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Absciscic Acid Metabolism and Lipid Accumulation of a Cell Suspension Culture of *Lesquerella fendleri*

Olesya A. Kharenko, L. Irina Zaharia, Michael Giblin, Vera Čekić, David C. Taylor, C. Don Palmer, Suzanne R. Abrams, Michele C. Loewen*

Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place,
Saskatoon, SK, S7N 0W9

*Corresponding Author, Tel: (306) 975-6823, Fax: (306) 975-4839, Email:
Michele.Loewen@nrc-cnrc.gc.ca

ABSTRACT

Lesquerella fendleri (commonly known as “Fendler’s bladderpod” or “yellowtop”) is a member of the Brassicaceae and is an important seed oil- producing plant. The lipid profile of *L. fendleri* seed indicates potential for producing a high quality replacement for castor oil. In this work, characterization of the lipid content of a suspension cell culture, derived from seedlings of *L. fendleri*, is provided. Under the described suspension cell culture conditions, 16:0, 18:1 Δ 9, 18:2 Δ 9, Δ 12 and 18:3 Δ 9, Δ 12, Δ 15 fatty acids were found to accumulate in the cells, while 16:0, 26:0 and 28:0 fatty acids were predominant in the culture medium. Subsequently, the effect of application of abscisic acid (ABA), which modulates lipid accumulation, was assessed. Exogenously applied ABA was taken up by the cells and metabolized via the conjugation pathway, resulting in the accumulation of ABA-glucose ester. Preliminary tests demonstrate the cell line is responsive to exogenous ABA, resulting in increased cellular lipid content and increased accumulation of lipids in the culture medium. This novel *L. fendleri* suspension culture presents a valuable model system for efficient characterization of mechanisms associated with ABA-induced accumulation of lipids.

KEYWORDS

Lesquerella fendleri; suspension cell culture; lipid profiles; abscisic acid (ABA)

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
ABA	Absciscic acid
ABA-GE	Absciscic acid glucose ester

DW	dry weight
FAMES	Fatty acid methyl esters
MS	Murashige and Skoog medium
PA	Phaseic acid
TAG	Triacylglycerol

INTRODUCTION

Plant oils are extremely important in commercial applications related to nutritional, industrial, and pharmaceutical industries. A number of species in the Brassicaceae are especially useful for the production of seed oil. For example *Brassica napus*, which produces canola oil, is ranked number two, after soybean, in the world production of seed oil (Gunstone 2001; <http://www.lipidlibrary.co.uk>). Canola oil is widely used for human consumption, animal feed, in numerous industrial applications and as a biodiesel fuel. Another member of the Brassicaceae, *Lesquerella fendleri* (Gray) S. Wats., commonly referred to as desert mustard, produces seed oil high in hydroxy fatty acids (Carlson et al., 1990; Skarjinskaia 2003). Oils high in hydroxy fatty acids can replace castor oil, which is used extensively in industrial applications including cosmetics, plastics and coatings (Redd et al., 1997; Dykinga 1999)

The quality of plant oils and their industrial value are determined by fatty acid content and composition (Scarath and Tang 2006). Both traditional plant breeding and genetic engineering have been employed to modify and improve oil content in oilseed crops (Holbrook et al., 1992; Perry and Harwood 1993; Scarath and Tang 2006; Jiang et al. 2009; Taylor et al., 2009). The successful development of such modulating strategies relies not only on selecting the appropriate plant line, but also on understanding the mechanisms regulating lipid biosynthesis and oil deposition as they relate to agricultural conditions and stresses.

It is known that different exogenous factors, such as temperature, osmotic potential, and externally applied plant hormones, have an effect on the storage lipids including: triacylglycerol (TAG) content and fatty acid composition in various plant species (Finkelstein and Somerville 1989; Weselake et al., 1998; Weselake 2000; Aly et al., 2008). Absciscic acid (ABA) in particular, is a plant hormone involved in many physiological responses including mediation of

