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Recent developments in genetic engineering in the CRISPR Technology and plant sciences with modern applications

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Abstract

The CRISPR toolbox can perform precise programmable genetic sequence insertions to the development of new technologies. The Cas9 nuclease is one of the proteins that make up the so-called genetic scissors, a system of directed cutting of foreign DNA and, RNA that is the subject of extensive research among all known Cas proteins. The correction of alpha1 antitrypsin deficiency and cancer immunotherapy are two important therapeutic development scenarios where HDR-mediated CRISPR-Cas9 editing has demonstrated encouraging outcomes in the clinical testing. Protospacers are DNA segments that are targeted by the prokaryotic immune system and resemble the corresponding spacers at the CRISPR locus, with the exception of the PAM motif. The transpiration is the loss of water by a plant, mostly through the stomata on its leaves. Drought is a dangerous occurrence that significantly affects how well plants perform. It sets off physiological and biochemical processes that help the plant withstand drought by lessening its impacts. Medicinal plants and other soil-grown agricultural goods are often impacted by oil pollution. The existence of hazardous heavy metals in the soil, such as cadmium, mercury, lead, chromium, arsenic, nickel, copper, and zinc, is one of the many other explanations for why some bacteria and germs have become resistant to medications this century.

Keywords: The CRISPR toolbox, precise programmable, genetic sequence, Cas9 nuclease, RNA.

INTRODUCTION

The Cas9 nuclease is one of the proteins that make up the so-called genetic scissors, a system of directed cutting of foreign DNA and as was subsequently discovered, RNA that is the subject of extensive research among all known Cas proteins. Initially identified as Cas-editing tool, this protein was first reported in relation to its link with CRISPR repeats. The HNH motif (His-Asn-His, which is present in other nucleases as well [1, 2]. The identification of a certain pattern on one side of the CRISPR arrays' described spacers' nucleotide sequences; nevertheless, the function of this pattern wasn't fully understood until afterward. Protospacer adjacent motifs, or PAMs, are brief motifs that were not a part of the original spacers of the CRISPR locus but are now found close to protospacers. Protospacers are DNA segments that are targeted by the prokaryotic immune system and resemble the corresponding spacers at the CRISPR locus, with the exception of the PAM motif. These motifs are essential for identifying potentially dangerous genetic information because, in contrast to DNA sequences that are stored in the CRISPR locus as spacers and do not contain PAM motifs, their presence at the end of a sequence indicates that the DNA fragment is foreign [1, 2].

The CRISPR toolbox can now perform precise programmable genetic sequence insertions to the development of new technologies. Over the next ten years, one major challenge will be to effectively refine and apply these technologies for genome engineering applications. By using HDR to insert genetic material from a co-delivered donor template into the target site, traditional Cas9 editing can introduce transgenes. This is currently being applied extensively in several genome engineering domains. The use of targeted integration to fluorescently tag over 1000 human proteins for investigate their interactions and localization is a noteworthy recent example of its application. The correction of alpha1 antitrypsin deficiency and cancer immunotherapy are two important therapeutic development scenarios where HDR-mediated CRISPR-Cas9 editing has demonstrated encouraging outcomes in preclinical and clinical testing. Despite these achievements, HDR-mediated CRISPR-Cas9 editing is not without its drawbacks. These include the inability to edit beyond cell division, the complexity of delivering donor templates, and the difficulties associated with accuracy that the DSB presents. High-precision alternatives to HDR-mediated CRISPR-Cas9 are needed because many human pathogenic genetic variations need a short sequence insertion to repair an indel, even if certain single-nucleotide alterations may be addressed by base editing [3, 4].

