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FATTY ACID AND TRANSCRIPT PROFILING IN DEVELOPING SEEDS OF THREE *BRASSICA NAPUS* CULTIVARS

ABSTRACT

Fatty acid levels and gene expression profiles for selected genes associated with the synthesis of fatty acids (FA), triacylglycerol, and oil body proteins were examined in three oilseed rape (*Brassica napus*) cultivars that have utility for cultivar development in our spring canola breeding program. The seed oil content of Bronowski, Q2, and Westar was 39.0, 40.1, and 40.6%, respectively at 40 days after flowering (DAF). During the 20 to 40 day period of seed development, cultivars had varying levels of palmitic, stearic, oleic, linoleic, α -linolenic, eicosenoic, and erucic acid. In general, the percentage of each FA was similar among the cultivars during seed development. However, the level of oleic acid was lower and the levels of eicosenoic acid and erucic acid were higher in Bronowski than in Q2 and Westar seeds; linoleic acid also tended to be lower in Bronowski. Gene expression among the cultivars was similar from 10 to 40 DAF. The few exceptions were that expression of *KAS1* and *SAD* were higher in Westar and Q2 than in Bronowski at 25 DAF, *SAD* was highest in Q2, intermediate in Westar, and lowest in Bronowski at 35 DAF, *FAD2* was higher in Q2 than in Bronowski at 35 DAF, *FAD3* was higher in Q2 than in Bronowski at 15 DAF and Q2 and Westar at 25 and 30 DAF, and *FAE1* was higher in Westar and Q2 than in Bronowski at 30 DAF. Correlation analysis for gene expression against DAF for each genotype supported a common trend in gene expression among the three cultivars with gene expression tending to decrease over time; except for *LPAAT*, which tended to increase. The correlation between the level of FAs and expression of genes by genotype indicated no general trend; rather correlations seem to depend on the genotype.

Key words: *Brassica napus*, canola, fatty acid, gene expression, oilseed, rapeseed, seed.

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INTRODUCTION

Brassica napus (L.) is commonly referred to as canola, rapeseed, or oilseed rape. Canola itself was bred from rapeseed in Canada to develop a nutritious oil low in glucosinolates and erucic acid (Stefansson *et al.* 1961; Stefansson and Kondra 1970), which are anti-nutritional components for humans and livestock. Canola is the second largest vegetable oilseed crop worldwide behind soybean (http://www.ers.usda.gov/data-products/oil-crops-yearbook.aspx). The U.S. ranks eighth in worldwide oilseed rape production (http://apps.fas.usda.gov/psdonline/psdQuery.aspx), valued at approximately $483 million in 2011/2013, yet the U.S. remains a primary importer of canola oil and meal (http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-14-2014.pdf). Because 80% of the U.S. canola production is in the state of North Dakota, a public spring canola improvement project was initiated to develop germplasm adaptable to the Northern Plains of the U.S.

High oil yield and quality are fundamental to developing adapted germplasm. The biosynthesis and regulation of oil production in oilseeds is complex encompassing several steps and organelles within the cell (Baud and Lepiniec 2010; Bates *et al.* 2013; Li-Beisson *et al.* 2013). Rapeseed or canola oil is a mixture of triacylglycerols (TAG) that account for about 40-45% of the seed dry weight (Troncoso-Ponce *et al.* 2011). Initially, compounds like sucrose are imported into the plastid, and through a number of enzymatically mediated steps beginning with a multisubunit heteromeric acetyl-CoA carboxylase (HtACCase), free fatty acids (FA) of 16 to 18 carbons are synthesized. Long-chain FA are then exported to the endoplasmic reticulum (ER) for modification in the form of desaturation and elongation and assembly of TAG, which are esters of glycerol and FA. The formation of very long-chain FA (VLCFA) such as erucic acid (22:1), a major component of non-canola quality rapeseed oil, is enzymatically mediated by the fatty acid elongase complex (FAE), with fatty acid elongation1 (FAE1) being the first of four enzymes that comprise FAE. Synthesis of polyunsaturated FA such as linoleic (18:2) and α-linolenic acid (18:3) is mediated by fatty acid desaturase (FAD) enzymes. De novo assembly of TAG occurs by various routes in the ER, with the relatively straight forward one being the Kennedy pathway (Baud and Lepiniec 2010). This pathway encompasses a series of sequential acylation of a glycerol-3-phosphate backbone culminating with the third acylation catalyzed by diacylglycerol acyltransferase (DGAT). Finally, TAGs are stored in oil bodies composed of a matrix of TAGs and various proteins such as oleosins and steroleosin (Baud and Le-

piniec 2009). The TAGs are important as they act as a reserve for post-germination growth prior to achieving sufficient photosynthetic capacity and comprise the tremendous economic value for oilseed crops such as canola. Thus, it is important to understand the link between various genes involved in the oil biosynthesis during development and composition of seeds as a prelude to germplasm development, as well as to understand factors related to oilseed quality improvement.

While the FA composition in rapeseed oil has been documented (Canvin 1965), employing genomic techniques to evaluate expression of gene transcripts in relation to FA composition during rapeseed development is more recent. Hu *et al.* (2009) used quantitative reverse transcription (qRT-PCR) to examine transcript levels of 32 genes involved in the biosynthesis of FA, TAG, storage proteins, and in other physiological processes during seed development of an older Chinese high erucic acid cultivar and a descendent, low erucic acid cultivar. They determined that the transcription profiles were similar for both cultivars, while selection pressure for no erucic acid, low glucosinolates, high oleic acid, oil content, and yield affected the expression levels of several genes. In turn, they determined FA levels during seed development and correlated those with the gene transcripts. In another investigation, comparative transcriptome analysis in developing oilseeds of multiple species, including *B. napus*, relied on expressed sequence tag (EST) database development through pyrosequencing (Troncoso-Ponce *et al.* 2011). A notable outcome of this study was that regardless of the species ESTs representing almost all reactions of FA biosynthesis had comparable stoichiometry and consistent temporal profiles. This outcome and related results from EST sequencing and gene and protein expression studies suggest it is valid to make some cross species comparisons such as between *Arabidopsis thaliana* and *B. napus* (Niu *et al.* 2009; Venglat *et al.* 2013).

The first canola-type cultivar of summer rape released in Canada (Stefansson and Kondra 1975) was derived from a complex series of crosses that include selections from Liho and Bronowski to impart low erucic acid and glucosinolates, respectively. Likewise, subsequent canola quality summer rape cultivars, such as Westar and Q2, with superior agronomic and disease resistance traits (Klassen *et al.* 1987; Stringam *et al.* 1999) relied on series of crosses using germplasm with low erucic acid and glucosinolates. Westar, released in 1982 by Agriculture and Agri-Food Canada, has been widely cultivated, modified, and used as a baseline for subsequent germplasm development (Juska *et al.* 1997). However, it is susceptible to a serious disease of canola called blackleg caused by the fungus *Leptosphaeria maculans*. Q2 released in 1998 by University of Alberta is resistant to blackleg disease and relatively resistant to lodging. We cultivated Bronowski, Westar, and Q2 in the greenhouse to examine for potential traits that could be used in our spring canola breed-

ing program. Thus, the objectives of this research were to examine fatty acid levels and expression profiles for selected genes associated with the synthesis of FA, TAG, and oil body proteins during seed development in the three cultivars.

MATERIALS AND METHODS

Plant Materials

Oilseed rape (*Brassica napus*) cultivars, Bronowski, Westar, and Q2 were grown in a greenhouse at North Dakota State University, Fargo, ND, USA, at 22±4°C (day and night). The seeds were sown in 15 cm (diameter) by 15 cm (depth) pots filled with Sunshine-Mix-1 (Sun Gro Horticulture). The plants were watered daily and fertilized with water soluble 20 N–20 P–20 K fertilizer. Light in the greenhouse was provided with a 16-h photoperiod by natural sunlight supplemented with 400 W HPS PL 2000 lights (P.L. Light Systems Inc. ON, Canada). During the flowering stage, plants were bagged (microperforated polybag, Crawford Provincial, ON, Canada) and allowed to self-pollinate. Developing pods were harvested at 5-day intervals, 10 to 40 days after flowering (DAF). Seeds were harvested into liquid nitrogen and stored at -80°C for gas chromatographic analysis and RNA extraction. There were three biological replications per treatment.

Determination of Oil and Fatty Acid Profile

At 40 d after flowering (DAF), oil was extracted from seeds with n-hexane using accelerated solvent extraction (Dionex ASE 200, Thermo Scientific, Sunnyvale, CA) according to the methods of Haagenson *et al.* (2010) for oil content determination. One gram canola seed was oven dried for 4 h at 70°C. Seed was milled in a coffee grinder with 3.5 g diatomaceous earth, and samples were loaded into 11 ml stainless steel cells. Any remaining extraction cell void volume was filled with diatomaceous earth prior to extraction. Extractions were performed at 100°C, 6.7 MPa with a 5 min equilibration time and three 10 min static cycles having a 100% flush volume and 60 s purge time. The solvent containing extracted oil was collected in pre-weighed vials, and solvent was evaporated to dryness with a stream of dry air (-70°C dew point). Extracted samples were air dried, and reground for a second extraction and the total oil recovery from the two extractions was recorded. Oil is reported as a percent of seed dry weight.

At 5 d intervals from 20 to 40 DAF, fatty acid profiles were determined on seeds air dried overnight at room temperature in a fume hood. Samples of 0.1 to 0.3 g of dried seed were ground in a mortar and pestle and vortexed in 0.5 to 2 ml of hexane -chloroform-sodium methoxide (HCSM) derivatization reagent to produce fatty acid methyl esters (FAMEs). The HCSM reagent was freshly prepared by mixing 75 ml hexane, 20 ml chloroform (pentene stabilized), and 5 ml 0.5 M sodium methoxide in methanol (Sigma #403067). Analysis of the FAMEs was carried out on

a Hewlett-Packard 5890 Series II gas chromatograph with a flame-ionization detector. Split injections of 1 µl of the FAMEs were separated on a J & W Scientific DB-23, 30 m by 0.25 mm, 0.25 µm film column with helium carrier gas at 29 psi (1.9 ml/minute) and split flow at 50-100 ml/minute. The column was temperature programmed at 190°C for 4 min then to 220°C at 15°C/min and held 1 min, then to 240°C at 25°C/min and held 1 min. Inlet temperature was 230°C and detector temperature was 250°C with air at 345 ml/minute, hydrogen at 36 ml/minute, and helium makeup gas at 35 ml/minute. Nu-Check 21A and 411 standards were used to identify the FAMEs.

