use of ABA analogs may be able to overcome these problems. We have noted differences in the plant physiological and molecular responses that result from changes to the structure of the ABA molecule (Churchill et al. 1992, Gusta et al. 1992, Walker-Simmons et al. 1992, Wilen et al. 1993). Bromegrass suspension culture cells increased in freezing tolerance following the addition of certain ABA analogs (Churchill et al. 1992). Some analogs were shown to be more effective at inhibiting seed germination than ABA (Gusta et al. 1992), whereas others displayed differential effects on seed germination and embryo-specific gene expression (Walker-Simmons et al. 1992, Wilen et al. 1993). We report the effects of applying ABA and ABA analogs on the freezing tolerance, low-temperature growth, and flowering in *B. napus* and *B. campestris*.

## Materials and Methods

### *Chemical Synthesis*

Racemic ABA (Fig. 1, compound 1) was purchased from commercial sources. The syntheses of methyl abscisate (compound 2), ABA aldehyde (compound 3), 2',3' dihydro ABA ([1a,1(2Z,4E),6β]-5-(1-hydroxy-2,2,6-trimethyl-4-oxocyclohexyl)-3-methyl-2,4-pentadienoic acid; compound 4),2',3' acetylenic dihydro ABA ([1a,1(2Z,4E),6β]-5-(1-hydroxy-2,2,6-trimethyl-4-oxocyclohexyl)-3-methylpent-2-en-4-ynoic acid; compound 5), divinyl methyl ABA (methyl-(2Z,4E)-5-(2,6-dimethyl-1-hydroxy-4-oxocyclohexa-2,5-dienyl)-3-methylpent-2,4-dienoate; compound 6), and acetylenic divinyl methyl ABA (methyl-(2Z,4E)-5-(2,6-dimethyl-1-hydroxy-4-oxocyclohexa-2,5-dienyl)-3-methylpent-2en-4-ynoate; compound 7) were as reported elsewhere (Blake et al. 1990, Lamb and Abrams 1991, Lei et al. 1994, Mayer et al. 1976). All chemicals were dissolved in acetone and slowly added to distilled deionized (D.D.) $H_2O$ to a final concentration of 100 μM and 0.1% acetone. Cittowett (0.1%) was added to all solutions, as a surfactant, to aid in uptake of the analogs.

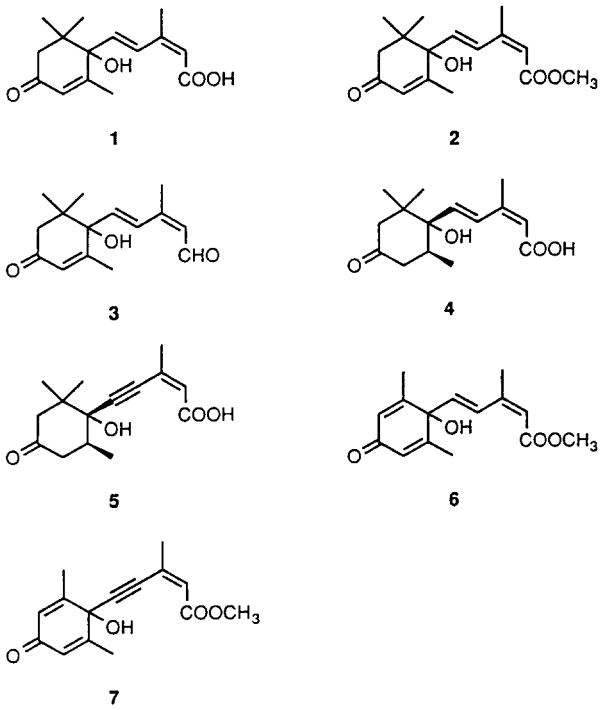


**Fig. 1.** Structural formulae of ABA and ABA analogs. **1:** (±)ABA, **2:** (±) methyl-ABA, **3:** (±)ABA aldehyde, **4:** (±)2',3' dihydro ABA, **5:** (±)2',3' acetylenic dihydro ABA, **6:** divinyl methyl-ABA, **7:** acetylenic divinyl methyl-ABA.

