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Review Article

LIPID METABOLISM IN DEVELOPING SOYBEAN SEEDS

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ABSTRACT
As because of short duration of seed development period variations of oil content and quality is known to be a phenomenon which is further enhanced by abiotic stresses. Formation of fatty acids and oil bodies are influenced by environmental factors. Though varietal improvements had been made on quality fatty acids but triglyceride improvement is of little success. In this context seed growth, metabolic changes, effects of abiotic stresses and molecular progress have been described briefly.

Keywords: *Soybean, Lipid Metabolism, Seed Development, Abiotic Stresses, Fatty Acids*

INTRODUCTION
Plant oils have been an important and integral part of our economy. They are important as feedstock, for food uses and for an abundance of industrial applications such as biodiesel fuel, lubricants, engine oils, polyesters, pesticides or inks. Vegetable oils have been identified as a potential replacement for fossil oils. Vegetable oil utilization is determined by its fatty acid composition. In soybean and other grain crops, during the seed development oil accumulation is important trait for value in food or industrial applications. Seed development is relatively short and sensitive to unfavorable abiotic conditions. These stresses can lead to a numerous undesirable qualitative as well as quantitative changes in fatty acid production. Fatty acid manipulation which targets a higher content of a specific single fatty acid for food or industrial application has gained more attention. Despite several successes in modifying the ratio of endogenous fatty acids in most domesticated oilseed crops, numerous obstacles in FA manipulation of seed maturation are yet to be overcome.

Soybean (Glycine max, Gm) is one of the most important oilseed crops, contributing to 59% of all the world oilseed production in 2014 (Soystats International, 2015) and is one of the world's most widely used and healthy edible oils. In addition to this, the industrial products and uses for soybean oil are becoming increasingly popular and diverse.

Soybean seeds contain about 21 % oil, 40 % protein, 34 % carbohydrates and 5 % ash (Burton, 1997), although cultivars with less than 18 % oil or over 50 % protein may be found (Yadav, 1996). The oil fraction is essentially composed of triacylglycerols, and the composition and distribution of fatty acids in the triacylglycerol molecules determine the oil quality, its nutritional value, flavor and physical properties, such as oxidative stability and melting point. Soybean oil, like most edible oils, is composed of palmitic (C16:0), stearic (C 18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Oleic, linoleic and linolenic acids are 18 carbon unsaturated fatty acids, containing one, two and three cis double bonds interrupted by a methylene group, respectively. The double bond positions in the acyl chain from the carboxyl terminal are 9 in C18:1, 9 and 12 (or 6, counting from the methyl terminal) in C18:2, and 9, 12 and 15 (or 3) in C18:3. Oleic acid is also referred to as monounsaturated fatty acid, while the linoleic and linolenic acids as polyunsaturated fatty acids (Yadav, 1996).

Soybean oil contains about 11 % palmitic, 4 % stearic, 24 % oleic, 54 % linoleic and 7 % linolenic acids (Kinney, 1996). The quality of the oil fraction varies considerably among these sources and it depends on the fatty acid composition and, specially, on the proportion of unsaturated fatty acids, mainly oleic, linoleic and linolenic acids (Somerville and Browse, 1991). Due to high levels of polyunsaturated fatty acids the quality of soybean oil is not ideal for industrial purposes, mainly due to its low oxidative stability. Currently, chemical hydrogenation is the industrial process used to increase the oxidative stability of the soybean oil (Hildebrand and Collins, 1998). However, this process also generates

significant amounts of trans fatty acids, which have been related with heart problems in animals and humans (Yadav, 1996). For this reason, there is a considerable interest on the genetic modification of soybean oil composition, by traditional breeding or by the use of molecular biology techniques. These modifications could avoid the production of the undesirable trans fatty acids and also produce oils with better nutritional and functional attributes (Wang and Hildebrand, 1988; Osório *et al*., 1995; Kinney, 1996).

After harvesting at the full maturity stage, R8, the dry weight of a soybean seed consists of the following elements: oil (20%), protein (40%), carbohydrates (30%), crude fiber (5%) and ash (5%) (Fehr and Caviness, 1977). Typically, soybean oil consists of approximately: 13% palmitic (C16:0), 4% stearic (C18:0), 20% oleic (C18:1), 55% linoleic (C18:2) and 8% linolenic (C18:3) acid at 13% moisture (Pham *et al*., 2010, 2011). In addition to these five major Fas, a numerous minor FAs, which may also have commercial value could be found in soybean oil such as myristic acid (C14:0), arachidic acid (C20:0), behenic acid (C22:0) or erucic acid (C22:1) (Jokic *et al*., 2013). Despite that seed development is a highly regulated process, dry matter accumulation at seed filling is affected by both genetic and environmental factors which lead to changes of oil and protein concentrations of crops. In soybean, the FA accumulation during seed maturation takes place in a short period about 4 to 6 weeks as opposed to those of other oil plants such as olive, oil palm or avocado. Soybean is therefore sensitive to stressful conditions during their short seed filling period, and this makes them susceptible to incurring permanent changes in oil content and FA profile as well as crop quality and productivity (Wang and Frei, 2011).