Template cDNA preparation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted at 5 d intervals from 10 to 40 DAF from canola seeds using the pine tree extraction protocol (Chang *et al.* 1993), and these samples were used to prepare template cDNA through reverse transcription following manufacturer's instructions (Invitrogen). Briefly, 5 µg of total RNA was DNase treated and then reverse transcription was performed in 20 µl total volume using a SuperScript First-Strand Synthesis Kit to produce total cDNA from each sample. After cDNA synthesis, each 20 µl reaction was diluted to 800 µl and stored at -80°C.

Gene expression by qRT-PCR was examined using template cDNA on a Roche LightCycler® 480 real-time PCR system. Primer pairs were synthesized based on sequences from Hu *et al.* (2009) (Electronic Supplementary Table S1). qRT-PCR parameters were described previously by Chao (2008) with some modification. The formula used to calculate the fold differences is similar to the comparative C_T method ($\Delta\Delta C_T$) except that no control sample is incorporated in the calculation. Thus, levels of different target gene expressions can be compared based on the expression of a reference SAND family gene, which served as a base line. A canola SAND family gene was used as a reference because it was verified to be stably expressed during seed development (see Electronic Supplementary Fig. S1 and Table S1 & S2). The modified formula for fold difference in gene expression of target vs. reference gene is:

$$\Delta C_T = \Delta C_{T\,target} - \Delta C_{T\,reference}$$

where, $\Delta C_{T,target}$ is the C_T value of the target gene, and $\Delta C_{T,reference}$ is the C_T value of the reference gene. SYBR green chemistry was used to produce fluorescent signal, and three technical replicates were used per sample for the RT-qPCR experiments. The C_T value of each gene is the average of three technical replicates. The difference in gene expression is designated as log2 value. Heatmap of the qRT-PCR results in Fig. S1 was created based on log2 values using Eisen Lab software, Cluster and TreeView as described by Eisen *et al.* (1998).

Statistical Analysis

The standard error (SE) of the mean difference of Ct values between the target and reference (SAND family) genes were calculated based on

$$S_{\bar{Y}_A-\bar{Y}_B} = \sqrt{\frac{S_A^2}{n_A} + \frac{S_B^2}{n_B} - 2 \cdot \hat{\rho}_{A,B} \cdot \frac{S_A}{\sqrt{n_A}} \cdot \frac{S_B}{\sqrt{n_B}}}$$

where S_A and S_B are the standard deviations and n_A and n_B are the sample sizes for samples *A* and *B*. $\hat{P}_{AB}$ is the estimated correlation of these pairs. The 95% confidence intervals were obtained based on the *mean difference* ±*t -value* × *SE*; the *t-value* with 2 degrees of freedom and 95% confidence is 4.303. The variance sum law was applied in the calculation of each reference gene normalized target gene SE and explains why the 95% confidence intervals for most of the target genes appeared very large.

MANOVA was used to compare FA profiles among cultivars using the manova function of the stats package in R (2015). The Wilks Lambda statistic was used to determine significant difference for the FA profiles (Johnson and Wichern 2007).

Pearson correlation coefficients between the FA and the gene expression by cultivar were computed using the cor function of the stats package in R (2015). Because only three biological replications were used in this study for each cultivar and DAF, only large effects and/or strong associations could be expected to be detected statistically.

RESULTS

Oil and Fatty Acids

The seed oil content of Bronowski, Westar, and Q2 grown in the greenhouse was 39.0, 40.6, and 40.1%, respectively. During the 20 to 40 d period of seed development, cultivars had varying levels of palmitic, stearic, oleic, linoleic, α-linolenic, eicosenioc acid, and erucic acid (Fig. 1). Levels of the two saturated FA, palmitic acid and stearic acid, were similar among the cultivars averaging 7.5% and 4.4% and 2.5% and 1.9%, respective at 20 and 40 DAF. The level of oleic acid was the same in Westar and Q2 over the 20 to 40 d period, although there was a trend for slightly high levels in Westar. In contrast, the mean level of oleic acid in Bronowski seeds was about 25% lower over the 20 d period relative to Westar and Q2. In general, the levels for two polyunsaturated FA, linoleic acid and α-linolenic acid, were similar among the cultivars averaging 22.2% and 15.7% and 10.8% and 6.1%, respective at 20 and 40 DAF. Nevertheless, the trend was for higher levels of linoleic acid in Q2 > Westar > Bronowski seeds from 20 to 40 DAF. The monounsaturated VLCFAs, eicosenioc acid and erucic acid, varied tremendously among the cultivars, particularly for Bronowski. Eicosenioc acid

levels over the 20 to 40 d period averaged 16.3%, 1.5% and 1.4%, respective for Bronowski, Westar, and Q2 seeds. Erucic acid was not detected in Westar seeds and the levels in Q2 were 3% and 1.1% at 35 and 40 DAF, respectively. In contrast, erucic acid levels in Bronowski seeds increased from 10.9% to 22.5% of total FAs over the 20 to 40 d period.

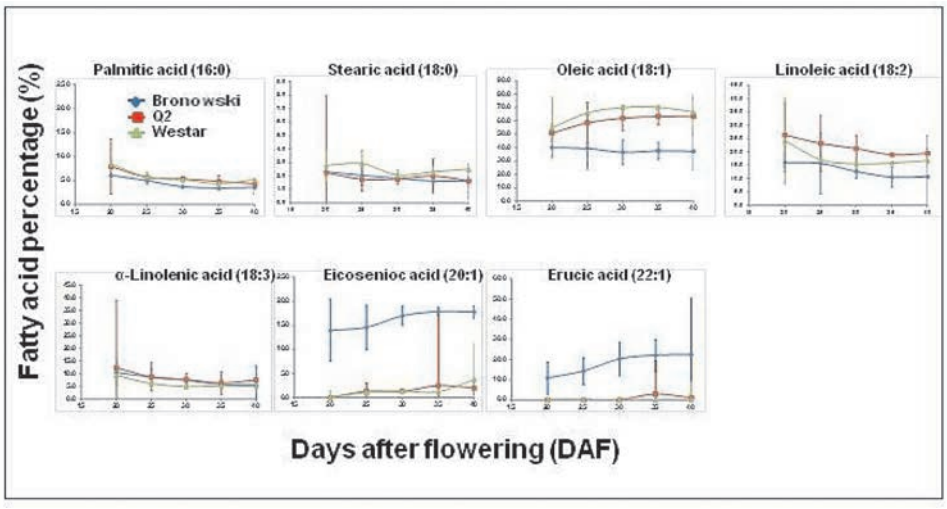

Fig. 1. Fatty acid accumulation during seed development.
The levels of 7 fatty acids (palmitic, stearic, oleic, linoleic, α-linolenic, eicosenioc, and erucic acid) were examined at 5-day intervals, 20 to 40 DAF in cultivars Bronowski, Westar, and Q2

Gene Expression

Gene designation, role, and location are provided in Table 1. Overall and as determined by the 95% confidence intervals, gene expression among the three cultivars was similar from 10 to 35 DAF (Fig. 2). We had only one data point for Bronowski genes at 40 DAF so confidence intervals could not be calculated. The few exceptions were that expression of *KAS1* and *SAD* were higher in Westar and Q2 than in Bronowski at 25 DAF, *SAD* was highest in Q2, intermediate in Westar, and lowest in Bronowski at 35 DAF, *FAD2* expression was higher in Q2 than in Bronowski at 35 DAF, *FAD3* expression was higher in Q2 than in Bronowski at 15 DAF and Q2 and Westar at 25 and 30 DAF, and *FAE1* expression was higher in Westar and Q2 than in Bronowski at 30 DAF. At its peak, expression of the gene for the seed storage protein napin was nearly 33,000 fold higher ($\log_2$ of 15) than the SAND gene. Conversely, lowest level of expression was the caleosin gene at 1/64 ($\log_2$ of -6) that of the SAND gene at 10 DAF.

Table 1

Gene designation, role, and location

Gene name	Gene annotation	Role	Location
ACCase	Homeomeric acetyl CoA carboxylase	Fatty acid biosynthesis	Cytosol
α-C7	Alpha carboxyltransferase	Fatty acid biosynthesis	Plastid
β-C7	Beta carboxyltransferase	Fatty acid biosynthesis	Plastid
BC	Biotin carboxylase	Fatty acid biosynthesis	Plastid
MCMT	Malonyl-CoA:ACP malonyltransferase	Fatty acid biosynthesis	Plastid
KAS1	Beta-ketoacyl-ACP synthase 1	Fatty acid biosynthesis	Plastid
KAS2	Beta-ketoacyl-ACP synthase 2	Fatty acid biosynthesis	Plastid
KAS3	Beta-ketoacyl-ACP synthase 3	Fatty acid biosynthesis	Plastid
HD/KACD	3-hydroxyacyl-ACP dehydratase	Fatty acid biosynthesis	Plastid
SAD	Stearoyl-ACP desaturase	Fatty acid biosynthesis	Plastid
FatA	Acyl-ACP thioesterasae	Fatty acid biosynthesis	Plastid
FatB	Palmiottoyl-ACP thioesterase	Fatty acid biosynthesis	Plastid
FAD6	Oleate desaturase	Acid editing	Plastid
FAD2	Oleate desaturase	Acid editing	Endoplasmatic reticulum
FAD3	Linoleate desaturase	Acid editing	Endoplasmatic reticulum
LPATT	Lysophosphatidic acid acyltransferase	Triacylglycerol biosynthesis	Endoplasmatic reticulum
DGAT2	Acyl-CoA:diacylglycerol acyltransferase	Triacylglycerol biosynthesis	Endoplasmatic reticulum
AAPT1	Aminoalcoholphosphotransferase	Triacylglycerol biosynthesis	Endoplasmatic reticulum
FAE1	Fatty acid elongase 1/3-ketoacyl-CoA synthase	Very long chain fatty acid biosynthesis	Endoplasmatic reticulum
KCR2	3-ketoacyl-CoA reductase	Very long chain fatty acid biosynthesis	Endoplasmatic reticulum
Oleosin	Oil nody associated protein	Storage protein	Oil body
Cruciferin	12S neutral oil body protein	Storage protein	Oil body
Napir	1.7S oil body protein	Storage protein	Oil body
Caleosin	Ca^{2+} binding oil body surface protein	Storage protein	Oil body