### *Freezing Tests*

*Brassica campestris* cv Tobin and *B. napus* cv Delta seeds were obtained from Dr. W. McNab (Pioneer Hi-Bred Production Ltd., Georgetown, ON). Seeds were germinated and seedlings grown at 10°C (16-h photoperiod, light intensity 550 $\mu mol \cdot m^2 \cdot s^{-1}$) in controlled environment chambers (Conviron). When the majority of the plants were at the one-leaf stage, they were thinned to 5–6 plants per seeding compartment, and then sprayed with a solution containing either 100 μM ABA or 100 μM of various ABA analogs. All solutions contained 0.1% acetone and 0.1% cittowett. Plants were also sprayed with a solution of acetone and cittowett as a treated control.

Freeze tests were conducted 2 days after application of the growth regulators. Plant trays were placed in a "styrofoam" box partially filled with a dry mixture of peatmoss and vermiculite to prevent freezing of the root tips growing from the bottom of the trays. The tops of the trays were adjusted to be level with the top of the "styrofoam" container. Plants were then placed in a chamber at −3°C and allowed to equilibrate for 1 h. Ice crystals were placed on plants to initiate extracellular freezing, and plants were then left at −3°C for an additional 60 min. The temperature was then lowered by 1°C $h^{-1}$, with samples removed at 1°C intervals. *Brassica napus* plants were frozen to −7°C, and *B. campestris* plants were frozen to −8°C. Plants were removed after 60 min at each temperature and transferred to 10°C for 7 days for survival evaluation. Plants were rated as surviving if the apex displayed no signs of frost damage and no more than 50% of the leaf area of the primary leaf and cotyledons were frost injured.

**Table 1.** Effect of ABA and ABA analogs on freezing tolerance in *Brassica napus* cv Delta and *B. campestris* cv Tobin.

| | *B. napus* (±SE) | | *B. campestris* (±SE) | |
|---|---|---|---|---|
| Treatment[a] | $LT_{50}$[b] | % Survival[c] | $LT_{50}$ | % Survival |
| Control | −3.9 ± 0.2°C | 32.8 ± 2.1 | −5.3 ± 0.2°C | 48.8 ± 2.1 |
| Acetone | −4.3 ± 0.3°C | 36.7 ± 4.2 | −5.3 ± 0.4°C | 47.8 ± 2.5 |
| ABA | −4.1 ± 0.4°C | 41.2 ± 5.4 | −6.0 ± 0.1°C | 57.1 ± 3.6 |
| Methyl (me)-ABA | −3.9 ± 0.5°C | 32.3 ± 6.3 | −6.0 ± 0.5°C | 56.4 ± 6.3 |
| ABA Aldehyde | −5.7 ± 0.3°C | 44.9 ± 3.9 | −5.6 ± 0.3°C | 54.7 ± 2.6 |
| Dihydro ABA | −5.9 ± 0.4°C | 59.4 ± 4.0 | −6.6 ± 0.4°C | 62.8 ± 2.0 |
| Acetylenic dihydro ABA | −4.9 ± 0.5°C | 46.4 ± 5.3 | −6.7 ± 0.2°C | 63.6 ± 1.8 |
| Divinyl me-ABA | −4.6 ± 0.2°C | 41.1 ± 2.3 | −6.0 ± 0.2°C | 54.3 ± 3.2 |
| Acetylenic divinyl me-ABA | −5.6 ± 0.1°C | 54.3 ± 4.2 | −6.7 ± 0.3°C | 62.8 ± 5.0 |

[a] All compounds were applied as a foliar spray to one-leaf-stage plants at a concentration of 100 μM.
[b] $LT_{50}$ was the temperature at which 50% of the plants were killed.
[c] Percentage of the plants that were alive at the conclusion of the experiment.

### *Low-Temperature Growth*

The plants were cultivated as described earlier for freeze tests. Immediately after treatment, one-fourth of the plants from each treatment were collected, and fresh and dry weights were recorded. At 7-day intervals, an additional 25% of the initial number of plants were harvested, and fresh and dry weights were measured until all plants were harvested (21 days after treatment). Eight seeding trays per treatment were used, and the experiment was repeated twice for each species. Plants were maintained at 10°C and watered daily for the duration of the experiment.

