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Proteomics, Food Borne Pathogen: Applications of Food Science in Rice, Proteomics of Yield Affected by Environmental Stress, Transgenic and Food-Safety Evaluation

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Abstract:
Farmers may tackle climate change, sustainability, and food insecurity by using molecular breeding techniques like genome editing and genetic manipulation in fodder crop species. Although the majority of genetically modified (GM) products and biomass end up in livestock feed, there is a dearth of data about the safety evaluation of GM forage crops designated solely for animal consumption. Taking into account the varied risk profiles of the two types of results, a comparable methodology developed for genetically modified (GM) food might be used to the evaluation of forage crops under the current regulatory review framework. Genomic modification (GM) feed and food can benefit from the same methods employed in environmental safety assessments, genetic engineering (GE), molecular characteriszation, and genetic tracking (HGT). Nevertheless, methodological tweaks may be necessary due to the fact that various sections of the genetically modified plant that animals consume may have varying amounts of the new proteins, hence altering their exposure. No clear guidelines exist for the conduct of nutritional, toxicological, or allergenicity research on genetically modified (GM) feed specifically for animals. The regulatory cost of ensuring the safety of genetically modified (GM) feed can be reduced by the development of more tailored measures that protect the species that will eat the crop. More effective use of resources and elimination of superfluous evaluation call for a rethinking of the risk assessment process for genetically modified (GM) food and feed. Ultimately, we need a better system to evaluate genetically modified (GM) innovative crops so that we can speed up the commercialisation of goods that may improve people's health and the economy.

Keywords : Food Science in Rice, Environmental Stress, Transgenic, Food-Safety, Evaluation

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Introduction

Nearly half of the calories that people consume come from four crops: rice, wheat, maize, and soybean seeds. Breeding new rice varieties and implementing an intensive farming strategy have allowed Asia to treble its rice production since 1961. Temperature extremes, floods, and droughts are now the most influential environmental variables on crop productivity and quality due to the changing global climate. Drought affected more than 25 million hectares in Asia. Because of its smaller genome compared to other cereals, rice is not only a very valuable agricultural crop but also a valuable model plant for biological research. Encompassing nearly all of the euchromatin and two full centromeres, the map-based, high-quality sequence generated by the Rice Genome Sequencing Project (The International Rice Genome Sequencing Project 2005) encompasses 95% of the 389 Mb genome of rice. There has been fast advancement in the annotation of the rice genome, and full-length cDNAs support the majority of the predicted genes [1, 2]. The plant research community has been facing the problem of determining the function and regulation of rice genes ever since the genome sequencing of rice was finished. Producing high-quality and abundant grains for consumption as a staple meal throughout Asia, particularly in the south-east region, is the primary goal of rice production. Furthermore, seeds are also very important to the rice lifecycle. In order to grow rice, one must first have seeds of good quality and viability. Studying rice seeds, particularly grain filling and seed germination, is one of the most active fields in rice biology, which is not surprising. Additionally, it is crucial for the breeding and production of rice to understand the regulatory mechanism of rice grain filling and seed germination. Thanks to proteomic technologies, which allow for large-scale protein profiling, the mechanism of these two complex processes has been better understood, revealing that the regulation occurred in separate pathways at various levels. Drought, flooding, extreme heat or cold, and salt are examples of abiotic factors that can hinder the germination of rice seeds and have a significant impact on the grain filling process, consequently reducing yield and quality.

Expression proteomics, functional proteomics, structural proteomics, proteome mining, and post-translational modifications are the six main categories of proteomic methods. Using proteomics, a novel and exciting method has emerged for the purpose of identifying proteins in food matrices and studying protein-protein interactions in both natural and artificially altered meals. It aids in predicting the stability and quality of food products by providing sensitive information on structural changes in proteins at certain amino acid residues throughout processing processes [3-5]. Protein and peptide analysis is crucial to proteomics, and one of the trickiest parts is isolating the compounds from the complex and ever-changing concentration range seen in food. Molecular markers for physiology and pathobiology, integrated foodomics, in-vivo protein digestion trails, identification of allergic proteins, diagnosis, targeted treatment, vaccine/drug development, characteriszation of rice proteins, and studies on changes in food quality are the primary uses of proteomic techniques.

Gastrointestinal Proteomics

A plant's seeds are a treasure trove of carbohydrates, proteins, and oils. Rice stores more carbs than other plant species, especially when compared to oilseeds and soybeans. The starch content makes up over 85% of the dry mass in fully grown rice grains. Grain filling is the stage of seed development when endosperm synthesises the majority of the starch. The period of time between six and twenty days following flowering is often considered grain filling in rice. Physiological, metabolic, and morphological changes occur in seeds at this stage [6, 7]. The synthesis and storage of starches, proteins, and minerals during grain filling primarily control the yield and nutrition, two significant economic aspects of rice grains. The fact that these traits can be drastically altered by manipulating several biosynthetic routes suggests that gene expression in these pathways is highly correlated. Several genes involved in starch and protein production and storage are selectively expressed during rice grain filling, according to mRNA expression studies. In addition to the fact that RNA cannot provide information regarding post-translational modification, gene expression at the RNA level is not necessarily congruent with protein level expression. The biosynthesis routes are catalysed by various enzymes, therefore studying them at the protein level would undoubtedly yield more direct evidence. Seed filling proteomic studies have been conducted in a wide variety of plant species, including Medicago truncatula, oilseeds, barley, wheat, rice, soybeans, and maize. Research shows that during seed filling, proteins involved in