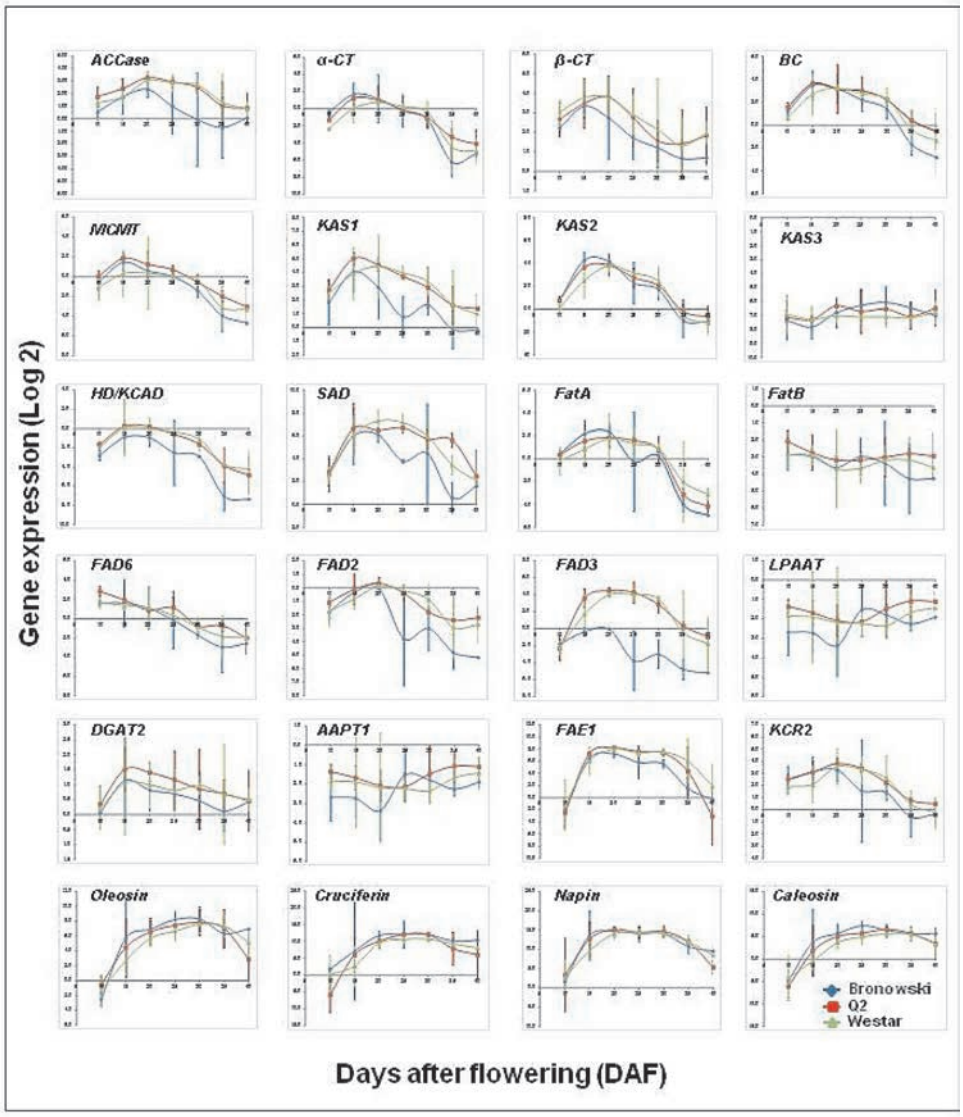

Fig. 2. Gene expression profiles during seed development.
The expression profiles of 24 genes were examined at 5-day intervals, 10 to 40 DAF in cultivars Bronowski, Westar, and Q2. Levels of different target gene expressions were compared based on the expression of a reference SAND family gene, which also served as a base line here. The fold difference is designated as log2 value. Gene designation is in Table 1. The 95% confidence intervals were obtained based on the mean difference ±t-value × SE

Table 2

The correlation coefficients between fatty acid (FA) accumulation and levels of gene expression in cultivars Bronowski, Westar, and Q2 across days after flowering (DAF)

Genotype	Fatty acid	ACCase	αCT	βCT	BC	MCAT	KAS1	KAS2	KAS3	HD/KACD	SAD	FatA	FatB
Brownowski	Palmitic	0.699	0.642	0.724	0.668	0.677	0.452	0.71	-0.084	0.605	0.489	0.61	-0.033
Brownowski	Stearic	0.662	0.589	0.734	0.938	0.605	0.543	0.694	0.117	0.64	0.499	0.607	-0.056
Brownowski	Oleic	0.603	0.603	0.484	0.61	0.666	0.457	0.597	-0.531	0.501	0.549	0.53	0.725
Brownowski	Linoleic	0.337	0.332	0.599	0.371	0.349	0.22	0.378	0.21	0.345	0.433	0.377	-0.119
Brownowski	Linolenic	0.978	0.735	0.885	0.728	0.765	0.527	0.751	-0.193	0.742	0.803	0.733	-0.148
Brownowski	Eicosenoic	-0.565	-0.627	-0.758	-0.623	-0.647	-0.286	-0.618	0.068	-0.539	-0.57	-0.589	-0.096
Brownowski	Erucic	-0.813	-0.834	-0.904	-0.836	0.865	-0.646	-0.828	0.272	-0.773	-0.835	-0.796	-0.456
Q2	Palmitic	0.708	0.749	0.789	0.678	0.722	0.808	0.792	0.186	0.783	0.681	0.729	-0.132
Q2	Stearic	0.299	0.314	0.43	0.369	0.387	0.485	0.431	-0.002	0.342	0.42	0.364	-0.327
Q2	Oleic	-0.622	-0.605	-0.752	-0.558	-0.629	-0.726	-0.686	-0.32	-0.67	-0.609	-0.637	0.062
Q2	Linoleic	0.743	-0.738	0.673	0.667	0.72	0.753	0.768	0.206	0.787	0.656	0.746	-0.004
Q2	Linolenic	0.499	0.481	0.839	0.369	0.451	0.595	0.535	0.532	0.557	0.473	0.481	-0.169
Q2	Eicosenoic	-0.682	-0.738	-0.726	-0.571	-0.601	-0.643	-0.68	-0.241	-0.747	-0.558	-0.655	0.127
Q2	Erucic	-0.537	-0.623	-0.457	-0.442	0.428	-0.441	-0.521	-0.152	-0.614	-0.424	-0.543	0.281
Westar	Palmitic	0.526	0.562	0.596	0.71	0.535	0.585	0.619	-0.066	0.561	0.554	0.522	-0.326
Westar	Stearic	0.22	0.317	0.45	0.443	0.353	0.429	0.35	0.065	0.308	0.425	0.268	-0.454
Westar	Oleic	-0.076	-0.17	-0.412	-0.106	-0.144	-0.119	-0.203	0.23	-0.095	-0.104	-0.021	0.405
Westar	Linoleic	0.273	0.323	0.342	0.363	0.253	0.266	0.351	-0.082	0.284	0.309	0.273	-0.388
Westar	Linolenic	0.45	0.502	0.51	0.487	0.412	0.409	0.522	-0.099	0.44	0.475	0.445	-0.426
Westar	Eicosenoic	-0.612	-0.598	-0.23	-0.75	-0.507	-0.583	0.579	-0.195	-0.624	-0.671	-0.716	0.056
Westar	Erucic	-0.566	-0.507	-0.144	-0.691	-0.449	-0.524	0.512	-0.218	-0.574	-0.612	-0.672	0

Table 2

Continued

Genotype	Fatty acid	FAD6	FAD2	FAD3	LPAAT	DGAT2	AAPT1	FAE1	KCR2	Oleosin	Cruciferin	Napin	Caleosin
Brownowski	Palmitic	0.805	0.693	0.586	-0.07	0.554	0.469	0.64	0.697	0.029	0.572	0.584	0.107
Brownowski	Stearic	0.745	0.667	0.57	-0.157	0.636	0.422	0.611	0.64	-0.018	0.733	0.604	-0.013
Brownowski	Oleic	0.685	0.522	0.577	-0.039	0.303	0.754	0.513	0.584	0.505	-0.288	0.496	0.22
Brownowski	Linoleic	0.489	0.37	0.299	-0.149	0.355	0.211	0.4	0.322	0.007	0.774	0.419	0.108
Brownowski	Linolenic	0.777	0.746	0.688	-0.373	0.509	0.601	0.71	0.708	0.069	0.552	0.658	-0.094
Brownowski	Eicosenoic	-0.745	-0.586	-0.529	0.037	-0.409	-0.483	-0.604	-0.592	-0.16	-0.557	-0.563	-0.246
Brownowski	Erucic	-0.949	-0.788	-0.77	0.217	-0.594	-0.8	-0.781	-0.791	-0.348	-0.399	-0.767	-0.118
Q2	Palmitic	0.672	0.705	0.681	-0.61	0.704	-0.125	0.62	0.746	0.278	0.338	0.528	0.298
Q2	Stearic	0.527	0.395	0.323	-0.207	0.151	-0.187	0.425	0.381	0.419	0.237	0.647	0.333
Q2	Oleic	-0.503	-0.624	-0.614	0.562	-0.651	0.168	-0.538	-0.649	-0.187	-0.289	-0.386	-0.235
Q2	Linoleic	0.513	0.681	0.719	-0.75	0.837	-0.058	0.58	0.745	0.15	0.339	0.307	0.234
Q2	Linolenic	0.292	0.486	0.471	-0.341	0.544	-0.332	0.434	0.483	0.092	0.297	0.197	0.184
Q2	Eicosenoic	-0.431	-0.58	-0.607	0.6	-0.783	0.123	-0.521	-0.649	-0.105	-0.388	-0.166	-0.216
Q2	Erucic	-0.289	-0.401	-0.451	0.505	-0.654	0.159	-0.438	-0.486	-0.155	-0.405	-0.161	-0.259
Westar	Palmitic	0.678	0.575	0.512	-0.306	0.121	-0.249	0.322	0.581	-0.265	-0.078	0.243	-0.21
Westar	Stearic	0.578	0.39	0.261	-0.064	0.004	-0.042	0.174	0.366	-0.246	0.112	0.177	-0.116
Westar	Oleic	-0.357	0.181	-0.142	0.13	0.148	0.155	0.032	-0.223	0.455	0.237	0.016	0.294
Westar	Linoleic	0.351	0.266	0.295	-0.332	-0.122	-0.337	0.242	0.335	-0.123	0.001	0.241	-0.044
Westar	Linolenic	0.521	0.452	0.473	-0.464	0.05	-0.323	0.388	0.534	-0.036	0.077	0.359	-0.004
Westar	Eicosenoic	-0.389	-0.476	-0.544	0.465	-0.208	0.358	-0.64	-0.52	-0.478	-0.407	-0.572	-0.339
Westar	Erucic	-0.295	-0.418	-0.493	0.429	-0.212	0.317	-0.614	-0.455	-0.537	-0.429	-0.55	-0.376