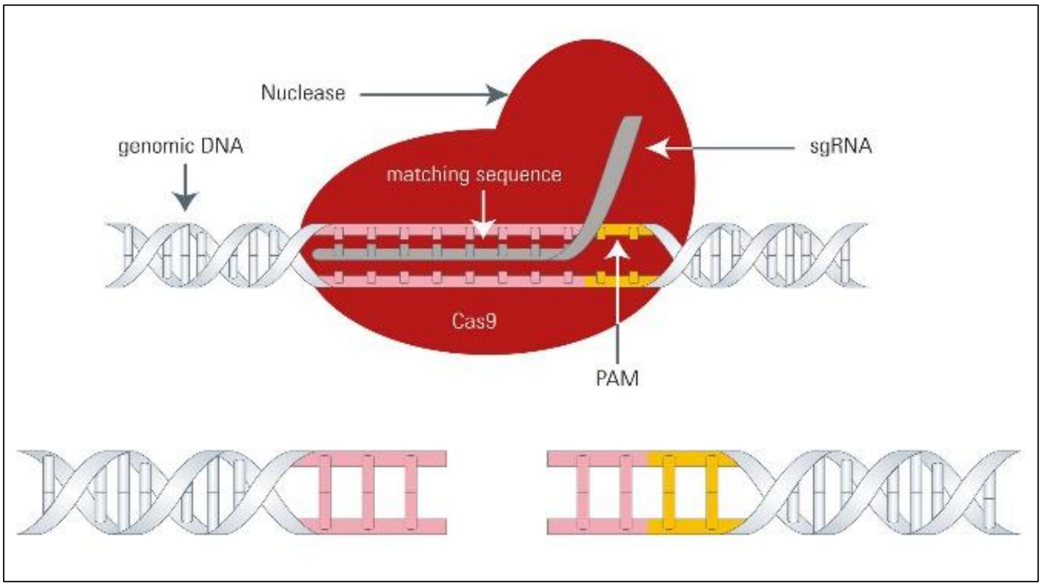

Fig-1: That revealed the principles of CRISPR editing

Mechanism, principles and applications

Animal growth is made up of connective tissues, intramuscular adipose tissues, and muscle fibers, skeletal muscle provides meat for human use or consumption. It has long been known that insulin-like growth factor I (IGF-I) and growth hormone (GH) play a key role in controlling body size in growing animals. Both before and after birth, development of muscle is dependent upon GH and IGF-I. Muscle growth and body mass are caused by the growth hormone-insulin-like growth factor (GH–IGF) axis, which is controlled by the pituitary gland and liver [5, 6]. GH, however, is present in practically all tissues. The only organ capable of producing serum IGF-I predominantly is the liver. GH, which is produced by the pituitary gland, promotes the growth of IGF-I in other tissues, including the muscle and liver.

The liver is the primary source of circulating IGF-I (cIGF-I), even if some is secreted from other tissues including muscle. One element of the negative feedback loop that regulates GH secretion is cIGF-I. IGF-1, which is produced in the liver and muscles, is essential for myogenesis. Simultaneously, a mutation in the regulatory function of the IGF-2 gene has been associated with greater muscle development in pigs. In recent years that the GH and IGF-1 genes may be effectively microinjected into pig zygotes. Eventually, the mass of two lines of transgenic GH-expressing pigs increased by 11.1 and 13.7 percent [6, 7].

Notwithstanding the noteworthy advancements in the use of CRISPR/Cas in fish farming, commercialization of CRISPR and other GE technological products depends on risk assessment (RA), regulatory approval, public and consumer acceptability, and consumer acceptance. Aside from the two fish that have been commercialized-the transgenic AAS and the GE tilapia-a number of additional fish are in advanced phases of research, but risk assessment frameworks, data, or experience to assess the safety of these goods. These have mostly concentrated on genetically engineered plants, which has caused a delay in the updating and amendment of frameworks for GE fish and other aquaculture goods. With an emphasis on CRISPR/Cas-edited fish, the outline some of the most significant problems that hinder the use of current frameworks for GE fish here and provide approaches that may help resolve them. In addition, several significant technical difficulties and relevant concerns about sustainability and acceptance of fish farming technology [8, 9]. In fact, by analyzing the effects of gene knockouts on the desired feature, able to pinpoint the suspected genes behind quantitative trait loci (QTL). This might facilitate the introduction of advantageous alleles or de novo variations into suitable fish species and aid in the correction of the desired phenotype by genome editing [9, 10].