Oil and protein concentrations of crops are sensitive to both genetic and environmental factors. The major stress factors that have been investigated are: drought, salinity, ozone and heat. The observed effects are variable and depend on the stress type, crop species, and experimental conditions, but some typical patterns can be characterized. A decrease in the lipid concentration has been reported in almost every study involving crops grown under unfavourable conditions. By contrast, these stresses usually stimulate higher protein concentration in the harvested fraction of crops, with only a few studies showing no effect or lower protein concentration (Wang and Frei, 2011).

The proportions of oil, protein, and carbohydrate in soybean (Glycine max) seeds influence their value, and the control of their accumulation has been studied extensively. Maternally supplied substrates (Fabre and Planchon, 2000; Nakasathien *et al*., 2000; Pipolo *et al.*, 2004) and seed genotype (Wilcox, 1998; Narvel *et al*., 2000; Hernandez-Sebastia *et al*., 2005) determine the oil and protein levels in the seed. Although, the fatty acid composition of soybeans has been successfully engineered (Damude and Kinney, 2007), molecular attempts to modify the proportions of oil and protein have resulted in only a few successes for related legumes (Rolletschek *et al*., 2005a, 2007). In part this reflects the complexity of metabolic networks (Egli, 1998) and the uncertain relationship between seed composition and seed metabolism.

Lipid Metabolism

The biosynthesis of seed storage oils containing the five major FAs occurs primarily in two subcellular compartments. FA biosynthesis occurs in the plastid of cells and involves the cyclic condensation of two-carbon units in which acetyl coenzyme A (acetyl-CoA) is the precursor. When conjugated to the acyl carrier protein (ACP), the FA chain is referred to as acyl-ACP. The first committed step in the pathway is the synthesis of malonyl-CoA from acetyl-CoA and CO2 by the enzyme acetyl-CoA carboxylase (Chapman and Ohlrogge, 2012; Li-Beisson *et al.*, 2013). In the following step, some 16:0-ACP is released from the FA synthase machinery, but most molecules that are elongated to 18:0-ACP are efficiently converted to 18:1-ACP by a desaturase enzyme (Figure 1) depicts the biosynthesis of the five common FAs present in the oil of annual oil crops and the main enzyme steps involved. The first three FAs (C16:0, C18:0 and C18:1) are produced by de novo synthesis and desaturation in the plastids (Li-Beisson *et al.*, 2013). Elongation and desaturation are carried out while the FAs are attached to an acyl carrier protein (ACP). After removal of the ACP group by acyl-ACP thioesterases (FatA or FatB), the FAs are exported from the plastid and incorporated into the cytosolic acyl-CoA by the action of an acyl-CoA synthetase (ACS). 18:1 is then acylated onto the membrane lipid phosphatidylcholine (PC), mainly by the action of

the lysophosphatidylcholine acyltransferase (LPCAT) (Li-Beisson *et al.,* 2013). Further desaturations of the 18:1 to 18:2 and 18:3 are catalysed by FA desaturase 2 (FAD2) and FAD3 while the acyl substrates are acylated to PC. Storage TAGs are synthesized by the Kennedy pathway (Figure 2) in developing seeds. The enzymes involved are probably located in the endoplasmic reticulum (ER) and act by the sequential acylation of the sn-1, -2 and -3 positions of glycerol-3-phosphate, with the removal of the phosphate group occurring before the final acylation step. The distribution of acyl groups on the glycerol backbone is often non-random because of the substrate selectivity of the acyltransferases for different FAs (Li-Beisson *et al.,* 2013). In detail, TAGs can be formed through three sequential acyl-CoA-dependent acylations of the glycerol backbone beginning with sn-glycerol-3-phosphate. The acylation of sn-glycerol-3-phosphate is catalyzed by acyl-CoA:sn-glycerol-3-phosphate acyltransferase (GPAT). The second acylation is catalyzed by acyl-CoA:lyso-phosphatidic acid acyltransferase (LPAAT). After removal of the phosphate group to generate sn-1,2-diacylglycerol (sn-1,2-DAG), the final acyl-CoA-dependent acylation is catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT) to form TAG (Chapman and Ohlrogge, 2012; Li-Beisson *et al.,* 2013).

Most of the polyunsaturated fatty acids production in developing seeds, occurs via desaturation of oleic and linoleic acids catalyzed by desaturases in the smooth endoplasmatic reticulum (Ohlrogge *et al.,* 1991; Somerville and Browse, 1991). The substrate for the desaturases is PC. The fatty acids linked to PC, which may become unsaturated, can subsequently be incorporated into storage triacylglycerol (TAG) molecules. The linoleic and linolenic acid levels in the oil depend on their biosynthesis rate and availabilities (Yadav, 1996). The polyunsaturated fatty acids can become available through two distinct mechanisms: (a) reversible reaction by which cholinephosphotransferase (CPT, CDP-choline:1,2–diacylglycerolcholine phosphotransferase, EC 2.7.8.2) converts phosphatidylcholine (PC) containing polyunsaturated fatty acids into diacylglycerols (DAGs), which can be used for oil synthesis via diacylglycerol acyltransferase (DAGAT) and (b) reversible reaction by which acyl-CoA: lysophosphatidylcholine acyltransferase (LPCAT, EC 2.3.1.23) catalyses the exchange of acyl groups, generally between oleoyl-CoA and a polyunsaturated acyl group linked to position 2 of PC.

