RECOMBINANT DNA TECHNOLOGY & ITS IMPORTANCE IN AGRICULTURE

Abstract

Food is the basic requirement of all human beings, food contains nutrients essential for repair and growth of body tissues, as the population increasing time to time the demand of people also increasing all over the globe. However productivity is reducing because of many difficulties in production. Throughout the mid 1970s the traditional practices of crop development/enhancement like resistant to pathogens, plant introduction, selection process, breeding in plants for high yield and draught animal practices were done. But nowadays there are many several few techniques are introduced and are used in agriculture sector like gene transfer, vector transfer, mutant breeding, DNA polymerase, PCR (polymerase chain reaction), protoplast culture etc. By the help of this technique, transgenic plants are growing effectively and resistant to pathogens, predators, parasites, and drought and can even grow in adverse condition with less uses of pesticides, herbicides and fertilizer etc. More than 5 million farmers are being benefitted by using transgenic plants in 60 hectares all over the globe. Recombinant DNA technology is also called genetic engineering, a technique that uses laboratory based technologies changes the genetic material by altering the genome of plants or by inserting foreign DNA. Genetically modified crops can be easily grown in agriculture by the help of genetically modified organisms. Nowadays, in the market genetically modified food, fruits and vegetables are available in large majority. As per the report all over the world farmer using less fertilizer and pesticide due to the establishment of recombinant DNA technology as well as it increases the productivity.

Keywords: Recombinant DNA, Genetically Modified Organisms (GMOs), TALENs, CRISPR-CAS9.

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I. INTRODUCTION

Before, the introduction of recombinant DNA technology in 1970s, it was just an imagination that desirable characteristics can be enhanced in the living bodies by controlling the expressions of target bodies. Recombinant DNA technology may be defined as DNA molecule that has been artificially created by combining genetically material form different organisms or changes the genetic material of an organism using various laboratory procedures [23]. In this method, Selection of specific genes or DNA occurs, cut-out from the original DNA and then inserted into a host DNA molecule resulting in a new DNA sequence that contains genetic information from multiple sources. Several enzymes are required such as restriction endonucleases and ligases. Restriction endonucleases also known as molecular scissors or restriction enzymes can recognise specific DNA sequences and cleave the DNA at specific sites or near sequence. It plays a vital role in rDNA technology by cutting the 3'end of one strand and the 5'end of another strand of same DNA. It allows manipulate DNA, enabling the insertion, deletion, or modification of genes. On another hand, ligase is an enzyme plays a crucial role in the process of DNA replication and repair. It catalysed the formation of phosphodiester bonds between adjacent nucleotides, joining the DNA strand together. The processing is essential for completing the replication of DNA strand or repairing damaged DNA molecules.

II. PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology, also referred as genetic engineering or gene splicing, is a set of techniques used to manipulate and modify the DNA of organisms. These techniques involve combining DNA molecules from different sources to create recombinant DNA molecules, which can then be introduced into host organisms to produce desired traits or perform specific functions. The principles of recombinant DNA technology include the following:

1. **Isolation of DNA:** Isolation of DNA is the first step in recombinant DNA technology from the donor organism and the host organism. This can be done by various methods such as cell lysis, lysogenic, organic extraction (phenol-chloroform method), non-organic and purification.

2. **Selection of Target Gene:** The target gene, which encodes the desired trait or protein, is identified and isolated from the donor organism's DNA. This gene can be obtained using restriction enzymes that cut DNA at specific recognition sites.

3. **Vector Selection:** A vector is a DNA molecule that is used to carry the target gene into the host organism. Commonly used vectors include plasmids, bacteriophages, and artificial chromosomes. The vector is chosen based on factors such as size, compatibility with the host organism, and ease of manipulation.

4. **Plasmid:** A small circular and extra chromosomal DNA and can replicate independently. They can be found in bacteria and used in genetic engineering. It can act as a vector of transforming genetic information from one host to another.

5. **Insertion of Target Gene:** The target gene is inserted into the selected vector using restriction enzymes which cuts the gene at specific site that create compatible ends between the gene and the vector. The gene and vector are then ligated together using DNA ligase, resulting in a recombinant DNA molecule.