### *Flowering*

Plants were grown as described in the Freezing Tests section. Following foliar application with either ABA or ABA analogs, plants were either left at 10°C for 14 days or were frozen 2 days after the foliar treatment. The plants were frozen as previously described and transferred to 10°C for an additional 12 days. Plants were transplanted into 15-cm pots (3 plants/pot) and transferred to the greenhouse (23°C; natural light supplemented with artificial lighting to maintain a 16-h photoperiod). After being transferred to the greenhouse, plants were watered daily and fertilized (20-20-20; NPK) weekly. Plants were monitored daily for flowering. After seed set, the plants were harvested and the flowering dates recorded. Each experiment consisted of 6–8 pots per treatment, and the experiment was replicated three times.

## Results

### *Effect of ABA, ABA Metabolites, and ABA Analogs on Freezing Tolerance*

Freeze tests indicated that the $LT_{50}$ for the *Brassica campestris* variety Tobin was on average 1°C lower than the *B. napus* variety Delta (Table 1). Application of (±)ABA and its methyl ester were ineffective in increasing the freezing tolerance of the Delta variety, but did result in a small gain in freezing tolerance for the *B. campestris* cv Tobin plants (Table 1). An approximately 2°C increase in freezing tolerance was observed 2 days following foliar application of ABA aldehyde, 2′,3′ dihydro ABA, or acetylenic divinyl methyl ABA to *B. napus* plants. In *B. campestris*, 2′,3′ dihydro ABA, 2′,3′ acetylenic dihydro ABA, and acetylenic divinyl methyl ABA all increased freezing tolerance by 1.5°C (Table 1). As well as lowering the $LT_{50}$, the effective treatments also increased the overall survival rate of the plants in both species (Table 1). Application of 2′,3′ dihydro ABA resulted in twice the frost survival rate (over the entire test temperature range) compared with control plants (59.4 vs. 32.8%) in *B. napus*.

### *Effect of ABA and ABA Analogs on Low-Temperature Growth*

Application of either ABA or some of the ABA analogs produced significant increases in both fresh weight and dry weight in both *Brassica* species (Fig. 2) 3 weeks following treatment and continued growth at 10°C. Differences were also noted in the tissue water content (data not shown). The rate of growth in untreated plants at 10°C decreased noticeably after the emergence of the third leaf, which occurred 1–3 weeks after the onset of the experiment. The observed decline in the growth rate after this stage was highest in the control plants.

Methyl ABA, ABA-aldehyde, dihydro ABA, and acetylenic divinyl methyl ABA were the most effective treatments in promoting both fresh weight and

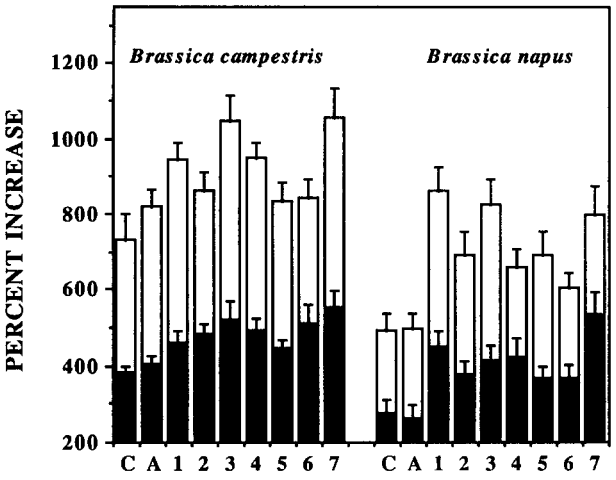


**Fig. 2.** The effect of 100 μM of either ABA or ABA analogs on fresh weight and dry weight of *Brassica campestris* cv Tobin and *B. napus* cv Delta seedlings grown at 10°C for 21 days. Dry weight increases are indicated by open bars, and increases in fresh weight are indicated by solid bars. Treatments were as follows: C, untreated control plants; A, acetone-treated plants; 1, (±)ABA; 2, (±)methyl-ABA; 3, (±)ABA aldehyde; 4, (±)2′,3′ dihydro ABA; 5, (±)2′,3′ acetylenic dihydro ABA; 6, divinyl ABA; and 7, acetylenic divinyl ABA. Standard errors are indicated.

dry weight in *Brassica campestris* at 10°C (Fig. 2). In *B. napus*, application of ABA, dihydro ABA, and acetylenic divinyl methyl ABA resulted in the largest gains in fresh weight, whereas ABA, ABA aldehyde, and acetylenic divinyl methyl ABA were the most effective treatments at increasing dry weight (Fig. 2). No significant differences were noted in fresh weight or dry weight between acetone-treated and control plants in either species (Fig. 2).