LPCAT catalyzes the reversible reaction between acyl groups of acyl-CoA cytoplasmatic pool and unsaturated acyl group linked to position 2 of PC. The reaction equilibrium is towards PC synthesis, i.e., lysophosphatidylcholine acylation, since it results in cleavage of an energy-rich thioester bond (Stymne and Stobart, 1984). The permutation between acyl groups is dictated by: (a) velocity and specificity of exchange of acyl groups between acyl-CoA and PC; (b) unsaturation rate of fatty acids in PC and (c) activity and specificity of LPCAT.

CPT catalyzes the reversible exchange between the PC pool and DAGs. Due to reaction reversibility, PC can act as a precursor of highly unsaturated molecular species of DAGs in seeds that accumulate polyunsaturated fatty acids in the oil fraction. It is a key enzyme in the metabolism of oilseeds, thus, its activity and regulation mechanisms are essential for understanding the fatty acid distribution for lipid synthesis (Vogel and Browse, 1996).

Oleoyl-CoA was a preferential substrate in the acyl-CoA pool for LPCAT activity while stearyl-CoA was completely excluded. It showed that during exchange of acyl groups, oleoyl-CoA enters position 2 of PC, liberating linoleate which is preferentially used in the acylation of position 2 of glycerol 3-phosphate. Thus, this enzyme regulates the type of acyl groups constituting the TAGs, which accumulate in developing seeds. The exchange of acyl groups between acyl-CoA and PC is a major step for regulating quality of polyunsaturated fatty acids in the acyl-CoA pool for oil synthesis in developing safflower seeds.

Up until few years ago, soybean produced more oil than any other crop plant, despite the fact that it is grown primarily for protein. Even today, soybean accounts for about 22% of the world production of oils and fats (Gunstone *et al*., 2007; http://lipidlibrary.aocs.org). Therefore, it was important to study oil accumulation in this crop. The soybean embryos used in a study have been shown to be an excellent system for studying transgenic and physiological influences on resource partitioning and have proven to be a very predictive model for seeds (Ttruong *et al*., 2013).

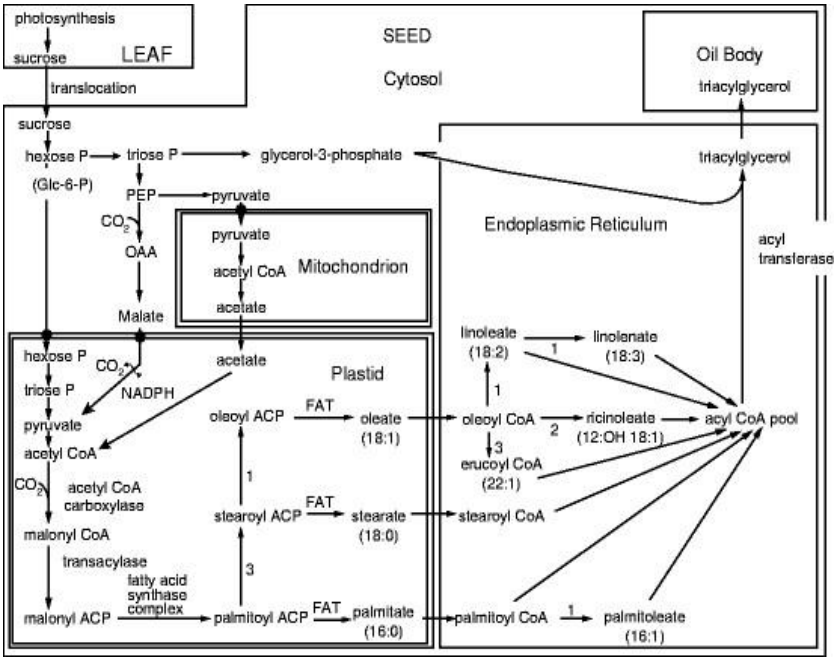

Figure 1: The Synthesis of Fatty Acids and Oils in Developing Seeds Involves the Participation of Enzymes in Several Cellular Compartments, the Cytosol, Mitochondrion, Plastid, and ER, the Latter Becoming Modified to Form the Oil Body; Numbered Stages Require the Following Enzymes: (1) FADs, (2) Hydroxylase, (3) Elongase Complex; Other Enzymes Occur in the ER of some Species to Produce Rarer Fatty Acids, e.g., by Epoxidation, Acetylenation, or Methylation. Biochemical Reactions in the ER are Intimately Associated with its Membrane, as are the Final Stages of TAG Production when Fatty Acids from the Acyl-CoA Pool are Added Sequentially by Acyltransferases to Glycerol-3-P. ER, Endoplasmic Reticulum; FAD, Fatty Acid Desaturases; Glc-6-P, Glucose-6-Phosphate; PEP, Phosphoenol Pyruvate; OAA, Oxaloacetate; ACP, Acyl Carrier Protein; CoA, Coenzyme A; FAT, Fatty Acyl Thioesterase

A detailed description of the lipid synthesis, compartment wise, can now be outlined below (Figure 2).

