**Malnutrition in Northeast India: Prevalence, Consequences, and Emerging Biotechnological Interventions**

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**Abstract**Excessive or insufficient nutritional intake is referred to as malnutrition. Vitamin D can be synthesis in the skin through a process that depends on exposure to sunshine or can come from a very small number of food sources. Such as greasy seafood and egg yolk. Those who live in mid- or high-latitude regions are most impacted by the vitamin D deficiency, which is caused by limited sun exposure.Although there is often a lot of sunshine exposure in tropical or subtropical regions, risk factors like darker skin color, air pollution, and covering one's skin for cultural or religious reasons can all contribute to vitamin D insufficiency. In North East 64.5 % people highly affected the vitamin D deficiency in India. People in North-East India highly depended on grain, particularly rice, to get their nourishment. Their primary grain preference is essentially rice, with maize coming in second. Vitamin A, D, B12, and C deficiencies found in cereal grains. The tribal people typically have low nutritional status, and they can’t take vitamin rich food item regularly. The region produces a number of valuable crops, including tomatoes, potatoes, saubergines, cauliflower, and cabbage. Tribal people can benefit from vitamin sources without changing their dietary patterns by using advanced biotechnology technologies tool like CRIPER/Cas to modify the plants genome through boost the quantity of vitamin D contained in plants.

**Key words:** Malnutrition, Vitamin D Deficiency, North East India, and CRIPER/Cas 9.

 **Introduction**

Malnutrition is the imbalance of macronutrients or micronutrients in the body, resulting in either excess or deficiency, leading to an imbalance in the body's nutritional status (1). Among these vitamin D insufficiency to be the most prevalent dietary deficit and frequently one of the most undetected illnesses. In the Indian subcontinent, between 50 and 90 % of young children suffer from vitamin D insufficiency (2). Less than 10 % of vitamin D comes from food, with the majority content synthesized by skin ultraviolet B rays (UVB) explored. The photochemical conversion of pro-vitamin D3 in the skin produces vitamin D3, however the required UVB rays (290–315 nm). Consuming vitamin D through food becomes crucial, however vitamin D not found in many foods. The general public is unable to achieve their vitamin D needs as a result of poor food intake and insufficient sun-derived vitamin D (3). The impact is especially severe in the Northeastern Indian states, where physiological vitamin D production is limited by traditional clothing, dietary habits, and less sun exposure. Despite growing awareness, supplementation and fortification food & awareness programs face logistical and economic barriers in these regions. According to the Das et., al (2020) research exhibit only 35.5 % of people had appropriate amount of vitamin D in their bodies, the rest individuals had vitamin insufficiencies (4). Vitamin D deficiency in adults leads to osteomalacia, osteoporosis, muscle weakness, and increased falls risk, and its lead to chronic diseases like infectious, autoimmune, cardiovascular, diabetes, and cancer. Therefore, worldwide institutions, such as the Endocrine Society of the United States, advise prevention and therapy of vitamin D insufficiency. Age-group-specific vitamin D requirements range from 600 to 800 IU/day. High dose vitamin D levels (50,000 IU/week for 8 weeks) are required to treat vitamin D insufficiency in people who already have it. After this treatment, a maintenance regimen of 1500–2000 IU/d is administered (5). In addition to having a vast store of different steroidal compounds, plants may also provide vitamin D3. Even yet, the amounts of vitamin D3 found in some plants and algae are far lower than those found in animal sources. A 7-dehydrocholesterol (7-DHC) is the direct precursor for the synthesis of vitamin D in plants (6). The majority of plant doesn’t contain much amount vitamin D3, *Solanaceae* family plants including tomatoes, potatoes, and egg plats are notable for their natural accumulation of elevated cholesterol levels in steroidal glycoalkaloids (SGA) pathway. The partial separation of the biosynthesis of cholesterol and phytosterols provides metabolic flexibility for the production of more specific stress chemicals, such SGAs, which have insecticidal, fungicidal, and antibacterial qualities, as well as essential hormones (brassinosteroids) (7). This chapter explores the potential of CRISPR-Cas9 gene editing in enhancing vitamin D3 precursor accumulation in *Solanaceous* crops, thereby with change their food habitat to enhance their Vitamin D3 level, helping poor and tribal communities proposing a food-based intervention to address this regional malnutrition crisis.