lipid accumulation in developing seeds (Wilén et al., 1990; Walker-Simmons et al., 1994; Sholi et al., 2009). These effects extend to related developing embryos and cell cultures (Browse et al., 1986; Finkelstein and Somerville 1989; Wilén et al., 1990; Holbrook et al., 1991; Attree et al., 1992; Holbrook et al., 1992; Weselake et al., 1998; Weselake 2000; Davoren et al., 2002). For example, zygotic embryos of *Brassica napus* L. cv. Nugget and L. cv Reston accumulate very-long-chain fatty acids in response to high osmoticum, achieved by the addition of high concentrations of sorbitol or additions of ABA (Finkelstein and Somerville 1989; Zou et al., 1995; Qi et al., 1998). Specifically, ABA treatments increased levels of eicosenoic (20:1 Δ 11) and erucic (22:1 Δ 13) acids three to four fold (Holbrook et al., 1992). The levels of TAGs in somatic embryos of *Picea glauca* also increased nine fold, in response to the addition of (+)-ABA (Attree et al., 1992). Developing wheat embryos (*Triticum aestivum* L.) showed a 55% TAG increase in response to supplementation with ABA or high osmoticum (400 mM mannitol) (Rodriguez-Sotres and Black 1994). Finally, increasing concentrations of sucrose were found to promote TAG accumulation in suspension cell cultures of *Brassica napus* L. cv Jet Neuf (Weselake et al., 1993, 1998; Weselake 2000). While, in this last instance it has been shown that the TAG accumulation is accompanied by increases in diacylglycerol acyltransferase activity and enhanced high levels of oleosin transcript, the mechanisms mediating these effects remain largely uncharacterized.

In the context of plant molecular studies, model systems are useful for circumventing the lengthy time frames associated with plant development and the limited availabilities of working materials for analysis. Because of the osmoticum and ABA induced modulation of TAG synthesis in plant embryos and tissue cultures, these could be good model systems for related mechanistic characterizations (Cui et al., 2010). Rapid growth and easy maintenance of

suspension cell cultures can provide a constant supply of relevant fresh material for analysis (Biesaga-Koscielniak et al, 2008). In this work we describe the development of suspension cultures derived from seedlings of *L. fendleri*, characterization of the fatty acid profiles, metabolism of exogenously administered ABA, and the effect of ABA and ABA metabolite treatments on lipid and fatty acid content.

MATERIALS AND METHODS

Chemicals

Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON, Canada. Solvents were of HPLC grade and were used as such. (+)-ABA was a gift from Valent Biosciences Corporation (Libertyville, IL, USA), while (+)-8' acetylene ABA was synthesized in the lab of S.R.A. as previously described (Rose et al. 1997).

Production of L. fendleri suspension culture

To establish a suspension culture of *L. fendleri*, seeds were surface-sterilized by washing with de-ionized water for 5 min followed by immersion in 70 % ethanol for 1 min. They were then surface-disinfected with a solution of sodium hypochlorite (1.0 %) containing 0.2 ml of Tween 20 for 25 min. After decanting, seeds were washed with 4 changes of sterilized de-ionized water before germination. The germination medium was $\frac{1}{2}$ Murashige and Skoog (MS) basal medium (all components $\frac{1}{2}$), solidified with 0.8 % Bacto agar but without growth regulators (Murashige and Skoog 1962). The medium was autoclaved at 121 °C for 25 min, cooled and poured into 100x15 mm Petri plates. Seeds were placed on the culture medium and the sealed plates maintained in the dark for 12 days.

The hypocotyl of 12- day- old seedlings was cut into 5-7 mm segments and incubated on a medium for callus initiation (MS basal medium with B5 vitamins (Gamborg et al., 1968), 3.0 % sucrose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg/l kinetin at a pH of 5.8. The medium was solidified with 0.8 % Bacto agar, sterilized by autoclaving at 121° C for 25 min, cooled and dispensed in 100x15 mm Petri plates). Twelve explants were placed in each dish, which was then sealed with plastic wrap and incubated in the dark. The progress of callus development was monitored for 28 days. After 28 days, the callus was sub-cultured by dividing each callus into 4 pieces, which were incubated on a medium of the same composition. The callus was allowed to grow for a further 21 days before suspension cultures were initiated.

