

SPRING 2026

Sunshine in a Cell: Biosynthetic Battles Against Vitamin D Deficiency

By Bella Zhang

AUTHOR BIOGRAPHY

Bella Zhang is a high school student attending Crofton House School in Vancouver, British Columbia. She hopes to conduct many more experiments and projects in the fields of plant sciences, synthetic biology, agriculture, and biodiversity.

ABSTRACT

Despite various forms of vitamin supplementation being available to consumers, the number of vitamin D-deficiency cases continues to increase. This review found that there are a plethora of health risks related to vitamin D deficiency, including many neurocognitive and bodily ailments, and that one of the most effective ways of combating vitamin D deficiency is by incorporating the micronutrient into one's diet. Furthermore, this paper explores two studies that utilize techniques such as transgenesis and mutagenesis to biosynthesize provitamin D₃ (the precursor of vitamin D₃). The first study discusses the use of horizontal gene transfer and the recombination technique—both transgenic techniques—on *Saccharomyces cerevisiae* (Baker's Yeast), which led to a significant improvement of 7-DHC production. Meanwhile, the second study centres on the use of CRISPR-Cas9, a mutagenesis technique, on *Solanum Lycopersicum* (Moneymaker Tomato) to redirect and increase the production of 7-DHC in the fruit. Both studies produce significant yields of provitamin D₃, suggesting that there is great potential for decreasing vitamin D-deficiency cases, as well as increasing market expansion through the genetic enhancement of food production and agriculture.

Keywords: *Vitamin D, provitamin D₃, deficiency, yeast, tomato, biofortification, transgenics, horizontal gene transfer, recombination, mutagenesis, CRISPR-Cas9, micronutrient, biosynthesis, food production, agriculture.*

SPRING 2026

PREVALENCE OF VITAMIN D DEFICIENCY GLOBALLY

Vitamin D deficiency is a global health concern; in the West alone, 24% of people in the US, 37% in Canada, and 40% in Europe are at risk of vitamin D deficiency (Morales-Villar et al., 2024). According to Cui et al. (2023), vitamin D deficiency remains notably high. Therefore, if the number of people with the deficiency continues to increase, the global burden of the disease will also increase, evaluated by its economic and health consequences (Biz & Bragazzi, 2022; Cui et al., 2023). Vitamin D deficiency and insufficiency clinical diagnoses are based on the levels of 25-hydroxyvitamin D in blood serum. In general, below 12 ng/mL is defined as vitamin D deficiency, while below 20ng/mL is insufficiency (National Library of Medicine, 2025). However, it should be noted that these are approximate figures, as there is no consensus on the definition (Lips et al., 2021).

Health Consequences of Deficient Vitamin D Levels

The risks of severe vitamin D deficiency include a large variety of symptoms such as musculoskeletal pain, joint pain and stiffness, fatigue, memory and mood problems, and—in extreme cases—fusing of spine vertebrae (Mayo Clinic, 2025; Banzal & Singhai, 2013). Furthermore, if left unchecked, vitamin D deficiency can also lead to osteoporosis, “micronutrient deficiencies, cancer, Parkinson’s disease, depression, neurocognitive decline, dementia, increased contraction of diseases such as COVID-19, and infection” (Li et al., 2022). There are a variety of causes of vitamin D deficiency (some of which overlap with the symptoms). Some of these include insufficient exposure to sunlight, darker skin pigmentation, malnutrition, certain medications, aging, or obesity (Holick & Chen, 2008).

At-risk Populations of Vitamin D Deficiency

However, diet is the predominant factor determining whether someone is vitamin sufficient or deficient. Therefore, individuals such as pregnant women, infants, children, teenagers, older adults, vegans, and malnourished people are at the greatest risk of vitamin D deficiency (National Library of Medicine, 2019). Additionally, individuals residing in lower-middle-income countries have been found to be more affected by vitamin D deficiency for much the same reasons as those in low-income households (Cui et al., 2023). Latitude and seasonality are also significant risk factors for vitamin D deficiency due to altered sun paths and prohibitive climates (Mayo Clinic, 2025). Given the many risk factors which are beyond human control, integrating biofortified foodstuffs into everyday meals is an optimal way to retain vitamin D in the global population, as it is generally more accessible and reduces individual burden compared to lifestyle changes and supplementation.

SPRING 2026

Bioengineering as a Potential Solution***Overview of Bioengineering Techniques in Addressing Micronutrient Deficiencies***

Genetic engineering (GE) is the application of advanced research technologies to manipulate and change an organism's DNA to enhance its characteristics (Smith, 2025). In fact, between 1996 and 2023, GE crops experienced a 121-fold expansion, meaning that they accounted for 13.38% of the total world farmland area (1,542 million hectares) and a planting area of over 3.4 billion hectares (Cheng et al., 2024). Currently, GE technologies in agriculture have been used to increase crop yields by modifying the characteristics of crops to increase their adaptability and nutritional content to address worldwide food insecurity (Phillips, 2008). GE encompasses many techniques, including transgenics, intragenics, and cisgenics. In this paper, we will discuss transgenic technologies (horizontal gene transfer and recombination) and mutagenesis (CRISPR-Cas9). Transgenics refers to the genetic modification of an organism by introducing a foreign DNA sequence(s) from another organism, while mutagenesis is when a mutation is introduced to an organism's DNA, in this case to affect the cholesterol or protein synthesized, or for phenotypic alterations (National Library of Medicine, 2022; National Human Genome Research Institute, 2025).

Purpose of the Review: Exploring Bioengineering Strategies to Mitigate Vitamin D Deficiency

The discussion of these GE methods will be in the context of *Saccharomyces cerevisiae* (*S. cerevisiae*, baker's yeast) and *Solanum lycopersicum* (*S. lycopersicum*, Monkeymaker tomato), both food staples commonly found in diets. The selection of these two organisms allows vitamin D synthesis to be contrasted between the complex tomato matrix, which has been less researched in GE, compared to the simpler yeast matrix, a well-established organism in experiments. This paper aims to answer the question: How can different transgenic and mutagenesis techniques enable different organisms to biosynthesize vitamin D₃?

THE BIOLOGICAL ROLE OF VITAMIN D**Mechanisms of Vitamin D Synthesis*****Difference Between Vitamin D₂ and D₃***

