

Primary carbon metabolism

5

OM5.1 MANIPULATION OF SOURCE AND SINK STRENGTH IN POTATO

In initial investigations of factors that determine potato tuber yield, researchers used genetic manipulation to alter the capacity of tubers to metabolize sucrose (**Figure OM5.1-1**). The capacity was increased by expression in the tuber apoplast of an invertase enzyme from yeast. Invertase hydrolyzes sucrose to glucose and fructose. Its presence

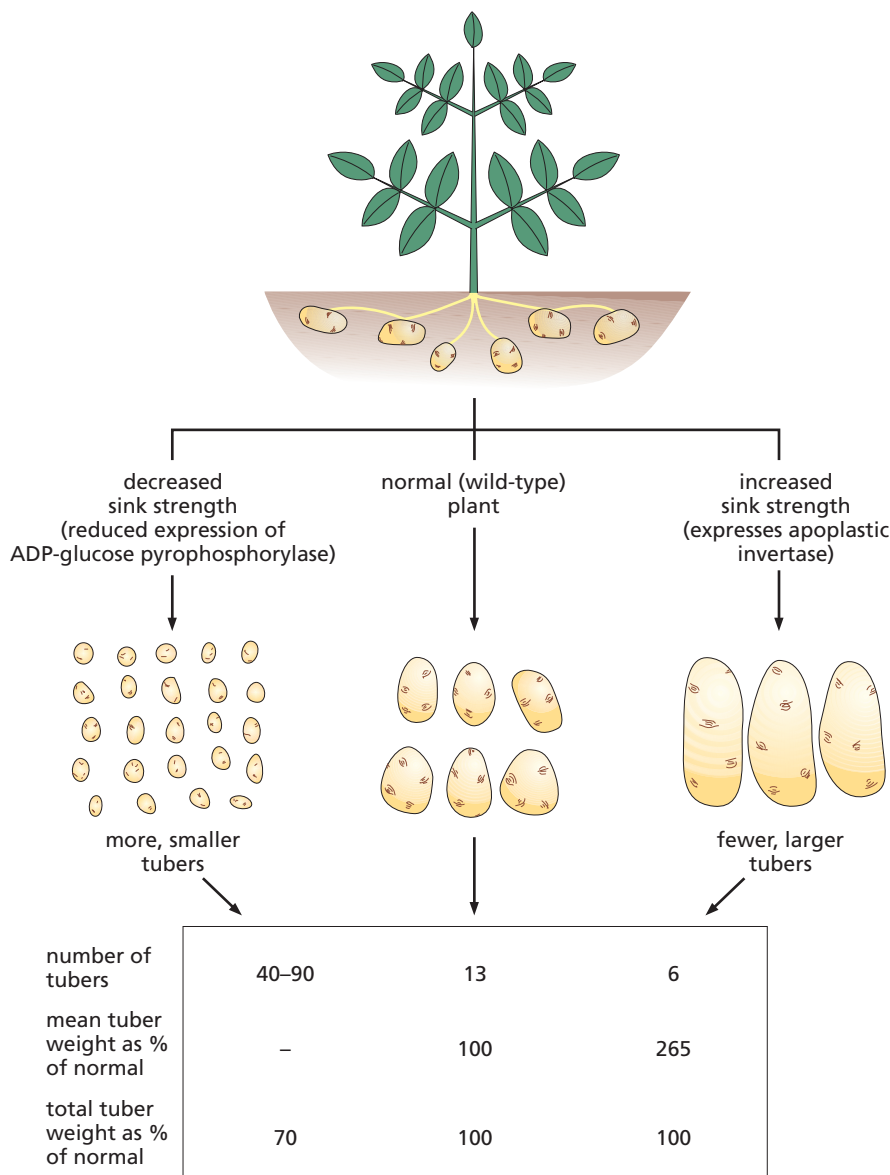


Figure OM5.1-1 Experiments demonstrating the importance of sink strength in potato. Altering the sink strength changed the partitioning of sucrose among tubers. (*Left*) At low sink strength achieved by reducing expression of ADP-glucose pyrophosphorylase many small tubers developed. (*Right*) At high sink strength achieved by expressing apoplastic invertase in tubers, high competition among tubers for available sucrose meant that fewer, larger tubers developed. The total tuber weight, however, was not strongly affected by the changes in sink strength: it was reduced only 30% (compared with wild type) when sink strength was low, and was unaltered when sink strength was high. Thus, in this case, the source organs were more important than the sink organs in determining how much sucrose was allocated to tubers.

increased tuber sink strength by lowering sucrose concentration in the apoplast, thus increasing the sucrose concentration gradient between the phloem and the tuber tissue. The invertase-expressing potatoes produced significantly larger but fewer tubers than the wild type. In other experiments, the capacity to metabolize sucrose was decreased by creating transgenic plants with reduced activity of ADP-glucose pyrophosphorylase. This enzyme is necessary for the synthesis of starch, the main fate for sucrose entering the tuber (see Section 5.3). Tubers of these transgenic plants had reduced rates of starch synthesis, leading to the accumulation of sucrose and a reduction in the sucrose concentration gradient between the phloem and the tuber tissue. This, in turn, decreased the rate of phloem unloading, leading to a reduction in the sink strength of individual tubers. The transgenic plants had many more, smaller tubers than normal plants. Overall, therefore, altering sink strength affected the numbers and sizes of tubers that formed, but had relatively little effect on the total yield of tubers.

The results from these experiments suggest that individual tubers compete with each other for sucrose from the phloem. When sink strength is very high, the first few tubers formed consume all of the available sucrose, and no further tubers develop. Conversely, when sink strength is very low, sucrose availability remains high and many tubers develop. Alterations in sink strength had relatively little effect on the total flux of carbon from source to sink, implying that an enhancement of source as well as sink strength would be required in order to achieve increased tuber yield. This proved to be the case: when increased sink strength was combined with increased source strength, tuber yield was doubled (**Figure OM5.1-2**).