The suspension culture medium was MS basal medium with B5 vitamins, 3.0 % sucrose, 1.0 mg/l 2,4-D, 0.1 mg/l kinetin. The pH of the medium was adjusted to 5.8 and 50 ml aliquots dispensed in 250 ml Erlenmeyer flasks, which were then closed with non-absorbent cotton plugs. The medium was autoclaved for 25 min at 121 °C before use. The medium was inoculated with *ca.* 350 mg of 28-day-old callus and the flasks placed on an orbital shaker at 145 rpm and maintained at 24 °C with a photoperiod of 16 h at a light intensity of 30 $\mu\text{E m}^{-2} \text{s}^{-1}$. The suspension was sub-cultured every 14 days by transferring 10 ml of the suspension to 40 ml of fresh medium of the same composition. At intervals, Evans Blue or FDA staining determined cell viability, and growth was monitored by packed cell volume determination. After two sub-cultures, the medium composition was altered by increasing the sucrose concentration to 5.0 % and the addition of 500 mg/l each of L-proline and casein hydrolysate. The concentration of growth regulators remained the same as in the previous cultures. To inoculate this culture, 14 day old cultures were sieved through 400-500 μm nylon mesh and 10 ml of the filtrate added to 90 ml of the modified medium. Growth and cell viability were determined and the suspension sub-

cultured at 21-day intervals by transferring 10 ml of un-sieved suspension to 90 ml of fresh medium. By this modification, the culture retained its growth potential for several years.

Propagation of L. fendleri suspension cell cultures and treatment with (+)-ABA and (+)-8'-acetylene ABA.

L. fendleri suspension cell cultures were maintained on MS basal medium containing 3 % sucrose (the culture did not contain auxin and cytokinin). For addition of ABA and analogs, cells were filtered and resuspended in fresh medium containing (+)-ABA or (+)-8'-acetylene ABA. (+)-ABA and (+)-8'-acetylene ABA were added to the cultures from 50 mM stocks dissolved in ethanol to give final concentrations of 10 or 100 μ M hormone in the medium. ABA treatments were performed in the dark at 25 °C for 4 and 7 days; controls were supplemented with corresponding amounts of ethanol only. The results represent the average of 3-5 replications.

Analysis of fatty acid content and composition

The plant cells were collected by vacuum filtration and the medium was saved for analysis. The cells were washed three times with water, freeze dried and the dry weight was recorded. Tripentadecanoin (15:0) dissolved in 2 : 1 dichloromethane: isopropanol was added to the cells as an internal standard (50.30 μ g) to allow quantitative fatty acid analysis and the samples were dried under nitrogen. Methanolic KOH (2 ml of a 10 % solution) was added to the samples which were heated at 80 °C for 2 hours, briefly cooled on ice, followed by the addition of 1 ml of 6 N HCl. The reaction mixtures were extracted three times with 2 ml of hexane. The hexane extracts were combined and evaporated to dryness. The samples were then transmethyalted with 2 ml of methanolic sulphuric acid at 80 °C for 2 hours. The reaction

mixtures were cooled on ice and 2 ml of 0.9 % (w/v) NaCl was added. The methyl esters of fatty acids (FAMES) were extracted with 1 ml of hexane three times, the extracts combined and dried under nitrogen gas. The samples were re-dissolved in 0.5 ml of hexane containing 10 µg of C17:0 methyl ester. FAMES were analyzed by gas chromatography on an Agilent model 6890 Gas Chromatograph using 30m DB23 x 0.25 mm id column (J & W Scientific Catalogue Number 122-2332) and FID detector, with a program of 160° for 1 min, followed by a temperature gradient of 4°/min until reaching 240°. Oil content (as a % of dry weight (DW)) was calculated as follows. From the total moles of FAMES, 3 mol of fatty acid and one mol of esterified glycerol backbone per triacylglycerol is assumed, from which an average TAG weight is estimated. Taking into account the DW of each sample, the oil content as a % DW is estimated. Values are plotted as means with standard deviation (n=3). For fatty acid analysis of medium samples, 50 ml of cell culture medium was extracted twice with 4 ml of dichloromethane, the organic extracts were combined, evaporated to dryness under a nitrogen stream and dissolved in dichloromethane with the addition of internal standard, transmethyated and analyzed as described above.

