- Spacers originate from invading sources of DNA, such as phages, that are copied and incorporated into the CRISPR locus when an infection occurs.
- Near this locus, a second locus includes a set of genes that encode CRISPR-associated endonucleases (Cas), which introduce cuts in the genome.
- When a repeat infection occurs from the same invading DNA, an RNA molecule (CRISPR RNA, or crRNA) will form a complex with Cas and a transactivating crRNA (tracrRNA) to guide the nuclease to the exogenous sequence.
- The Cas-RNA complex will recognize the DNA target that is complementary to the crRNA and adjacent to a specific 3-nucleotide locus (the protospacer-adjacent motif, or PAM).
- The complex then cuts and deactivates the invading DNA.
- Although different nucleases are associated with CRISPR activity in different bacteria, most of the topics in this review refer to Streptococcus pyogenes, which relies on the endonuclease Cas9.
- For this reason, the review refers to CRISPR-Cas9 technology. The CRISPR-Cas9 system requires 2 RNA molecules: crRNA, transcribed from the DNA spacers, and tracrRNA, whose interaction with crRNA may be structural requirement for the recruitment of Cas9. In a landmark study, these 2 RNAs were hybridized to create a single-guide RNA (sgRNA). This simplified Cas9-sgRNA system demonstrated gene editing properties

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2. Biosensors and Their Applications in Agriculture

KUMARASWAMY, H. H. AND USHA KIRAN, B.

ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Rajendranagar, Hyderabad-500 030, Telangana, India

Introduction

Biosensors have revolutionized the way data recording and analysis of biological material are dealt with. Various activities from research through quality control to quality monitoring involve precise and accurate assessment and ascertainment of quality and quantity of biomolecules including metabolites and microorganisms. This can be carried out in a precise and cost-effective manner using biosensors. In this article, we discuss basics, principles, types and applications of biosensors, with special reference to agriculture.

What are Biosensors?

Biosensors can be defined in many ways and the most accepted definition is this: according to Tothil and Turner (2003), "*biosensors* are analytical devices incorporating a biological material, a biologically derived material, or a biomimic as the recognition molecules, which is either intimately associated with or integrated within a physicochemical transducer or transducing microsystems".

Components of Biosensors

In essence, a biosensor is made up of two types of components: biological and physical. Biological component is constituted by biological substance that reacts with biological analyte (biological molecules, presence or concentration of which is to be detected) and physical components are comprised of three parts: (a) part of the biosensor that recognises the signal produced due to interaction between analyte and biological component of the biosensor, (b) part that converts the recognized biochemical or biophysical signal into electrical signal (the process is called transduction), and (c) biosensor's part that reads the transduced signal (the part called reader device) and gives either analog or digital output inferable by the experimenter/observer. Physically as well as functionally closer these components in the biosensor system, better would be the performance of the biosensor (Santoro and Ricciardi, 2016) due to least signal-loss and reduced background-noise. Biosensors fabricated using nanotechnological approach are called nanobiosensors (Aquino and Conte-Junior, 2020).

Principles of Biosensors

In fact, biosensors augment the human sensory capacity even beyond the limits of sensory organs: tasting capacity of tongue and smelling capacity of nose, for instance. When a biochemical reaction takes place between analyte and biological component of the biosensors; detector, transducer, and reader components of the biosensors mediate helping the experimenter to understand the presence or quantity of analyte. More the analyte, more would be the signal produced and corresponding output is given by the reader part of the biosensors. Thus, biosensors aid the human brain to understand the presence or quantity of the analyte by producing the type and amount of the signal without which it is impossible for human sensory organs to detect such a minute and otherwise incomprehensible signal that is produced by analyte upon interacting with the biological component of the biosensor.

Types of Biosensors

Based on the bio-physicochemical signal that is produced when analyte reacts with the biological component of the biosensors, different types of biosensors can be developed to detect these signals. Accordingly, biosensors are classified into the following broad types:

Electrochemical Biosensors

They measure electroactive chemical species produced or consumed by biological analyte upon interacting with the biosensors. Further, based on how electrochemical signal of these species is transduced, electrochemical biosensors have the following variants:

Potentiometric Biosensors

This type has two electrodes: reference electrode and working electrode. Sensing of analyte is based on measuring electric potential of working electrode in relation to reference electrode that has zero current flow. E.g., Biosensor for measuring sucrose concentration in soft drinks (Rotariu et al. 2002). This type has an advantage of detecting wide range of variations of analyte in the test samples.