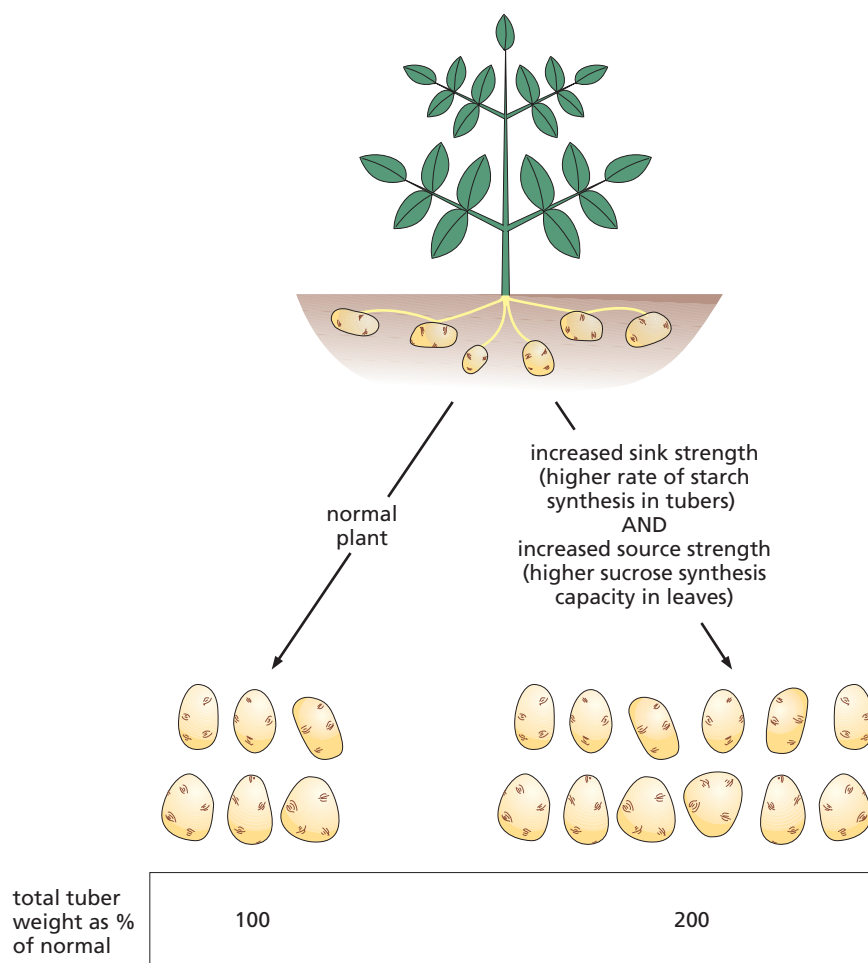


Figure OM5.1-2 Experiments demonstrating a yield increase when both sink and source strength are increased in potato. Altering both the sink and the source strength resulted in an increased total yield of tubers. Sink strength was increased by over-expressing genes encoding transporters that move precursors of starch synthesis into the plastids in tubers. Source strength was increased either by over-expressing genes encoding enzymes involved in sucrose synthesis in photosynthetic cells of the leaf or by manipulations that reduced chloroplastic starch synthesis and thus diverted extra assimilate to sucrose synthesis.

OM5.2 THERMOGENESIS IN THE ARUM SPADIX

Arum maculatum is native to Europe and parts of western Asia. The flower consists of a heat-generating organ – the **spadix** – subtended by male and female flowers. During development of the inflorescence, over a period of several weeks, the male and female flowers and spadix are enclosed in a leaf-like sheath (**Figure OM5.2-1A**). Sucrose is imported into the spadix from the leaves and converted to large amounts of starch. The capacity for glycolysis (the amounts of glycolytic enzymes) in the spadix increases to a high level, but the flux through this pathway is low. When the inflorescence is mature, the sheath opens to reveal the spadix (**Figure OM5.2-1B**). Within just a few hours, all of the starch is degraded to hexoses and metabolized via glycolysis and the tricarboxylic acid cycle. The very high rate of glycolytic flux and the engagement of the alternative oxidase pathway rather than the cytochrome pathway result in the generation of heat (thermogenesis). The spadix temperature can rise to several degrees above ambient (it feels very warm to the touch). The heat volatilizes amines produced in the spadix, and the smell attracts small insects. These crawl down into the base of the sheath, where they are trapped by a ring of downward-pointing hairs. Female flowers below these hairs are pollinated by insects that have visited other *A. maculatum* inflorescences. The photographs in **Figure OM5.2-1C** show a mature inflorescence cut away to reveal the downward-pointing hairs and male and female flowers enclosed in the sheath base (*left*) and an intact inflorescence in its native habitat in woodland (*right*). On the day following thermogenesis, the downward-pointing hairs shrivel and the insects can escape (**Figure OM5.2-1D**). The male flowers now shed pollen, which is carried by the escaping insects to other inflorescences. **Figure OM5.2-1E** outlines the heat-generating metabolism in the cytosol and mitochondrion of a spadix cell during thermogenesis. Phosphoenolpyruvate (PEP) generated from starch via glycolysis is converted to pyruvate by the enzymes PEP carboxylase, malate dehydrogenase, and malic enzyme. Pyruvate is oxidized via the tricarboxylic acid cycle, and the NADH generated donates electrons to the alternative oxidase pathway, resulting in the generation of heat. The spadix at this stage has no requirement for ATP or precursor molecules – it senesces when its starch supply is exhausted – and essentially all of the carbon derived from starch is converted to carbon dioxide and reducing power for heat production in the mitochondria.

Thermogenesis occurs in diverse groups of plants including cycads (gymnosperms), monocots, and dicots. The most extensively studied species are members of the Araceae (monocots; including the giant voodoo lily *Amorphophallus titanum* (**Figure OM5.2-1F top**), the eastern skunk cabbage *Symplocarpus foetidus*, and some other species of *Arum*) and the basal eudicot *Nelumbo nucifera* (sacred lotus) (**Figure OM5.2-1F bottom**). In the lower part of (F) on the left is a *N. nucifera* flower photographed in normal light; on the right, a *N. nucifera* flower photographed with an infrared camera (sensitive to heat). The blue regions (petals) are at **ambient** temperature, while the red region (the receptacle – female reproductive organ) is considerably above ambient temperature.

