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PLANT AS A PLATFORM FOR PRODUCTION OF VACCINE

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ABSTRACT

Edible vaccines is a new concept for the formulation and development department in the pharmaceutical industry because it is easily available from plant, cost-effective, easy-to-administer, easy-to-store, easy to deliver in the vaccines delivery system and culturally readily acceptable vaccine delivery system. It also involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. A variety of delivery systems have been developed. It has also found application in prevention of autoimmune diseases, cancer therapy, birth control, etc. They have passed the major hurdles in the path of an emerging vaccine technology. Various technical obstacles, regulatory and non-scientific challenges, this review is an attempt for introduce the vaccine which is prepared from plant.

Keywords: Edible Vaccine, Transplastomic Plants, Hepatitis B.

INTRODUCTION

About 57 million deaths approximately 15 million (25%) of per year across worldwide are estimated to be related directly to infectious diseases. High cost of vaccination makes it unaffordable for most people living in developing countries, as the daily average income for nearly one billion people is <US\$1. A 14-fold increase in the cost of vaccines over the past decade makes it necessary to investigate alternate strategies for their production and delivery. Vaccines represent an invaluable contribution in the field of biotechnology as they provide protection against various diseases.[1] Conventional vaccines are made up of live/attenuated vaccine and killed vaccine because according to WHO, various diseases are responsible for 80% of illness worldwide and cause more than 20 million deaths annually. [2]

HISTORY OF PLANT-DERIVED VACCINES [3]

- ✓ The first demonstration of expression of a vaccine antigen within plants occurred in 1990 when Curtiss and Cardineau expressed the *Streptococcus mutans* surface protein antigen A (SpaA) in tobacco. [4]
- ✓ This demonstration was closely followed by plant expression of the hepatitis B surface antigen (HbsAg), the *E. coli* heat-labile enterotoxin responsible for diarrhoea.[5]
- ✓ Proteins produced in these plants induced synthesis of antigen specific mucosal IgA and serum IgG when delivered orally to mice and humans.[6]

What is Edible Vaccine

Edible Vaccine involves introduction of selected desired genes into plant and then inducing these altered plants to manufacture the altered protein.[7]

How it work

Antigen in transgenic plant > Ingestion >Delivered by bioencapsulation >Taken up by Mcell > Pass on to the Macrophage > IgG,IgE responses > Local IgA response & Memorycells > Neutralize the attack by the real infectious agent.[8]

Potato [9]

Advantage

- ✓ It is Easily transformed.
- ✓ It is Easily propagated.
- ✓ It Stored for long periods without refrigeration.

Disadvantage

- ✓ Need cooking which denature antigen.

Banana [10]

Advantages

- ✓ It do not need cooking.
- ✓ Protein not destroyed even after cooking.
- ✓ It grown widely in developing countries.

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Disadvantages

- ✓ Trees take 2-3 to mature years.
- ✓ Spoils rapidly after ripening

Rice [11]

Advantages

- ✓ Commonly used in baby food.
- ✓ High expression of antigen.
- ✓

Disadvantages

- ✓ It grows slowly.
- ✓ It requires glasshouse condition.

ADVANTAGES OF EDIBLE VACCINE [12]

- ✓ Cost effective.
- ✓ Easy to administer.
- ✓ Easy to store and Heat stable.
- ✓ Acceptable to poor developing country.
- ✓ Activate both mucosal and systemic immunity.
- ✓ Do not required cold chain maintenance.
- ✓ No fear of contamination.

DISADVANTAGES

- ✓ One of the fears is that GM-pollen may outcross with sexually compatible plants which the affect biodiversity.
- ✓ Producing vaccines in plants also has a drawback because of the associated contamination risk for food crop production.[13]
- ✓ A soya field was found to be contaminated with transgenic maize producing the pharmaceutically-active substance trypsin causing the complete harvest of 13,500 tons of soya beans to be destroyed. Hence, research is moving towards non-foods such as tobacco.[14]
- ✓ There is also the risk that the pharma plants could be eaten by animals.
- ✓ The active substances could enter the groundwater and lead to harmful effects.
- ✓ Antibiotic resistance marker genes can spread from GM food to pathogenic bacteria.
- ✓ If a vaccine was consumed inadvertently, it could lead to desensitization so that vaccinations would eventually cease to work. [15]

STRATEGIES FOR THE PRODUCTION OF CANDIDATE VACCINE ANTIGENS IN PLANT TISSUES [16]