6. **Transformation:** The recombinant DNA molecule is introduced into the host organism, typically a bacterium or yeast, through a process called transformation. Once the recombinant DNA is inserted into the host cell and gets multiplied in the form of manufactured protein. This can be done using techniques such as heat shock, electroporation, or microinjection.

7. **Selection and Screening:** Once the recombinant DNA is introduced into the host organism, selection methods are employed to identify and isolate cells that have taken up the recombinant DNA. This can be achieved by incorporating selectable markers, such as antibiotic resistance genes, into the vector.

8. **Formulation of the Target Gene:** After successful integration of the recombinant DNA into the host organism's genome, the target gene can be expressed to produce the desired protein or trait. Gene expression can be control by the use of specific promoters and regulatory sequences.

9. **Redemption and Characterization:** Once the target protein is expressed, it can be purified and characterized using various biochemical (western blotting, PCR, ligation, gel filtration and electrophoresis) and biophysical techniques. This ensures that the protein is functional and suitable for further applications.

10. **Transcription and Translation of Inserted Gene:**Desired protein can be carried out by two processes *i.e.* transcription and Translation. Transcription means the copying of genetic material information from template strand of DNA into single stranded mRNA by RNA polymerase enzymes. There are three types of RNA polymerase enzymes (RP1, RP2, RP3). The region on DNA molecule to which RNA polymerase binds &initiates transcription is called Promoter. Promoter is located upstream *i.e.* 3’end of template strand and 5’end of coding strand. The area of template strand that is involved in transcription or formation of RNA called Structural gene. The area where the process of transcription stops called Terminator, located downstream *i.e.* 5’end of template strand.

    Translation means the process of formation of protein by using the codons of mRNA. It is also called protein synthesis. DNA polymerase is the enzyme required in translation process. Three types of DNA polymerase are there (DP1, DP2, DP3). All have different roles and importance. DNA polymerase synthesizes a new strand over m-RNA. Consequently it encodes the codon sequences of m-RNA in 3’to 5’ sequence and vice versa.

III. IMPORTANCE IN AGRICULTURE

Genetic engineering has now have numerous benefits faster and more precise breeding, higher crop yields, development of more nutritious food and decreased need for herbicides and pesticides. Genetically modified soybeans, corn, potatoes and wheat resist herbicides sprayed on farms to kill weeds [4]. The gene have been altered through the process of genetic engineering is called Genetically Modified Organisms. Flavr savr tomato produced by the Calgene Company which is first genetically modified crop [1] and long shelf life than natural growing tomatoes that means it takes longer period to ripen, it took from the branches unripened and ripened by means of chemical processes. It was first ever marketed in 1994. Disease resistance crops such as virus resistant papaya in Indonesia. Bt brinjal in India [2] was the first crop to get GEAC approval, it approves disease resistant. In recent years, it was to be known that people in India suffered from colour blindness which is caused by vitamin-A deficiency, thus golden rice was been introduced into the market with genetic engineering techniques to surpass the need of farmers by developing beta-carotene which is a precursor of vitamin A. With view knowledge GM crops allow farmers to reduce greenhouse gas emissions, since less herbicide is applied and the crops can be grown using low-or-no-till farming [11]. GM variety seeds along with protein rich rice, wheat, Tomato are been developed [16]. GMO has promise of giving safety and risk-free assessment of biotechnology derived foods developed by FAO (Food and Agricultural Organisation).