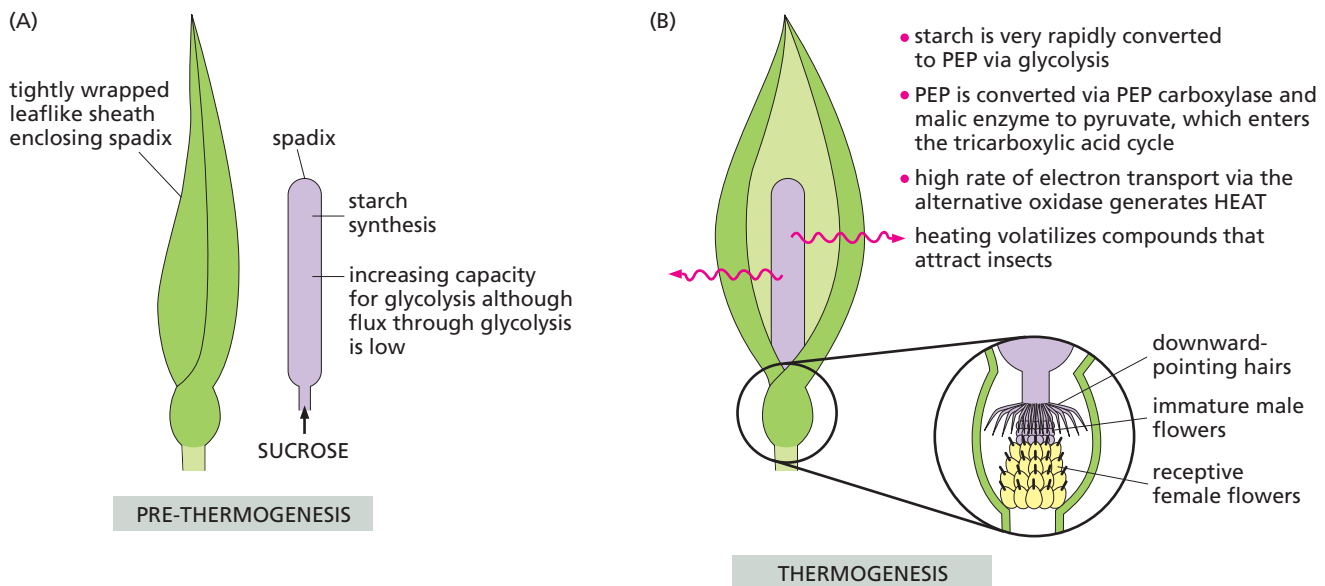
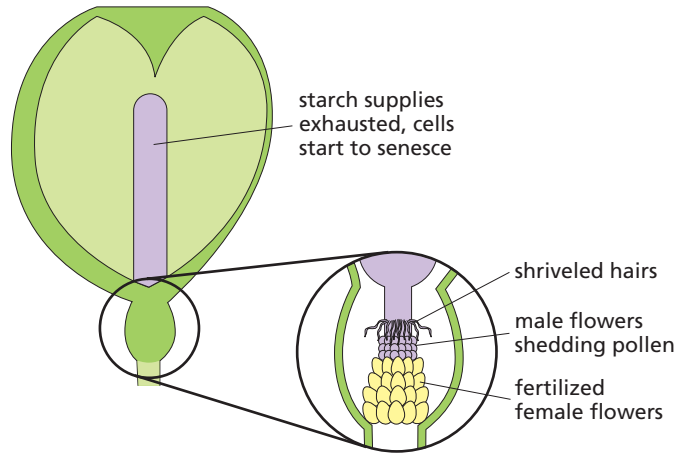


Figure OM5.2-1 Thermogenesis in the *Arum* spadix. See text for details. (Continued)

(C)

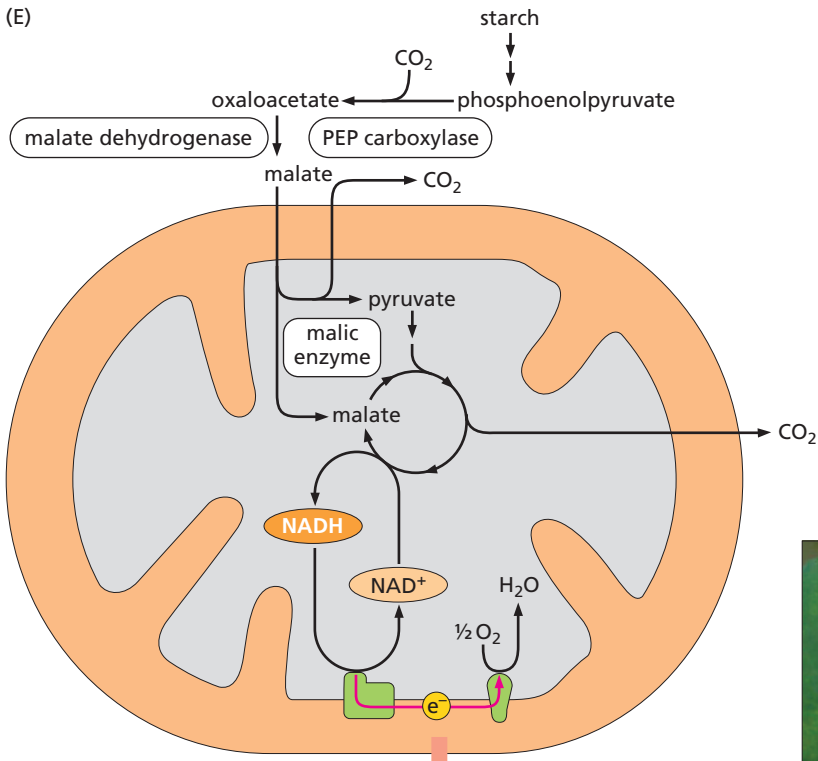


(D)



POST-THERMOGENESIS

(E)



(F)



Figure OM5.2-1 (Continued) (C, courtesy of Tobias Kieser. F top, from "Amorphophallus titanum with 3 flowers – Botanischer Garten Bonn". © Raimond Spekking. Licensed under CC BY SA 4.0 via Wikimedia Commons. F bottom, from Zou Y et al. (2020) *Int J Mol Sci* 21:3324. ©2020 by the authors. Licensee MDPI, Basel, Switzerland. Distributed under CC BY 4.0.)

OM5.3 METABOLIC DIFFERENTIATION IN CELLS OF THE ROOT TIP

Each cell type in the root arises from a meristem close to the apex (**Figure OM5.3-1**; further described in Section 10.3). Cells in this apical meristem divide and grow rapidly, generating a high demand for precursors for the biosynthesis of a wide range of cell constituents such as cell walls, membranes, and proteins, and for ATP and reducing power to drive these biosynthetic reactions. The flux through glycolysis and the tricarboxylic acid cycle is high, and **post-translational regulation** (regulation at the level of the enzyme protein) of enzyme activity at many steps maintains a balance between the removal of intermediates as precursors and the oxidation of sufficient substrate to produce the ATP required to meet the biosynthetic demand. About half of the carbon entering glycolysis is used as metabolic precursors, and is ultimately converted to structural and protein components of the cell. The other half is oxidized to carbon dioxide in the tricarboxylic acid cycle to drive ATP synthesis.