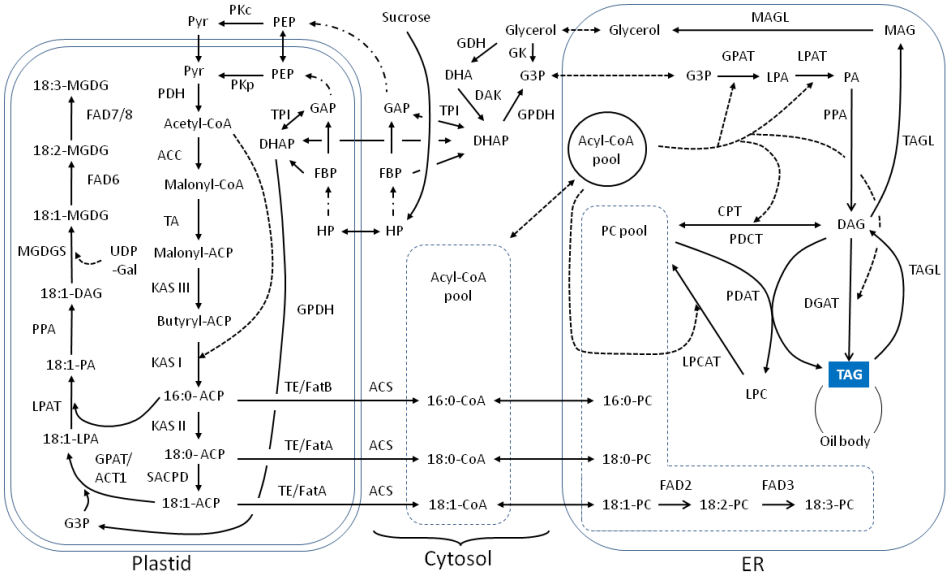

Figure 2: Lipid Metabolic Pathway

In the flux control experiments using [1-^{14}C] acetate to label fatty acids and [U-^{14}C] glycerol for incorporation into the backbone of complex lipids during assembly (Block B reactions). These two precursors are virtually specific for each type of incorporation (> 96%), as demonstrated for other plant oil tissues (Ramli *et al*., 2013; Tang *et al.,* 2012). The distribution of radioactivity into lipid classes during the linear period of incorporation (4 h) is shown that of the non-polar lipids, only TAG and diacylglycerol (DAG) were significantly labelled while phosphatidylcholine (PC) contained the bulk of radiolabel amongst the polar lipids. The latter is indicative of a cycling of carbon flux between DAG and PC as expected from the high activity of the "acyl editing" reactions in soybean (Bates *et al*., 2009). Since, the soybean cultures are non-photosynthetic and mimic developing seed metabolism of chloroplast lipids such as MGDG and, consequently, their labelling was minor. The relatively small accumulation of radioactivity in the Kennedy pathway intermediates, phosphatidate and, especially, lysophosphatidate, compared to DAG attests to the important control exerted by the final enzyme of the Kennedy pathway, DGAT, in soybean.

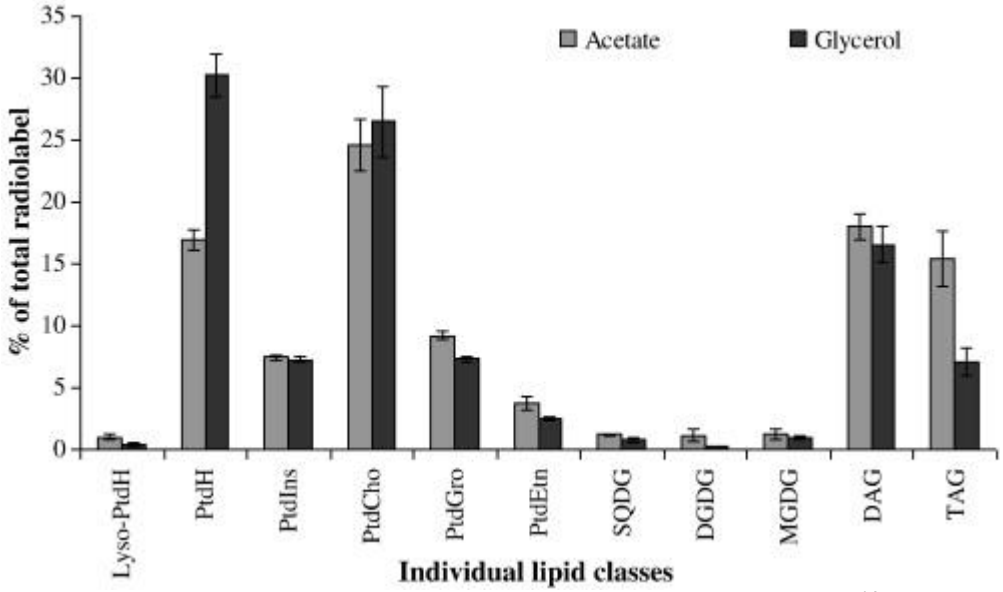

Figure 3: Labelling of Individual Polar Lipids in Soybean Cultures from [1-^{14}C] Acetate and [U-^{14}C] Glycerol
Abbreviation: PtdH, Phosphatidic Acid etc