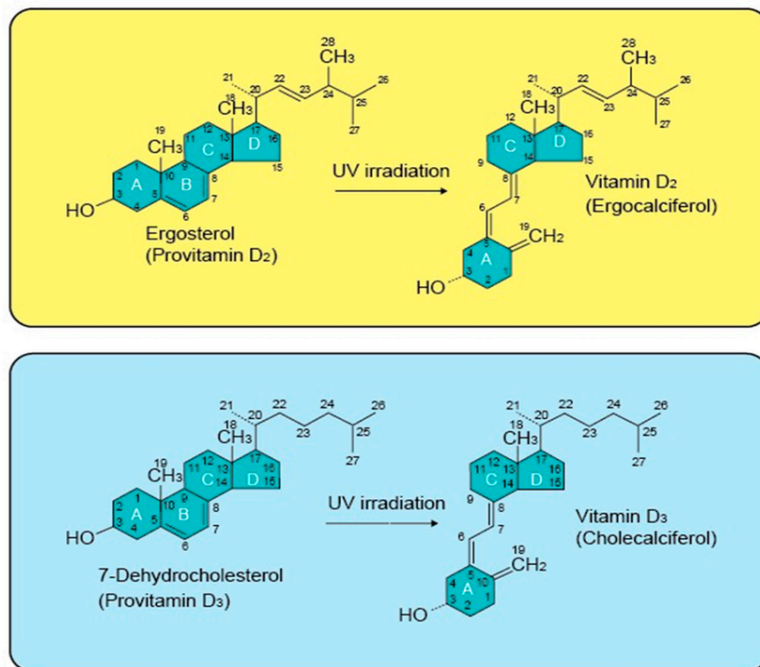
There are differences between both the molecular structure and sources of origin for vitamin D₂ and D₃. Unlike the molecular structure of vitamin D₃, D₂ has double bonds between carbon 22 and 23, and an additional methyl group (CH₃) attached to carbon 24 (Fig. 1). These changes are likely involved in arguments surrounding the bioequivalence of D₂ and D₃'s stability, metabolism, and binding affinity to vitamin D-binding

SPRING 2026

proteins has been a topic of numerous scientific arguments. Bouillon et al. (2016) suggest that vitamin D₃ contributes more effectively to maintaining blood 25-hydroxyvitamin D levels compared to vitamin D₂ when highlighting the differences in D₂ and D₃, and that this difference may influence their effectiveness in the human system. However, other sources argue that they are bioequivalent as they have similar biological activity depending on the context of supplementation. This suggests that opting for vitamin D through dietary means is not only a less harmful alternative compared to UVB exposure, but it also ensures the absorption of both D₂ and D₃, unlike UVB exposure. The sources of D₃ and D₂ also have variation, as both are present in fortified foods or supplementation; however, D₂ is also commonly found in plants and fungi as a less potent form of vitamin D, compared to D₃, which is commonly derived from sunlight and animal products (Mayo Clinic, 2025).

Figure 1.

Molecular Structure of Vitamin D₂ (Ergocalciferol) and Vitamin D₃ (Cholecalciferol)

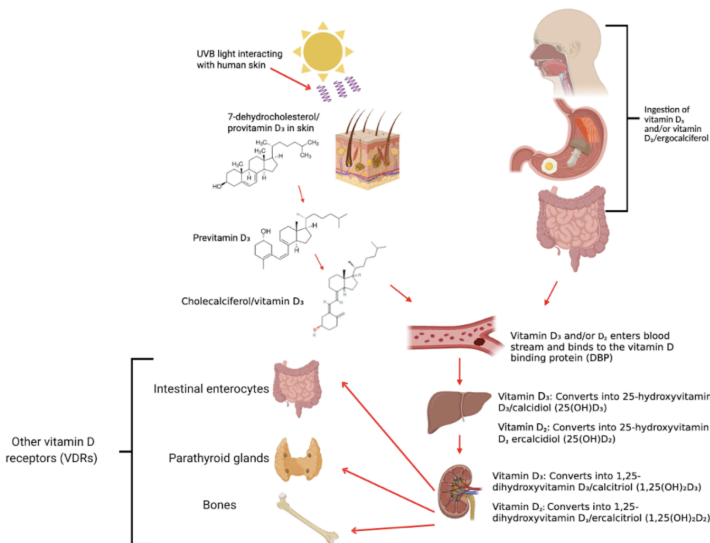


Reprinted from Sirajudeen, S., Shab, I., & Al Menhali, A. (2019). A Narrative Role of Vitamin D and Its Receptor: With Current Evidence on the Gastric Tissues. *International Journal of Molecular Sciences*, 20(15). <https://doi.org/10.3390/ijms20153832>

Natural Synthesis of Vitamin D Through UVB Exposure and Dietary Choices

The two main ways of generating vitamin D are skin exposure to ultraviolet B (UVB) light and dietary decisions. The most common way of retaining vitamin D is through vitamin D-rich foods, which provide the body with both D₂ and D₃ (Fig. 2). Once consumed, 25-hydroxylase enzymes obtained from the blood in the liver turn the vitamin D₂ and/or D₃ into calcidiol, which is released into the blood for the kidneys to convert it into calcitriol, the active form of the vitamin (Fig. 2). Afterward, the calcitriol molecules will bind to the vitamin D receptor (VDR) of cells in the intestine, kidneys, bone, and parathyroid gland to maintain blood calcium homeostasis and promote bone development (Lips, 2006). The second most common way of retaining vitamin D is through skin exposure to UVB light, which triggers the skin’s 7-dehydrocholesterol (7-DHC) synthesizing provitamin D₃, a precursor to previtamin D₃, then vitamin D₃ (Fig. 2). Then, the vitamin D₃ will enter the bloodstream and follow a similar pathway to consumed vitamin D-rich foods (Fig. 2). However, when retaining vitamin D₃ from the sun, the skin’s melanin and sunscreen that protects the skin from Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC), and/or Melanoma, absorbs 99% of UVB radiation, therefore, decreasing the skin’s synthesis of vitamin D₃ (Chen et al., 2008).

Figure 2.
Vitamin D₃ and D₂ Pathways of Retention Through Sunlight Compared to Diet



SPRING 2026

Fortified Foods*Types of Fortified Foods and Their Impact*

One key cause of vitamin D₂ and D₃ deficiency is a lack of vitamin D-rich foods in one's diet (Cashman, 2019). This is likely because vitamin D₂ and D₃ naturally occur in very few foods, for example: fatty fish (e.g. salmon), fish oils, and egg yolks. In a study by Chen et al. (2008), it is discovered that on average, every 100 g of wild salmon contains 500-1000 IU of vitamin D₃; however, only 100-250 IU of vitamin D₃ every 100 g is found in farmed salmon (Chen et al., 2008). They conclude that although vitamin D₃ occurs naturally in our food chain, it is limited in the pelleted food fed to farmed salmon. Although farmed seafood sources are lower in vitamin D₃ compared to naturally caught fish, they still have a generally high amount of vitamin D₃ compared to other sources (Schmid & Walther, 2013).