IV. APPLICATION OF RECOMBINANT DNA TECHNOLOGY

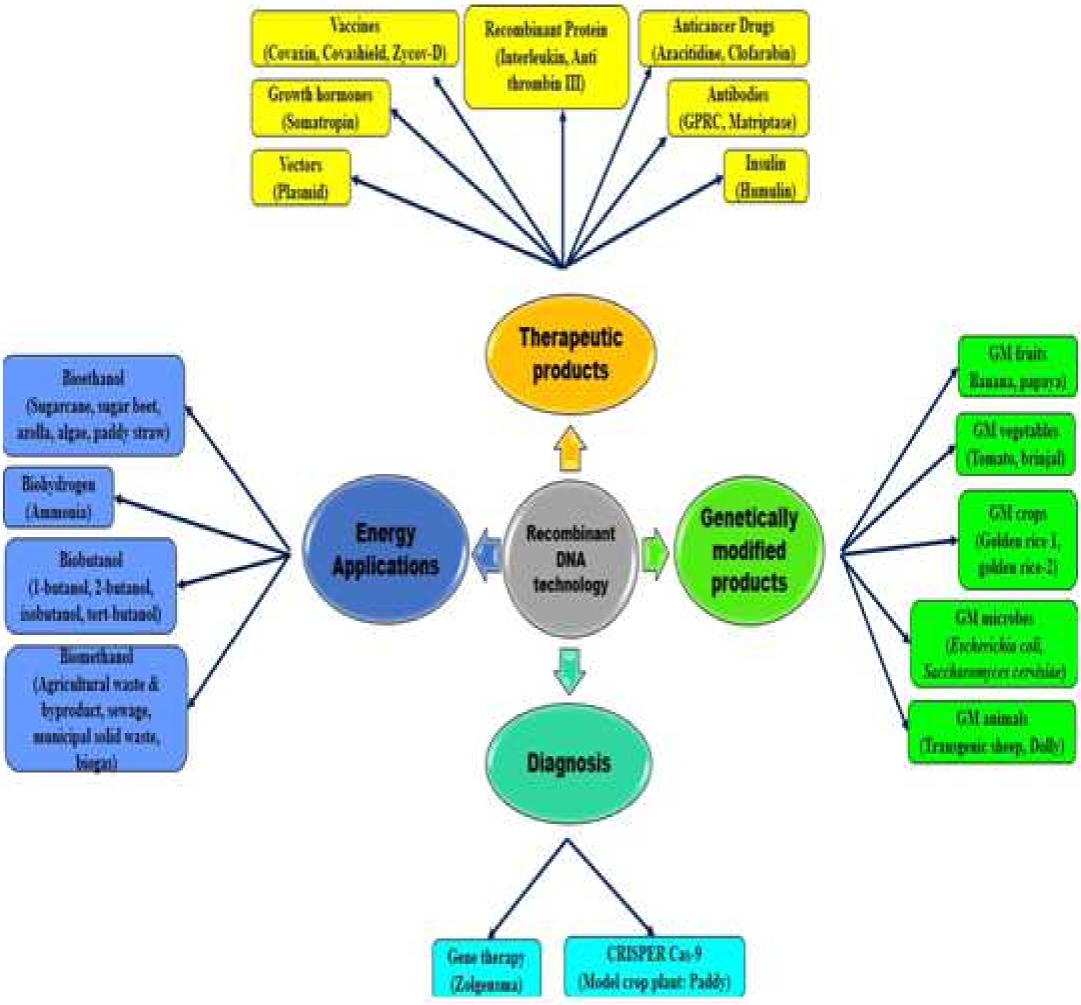

Figure 1: Application of Recombinant DNA Technology

Molecular marker are defined as a biological molecule which can be termed as measurable indicator to indicate where the vector is placed at recognise site of the host. Higher yields also benefit the consumer, as more food is available at lower prices. GM foods are the new normal. The pioneering work of Paul Berg, Herbert Boyer, and Stanley when in the early 1970s led to the development of recombinant DNA technology, which has permitted biology to move from an exclusively analytical science to a synthetic one. Recombinant DNA technology is used for many aspects in research, medicine agriculture and industry. Human insulin was one of the first therapeutic proteins that was genetically cloned. Recombinant DNA based drugs were being tested for safety and efficacy [5]. This technology is also important tool in agriculture being fused to improve plants resistance to pests and increase crop yields. Higher yields also benefit the consumer, as more food is available at lower prices. GM foods are the new normal [7].

1. **Application of Recombinant DNA Technology in Agriculture:** Agriculture is the backbone of India. Antibiotics produced by microorganisms are very effective against different viral, bacterial, in protozoan disease. Enzymes are encoded by genes, if there are changes in genes then definitely the enzyme structure also changes. Some commercial crops, such as soy, maize, sorghum, canola, cotton etc are grown with DNA thus increases resistance to herbicides used in the agricultural process. Recent developments have enabled plants to express a recombinant form of Bt toxin protein usually produced by *Bacillus thuringeiensis* bacteria introduced in India [2], Bangladesh [20] and the Philippines [3]. Bt toxin introduced to plants to resistance against insects and this practice has been widely developed in gardening and agriculture. Recombinant DNA technology in agriculture provides with helping in human research in future by provision of developing new transgenic crops. Last few years, the harvest and the output was less, has now overcomes. Nutritious plants with drought resistance are developed certain genes are introduced responsible flavour and aroma Compounds can be enhanced improving the taste of fruits and vegetables. It’s possible to modify genes to slow down the ripening process of fruits, extending their shelf life (Flavr Savr). DNA technology can be used to produce bio pesticides reducing chemical pesticides plants can be modified to clean up soil by absorbing pollutants and contaminants.