Amperometric Biosensors

In this type, constant potential applied between working and reference electrodes results in redox reactions in the test solution leading to net current flow. The amount of current flow is directly proportional to the concentration of the electro-active chemical species and, therefore, to that of analyte. Bothe oxidizing (at anode) and reducing (at cathode) reactions can be measured using this type. Both disposable as well as multi-measure variants are available commercially. E.g., biosensors that deploy oxidase and hydrogenase enzymes (Terry et al. 2005).

Calorimetric Biosensors

This type of biosensors detects the increase or decrease in the temperature in response to biochemical reaction that takes place due to presence of the analyte in the test solution. E.g., Biosensors that detect metabolites for food quality analysis (Thavarungkul et al., 1991).

Optical Biosensors

Optical biosensors detect the presence or amount of the analytes based on the optical properties including phosphorescence, surface plasmon resonance, absorbance, emittance, and chemiluminescence. E.g., Biosensors designed to detect ovalbumin in foods (Mohamad et al., 2019).

Acoustic Biosensors

They are piezoelectric (i.e., property of some material to generate electric charge in response to mechanical stress) property-based biosensors where antibodies, for instance, when bound to specific analytes undergoes structural modification creating stress on the surface of the crystal, onto which antibodies are bound, causing piezoelectrical changes that can be detected and measured as acoustic sound with the help of the acoustic biosensor systems. E.g., Biosensors used for cell-cell interactions in cell biology research (Damiati, 2020).

Immunosensors

This type of biosensors is based on immunological property that specific antibody binds to specific antigen. They are more rapid and accurate compared to immunoassay such as enzyme-linked immunosorbent assay (ELISA). E.g., Biosensors that detect pollutants in environment (Lim et al., 2019)

Application of Biosensors in Agriculture

The following are the illustrative applications of biosensors in agriculture.

Food/fruit quality control: By measuring various components/constituents of foods biosensors enable to satisfy consumers and regulatory requirements rapidly and accurately. They can be developed and applied to assess and ascertain biochemical composition, maturity, ripening and quality of fruits by measuring specific organic acids and other related biochemicals. Thus, biosensors empower consumers to buy food/fruits having quality and taste of their choice and, also, help farmers to obtain premium prices for such commodities as their quality can be easily ascertained on-site. Also, biosensors help to monitor moisture stresses caused by drought and flood conditions.

For instance, Im et al. (2018) have developed a flexible polyimide-based-biosensors for real-time monitoring of plant response to drought-stress, and it can be applied in smart farm management.

Conclusion and Future Perspectives.

The rapid advancement in the biosensor development and validation, empowered by nanotechnological applications in fabricating nanobiosensors, have resulted in the entry of biosensors to every field including agriculture and foods. In the immediate future, one can witness every quality-conscious consumer and representative of regulatory authority walking with handheld biosensors across the streets of market as well as floors of supermarkets. However, more focused research needs to be undertaken on development, validation, and fabrication of biosensors particularly for enhancing broad range of detection limits and for reducing cost of manufacturing so that biosensors become affordable by everyone and everywhere: researchers to consumers as well as lab desk to dining table.

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3. Machine Transplanting in Paddy Cultivation

G. SENTHIL KUMAR

Assistant Professor (Agronomy), Department of Rice, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu, India.

Mechanical transplanting of rice is the process of transplanting young rice seedlings, which have been grown in a mat nursery, using a self-propelled rice transplanter. In conventional manual transplanting practice, 8-12 labourers are required to transplant one acre. However, if a self-propelled rice transplanter is used, three people can transplant up to four acres in a day.

A rice transplanter is a specialized transplanter fitted to transplant rice seedlings on to paddy field. Mainly two types of rice transplanter are available i.e., riding type and walking type. Riding type is power driven and can usually transplant six lines / eight lines in one pass. On the other hand, walking type is manually driven and can usually transplant four lines in one pass.

A common rice transplanter comprises of

- Seedling tray like a shed roof on which mat type rice nursery is set;