Issues with Accessibility and Consumption

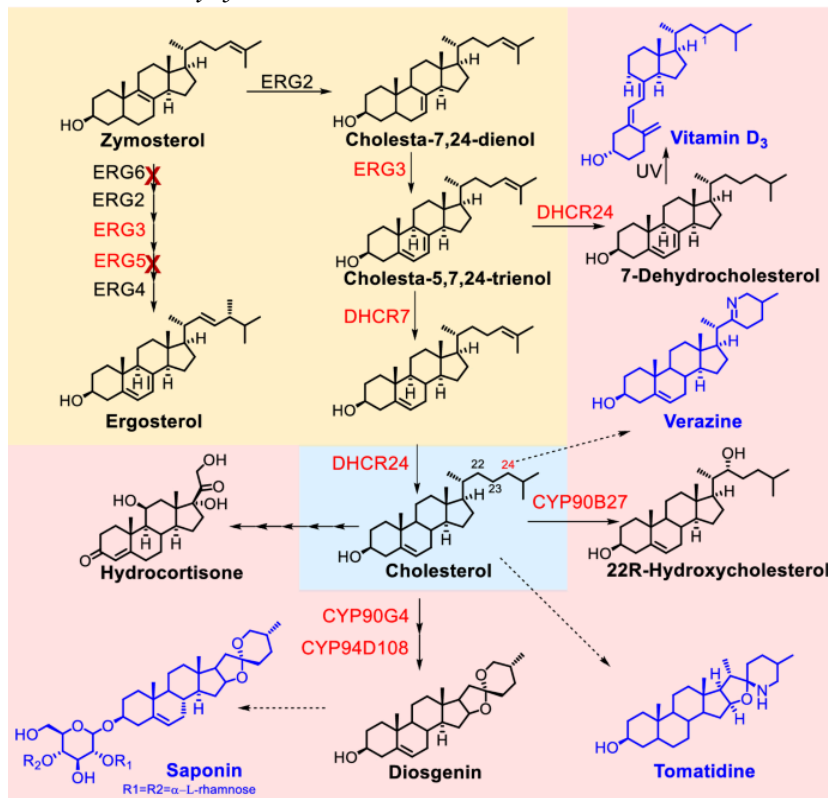
However, farmed seafood sources are more expensive than other protein options, causing lower-income households to opt for more affordable (though vitamin D-scarce) alternatives, leading to decreased vitamin D intake in lower-income households (Love et al., 2022). Additionally, due to the relative scarcity of natural vitamin D₃, many governments biofortify foods such as plants and fungi to contain vitamin D₂, and there are many D₂ supplements available to the public through pharmacies and online retailers (Wilson et al., 2017). In this way, even lower-income communities can afford other vitamin D-containing alternatives. However, despite these public services, many consumers and sources online have mixed opinions regarding the safety, nutritional value and regulation of biofortified foods and supplements (Bawa & Anilakumar, 2012). Thus, education and awareness are necessary to address public skepticism.

Genetic Engineering Approaches to Biofortify Organisms with Vitamin D

Saccharomyces cerevisiae (*S. cerevisiae*), also known as baker's yeast, is already commonly used in foods, drinks and supplements, suggesting that vitamin D would be easy to integrate into consumer products, unlike bacteria that may cause more concern from consumers. Although using bacteria like *Escherichia coli* (*E. coli*) is very popular in genetic modification research projects, its lack of the essential metabolic pathways helpful for vitamin D₃ synthesis means that extra effort is required to insert several genes into a plasmid. Unlike *E. coli*, *S.cerevisiae* is one of the most promising options for vitamin D₃ biosynthesis, because the organism already contains a metabolic pathway that can be manipulated to produce provitamin D₃. On top of that, yeast is an eukaryotic organism, allowing it to utilize the enzymes required for sterol transformation, as it can synthesize

more complex human-like proteins. In fact, Guo et al successfully engineered yeast to produce 7-DHC by modifying its existing sterol pathway and adding human genes like *DHCR7*. With the optimization and modification of other regulatory genes, they manage to increase the cell's production of 7-DHC to reach 1.07 g/L in a 5-L bioreactor, obtaining a 26.9-fold improvement compared with the initial strain (Guo et al, 2018) (Fig. 3).

Figure 3.
The Sterol Pathway of Yeast



Reprinted from Xu, S., & Li, Y. (2020). Yeast as a promising heterologous host for steroid bioproduction. *Journal of Industrial Microbiology and Biotechnology*, 47(9-10), 829–843. <https://doi.org/10.1007/s10295-020-02291-7>

Recombination Technology and Horizontal Gene Transfer for Vitamin D Synthesis in *S. Cerevisiae*

Ergosterol, also known as provitamin D2, is a vital fungal sterol lipid for fungal cell membranes' regulation, stimulation, proliferation, and inducement of host cell pyroptosis in fungi (Bhattacharya, 2021;

SPRING 2026

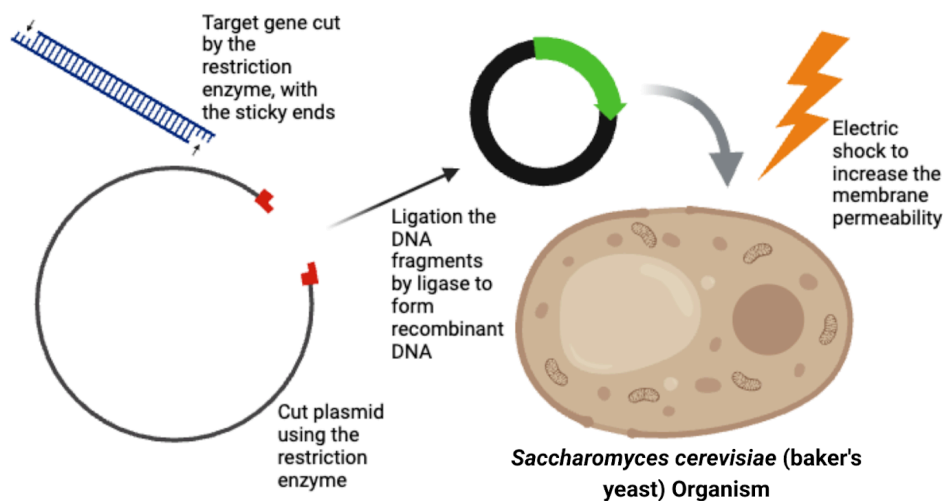
Jordá & Puig, 2020). Importantly, provitamin D₂ is a natural product of fungi and a precursor to vitamin D₂ (ergocalciferol) following exposure to ultraviolet radiation (Bhattacharya, 2021; Jordá & Puig, 2020; Rodrigues, 2018).

This suggests that, when exposed to UV radiation, high-yielding ergosterol strains converted into vitamin D₂ can be artificially selected or genetically engineered. For example, genetic and metabolic engineering are required to produce vitamin D₃ in *S. cerevisiae* as it cannot synthesize 7-dehydrocholesterol (7-DHC)—a key precursor of vitamin D₃. In this case, researchers employ techniques such as horizontal gene transfer to allow vitamin D₃ biosynthesis in *S. cerevisiae*. This process looks like introducing foreign genes from animals. For example, in yeast genomes, by introducing genes encoding cholesterol biosynthesizing enzymes, 7-DHC can be synthesized, allowing for it to be converted into vitamin D₃ upon UV exposure (Kessi-Pérez et al., 2022).

Typically, plasmid-based transformation techniques are used for the genetic modification of yeast. A plasmid is a small, circular, double-stranded DNA molecule that exists independently of the chromosomal DNA in bacterial and some eukaryotic cells (Helinski, 2022). It can be used as a carrier for foreign genetic material and transferred between bacteria through horizontal gene transfer. Scientists can use plasmids as vectors to insert, carry, or even express foreign genes in the target cell by introducing desired traits, leading to improved cellular functions. To construct a plasmid, suitable promoters are necessary to insert target genes (such as those coding for cholesterol biosynthesizing enzymes) into expression vectors. The promoter is necessary because it allows the RNA polymerase to bind to DNA, enabling the synthesis of the primary transcript (e.g. mRNA), which is then translated into the desired proteins or enzymes on ribosomes. Before the insertion of the DNA, a polymerase chain reaction (PCR) is required for gene amplification, allowing both specific target DNA sequences flanked by DNA primers to be copied by Taq DNA polymerase, and denaturing into strands at high temperatures (depending on the DNA's length and composition) (NIH, 2025). Once denatured, the amplified DNA is cut using restriction enzymes (proteins that can recognize and cut specific DNA sequences, such as palindromic DNA) before being inserted into bacterial plasmids (Helinski, 2022). Before introducing the DNA fragments into the host bacteria via transformation, the fragments are ligated using DNA ligase, allowing the bacteria to internalize the foreign recombinant DNA (Fig. 4).