One of the exemplary cases is the farming of Atlantic salmon (*S. salar*), where the aquaculture industry's growth is restricted by two main obstacles. Open sea cages are used to produce the majority of Atlantic salmon, a practice that raises concerns about sustainability due to the spread of diseases from farmed to wild fish and vice versa, as well as the impact of escaping farmed fish on wild populations. One way to stop wild introgression in Atlantic salmon might be to produce and employ sterile fish in production. This was achieved by using CRISPR/Cas9 to perform targeted mutagenesis against dnd, producing a sterile fish with altered genes [10-12].

Stomatal control from the interaction of stomatal architecture and physiological behavior. When two presumably biochemically equivalent plants with the same A% values are compared, the species with a higher density of smaller stomata should show quicker responses in terms of stomatal conductance. This includes the capacity to transfer ions across the guard cell plasma membrane and stomatal signaling. This is so that the guard cell water potential may be adjusted more quickly due to the reduced guard cell surface area. There seems to be a quicker rate of increase in stomatal conductance during stomatal opening in closely related species with smaller kidney-shaped stomatal morphologies. More species with kidney-shaped stomatal complexes have a lower rate of stomatal conductance loss, a measure of the pace of stomatal expansion. This relationship vanishes when species with dumbbell-shaped stomatal complexes are considered. Nevertheless, even while low SS and high SD may not necessarily correspond to a high degree of physiological stomatal responsiveness, they do indicate that low densities of large stomata are associated with relatively delayed physiological responses [12, 13]. Stomata, more than any other single vegetative structure in plants, have perhaps attracted greater attention because they regulate the flow of gases between the plant and its environment. The minute pores on the surfaces of angiosperm flowers, fruits, leaves, and stems are represented by these characteristics. Because of the control over the flow of CO2 and water vapor from the inside of the leaf to the outside, several plant functions. Multiple cellular processes must be coordinatedly controlled to regulate the stomatal aperture, and many environmental cues have an impact on the morphogenesis of the aperture. Due to genetic makeup, leaf morphology, and environmental influences throughout growth, stomatal traits the number of stomata per unit area and their shape vary across and within species. The arrangement of stomata, which may vary greatly, is another feature. They can be arranged in regular rows down the length of the leaf, in patches, or very equally across the leaf. It displays the stomatal characteristics of four distinct plant species [14, 15].