### *Promotion of Flowering by Frost and Analog Treatment*

Subjecting plants to a mild nonlethal frost of −3 or −4°C reduced the time to flowering by 1–2 days compared with nonfrozen plants of either *Brassica campestris* or *B. napus* (Table 2). However, as the severity of the frost increased, the early flowering benefit observed from the mild frost was lost in both species (Table 2). The percentage of control plants that survived freezing temperatures of −5°C in *B. napus* and −6°C in *B. campestris* was extremely small; therefore, only temperatures warmer than these were examined for effects on flowering.

Application of ABA and ABA analogs also effected the date of flowering (Table 2). Dihydro ABA, divinyl methyl ABA, and acetylenic divinyl methyl ABA promoted flowering in *B. campestris* by approximately 2.5 days (Table 2). Only dihydro ABA and acetylenic divinyl methyl ABA significantly reduced the time to flowering in *B. napus* (Table 2). A combination of a mild frost and an application of ABA or ABA analogs had a synergistic effect on reducing the time to flowering. The average time to flowering was reduced by as much as 4 days by application of ABA, dihydro ABA, acetylenic dihydro ABA, and acetylenic divinyl methyl ABA in combination with a frost treatment of −3 or −4°C for *B. napus* and −4 or −5°C for *B. campestris* (Table 2).

## Discussion

The concept of using ABA and/or ABA analogs to increase frost resistance has been suggested (Flores et al. 1988, Gusta et al. 1990). Similar to the results obtained by Flores et al. (1988) for winter wheat, application of ABA and several ABA analogs resulted in enhanced recovery from freezing in both *Brassica campestris* and *B. napus*. This is shown by an increased survival rate of frozen plants (Table 1) and a reduced time to flowering following a frost in ABA- and ABA-analog-treated plants (Tables 1 and 2). However, in contrast to the results of Flores et al. (1988) with winter wheat, treatment of rapeseed plants with ABA analogs did result in an increase in freezing tolerance (Table 1). A possible explanation may be that winter wheat has reached its maximum achievable level of frost tolerance, an idea that is supported by the inability of plant breeders to significantly increase the cold hardiness of this crop for over 75 years (Fowler et al. 1993). In addition, the genes encoding the major proteins that accumulate during cold acclimation in winter wheat are not responsive to exogenous ABA (Houde et al. 1992, Ouellet et al. 1993). In contrast, *B. napus* cell cultures have been shown to increase in freezing tolerance in response to (±)ABA (Johnson-Flanagan et al. 1991, Orr et al. 1986). Also, a number of cold-regulating genes, which have been isolated from several crucifer species, are induced by exogenous (±)ABA (Kurkela and Franck 1990, Orr et al. 1992). Our studies suggest there is some potential to increase the frost tolerance of rapeseed with ABA and analogs of ABA.

Both *Brassica* species demonstrated increased rates of growth at 10°C following treatment with ABA and ABA analogs. ABA is generally associated with reduced shoot growth (Biddington and Dearman 1982, Creelman et al. 1989, Rehm and Cline 1973, Watts et al. 1981); however, these reports are based on optimal growth temperatures. Low temperature is a form of stress. Thus, applica-

**Table 2.** Effect of ABA and ABA analogs on time to flowering in frozen and nonfrozen *Brassica campestris* cv Tobin and *B. napus* cv Delta plants.