2. **Food Quality:** The aroma, flavour and texture mostly attracts the Consumers. Food is the basic need of humans, however the quality of food matters in every conditions. Recombinant DNA technology has enabled improvements in food quality within agriculture. This technology allows scientists to modify the genetic makeup of crops to enhance nutritional content, shelf life, and resistance to pests or diseases. For example, crops can be engineered to have higher levels of specific nutrients like vitamins or minerals, leading to improved food quality. However, public concerns about GMOs and ethical considerations should also be taken into account when implementing these technologies. Recombinant DNA technology has led to the development of genetically modified organisms within agriculture. Some examples of quality foods are Bt-cotton, Bt-corn and high drought resistance crops are developed [13]. Tissue culture was scared in the year 1950s and became famous in 1960s. Today micro propagation and in vitro conservation are standard techniques in significant crops. Transgenic engineered plants remained a promise of the future, at the end of 1970s. Many food industries like Kellogg’s company was established for the production of corn flakes snacks, this industry has get there substituted products from the original high drought resistance corn field and

later on processing made on it available. In 1996 more than 1 million transgenic crops were grown in all over the world especially in North America with an excess of 150-million-acre area. Following countries has also participated with America such as Argentina, China etc. Now Argentina is the leading producer of transgenic herbicide-resistant soybean. Nestle is researching to develop a genetic engineering system for soybeans and corn, collaborate with the biotechnology company under the licence of U. S. Food and Drug Administration present in California,USA. Protein content cereals, Roundup soybeans, iron content seeds, vitamin content tomato by introducing sense [19] and antisense [18] properties are developed.

3. **Enhanced Resistance:** Transgenic crops are now developed by using transgenic technique possessing humidity, temperature, salinity, high drought, and cold resistance [15]. C-repeat binding factor (CBF) possess significantly tolerant to abiotic and environment stresses. Thus, rice is kharif crop cultivated abundantly in June-July month, however now even rice are cultivated in winter season by introducing resistance to cold climate. It is important, therefore, to look for alternative strategies to develop cold stress tolerant crops. *Bacillus thuringiensis* which is called a bacteria introduced in cotton *i.e.*, Bt cotton developed resistance against pest like lepidopterans, coleopterans etc. Bt (cry1Ab) protein introduced in corn plant possess resistance against pest such as American bollworm *i.e. Hemicoverpa armigera* and pyralideae family. Tobacco are generally shows resistant against nematodes and these certain crops are grown in many countries. Also using CRISPR-cas9 [14], to modify plant genes for improved resilience. Golden rice and drought-tolerant corn were developed early by transgenic techniques. The crops are developed shows biotic as well as abiotic stress tolerant, fertility, yield, and quality improvement.

4. **Nutritional Quality:** During crop domestication, humans have learned to improve only crops, however green revolution has impacted in humans and considerably increased nutritional quality, crop yield etc. It is obvious that nutritional quality of a crop attracts people. Fortified crops such as spinach with calcium content, tomato with protein content has been introduced by breeding and cultivation. The development of golden rice encoding beta-carotene which is a pro-vitamin in transgenic rice [22]. CRISPR has evolved to perform small mutation at target sequences. CRISPR Cas-9 genome editing protocol has successfully introduced in onion. These resources rapidly increases the creativity of genome editing tools, enabling the induction of precise mutation in crop genomes [10]. Recently several research groups have successfully synthesizes EPA & DHA in Arabidopsis, mustard, and canola etc. [17]. Zinc and Iron deficiencies are the major problem nowadays thus plant up taking these nutrients around surroundings which is made possible by transgenic crops. Transgenic lettuce, fortified wheat with high mineral content reduces cancer. Tomatoes with folate level that provides a complete adult daily requirement. Food security and heath safety can be induced by transgenic approaches.