Time-course study and metabolism of (+)- and (-)-ABA in L. fendleri cell suspension culture media

Three 250-ml Erlenmeyer flasks containing each *ca.* 1 g *L. fendleri* cell suspension culture cells in 50 mL fresh MS media were used. An ethanolic solution of either (+)-ABA or (-)-ABA was added to each flask, such that the final concentration in the medium was 100 µM. Control flasks contained equivalent amounts of ethanol added to the cell cultures. Samples (2 ml medium, no cells) were withdrawn from each flask immediately after adding the compound.

Subsequently, 2 ml samples were taken after 6 h, 1, 2, 3 days and 4 days of incubation. Each sample was extracted twice with 2 ml hexane. The remaining aqueous portions were concentrated under reduced pressure, redissolved in a 250 μ l mixture of H₂O–CH₃CN (1:1, v/v) and analyzed by HPLC. Analytical HPLC analysis was carried out with an Hewlett Packard liquid chromatograph 1100 series (Hewlett Packard, Palo Alto, CA) equipped with a quaternary pump, automatic injector, diode array detector (wavelength range 190-600 nm), degasser, and a Supelcosil™ LC-18 column (4.6 mm i.d. x 3.3 cm, 3 μ m particle size silica), equipped with an in-line filter. The chromatographic peaks for ABA and ABA metabolites were monitored at 262 nm. The retention time (R_t) is reported for an isocratic elution with 0.1 % HOAc in H₂O -0.1 % HOAc in CH₃CN, 85:15 (v/v) for 20 min, at a flow rate of 1.0 ml/min. Under these chromatographic conditions (+)-ABA and (-)-ABA have a R_t =5.5 min, while ABA-GE has a R_t =1.9 min.

RESULTS AND DISCUSSION

Lipid profiling of the L. fendleri suspension cell culture.

A novel *L. fendleri* (Gray) S. Wats. suspension cell culture was produced from callus derived from hypocotyl segments of freshly germinated seeds, as described in Materials and Methods. A complete description of the production and growth characteristics of this cell line is being published elsewhere. The fatty acid composition of both the cells and medium samples of cultures of this *L. fendleri* cell line are shown in Figures 1 and 2. In the cell samples, the most predominant fatty acids were palmitic 16:0, oleic 18:1 Δ 9, linoleic 18:2 Δ 9, Δ 12 and linolenic 18:3 Δ 9, Δ 12, Δ 15 acids (numbers indicate carbon chain length : # of unsaturated bonds followed

by position(s) of unsaturated bond(s) respectively numbered with the carbon atom of the carboxyl group as C-1). The media samples were also found to contain fatty acids, among which the most predominant ones were palmitic 16:0, cerotic 26:0 and montanic 28:0 acids. Control samples of fresh media, as well as fresh media treated with ABA only (no cells) were also analyzed and found to contain no fatty acids. It can be seen that the higher molecular weight long chain fatty acids ($>C_{18}$) are present in both the cell and the media samples.. The presence of such fatty acids in microspore embryos has been considered a marker for seed-specific metabolism, highlighting possible conservation of seed-like metabolic events in this cell culture (Taylor et al., 1990). These *L. fendleri* cell culture profiles showed that the fatty acids 18:2, and 18:3, were the most predominant and there were no measureable quantities of hydroxyl fatty acids, in contrast to studies of mature seeds of this *Lesquerella* genotype reported previously, wherein the very long chain hydroxy fatty acid 20:1 Δ 14-OH comprised 62% of the total fatty acids (Reed et al., 1997). That this hydroxy fatty acid which is of great interest for industrial applications, was not detected in the cell culture suggests that the metabolic requirements for production of ricinoleic acid followed by its elongation to 20:1 Δ 14-OH (Reed et al., 1997) is not preserved in the cell culture line. This suggests that the hydroxylase and elongase genes/enzymes responsible for this fatty acid modification pathway are inactive in cell culture. A similar lack of “unusual” fatty acids in somatic cell cultures of other oilseed species is commonly observed (Weber, et al., 1992). For example, cell cultures of *Tropaeolum majus* (garden nasturtium), the seed of which produces large proportions of 22:1, do not accumulate any erucic acid, but instead, only palmitic and oleic fatty acids. Similarly, cell cultures of *Petroselinum crispum* (parsley), which contains 79% petroselinic acid (18:1 Δ 6) in its seed oil, synthesizes less than 4% in cell culture (Weber et al., 1992). Further study of modifications to culture conditions may

provide insight into regulation of these metabolic pathways, specifically gene and/or enzyme induction.