| | Average days to flower from date of seeding (±SE) | | | | | |
|---|---|---|---|---|---|---|
| | *B. campestris* | | | *B. napus* | | |
| Treatment | 10°C | −4°C | −5°C | 10°C | −3°C | −4°C |
| Control | 58.0 ± 0.6 | 56.4 ± 0.4 | 58.7 ± 0.7 | 65.5 ± 1.0 | 64.9 ± 0.5 | 66.8 ± 1.1 |
| Acetone | 57.1 ± 0.6 | 55.9 ± 0.7 | 58.4 ± 0.6 | 65.1 ± 0.9 | 65.5 ± 0.7 | 66.3 ± 0.9 |
| ABA | 56.7 ± 0.6 | 54.4 ± 0.7 | 56.5 ± 0.6 | 64.6 ± 0.6 | 63.6 ± 0.7 | 64.9 ± 0.8 |
| MeABA | 57.3 ± 0.9 | 55.4 ± 0.4 | 57.3 ± 0.8 | 65.2 ± 0.9 | 63.8 ± 0.5 | 65.7 ± 1.0 |
| ABA aldehyde | 57.1 ± 0.7 | 56.0 ± 0.9 | 58.5 ± 0.7 | 64.7 ± 0.6 | 62.8 ± 0.7 | 64.1 ± 0.8 |
| Dihydro ABA | 55.5 ± 0.5 | 55.2 ± 0.9 | 54.7 ± 0.6 | 63.1 ± 0.3 | 61.9 ± 0.4 | 62.0 ± 0.6 |
| Acetylenic dihydro ABA | 56.3 ± 1.1 | 56.1 ± 0.5 | 55.1 ± 0.5 | 64.8 ± 0.3 | 62.8 ± 0.6 | 62.6 ± 1.1 |
| Divinyl me-ABA | 55.4 ± 0.4 | 56.2 ± 0.8 | 58.7 ± 0.8 | 64.4 ± 0.3 | 63.0 ± 0.5 | 65.1 ± 0.9 |
| Acetylenic divinyl me-ABA | 54.2 ± 0.4 | 54.6 ± 0.6 | 54.9 ± 0.6 | 62.9 ± 0.5 | 60.8 ± 0.5 | 61.8 ± 1.2 |

tion of ABA, which is generally acknowledged as a stress regulator (Zeevaart and Creelman 1988), may partially alleviate this stress and allow the plants to adapt to growth under these conditions.

The reduction in the time to flower observed in both species may be related to the enhanced low-temperature growth rate induced by ABA and ABA-analog treatment. The increases in dry weight indicate that active photosynthesis is occurring at substantially higher rates in the treated plants compared with the control plants. This stored dry matter would then be available for rapid growth when temperatures become optimal. The treated plants were left for 14 days after treatment at 10°C. Thus, the promotion of flowering may reflect the increased accumulation of dry matter measured in treated plants (Fig. 2), which was then utilized when the plants were transferred to the greenhouse.

The promotion of flowering by these treatments may also be related to stress. Several stresses, including cold temperature, have been reported to promote flowering (Fontes et al. 1967, Miller et al. 1985). The ABA treatments may induce a similar set of proteins to these imposed stresses, which in turn induces the flowering response. The induction of similar patterns of gene expression by ABA and various environmental stresses is well documented (Bray 1988, Close et al. 1989, Heino et al. 1990, Skriver and Mundy 1990).

The basis for the selection of analogs used in this study were results obtained in previous studies. The 2′,3′ dihydro ABA molecule has been shown to have strong ABA-like activity in induction of freezing tolerance (Churchill et al. 1992), promotion of cotyledon abscission (Suttle and Abrams 1993), induction of embryo-specific gene expression (Walker-Simmons et al. 1992), inhibition of seed germination (Gusta et al. 1992, Walker-Simmons et al. 1992), and stomatal closure (Oritani and Yamashita 1982). Acetylenic and methyl ester analogs of ABA have also been demonstrated to have ABA-like activity (Churchill et al. 1992, Suttle and Abrams 1993, Walker-Simmons et al. 1992, Wilen et al. 1993). Thus, neither the ring nor side chain bond order appear to be strictly required for activity.

The ability of some ABA analogs to have a greater effect than ABA may be related to metabolism. ABA is degraded to phaseic and dihydrophaseic acid in vivo, and the application of ABA induces its own metabolism (Uknes and Ho 1984). The 2′,3′ dihydro ABA analogs are not converted to phaseic acid in cell cultures (Lamb et al. 1993). The two divinyl analogs were also selected for this study because they potentially would not be metabolized as rapidly as natural ABA. The absence of the 8′ methyl group in the divinyl ABA analogs would prevent the formation of phaseic acid. Thus, the increased activity that is apparent with these analogs may be related to a slower rate of their metabolism. In all three assays conducted in this study, 2′,3′ dihydro ABA and acetylenic divinyl methyl ABA were the most active.