After its final division, a cell destined to become part of the cortical parenchyma undergoes dramatic elongation. The huge increase in cell volume is dependent on the development of a central vacuole, which fills most of the internal volume of the cell, and on the synthesis of new membrane and cell wall material (see Section 8.1). These elongating cells have a high requirement for ATP for the ion pumps in the plasma membrane and tonoplast that drive the filling of the vacuole. Much of the carbon entering glycolysis is ultimately oxidized to carbon dioxide to fuel the ATP demand, and the yield of ATP per sucrose molecule oxidized is closer to the theoretical maximum (about 60–64 ATP per sucrose) than it is in meristematic cells. At the same time, the demand for precursors for the synthesis of cellulose, pectins, and membrane lipids for wall and membrane extension must be met.

Having reached maturity and ceased expansion, the parenchyma cell is less metabolically active. Cellular structures and functions are maintained: lipids and proteins are replaced as they are turned over, and ATP is supplied to the ion and proton pumps that maintain the ionic, pH, and turgor (internal pressure) balance of the cell. However, overall demand for ATP, reducing power, and biosynthetic precursors – and hence the rate of utilization of sucrose – is much lower than in the earlier stages of the cell's life.

A cell differentiating to form a xylem vessel undergoes massive thickening of the cell wall brought about by the deposition of cellulose, and the cell wall also becomes lignified. Cellulose is synthesized from UDP-glucose (see Section 6.3). Lignin is synthesized from monolignol monomers derived from the aromatic amino acid phenylalanine (see Section 6.5), for which the precursors are the glycolytic intermediate PEP and erythrose

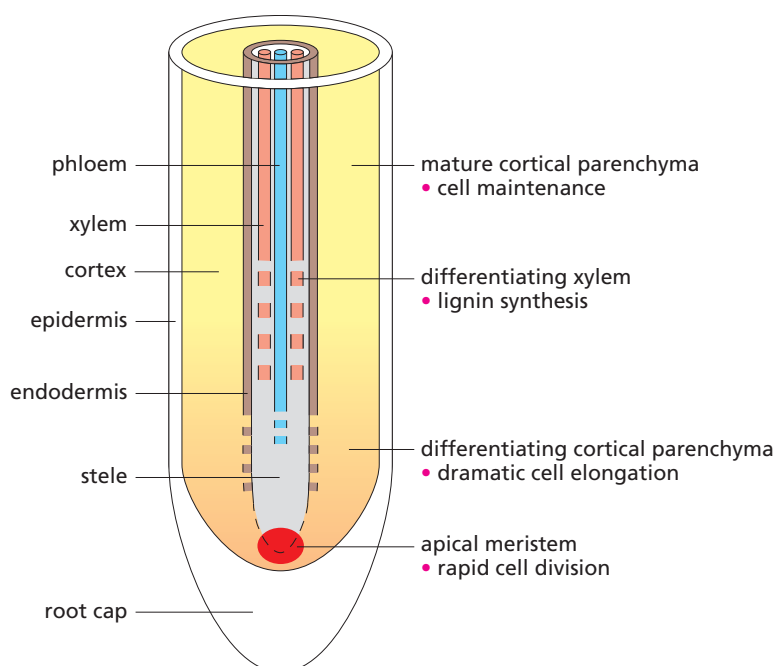


Figure OM5.3-1 Functions of major cell types in a root tip. Labels on the *left* show features of root anatomy that are further described in Section 10.3. The four major cell types are indicated on the *right*.

4-phosphate (an intermediate of the oxidative pentose phosphate pathway: see Figure 5.10). Monolignol synthesis requires reducing power in the form of NADPH, which is generated in the oxidative pentose phosphate pathway. Each phenylalanine used is deaminated producing ammonia, which is reassimilated via the GS-GOGAT (glutamine synthase-glutamine-2-oxoglutarate aminotransferase) pathway, which requires ATP and NAD(P)H. Because of these specific demands on the oxidative pentose phosphate pathway, a higher proportion of the carbon from sucrose enters this pathway in the differentiating cell than in the meristematic cell.

The changes in flux through metabolic backbone pathways during differentiation to form xylem are facilitated both by post-translational regulation of enzyme activities and by changes in gene expression. For example, the increased proportion of carbon from sucrose that enters the oxidative pentose phosphate pathway is accompanied by an increased capacity for the pathway as a whole (that is, increased amounts of all pathway enzymes resulting from increased expression of the genes that encode them) and by modulation of the activities of some of the enzymes, for example by the NADPH:NADP⁺ ratio in the cell.

Other important aspects of the flexibility of the metabolic backbone are discussed in Chapters 5, 6, and 7. These include the capacity to store carbon as sucrose and to remobilize this carbon; division of biosynthetic pathways between the cytosol and the plastid; and coordination of carbon metabolism with the requirements of the assimilation of nitrogen and of other essential nutrients.

OM5.4 FRUCTANS

The fructans are a complex series of soluble fructose polymers. Fructans are synthesized inside vacuoles, from sucrose imported from the cytosol (**Figure OM5.4-1A**). Fructan synthesis starts with the transfer of a fructosyl (Fru) unit (*pink*) from one sucrose molecule to another, yielding glucose and a glucose-fructose-fructose molecule called kestose. Synthesis proceeds by transfer of the fructosyl residue of sucrose onto the fructan chain. Several different types of fructosyl transferase enzyme may be involved, depending on the type of fructan (**Figure OM5.4-1B**). The glucose residue of the sucrose is exported from the vacuole to the cytosol, is phosphorylated, and is recycled into sucrose (Figure OM5.4-1A). Fructans retain a glucosyl (Glc) unit (*blue*) from the original sucrose acceptor molecule.

Fructan synthesis in grass leaves takes place when the rate of sucrose synthesis exceeds the rate of export; high sucrose levels induce the expression of genes encoding fructan-synthesizing enzymes. When sucrose levels fall, fructans are enzymatically degraded to release fructose from the ends of their polymer chains. The fructose is exported from the vacuole to the cytosol, where it is phosphorylated and converted to sucrose. Fructans in non-photosynthetic storage tissues – such as the stems of perennial grasses, bulbs of onions, roots of chicory, and tubers of Jerusalem artichoke – fulfill the same role as starch in similar organs of other species: they are synthesized during active growth of the plant, persist during periods when photosynthesis is reduced or absent, and are degraded to provide carbon for regrowth when conditions become favorable.

