Advances in Crop Science and Technology

Sticklen, Adv Crop Sci Tech 2013, 1:2
http://dx.doi.org/10.4172/2329-8863.1000e104

Editorial Open Access

An Advance Crop Science and Technology: Potentials for Producing the High-Value High-Calorie Triacyglycerols Commodity in Crop Vegetative Wastes

Mariam Sticklen*

Michigan State University, East Lansing, MI 48824, USA

Crops that contain high levels of lipids in their fruits or seeds are considered oil crops. According to Hildebrand [1], the lipids content per dry weight of oil palm fruit and soybean seeds are 75% and 60%, respectively. Crops that are not considered oil crops produce much lower amount of lipids in their tissues. For example, maize (*Zea mays*) is not considered an oil crop because its seeds (commercial hybrid grown in the Corn Belt) only contain 3-5% lipids.

About 5-6% per dry weigh of crop lipids is in forms of phospholipids and oleosins, and 94-95% per dry weight in form of Triacyglycerols (TAG). At present, the crops TAG is generating $25 billion/year, being considered a very valuable commodity [1].

A few groups of scientists have recently tried to increase the TAG content of the vegetative tissues of non-oil model plants. The idea is to potentially produce TAG in abundantly available crop vegetative residues such as corn stover, and rice or wheat straws. In fact, certain crop residues such as rice straws and sugarcane stems and leaves are presently burned to waste in the field creating environmental contaminations and health problems such as asthma [2]. It is expected that TAG commodity could be produced as a value-added product in relatively large quantity in the crops wastes such as in ice straws and husks and in maize stover for extractions and use.

The TAG produced from crop residues is a value-added product, and therefore farmers can sell their crop leftover such as corn residues. Furthermore, after TAG is extracted from transgenic crop leftover, the leftover biomass can be used as "press cake" for "biogas" to replace the non-renewable and toxic "natural gas".

Recent advances in understanding the crop TAG biosynthesis, accumulation and breakdown along with up-regulating of some of the genes involved in TAG biosynthesis and accumulation, and down-regulating of the genes involved in TAG breakdown are most recent technologies of this decade.

The Diacylglycerol Acetyltransferase (DGAT) enzyme is the unique and the last enzyme involved in TAG biosynthesis. Overexpression of DGAT1 and DGAT2 in plants has proven to increase TAG content in model plants without any side effects. Also, several transcription factors including LEC1, LEC2 (leafy cotyledon 1 and 2) and WR (wrinkled) are known to be associated with TAG biosynthesis, and therefore their overexpression can increase TAG production in crop tissues.

ATAG is usually synthesized in endoplasmic reticulum (ER). However, Kaup et al. [3] reported that TAG in senescing leaves is synthesized in the chloroplasts and their elongation takes place in ER.

Zhang et al. [4] reported that in *Arabidopsis*, DGAT1 is in charge of TAG biosynthesis, and DGAT2 is involved in shifting of the fatty acids. Therefore, it might be possible to increase the level of TAG in plant via the overexpression of the DGAT1, and to shift the composition of TAG in transgenic plants by overexpressing of DGAT2. Andrianov et al. [5] reported that the overexpression of *Arabidopsis* DGAT2 in tobacco green tissues resulted in a major shift in fatty acid compositions, and the report by Zhang et al. [4] indicates that by overexpressing of the DGAT2, percentage of plant linolenate was reduced from 67% in the wild-type control to 35% in transgenic plants. The second shift was a dramatic increase in total extracted FA from 1.5% to about 25%. Also, TAG linolenate was reduced from 20% to 12% and oleate of TAG fraction was increased from 18% to 44% in transgenic lines.

Sabudak [6] studied the fatty acid composition of domesticated maize leaf oil and seed oil, and reported that the caproic, behenic, and lignoseric acids were much higher in the maize leaf oils but very little in the maize seed oils. He also reported that the carbons: cis-double bonds in maize leaf oil are in form of unsaturated fatty acids of 6:0, 12:0, 14:0, 16:0, 18:0, 18:2, 18:3, 20:0, and 22:0. Maize genome does not contain DGAT2, but it contains the DGAT1 in form of acyl-CoA: diacylglycerol acyltransferase (DGAT1-2; EC: 2.3.2.20; GenBank: EU039830.1). It has been reported [7] that the ectopic expression of the high-oil DGAT1-2 allele increases oil content by 41% and the oleic-acid content by up to 107%.