Single manipulation used the addition of oleate. We felt that was entirely appropriate for soybean which accumulates this fatty acid as a major component of its oil (~ 25%) and uses oleate to produce the main fatty acid, linoleate (~ 50%). Calculation of changes induced by the addition of oleate gave group flux controls for Block A and Block B of 0.63 and 0.37, respectively. In fact, the data showed that oleate reduced labelling of fatty acids from [1-^{14}C] acetate and enhanced that of lipids from [U-^{14}C] glycerol. This is most simply interpreted as product inhibition (by oleate) of the fatty acid biosynthesis block while constraints caused by limitation in fatty acid supply are alleviated by oleate addition. Product inhibition may be similar to the reduction of acetyl-CoA carboxylase activity by oleoyl-ACP observed in oilseed rape seeds (Andre *et al*., 2012).

Seed Development

Much work has been done in legumes and in particular on Arabidopsis, which strongly implicates metabolite and hormone responsive pathways as key contributors (Finkelstein, 2002; Gibson, 2004; Wobus and Weber, 1999). Soybean Prestorage or morphogenesis begins with the fertilization of the first flower, follows on to the completion of embryogenesis, and ends once pod development has been

achieved. Prestorage includes GS R1-R4. Moreover, the zygote undergoes extensive cell divisions, and resembles the globular heart stage. This cell differentiation subsequently results in the tissue types required to form the root-shoot axis (Berger, 2003) and large cotyledon where oil, protein and starch reserves are localized during seed maturation. In the early stage of embryogenesis, the embryo is supported by a temporary organ called a suspensor, which provides a connection for the embryo to the surrounding nutrient-providing tissues. Measurements of endogenous hormone concentrations during morphogenesis have shown that cytokinins (CKs), abscisic acid (ABA), gibberellin (GA) and indole-3-acetic acid (IAA) are all transiently high and significantly active (Finkelstein, 2002; Audran *et al*., 2001; Bewley *et al.,* 2012). Tissue culture studies involving Phaseolus (common bean) have shown that the addition of exogenous GA can substitute.

Reproductive stages and development: R1 Beginning bloom: One flower at any node. R2 Full bloom: Open flower at one of the two uppermost nodes. R3 Beginning pod: Pod 0.5 cm (1/4 inch) long at one of the four uppermost nodes. R4 Full pod: Pod 2 cm (3/4 inch) long at one of the four uppermost nodes. R5 Beginning seed: Beans beginning to develop at one the four uppermost nodes. R6 Full seed: Pod containing a green seed that fills the pod cavity at one of the four uppermost nodes. R7 Beginning maturity: One pod anywhere with its mature color. R8 Full maturity: 95% of the pods have reached their mature color. A generalized graph showing the relative levels of water, dry weight (DW), and hormones during the stages of seed development for a detached suspensor in promoting embryonic growth, suggesting that the suspensor may normally provide GAs as well as nutrients to the developing embryo. Similarly, other studies involving a focus on either exogenous hormone addition, genetic responses or exudates from tissue culture all suggest that the roles of GAs and CKs are primarily nutritive. IAA has shown to play a major role in establishing the embryonic body-plan via effects on apical-basal polarity/pattern formation and vascular development (Souter and Lindsey, 2000; Vogler and Kuhlemeier, 2003). ABA can act to prevent seed abortion and promote embryo growth during the early embryogenesis (Cheng, 2002; Frey *et al.,* 2004). Despite the low levels of ABA generally detected during early embryogenesis, the ABA biosynthetic pathway is apparently active at this stage. In agreement, high ABA levels have been found in the pedicel/placento-chalazal complex of maize kernels (Jones and Brenner, 1987). CKs have been implicated in a number of processes including support of suspensor function, significant promotion of embryonic growth to reduce seed abortion, and enhancement of grain filling and seed yield via the promotion of cell division, especially within the cotyledons (Emery *et al.,* 2000; Zalewski *et al*., 2010). In dicots such as soybean, prestorage cell division is critical as it dictates the total number of cells that will exist within, and in doing so lays down the ground work for cell enlargement during maturation. Moreover, once the number of embryonic cells has been defined by the key contributors, the seed cotyledon will enlarge and accumulate the important constituents based upon the available number of cells, assimilate supply, and regulatory signals. Accumulation of oils/FAs and proteins occurs throughout cell enlargement and is central to cotyledon development. Inside the cells of cotyledons, oil is stored in small discrete oil bodies in the form of triacylglycerols (TAGs) (Ohlrogge and Kuo, 1984). It is believed that the more intracellular volume is available, the more space oil bodies can occupy. However, this limited available intracellular space must be shared between both protein bodies and TAGs. Thus, it is well-known that the production of TAGs and protein bodies is inversely correlated (Chung *et al*., 2003).