Figure 4.

Internalization of Foreign Recombinant DNA Using Ligation in Plasmid and Electroporation



Created in BioRender. Zhang, B. (2026) <https://BioRender.com/ocsdn67>

Additionally, electroporation is when the cell is electrically shocked to create small pores in the cell membrane to increase cell permeability when accepting foreign DNA (Fig. 4) (Goldberg, Rubinsky, 2013). For yeast cells, electroporation can transiently disrupt the cell membrane using an electrical pulse to increase cell competency needed for absorbing the plasmids encoding target genes for a successful transformation (Rems et al., 2020). This procedure is demonstrated when Xiao-Jing Gou and his colleague create a plasmid using *DH5 α* genes from the bacteria *E. coli*. The popular *E. coli* strain, *DH5 α* , is effective at genetic cloning and plasmid propagation (Zhou et al. 2015). After transformation, the plasmid is expressed in the recombinant yeast cells with an inducible promoter and is activated in the presence of galactose. With the optimization and modification of other regulatory genes, they manage to increase the cell's production of 7-DHC to reach 1.07 g/L in a 5-L bioreactor, obtaining a 26.9-fold improvement compared with the initial strain (Guo et al., 2018).

CRISPR-Cas9 for Vitamin D Synthesis in the Solanum Lycopersicum (Moneymaker Tomato)

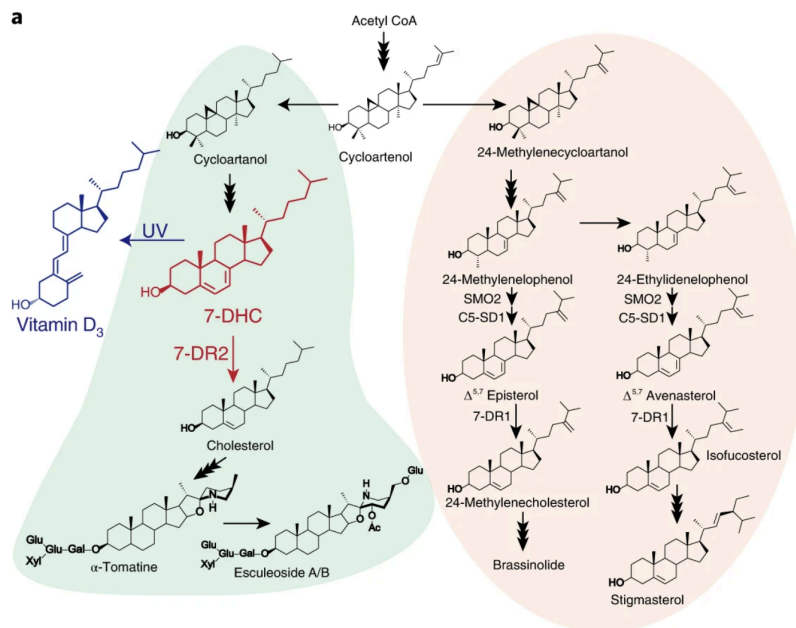
In addition to promoting biosynthesis of vitamin D from microorganisms, scientists also explore methods of increasing provitamin D₃ concentrations in plants through genetic modifications and metabolic engineering. Plants are the major component of the human diet, yet they are typically an insufficient source of vitamin D (Li et al., 2022). However, phytosterol, unlike ergosterol, is a sterol compound naturally produced by

SPRING 2026

plants that is structurally similar to cholesterol (Linus Pauling Institute, 2016). It serves both as a stabilizer for plant cell membranes' phospholipid bilayers, a structural support for the cell membranes (Moreau et al., 2002), and is a key precursor in plant sterol biosynthesis. Detailed investigation of the phytosterols biosynthesis pathway shows that 7-dehydrocholesterol (7-DHC)—also known as provitamin D₃—serves as a key intermediate of the pathway (Fig. 5). In tomatoes, for example, the Sl7-DR2 enzyme converts 7-DHC into cholesterol, which can then be used for the biosynthesis of steroidal glycoalkaloids (SGAs). As a result, 7-DHC typically does not accumulate significantly in tomato fruits. However, GE techniques, such as CRISPR-Cas9, can be used to disrupt this enzymatic step, redirecting metabolic flux toward the accumulation of 7-DHC with minimal impact on the biosynthesis of phytosterol and brassinosteroid (Li et al., 2022). Since 7-DHC is the direct precursor to vitamin D₃ following UVB exposure, enhancing its concentration is a promising strategy for fortifying plant-based foods with vitamin D.

Figure 5.

7-DHC pathway in Solanum lycopersicum (Moneymaker tomato) from Sl7-DR2-homozygous-knockout lines.

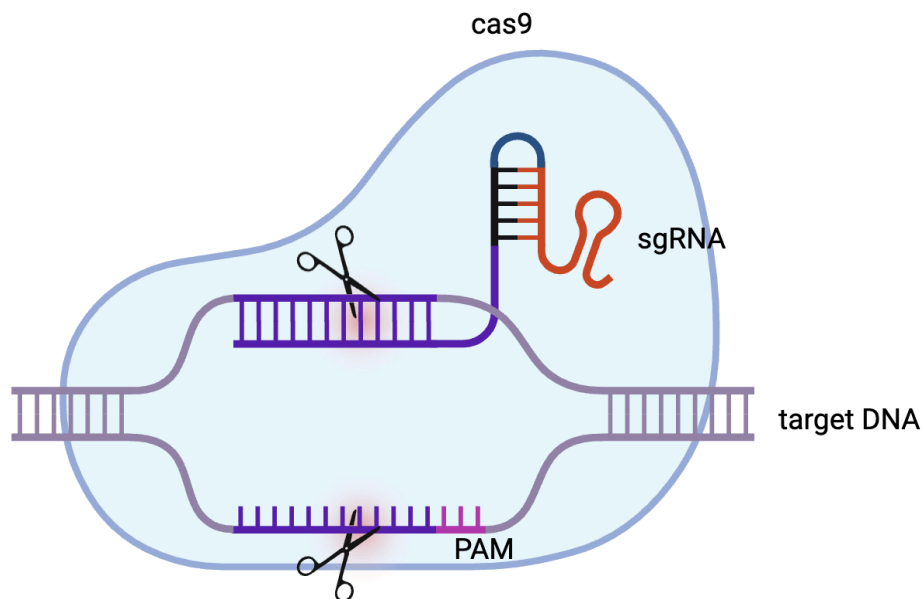


Reprinted from Li, J., Scarano, A., Gonzalez, N. M., Fabio D'Orso, Yue, Y., Nemeth, K., Gerhard Saalbach, Hill, L., Carlo, Moran, R., Santino, A., & Martin, C. (2022). Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants*, 8(6), 611–616. <https://doi.org/10.1038/s41477-022-01154-6>

SPRING 2026

To achieve this metabolic influx of 7-DHC, scientists utilize the CRISPR-Cas9 to edit the target gene involved in sterol biosynthesis (gene *Sl7-DR2*). When the tomato is first infected by the foreign DNA carried by the bacteria, the bacteria take a segment of the tomato's DNA sequence and insert it into the CRISPR spacer region. Then, the bacteria transcribe the CRISPR region into RNA to match the homologous DNA. Once a single guide RNA (sgRNA) is generated, the Cas9 endonuclease enzyme cleaves the DNA strand at the homologous spacer region, recognizing previous invaders (virus or plasmid) in the future. This recognition process requires protospacer-adjacent motifs (PAMs), short guanine-enriched sequences that act as restriction sites for the Cas9 (Li et al., 2023). The *Streptococcus pyogenes* Cas9, also known as SpCas9, prefers the NGG, a type of PAM, when guiding the Cas9 (Fig. 6). This process shows CRISPR-Cas9's precision in genome editing when it modifies the nucleotide sequence of a small segment of guide RNA.