An interesting research by Slocombe et al. [8] resulted by 10 to 20-fold increase in the level of TAG in *Arabidopsis* leaves via blocking of TAG hydrolysis (breakdown). There are a few enzymes associated with TAG hydrolysis in plants. For example, Eastmond [9] reports that the adipose triglyceride lipase like (SDP1L) enzyme found in double and triple mutants of *Arabidopsis* showed to be 95% responsible for all of the residual TAG hydrolysis. Furthermore, in-vitro assays confirmed that SDP1L was able to hydrolyze TAG, and such hydrolysis was not essential for seed germination or seedling establishment [10]. Therefore, it might be possible to increase the level of TAG accumulation in certain crops by blocking of its also reported that the generation of a double mutant in fatty acid breakdown and DGAT1 resulted in a severe vegetative growth phenotype proposing that channeling of fatty acids to TAG in leaves is mostly done by the acyltransferase. When LEC2 was ectopically expressed during senescence in the fatty acid breakdown mutant COMATOSE (cts2), it resulted in accumulation of seed oil type species of TAG in senescing tissue, suggesting that the recycled membrane fatty acids during senescence can be re-directed to TAG or by blocking of the fatty acid breakdown.

Several transcription factors have been found to be associated with TAG biosynthesis. These include, but are not limited to LEC and

***Corresponding author:** Mariam Sticklen, Michigan State University, East Lansing, MI 48824, USA, E-mail: stickle1@msu.edu

Received May 28, 2013; **Accepted** May 29, 2013; **Published** June 06, 2013

Citation: Sticklen M (2013) An Advance Crop Science and Technology: Potentials for Producing the High-Value High-Calorie Triacyglycerols Commodity in Crop Vegetative Wastes. Adv Crop Sci Tech 1: e104. doi:10.4172/2329-8863.1000e104

WR genes. For example, the overexpression of WR1 is documented to increase TAG accumulation in plants [11]. The overexpression of transcription factors in plants may or may not have down sides. For example, the overexpression of maize LEC1 in soybean seed increased seed oil by up to 48%, but it reduced plant growth. In contrast, the overexpression of maize WRI1 in maize seed resulted in an increase in maize seed oil without any side effect on its plant growth. This might be because the overexpression of WRI1 does not increase DGAT1 expression, but the overexpression of LEC1 increased the DGAT1 expression [1]. The over expression of the LEC2 has resulted in accumulation of TAG in *Arabidopsis* leaf tissues [12,13]. It is also reported that LEC2 induces other transcription factors including FUS3 resulting in an increase in TAG biosynthesis in plant leaves. The overexpression of LEC2 transcription factor also has caused lipid accumulation in crop leaf tissues [14].

It is possible to increase TAG in crop vegetative tissues. For example, constitutive expression of LEC1 or LEC2 in *Arabidopsis* induced "seed-like structure" in plant green vegetative tissues, and transgenic *Arabidopsis* expressing LEC2 in their leaves showed transcripts of seed-specific mRNAs along with extra oil accumulation [1]. In a report by Andrianov et al. [5], the overexpression of *Arabidopsis* DGAT and LEC2 genes regulated by ribulose-biphosphate carboxylase small subunit (rbcS) promoter in tobacco increased the accumulation of lipids in tobacco plant green tissues, and also shifted the composition of lipids in its green tissues, increasing the leaf TAG by 20-fold in tobacco leaf (i.e., 2-fold extractable fatty acids up to 5.8-6% of dry matter). Such TAG increase was reported to be due to a shift in extractable fatty acid composition, in favor of biodiesel quality oil. The same group expressed the *Arabidopsi*s LEC2 in tobacco under an ethanol triggered inducible promoter [15] and found 6.8% per dry weight of total extracted fatty acids.

Another interesting report [16] documented that the disruption of the homolog of human CGI-58 gene (i.e., the human defective gene in the neutral lipid storage Chanarin-Dorfman syndrome disease resulting in extracellular lipid droplets accumulation in skin and blood cells) in mutant *Arabidopsis thaliana* resulted in the accumulation of neutral lipid droplets containing a 10-fold increased level of TAG with leaf specific fatty acids in mature leaves, while the oil storage of seeds of the same mutants did not show any increase in lipid levels.

The fundamental study options for increasing the TAG content of crop vegetative tissues might include, but is not limited to the overexpression of any of the above enzymes. The genes used can be heterologous of homologous to the host crop, and the promoters used could include the green-specific rbcS promoter. One can also use an inducible promoter to only produce high concentrations of TAG after plant growth is completed and just prior to or at the senescence.