Red and green colored coefficients display statistically significant (α=0.05 Bonferroni adjusted for multiple tests within each gene and cultivar) positive and negative correlations, respectively

Correlations

We examined the correlation between FA levels and gene expression across DAF for each cultivar. The data support a common trend in gene expression among the three cultivars with gene expression tending to decrease over time; except for *LPAAT*, which tended to increase (Electronic Supplementary Table S3). In our subsequent determination of the correlation between the level of FAs and expression of genes by individual cultivar, we observed no consistent relationship between FA and gene expression, rather these correlations seem to depend on the individual cultivar (Table 2). Forty-eight (red) and 27 (green) coefficients displayed significant ($P<0.05$) positive and negative correlations, respectively. Of these, 43, 29, and 3 were associated with Bronowski, Q2, and Westar, respectively. The correlation coefficients ranged from a positive correlation of 0.88 for *β-CT* and α-linolenic acid in Bronowski, to no correlation between *FatB* and erucic acid in Westar, to a highly negative correlation of -0.95 between *FAD6* and erucic acid in Bronowski. Interestingly, for Bronowski and Q2, 52% ($r^2=0.72$) or more of the variation in the expression of *β-CT* was related to variation in the level of palmitic acid, α-linolenic acid, and eicosenioc acid. In this case for Bronowski and Q2, expression of *β-CT* was positively correlated with levels of palmitic acid and α-linolenic acid, whereas levels of eicosenioc acid were negatively correlated with *β-CT*. For oleic acid, the correlations coefficients for most genes were positive for Bronowski but negative for Q2 and Westar; although most of the correlation coefficients were not significantly different. There were no significant correlations between the level of any fatty acid for the three cultivars and expression of *KAS3*, *Oleosin*, and *Caleosin* genes when correlations were computed across DAF.

DISCUSSION

We designed this investigation of *B. napus* seed development after a similar study by Hu *et al.* (2009). They examined an older high erucic acid cultivar Zhongyou 821 (ZY821) and a low erucic acid descendant of ZY821, Zhongshuang 9 (ZS9). In turn, we employed several cultivars important for breeding improved germplasm adapted to a region of the U.S. where 80% of the spring canola is produced. The level of seed oil at maturity in our three greenhouse grown cultivars was 2.7 to 7.7% lower than the content reported for seeds of these cultivars from field grown plants (Bronowski 41.7%, Westar 43.3%, and Q2 47.8%), but were similar to the level in rapeseed cultivars (ZY821 39.8% , ZS9 42%, respectively).

The abundance of FA in our three cultivars was typical relative to another report with oleic acid > linoleic > α-linolenic (Vuorinen *et al.* 2014). The VLCFAs, eicosenioc acid and erucic acid, as reported previously Finlayson *et al*., 1973, were higher in Bronowski at the expense of oleic acid, which is the

economically important FA component. In contrast to the level of eicosenioc acid in Bronowski (17%), the level in ZY821 was reduced by about one-half (9.75%); whereas, the level of erucic acid in Bronowski (21%) was reduced by about one-half the level in ZY821 (42%) (Hu *et al.* 2009). These difference likely reflect dissimilarity in genetic background of the cultivars.

We examined nearly the same set of genes as reported by Hu *et al.* (2009), but direct comparison is problematic because different reference genes (*β-actin* vs. *SAND*) were utilized for qRT-PCR normalization, different statistical procedures were employed, and we expressed our data on a log2 scale. To compare fold differences between relative copy number data (Hu *et al.* 2009) and our log2 scale data would require numerical data for the qRT-PCR done by Hu *et al.* (2009). This is because fold estimates are not possible when the relative copy number value is close to zero as is the case for many of the genes they examined. Nevertheless, similarities and differences in general trends can be discerned (see Fig. 2 (Hu *et al.* 2009)). For example, the biosynthesis of FAs begins with ACCase catalyzing the carboxylation of acetyl-CoA to malonyl-CoA. Expression of *β-CT*, a gene encoding for β-carboxyltransferase, one of four components of the heteromeric ACCase, is nearly the same in the two studies, increasing by about 2 fold from 10 to 15 DAF and thereafter decreasing about 2 fold by 35 DAF. Expression of *FAE1*, a component of a multienzyme complex involved in VLCFA biosynthesis, increases by 5 fold from 25 to 40 DAF in ZY821 and thereafter decreased to the 25 DAF level; whereas, the expression peaks (log2 = 7) around 15 DAF in Bronowski and thereafter decreases 128 fold by 40 DAF (log2 = 0).

The expression profile of the seed storage proteins was similar among Westar, Q2, and Bronowski, which was similar to that observed based on a comparison between Westar and Reston (Katavic *et al.* 2002), another high erucic acid (26%) low oleic acid (30%) cultivar similar to Bronowski. However, the expression profiles of the seed storage proteins between our cultivars and ZY821 and ZS9 differed (see Fig. 2 (Hu *et al.* 2009)). For example, oleosin, which is the major protein component of oil bodies, narrowly peaked at 40 DAF in ZY821 with a relative copy number of 25,000 (log2 =14.6) and 12,500 for ZS9 (log2 = 13.6); whereas in the cultivars we examined the broad peak occurred around 25 DAF with a log2 = 7. However, the *napin* gene, which accounted for over 75% of total transcription from all 32 genes assessed by Hu *et al.* (2009), and displayed the highest level of expression among the genes we assessed, had nearly the same level of expression at its peak; ZY821 and ZS9 peaked at 40 and 35 DAF, respectively with a similar relative copy number of 175,000 (log2 =17.4); whereas in the cultivars we examined the broad peak occurred around 25 DAF with a log2 = 13. In any event, the seed storage protein genes in both studies generally displayed the highest level of expression of the genes assessed.

Some of the genes we appraised were significantly correlated with fatty acid accumulation, especially for the Bronowski and Q2 cultivars. In particular, the level of several FAs was correlated with *β-CT* expression. *β-CT* encodes for one of the subunits (α-CT, β-CT and BC) for the plastid localized heteromeric ACCase, which catalyzes the first committed step of fatty acid biosynthesis. This gene is thought to be unique in that it is the only known lipid metabolism gene that is encoded by the plastid genome (Elborough *et al.* 1996; Li-Beisson *et al.* 2013). In the high erucic acid cultivars Bronowski and ZY821, *β-CT* expression was negatively correlated with erucic acid, whereas *β-CT* expression was positively correlated with palmitic acid, steric acid, α-linoleic acid in Bronowski, but not in ZY821 (Hu *et al.* 2009). Perhaps there is a negative correlation between erucic acid levels and *β-CT* expression because expression of this gene is declining, while erucic acid levels increase after 25 DAF.

Different patterns of gene expression exists for *FAE1* between the high erucic acid cultivars Bronowski and ZY821 (Hu *et al.* 2009). FAE1 is a component of the multi-enzyme complex involved in VLCFA biosynthesis; mutations in the *FAE1* gene are responsible for the low erucic acid trait (Puyaubert *et al.* 2005). Erucic acid levels peak by 30 DAF in Bronowski, whereas the levels substantially increases in ZY821 until 40 DAF. Thus, the negative correlation coefficient (-0.78) that we observed for *FAE1* and erucic acid in Bronowski is consistent with a large fold decrease in gene expression and slightly increased level of erucic acid as Bronowski seeds mature. However, the positive correlation (0.78) between erucic acid and *FAE1* for ZY821 is likely explained by the much different temporal pattern of *FAE1* expression and erucic acid accumulation (see Fig. 2 (Hu *et al.* 2009)). The high level of *FAE1* expression in low erucic acid cultivars such as Q2, ZS9 and other cultivars (Hu *et al.* 2009; Vuorinen *et al.* 2014) might seem inconsistent with the absence of VLCFAs. However, as mentioned, *FAE1* gene contains a mutation that result in the absence of 3-ketoaacyl-CoA synthase protein, thus preventing the synthesis of VLCFAs (Puyaubert *et al.* 2005; Wu *et al.* 2008). Interestingly, Westar contains a point mutation while ZS9 contains a point mutation and four base pair deletion (Katavic *et al.* 2006; Wu *et al.* 2008). Overall, appraisal of the correlation coefficients, which are sometimes different between our cultivars and the Chinese cultivars investigated by Hu *et al.* (2009), is instructive of the different patterns of gene expression in relation to a particular FA or storage protein.

The results of this investigation, which employed three publically available cultivars from Canadian breeding programs, provide background data into the transcriptional network for FA, TAG, and seed storage proteins. By comparing the outcome of our investigation to that of Hu *et al.* (2009), we further demonstrated that genetic background of the cultivars from different breeding programs affects important metabolic and molecular responses during oilseed development. In any event, these insights and benchmark data will be important

for the success of the recent public spring canola improvement project we initiated to develop germplasm adaptable to the Northern Plains of the U.S.