Figure 6.
CRISPR-Cas9 Editing Sl7-DR2 gene for Sterol Biosynthesis



Created in BioRender. Zhang, B. (2026) <https://BioRender.com/79u2dgc>

In the transgenic tomato study above, CRISPR-Cas9 plays a pivotal role in knocking out five independent *Sl7-DR2* genes to increase 7-DHC synthesis with few disruptions to phytosterol and brassinosteroid biosynthesis (Li et al., 2022). Soon after, when the fortified tomatoes are exposed to UVB radiation, they yield nearly 200 μg of vitamin D_3 /per gram of dry weight, and the green and red fruits produce 0.3 $\mu\text{g}/\text{g}$ and 0.2 $\mu\text{g}/\text{g}$, respectively (Li et al., 2022). From the *Sl7-DR2*-mutant lines, a medium-sized green fruit

SPRING 2026

tomato with a dry weight of 8–10 g produces around 30% of the recommended daily allowance (RDA) of vitamin D₃, and in red fruit, it could produce 20% of the RDA (Li et al., 2022). Further UVB exposure of the 7-DHC through sun drying could likely further increase its vitamin D₃ levels (Li et al., 2022).

APPLICATIONS OF VITAMIN D-FORTIFIED ORGANISMS

Vitamin D Fortified Foodstuffs and Potential Concerns

The benefit of genetically engineered yeast strains lies in their application to commercial baking or brewing, wherein they produce vitamin D-enriched foods or beverages, such as beers and bread. It can also be fermented to produce yeast-based vitamin D-enriched sustenance or added directly to foods as a umami flavour enhancer, a vegan-friendly alternative to traditional animal-derived vitamin D₃ supplements such as tablets and or capsules. However, these genetic modifications require experimental investigations as they could risk metabolic imbalance in yeast, potentially affecting its viability or productivity in the environment (Kessi-Pérez et al., 2022). Furthermore, the risks of vitamin D fortified foods include vitamin D toxicity resulting in mild hypercalcemia symptoms, such as fatigue, reduced appetite, musculoskeletal pains, and severe symptoms like apathy, agitation, or even problems with movement and staying conscious (National Library of Medicine, 2023).

Li et al.'s (2022) experiment shows that enriching tomatoes with vitamin D is an accessible dietary alternative to addressing vitamin D deficiency. Their verification of the high provitamin D levels in tomato leaves compared to its fruit offers the potential for vitamin D synthesis upon UVB radiation (Reuters, 2022). This synthesized vitamin D from tomato leaves can be further extracted and made into vitamin D₃ supplements, or be used for food fortification, promoting a zero-waste culture where the entirety of the tomato plant is utilized (Reuters, 2022). Crucially, other plants in the nightshade family contain similar phytosterol biochemical pathways, suggesting that there is potential for the application of similar fortification strategies on “closely related plants such as aubergine, potato, and pepper” (Reuters, 2022). Despite this, there is some worry regarding the credibility of accurately analyzing vitamin D₃ levels in complex plant matrices, and toxicity levels in tomato leaves may hinder their viability as a source of vitamin D₃ (Black et al., 2017).

Lastly, genetically modified bacteria are also a solution to vitamin D deficiency because they can be manufactured into different forms of vitamin D supplementation, such as suspensions, powders and capsules. This is closely related to an experiment done by Dyrda-Terniuk et al. (2025), testing different vitamin D₃ bioavailability in lactic acid bacteria (LAB) samples, a natural probiotic for gut health. Their test samples include *Lactiplantibacillus plantarum* B/00401 strain in a biomass form and a lyophilized powder form. Their results indicate that independent variables such as the light/dark exposure, mixing/non-mixing, cream/bacteria ratio, and incubation time have a great effect on enhancing vitamin D₃. In particular, non-mixing conditions and light

SPRING 2026

exposure have the greatest effect. This connects to how transgenic techniques can be paired with different environmental factors (e.g., UVB light) to increase vitamin D₃ bioavailability in organisms. Therefore, the application of transgenic techniques could also be employed in the LAB, allowing for vitamin D₃ fortified lactic acid necessary for yogurt production. However, changes in the gut microbiomes' bacterial composition will occur because the high vitamin D intake can affect the species' richness. For example, when Bellerba et al. increase vitamin D supplementation levels, *Veillonellaceae* and *Oscillospiraceae* families in the *Firmicutes* phylum decrease. Hence, more research is necessary to understand how to balance vitamin D intake and gastrointestinal microbiota health (Bellerba et al., 2021).

Although transgenic and mutagenesis techniques used in genetically modified (GM) crops offer promising outcomes, concerns from both ethical and public perspectives arise regarding their technological limitations, cost, accessibility, and long-term sustainability.

IMPLICATIONS OF LARGE-SCALE BIOFORTIFIED FOOD PRODUCTION FOR VITAMIN D SUPPLY

Technological Limitations

By 2030, it is estimated that the biotechnology industry's market will have made \$30 trillion, meaning their production rates will be $\frac{1}{3}$ of manufacturing companies worldwide (Candelon et al., 2022). However, the industry's rapidly increasing production rate using commercial reactors while maintaining performance levels is one of the most difficult challenges industries face (Abbate et al., 2023).

These technical hurdles when scaling up production often come at the expense of production quality, especially when there are biological limitations to the host cell. For example, Yang et al.'s experiment is conducted on the melatonin production in *E. coli* to investigate increasing melatonin rates. The conclusion is that increasing production rates is difficult because melatonin inhibits *E. coli* growth (Yang et al., 2022). A hurdle such as this is similar to the hurdles experienced by Black et al. (stated above), and hence, further research into the biological limitations of the experiments is necessary.

Cost and Accessibility

Economic Feasibility of Bioengineered Solutions for Vitamin D. In addition to technical hurdles, there are harms and benefits of GM crops to the socioeconomics of countries. On the one hand, bioengineering companies have started monopolizing the farming economy by controlling the yearly repurchasing of expensive GM crop seeds (or risk patent infringement). As a result, sustainable practices such as seed-saving are punished, and pest- or blight-resistant biodiversity is discouraged.