5. **Fertilizer:** Genetic engineering holds the beliefs of many farmers, crops require fertilizers for better results however transgenic techniques has made possible to know the precise amount of fertilizer requirement for growth and development of crops. By using genetic information farmers can accurately clarify the use efficiency, working speed, site of action, up taking path nutrients of growing crops. Soil fertilizers and foliar application

can be used early crop sowing or post emergence of crops. Fertilizer production by using the tools of molecular biotechnology like recombinant DNA technology can improve the metabolism of plant crops. Soil fertilizer with foliar fertilizer application can be used simultaneously for crop improvement. This gives different fertility at different time such as at reproductive period.

6. **Microorganisms:** Since years Microorganisms played a very vital role in food production. Microorganisms such as bacteria and moulds are used in the brewing industry for making bread and alcohol, one of them is *Saccharomyces cerevisiae* [6]; [9]. With great concerns, scientists manipulating microorganisms in the production of food. Nowadays, modern day industries use microorganisms for the preparation of fermented foods, this emphasizes various companies. *Saccharomyces cervisae* is an attractive microbial species which is widely used for baking, alcohol and beverages. It is also known as bakers yeast. The working properties of microorganisms can be changed by genetic engineered techniques such as the DNA sequences. In fact, *Saccharomyces cerevisiae* officially introduced form the skin of grapes, now abundantly used in industries. In dairy products such as cheese and fermented milks, the lactobacilli converts the Lactose into lactic acid have multiple effects depending on the species, adequate strain and viable product. It is an era of science period, thus GM bacteria increases the activities of maltase and fructose of brewer's yeast. Lactic acid bacteria are used to ferment many vegetables like tomato, cabbage, onion which is widely accepted in Asian countries [4]. A fermented tea beverages are produces by the symbiotic cultures of bacteria and yeast (SCOBY) which is called Kombucha. *Aspergillus oryzae* use as the starter for preparation of koji or soy sauce.

7. **Enzymes:** Enzymes can be easily obtained from plants and culturable microorganisms through simple extraction processes [12]. Enzymes are biocatalyst which conduct biochemical reactions. Many microorganism like bacteria and their enzymes quietly used in food production and processing of bread, wine, beer and dairy products. In bakery industry it is a natural way to keep bread softer, moist for longer period and establishing tolerant properties. The uses of enzymes can enhance the shelf life, reducing additives and energy usage, food loss and food waste, with sustainability benefits without causing any harm to the environment. Maltogenic amylase allows to keep bread softer for longer, to extend shelf life, by improving product sensory characteristics through genetic engineering such as "softness", "texture", "crumb structure", "taste", "aeration", "emulsification" and "colour". The sugar found in dairy products is called lactose, can cause gastrointestinal discomforts, indigestion, bloating and many other infections. With that knowledge lactase is an enzyme that cleaves lactose into these two smaller sugars galactose and glucose. Many novel enzymes are induced by recombinant DNA Technology with which increases the vitality of food condition. Nowadays enzymes widely used in pharmaceutical, nutraceutical industries. Amylases and lipases are the food processing enzymes with properties of microbial production strains and Beta oxidase increasing storage life by removing oxygen during food packaging.

8. **Current Research Progress:** Fibroblast Growth factor (FGF) is a type of protein that plays a crucial role in cell growth, proliferation, and differentiation in various organisms, including plants. In agriculture FGFs can be used to enhance plant growth, development, and abiotic and biotic stress tolerance. They can stimulate root and shoot growth, improve