The precise structure of the polymer is species-dependent. In the simplest case, polymers are linear chains of either β 1,2-linked or β 2,6-linked fructose residues. Other types of fructans contain a mixture of linkage types and may be branched. Figure OM5.4-1B shows three examples of fructans: levan, inulin, and graminan types. Species that accumulate fructans include *Allium cepa* (onion: inulin and graminan-like fructans), *Chicorium intybus* (chicory: inulin), *Helianthus tuberosus* (Jerusalem artichoke: inulin), and *Lolium* spp. (rye grass: levan) (**Figure OM5.4-1C**).

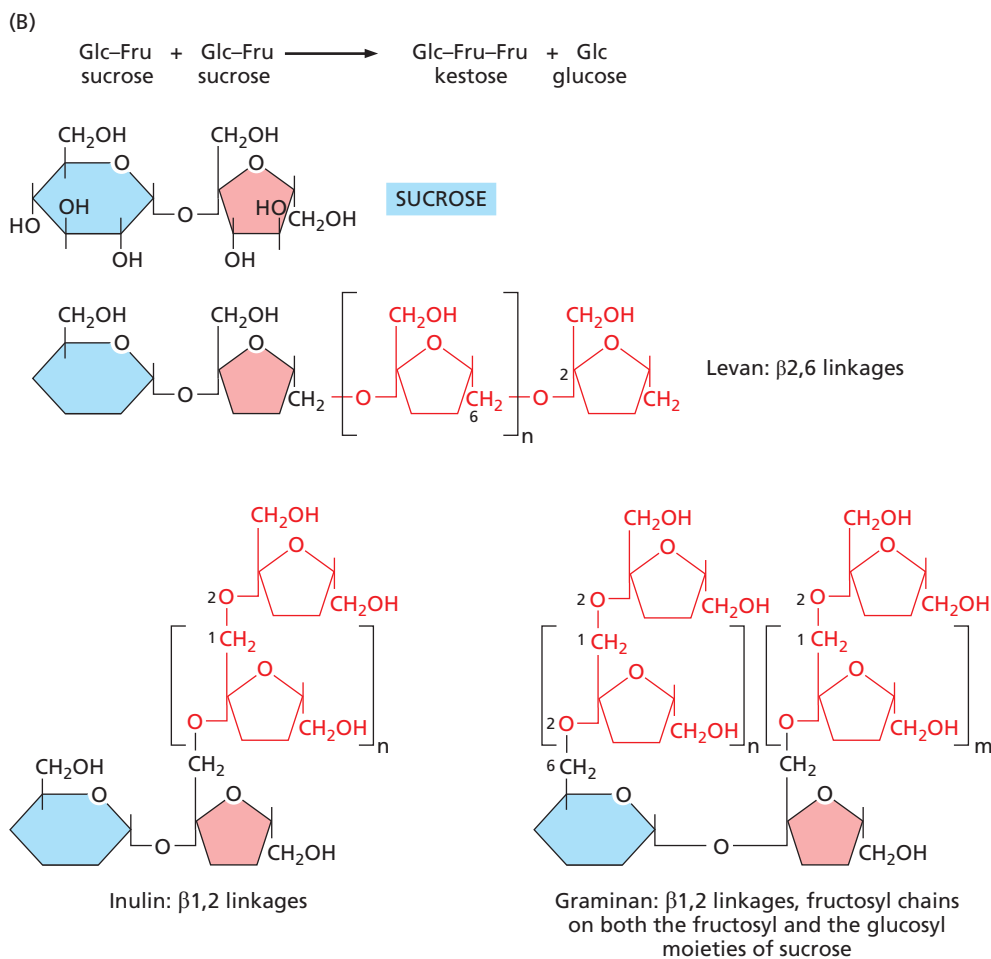
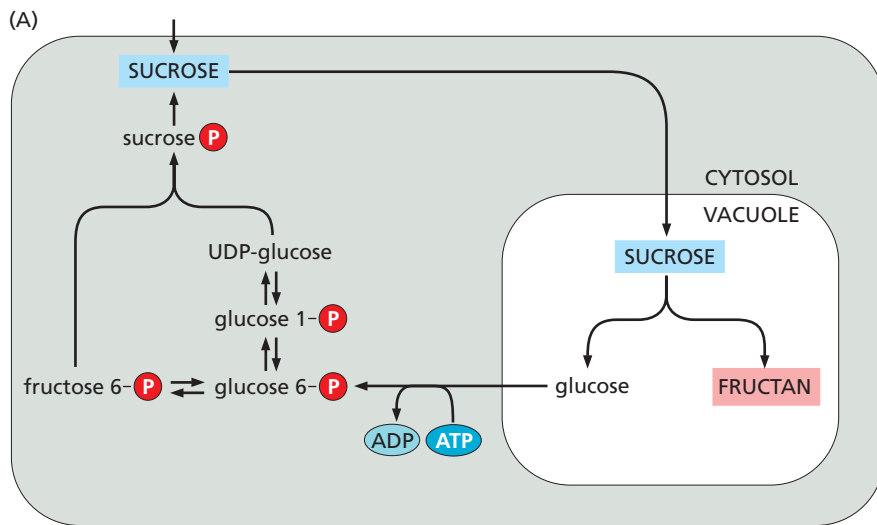


Figure OM5.4-1 Synthesis of fructans. (A) Use of sucrose for fructan synthesis in the vacuole releases glucose, which is exported to the cytosol where it can be phosphorylated by hexokinase and recycled back into sucrose (see Figure 5.8). (B) Fructan synthesis starts with the transfer of a fructosyl (Fru) unit (pink) from one sucrose molecule to another, yielding glucose and kestose. Fructans retain a glucosyl (Glc) unit (blue) from the original sucrose acceptor molecule. The number of fructosyl units and the types of linkage in the fructan molecule vary with species and developmental stages. Three classes of fructans are shown here. Fructans with β 2,6 bonds are commonly known as levans. Fructans with a predominance of β 1,2 bonds are known as inulins. Fructans with β 1,2-linked fructan chains attached to both the fructosyl and the glucosyl moieties of sucrose are known as graminans, and are found in grasses. (C) Some fructan-accumulating species. From top to bottom: Onion (fructans stored in swollen leaf bases), flowers of chicory (fructans stored in roots), flowers of Jerusalem artichoke (fructans stored in tubers), and rye grass in a stadium (*Lolium* sp.; fructans accumulated in leaves in high light). (C top, upper middle, courtesy of Tobias Kieser. C lower middle, courtesy of David G. Smith. C bottom, from "Estádio do Dragão – Porto, 2009" by Angela Antunes, Dublin, Ireland. Licensed under CC BY 2.0 via Wikimedia Commons.)