On the other hand, GM crops like *Bacillus thuringiensis* (Bt) corn—with specific pest resistance—increase farmers' crop yields through reduced damage to crops (Aid, 2016; Bt Corn - UT Crops, 2025). In fact, in developing countries such as India, China and South Africa, crop yields increase when GM rice and corn are used in regions with high pest populations and inadequate pest management systems (Lemaux, 2009). However, while Bt corn is resistant to some pests, that resistance is specifically engineered and cannot react to new threats.

Barriers to Equitable Distribution in Developing Countries. Other considerations when using GM crops include national biosafety regulations, consumer attitudes toward GM products, dietary choices, and crop conditions (Edmeades & Smale, 2006). As with GM corn and rice, these considerations will affect which different strains are inserted into the crop's genome. For example, in humid climates with higher pest populations, pest resistance will be prioritized. Likewise, in countries that are large exporters of a certain crop, priority will be given to herbicide resistance. Finally, in countries struggling with vitamin D insufficiency or deficiency, the vitamin-D-producing (7-DHC) strains in agriculture will be prioritized.

However, private companies that develop GM crops have lowered incentives to help smallholder farmers. The lowered incentives mean that developing countries will lack access to tailored agricultural benefits, given that smallholders play a vital role in the agricultural production of these countries (Dowd-Uribe et al., 2023). Even with the significant impact of GM crops, issues with their equitable distribution will disproportionately impact smallholder farmers. To address this barrier, governments and private companies collaborate in systems such as public-private partnerships (PPP). In African countries such as Morocco, Egypt, and Sudan, some suitable GM crops have already been equitably distributed by such PPPs (Dowd-Uribe et al., 2023). Similar systems can be enforced in other developing countries for suitable GM crop strains to ensure equitable distribution worldwide.

SPRING 2026

Long-Term Sustainability

Concerns about Dependency on Bioengineering Solutions. As bioengineering solutions become more integrated in agriculture and food production, fears surrounding the environmental and economic consequences of society's gradual overdependence on these technologies increase (Abdul Aziz et al., 2022). Indeed, bioengineering technologies have been found to decrease crop biodiversity when genetic pollution occurs. Put differently, bioengineering has a detrimental effect on the process in which pollinators cross-pollinate between a GM crop and a non-GM crop (Dida, 2022). As a result, the genetic differences between plants decrease, and further genetic modification becomes ineffective, as happened in India in 2002.

In 2002, India's GMO cotton was modified to produce a Bt chemical for pest resistance, specifically to the cotton bollworm (Pt et al., 2022). Unlike the GM cotton, non-GM cotton varieties lack the Bt chemical, ensuring that the cotton bollworm cannot develop resistance to the Bt chemical (Pt et al., 2022). However, because 5-30% of cotton strains cross-pollinate, many non-GM cotton varieties develop the Bt chemical, undermining long-term pest resistance (Pt et al., 2022). This cross-pollination eventually creates a monoculture that is ineffective at resisting the ever-evolving insects, and the risk of blights or crop die-offs increases.

Environmental and Economic Sustainability of Large-scale Implementation. To prevent genetic pollution, several methods are currently under development. These new methods will likely be applied to ensure the sustainability of all GM crops, including vitamin D-synthesizing products. These methods include male and seed sterility, transgene excision, and delayed flowering, which either stop or reduce the GM crops' ability to produce pollen or misalign the pollination timeframe of non-GM and GM crops (Stockdale et al., 2023). Through such efforts, GM crops will be able to tackle the prevalent issues countries face while addressing the fears surrounding their sustainability.

Future Directions

As the world of agriculture and food production is rapidly growing, so is synthetic biology and biotechnology. Innovative projects continue to drive meaningful change in quality of life and sustainability goals, from developed initiatives like the vitamin A-producing golden rice experiment addressing malnutrition, to emerging technologies like precision fermentation to protein-engineer food ingredients for food waste reduction, or cultivated meat made from stem cells that could potentially reduce that can reduce 92% of greenhouse gas emissions (Mirsalami & Mirsalami, 2025; Swartz, 2021). If biotechnologies continue to advance along these lines, global problems such as food production and the loss of viable farmland could be addressed and even eliminated.

SPRING 2026

Emerging technologies have the potential to further enhance agriculture and food production, creating numerous opportunities for continued market expansion. Furthermore, because vitamin D deficiency and insufficiency are pressing issues that many countries face, this market expansion suggests similar prospects for vitamin D biofortification. If vitamin D were to be integrated into many staple foods, such as baked goods and fermented drinks, populations could be assured that vitamin D would be accessible even in developing countries that struggle to implement expensive or culturally taboo policies, such as many countries in the Middle East and South Asia (Siddiquee et al., 2021).

CONCLUSION

Key bioengineering advancements, including transgenic yeast using horizontal gene transfer and recombination techniques and mutant tomatoes using CRISPR-Cas9, fortify efforts to eliminate vitamin D insufficiency and deficiency. These synthetic biological and bioengineering experiments demonstrate that the modification of microorganisms and plants is a viable strategy for addressing vitamin D deficiency and insufficiency. Thus, in order to increase the world community's quality of life, new research must include multidisciplinary approaches so that effective, sustainable solutions (that are aligned with the contexts and frameworks of different countries) can be implemented as soon as possible.

REFERENCES

- Abbate, E., Andrión, J., Apel, A., Biggs, M., Chaves, J., Cheung, K., Ciesla, A., Clark-El Sayed, A., Clay, M., Riarose Contridas, Fox, R., Hein, G., Held, D., Horwitz, A., Jenkins, S., Karolina Kalbarczyk, Krishnamurthy, N., Mirsiaghi, M., Noon, K., & Rowe, M. (2023). Optimizing the strain engineering process for industrial-scale production of bio-based molecules. *Journal of Industrial Microbiology & Biotechnology*, 50(1). <https://doi.org/10.1093/jimb/kuad025>
- Abdul Aziz, M., Brini, F., Rouached, H., & Masmoudi, K. (2022). Genetically engineered crops for sustainably enhanced food production systems. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.1027828>
- Aziz, M. A., Faical Brini, Hatem Rouached, & Khaled Masmoudi. (2022). Genetically engineered crops for sustainably enhanced food production systems. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.1027828>
- Bawa, A. S., & Anilakumar, K. R. (2012). Genetically modified foods: safety, risks and public concerns—a review. *Journal of Food Science and Technology*, 50(6), 1035–1046. <https://doi.org/10.1007/s13197-012-0899-1>
- Bellerba, F., Muzio, V., Gnagnarella, P., Facciotti, F., Chiocca, S., Bossi, P., Cortinovis, D., Chiaradonna, F., Serrano, D., Raimondi, S., Zerbato, B., Palorini, R., Canova, S., Gaeta, A., & Gandini, S. (2021). The