Except for the nonchiral divinyl compounds, the growth regulators used in this study were racemic mixtures while naturally occurring ABA exists as the (S) enantiomer. Studies have shown differences in the activity and the metabolism of the different enantiomers of ABA and various analogs (Cummins and Sondheimer 1973, Lamb et al. 1993, Wilen et al. 1993). It would be of interest to examine the relative activities of the (R) and (S) enantiomers ABA, ABA aldehyde, and the two dihydro analogs to establish whether ABA receptors consistently display enantio selectivity.

These results indicate a potential for the application of ABA and other growth regulators to improve

the stress tolerance of canola. If the problem of low-temperature germination can be overcome, it may be possible to seed rapeseed earlier in the spring and subsequently avoid the hazards of early fall frosts. Earlier seeding and increased low-temperature growth could also result in seed set occurring before the hottest part of the summer, which also impacts on seed quality. We are currently conducting field trials with these growth regulators to determine if commercial application is feasible.

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## References

Abrams SR, Gusta LV (1989) Abscisic acid–related plant growth regulators—Germination promoters. European Patent No. 89403331.5

Biddington NL, Dearman AS (1982) The effect of abscisic acid on root and shoot growth of cauliflower plants. Plant Growth Regul 1:15–24

Blake TJ, Tan W, Abrams SR (1990) Antitranspirant action of abscisic acid and ten synthetic analogs in black spruce. Physiol Plant 80:365–370

Bray EA (1988) Drought- and ABA-induced changes in polypeptide and mRNA accumulation in tomato leaves. Plant Physiol 88:1210–1214

Chen HH, Li PH, Brenner MI (1983) Involvement of abscisic acid in potato cold acclimation. Plant Physiol 71:362–365

Chen THH, Gusta LV (1983) Abscisic acid–induced freezing resistance in cultured plant cells. Plant Physiol 73:71–75

Churchill GC, Ewan B, Reaney MJT, Abrams SR, Gusta LV (1992) Structure–activity relationships of abscisic acid analogs based on the induction of freezing tolerance in bromegrass (*Bromus inermis* Leyss) cell cultures. Plant Physiol 100:2024–2029

Close TJ, Kortt AA, Chandler PM (1989) A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13:95–108

Creelman RA, Mason HS, Bensen RJ, Boyer JS, Mullet JE (1989) Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: Analysis of growth, sugar accumulation, and gene expression. Plant Physiol 92:205–214

Cummins WR, Sondheimer E (1973) Activity of asymmetric isomers of abscisic acid in a rapid bioassay. Planta 111:365–369

Daie J, Campbell WF, Seeley SD (1981) Temperature-stress-induced production of abscisic acid and dihydrophaseic acid in warm and cool season crops. J Am Soc Hort Sci 106:11–13

Dörffling K, Schulenburg S, Lesselich G, Dörffling H (1990) Abscisic acid and proline levels in cold hardened winter wheat leaves in relation to variety-specific differences in freezing resistance. J Agr Crop Sci 165:230–239

Flores A, Grau A, Laurich F, Dörffling K (1988) Effect of new terpenoid analogues of abscisic acid on chilling and freezing resistance. J Plant Physiol 132:362–369

Fontes MR, Ozbun JL, Sadik S (1967) Influence of temperature on initiation of floral primordia in green sprouting broccoli. Proc Am Soc Hort Sci 91:315–320

Fowler DB, Limin AE, Robertson AJ, Gusta LV (1993) Breeding for low temperature tolerance in field crops. In: Buxton DR, et al. (eds) International crop science, vol 1. Crop Science Society of America, Madison, pp 357–362

Gusta LV, Ewan B, Reaney MJT, Abrams SR (1992) The effect of abscisic acid and abscisic acid metabolites on germination of cress seed. Can J Bot 70:1530–1535