Following the first phase, the reserve accumulation is the next critical period in soybean seed production. Soybean seed value is determined in this phase as lipid bodies and proteins are synthesized and stored throughout development stage of R5 until the end of R6. This is one of the last two phases of embryonic development and is sometimes collectively referred to as “maturation”. At that time, seeds acquire the ability to survive desiccation and become ready to initiate growth of the next generation, independent of the maternal plant. Seed maturation begins when developing embryos cease growth by cell division; this coincides with an increase in seed ABA, a hormone which induces expression of a cyclin-dependent kinase inhibitor (ICK1) that could lead to cell cycle arrest at the G1/S transition (Finkelstein, 2002). As demonstrated in the Arabidopsis seed model, ABA, classically associated with seed maturation, is

produced first in maternal tissues and later in the embryo (Karssen *et al*., 1983). Maternal ABA, synthesized in the seed coat and translocated to the embryo, promotes its growth and prevents abortion (Frey *et al.,* 2004). A major increase in ABA levels occurs during the maturation phase corresponds to the positive regulation of a number of genes for seed reserves (Finkelstein *et al*., 2002; Audran *et al.,* 1998). The middle stage of seed development is a period of massive reserve accumulation and cell enlargement as cells fill with protein and lipid bodies (Goldberg *et al.,* 1994; Harada, 1997). Multiple seed mass and composition studies on "Williams 79" soybean seeds by Dornbos and McDonald (1986) demonstrated that stages R5 and R7 corresponded to seed filling initiation and physiological maturity, respectively. Between those phases, water content (% fresh weight - FW) declines steadily although the total amount of water per embryo is still increasing. The most abundant hormone at this stage is ABA, which reaches peak levels during the period of maximal seed weight gain. In the late-developmental stage, ABA induces dormancy and inhibits germination in the matured seeds by upregulating its own levels and down-regulating GA synthesis (Gazzarrini *et al.,* 2004; Nambara *et al.,* 2000; Nambara and Marion-Poll, 2005; Wilkinson *et al.,* 2010). During the final phase of seed development, embryos become desiccation tolerant, lose water, and become relatively metabolically inactive. A decrease in the ABA level during the desiccation phase is also expected to result from decreased ABA synthesis (Audran *et al.,* 1998).

Lipids accumulate as triacylglycerides that are found in oil storage bodies surrounded by the protein oleasin or occasionally as oil droplets in the cytosol. Predominant fatty acids in triacylglycerides are palmitate (16:0), stearate (18:1), linoleate (18:2) and linolenate (18:3). In soybean, cell division in the seed is completed at an early stage of development (20-25 DAF) while the embryo is still quite small (Goldberg *et al.,* 1994). The major increase in seed size which occurs between 25 to 60 days after flowering (DAF) is brought about through enlargement of pre-existing cells. The majority of oil, protein and carbohydrate synthesis and storage occur during this period by simultaneous partitioning of the photosynthates among those three major reserves (Ohlrogge and Kuo, 1984). It was reported that by 26 DAF starch, lipid and protein bodies were present in the cytosol of soybean cotyledons. As the seed developed, the cells of the cotyledons became packed with the lipids, protein and starch bodies. However, the starch bodies disappeared just prior to maturation. Developing soybean seeds contained 5% oil at 25 DAF. The oil percentage increased slightly to around 20% by 40 DAF and remained essentially constant during the remaining period of seed development.

Abiotic Streses

Consequences of exposure to abiotic stresses include various physiological changes in crop plants, such as: alterations in the photosynthetic gas exchange and assimilate translocation (Morgan *et al.,* 2004), altered water uptake and evapotranspiration, effects on nutrient uptake and translocation (Sanchez-Rodriguez *et al*., 2011), antioxidant reactions (Apel and Hirt, 2004), programmed cell death (Kangasjari *et al*., 2005), and altered gene expression and enzyme activity. These exposures are likely to have numerous effects on the chemical composition of crops and, consequently, the quality of agricultural products.

Oil and protein concentrations of crops are sensitive to both genetic and environmental factors. The major stress factors that have been investigated are: drought, salinity, ozone and heat. The observed effects are variable and depend on the stress type, crop species, and experimental conditions, but some typical patterns can be characterized. A decrease in the lipid concentration has been reported in almost every study involving crops grown under unfavorable conditions. By contrast, these stresses usually stimulate higher protein concentration in the harvested fraction of crops, with only a few studies showing no effect or lower protein concentration (Wang and Frei, 2011).

The FA profile of soybean oil is a fundamental quality attribute. Genotype is the main determinant of FA composition, but environmental factors such as climate conditions have been linked to variations in oil quality and yield. The majority of the studies reported decreases in the lipid concentration when crops were grown under stressful conditions. Liu *et al.,* (2013) indicated UV-B radiation decreased total biomass and seed yield per plant. These losses were mainly attributed to the change of pod number per plant and seed size. In a report on seed development gene expression, Fatihi *et al.,* (2013) indicated that a