ABA metabolism in L. fendleri suspension cell culture

In order for ABA to elicit an effect on lipid metabolism in the *L. fendleri* suspension cell culture, it must be internalized by the cells. To confirm whether exogenously supplied ABA was taken up and catabolized by the *L. fendleri* cell culture, ABA biotransformation by *Lesquerella fendleri* cell culture was investigated. It is known that (+)-ABA can be catabolized through various pathways, the preference for which depends on the plant species, plant part, different physiological conditions and the plant developmental stage (Balsevich et al., 1994; Zhou et al., 2004; Zaharia et al., 2005a). The catabolism of (+)-ABA occurs through oxidation and reduction or conjugation to glucose (Priest et al., 2005). The major oxidation/reduction pathway occurs through hydroxylation of the 8'-methyl group of ABA, leading to the formation of the intermediate product (+)-8'-hydroxy ABA which further rearranges to phaseic acid (PA) (Zou et al., 1995; Zhou et al., 2004). The major conjugation pathway leads to the formation of ABA glucose ester (ABA-GE) (Priest et al., 2005). On the other hand, metabolism of (-)-ABA occurs through other catabolic pathways than (+)-ABA. For example, in bromegrass cell cultures (-)-ABA was transformed principally to (-)-7'-hydroxy ABA (Hampson et al. 1992), while in tomato it was converted predominantly to (-)-ABA-GE (Vaughan and Milborrow 1984).

In a time course experiment aimed at studying the biotransformation of exogenously applied (+)- or (-)-ABA, the *L. fendleri* culture medium was supplemented with either ABA enantiomer (as described in Materials and Methods) and the presence of ABA in the culture medium was monitored by HPLC over a 4 day time period. Figure 3 shows the HPLC profiles of

the medium samples from the time course experiment in which (+)-ABA was supplied to *L. fendleri* cell suspension cultures.

The results show that over the 24 h of treatment, the peak for (+)-ABA ($R_t=5.5$ min) is decreasing while that of (+)-ABA-GE is increasing ($R_t=1.9$ min), suggesting the formation of the conjugated product from the exogenously-supplied (+)-ABA. The formation of the PA was not observed, indicating catabolism of (+)-ABA occurs strictly through the conjugation pathway. Within four days of treatment, (+)-ABA was no longer detected in the medium and entirely transformed to (+)-ABA-GE. Treatments with (-)-ABA led to similar results, with the (-)-ABA undergoing biotransformation to (-)-ABA-GE (major product) and (-)-7'-hydroxy ABA (minor), albeit at a much slower rate than (+)-ABA (data not shown). The identity of the metabolic products was confirmed by comparison with authentic synthetic standards, that is, they had identical retention times and UV spectrum. Control samples of media treated with either (+)- or (-)-ABA showed that both enantiomers were stable in the media solutions. Also, controls of untreated cells did not show the presence of possible ABA catabolites. Overall these results indicate that exogenously applied ABA is taken up, metabolized by the *L. fendleri* cell culture and released/excreted back into the media. At the same time, this system presents a novel source of ABA-GE, which otherwise must be produced synthetically, with a low overall yield of 29 % (Balsevich et al. 1996). This is particularly important for preparative purposes, since biotransformation of either (+) or (-)-ABA in *Zea mays* L. cv. Black Mexican Sweet maize suspension cultures, an important source for obtaining some of the ABA metabolites (Zaharia et al., 2005b) did not yield quantity amounts of ABA-GE (Balsevich et al., 1994).

Effect of exogenous application of (+)-ABA and (+)-8'-acetylene ABA on lipid profiles of the L.

fendleri suspension cell culture.

In light of the observed uptake and metabolism of ABA by the *L. fendleri* cell culture, related effects on lipid biosynthesis were assessed. Specifically, a series of treatments of (+)-ABA and its biologically active analog, (+)-8'-acetylene ABA were performed. The latter analog is known to be more resistant to metabolism through the oxidation/reduction pathway because of the 8' acetylene substituent (Huang et al. 2008; Rose et al. 1997). A slower metabolizing analog might increase effects on lipid content through longer exposure to a biologically active molecule.