nutrient uptake of wheat, rice, corn (maize), soybeans, cotton, coffee etc. [20]. Zinc finger nucleases (ZFNs) are a type of genome editing used in agriculture to modify the DNA of plants and animals. ZFNs is a very laborious work and excellent fidelity. The ZFN-based gene editing system consists of a DNA recognition element based on the zinc finger (ZF) protein and a DNA cleavage domain of the FokI nucleic acid endonuclease. This allows for precise modifications of genes. One example of using ZFNs in agriculture is in creating crops with improved traits. For instance, researchers have used ZFNs to modify crops to be more resistant to pests, diseases or abiotic and biotic conditions. Another example is the enhancement of nutrients content in crops, such as increasing the levels of vitamin or minerals. ZFNs have been used for those purposes, newer genome editing technologies like CRISPR-Cas 9 have gained more prominent due to their case of efficiency and originality. CRISPR *i.e.,* clustered regularly Inter spaced short Palindromic repeats [14] is a biotechnology that has been developed to accurately modify the genes of living organisms. The associated Cas operon encodes the interfering several Cas proteins [21]. Initially discovered as like Recombinant DNA, gene-editing techniques can insert genetic material I to the genome but it can also make edits without using foreign DNA. As per 2023 report, scientists from the Directorate of Onion and garlic Research (ICAR-DOGR, Pune), India and Iowa State University, USA are reporting that a CRISPR Cas 9 genome editing protocol was successfully established in onion. Compare to plant and mammalian cells to beta Ken as hosts, microbial structures supply less problematic equipment which subsequently enhances the overall performance.

V. RECOMBINANT DNA TECHNOLOGY MARKET ANALYSIS

Recombinant DNA technology market plays a concern role during pandemic period. There has been a rapid growth in the rDNA technology market. The demand of this technology increases day by day with concern for the diagnosis, Prevention and therapies of today's patient. Since the COVID-19 outbreak in December 2019, more than 100 countries has been affected around the world and it has declared as public health emergency by World Health Organisation (WHO) on January 30, 2020. As the population increases day by day, people are more concern with their health life they preferred to be healthy as much as possible, so the demand of monitoring tools, pharmaceutical products, drugs, vaccines are produced in the pharmaceutical industry. RDT was very useful in the research and development purposes for developing COVID-19 vaccines. This allows scientists to manipulate this technology for production of vaccines. Due to nationwide lock down declaration on March 20, 2020 onwards various countries like China, Japan, India, Egypt and other countries facing problems with regards to the transportation of drugs from one place to another. By using this technology COVID -19 and many vaccines are expected to effective and has higher safety concerns against the people. Developing country like India, has began the administration of COVID-19 vaccines on 16 January 2021. India has developed more than 2 billion approved vaccines doses manufactured under license by serum Institute of India under the trade name Covishield, and Covaxin *i.e.* a vaccine developed locally by a company called Bharat Biotech. The budget was 35000 crore INR, and target is to immunise Indians against COVID-19. "**Prime Minister Narendra Modi has confirmed that every Indian will be vaccinated and no one will left behind until the vaccine has developed**". Later on 238,367,228 people has administered booster dose of Covaxin or Oxford-Astra Zeneca. Influenza vaccines wired rightly developed in 2017 under the licence of US Food and Drug Administration (USFDA). Also genetically modified vaccines are developed for the diagnosis and cure of the increasing population.

VI. CONCLUSION

Since 1970s, RDT was introduced to the world. This technology was used only for the production and betterment of food, enhancing food with nutritional content, mineral like protein, carbohydrate, calcium based, without harming the environment. Several conclusion can be made from the RDT or genetic engineering. The risks of introducing Recombinant DNA engineered organisms or genetically modified organisms into the environment should be based on the nature of the organisms and the environment into which it is introduced, not on the method by which it was produced. However, in recent years this allows scientists to manipulate with the technology for the advance strategists of biomedical applications such as for cancer treatment, AIDS, diabetics, various plant diseases. Several Vaccines and serum has been produced under the concern of RDT. Thus vaccines also has been marketed with lower life risk and sustainable to environment. The transgenic crops introduced are Golden rice has beta -carotenoid a precursor of vitamin A. Golden rice is also called genetically modified crop. By developing product not carrying only positive factor as well as various challenging factors to the community. The introduction of genetic information from only one source should brought a negative impact. Moreover, there are several concerns over development of genetically engineered cross breed plants and other products. Recombinant DNA technology has significantly impacted agriculture by allowing scientists to develop genetically modified crops with enhanced traits such as improve yield, pest resistance, insect resistance, nutritional value food per capita content. This technology has the potential to address food security challenges and increases agricultural productivity. This technology holds promise, careful regulation and thorough assessment of its long-term effects are essential for it's responsible and sustainable use in agriculture. Intensive use of genetical techniques has been central to the improvement of nutrition and health throughout the world. Investigation and guidance provide by the scientific community to the investigators and regulators in evaluating planned introduction of modified organisms from an ecological prospective.

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