- Association between Vitamin D and Gut Microbiota: A Systematic Review of Human Studies. *Nutrients*, 13(10), 3378–3378. <https://doi.org/10.3390/nu13103378>
- Bhattacharya, S. (2021). Ergosterol Synthesis. *Elsevier eBooks*, 230–238. <https://doi.org/10.1016/b978-0-12-819990-9.00070-6>
- Biz, C., & Bragazzi, N. L. (2022). Big data in orthopedics. *Big Data Analytics for Healthcare*, 291–300. <https://doi.org/10.1016/b978-0-323-91907-4.00010-8>
- Black, L., Lucas, R., Sherriff, J., Lars Björn, & Bornman, J. (2017). In Pursuit of Vitamin D in Plants. *Nutrients*, 9(2), 136–136. <https://doi.org/10.3390/nu9020136>
- Bouillon, R., Verlinden, L., & Annemieke Verstuyf. (2016). Is Vitamin D2 Really Bioequivalent to Vitamin D3? *Endocrinology*, 157(9), 3384–3387. <https://doi.org/10.1210/en.2016-1528>
- Candelon, F., Gombeaud, M., Stokol, G., Patel, V., Gourévitch, A., & Goedel, N. (2022, February 10). *Synthetic Biology Is About to Disrupt Your Industry*. BCG Global. <https://www.bcg.com/publications/2022/synthetic-biology-is-about-to-disrupt-your-industry>
- Carter, M., & Shieh, J. C. (2010). Gene Delivery Strategies. *Guide to Research Techniques in Neuroscience*, 229–242. <https://doi.org/10.1016/b978-0-12-374849-2.00010-0>
- Cashman, K. D. (2019). Vitamin D Deficiency: Defining, Prevalence, Causes, and Strategies of Addressing. *Calcified Tissue International*, 106(1), 14–29. <https://doi.org/10.1007/s00223-019-00559-4>
- Chen, T. C., Farhad Chimeh, Lu, Z., Mathieu, J., Person, K. S., Zhang, A., Kohn, N., Martinello, S., Berkowitz, R., & Holick, M. F. (2007). Factors that influence the cutaneous synthesis and dietary sources of vitamin D. *Archives of Biochemistry and Biophysics*, 460(2), 213–217. <https://doi.org/10.1016/j.abb.2006.12.017>
- Cheng, X., Li, H., Tang, Q., Zhang, H., Liu, T., & Wang, Y. (2024). Trends in the global commercialization of genetically modified crops in 2023. *Journal of Integrative Agriculture*, 23(12), 3943–3952. <https://doi.org/10.1016/j.jia.2024.09.012>
- Cleveland Clinic. (2025, May). *Breaking Down Vitamin D, D2 and D3*. Cleveland Clinic. <https://health.clevelandclinic.org/vitamin-d-vs-d3>
- Cui, A., Zhang, T., Xiao, P., Fan, Z., Wang, H., & Zhuang, Y. (2023). Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. *Frontiers in Nutrition*, 10. <https://doi.org/10.3389/fnut.2023.1070808>
- Dida, G. (2025). Ecological and Evolutionary Consequences of Gene Flow through Pollen from Transgenic Crops to Their Wild Relatives. *Journal of Ecology & Natural Resources*, 6(4). <https://doi.org/10.23880/jenr-16000315>
- Dowd-Urbe, B., Rock, J. S., Spreadbury, T., Chiril, P., & Uminsky, D. (2023). Bridging the gap? Public–private partnerships and genetically modified crop development for smallholder farmers in Africa. *Plants People Planet*, 6(2), 437–451. <https://doi.org/10.1002/ppp3.10453>

SPRING 2026

-
- Dyrda-Terniuk, T., Railean, V., Florkiewicz, A. B., Walczak-Skierska, J., Kolankowski, M., Rudnicka, J., Białczak, D., & Pomastowski, P. (2025). The impact of *Lactiplantibacillus plantarum* on the cream composition: Insight into changes of vitamin D3 content and fatty acid composition. *International Dairy Journal*, 161, 106118. <https://doi.org/10.1016/j.idairyj.2024.106118>
- Edmeades, S., & Smale, M. (2006). A trait-based model of the potential demand for a genetically engineered food crop in a developing economy. *Agricultural Economics*, 35(3), 351–361. <https://doi.org/10.1111/j.1574-0862.2006.00167.x>
- Farm Aid. (2016, March 17). *GMOs — Top five concerns for family farmers – Farm Aid*. Farm Aid. <https://www.farmaid.org/issues/gmos/gmos-top-5-concerns-for-family-farmers/>
- Golberg, A., & Rubinsky, B. (2010). The Effect of Electroporation Type Pulsed Electric Fields on DNA in Aqueous Solution. *Technology in Cancer Research & Treatment*, 9(4), 423–430. <https://doi.org/10.1177/153303461000900412>
- Guo, X.-J., Xiao, W.-H., Wang, Y., Yao, M.-D., Zeng, B.-X., Liu, H., Zhao, G.-R., & Yuan, Y.-J. (2018). Metabolic engineering of *Saccharomyces cerevisiae* for 7-dehydrocholesterol overproduction. *Biotechnology for Biofuels*, 11(1). <https://doi.org/10.1186/s13068-018-1194-9>
- Helinski, D. R. (2022). A Brief History of Plasmids. *EcoSal Plus*, 10(1). <https://doi.org/10.1128/ecosalplus.esp-0028-2021>
- Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: a worldwide problem with health consequences. *The American Journal of Clinical Nutrition*, 87(4), 1080S1086S. <https://doi.org/10.1093/ajcn/87.4.1080s>
- Jordá, T., & Puig, S. (2020). Regulation of Ergosterol Biosynthesis in *Saccharomyces cerevisiae*. *Genes*, 11(7), 795–795. <https://doi.org/10.3390/genes11070795>
- Kessi-Pérez, E. I., González, A., Palacios, J. L., & Martínez, C. (2022). Yeast as a biological platform for vitamin D production: A promising alternative to help reduce vitamin D deficiency in humans. *Yeast*, 39(9), 482–492. <https://doi.org/10.1002/yea.3708>
- Lemaux, P. G. (2009). Genetically Engineered Plants and Foods: A Scientist’s Analysis of the Issues (Part II). *Annual Review of Plant Biology*, 60(1), 511–559. <https://doi.org/10.1146/annurev.arplant.043008.092013>
- Li, J., Scarano, A., Gonzalez, N. M., Fabio D’Orso, Yue, Y., Nemeth, K., Gerhard Saalbach, Hill, L., Carlo, Moran, R., Santino, A., & Martin, C. (2022). Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants*, 8(6), 611–616. <https://doi.org/10.1038/s41477-022-01154-6>
- Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., He, X., Liu, M., Li, P., & Yu, T. (2023). CRISPR/Cas9 therapeutics: progress and prospects. *Signal Transduction and Targeted Therapy*, 8(1). <https://doi.org/10.1038/s41392-023-01309-7>
- Lips, P. (2006). Vitamin D physiology. *Progress in Biophysics and Molecular Biology*, 92(1), 4–8. <https://doi.org/10.1016/j.pbiomolbio.2006.02.016>

