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Genetic Enhancement of Soybean Oil for Industrial Uses: Prospects and Challenges

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Soybean oil is a renewable raw material for a wide variety of industrial products, including inks, plasticizers, and paints. The range of potential applications can be expanded by genetic improvements that alter the fatty acid composition of soybean oil. Technical challenges and costs associated with the development and production of these new oil traits are the major hurdles that currently limit the commercial potential of this research.

Key words: soybean, oil modification, fatty acid, genetic engineering.

Introduction

More than 600 million pounds of the soybean oil produced annually in the United States are used for nonedible applications, including the production of industrial materials (American Soybean Association, 2001). More than half of this use of soybean oil falls in the category of fatty acids, soaps, and feed. The remainder of the nonedible soybean market is used for the manufacture of inks, paints, varnishes, resins, plastics, and biodiesel. Although the use of soybean oil for industrial purposes is economically significant, it represents only 4% of the total consumption of US soybean oil. By contrast, more than 13 billion pounds of US soybean oil are used annually in foods and food processing. (For more information, see SoyStats 2001 at http://www.unitedsoybean.org/soystats2001.)

Improved Industrial Properties by Genetic Enrichment of Specific Fatty Acid Components of Soybean Oil

Considerable potential exists for an expanded use of soybean oil as a renewable chemical feedstock. However, the physical and chemical properties of conventional soybean oil limit its use for many industrial applications. Soybean oil is a complex mixture of five fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids) that have vastly differing melting points, oxidative stabilities, and chemical functionalities. In this regard, genetic engineering approaches can be applied to enrich the content of soybean oil for a particular fatty acid or class of fatty acids. The most notable example, developed by researchers at DuPont, is the transgenic production of soybean seeds with oleic acid content of approximately 80% of the total oil (Kinney, 1997). Conventional soybean oil, by comparison, contains oleic acid at levels of 25% of the total oil. The high oleic acid trait was obtained by down-regulating the expression of *FAD2* genes that encode the enzyme that converts the monounsaturated oleic acid to the polyunsaturated linoleic acid. More recently, soybean seeds with oleic acid contents of greater than 85% of the total oil have been generated by down-regulating the expression of *FAD2* genes together with genes that control the production of palmitic acid (i.e., *FATB* genes; Buhr et al., 2002). In addition to the enhanced oleic content, these oils contain relatively low levels of the polyunsaturated fatty acids linoleic and linolenic acids. For example, polyunsaturated fatty acids account for 3-5% of the total oil in genetically engineered seeds, with oleic acid content approaching 90% of the total oil. Polyunsaturated fatty acids, in contrast, typically compose 60-65% of conventional soybean oil, most of which is in the form of linoleic acid. High-oleic oils with elevated oleic acid content are generally considered to be healthier oils than conventional soybean oil, which is an omega-6 or linoleic acid-rich oil. From an industrial perspective, the high content of oleic acid and low content of polyunsaturated fatty acids result in an oil that has high oxidative stability. In addition, soybean oil is naturally rich in the vitamin E antioxidant gamma-tocopherol, which also contributes to the oxidative stability of high oleic acid soybean oil. High oxidative stability is a critical property for lubricants. Currently, the DuPont high-oleic soybeans are used commercially for biodegradable lubricant formulations produced by Environmental Lubricants Manufacturing, Inc. of Waverly, Iowa. High-oleic soybeans are the only soybeans with genetically modified oil compositions that are now commercially used for industrial applications.

In contrast to the oxidative stability of high oleic acid oils, genetic engineering can also be used to produce soybean oil with high levels of linolenic acid, a polyunsaturated fatty acid with low oxidative stability. On a research scale, soybean seeds with linolenic acid content in excess of 50% of the total oil have been generated by increasing the expression of the *FAD3* gene, which encodes the enzyme that converts linoleic acid to linolenic acid (Cahoon, 2001). The linolenic acid content of conventional soybean oil, in contrast, is approximately 10% of the total oil. These genetically modified soybean lines have linolenic acid levels that are comparable to linseed oil. The low oxidative stability associated with high linolenic acid oil is a desirable property for drying oils that are used in coating applications, such as paints, inks, and varnishes.