reduced seed size is primarily associated with reduced TAG content in the embryos of Arabidopsis. In case of the drought stressed crops, almost all studies reported a decrease in the lipid concentration of the harvested products compared to that of the sufficiently watered plants. It is important to note that, in seeds of annual crops, such as soybean and sunflower, oil accumulates at a high rate during a short period of time (between 30 and 45 days). On the other hand, in olive fruit – similar to those of oil palm and avocado – oil accumulates principally in the mesocarp at low rate, over a long period (100 to 140 days). Thus, it is possible that greater opportunities for recovery to normal values after a high-temperature event might exist in olives. A similar trend towards declining oil concentration was seen under salinity and heat stress, for which only a few studies reported increases or no effects on lipid concentrations. In contrast, ozone stress seemed to be an exception, as the available studies reported either no effect, or even an increase in lipid concentration (Wang and Frei, 2011). Temperature effects on seed growth (Wardlaw *et al.*, 2002) are well documented in annual crops, including oil-seed species. Seed oil concentration decreased in response to high temperatures during the period of oil synthesis (Roudanini *et al.*, 2003). Processes indirectly linked to oil synthesis such as photosynthesis or respiration could also be simultaneously modulating the oil concentration. Photosynthesis of both leaves and fruit are likely negatively affected by exposure to high temperatures. Increases in leaf temperature above 32o C in growth chambers resulted in a decline in photosynthetic rate (Nambara and Marion-Poll, 2005). The high temperature stress decreases the duration of seed filling period via accelerated leaf senescence, and consequently oil accumulation is stopped before fulfilling seed oil capacity, when the seed is ready for desiccation. The environmental stresses not only change the oil contents of oil crops but also affect oil composition. A general trend indicated an increase in the saturation level of the oil fraction due to various abiotic stresses has been reported (Wang and Frei, 2011). The proportion of polyunsaturated FAs (PUFA) in soybean oil dropped considerably under heat stress (Dornbos and Mullen, 1992). The same pattern was observed under drought stress in the oil fractions of sunflower (Flagella *et al*., 2002), groundnut (Dwivedi *et al*., 1996) and under salt stress in sunflower (Di Caterina *et al.*, 2007), cotton (Ahmad *et al.*, 2007), sage (Taarit *et al.*, 2010), and coriander (Neffati and Marzouk, 2008). These decreases in PUFA (especially linoleic acid, C18:2) were consistently accompanied by increases in the proportion of oleic acid (C18:1) (Osório *et al.*, 1995; Pham *et al.*, 2010; 2011). FA composition varies depending on the timing of the high temperature event. For example, in sunflower (Roudanini *et al.*, 2003), when high temperature was applied during the final portion of oil accumulation phase, the proportion of C18:1 increased while that of C18:2 decreased. In soybean, as well as in sunflower, lower latitudes leading to the increase of temperature have been associated with high C18:1 oils (Taarit *et al.*, 2010). A high C18:1 concentration in sunflower was shown to be associated with increased temperature also when heat was applied to the plants as an experimental factor (Maestri *et al.*, 1998). In addition, it has been demonstrated that the differences in night temperatures are better indicator of the changes in FA composition than daily average temperatures in annual oil-seed crops (sunflower: (Izquierdo *et al.*, 2006; Pereyra-Irujo and Aguirrezbal, 2007); soybean: Gibon and Mullen, 1996). The observed changes in FA composition are believed to be a result of the activity of enzymes involved in lipid synthesis and conversion (Bouchereau *et al.*, 1996).

FA synthesis in oil seeds starts its early steps in the plastids and then C18:1 as the main product of plastidal lipid synthesis is exported to the cytosol. The enzyme activity in which oleate desaturase (OD) moderates the cytosolic desaturation of C18:1 to form PUFA (i.e. C18:2) is believed as an explanation for shifts in C18:1/C18:2 ratio in several crops under various types of stress, including: salinity, drought, and heat (Hernandez *et al.*, 2009). A numerous studies have demonstrated the temperature dependence of this enzyme (Esteban *et al.*, 2004). In sunflower, the highest OD activity was observe at 20o C and its activity dropped considerably at higher temperatures. In contrast in safflower OD was more heat stable and maintained its full activity up to 30o C. Two factors including (i) the heat stability of the enzyme, and (ii) the effects of temperature on the internal oxygen concentration of seeds, which is a key regulator of OD activity (Rolletschek *et al.*, 2007) have been proposed in order to explain this temperature-dependent decline in enzyme activity. Besides enzymatic desaturation of FAs, transport of plastidal FAs to the

cytosol is potentially affected by environmental stresses (Flagella *et al.,* 2002). It is generally considered that the common stress factors including drought, heat and tropospheric ozone result in an increased protein concentration in wheat grains and soybean seeds.