OM5.5 THE *RUGOSUS* MUTATION OF PEA, AND OTHER COMMERCIALY VALUABLE MUTATIONS AFFECTING STARCH IN CROPS

The *rugosus* (*r*) mutant of pea was one of the characters of peas that provided the basis of Gregor Mendel's discovery of the laws of heredity in the mid 19th century. Seeds of normal (wild-type) peas are round, whereas those of *rugosus* mutants are wrinkled. This is because the reduction in starch-branching enzyme activity in mutant relative to wild-type peas during their development causes a reduction in overall starch synthesis and hence a build-up of sucrose and a concomitant high water content. When the seed dries out at maturity, the seed coat of the mutant wrinkles because of the large loss of volume of the embryo. Mendel observed that in the F₂ generation from a cross between round- and wrinkled-seeded peas, one-quarter of the plants were wrinkled-seeded. He proposed that the seed shape character was determined by two factors, a "round" and a "wrinkled" factor, and that each plant inherited one factor from each parent. The round factor was dominant over the wrinkled factor, so that a plant with two round factors or one round and one wrinkled factor would have round seeds, and a plant with two wrinkled factors would have wrinkled seeds. We now know that Mendel's factors are alleles of the gene encoding one isoform of starch-branching enzyme. The round factor is the wild-type allele; the wrinkled factor is a mutant allele that does not give rise to an active starch-branching enzyme (Figure OM5.5-1).

Mutant plants that lack isoamylase contain both starch and another glucose polymer, phytyloglycogen. The best known of these mutants is the *sugary-1* mutant of maize, which was the original sweetcorn. It was discovered in 1901 as a mutant in a commercial maize line, identifiable by its shrunken mature kernels and the sweet taste of the immature kernels. Phytyloglycogen is, like amylopectin, an α 1,4-, α 1,6-linked glucose polymer, but it is more highly branched. It is not organized to form a granule and is found in plastids in a soluble form (as shown for the isoamylase-deficient mutant of Arabidopsis, *isa2*, in Figure OM5.5-2).

Mutant plants that have altered starches are of commercial and nutritional value as well as of academic interest. Starch extracted from crops such as maize, wheat, and cassava has a host of industrial uses because it swells to form gels or pastes when cooked in water. These gels and pastes are used in many processes including the manufacture of processed food, paper, and biodegradable plastics. Starches with particularly low or high amylose contents have different physical properties from normal starches: they differ in the extent to which they swell on cooking and in the type of gels or pastes that are formed. These properties have specific industrial applications, and both waxy and amylose-extender mutants of maize are grown commercially for starch extraction. Starch is the major source of calories in the human diet. Because most starches are rapidly broken down to glucose in the gut, over-consumption of starchy foods can result in obesity and increased risk of diseases including diabetes and heart conditions. Consumption of foods that contain less digestible starches could reduce the prevalence of these diseases. High-amylose starches in particular are less susceptible to rapid digestion, and biotechnologists are investigating ways of increasing the amylose contents of common foods. Because of its high sugar content, the *rugosus* mutant is the basis of many pea cultivars grown for human consumption.

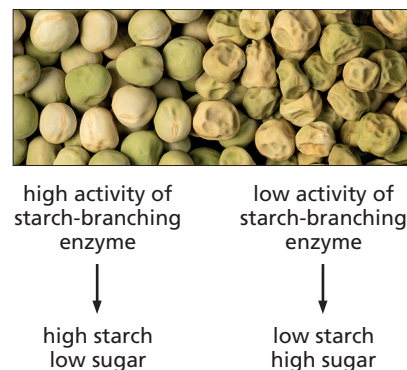


Figure OM5.5-1 The *rugosus* mutant of pea. The peas on the left are wild type: they contain high amounts of starch and have a round shape. The peas on the right are mutants lacking one isoform of starch-branching enzyme (*rugosus* or *r* mutants). Starch synthesis is reduced in these mutants, and as a result sugar accumulates during seed development. High sugar draws large amounts of water into the developing seed, stretching the seed coat. The seed coat then wrinkles due to the large loss of volume when the seeds dry out upon reaching maturity. Because of the high sugar contents of their seeds, many of the varieties of pea grown for direct human consumption of developing seeds are *rugosus* mutants. (Courtesy of Claire Domoney, Tracey Rayner, and Phil Robinson, John Innes Centre.)

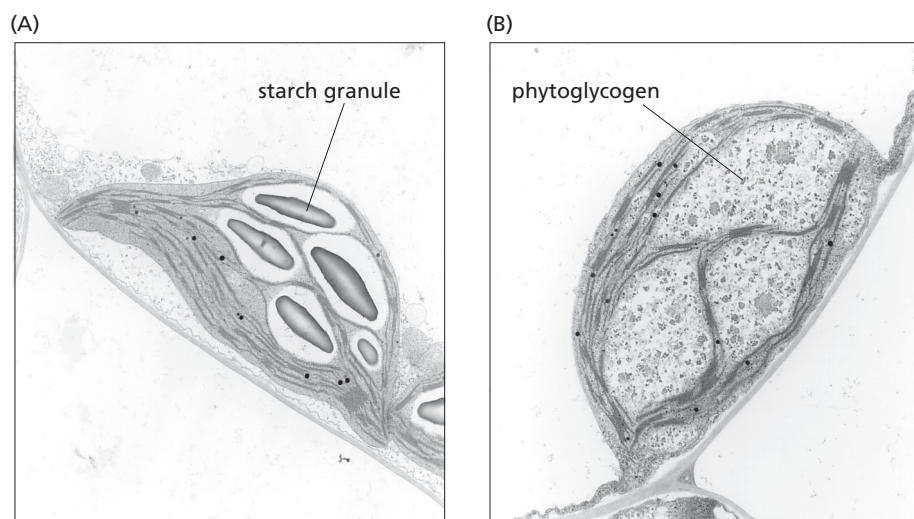


Figure OM5.5-2 Accumulation of phytyloglycogen in the chloroplasts of an Arabidopsis mutant that lacks isoamylase. (A) Chloroplast from a wild-type plant, containing starch granules. Chloroplast starch granules are typically 1–2 μ m in diameter. (B) Chloroplast from an isoamylase (*isa2*) mutant, containing residual starch and a large quantity of soluble and semi-soluble phytyloglycogen molecules. (A, from Zeeman SC *et al.* (2004) *New Phytol* 163:247–261. © New Phytologist Trust. With permission from John Wiley & Sons, Inc.)