The high oleic acid and high linolenic acid oils described above exemplify the ability to enrich soybean seeds for specific fatty acids using standard genetic engineering techniques. These types of oils may also complement ongoing research aimed at improving the industrial value of soybean oil through "green chemistry." Significant progress has been made in the development of chemical methods for enhancing the functionality of soybean oil. One of the most notable examples of this research has been the discovery of methods for producing polyols from soybean oil (Crandall, 2002). Soybean-derived polyols can be used in a number of industrial applications, including the production of polyurethanes. These methods typically require chemically epoxidized soybean oil as the starting material. By using soybean oils with high levels of specific unsaturated fatty acids (e.g., oleic acid), epoxidized soybean oil with more homogeneous compositions can be obtained. This may ultimately lead to the production of higher quality industrial chemicals with more uniform properties from soybean oil.

Improved Industrial Properties by Transgenic Production of Novel Fatty Acids in Soybean Oil

Genetic engineering also allows for genes to be transferred across plant species. In this regard, many plant species produce high levels of novel fatty acids with potential industrial value in their seeds. These plants typically have limited agronomic potential. Research efforts have been directed towards the identification of genes associated with the synthesis of these novel fatty acids with the eventual goal of transferring these genes to existing oilseed crops such as soybean. Examples of this research include the identification of the gene from pot marigold (*Calendula officinalis*) encoding an enzyme that introduces conjugated double bonds into polyunsaturated fatty acids. Expression of this gene in soybean seeds resulted in the accumulation of calendic acid, a novel conjugated polyunsaturated fatty acid, to amounts of 20-25% of the total seed oil (Cahoon, Ripp, Hall, & Kinney, 2001). Oils rich in conjugated fatty acids (such as calendic acid) have superior properties as drying oils in coating applications. Such oils, in fact, are more oxidatively unstable than linolenic acid-rich oils. Progress has also been made in the transfer of the biosynthetic pathway for 20-carbon monounsaturated fatty acids from seeds of meadowfoam (*Limnanthes alba*) to soybean (Cahoon et al., 2000). Oils that contain high levels of this fatty acid can potentially be used as high-value lubricants and as precursors of industrial compounds such as estolides and delta-lactones (Burg & Kleiman, 1991; Erhan, Kleiman, & Isbell, 1993). Research has also been directed towards the transgenic production of industrially valuable epoxy and hydroxylated fatty acids in the seed oil of soybean (Cahoon, Ripp, Hall, & McGonigle, 2002). The current challenge of this line of research is to obtain high levels of novel fatty acid accumulation in soybeans without negatively affecting the agronomic quality of the transgenic seed. In this regard, amounts of novel fatty acids produced in soybean seeds, to date, have been significantly less than amounts present in seeds of the nonagronomic species from which fatty acid modification genes have been isolated. For example, the calendic acid content of the seed oil of pot marigold is 55%, or two to three times more than levels accumulated in transgenic soybean seeds.

A more long-term prospect is the use of enzyme crystallographic data to design fatty acid modifying activities that are not found in nature. Through the use of this technology, it should be possible to generate fatty acid modifying enzymes that introduce double bonds or functional groups (e.g., hydroxyl or epoxy groups) in novel positions within fatty acid chains. The chemical processes involved in the production of many specialty industrial compounds require fatty acids with very specific structures. For example, the production of 12-carbon nylon monomers can be achieved using a monounsaturated fatty acid with a double bond at the twelfth carbon atom. Such a fatty acid does not exist in nature, but an enzyme could be potentially designed to produce this fatty acid based on sufficient structural information from known fatty acid desaturation enzymes. Limited progress has been made to date in the rational design of fatty acid modifying enzymes for the production of novel seed oils (Cahoon & Shanklin, 2000).