The medium of *L. fendleri* cell cultures was supplemented with 10 or 100 μ M (+)-ABA or 10 μ M (+)-8'-acetylene ABA and grown for an additional 4 or 7 days. Total oil content (fatty acids + glycerol) of the cells (Figure 4) and the medium (Figure 5) was compared. In control samples the total oil content of the cells comprising the culture was found to be approximately 3.5 % of DW. Addition of (+)-ABA yielded a 57% increase in total cellular oil content (approximately 5.5 % of DW) after 4 days. Application of (+)-8'-acetylene ABA had a similar effect leading to a 42 % increase in total cellular oil content at 4 days (5% of DW). In both cases fatty acid profiles remained the same as in uninduced cultures. At 7 days, effects on total oil were reduced, likely due to metabolism of the applied ABA and related molecules. Analysis of total fatty acid content in the medium samples revealed that addition of exogenous (+)-ABA provoked a 250 % (2.5-fold) increase of fatty acid content in the medium of *L. fendleri* suspension cell cultures relative to controls cultured in the absence of any ABA.

Conclusions

Exogenous application of ABA and a related analog, (+)-8'-acetylene ABA, both mediate induction of lipid accumulation in the seedling-derived *L. fendleri* suspension culture described

in this work. This result indicates that this *L. fendleri* suspension culture could be useful for studying the effects of different culture conditions, including exogenously applied phytohormones or metabolic inhibitors, as well as mutagenic agents, on lipid metabolic pathways. The insights gained from this experimental cell system could then be applied to more complex plant systems where lengthy time frames associated with plant development and where limited availabilities of working materials for analysis preclude broad screening and molecular investigations. Further study will also provide valuable insight into the regulation of lipid metabolism in this industrially and economically valuable plant system.

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REFERENCES

- Aly MAM, Amer EA, Al-Zayadneh,WA, Eldin AEN (2008) Growth regulators influence the fatty acid profiles of *in vitro* induced jojoba somatic embryos. Plant Cell Tiss Organ Cult 93:107-114
- Attree SM, Pomeroy MK, Fowke LC (1992) Manipulation of conditions for the culture of somatic embryos of white spruce for improved triacylglycerol biosynthesis and desiccation tolerance. Planta 187:395-404
- Balsevich J, Bishop G, Jacques SL, Hogge LR, Olson DJH, and Laganieri N (1996) Preparation and analysis of some acetosugar esters of abscisic acid and derivatives. Can J Chem 74:238-245
- Balsevich JJ, Cutler AJ, Lamb N, Friesen LJ, Kurz EU, Perras MR, Abrams SR (1994) Response of cultured maize cells to (+)-abscisic acid, (-)-abscisic acid, and their metabolites. Plant Phys 106:135-142

- Biesaga-Koscielniak J, Koscielniak J, Filek M, Janeczko A (2008) Rapid production of wheat cell suspension cultures directly from immature embryos. *Plant Cell Tiss Organ Cult* 94: 139-147
- Browse J, McCourt PJ, Somerville CR (1986) Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissues. *Anal Biochem* 152:141-145
- Carlson KD, Chaudhry A, Bagby MO (1990) Analysis of Oil and Meal from *Lesquerella fendleri* Seed. *J Am Oil Chem* 67:438-442
- Cui X-H, Murthy HN, Wu C-H, Paek K-Y (2010) Sucrose-induced osmotic stress affects biomass, metabolite, and antioxidant levels in root suspension cultures of *Hypericum perforatum* L. *Plant Cell Tiss Organ Cult* 103:7-14
- Davoren JD, Nykiforuk CL, Laroche A, Weselake, RJ (2002) Sucrose-induced changes in the transcriptome of cell suspension cultures of oilseed rape reveal genes associated with lipid biosynthesis. *Plant Physiol and Biochem* 40:719-725
- Dykinga J (1999) A Storybook Future for *Lesquerella*? *Ag Res Magazine* 47:14-15
- Finkelstein R, & Somerville C (1989) Absciscic acid or high osmoticum promote accumulation of long-chain fatty acids in developing embryos of *Brassica napus*. *Plant Sci* 67:213-217
- Gamborg O, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp Cell Res* 50:151-158
- Gunstone FD (2001) Production and consumption of rapeseed oil on a global scale. *European J Lipid Sci and Tech* 103:447-449
- Hampson CR, Reaney MJT, Abrams GD, Abrams SR, and Gusta LV (1992) Metabolism of (+)-abscisic acid to (+)-7'-hydroxyabscisic acid by bromegrass cell cultures. *Phytochem* 31:2645-2648
- Holbrook LA, Magus JR, Taylor DC (1992) Absciscic acid induction of elongase activity, biosynthesis and accumulation of very long chain monounsaturated fatty acids and oil body proteins in microspore-derived embryos of *Brassica napus* L. cv Reston. *Plant Sci* 84:99-115
- Holbrook LA, Rooijen GJH, Wilen RW, Moloney MM (1991) Oilbody Proteins in Microspore-Derived Embryos of *Brassica napus*. *Plant Phys* 97:1051-1058
- Huang D, Wu W, Abrams SR, Cutler AJ, (2008) The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. *J Exp Bot* 59:2991-3007