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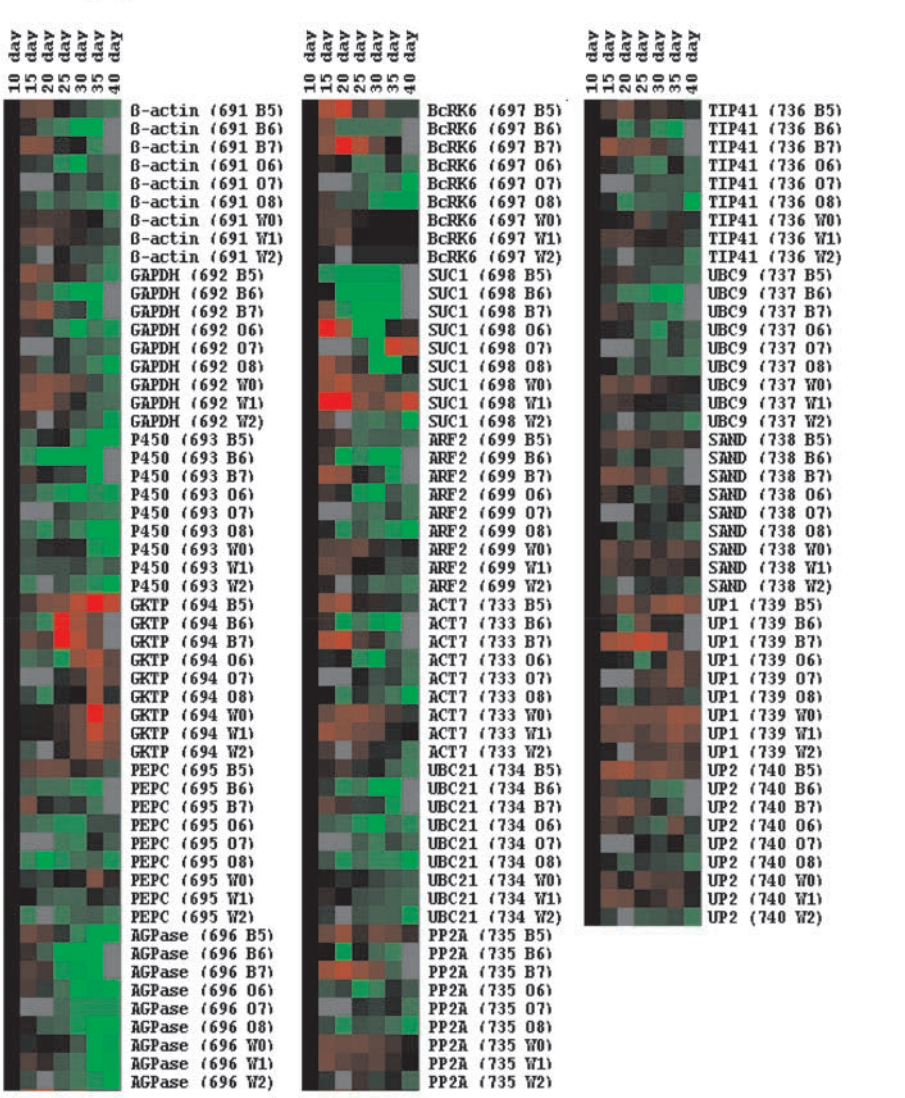
SUPLEMENTARY FIGURE AND TABLES



Heat map of various genes including SAND family gene. The formula used to calculate the fold differences is similar to the standard comparative C_T method ($\Delta\Delta C_T$) except that no endogenous reference gene is incorporated in the calculation since we want to determine stably expressed genes before normalization. The modified formula for fold difference in gene expression of test vs control sample is $\Delta C_T = \Delta C_{T,test} - \Delta C_{T,control}$. Here, $\Delta C_{T,test}$ is the C_T value of the test sample, and $\Delta C_{T,control}$ is the C_T value of the control sample, a 10 day sample (see Supplementary Table S1 for gene designation and primer sequences).

Suplementary Fig S1

Supplementary Table S1: Primers used for qRT-PCR analysis

Primer set	Canola Primer pairs				Primer name	Primer sequence	Amplicon size(bp)	PCR efficency
	Categry	Accession number	Gene name	Gene annotation				
1	2	3	4	5	6	7	8	9
667a	ACCase	X77382	*ACCase*	Homomeric acetyl CoA carboxylase	**Bn_ACCase_F**	F:5'AGGACTTGCCAATCTTCTAAAC3'	157	0,973
667b					**Bn_ACCase_R**	R:5'AGCTTCTTTCACCGTAGGACAC3'		
668a		AY538675	*α-CT*	alpha-carboxyltransferase	***Bn_α-CT_F***	F:5'CTTGTCCACCCTATTCTGATTG3'	106	0,958
668b					***Bn_α-CT_R***	R:5'ATGTCCAGCTTAGATTTGAGGC3'		
669a		Z50868	*β-CT*	Beta-carboxyltransferase	***Bn_β-CT_F***	F:5'CAGCAAGTTTGGGTATGTTGGG 3'	116	1,03
669b					***Bn_β-CT_R***	R:5'GTGAACCTTCAGGCACGGCTTT3'		
670a		AY034410	*BC*	Biotin carboxylase	***Bn_BC_F***	F:5'AGGACCCATTCAAAGGATTCAG3'	118	1
670b					***Bn_BC_R***	R:5'GCTTGGAGGAACAACATAGTCG3'		
671a	Desaturase	AY642537	*SAD*	Stearoyl-ACP desaturase	***Bn_SAD_F***	F:5'GTTTACACTGCCAAAGACTATGCG3'	135	0,937
671b					***Bn_SAD_R***	R:5'CCTGATTCTCGGAGTCAACCCAC3'		
672a		AY592975	*FAD2*	Oleate desaturase	***Bn_FAD2_F***	F:5'AGGCGATAAAGCCGATACTTGG3'	107	1,095
672b					***Bn_FAD2_R***	R:5'CCTATCCGGTTCAACATAGATACACT3'		
673a		AY599884	*FAD3*	Linoleate desaturase	***Bn_FAD3_F***	F:5'TTCCCACAAATCCCTCACTATCA3'	132	0,936
673b					***Bn_FAD3_R***	R:5'ACTTGCCACCAAACTTTCCACC3'		
674a		AY642535	*FAD6*	Oleate desaturase	***Bn_FAD6_F***	F:5'ATCACATAAGCCCAAGGATACCG3'	116	0,953
674b					***Bn_FAD6_R***	R:5'TCGTCTTCATCAACCGCCAATT3'		

Supplementary Table S1—continued

1	2	3	4	5	6	7	8	9
675a		AJ007046	*MCMT*	Malonyl CoA-ACP malonyltransferase	***Bn_MCAT_F***	F:5'ATCATAGGGTTGGACTCAGAAA 3'	116	0,955
675b					***Bn_MCAT_R***	R:5'ACTGCGTAGTTACCCGGACATA 3'		
676a		AF244519	*KAS1*	Beta-ketoacyl-ACP synthase 1	***Bn_KAS1_F***	F:5'ACACGGTCGCAAACGAGAAGAA3'	204	0,976
676b					***Bn_KAS1_R***	R:5'GAAGATAATGGTGATGGAGCAG3'		
677a		AF244520	*KAS2*	Beta-ketoacyl-ACP synthase 2	***Bn_KAS2_F***	F:5'GGAGTACCAAGCCCTTGCTCAC3'	133	0,812
677b					***Bn_KAS2_R***	R:5'TCCTTATGGCCTGCACAGTTGC3'		
678a	Elongase	AF179854	*KAS3*	Beta-ketoacyl-ACP synthase 3	***Bn_KAS3_F***	F:5'GGATGATGGGTTATTTAGTTTC3'	108	0,918
678b					***Bn_KAS3_R***	R:5'CCAAAGGGTAAAGCAGGAGAAG3'		
679a		AF009563	*FAE1*	Fatty acid elongase 1/3-ketoacyl-CoA synthase	***Bn_FAE1_F***	F:5'GTCAGGCTTTAAGTGTAACAGTGCA3'	159	0,957
679b					***Bn_FAE1_R***	R:5'TTATTAGGACCGACCGTTTTGG3'		
680a		AF382146	*HD/KACD*	3-keto-acyl-ACP dehydratase	***Bn_KACD_F***	F:5'GATAGCGAAAATGGAAGGGAAAG3'	115	0,958
680b					***Bn_KACD_R***	R:5'AAAGCAAAAGGCACGAGAACATA3'		
681a		AY196197	*KCR2*	3-ketoacyl-CoA reductase	***Bn_KCR2_F***	F:5'TGAGTACAAGAAAAGTGGGATTG3'	101	0,983
681b					***Bn_KCR2_R***	R:5'GAGATGCCACTAAGAAAGATGCT3'		
682a		BRU17098	*FatA*	Acyl-ACP thioesterase	***Bn_FatA_F***	F:5'GGGACCAATGGCTCTGCATCAT3'	121	0,965
682b	Thioesterase				***Bn_FatA_R***	R:5'GGCTTCTTTCTCCACAGGGTTG3'		
683a		DQ847275	*FatB*	Palmitoyl-ACP thioesterase	***Bn_FatB_F***	F:5'AGTTTGTGGGTGATGATGAATA3'	107	0,944
683b					***Bn_FatB_R***	R:5'GCAAGGATAGGGTCAGAGTTCA3'		