Although might not be necessary, targeting of the DGAT and some of the other above transgenes sub-cellular compartments such as to ER might help in the processes of TAG biosynthesis and accumulation.

If TAG is used as biodiesel, it has the advantage of being non-toxic and biodegradable, while diesel is toxic and non-biodegradable. It might e possible to produce crop vegetative TAG with fatty acids similar to vegetable oil with higher oleate contents. Therefore if the new TAG produced in crop wastes can have the fatty acids of vegetable oil, such TAG might bring higher revenue and could increase the food calorie contents i.e. important to the people of the developing nations. The new crop with high TSG content can also be used as silage to livestock for a faster livestock growth. If successful, the potential commercial application of producing TAG in crop vegetative tissues will be enormous. The author's laboratory is working on producing relatively high percentage of the TAG commodity in cereal crop vegetative tissues for its use as biofuel as well as for food and feed energy.

References

1. Ashihara H, Crozier A, Komamine A (2011) Plant Metabolism and Biotechnology. (1stedn). John Wiley and Sons.
2. Oraby H, Venkatesh B, Dale B, Ahmad R, Ransom C, et al. (2007) Enhanced conversion of plant biomass into glucose using transgenic rice-produced endoglucanase for cellulosic ethanol. Transgenic Res 16: 739-749.
3. Kaup MT, Froese CD, Thompson JE (2002) A Role for Diacylglycerol Acyltransferase during Leaf Senescence. Plant Physiol 129: 1616-1626.
4. Zhang M, Fan J, Taylor DC, Ohlrogge JB (2009) DGAT1 and PDAT1 Acyltransferases have overlapping functions in *Arabidopsis* triacylglycerol biosynthesis and are essential for normal pollen and seed development. Plant Cell 21: 3885-3901.
5. Andrianov V, Borisjuk N, Pogrebnyak N, Brinker A, Dixon J, et al. (2010) Tobacco as a production platform for biofuel: overexpression of Arabidopsis DGAT and LEC2 genes increases accumulation and shifts the composition of lipids in green biomass. Plant Biotechnol 8: 277-287.
6. Sabudak T (2005) Fatty acid composition of seed and leaf oils of pumpkin, walnut, almond, maize, sunflower and melon. Chem Nat Compounds 43: 465-467.
7. Zheng P, Allen WB, Roesler K, Williams ME, Zhang S, et al. (2008) A phenylalanine in DGAT is a key determinant of oil content and composition in maize. Nat Genet 40: 367-372.
8. Slocombe SP, Cornah J, Pinfield-Wells H, Soady K, Zhang Q, et al. (2009) Oil accumulation in leaves directed by modification of fatty acid breakdown and lipid synthesis pathways. Plant Biotechnol J 7: 694-703.
9. Eastmond PJ (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating *Arabidopsis* seeds. Plant Cell 18**:** 665-675.
10. Eastmond PJ (2011) Triacylglycerol breakdown in *Arabidopsis* seedlings. Plant Physiology Preview.
11. Cernac A, Benning C (2004) WRINKLED1 encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in *Arabidopsis*. Plant J 40: 575–585.
12. Baud S, Lepiniec L (2009) Regulation of *de novo* fatty acid synthesis in maturing oilseeds of Arabidopsis. Plant Physiol Biochem 47: 448-455.
13. Baud S, Wuillème S, To A, Rochat C, Lepiniec L (2009) Role of WRINKLED1 in the transcriptional regulation of glycolytic and fatty acid biosynthetic genes in Arabidopsis. Plant 60: 933-947.
14. Shen B, Allen WB, Zheng P, Li C, Glassman K, et al. (2010) Expression of *ZmLEC1* and *ZmWRI1* increases seed oil production in maize. Plant Physiol 153**:** 980-987.
15. Caddick MX, Greenland AJ, Jepson I, Krause KP, Qu N, et al. (1998) An ethanol inducible gene switch for plants used to manipulate carbon metabolism. Nature Biotechnol 16: 177-180.
16. James CN, Horn PJ, Case CR, Gidda SK, Zhang D, et al. (2010) Disruption of the Arabidopsis CGI-58 homologue produces Chanarin-Dorfman-like lipid droplet accumulation in plants. Proc Natl Acad Sci U S A. 107: 17833-17838.

Citation: Sticklen M (2013) An Advance Crop Science and Technology: Potentials for Producing the High-Value High-Calorie Triacyglycerols Commodity in Crop Vegetative Wastes. Adv Crop Sci Tech 1: e104. doi:10.4172/2329-8863.1000e104