- Jiang JJ, Zhao XX, Tian W, Li TB, Wang YP (2009) Intertribal somatic hybrids between *Brassica napus* and *Camelina sativa* with high linolenic acid content. *Plant Cell Tiss Organ Cult* 99:91-95
- Murashige T, Skoog F (1962) A revised medium for the rapid growth and bioassays with tobacco tissue cultures. *Phys Plantarum* 15:473-497
- Perry HJ, Harwood JL (1993) Changes in the lipid content of developing seeds of *Brassica napus*. *Phytochem* 32:1411-1415
- Priest DM, Jackson RG, Ashford DA, Abrams SR, Bowles DJ (2005) The use of abscisic acid analogues to analyse the substrate selectivity of UGT71B6, a UDP-glycosyltransferase of *Arabidopsis thaliana*. *FEBS Letters* 579:454-4458
- Qi Q, Rose PA, Abrams GD, Taylor DC, Abrams SR, Cutler AJ (1998) (+)-Abscisic acid metabolism, 3-ketoacyl-coenzyme A synthase gene expression, and very-long-chain monounsaturated fatty acid biosynthesis in *Brassica napus* embryos. *Plant Phys* 117:979-987
- Reed DW, Taylor DC, Covello PS (1997) Metabolism of Hydroxy Fatty Acids in Developing Seeds in the Genera *Lesquerella* (Brassicaceae) and *Linum* (Linaceae). *Plant Phys* 114:63-68
- Rodriguez-Sotres R, Black M (1994) Osmotic potential and abscisic acid regulate triacylglycerol synthesis in developing wheat embryos. *Planta* 192:9-15
- Rose PA, Cutler AJ, Irvine NM, Shaw AC, Squires TM, Loewen MK, and Abrams SR (1997) 8'-Acetylene ABA: an irreversible inhibitor of ABA 8'-hydroxylase. *Bioorg Med Chem Lett* 7:2543-2546
- Scarth R, Tang J (2006) Modification of *Brassica* oil using conventional and transgenic approaches. *Crop Sci* 46:1225-1236
- Sholi NJY, Chaurasia A, Agrawal A, Sarin NB, (2009) ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* sp.). *Plant Cell Tiss Organ Cult* 99:133-140
- Skarjinskaia M, Svab Z, Maliga P (2003) Plastid Transformation in *Lesquerella Fendleri*, an Oilseed Brassicacea. *Trans Res* 12:115-222
- Taylor DC, Weber N, Underhill EW, Pomeroy MK, Keller WA, Scowcroft WR, Wielen RW, Moloney MM, Holbrook LA (1990) Storage-protein regulation and lipid accumulation in microspore embryos of *Brassica napus* L. *Planta* 181, 18-26
- Taylor DC, Zhang Y, Kumar A, Francis T, Giblin EM, Barton DL, Ferrie JR, Laroche A, Shah S, Zhu W, Snyder CL, Hall L, Rakow G, Harwood JL and Weselake RJ (2009) Molecular Modification of Triacylglycerol Accumulation under Field Conditions to Produce Canola with Increased Seed Oil Content. *Botany* 87: 533-543