**Metabolic pathway of Vitamin D3**

Cholecalciferol, the primary type of vitamin D produced by human skin, is produced when exposed to UVB radiation. Three double bonds (C5=C6, C7=C8, **C9=C10**) will break during this process, converting 7-DHC into pre-vitamin D. After that pre-vitamin D undergo the non-catalytic transformation to synthesis vitamin D3, it will metabolism in the liver and kidney (8). The vitamin D after transform into 25-hydroxyvitamin D and hormonal form (calcitriol), as well as in other tissues. Where the calcitriol produced has paracrine or autocrine functions, the main regulators of renal 1-hydroxylase (CYP27B1, the enzyme that produces calcitriol) are parathyroid hormone, FGF23, calcium, and phosphate, cytokines are involved in the control of extra renal 1-hydroxylase, which is different from that in the kidney (9). Similar to 1-hydroxylase, 24-hydroxylase is the primary enzyme responsible for catabolizing 25(OH)D and calcitriol. Although it is widely distributed in other tissues where its control is different from that of the kidney, it is closely regulate in the kidney in the opposite manner (10).

**Geographical, Climatic, and Cultural Contributors to Vitamin D3 Deficiency**

North East India states such as Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura highly affected the vitamin d deficiency in India, due the lack of consistent sunlight exposure, so low amount of vitamin D3 synthesized body when skin exposed through UVB rays (11). Their people lifestyle also influenced vitamin D deficiency and clothing, which may limit sun exposure, are frequently governed by cultural and societal taboos. The energy content of cereal grains is around 300–350 kcal/100g. Vitamin A, D, B12, and C deficiencies found in cereal grains. People in North-East India rely on grain, particularly rice, to get their nourishment. Their primary grain preference is essentially rice, with maize coming in second. Cereals are grown in 3714 thousand hectares of total land, which makes about 60 % of the region's cultivated area (12). Individuals from underprivileged backgrounds typically have low nutritional status. Most Indians cannot afford the fortified and supplements food sources of vitamin D. The prevalence of Vitamin D deficiency and insufficiency among young adult female nursing students in Northeast India was very high at 98.4%. Despite being urban, educated, and living in a progressive society with minimal cultural restrictions like veiling, most of students had sun exposure of less than one hour daily. Although the majority were non-vegetarian and aware that sunlight is a key source of Vitamin D, their practices-such as using sunscreen (SPF >15), limited outdoor activity, and low supplement use contributed to the deficiency. This research conducted during summer month, the study likely reflects a best-case scenario, with even worse rates expected in other seasons (13). These findings suggest sociocultural and dietary habits still strongly influence Vitamin D status. A study in East Khasi Hills, Meghalaya, found a high prevalence of vitamin D deficiency, especially in Jatah village (Mawkynrew block), where rickets cases among children were founded. Despite many rural participants engaging in outdoor physical work, deficiency persisted-likely due to limited UVB exposure from high altitude, cold winters, and frequent cloud cover. A weak positive correlation (r = 0.256) was observed between sun exposure and serum vitamin D levels. The tribal people doesn’t have awareness about vitamin D, 86.9 % didn’t recognize sunlight as a vitamin D source, and 85.9 % had no general knowledge about it. Dietary habits were also inadequate 60.4% didn’t consume milk, 69.8 % consumed only 1-4 eggs weekly or monthly, and 43.5 % ate rice with minimal red meat daily. Demographically, 30.6% were aged 30-35, 78.8% were Christians, 92.3% belonged to the Khasi ethnic group, and 52.3% had completed high school. Although 65.5% had physically active jobs with sun exposure over 30 minutes daily, deficiency remained common, suggesting environmental and dietary factors played a greater role than sun exposure alone (14). Due to the active transplacental transfer of calcium to the growing fetus, the issue is likely to get worse during pregnancy in a population that already has a high prevalence of vitamin D deficiency and inadequate dietary calcium intake. Skin pigmentation may exacerbate this issue, as growing urbanization leads to less outside exercise and increased pollution. In addition, milk the main source of calcium is a costly food in India. Most of people can’t take milk regularly because of poverty (15). A another study from North-Eastern part of India reported that 41 % of people **deficiency** and 14 % had vitamin D insufficiency, while 20 % of the controls had both conditions. Levels of 25(OH)D were significantly correlated with sun exposure, sunscreen usage, and vegetarianism. Dietary calcium consumption and multivitamin supplementation did not correlate with 25(OH)D levels (16). Research on vitamin D insufficiency in school children in Guwahati, Assam. In this study, they discovered that 14.2 % of students had vitamin D insufficiency and 8.4 % percent had deficiencies (17). Guwahati is 54 m above sea level and situated at 26 ̊ 06'N and 91 ̊ 35'E. An average of 6.23 hours of sunlight per day, or 2276.9 hours per year. These studies illustrates how diet, outdoor activities, and both rural and urban populations cause vitamin D deficiency. According to another study, 15.71 % of expectant mothers get enough vitamin D. The remaining 22.8 % of pregnant women had inadequate vitamin D, and 61.43% had a deficit. The majority of pregnant women's social and economic circumstances have an impact on vitamin deficiencies. According to this finding, a vitamin shortage during pregnancy may have an impact on the newborn's height, weight, head size, and chest circumference. Additionally, perinatal outcomes including Neonatal Intensive Care (NICU) hospitalization and Respiratory Distress Syndrome were linked to the mother's vitamin D level (18).