Supplementary Table S1—continued

1	2	3	4	5	6	7	8	9
684a		AF155224	*DGAT2*	Acyl-CoA: diacylglycerol acyltransferase	***Bn_DGAT2_F***	F:5'CATGACCTGATGAACCGCAAAG3'	111	0,985
684b					***Bn_DGAT2_R***	R:5'ACGGCTACCAAAAGGATACAAAA3'		
685a	TAG synthesis	AF111161	*LPAAT*	Lysophosphatidic acid acyltransferase	***Bn_LPAAT_F***	F:5' CGAAGAGGCGAGAAACAAGATAG3'	100	0,97
685b					***Bn_LPAAT_R***	R:5'TGGTTTAGCCTTCTCATTGTTCA3'		
686a		AY179560	*AAPT1*	Aminoalcoholphosphotransferase	***Bn_AAPT1_F***	F:5'TGGTGCTTCTTGGTTATTGTAT3'	156	0,821
686b					***Bn_AAPT1_R***	R:5'GGATTTGCATTATCCTCCCTTG3'		
687a		AY570250	*Napin*	1.7S oil body protein	***Bn_Napin_F***	F:5'GACCCTCGATGGTGAGTTTGA3'	148	0,958
687b					***Bn_Napin_R***	R:5'CTTTGGATGCTCCTTTCAAGGT3'		
688a		AY966447	*Caleosin*	Ca2+-binding oil body surface protein	***Bn_Caleosin_F***	F:5'GTAATCAATTTGGCCCTTAGCT3'	116	0,952
688b	Oil body protein				***Bn_Caleosin_R***	R:5'CTCAAGATTCACAGGCATAAAC3'		
689a		X58000	*Oleosin*	oil body associated protein	***Bn_Oleosin_F***	F:5'CTGGGAGGCAAAGTTCAGGATA3'	122	0,969
689b					***Bn_Oleosin_R***	R:5'CATGGCGTAATTTAGGTAGTGT3'		
690a		M16860	*Cruciferin*	12S neutral oil bodyprotein	***Bn_Cruciferin_F***	F:5'GAGGAGTCAGAGACCGCAGGA3'	165	0,969
690b					***Bn_Cruciferin_R***	R:5'AAGGAAGCGAAGGATGGGGAGA3'		
691a		AF111812	*β-actin*	Housekeeping gene	***Bn_β-actin_F***	F:5'CTGGAATTGCTGACCGTATGAG 3'	145	1,001
691b	Housekeep genes				***Bn_β-actin_R***	R:5'ATCTGTTGGAAAGTGCTGAGGG 3'		
692a		DQ097338	*GAPDH*	Glyceraldehyde-3-phosphate dehydrogenase	***Bn_GAPDH_F***	F:5'GCTATCAAGGAGGAATCTGAGGAC3'	146	0,936
692b					***Bn_GAPDH_R***	R:5'CTTCACGAAATTGTCACTCAACG3'		
693a		DQ167182	*P450*	Cytochrome P450	***Bn_P450_F***	F:5'ATGGATCTCGGGATCGGACAGT3'	156	0,954
693b	Others				***Bn_P450_R***	R:5'GTCAAGCGATGACGGAGCAAAA3'		
694a		X93015	*GKTP*	Glyoxysomal beta-ketoacyl-thiolase precursor	***Bn_GKTP_F***	F:5'GTTGGTCCAGCAGTTGCCATTC3'	159	0,934
694b					***Bn_GKTP_R***	R:5'CGCCTCCGTTGACATTGATTTT3'		

Supplementary Table S1—continued

1	2	3	4	5	6	7	8	9
695a		AJ223497	*PEPC*	Phosphoenolpyruvate carboxylase	***Bn_PEPC_F***	F:5'GGTTGGGTTTATTGGTTTGTTTATG3'	134	0,934
695b					***Bn_PEPC_R***	R:5'ATTCCCTTGCTCGGTTTTGTTA3'		
696a		AJ271162	*AGPase*	ADP-glucose pyrophosphorylase small sub-	***Bn_AGPase_F***	F:5'AGACACCACCACCCCGTTTGAC3'	129	0,974
696b					***Bn_AGPase_R***	R:5' TTTAGGGATAAGGCAGGAGGAT3'		
697a		AB041622	*BcRK6*	Receptor kinase 6	***Bn_BcRK6_F***	F:5'AGGTTAAGTGACGGGCAAGAAA3'	143	1,019
697b					***Bn_BcRK6_R***	R:5'TTGAACGCAACAGCCAAGAAGT3'		
698a		AY065839	*SUC1*	Sucrose transporter	***Bn_SUC1_F***	F:5'GCCAAGGACTGTCGTTAGGAGTTT3'	133	0,97
698b					***Bn_SUC1_R***	R:5'TGCGATTGCTCCGACTATAAATG3'		
699a		AJ716227	*ARF2*	Auxin Response Factor2	**Bn_ARF2_F**	F:5'ACCACTAGTATTCCTCGCCCTGAT3'	171	
699b					**Bn_ARF2_R**	R:5'TGCCTTAGATGAGCCTTCCCTTAT3'		
733a		EV116054	*ACT7*	Actin	***ACT7F***	5'-TGGGTTTGCTGGTGACGAT	63	
733b					***ACT7R***	5'- TGCCTAGGACGACCAACAATACT		
734a	Others	EV086936	*UBC21*	Ubiquitin conjugating enzyme 21	***UBC21F***	5'- CCTCTGCAGCCTCCTCAAGT	77	
734b					***UBC21R***	5'- CATATCTCCCCTGTCTTGAAATGC		
735a		EV051005	*PP2A*	Regulatory subunit of protein phosphatase 2A	***PP2AF***	5'- TGGCTTCAGTTATAATGGGAATGG	75	
735b					***PP2AR***	5'- GAAAGATTGGAAGGAGATGCTCAAT		
736a		EV222761	*TIP41*	TIP41-like family protein	***TIP41F***	5'- AGAGTCATGCCAAGTTCATGGTT	69	
736b					***TIP41R***	5'- CCTCATAAGCACACCATCAACTCTAA		
737a		EV002123	*UBC9*	Ubiquitin conjugating enzyme 9	***UBC9F***	5'- GCATCTGCCTCGACATCTTGA	68	
737b					***UBC9R***	5'- GACAGCAGCACCTTGGAAATG		
738a		EV084276	*SAND*	SAND-family protein	***SANDF***	5'- GCTGGGTCACTCCAGATTTTG	63	
738b					***SANDR***	5'- CCATCGCCTTGTCTGCAAG		
739a		EE450388	*UP1*	Unknown protein	***UP1F***	5'- AGCCTGAGGAGATATTAGCAGGAA	87	
739b					***UP1R***	5'- ATCTCACTGCAGCTCCACCAT		
740a		EV116750	*UP2*	Unknown protein	***UP2F***	5'- AAATTCCTGGGAGGGAAGCTAT	70	
740b					***UP2R***	5'- TTCTGTCTCAGGAGCGAAGTCAT		

Supplementary Table S2: Cycle treshold (CT) values for reference genes 10 d value as a baseline

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
β-actin (691 B5)	*β-actin*	(691	B5)	Bronowski 1805	21.833	20.950	20.770	21.737	22.250	22.927	23.280
β-actin (691 B6)	*β-actin*	(691	B6)	Bronowski 1806	20.803	20.573	22.037	22.690	24.260	24.580	no cDNA
β-actin (691 B7)	*β-actin*	(691	B7)	Bronowski 1807	21.883	20.490	20.570	22.110	21.953	23.870	no cDNA
β-actin (691 Q6)	*β-actin*	(691	Q6)	Q2 1966	20.970	21.290	21.473	23.070	24.820	21.920	22.177
β-actin (691 Q7)	*β-actin*	(691	Q7)	Q2 1967	21.060	no cDNA	no cDNA	21.327	21.980	21.463	22.313
β-actin (691 Q8)	*β-actin*	(691	Q8)	Q2 1968	20.870	21.307	22.443	21.623	22.010	22.597	23.313
β-actin (691 W0)	*β-actin*	(691	W0)	Westar 2030	22.173	21.843	21.663	21.710	21.987	22.147	23.350
β-actin (691 W1)	*β-actin*	(691	W1)	Westar 2031	22.220	21.380	21.207	21.870	22.020	22.577	22.783
β-actin (691 W2)	*β-actin*	(691	W2)	Westar 2032	21.187	21.680	no cDNA	21.277	21.817	21.837	22.970
GAPDH (692 B5)	*GAPDH*	(692	B5)	Bronowski 1805	23.110	21.717	22.017	22.947	22.767	24.327	25.257
GAPDH (692 B6)	*GAPDH*	(692	B6)	Bronowski 1806	21.980	21.330	22.970	24.310	25.343	25.757	no cDNA
GAPDH (692 B7)	*GAPDH*	(692	B7)	Bronowski 1807	22.900	21.720	21.300	23.500	23.357	25.770	no cDNA 0!
GAPDH (692 Q6)	*GAPDH*	(692	Q6)	Q2 1966	22.183	21.813	22.480	24.037	26.000	23.803	24.757
GAPDH (692 Q7)	*GAPDH*	(692	Q7)	Q2 1967	22.357	#DZIEL/0!	no cDNA	22.607	23.567	23.233	23.780
GAPDH (692 Q8)	*GAPDH*	(692	Q8)	Q2 1968	22.443	21.977	23.430	22.707	23.410	24.297	25.493
GAPDH (692 W0)	*GAPDH*	(692	W0)	Westar 2030	23.617	22.503	22.200	22.443	22.903	24.193	25.200
GAPDH (692 W1)	*GAPDH*	(692	W1)	Westar 2031	23.450	22.010	21.960	22.833	23.620	24.613	24.757
GAPDH (692 W2)	*GAPDH*	(692	W2)	Westar 2032	22.607	22.177	no cDNA	22.370	23.273	23.673	25.367
P450 (693 B5)	*P450*	(693	B5)	Bronowski 1805	27.450	28.680	27.657	27.517	29.230	31.210	32.880

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
P450 (693 B6)	*P450*	(693	B6)	Bronowski 1806	26.007	27.937	29.440	29.237	35.000	35.000	no cDNA
P450 (693 B7)	*P450*	(693	B7)	Bronowski 1807	27.993	27.437	28.087	28.410	28.503	32.383	no cDNA
P450 (693 Q6)	*P450*	(693	Q6)	Q2 1966	28.080	28.767	29.800	30.547	35.000	30.213	30.750
P450 (693 Q7)	*P450*	(693	Q7)	Q2 1967	27.863	no cDNA	no cDNA	28.260	28.873	29.290	29.587
P450 (693 Q8)	*P450*	(693	Q8)	Q2 1968	27.883	29.040	30.033	28.515	29.723	32.267	32.307
P450 (693 W0)	*P450*	(693	W0)	Westar 2030	29.090	29.790	29.277	28.970	29.257	31.557	32.050
P450 (693 W1)	*P450*	(693	W1)	Westar 2031	29.027	29.810	29.710	29.703	29.490	30.560	29.903
P450 (693 W2)	*P450*	(693	W2)	Westar 2032	28.173	29.913	no cDNA	28.880	29.660	30.360	33.193
GKTP (694 B5)	*GKTP*	(694	B5)	Bronowski 1805	24.620	24.170	23.393	23.300	22.370	21.487	22.240
GKTP (694 B6)	*GKTP*	(694	B6)	Bronowski 1806	23.537	24.400	25.523	20.483	21.647	22.577	no cDNA
GKTP (694 B7)	*GKTP*	(694	B7)	Bronowski 1807	24.463	23.470	23.837	21.127	22.233	23.470	no cDNA
GKTP (694 Q6)	*GKTP*	(694	Q6)	Q2 1966	24.317	25.620	25.207	26.777	22.650	22.207	23.513
GKTP (694 Q7)	*GKTP*	(694	Q7)	Q2 1967	24.050	no cDNA	no cDNA	24.283	23.627	22.347	23.167
GKTP (694 Q8)	*GKTP*	(694	Q8)	Q2 1968	24.567	25.140	26.533	24.663	24.590	22.843	24.793
GKTP (694 W0)	*GKTP*	(694	W0)	Westar 2030	25.657	25.770	25.553	25.337	24.673	22.607	24.187
GKTP (694 W1)	*GKTP*	(694	W1)	Westar 2031	25.587	25.653	25.503	25.337	24.607	23.180	24.253
GKTP (694 W2)	*GKTP*	(694	W2)	Westar 2032	25.023	25.807	no cDNA	24.870	24.023	23.473	23.977
PEPC (695 B5)	*PEPC*	(695	B5)	Bronowski 1805	29.107	28.363	28.063	28.097	28.907	28.660	29.953
PEPC (695 B6)	*PEPC*	(695	B6)	Bronowski 1806	27.627	28.557	28.947	28.987	29.647	30.010	no cDNA