They have consigned smallpox to history and should soon do the same for polio. By the late 1990s an international campaign to immunize all the world's children against six devastating diseases was reportedly reaching 80 percent of infants and was reducing the annual death toll from those infections by roughly three million.[18] The 20 percent of infants still missed by the six vaccines—against diphtheria, pertussis (whooping cough), polio, measles, tetanus and tuberculosis—account for about two million unnecessary deaths each year, especially in the most remote and impoverished parts of the globe. [19]

When environmental or social disasters undermine sanitation systems or displace communities bringing people with little immunity into contact with carriers infections that have been long gone from a population can come roaring back. In the early 1990s Charles J. Arntzen, then at Texas A&M University, conceived of a way to solve many of the problems that bar vaccines from reaching all too many children in developing nations. Soon after learning of a World Health Organization call for inexpensive, oral vaccines that needed no refrigeration. [20]

EXAMPLES OF EDIBLE VACCINES [21]

SOME PATENTS OF EDIBLE VACCINES [22]

Plant-based vaccine Antigen selection [23]

- ✓ Is the antigen safe and non-pathogenic in all circumstances.
- ✓ Can the antigen induce a protective immune response.
- ✓ Is the antigen suitable for expression in plants?
- ✓ Efficiency in model systems
- ✓ Does the antigen accumulate in plants in sufficient quantities
- ✓ Is the plant-derived antigen immunogenic
- ✓ Do trial animals develop protective immune responses
- ✓ Possible plant cell interference with antigen presentation
- ✓ Possible induction of immune tolerance
- ✓ Choice of plant species for vaccine delivery, Best food plant, Ability to be eaten raw and unprocessed

SUITABLE FOR INFANTS [24]

Easily stored Resistant to spoiling. Amenable to transformation and regeneration, Possible cost to plant of multiple transgenes.

Delivery and dosing issues [25]

Requirement of mucosal adjuvants for protective response can a large enough dose be delivered by simply eating the plant Number of doses required.

Safety issues [26]

- ✓ Allergenic and toxic (e.g., glycans, nicotine, etc.) potential of plant components
- ✓ Potential for interference
- ✓ Production of oral tolerance?
- ✓ Risk of atypical measles (in plants with cloned measles virus genes)
- ✓ Health and environmental risks of genetically modified organisms
- ✓ Prevention of misuse/overuse

Public perceptions and attitudes to genetic modification [27]

Will negative attitudes to genetically modified organisms influence vaccine acceptability Legal and ethical considerations regarding products from plants with status like tobacco.

Quality control and licensing

Can antigen expression be consistent in crops Who will control vaccine availability and production.

DIFFERENT METHOD OF VACCINE PRODUCTION [28]

TRADITIONAL VACCINES

Traditional vaccines are live, killed or otherwise attenuated/modified pathogens (e.g., influenza vaccines produced in specific pathogen free-eggs). However, they have been increasingly supplanted by recombinant subunit vaccines produced in genetically modified cells because they offer increased safety, less antigenic competition, the ability to target vaccines to specific sites and the ability to differentiate between infected and vaccinated animals.

TRANSPLASTOMIC PLANTS

Instead of introducing transgenes into the nuclear genome, they can be targeted to the chloroplast genome using particle bombardment or other physical DNA delivery techniques, ensuring that the transgene is embedded in a chloroplast DNA homology region.[29]

PRODUCTION SYSTEMS FOR RECOMBINANT SUBUNIT VACCINES

Recombinant subunit vaccines currently on the market are produced in bacteria, yeast or insect cells and mammalian cells.[30]

Bacterial systems for vaccine production cannot produce more complex proteins that folds correctly and undergo forms of post-translational modifications (e.g. N-glycosylation). Furthermore, the presence of endotoxins and other pyrogens has limited their use. Since *yeast cells* are eukaryotes, they can fold and assemble complex recombinant proteins and carry out N-glycosylation however, the glycan structures often differ to those found in mammals. [31]

Insect cells culture medium is much less expensive and tend not to harbor mammalian pathogens. However, insect cells have limited scalability and major differences in glycan structures could raise challenges for the production of some recombinant proteins. In case of *mammalian cells* factors such as the cost of infrastructure and consumables, and the need for extensive product validation to prove that the final product is pathogen-free and does not contain oncogenic agents make this platform commercially unfeasible for vaccines required on a large scale. [32]

ADVANTAGES OF PLANT MADE VACCINE [33]

- ✓ Plant-derived vaccines have the potential to be used as oral vaccine, thus evading the costs related to sterile needles and trained medical staff.
- ✓ Plants-derived vaccines are likely to be more stable. Proteins expressed in certain plant tissues (e.g., cereal seeds)

remain stable for years at ambient temperatures without loss of activity. [34]

