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Mini Review

Unlocking the Role of Flavonoids as Molecular Biosensors

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Abstract

The specialized metabolites found in plants have a wide spectrum of bioactivities and several uses in daily life. Significant progress has been made in recent years in both the identification of numerous such metabolites in various plant species and the clarification of their biosynthesis routes. The biological activities of plant-specific metabolites are still unclear, and proposed uses need a clear molecular basis. The comprehension of the functions of flavonoids is often impeded by their fluctuating synthesis and their distinct spatial and temporal deposition within plant structures and systems over the course of a plant's developmental stages. The present review suggests the utilization of synthetic biology techniques to develop and enhance genetically encoded biosensors for the identification of diverse types of flavonoids in a consistent and efficient manner. This methodology will aid in identifying the exact localization of flavonoids within tissues and individual cells. The acquisition of this information would prove valuable in the creation of comprehensive system-level models pertaining to flavonoid biosynthesis. These models would ultimately showcase the integration of flavonoid biosynthesis with the fundamental processes of plant growth and development.

INTRODUCTION

Flavonoids are a class of polyphenolic compounds that are synthesized as secondary metabolites in various plant species. Flavonoids are a ubiquitous class of compounds present in a variety of food crops including fruits and vegetables. These substances exhibit beneficial biochemical properties in relation to various ailments, including cardiovascular disease and atherosclerosis. Additionally, they possess other bioactive properties, such as anti-inflammatory and anti-aging effects [1-5]. The different flavonoids have diverse biological functions, including protection against ultraviolet (UV) radiation and phytopathogens, signaling during nodulation, male fertility, auxin transport, as well as the coloration of flowers as a visual signal that attracts pollinators [6-10]. Flavonoids exhibit a wide range of biological activities, such as safeguarding against ultraviolet (UV) radiation and phytopathogens, participating in nodulation signaling, regulating male fertility, facilitating auxin transport, and contributing to the pigmentation of flowers as a visual cue for pollinator attraction. Furthermore, flavonoids are a crucial component in numerous nutraceutical formulations. The predominant focus of research on flavonoids pertains to their antioxidant properties, which constitute their primary biological activity. The antioxidant activity of flavonoids has the potential to mitigate harm resulting from free radicals by means of scavenging reactive oxygen species (ROS), stimulating antioxidant enzymes, restraining oxidases, and decreasing α-tocopherol radicals [11,12].

The phenomenon of metabolic channeling in the secondary metabolism of plants facilitates the efficient production of distinct natural compounds, thereby circumventing metabolic crosstalk. The presence of metabolons related to cytochrome P450 monooxygenases (P450s) has been established. P450 enzymes in the phenylpropanoid, flavonoid, cyanogenic glucoside, and other biosynthetic pathways are characterized by both direct and indirect experimental data [13-21]. The regulation of flavonoid biosynthesis genes involves the interplay of various families of transcription factors. The regulation of genes within the anthocyanin pathway is distinct between monocotyledonous and dicotyledonous species, and is mediated by transcription factors of the R2R3 MYB family, as well as basic helix-loop-helix (bHLH) and WD40 proteins [22-25]. The flavonoids scaffold and its various derivatives play crucial roles in maintaining plant structural integrity, providing protection against UV radiation, regulating plant cell physiology and signaling, and facilitating reproduction [26-30], [12]. Flavonoids play a crucial role as chemical modulators in the communication between plants and insects or microbes. They can function as either attractants or repellents, as well as phytoalexins against herbivores and pathogens. Additionally, flavonoids can attract pollinators through their influence on flower color. Symbiotic

nitrogen-fixing rhizobia can induce root nodulation through the excretion of certain compounds [31, 32, 4, 33]. Certain flavonoids exhibit stress-protective properties by functioning as scavengers of free radicals, including reactive oxygen species (ROS), and by chelating metals that produce ROS through the Fenton reaction. Maize's resistance to aluminum toxicity is also attributed to the involvement of flavonoids. The exudation of phenolic compounds, namely catechin and quercetin, by the roots of maize plants that were subjected to aluminum exposure suggests an in vivo mechanism for the chelation of metals, potentially serving as a means to mitigate aluminum toxicity [34-37].