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
PEPC (695 B7)	*PEPC*	(695	B7)	Bronowski 1807	28.473	27.253	28.610	28.950	28.793	29.970	#DZIEL/0 !
PEPC (695 Q6)	*PEPC*	(695	Q6)	Q2 1966	25.587	no cDNA	27.043	no cDNA	26.503	no cDNA	25.200
PEPC (695 Q7)	*PEPC*	(695	Q7)	Q2 1967	no cDNA	no cDNA	no cDNA	26.560	no cDNA	25.320	no cDNA
PEPC (695 Q8)	*PEPC*	(695	Q8)	Q2 1968	25.193	no cDNA	26.930	no cDNA	25.613	no cDNA	26.867
PEPC (695 W0)	*PEPC*	(695	W0)	Westar 2030	28.377	29.543	29.700	no cDNA	26.213	no cDNA	28.810
PEPC (695 W1)	*PEPC*	(695	W1)	Westar 2031	27.887	27.690	28.513	28.813	28.600	27.737	28.233
PEPC (695 W2)	*PEPC*	(695	W2)	Westar 2032	27.040	29.207	no cDNA	28.943	27.983	27.887	28.953
AGPase (696 B5)	*AGPase*	(696	B5)	Bronowski 1805	24.490	23.467	23.803	24.837	26.433	28.497	29.183
AGPase (696 B6)	*AGPase*	(696	B6)	Bronowski 1806	23.280	22.943	24.307	28.877	30.750	30.673	no cDNA
AGPase (696 B7)	*AGPase*	(696	B7)	Bronowski 1807	24.307	23.660	23.890	26.827	27.207	29.320	no cDNA
AGPase (696 Q6)	*AGPase*	(696	Q6)	Q2 1966	22.760	22.217	23.137	24.193	30.883	25.650	27.410
AGPase (696 Q7)	*AGPase*	(696	Q7)	Q2 1967	22.727	no cDNA	no cDNA	23.913	25.083	25.117	25.110
AGPase (696 Q8)	*AGPase*	(696	Q8)	Q2 1968	23.007	22.867	23.940	24.073	25.493	26.847	27.250
AGPase (696 W0)	*AGPase*	(696	W0)	Westar 2030	23.440	23.650	23.373	23.520	24.257	26.970	26.293
AGPase (696 W1)	*AGPase*	(696	W1)	Westar 2031	23.690	23.073	23.447	24.490	25.207	27.703	26.673
AGPase (696 W2)	*AGPase*	(696	W2)	Westar 2032	22.677	23.340	no cDNA	23.713	24.297	25.010	27.977
BcRK6 (697 B5)	*BcRK6*	(697	B5)	Bronowski 1805	34.470	32.487	31.853	33.930	33.130	35.000	35.000
BcRK6 (697 B6)	*BcRK6*	(697	B6)	Bronowski 1806	33.597	32.373	35.000	35.000	35.000	35.000	no cDNA
BcRK6 (697 B7)	*BcRK6*	(697	B7)	Bronowski 1807	35.000	33.540	32.003	32.823	34.097	35.000	no cDNA

\Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
BcRK6 (697 Q6)	*BcRK6*	(697	Q6)	Q2 1966	33.380	33.093	33.437	35.000	35.000	33.617	35.000
BcRK6 (697 Q7)	*BcRK6*	(697	Q7)	Q2 1967	31.813	no cDNA	no cDNA	32.693	33.643	33.303	35.000
BcRK6 (697 Q8)	*BcRK6*	(697	Q8)	Q2 1968	32.200	31.653	32.920	32.933	34.583	35.000	35.000
BcRK6 (697 W0)	*BcRK6*	(697	W0)	Westar 2030	35.000	34.373	34.383	34.857	35.000	35.000	35.000
BcRK6 (697 W1)	*BcRK6*	(697	W1)	Westar 2031	35.000	34.450	34.113	35.000	35.000	35.000	35.000
BcRK6 (697 W2)	*BcRK6*	(697	W2)	Westar 2032	34.850	35.000	no cDNA	35.000	35.000	35.000	35.000
SUC1 (698 B5)	*SUC1*	(698	B5)	Bronowski 1805	32.267	34.497	35.000	35.000	35.000	35.000	no cDNA
SUC1 (698 B6)	*SUC1*	(698	B6)	Bronowski 1806	32.020	31.993	35.000	35.000	35.000	35.000	no cDNA
SUC1 (698 B7)	*SUC1*	(698	B7)	Bronowski 1807	31.133	29.930	33.387	35.000	34.813	34.803	no cDNA
SUC1 (698 Q6)	*SUC1*	(698	Q6)	Q2 1966	31.713	28.647	29.783	34.857	35.000	31.883	31.403
SUC1 (698 Q7)	*SUC1*	(698	Q7)	Q2 1967	30.797	no cDNA	no cDNA	31.713	33.833	28.193	28.573
SUC1 (698 Q8)	*SUC1*	(698	Q8)	Q2 1968	31.270	29.473	30.337	30.977	35.000	35.000	31.357
SUC1 (698 W0)	*SUC1*	(698	W0)	Westar 2030	31.947	30.270	29.843	30.867	30.873	32.413	32.833
SUC1 (698 W1)	*SUC1*	(698	W1)	Westar 2031	33.150	29.797	29.503	31.663	32.227	31.637	30.633
SUC1 (698 W2)	*SUC1*	(698	W2)	Westar 2032	31.333	30.397	#DZIEL/0!	32.607	32.920	32.377	35.000
ARF2 (699 B5)	*ARF2*	(699	B5)	Bronowski 1805	26.983	26.717	26.370	28.270	27.957	27.917	28.340
ARF2 (699 B6)	*ARF2*	(699	B6)	Bronowski 1806	26.257	25.763	30.383	28.307	30.953	31.850	no cDNA
ARF2 (699 B7)	*ARF2*	(699	B7)	Bronowski 1807	27.607	25.837	26.093	27.717	28.140	30.193	no cDNA
ARF2 (699 Q6)	*ARF2*	(699	Q6)	Q2 1966	28.477	28.033	28.670	31.023	32.257	29.217	30.027
ARF2 (699 Q7)	*ARF2*	(699	Q7)	Q2 1967	28.190	no cDNA	no cDNA	29.150	29.607	28.617	29.730

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
ARF2 (699 Q8)	*ARF2*	(699	Q8)	Q2 1968	28.093	28.237	30.313	28.793	29.817	30.530	31.420
ARF2 (699 W0)	*ARF2*	(699	W0)	Westar 2030	30.313	29.657	29.587	29.357	30.067	30.667	30.437
ARF2 (699 W1)	*ARF2*	(699	W1)	Westar 2031	30.013	29.073	29.213	30.003	30.040	30.683	30.590
ARF2 (699 W2)	*ARF2*	(699	W2)	Westar 2032	29.333	29.160	no cDNA	29.743	30.617	30.353	31.120
ACT7 (733 B5)	*ACT7*	(733	B5)	Bronowski 1805	25.550	24.060	23.727	25.397	25.290	25.820	26.377
ACT7 (733 B6)	*ACT7*	(733	B6)	Bronowski 1806	24.163	23.810	26.860	25.667	26.817	27.317	no cDNA
ACT7 (733 B7)	*ACT7*	(733	B7)	Bronowski 1807	25.580	23.600	23.233	24.947	25.283	26.923	no cDNA
ACT7 (733 Q6)	*ACT7*	(733	Q6)	Q2 1966	25.480	25.697	25.403	27.643	28.577	26.070	26.857
ACT7 (733 Q7)	*ACT7*	(733	Q7)	Q2 1967	25.230	no cDNA	no cDNA	25.313	25.870	25.610	26.800
ACT7 (733 Q8)	*ACT7*	(733	Q8)	Q2 1968	25.163	25.173	27.073	25.397	25.950	26.753	28.263
ACT7 (733 W0)	*ACT7*	(733	W0)	Westar 2030	27.830	26.893	26.377	26.267	26.743	27.333	28.163
ACT7 (733 W1)	*ACT7*	(733	W1)	Westar 2031	27.877	26.187	26.440	26.840	26.977	28.020	27.907
ACT7 (733 W2)	*ACT7*	(733	W2)	Westar 2032	26.383	25.860	#DZIEL/0!	25.677	26.590	26.643	28.063
UBC21 (734 B5)	*UBC21*	(734	B5)	Bronowski 1805	27.157	26.960	26.560	27.473	27.267	28.187	29.000
UBC21 (734 B6)	*UBC21*	(734	B6)	Bronowski 1806	26.237	26.607	28.350	27.797	30.243	30.230	no cDNA
UBC21 (734 B7)	*UBC21*	(734	B7)	Bronowski 1807	27.057	26.153	26.610	27.543	27.450	29.567	no cDNA
UBC21 (734 Q6)	*UBC21*	(734	Q6)	Q2 1966	26.737	27.640	27.990	29.083	30.683	28.040	28.863
UBC21 (734 Q7)	*UBC21*	(734	Q7)	Q2 1967	26.837	no cDNA	no cDNA	27.737	28.597	27.410	28.043
UBC21 (734 Q8)	*UBC21*	(734	Q8)	Q2 1968	26.460	27.210	28.783	27.730	28.473	28.523	29.403
UBC21 (734 W0)	*UBC21*	(734	W0)	Westar 2030	27.740	27.673	28.020	27.837	28.220	28.500	28.697
UBC21 (734 W1)	*UBC21*	(734	W1)	Westar 2031	27.717	27.433	27.700	28.333	28.463	28.710	29.017