**Limitations of Current Interventions**

Recent research on vitamin D showed that those who are physically wealthy will take supplements, and their vitamin levels will significantly rise as a result. However, many people's levels remained inadequate based on their health condition. In the Indian market, vitamin D capsules with a dosage of 60,000 IU are the most popular form. It is available in powder form in sachets or oil-based capsules also recommended to be uptake once a week. However, regular weekly doses may lead to toxicity problems. Supplements with lesser dosages are more dependable for those who require 4000 IU daily to compensate for their vitamin D deficiency. Without understanding the patient's health status, the majority of pharmacists in India just prescribe medications, which might occasionally result in incorrect prescriptions, it will directed get health problems such vascular calcification and hypercalcemia (19). In India, doctors prescribe vitamin D3 medicine 60,000 IU per week for 8 weeks to treat vitamin D deficiency. Twenty-two healthy Indians with subnormal serum 25(OH)D levels were supplemented with oral D3 60,000 IU/week and calcium 1 gm/day for 8 weeks. At 8 weeks, mean 25(OH)D levels increased, and serum Parathyroid Hormone (PTH) normalized. However, all subjects were vitamin D deficient at 12 months (20). Since meat and dairy products provide the majority of the vitamin D supply, it might be difficult for vegetarians to maintain adequate vitamin D levels. Vitamin D supplements are not feasible for population-based approaches, and fortifying staple foods with vitamin D is the only viable solution, with minimal toxicity risk. When focusing on the people who need it the most-women (including non-pregnant, pregnant, and nursing women), babies, children (particularly girls, who are typically marginalized in India), and older citizens-food fortification may be a better option than supplementing options (5). Although supplementation and food fortification work in cities, they are not practical or trusted solutions in remote and tribal areas of Northeast India (21). Food Safety and Standards Authority of India (FSSAI), established under Food Safety and Standards, 2006, is working on fortification of staple food products in India to combat micronutrient deficiencies like anemia and goiter. The organization has started fortifying wheat, rice, oil, salt, and milk to combat these diseases. The Food Fortification Resource Centre (FFRC), initiated by stakeholders, aims to impart information, emphasize standards, quality assurance, food safety, and management processes. It encourages the food industry to adopt food fortification as an obligatory norm. FSSAI also sponsored an international conference on ‘‘Recent Advances in Food Fortification with Emphasis on Vitamin D Deficiency in Human Health’’ in 2018, focusing on Vitamin D deficiencies in human health. The following is a list of foods fortified with micronutrients that are sold in Indian markets: Tru (MDVL forms), Soya milk (Danone), Biscuit (Britannia), Oil (Patanjali, Fortune, and Cargill), Orange juice (Minute maid), Milk (Amul). In addition, foods like rice, potatoes, and tomatoes that are frequently using in cooking and are primarily consume by Indians must to be fortified and make available in market with low cost to every one for easily getable (22).

**Vitamin D pathway in potatoes and tomatoes**

The pre-cholesterol and post-cholesterol pathways are production route of SGAs, with cholesterol acting as an intermediary marker (23). The pre-cholesterol pathway is a common component in the sterol synthesis of all plants, while the postcholesterol pathway is essential for generating diverse. The pre-cholesterol and post-cholesterol pathways are production route of SGAs, with cholesterol acting as an intermediary marker. Acetyl-coenzyme A initiate the reaction conversation of isoprenoid pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP) thorough the mevalonate pathway. The cycloartenol serves as a junction for two metabolic pathways, the C-24 alkyl phytosterols pathway and the cholesterol synthesis pathway, which are mediated by sterol side chain reeducates 2. The oxidizing process converts squalene to 2,3-oxidosqualene and finally to lanosterol. 7-DHC synthesized by lanosterol, meanwhile it converted into cholesterol through additional enzymatic actions. This pathway is essential for the production of sterols that serve as precursors for SGA in potatoes (24).