OM5.6 THE FATTY ACID DESATURASES FAD2 AND FAD3

Two **fatty acid desaturases** – FAD2 and FAD3 – are considered as examples of the different specificities of these enzymes. They are located in the endoplasmic reticulum and catalyze the desaturation of the fatty acids of phosphatidylcholine molecules (**Figure OM5.6-1**). FAD2 introduces a double bond between carbon-12 and carbon-13 of the 18:1 fatty acid in which the existing double bond is between carbon-9 and carbon-10, thus converting an 18:1^{Δ9} fatty acid to an 18:2^{Δ9,12} fatty acid (the Δ indicating the position of the double bond). FAD3 then converts this product to an 18:3^{Δ9,12,15} fatty acid. These 18:1, 18:2, and 18:3 fatty acids (oleic, linoleic, and α-linolenic acids, respectively) are of almost universal occurrence in plants as major constituents of membrane lipids, and are also found in triacylglycerols. Linoleic and α-linolenic acids are so-called “essential fatty acids.” They are not synthesized in the human body yet are essential precursors for some membrane lipids and cell-signaling molecules. All of the linoleic and α-linolenic acid required by humans comes from the diet, largely from seeds and leafy vegetables.

Some plants synthesize highly unusual, species-characteristic, desaturated fatty acids. For example, the oils of borage (*Borago officinalis*) (**Figure OM5.6-2A**) and evening primrose (*Oenothera* spp.) are rich in γ-linolenic acid (18:3^{Δ6,9,12}), a fatty acid believed to have important benefits for human health. These species contain a Δ6 desaturase that introduces a double bond into an 18:2^{Δ9,12} fatty acid of a phosphatidylcholine molecule. The oils of coconut and oil palm (**Figure OM5.6-2B**) are rich in a fatty acid of 12 carbon atoms in length (lauric acid), formed by the early release of fatty acid (acyl) chains from the fatty acid synthase complex in the plastid. Fatty acids with more than 18 carbons are formed by elongase complexes bound to the endoplasmic reticulum membrane (**Figure OM5.6-3**). In a series of reactions involving at least four different proteins, one or more two-carbon units are added to an 18-carbon substrate. The triacylglycerols of many crucifers contain a high proportion of these longer fatty acids. Long-chain fatty acids are also found in the waxes that form the cuticle on the aerial surface of plants (see Section 6.3).

The substrate preferences of the acyltransferases that add the fatty acids to the glycerol backbone are also important in determining the fatty acid composition of triacylglycerols. The acyltransferase that adds the fatty acid chain to position 1 of glycerol has a low selectivity for the type of fatty acid, and many different sorts of fatty acid appear in this position in triacylglycerols. The transferase acting at position 2 of glycerol preferentially uses unsaturated fatty acids. The chain-length preferences of this enzyme differ among species. For example, in *Brassica napus* (oilseed rape), the position 2 transferase will not accept erucic acid (22:1) but the acyltransferases acting at positions 1 and 3 will. In the seed oil of *Limnanthes* (**Figure OM5.6-2C**), long-chain fatty acids appear at all three positions on the glycerol backbone.

Plant triacylglycerols have a wealth of food and industrial uses – for example as detergents, soaps, lubricants, biofuels, cooking oils and food ingredients, and additives – and they are increasingly seen as direct, renewable replacements for fossil hydrocarbons as fuel and in manufacturing processes. Global production of vegetable oils in 2020–2021 was over 200 million metric tonnes, the bulk of which was extracted from soybean, oilseed rape (rapeseed, canola), and oil palm. A large fraction of these products was consumed by the food industry. The most widely used vegetable oil is extracted from oil palm fruits. It contains a much larger fraction of saturated fatty acids than soybean or rapeseed oil (almost 50%, largely as palmitoyl chains, compared with 15% and 7% for soybean and canola, respectively). The high degree of saturation renders palm oil semi-solid at room temperature, and very stable during frying and other food uses. A great increase in demand for palm oil in the last few decades has led to very large-scale expansion of plantations of oil palm in moist tropical regions. Expansion has often been achieved through destruction of rainforests, with adverse consequences for climate change and biodiversity.

Numerous attempts have been made to engineer or breed major oil crops that contain large amounts of specific, high-value triacylglycerols, by manipulation of enzymes that alter chain lengths or saturation levels of fatty acids. Attempts to produce long-chain polyunsaturated fatty acids important in the human diet through manipulation of the oilseed crop *Camelina sativa* are described as an example in Online Material 5.7.

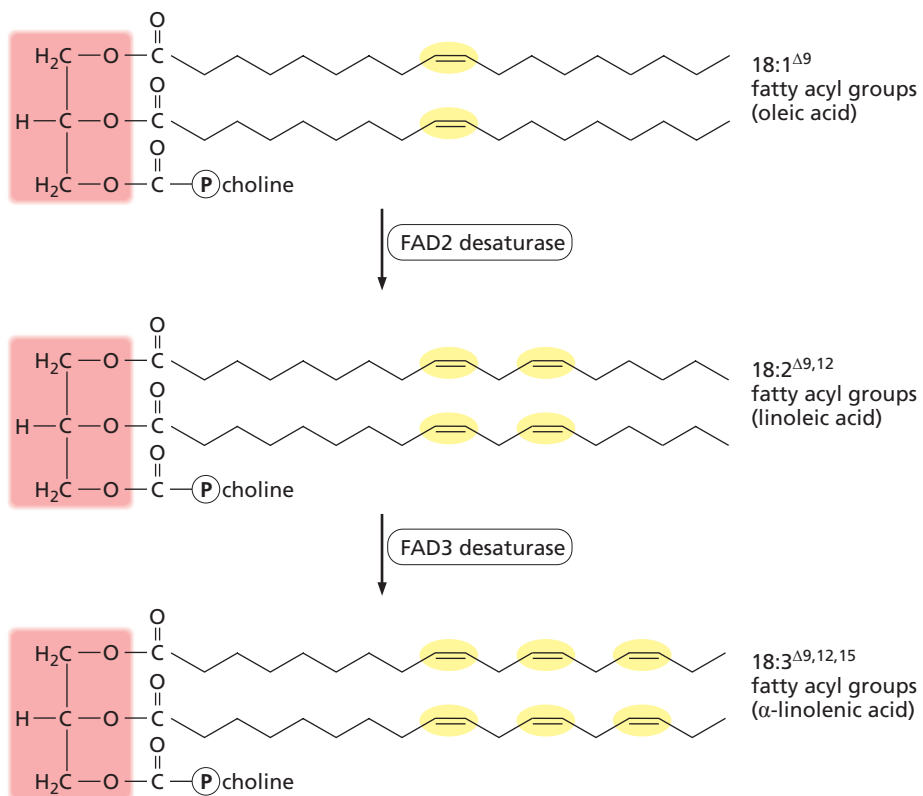


Figure OM5.6-1 Desaturation of fatty acyl groups in phosphatidylcholine.