- ✓ Plant-derived vaccines survive in the stomach through bioencapsulation, which allows gradual release and, in some, cases this makes the vaccine more efficacious than the same subunit delivered through the parenteral route. [35]
- ✓ With plants, there is no need to build and run expensive fermenters, hire skilled workers and pay for expensive culture media.
- ✓ Very high yields of recombinant proteins can be achieved by chloroplast transformation. In two recent reports, approximately 70% and 72% of total soluble protein (TSP) has been obtained by plastid transformation, respectively.
- ✓ Growth of plants can be scaled up according to the required amount of protein. [36]
- ✓ Plants tend not to harbor human pathogens and any colonizing bacteria or animal-derived material can be removed using appropriate sanitary measures before processing.
- ✓ Transgenic plants can be grown at the site where the vaccine is needed. This advantage can save the costs related to transportation and cold storage

PROCEDURE OF PLANT-DERIVED VACCINES [37]

- ✓ A DNA molecule carrying the genetic information for a pharmaceutical substance is introduced into the plant genome.
- ✓ This process is called transformation. The genes can be incorporated permanently (stable transformation) or for a short period of time (transient transformation). The transformed plant acts as a bioreactor producing large quantities of the pharmaceutical using its protein making machinery.
- ✓ Through industrial processing, the pharmaceutically active substance is extracted from the plant and made into in a formulated product, for example a pill.

BANANA TREES AND TOMATO PLANTS [38]

Most children baby by offering a piece of banana. Boyce Thompson Institute for Plant Research at Cornell university have been genetically engineered to produce vaccines in their fruit. Bananas are particularly appealing as vaccines because they grow widely in many parts of the developing world. Plant biologists had already devised ways of introducing selected genes into plants and inducing the altered, or “transgenic,” plants to manufacture the encoded proteins advantages plants could be grown locally, cheaply, using the standard growing methods of a given region. Because many food plants can be regenerated readily, the crops could potentially be produced Homegrown vaccines would also avoid the logistical and economic problems posed by having to transport traditional preparations over long distances, [38] the vaccines would require no syringes which, aside from costing something, can lead to infections if they become contaminated. Among the autoimmune disorders that might be prevented or eased

are type I diabetes, multiple rheumatoid arthritis, and sclerosis.

Plant-Based Vaccine Against Hepatitis B: [39]

- Gene encoding HBsAg expressed in tobacco plants
- Targeted to the chloroplast
- Genetic constructs-
 - 1) driven by CaMV 35S promoter
 - 2) signal peptide from soybean VSP
 - 3) A plastid transit peptide

Asexual Blood Stage of Plasmodium Plant-Made Antigens. [40]

A small number of merozoite surface proteins have been analyzed as vaccines against the asexual blood stage of *Plasmodium* by inducing an immune response that either blocks invasion of erythrocytes or inhibits its subsequent multiplication. Apical membrane antigen-1 (AMA1), merozoite surface protein-1 (MSP1), and erythrocyte surface antigens are under clinical trials. Some of them have been expressed in plants, and their immunogenicity assayed. the first malaria antigen expressed in plants by stable transformation, with a very low expression level (~0.0035% of the total soluble protein (TSP)) flowed by a plant-codon-optimized version of the 42 kDa C-terminal fragment of Pf MSP1 (PfMSP142) was designed and synthesized, and expressed in transgenic *Arabidopsis thaliana* seeds Also, for protein stable accumulation, the plant-optimized PfMSP142 gene was fused to the phaseolin peptide signal or to the lysinerich protein. These strategies resulted in a substantial improvement of PfMSP142 expression plant codon optimization has also been implemented to improve the

expression level of *Plasmodium yoelii* merozoite surface protein 4/5 (PyMSP4/5), another important vaccine candidate with high effectiveness in protecting mice against a lethal challenge. In this case, this optimized PyMSP4/5 version was expressed in transgenic tobacco plants and its expression level was six times greater than that obtained with the native protein rare codons, AU-rich destabilizing sequences and putative polyadenylation and splicing signals may contribute to rapid mRNA decay, thus limiting the expression of foreign genes in plants. In the plant codon-optimized PyMSP4/5 antigen, the A+T content is reduced from 67% to 53%, and the AT-rich regions are disrupted, allowing the reduced A+T gene version to be more efficient in antigen production than the native version. Codon optimization has also been implemented in protozoan parasite *T. gondii* antigens expressed in plants.

FUTURE PERSPECTIVES [41]

- ✓ The main challenges that plant made vaccines will face in becoming commercially available, is gaining approval from regulatory agencies.
- ✓ Limited resources in research and lack of interest/investment from companies are also further reasons for the slow advancement of plant-based vaccines.
- ✓ Other hurdles are the costs related to clinical trials, a small number of plants made vaccines, such as vaccines for Norwalk virus, Hepatitis B, and rabies are currently in Phase I clinical trials.[42]
- ✓ However, major developments still need to be made to make these vaccines more cheaply produced and easy to distribute in the market.