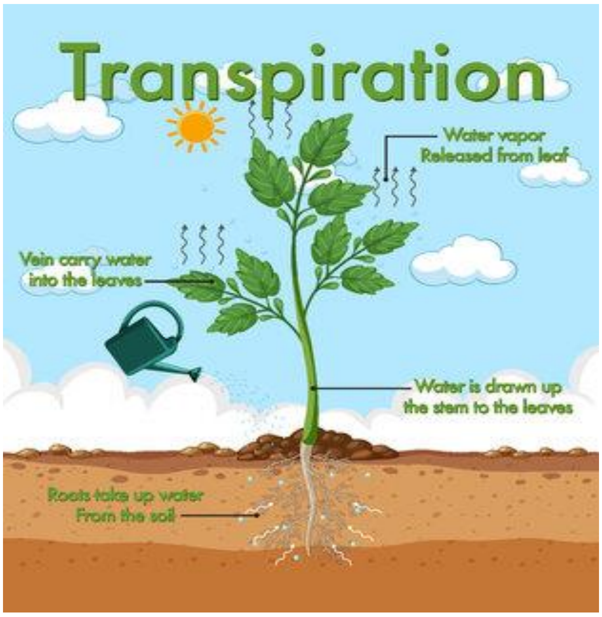

Fig-2: The transpiration in the plants with different events

In botany, transpiration is the loss of water by a plant, mostly through the stomata on its leaves. In order for carbon dioxide to enter the leaf's core and for oxygen to exit during photosynthesis, stomatal apertures are required. As a result, transpiration is typically viewed as only an inevitable byproduct of the stomata's actual duties. Though these views have been contested, transpiration gives plants the energy to move water throughout them and may also help plants dissipate heat in direct sunshine by allowing water to evaporate. An excessive amount of transpiration may be very harmful to a plant. When a plant loses more water than it takes in, its development may be stunted and eventually die from dehydration [14, 15]. Drought is a dangerous occurrence that significantly affects how well plants perform. It sets off physiological and biochemical processes that help the plant withstand drought by lessening its impacts. In order to enhance water availability and facilitate water absorption from deeper soil layers, root system growth and development are encouraged. Additionally, this is done at the price of the sections of the plant that are above ground, where a lack of water causes the leaf cells to lose their turgor and wilt. By aligning the leaf parallel to the incident light rays, certain plants such *Macroptilium atropurpureum* may regulate the movement of their leaf blades, hence decreasing water loss within the plant. Guard cells function to prevent excessive water loss by opening when the weather is more favorable and closing on hot, dry, or windy days. As night falls, there is another dip after recovery and reopening. To minimize water loss through evapotranspiration, stomata of plants that photosynthesize using the CAM carbon fixation pathway such as bromeliads and Crassulaceae family members open at night. Drought also causes disruptions to the photosynthetic process because the plant's stomata close, allowing less CO_2 to enter the plant. When there is a lack of water in the soil, stomata shut because they reduce transpiration, which reduces water loss and helps prevent drought. When a root expands, more effort is needed to push soil particles out of the way, causing mechanical resistance. The consequence is an increase in root diameter behind the root tip [16-18].

Strong soils were more easily pierced by dicotyledons (with larger diameters) than by graminaceous monocotyledons with smaller diameters. This implies that dicotyledonous species may apply higher axial growth pressures than monocotyledonous species. Nonetheless, no correlation has been observed between the maximum axial root growth pressure (σmax) and the capacity to withstand dense soil in investigations that have directly examined it. This includes directly compared monocotyledons and dicotyledonous species. Hence, root diameter may instead be directly correlated with the capacity to pierce dense soils. The significance of ethylene in control demonstrated by the addition of many inhibitors of ethylene action and synthesis, which helped restore root elongation rates in these mechanically inhibited roots [19-21].

The extra features when dealing with the size, depth, and complexity of manipulating the roots of certain perennial plants. These difficulties are examined in-depth in a plant system that covers the intricate physiology of deep roots and the numerous roles they play at different depths. Popular functions of deep roots are critically examined by the writers. The majority of the models in use today are not well supported by data, and they offer priority areas for further study to enhance these models. For the permanent pastures that are essential to the grazing systems that sustain the production of milk and meat, rooting depth is crucial. One of the most significant fodder plants in temperate regions, white clover (Trifolium repens), is vulnerable to drought stress because of its very [19, 20]. Their tactics involved crossing white clover to a variety of *Trifolium uniflorum* accessions in an effort to improve the root structure of the plant. The backcross hybrids' root length density and depth were two of their changed features. The using focused breeding techniques, root architecture may be changed to increase pasture productivity. Supporting and stabilizing plant shoots is one of the roots' key functions. Using tendrils, which are specialized organs with a filamentous structure that emerge from leaf axils, cucurbitaceous crop species may climb. Tendrils give plants a winding support that enables them to reach higher or more opportune places for the purpose of absorbing more light or other helpful resources. Cucurbitaceous crop species' modified branches are called tendrils. Cucumber and melon tendrils are branchless [21-23].

To host the plant shoot toward the attachment site, tendrils coil by producing two opposed helices with around ten twists on either side of a perversion point. The lignified gelatinous fiber ribbons are only present on the ventral side of tendrils. As a result, in cucumbers, the tendrils coil and the ventral side shrinks lengthwise in comparison to the dorsal side. Because tendrils may climb, growing cucumbers in sheltered areas can become unruly and require more crop management. As a result, tendrils must be physically removed as soon as possible, and the primary vines typically develop in [24, 25].