SPRING 2026

-
- Lips, P., de Jongh, R. T., & van Schoor, N. M. (2021). Trends in Vitamin D Status Around the World. *JBMR Plus*, 5(12). <https://doi.org/10.1002/jbm4.10585>
- Love, D. C., Thorne-Lyman, A. L., Conrad, Z., Gephart, J. A., Asche, F., Godo-Solo, D., McDowell, A., Nussbaumer, E. M., & Bloem, M. W. (2022). Affordability influences nutritional quality of seafood consumption among income and race/ethnicity groups in the United States. *The American Journal of Clinical Nutrition*, 116(2), 415–425. <https://doi.org/10.1093/ajcn/nqac099>
- Mayo Clinic. (2025). Polymyalgia rheumatica-Polymyalgia rheumatica - Symptoms & causes. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/polymyalgia-rheumatica/symptoms-causes/syc-20376539>
- Mayo Clinic. (2025) Rickets-Rickets - Symptoms & causes. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/rickets/symptoms-causes/syc-20351943>
- Mirsalami, S. M., & Mirsalami, M. (2025). Advances in Genetically Engineered Microorganisms: Transforming Food Production through Precision Fermentation and Synthetic Biology. *Future Foods*, 100601–100601. <https://doi.org/10.1016/j.fufo.2025.100601>
- Morales-Villar, A. B., Maldonado-Hernández, J., Eduardo Álvarez-Licona, N., Piña-Aguero, M. I., Villalpando-Hernández, S., Robledo-Pérez, R. M., Díaz-Rangel, I., Barbosa-Cortés, M. de L., & Núñez-García, B.-A. (2024). Determinants of Vitamin D Status in Healthy Young Adults from Mexico City. *Archives of Medical Research*, 55(3), 102968. <https://doi.org/10.1016/j.arcmed.2024.102968>
- Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*, 41(6), 457–500. [https://doi.org/10.1016/s0163-7827\(02\)00006-1](https://doi.org/10.1016/s0163-7827(02)00006-1)
- National Human Genome Research Institute. (2025). *Transgenic*. Genome.gov. <https://www.genome.gov/genetics-glossary/Transgenic#:~:text=Transgenic%20refers%20to%20an%20organism,the%20laboratory%20for%20research%20purposes>.
- National Library of Medicine. (2019). Public Health Aspects in the Prevention and Control of Vitamin Deficiencies. *Current Developments in Nutrition*, 3(9), nzz075. <https://doi.org/10.1093/cdn/nzz075>
- National Library of Medicine. (2022, September 19). *Genetics, Mutagenesis*. Nih.gov; StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK560519/>
- National Library of Medicine. (2023a, March 6). *Polymerase Chain Reaction (PCR)*. Nih.gov; StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK589663/>
- National Library of Medicine. (2023b, May 24). *Vitamin D Toxicity*. Nih.gov; StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK557876/#:~:text=History%20and%20Physical&text=A%20dietary%20history%20is%20essential,maintained%20based%20on%20historical%20information>
- National Library of Medicine. (2025, February 15). *Vitamin D Deficiency*. Nih.gov; StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK532266/>

-
- Phillips, T. (2008). *Genetically Modified Organisms (GMOs)*. Nature.com. <https://www.nature.com/scitable/topicpage/genetically-modified-organisms-gmos-transgenic-crops-and-732/#:~:text=Some%20benefits%20of%20genetic%20engineering,to%20the%20world%27s%20growing%20population.>
- Pt, P., Ta, D., Ya, V., & Kg, K. (2022). Genetic pollution in plants: A review. ~ 331 ~ *the Pharma Innovation Journal*, 11(5), 331–334. <https://www.thepharmajournal.com/archives/2022/vol11issue5/PartD/11-4-347-314.pdf>
- Rems, L., Kasimova, M. A., Testa, I., & Delemotte, L. (2020). Pulsed Electric Fields Can Create Pores in the Voltage Sensors of Voltage-Gated Ion Channels. *Biophysical Journal*, 119(1), 190–205. <https://doi.org/10.1016/j.bpj.2020.05.030>
- Reuters. (2022, May 24). *No sun no problem: Scientists gene-edit tomatoes with vitamin D*. Daily Sabah. <https://www.dailysabah.com/life/science/no-sun-no-problem-scientists-gene-edit-tomatoes-with-vitamin-d>
- Rodrigues, M. L. (2018). The Multifunctional Fungal Ergosterol. *MBio*, 9(5). <https://doi.org/10.1128/mbio.01755-18>
- Schmid, A., & Walther, B. (2013). Natural Vitamin D Content in Animal Products. *Advances in Nutrition*, 4(4), 453–462. <https://doi.org/10.3945/an.113.003780>
- Siddiquee, M. H., Bhattacharjee, B., Siddiqi, U. R., & Rahman, M. M. (2021). High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis. *BMC Public Health*, 21(1). <https://doi.org/10.1186/s12889-021-11888-1>
- Singhai, A., & Banzal, S. (2013). Severe Vitamin D deficiency causing kyphoscoliosis. *Journal of Family Medicine and Primary Care*, 2(4), 384. <https://doi.org/10.4103/2249-4863.123930>
- Sirajudeen, S., Shah, I., & Al Menhali, A. (2019). A Narrative Role of Vitamin D and Its Receptor: With Current Evidence on the Gastric Tissues. *International Journal of Molecular Sciences*, 20(15), 3832–3832. <https://doi.org/10.3390/ijms20153832>
- Smith, M. (2025). *Genetic Engineering*. Genome.gov. <https://www.genome.gov/genetics-glossary/Genetic-Engineering>
- Stockdale, J. N., & Millwood, R. J. (2023). Transgene Bioconfinement: Don't Flow There. *Plants*, 12(5), 1099–1099. <https://doi.org/10.3390/plants12051099>
- Swartz, E. (2021, January 28). *The science of cultivated meat*. The Good Food Institute. <https://gfi.org/science/the-science-of-cultivated-meat/>
- The University of Tennessee Institute of Agriculture (2025). Bt Corn. <https://www.google.com/url?q=https://utcroplab.com/corn/insects/bt-corn/&sa=D&source=docs&ust=1751238041606441&usq=AOvVaw37YrC2C9ip83Xd7U90LjCj>

SPRING 2026

- Wilson, L. R., Tripkovic, L., Hart, K. H., & Lanham-New, S. A. (2017). Vitamin D deficiency as a public health issue: using vitamin D₂ or vitamin D₃ in future fortification strategies. *Proceedings of the Nutrition Society*, 76(3), 392–399. <https://doi.org/10.1017/s0029665117000349>
- Xu, S., & Li, Y. (2020, October 1). *Yeast as a promising heterologous host for steroid bioproduction*. *Journal of Industrial Microbiology and Biotechnology*; Oxford Academic Platform. <https://academic.oup.com/jimb/article/47/9-10/829/6046084>
- Yang, L., Malla, S., Özdemir, E., Kim, S. H., Lennen, R., Christensen, H. B., Christensen, U., Munro, L. J., Herrgård, M. J., Kell, D. B., & Palsson, B. Ø. (2022). Identification and Engineering of Transporters for Efficient Melatonin Production in *Escherichia coli*. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.880847>
- Zhang, B (2026a). CRISPR-Cas9 Editing Sl7-DR2 gene for Sterol Biosynthesis. <https://BioRender.com/79u2dgc>
- Zhang, B (2026b). Internalization of Foreign Recombinant DNA Using Ligation in Plasmid and Electroportation. <https://BioRender.com/ocsdn67>
- Zhang, B (2026c). Vitamin D₃ and D₂ Pathways of Retention Through Sunlight Compared to Diet. <https://BioRender.com/e9y9k2b>
- Zhou, H., Vonk, B., Roubos, J. A., Bovenberg, R. A. L., & Voigt, C. A. (2015). Algorithmic co-optimization of genetic constructs and growth conditions: application to 6-ACA, a potential nylon-6 precursor. *Nucleic Acids Research*, gkv1071. <https://doi.org/10.1093/nar/gkv1071>