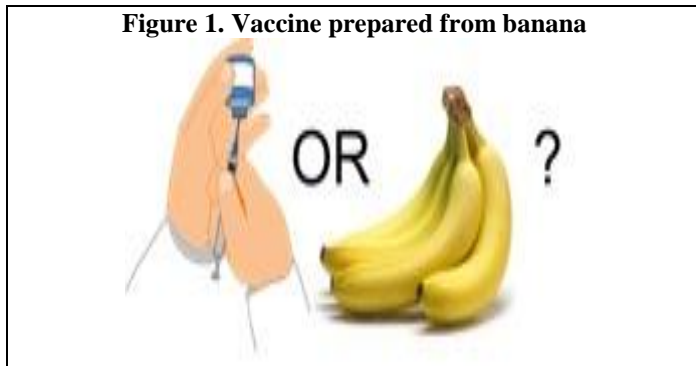


Figure 1. Vaccine prepared from banana

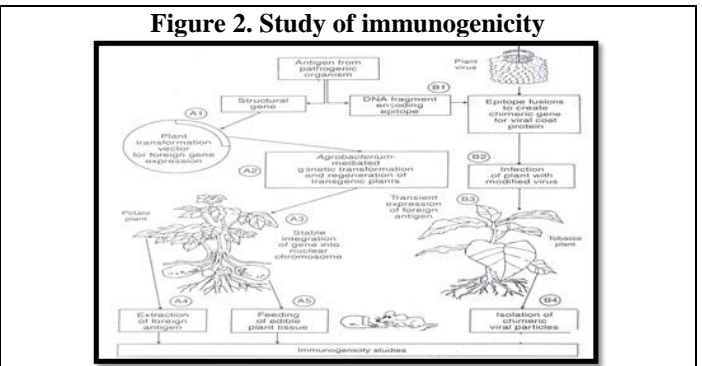


Figure 2. Study of immunogenicity

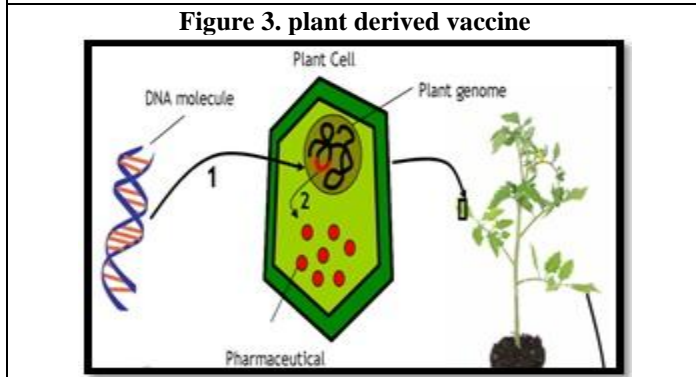


Figure 3. plant derived vaccine

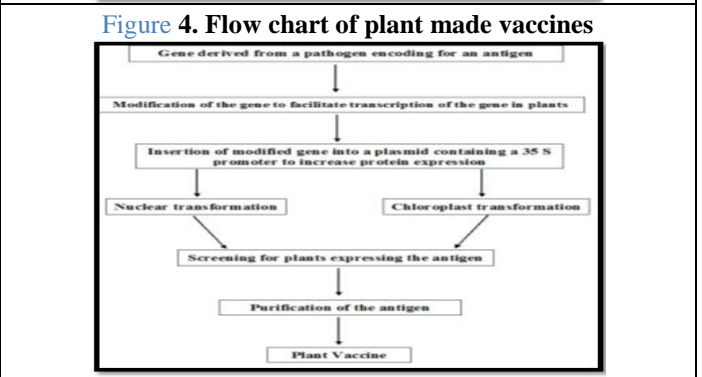


Figure 4. Flow chart of plant made vaccines

Banana Trees and Tomato Plants



Figure 6. Tamoto plant



Figure 7. Banana plant



CONCLUSION

Plant made vaccines have a new concept in the formulation and development because it is cheap, safe, therapeutic effectiveness, and highly accessible alternative to current methods of vaccine production. In the India or apart from India millions of lives every year. The technology has shown to have numerous advantages over current methods of vaccine production as well as a few disadvantages. While the challenges facing plant made vaccines have inhibited efforts to make the technology

viable for human use. Thus, the technology is highly promising and will in all likelihood be a major contender in vaccine production for the 21st century.

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