Gusta L, Reaney MJT, Abrams SR, Robertson AJ, Abrams GD (1990) Abscisic acid analogs: Biological activity and interactions with the growth regulator abscisic acid. Com Agricul Food Chem 2:143–169

Heino P, Sandman G, Lang V, Nordin K, Palva ET (1990) Abscisic-acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 79:801–806

Houde M, Danyluk J, Laliberté JF, Rassart E, Dhindsa R, Sarhan F (1992) Cloning, characterization and expression of a cDNA encoding a 50 kD protein specifically induced by cold acclimation. Plant Physiol 99:1381–1387

Johnson-Flanagan AM, Huiwen Z, Thiagarajah MR, Saini HS (1991) Role of abscisic acid in the induction of freezing tolerance in *Brassica napus* suspension-cultured cells. Plant Physiol 95:1044–1048

Kurkela S, Franck M (1990) Cloning and characterization of a cold and ABA-inducible *Arabidopsis* gene. Plant Mol Biol 15:137–144

Lamb N, Abrams SR (1990) Synthesis of optically active cyclohexanone analogues of the plant hormone, abscisic acid. Can J Chem 68:1151–1162

Lamb N, Shaw AC, Abrams SR, Reaney MJT, Ewan B, Robertson AJ, Gusta LV (1993) Oxidation of the 8′-position of a biologically active abscisic acid analog. Phytochemistry 34:905–917

Lang V, Heino P, Palva ET (1989) Low temperature acclimation and treatment with exogenous abscisic acid induce common polypeptides in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 77:729–734

Lei B, Abrams SR, Ewan B, Gusta LV (1994) Non-chiral cyclohexadienone analogs of abscisic acid: Synthesis and biological activity. Phytochemistry (in press)

Mayer HJ, Rigassi N, Schwieter U, Weedon BCL (1976) Synthesis of abscisic acid. Helv Chim Acta 59:1424–1427

Miller CH, Konsler TR, Lamont WJ (1985) Cold stress influence on premature flowering of broccoli. Hort Sci 20:193–195

Oritani T, Yamashita K (1982) Synthesis and biological activity of (±)-2′,3′-dihydroabscisic acid. Agric Biol Chem 46: 817–818

Orr W, Iu B, White TC, Robert LS, Singh J (1992) Complimentary DNA sequence of low temperature-induced *Brassica napus* gene with a homology to the *Arabidopsis thaliana kin1* gene. Plant Physiol 98:1532–1534

Orr W, Keller WA, Singh J (1986) Induction of freezing tolerance in an embryogenic cell suspension culture of *Brassica napus* by abscisic acid at room temperature. J Plant Physiol 126:23–32

Ouellet F, Houde M, Sarhan F (1993) Purification, characterization and cDNA cloning of the 200 kDa protein induced by cold acclimation in wheat. Plant Cell Physiol 34:59–65

Rehm MM, Cline MG (1973) Rapid growth inhibition of *Avena* segments by abscisic acid. Plant Physiol 85:8–9

Skriver K, Mundy J (1990) Gene expression in response to abscisic acid and osmotic stress. Plant Cell 2:502–512

Suttle JC, Abrams SR (1993) Abscission-promoting activities of abscisic acid and five abscisic acid analogs in cotton seedlings and explants. Plant Growth Regul 12:111–117

Uknes SJ, Ho T-HD (1984) Mode of action of abscisic acid in barely aleurone layers: Abscisic acid induces its own conversion to phaseic acid. Plant Physiol 75:1126–1132

Walker-Simmons MK, Anderberg RJ, Rose PA, Abrams SR (1992) Optically pure ABA analogs: Tools for relating germination inhibition and gene expression in wheat embryos. Plant Physiol 99:501–507

Watts S, Rodriguez JL, Evans SE, Davies WJ (1981) Root and shoot growth of plants treated with abscisic acid. Ann Bot 47:595–602

Wilen RW, Hays DB, Mandel RM, Abrams SR, Moloney MM (1993) Competitive inhibition of abscisic acid-regulated gene expression by stereoisomeric acetylenic analogs of abscisic acid. Plant Physiol 101:469–476

Zeevaart JAD, Creelman RA (1988) Metabolism and physiology of abscisic acid. Ann Rev Plant Physiol Plant Mol Biol 39:439–473