In tomatoes, SGAs synthesis from cholesterol through oxidation, glycosylation, and other modifications. The biosynthesis of SGA initiate with squalene, which it was converted to cycloartenol theorugh mevalonate pathway, and subsequently transformed into cholesterol through a series of enzymatic steps involving Sterol Methyl Oxidase 2, C-5 Sterol Desaturase 1, and Solanum lycopersicum 7-Dehydrocholesterol Reductase 2. Cholesterol was common precursor for both SGAs and 7-DHC. SGAs such as α-tomatine accumulate in green fruits and are later converted into esculeosides during ripening, reducing their bitterness and toxicity. Simultaneously, 7-DHC is synthesized as a branch product of the cholesterol pathway, where the 7-DHC double bond is introduced by dehydrogenation. Under normal conditions, 7-DHC is rapidly reducing to cholesterol by the enzyme Sl7-DR2, maintaining a low steady-state level (25).

**CRISPR/Cas Genome Editing**

CRISPR/Cas9 protein is a bacterial adaptive immunity system that combats viruses or phages. It is classified into c lass 1 and class 2, with Cas9 being the most widely used machinery in crop improvement. It uses specific nucleases to cause double-stranded breaks in DNA, which are repair using mechanisms like non-homologous end joining (NHEJ) or homology-directed repair (HDR)/homologus recombination (HR). However, NHEJ is error-prone and may cause gene knock-out mutations. HDR generates point mutations or deletions caused by gene knock-in, but has a low success rate. CRISPR/Cas9 simplifies breeding by producing gene knock-out mutants for desired traits. However, it has limitations, such as the availability of Next-Generation Genotyping (NGG) protospacer adjacent motif (PAM) modified version of CRISPR/Cas9 (26). Through gRNA-DNA pairing between one DNA strand (the complementary stand of the protospacer) and the 5'-end sequence of the gRNA spacer, the gRNA-Cas9 complex recognizes the target sequence. The PAM sequence at the target site is necessary for Cas9. containing the use of the internet tools, it would be easy to program the about 20 nucleotide long gRNA spacer sequence to target DNA locations containing PAM (28). CRISPR direct is a free online resource for sgRNA creation and quality assessment. Due to its ease of use, multiplexing, affordability, high efficiency, and few off-targets, CRISPAR/Cas9 has currently transformed plant research. CRISPR/Cas, especially site-directed nucleases (SDN1 and SDN2), modifies the current genome without introducing new genes, in contrast to genetically engineered species. Therefore, transgene-free CRISPR/Cas is anticipated, and biosafety laws are being considered in a number of nations (27). Researchers have used CRIPER/Cas9 tool to blocking 7-dehydrocholesterol reductase (Sl7-DR2) activity in tomato plants to biofortify them with provitamin D3. They used CRISPR-Cas9 genome editing to recover five independent knockout alleles of the Sl7-DR2 gene. The study found that the loss of Sl7-DR2 activity did not affect tomato growth, development, or yield. However, it resulted in substantial increases in 7-DHC levels in leaves and green fruit. The levels were lower in ripe fruit but still high enough to be converted to vitamin D3 by UVB treatment (26). In northeastern India, horticulture is progressively becoming an economically viable occupation since it permits diversification and ensures a steady stream of revenue all year long. Tomato, potato, cabbage, cauliflower, aubergine, and onion are among the marketable excess crops produced in the area. Since these crops are primarily organic, they are becoming more widely available both domestically and internationally, particularly in neighboring and Middle Eastern nations (28). Utilizing cutting-edge biotechnology tools such as CRIPER/Cas 9 to modify plant genetics to increase the amount of vitamin D stored in plants is beneficial for supplying vitamin sources to tribal people without altering their eating habits.

**Conclusion**

Human skin synthesized the majority of vitamin D in the form of cholecalciferol, and when exposed to UVB rays. Most plants are low in vitamin D3. However, plants in the Solanaceae family, such as tomatoes, potatoes, and egg plates, are renowned for naturally accumulating high cholesterol levels in the SGA pathway. The commercial surplus crops grown in northeastern India include tomato, potato, and egg plants. In order to create a food crop that is enriched with vitamin D, advanced biotechnology technologies such as CRIPER/Cas 9 editing (knocking in or out) the plant genome are used to create the fortified crop.

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