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
UBC21 (734 W2)	*UBC21*	(734	W2)	Westar 2032	27.123	27.493	#DZIEL/0!	27.820	28.147	28.147	29.790
PP2A (735 B5)	*PP2A*	(735	B5)	Bronowski 1805	27.163	26.190	25.520	26.830	26.050	26.237	26.837
PP2A (735 B6)	*PP2A*	(735	B6)	Bronowski 1806	25.863	25.817	28.537	25.953	27.040	27.673	no cDNA
PP2A (735 B7)	*PP2A*	(735	B7)	Bronowski 1807	27.410	25.693	25.210	25.823	26.277	28.487	no cDNA
PP2A (735 Q6)	*PP2A*	(735	Q6)	Q2 1966	26.803	27.660	27.290	29.393	28.630	27.040	27.620
PP2A (735 Q7)	*PP2A*	(735	Q7)	Q2 1967	26.833	no cDNA	no cDNA	27.097	27.273	27.000	28.393
PP2A (735 Q8)	*PP2A*	(735	Q8)	Q2 1968	26.523	27.123	28.757	27.190	27.303	27.947	29.083
PP2A (735 W0)	*PP2A*	(735	W0)	Westar 2030	29.173	28.090	28.187	28.140	28.400	28.713	29.090
PP2A (735 W1)	*PP2A*	(735	W1)	Westar 2031	29.100	27.937	28.353	28.550	28.357	28.823	29.107
PP2A (735 W2)	*PP2A*	(735	W2)	Westar 2032	28.290	28.610	#DZIEL/0!	27.893	28.793	28.427	29.450
TIP41 (736 B5)	*TIP41*	(736	B5)	Bronowski 1805	29.100	28.447	27.917	28.833	28.600	28.867	29.383
TIP41 (736 B6)	*TIP41*	(736	B6)	Bronowski 1806	27.577	27.610	29.573	28.053	29.613	30.270	no cDNA
TIP41 (736 B7)	*TIP41*	(736	B7)	Bronowski 1807	29.063	27.453	28.017	27.933	28.480	29.813	no cDNA
TIP41 (736 Q6)	*TIP41*	(736	Q6)	Q2 1966	28.523	28.340	29.083	30.070	30.330	28.420	29.713
TIP41 (736 Q7)	*TIP41*	(736	Q7)	Q2 1967	27.847	no cDNA	no cDNA	28.387	28.620	28.627	29.067
TIP41 (736 Q8)	*TIP41*	(736	Q8)	Q2 1968	27.913	28.093	29.893	28.940	28.880	28.693	31.903
TIP41 (736 W0)	*TIP41*	(736	W0)	Westar 2030	29.433	29.480	29.323	28.660	29.917	29.163	29.700
TIP41 (736 W1)	*TIP41*	(736	W1)	Westar 2031	29.090	28.493	28.423	28.913	28.947	28.827	29.700
TIP41 (736 W2)	*TIP41*	(736	W2)	Westar 2032	28.263	28.663	no cDNA	28.757	28.707	28.687	30.030
UBC9 (737 B5)	*UBC9*	(737	B5)	Bronowski 1805	22.280	22.023	21.743	22.730	23.207	23.177	23.733

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
UBC9 (737 B6)	*UBC9*	(737	B6)	Bronowski 1806	21.383	21.890	23.087	23.323	24.170	24.520	no cDNA
UBC9 (737 B7)	*UBC9*	(737	B7)	Bronowski 1807	22.333	21.723	22.000	23.283	23.173	24.273	no cDNA
UBC9 (737 Q6)	*UBC9*	(737	Q6)	Q2 1966	22.800	22.700	22.990	23.990	25.077	23.123	23.850
UBC9 (737 Q7)	*UBC9*	(737	Q7)	Q2 1967	22.340	no cDNA	no cDNA	22.917	23.623	22.687	23.660
UBC9 (737 Q8)	*UBC9*	(737	Q8)	Q2 1968	22.427	22.483	23.533	22.820	23.677	23.853	24.157
UBC9 (737 W0)	*UBC9*	(737	W0)	Westar 2030	23.800	22.810	23.093	22.723	23.410	23.610	23.930
UBC9 (737 W1)	*UBC9*	(737	W1)	Westar 2031	23.490	22.960	23.133	23.403	23.567	23.723	23.830
UBC9 (737 W2)	*UBC9*	(737	W2)	Westar 2032	22.630	23.033	no cDNA	23.020	23.550	23.480	24.507
SAND (738 B5)	*SAND (2)*	(738	B5)	Bronowski 1805	27.927	27.280	26.907	27.380	27.543	27.343	27.650
SAND (738 B6)	*SAND (2)*	(738	B6)	Bronowski 1806	27.037	26.740	27.947	27.130	27.927	27.973	no cDNA
SAND (738 B7)	*SAND (2)*	(738	B7)	Bronowski 1807	28.060	26.833	26.893	27.500	27.050	28.540	no cDNA
SAND (738 Q6)	*SAND (2)*	(738	Q6)	Q2 1966	28.100	27.680	28.013	29.020	28.627	27.810	28.250
SAND (738 Q7)	*SAND (2)*	(738	Q7)	Q2 1967	27.743	no cDNA	no cDNA	27.863	28.143	27.693	28.200
SAND (738 Q8)	*SAND (2)*	(738	Q8)	Q2 1968	27.823	27.777	28.793	28.003	28.117	28.373	29.133
SAND (738 W0)	*SAND (2)*	(738	W0)	Westar 2030	28.550	27.563	28.127	27.720	28.240	27.703	27.980
SAND (738 W1)	*SAND (2)*	(738	W1)	Westar 2031	28.287	27.513	27.873	28.133	27.803	27.943	28.130
SAND (738 W2)	*SAND (2)*	(738	W2)	Westar 2032	27.493	28.143	no cDNA	27.840	27.697	27.887	28.613
UP1 (739 B5)	*UP1 (2)*	(739	B5)	Bronowski 1805	29.420	28.950	28.107	28.900	28.037	27.690	28.170
UP1 (739 B6)	*UP1 (2)*	(739	B6)	Bronowski 1806	28.757	28.613	30.457	27.710	28.573	28.937	no cDNA

Supplementary Table S2—continued

Gene name Primer # Bio Rep	Gene name	Primer #	Bio Rep	Variety	10 day	15 day	20 day	25 day	30 day	35 day	40 day
UP1 (739 B7)	*UP1 (2)*	(739	B7)	Bronowski 1807	30.027	28.287	27.997	27.590	27.847	29.497	no cDNA
UP1 (739 Q6)	*UP1 (2)*	(739	Q6)	Q2 1966	29.447	29.503	29.610	31.270	29.623	28.140	28.853
UP1 (739 Q7)	*UP1 (2)*	(739	Q7)	Q2 1967	29.547	no cDNA	no cDNA	29.037	28.870	28.130	28.963
UP1 (739 Q8)	*UP1 (2)*	(739	Q8)	Q2 1968	29.417	29.590	30.757	29.637	28.910	28.990	30.123
UP1 (739 W0)	*UP1 (2)*	(739	W0)	Westar 2030	30.767	29.517	29.643	29.683	29.683	29.060	29.157
UP1 (739 W1)	*UP1 (2)*	(739	W1)	Westar 2031	30.747	29.793	29.730	30.153	29.830	29.237	29.753
UP1 (739 W2)	*UP1 (2)*	(739	W2)	Westar 2032	30.213	30.117	no cDNA	29.700	29.677	29.257	29.840
UP2 (740 B5)	*UP2*	(740	B5)	Bronowski 1805	29.593	27.963	27.737	28.373	28.153	28.467	29.073
UP2 (740 B6)	*UP2*	(740	B6)	Bronowski 1806	28.273	27.667	28.763	28.730	29.653	29.933	no cDNA
UP2 (740 B7)	*UP2*	(740	B7)	Bronowski 1807	29.190	28.207	27.783	28.513	29.010	30.157	no cDNA
UP2 (740 Q6)	*UP2*	(740	Q6)	Q2 1966	28.637	28.033	28.333	29.343	29.870	27.610	29.020
UP2 (740 Q7)	*UP2*	(740	Q7)	Q2 1967	27.780	no cDNA	no cDNA	27.803	28.173	27.727	28.577
UP2 (740 Q8)	*UP2*	(740	Q8)	Q2 1968	27.797	27.913	28.807	28.103	28.340	28.420	29.363
UP2 (740 W0)	*UP2*	(740	W0)	Westar 2030	29.657	28.907	29.520	28.817	29.080	29.067	29.530
UP2 (740 W1)	*UP2*	(740	W1)	Westar 2031	29.097	28.023	28.163	28.963	28.777	28.327	29.240
UP2 (740 W2)	*UP2*	(740	W2)	Westar 2032	27.833	28.303	no cDNA	28.780	28.647	28.457	29.493

Supplementary Table S3. In the test for statistical significance, red and green coefficients displayed positive and negative correlations, respectively; non-colored coefficients statistically non-significant

Genotype	*ACCase*	*α-CT*	*β-CT*	*BC*	*SAD*	*FAD2*	*FAD3*	*FAD6*	*MCMT*	*KAS1*	*KAS2*	*KAS3*
Bronowski	-0,76	-0,77	-0,85	-0,83	-0,73	-0,84	-0,77	-0,84	-0,79	-0,63	-0,85	-0,01
Q2	-0,72	-0,78	-0,74	-0,73	-0,81	-0,62	-0,67	-0,6	-0,74	-0,8	-0,86	-0,13
Westar	-0,83	-0,91	-0,83	-0,94	-0,91	-0,86	-0,88	-0,91	-0,9	-0,89	-0,95	0,27
Genotype	***FAE1***	***HD/KACD***	***KCR2***	***FatA***	***FatB***	***DGAT2***	***LPAAT***	***AAPT1***	***Napin***	***Caleosin***	***Oleosin***	***Cruciferin***
Bronowski	-0,79	-0,84	-0,84	-0,81	-0,23	-0,73	0,39	-0,64	-0,80	-0,05	-0,15	-0,63
Q2	-0,72	-0,82	-0,8	-0,79	0,36	-0,72	0,72	0,34	-0,52	-0,52	-0,44	-0,57
Westar	-0,64	-0,91	-0,92	-0,89	0,08	-0,35	0,62	0,36	-0,56	0,04	-0,03	-0,09