Flavonoids interacting plant world as defensive agents

The UV-absorbing properties of flavonoids have been widely regarded as substantiation for the involvement of flavonoids in safeguarding against UV radiation. The primary structural characteristic responsible for chelating metal ions, including iron, copper, zinc, and aluminum, and thereby inhibiting the formation of free radicals and reducing ROS, is the presence of the OH group in the 3-position of the flavonoid skeleton. As a result, it has been postulated that flavonols may have undefined functions in the response to UV stress [38-42]. Flavonoids have been identified as a class of compounds that provide protection to plants against both pathogenic microorganisms and herbivorous animals. The phytochemical co-evolution theory posits that plant-insect interactions are primarily mediated by secondary metabolites. Consequently, the coevolution of plants and insect herbivores has resulted in the development of plant defense mechanisms, such as the production of plant secondary metabolites, and herbivore offence mechanisms, such as the ability to detoxify these metabolites [43,44]. The induction of ultraviolet (UV)-absorbing compounds is a phenomenon that is observed in response to various stressors in plants, including herbivore or infection by pathogens. This induction can have either a positive or negative effect on the levels of phytochemical production [7,45-48]. The role of flavonoids as antioxidants has been identified in the process of detoxifying and scavenging reactive oxygen species (ROS) that are generated as by-products of oxidative metabolism during abiotic stresses such as salt and drought. The buildup of said metabolites occurs in response to environmental stressors experienced by plants. An increase of 40% in anthocyanin accumulation was noted in response to salt stress, suggesting a potential phytochemical mechanism to mitigate the effects of salt stress and its associated toxic reactions. Furthermore, a comparative analysis was conducted to evaluate the protective properties of anthocyanin in two distinct categories of rice genotypes, namely those that are susceptible to salt and those that are resistant to salt.

Promiscuity of flavonoid suits as potential biosensors

Biosensors present a non-invasive substitute to the metabolomics analysis methodologies delineated earlier. Due to their high selectivity, these sensors are not suitable for the profiling of a broad spectrum of metabolites. Conversely, they provide a notably sensitive and discerning instrument for instantaneous

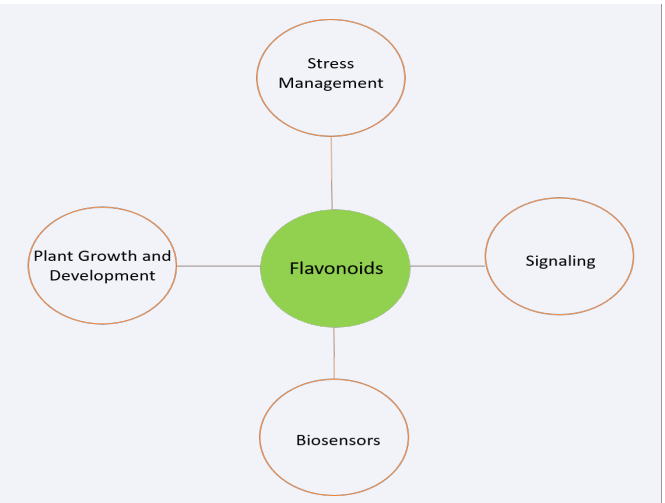

Figure 1 Flavonoid plasticity involving multiple biological process in plants.