cellularization and cell division are lowered, while proteins involved in cell growth and reserve accumulation are elevated. To this day, Xu et al. (2008) hold the record for the most thorough proteomic investigation of rice grain filling. Among the 345 proteins that were found to be differently expressed, 45% were associated with various metabolic pathways, and 20% with protein production and its final destination. Proteins involved in starch synthesis rose during the grain filling stage and reached a peak just before filling was complete; these proteins can improve starch production and accumulation [8, 9]. Similar to maize, a shift from central carbon metabolism to fermentation occurs in the late stage of grain filling as a result of starch buildup, which can lead to anoxic conditions. Grain quality is affected by the production of starch, its components, the structure and organisation of starch granules, and other factors. Two primary components of starch are branched amylopectin (a-1, 6-branched polyglucans) and unbranched amylose (linear a-1, 4-polyglucans). One factor that determines the quality of rice grains is the ratio of amylose to amylopectin. The quality of the seeds is negatively affected because mutants that cause a low amylose content (<2%) would result in seeds with opaque endosperm. Enzymes involved in starch production were found to be at their most active fifteen days following flowering, according to reports. The quality of the grains improves as the duration of the activities increases. Transcriptional factor MADS-box The rate-limiting step enzyme in the starch synthesis pathway, ADP-glucose pyrophosphorylase, is encoded by genes that OsMADS6 regulates, whereas OsMADS6's own expression is controlled by epigenetic mechanisms. Kim et al. (2009), Zhang et al. 2010b, and Zhu et al. (2011) found that phytohormone cytokinins can improve rice grain fullness, while ABA, ethylene, and methyl-jasmonic acid (JA) can have the opposite effect. Ethylene and ABA inhibit the expression of genes involved in starch synthesis, which can lead to inferior spikelets with poorly filled grains. Methyl-JA may inhibit starch synthesis gene expression by inducing ABA biosynthesis [10-13]. Grain filling can be reduced by drought due to the action of methyl-JA. Gene regulatory element AP37 has the ability to restore grain filling after a drought. One possible strategy to satisfy the grains and boost production is to postpone the action of ABA and ethylene.

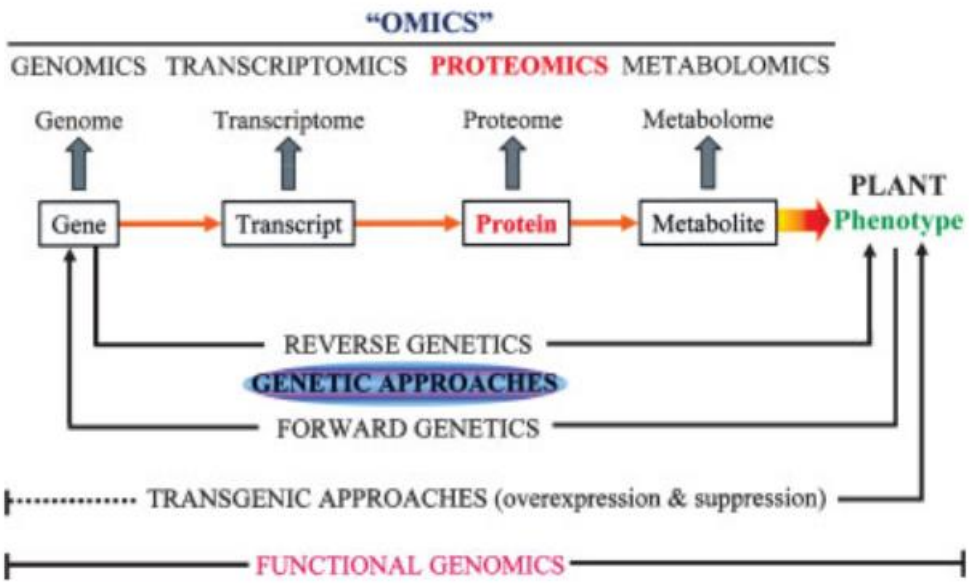

Figure 1. "Omics" software at the age of functional genomics. To definitively identify the role of a gene responsible for a specific phenotype, a combination of genetic (reverse and forward), transgenic, and other methods will be required. Understanding the plant as a whole will be possible with the use of proteomics and other profiling technologies, which will provide light on the roles played by every gene and the networks that encode them.