By overexpressing the Zea mays CKX gene in Nicotiana tabacum, which is stimulated by the CaMV35S promoter, transgenic plants were created. Transgenic plants exhibited greater levels of enzyme activity than their wild counterparts, which resulted in a reduction in cytokinin levels, suppression of stem development, and an increase in root growth. In a follow-up investigation, the scientists demonstrated that cytokinins have crucial and conflicting functions in roots and shoots: they are a negative regulator of root meristem activity and a positive regulator of stem meristem activity. However, cambial activity of the mutant stem is entirely recovered by grafting a wild-type scion or rootstock. This implies that, in the context of cambial growth regulation, cytokinins serve as a mobile, bidirectional signal [26-28].

The polyketide or shikimate pathway in plants produces polyphenols, which are secondary metabolites that are frequently found in fruits, nuts, seeds, and vegetables. They have many phenol units (C6H5OH) connected to the aromatic benzene ring by hydroxyl groups (OH). It has been discovered that polyphenols may have medicinal benefits. Numerous experimental and clinical studies have demonstrated the significant modulatory effects of a diet rich in polyphenols on the pathophysiological mechanisms of many underlying chronic diseases, particularly in diabetes, cardiovascular, and neurodegenerative diseases, indicating their significant prophylactic and therapeutic potential. The polyphenolic chemicals lowers the chance of developing neurodegenerative diseases. It has been discovered that a number of these substances can protect cells from oxysterols (such as 7-ketocholesterol), reduce mitochondrial malfunction, and lessen cell damage. Polyphenols such as apigenin, quercetin, and resveratrol have been shown to be able to scavenge reactive oxygen species (ROS) that are formed by oxysterols, hence reducing the effects of ROS. They have strong antioxidant properties because of their capacity to scavenge free radicals and their iron-chelating properties. The antiviral, antibacterial, anti-inflammatory, anticarcinogenic, and neuroprotective. Most soils, especially those in Africa, are deficient in organic matter. The important chemical components of the SOM include phenols, polysaccharides, peptides, hydrocarbons, lipids, sterols, lignins, bound and free fatty acids, nitriles, and suberin. Additionally, SOM support microbiological activity, physical soil protection, root development, and erosion prevention [29, 30].

The influence of pH and the amount of nanoparticles (NPs) scattered throughout the soil based on the particular usage of the land are two primary indicators used to evaluate the health and quality of the soil. Fertilizer additions have an impact on the pH of the soil and the plants in different agricultural settings. The availability of plant nutrients, their medicinal value, the quality of food crops, and the general activity of microorganisms are all impacted by the pH of the soil. Once again, a variety of additional parameters, such as amount, duration, intrinsic toxicity, experimental settings, treated microbiological species, and other soil characteristics (pH, SOM, water content), affect how the microorganisms in the soil react to different types of NPs [31, 32].

CONCLUSION

Medicinal plants and other soil-grown agricultural goods are often impacted by oil pollution. The factors behind the decrease in the chemical activity of medications derived from these medicinal plants is necessary due to the increasing resistance of infections to currently available treatments. To combat microorganisms that are resistant to several drugs, new and potent antibiotics are thus required. The existence of hazardous heavy metals in the soil, such as cadmium, mercury, lead, chromium, arsenic, nickel, copper, and zinc, is one of the many other explanations for why some bacteria and germs have become resistant to medications this century. These techniques allow genetic material to be added, removed, or modified at certain genomic locations. A plethora of techniques have been developed to alter DNA. One well-known example is CRISPR-Cas9, short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. Because the CRISPR-Cas9 system provides numerous benefits over current genome editing approaches, including speed, cost, precision, and efficiency, the scientific community has been highly enthused about the technology.

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