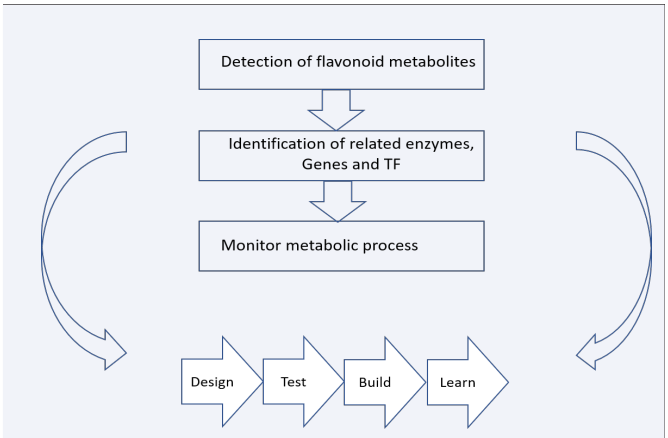

Figure 2 The utilization of biosensors in the discovery of natural products, with a specific focus on the Design-Build-Test-Learn (DBTL) module for the development of flavonoid metabolite biosensors in plants.

assessments of a specified metabolite, and thus are indispensable for comprehending the metabolism (accumulation and kinetics) of a metabolite within singular cells. This could potentially offer significant insights into comprehending the mechanism of action of the metabolite. The capacity of the luminescent moiety to specifically bind and sense the target metabolite of interest, or the presence of a protein metabolite binding entity fused to a fluorescent reporter molecule whose fluorescent properties are altered upon metabolite binding, determines whether a protein biological sensor is intrinsic or external to the protein [49]. The capacity of the luminescent moiety to specifically bind and sense the target metabolite of interest, or the presence of a protein metabolite binding entity fused to a fluorescent reporter molecule whose fluorescent properties are altered upon metabolite binding, determines whether a protein biological sensor is intrinsic or external to the protein [50]. Nucleic acid biosensors represent a potentially viable substitute for protein biosensors. Biosensors of this nature are composed of DNA or RNA aptamers and riboswitches. Aptamers, which are short single-stranded DNA or RNA molecules, possess the capacity to bind to

a specific target with exceptional selectivity and affinity, thereby generating a detectable signal through the use of a probe or other signal transducer [51]. Another potential avenue for engineering intrinsic biosensors in the field of engineering involves the utilization of synthetic promoters, either independently or in conjunction with synthetic transcription factors (TFs) that have been designed to detect particular flavonoid metabolites [52].

The issue of scale must be addressed when utilizing extrinsic biosensors for the visualization of flavonoids. These specialized metabolites represent a substantial category of chemical compounds, in contrast to primary metabolites and hormones. The above three methods outlined for developing a genetically encoded biosensor require a meticulous and arduous procedure of identifying prospective binding domains for specialized metabolites. Furthermore, it is common practice to perform multiple rounds of sequence mutagenesis and alteration on promising candidates, even when a binding domain for the target metabolite has been identified, in order to develop a functional biosensor. The utilization of integrated omics technologies presents a promising avenue for the identification of metabolite binding sites. Additionally, the principles of Synthetic Biology offer a valuable opportunity for the development of a standardized and expeditious approach to overcome this bottleneck. The application of Synthetic Biology principles, namely standardization and Design-Build-Test-Learn (DBTL) cycles, can be directly employed in the study of flavonoid metabolites. By employing automated design based on data and conducting high-throughput assembly and testing, it is possible to standardize and optimize the process. The utilization of tools and technologies is imperative throughout all phases of the design, construction, and testing process.

CONCLUSIONS AND FUTURE PERSPECTIVES

The integration of modular cloning techniques with efficient single-cell screening platforms has the potential to mitigate the primary hindrance in the development of genetically encoded biosensors. The utilization of these fundamental yet potent concepts can enhance the feasibility of biosensor development and authentication as a means of screening complete plants for the accumulation of specialized metabolites. This is due to the fact that biosensors that are genetically encoded can overcome the challenges associated with the dynamic production, accumulation, and transportation of metabolites in response to stimuli, which impede the analysis of bulk extracts. In addition, biosensors facilitate the non-invasive tracking of specific metabolites in singular cells, as opposed to a singular measurement of their concentration under a particular condition. The utilization of biosensors can facilitate precise monitoring and quantification of specialized metabolites within living organisms, thereby enhancing our comprehension of the distinct functions that these compounds serve in plants.

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