- Vaughan GT, and Milborrow BV (1984) The resolution by HPLC of RS-[2-14C]Me 1',4'-cis-diol of abscisic acid and the metabolism of (-)-R- and (+)-S-abscisic acid. *J Exp Bot* 35:110-120
- Walker-Simmons MK, Rose PA, Shaw AC, Abrams RS (1994) The 7'-Methyl Group of Absciscic Acid is Critical for Biological Activity in Wheat Embryo Germination. *Plant Phys* 106:1279-1284
- Weber N, Taylor DC, Underhill EW (1992) Biosynthesis of Storage Lipids in Plant Cell and Embryo Cultures. *Adv Biochem. Engineering/Biotech*, Feichter, A. (ed.), Springer-Verlag Berlin Vol. 45, pp 99-131.
- Weselake RJ (2000) Lipid biosynthesis in cultures of oilseed rape. *In vitro Cell Dev Biol* 36:338-348
- Weselake RJ, Byers SD, Davoren JM, Laroche A, Hodges DM, Pomeroy MK, Furukawa-Stoffer TL (1998) Triacylglycerol biosynthesis and gene expression in microspore-derived cell suspension cultures of oilseed rape. *J Exp Bot* 49:33-39
- Weselake RJ, Pomeroy KM, Furukawa TL, Goldwin JL, Little DB, Laroche A (1993) Developmental profile of diacylglycerol acyltransferase in maturing seeds of oilseed rape and safflower and microspore-derived cultures of oilseed rape. *Plant Phys* 102:565-571
- Wilén RW, Mandel RM, Pharis RP, Holbrook LA, Moloney MM (1990) Effects of abscisic acid and high osmoticum on storage protein gene expression in microspore embryos of *Brassica napus*. *Plant Phys* 94:875-881
- Zaharia LI, Walker-Simmons MK, Rodriguez CN, Abrams SR (2005a) Chemistry of abscisic acid, abscisic acid catabolites and analogs. *J Plant Growth Regul* 24:274-284
- Zaharia LI, Galka MM, Ambrose SJ, Abrams SR (2005b) Preparation of deuterated abscisic acid metabolites for use in mass spectrometry and feeding studies. *J Labeled Comp Rad* 48:435-445
- Zhou R, Cutler AJ, Ambrose SJ, Galka MM, Nelson KM, Squires TM, Loewen MK, Jadhav AS, Ross ARS, Taylor DC, Abrams SR (2004) A new abscisic acid catabolic pathway. *Plant Phys* 134:361-369
- Zou JAG, Barton DL, Taylor DC, Pomeroy MK, Abrams SR (1995) Induction of lipid and oleosin biosynthesis by (+)-Abscisic acid and its metabolites in microspore-derived embryos of *Brassica napus* L. cv Reston. *Plant Phys* 108:563-571

FIGURE LEGENDS

Figure 1. Fatty acid composition of *L. fendleri* suspension cells. Cells were maintained in basal MS medium for seven days and then harvested. The lipids were extracted, transmethylated and the FAMES analyzed by gas chromatography.

Figure 2. Fatty acid composition of *L. fendleri* suspension culture medium. Cells were grown in basal MS medium for seven days. The resulting medium was collected and the lipids analyzed quantitatively.

Figure 3. Time course of (+)-ABA metabolism in *L. fendleri* suspension cell culture medium. The cultures were grown in the presence of 100 μ M (+)-ABA and monitored by HPLC.

Figure 4. Effect of (+)-ABA and (+)-8'-acetylene ABA on total lipid content in *L. fendleri* cells. The cells were grown for 7 days in basal MS medium and then transferred to fresh MS medium containing 10 or 100 μ M (+) ABA or 10 μ M 8'-acetylene (+) ABA. After 4 or 7 days of treatment the cells were filtered, and total oil content (fatty acid + glycerol) of cell samples was determined (mg oil / mg cell dry weight %).

Figure 5. Effect of (+) ABA on fatty acid content in *L. fendleri* culture medium. The cells were grown in the basal MS medium, transferred to fresh MS medium supplemented with exogenous 10 or 100 μ M (+) ABA for 4 and 7 days after which the cells were filtered, the media collected and the fatty acid content was analyzed.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5