Outlook

From a research perspective, significant progress has been made in the genetic improvement of soybean oil for industrial uses, and the prospects for continued progress are considerable. In addition, it should be emphasized that it is extremely difficult or impossible to obtain the traits described above through conventional plant breeding rather than through genetic engineering. In decades of plant breeding efforts, for example, soybean oil with 50% linolenic acid content has never been achieved. It is also not possible to transfer genes to soybean (as described above) for the production of novel fatty acids by conventional breeding.

Despite the technical successes to date, the wide use of genetic engineering to produce soybean oils with enhanced industrial properties faces an uncertain future due to costs associated with regulatory approval and identity preservation. It has been estimated that the costs for generating the necessary data and information for regulatory agencies is in the range of at least $1 million to $10 million for a given transgenic trait (Parrot & Clemente, in press). In addition, transgenic soybeans with modified oil composition are not likely to be grown on a commodity scale and will therefore require identity preservation, which will increase the production costs. As a result of these expenses, the commercial promise of genetic engineering for industrial soybean oil improvement may only be realized for oil traits with high value.

References

American Soybean Association. (2001). *SoyStats 2001*. Available on the World Wide Web: http://www.unitedsoybean.org/soystats2001.

Buhr, T., Sato, S., Ebrahim, F., Xing, A.Q., Zhou, Y., Mathiesen, M., Schweiger, B., Kinney, A., Staswick, P., & Clemente, T. (2002). Nuclear localization of RNA transcripts down-regulate seed fatty acid genes in transgenic soybean. *Plant Journal*, *30*, 155-163.

Burg, D.A., & Kleiman, R. (1991). Preparation of meadowfoam dimer acids and dimer esters and their use as lubricants. *Journal of the American Oil Chemists Society*, *68*, 600-603.

Cahoon, E.B. (2001). [Metabolic engineering of soybean oil composition for increased linolenic content]. Unpublished data.

Cahoon, E.B., & Shanklin, J. (2000). Substrate-dependent mutant complementation to select fatty acid desaturase variants for metabolic engineering of plant seed oils. *Proceedings of the National Academy of Sciences*, *USA*, *97*, 12350-12355.

Cahoon, E.B., Marrillia, E.-F., Stecca, K.L., Hall, S.E., Taylor, D.C., & Kinney, A.J. (2000). Production of fatty acid components of meadowfoam oil in somatic soybean embryos. *Plant Physiology*, *124*, 243-251.

Cahoon, E.B., Ripp, K.G., Hall, S.E., & Kinney, A.J. (2001). Formation of conjugated delta 8, delta10 double bonds by delta12-oleic acid desaturase related enzymes: Biosynthetic origin of calendic acid. *Journal of Biological Chemistry*, *276*, 2637-2643.

Cahoon, E.B., Ripp, K.G., Hall, S.E., & McGonigle, B. (2002). Transgenic production of epoxy fatty acids by expression of a cytochrome P450 enzyme from Euphorbia lagascae seed. *Plant Physiology*, *128*, 615-624.

Crandall, L. (2002). Bioplastics: A burgeoning industry. *Inform*, *13*, 626-630.

Erhan, S.M., Kleiman, R., & Isbell, T.A. (1993). Estolides from meadowfoam oil fatty acids and other monounsaturated acyl moieties in developing seeds. *Journal of the American Oil Chemists Society*, *70*, 460-465.

Kinney, A.J. (1997). Genetic engineering of oilseeds for desired traits. In J.K. Setlow (Ed.), *Genetic engineering: Vol. 19* (pp. 149-166). New York: Plenum Press.

Parrot, W.A., & Clemente, T.A. (in press). Transgenic soybean. In J. Specht & R. Boerma (Eds.), *Soybeans: Improvement, production, and uses* (3rd Ed., Agronomy Monograph No. 16). Madison, WI: American Society of Agronomy.