Molecular Aspects

Soybean is an important economic crop and provides oil and proteins for human and animals. Increasing the FA (fatty acid) contents and improving the oil quality are closely related to our daily life. So far, numerous efforts have been made to meet the needs of human food and industry production by changing the fatty acid content in seeds (MacKenzie, 1995). However, the extracted fatty acids from the existing oil plants are far from enough, and hence traditional breeding methods and transgenic approaches manipulating fatty acid biosynthesis pathway are used to increase oil content in soybean. In plants, the pathways for lipid biosynthesis and oil accumulation had been studied and the genes related to fatty acid biosynthesis have been characterized. There are several key genes in the process of fatty acid biosynthesis. One is *ACCase* encoding acetyl CoA carboxylase in the first key step of fatty acid biosynthesis, and malonyl-CoA is produced (Ohlrogge and Jaworski, 1997). The second one is *KASIII*, which encodes 3-ketoacyl-ACP synthase III to catalyze the formation of a 4-carbon product (Clough *et al.,* 1992). The carbon number of fatty acid is increased by two in acyl chain, and elongation of the acyl chain from six to 16 carbon molecules is catalyzed by an enzyme named KAS1 (Shimakata and Stumpf, 1982). Without KAS1, FA contents would be sharply reduced, and plant growth and development would be strongly affected (Wu and Xue, 2010). The genes related to FA biosynthesis such as *Pl-PKβ1 (pyruvate kinase*), *PDHE1*α (*pyruvate dehydrogenase E1 alpha subunit*), *BCCP2 (acetyl-CoA carboxylase*), *ACP1 (acyl carrier protein*), and *KAS1* have similar expression pattern with *WRI1 (WRINKLED1*), and the FA biosynthesis-related genes were up-regulated in the *WRI1*-overexpressing plants (Ruuska *et al.,* 2002). WRI1 is an AP2-type transcription factor (TF) with two AP2 DNA-binding domains (Cernac and Benning, 2004), and it appears to be a master regulator of *FAS* (fatty acid synthesis) genes in expression level. There is a specific sequence motif AW-box in the promoter regions of the *FAS* genes, and WRI1 binds to this motif in *Arabidopsis* (Maeo *et al.,* 2009). Over expression of *WRI1* enhanced the oil content in transgenic *Arabidopsis* (Liu *et al.,* 2010) and maize (Pouvreau *et al.,* 2011). In castor bean, there are WRI1 binding consensus sites in the promoter region of *RcBCCP2* and *RcKAS1*, and RcWRI1 possibly binds to these sites to play a pivotal role in fatty acid biosynthesis (Tajima *et al.,* 2013). Over expression of a single transcription factor gene *WRI* can increase the seed oil contents while manipulating a single fatty acid biosynthesis gene had only very limited effect on the oil content (Dehesh *et al.,* 2001).

Transcription factors can regulate expression of genes involved in a wide range of plant processes and have a cascade amplification effect (Riechmann and Meyerowitz, 1998). Therefore, transcription factors are the promising targets to improve oil contents in plants. Several candidate transcription factors involved in fatty acid biosynthesis and accumulation have been characterized, including *WRI1* (Baud *et al*., 2007) and *LEC2 (leafy cotyledon2*) (Santos-Mendoza *et al.*, 2008) in *Arabidopsis*. *WRI1* is a target of LEC2. The transcription factors regulating fatty acid contents have been identified from soybean in our lab. Two Dof-type (DNA-binding one zinc finger) genes *GmDof4* and *GmDof11* were found to increase the content of total fatty acids in their transgenic *Arabidopsis* seeds by activating the ACCase and ACSL (long-chain-acyl CoA synthetase) genes respectively (Wang *et al*., 2007). Through microarray analysis, a MYB-type gene *GmMYB73* was identified and this gene can suppress expression of *GL2 (GLABRA 2*), a negative regulator of oil accumulations (Liu *et al.*, 2014). Over expression of *GmMYB73* enhanced lipid contents in seeds of transgenic *Arabidopsis* through release of GL2-inhibited *PLDα1* (phospholipase D) expression (Shi *et al.*, 2012). Over expression of *GmbZIP123* also enhanced lipid content and oil accumulation by regulating two sucrose transporter genes *SUC1* and *SUC5*, and three cell-wall invertase genes *cwINV1*, *cwINV3* and *cwINV6* (Song *et al.,* 2013).

Recently, through RNA-seq analysis, gene co-expression networks have been identified for soybean seed trait regulation and *GmNFYA (nuclear transcription factor Y alpha*) is found to enhance seed oil

contents in transgenic *Arabidopsis* plants (Lu *et al.*, 2016). A DREB-type (dehydration-responsive element-binding) transcription factor gene *GmDREBL,* was cloned and found to increase the seed lipid content in the transgenic plants. GmDREBL directly activates the expression of *WRI1* to promote fatty acid accumulation.

Transcriptomic analyses of RHA1 grown under conditions of N-limitation and N-excess revealed 1,826 dysregulated genes. Genes whose transcripts were more abundant under N-limitation included those involved in ammonium assimilation, benzoate catabolism, fatty acid biosynthesis and the methylmalonyl-CoA pathway. Of the 16 *atf* genes potentially encoding diacylglycerol *O* acyltransferases, *atf8* transcripts were the most abundant during N-limitation (~50-fold more abundant than during N-excess). Consistent with Atf8 being a physiological determinant of TAG accumulation, a Δ*atf8* mutant accumulated 70% less TAG than wild-type RHA1 while *atf8* over expression increased TAG accumulation 20%.

Triacylglycerol (TAG) is the main storage lipid in plant seeds and the major form of plant oil used for food and increasingly, for industrial and biofuel applications. Several transcription factors including FUSCA3 (At3, g26790, FUS3) are associated with embryo maturation and oil biosynthesis in seeds.

Conclusion

Precise knowledge of soybean seed development till maturity is required. Unsaturated fatty acid enrichment is essential for quality and human health. Varietal improvement and good practices to be made available for further examinations. Genetic efficiency in producing of high level of triglycerides to be found out in near future.

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