The FAD2 desaturase acts on the oleic acid groups of phosphatidylcholine to introduce a second double bond in each, and FAD3 desaturase introduces a third double bond in each. The resulting linoleic or α-linolenic fatty acyl groups can be introduced into triacylglycerols using the pathway shown for phosphatidylcholine in Figure 5.34.



Figure OM5.6-2 Some species that produce high proportions of unusual fatty acids. (A) Borage (*Borago officinalis*): oil from seeds contains a high proportion of γ-linolenic acid (18:3^{Δ6,9,12}). **(B)** Oil palm (*Elaeis guineensis*): oil from the outer part of the fruit contains about 50% palmitic acid, whereas oil from the inner part (kernel oil) contains 50% lauric acid. **(C)** Meadow foam (*Limnanthes douglasii*): oil from the seeds contains a high proportion of 20:1, 22:1, and 22:2 fatty acids. (C, courtesy of Tobias Kieser.)

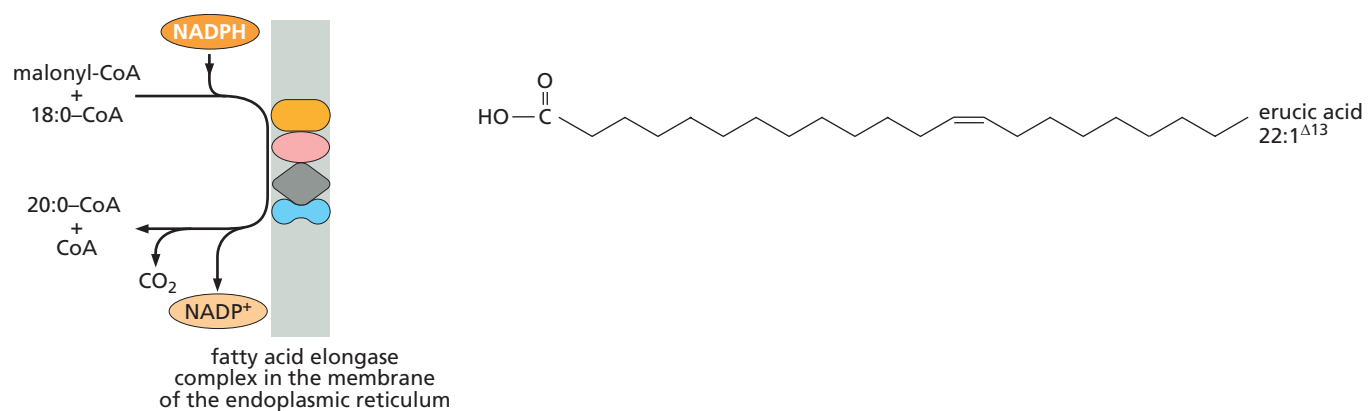


Figure OM5.6-3 Fatty acid elongation by an elongase. Fatty acids of 20 or more carbons are produced by membrane-bound elongase complexes (*left*). These enzymes catalyze the addition of two-carbon units to a fatty acyl-CoA (here, stearic acid, 18:0), using malonyl-CoA as substrate. This reaction is analogous to the elongation of acyl-ACP by fatty acid synthase in the plastid, using malonyl-ACP as substrate (see Section 6.1). The 22-carbon erucic acid (*right*) is synthesized by elongation of oleic acid (18:1^{Δ9}). It is found in the triacylglycerols of some crucifers, including some cultivars of oilseed rape.

OM5.7 PRODUCTION OF “FISH OIL” IN OILSEED CROPS

The long-chain fatty acids eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids are essential for human health, particularly because of their beneficial effects on neural development and in the prevention of various chronic diseases. These long-chain polyunsaturated fatty acids (LC-PUFAs) are also known as omega-3 fatty acids because they have a double bond three carbon atoms in from the methyl terminus (the terminal carbon is called the omega carbon) of the fatty acid chain. LC-PUFAs can be synthesized in the human body, but not in sufficient quantities to meet physiological requirements. It is estimated that the mean intake of omega-3 fatty acids is far below the required level for a healthy diet. This shortfall is in large part because almost the only source of these fatty acids in the diet is from oily fish. Many people do not have access to oily fish or choose not to eat them, and farmed fish have lower omega-3 fatty acid contents than wild-caught fish. Over-fishing and unsustainable fishery practices exacerbate the problem of human access to omega-3 fatty acids.

To boost readily accessible supplies of LC-PUFAs for human consumption, several research groups have attempted to create transgenic oilseed rape (*Brassica napus*) and *Camelina sativa* (a related species that is a minor crop in Europe and North America) that accumulate omega-3 fatty acids in their seeds. The introduction of genes to convert endogenous seed fatty acids into EPA and DHA has required the identification and cloning of desaturase and elongase genes from marine microalgae, oomycetes, and diatoms. Although the omega-3 fatty acids accumulate in fish, they are not synthesized by fish but are acquired by fish through their consumption of omega-3 fatty acid-synthesizing microalgae and diatoms.

EPA and DHA were produced in oilseeds at an early stage in these studies. However, to maximize production, achieve an optimal ratio of EPA to DHA for human health, and ensure that production of these compounds was spatially and temporally controlled within the developing seed, many subsequent adjustments to the expression of introduced and endogenous genes encoding enzymes of fatty acid synthesis were required.

These efforts over two decades have been successful. Consumption of oil from the transgenic seeds has the anticipated beneficial impacts on human health, and meal from the seeds can also bolster levels of omega-3 fatty acids when fed to farmed fish.