Taken together, these results suggest that nuclear proteomic research could shed light on the process of grain filling. To my knowledge, no one has ever reported anything similar in rice till today. During the early stages of seed filling in Medicago truncatula, proteins that are involved in transcription control [14, 15], RNA processing and transport, chromatin modification, RNA interference, and RNA-directed DNA methylation were discovered to be deposited in the nucleus. Based on these findings, it appears that active regulation of gene expression occurred at multiple levels throughout seed formation. Proteins that bind RNA are essential for many cellular processes, including RNA

processing in the nucleus and RNA transport, localisation, translation, storage, and destruction in the cytoplasm. Many proteins involved in metabolism were also discovered to be cytosolic RNA binding proteins, in addition to those famous RNA binding proteins. It is unclear why these metabolic proteins should bind to RNAs. Proteins, which make up about 6-10% of the dry mass of rice grains, are essential for the grain's nutritional value, cooking capabilities, and brewing qualities, alongside carbohydrate. Glutamins, prolamins, globulins, and albumins are only a few of the rice storage proteins. Many ribosomal proteins are stored in the nucleus in Medicago truncatula, according to studies, in anticipation of the subsequent intense protein synthesis. All of these points to the stocking proteins being the primary reserves (at around 40%) in Medicago truncatula. While filling their seeds, various plants clearly synthesise and store different amounts of reserves [16-18]. Since many biosynthetic pathways often share the same carbon precursors, it is crucial to understand how the plant regulates the synthesis of its various reserves. The binding of glutelin to an RNA binding protein and

RNAs that are prolamine in living organisms. This protein may be involved in the regulation of storage protein gene expression and is transported to many cellular compartments. Two proteins, lipoxygenase and SBPs, were shown to be over-represented in developing soybean and Medicago trunctula seeds, but under-represented in non-leguminum plants like rapeseed, wheat, maize, and rice, according to proteomic research. It is possible that these two proteins help legume seeds store stocking proteins. The proteome data of growing seeds from various plant species should be compared thoroughly.

Anxieties and Food-Borne Infectious Agents

Allergies, in their broadest sense, are the body's negative reactions to substances, whether they originate from outside the body or from within. The allergen is the potential culprit that explains the reactions in the body. A variety of physiological reactions, including elevated levels of immunoglobulin E (IgE), histamines, cytokines, and others, can trigger an allergic reaction in the body. The majority of food allergy illnesses are caused by problems that are mediated by IgE. There has been a rise in the demand for molecular methods to identify food allergies, as well as to predict how the body would react to them. In order to identify dietary allergens, Sommergruber (2016) conducted a comprehensive analysis of both plant and animal sources. He has mentioned numerous significant food allergies, including parvalbumin in fish, caseins in cow's milk, tropomyosins in shellfish and crustaceans, and Bet v 1 related proteins in monocot and dicot plants. The importance of developing sensitive detection/quantification methods for allergy diagnosis, therapy, risk assessment, and the reinforcement of current regulations on the issue necessitates the extensive translation of proteome research into the field of food allergens [19-21]. Determination of allergenic proteins or allergenic disease genes. The impact of food on consumer health has propelled food safety and quality to the forefront of global health organisations' agendas. Microbial or enzymatic decomposition can quickly ruin the food. The social, economic, and public health significance of food rotting is impacted by it, in addition to the individual's health. The discovery of molecular markers for particular food spoilage or pathogenic micro-organisms is crucial for the use of proteomics in nutritional quality of food analysis. Predicting the quality of the fermented end-product from the proteome or metabolome of the starting culture is possible for fermented foods that comprise various microorganisms and complicated substrates. Microbes, which include mostly bacteria and fungus but also viruses, prions, parasites, and protozoa, disrupt human homeostasis, which in turn can cause illness, abnormalities, or even death. Sometime before, during, or after harvesting is when these microorganisms get into the food [22-25]. Before they reach the consumer, they can already be present due to improper storage or the presence of infectious food handlers. Food intoxication, food infection, and toxico-infection are the three main categories of food-borne illnesses. Toxins produced by bacteria or fungi can endure many food processing methods and can be released either externally or internally. Martinović et al. (2016) evaluated the use of proteomics to identify such factors in food. His research centred on the use of several proteomic methods for the identification of food-borne pathogens and toxins.

Proteomics in the Maturation Process

The majority of offspring of higher plants are born from seeds. The plant lifecycle relies on the regulation of seed germination. Germination is defined as the physiological process that begins with the dry seeds absorbing water and culminates in the radicle's protrusion. There are three distinct stages to water intake throughout germination. The first step, known as fast water absorption, is followed by the second, more gradual stage. Phase II is characterised by a

nearly complete cessation of water absorption. Once germination is complete, the third stage, which involves rapid water uptake, can begin. In order to enter phase III, dormant seeds must be broken. Rice seeds are active. Once the right circumstances are met, it will begin to germinate. Various studies have examined the transcriptomic and proteomic alterations in Arabidopsis and rice seed germination genome expression profiles. Germination was characterised by the preferential expression of genes involved in translation, protein breakdown, and cell wall remodelling, according to these findings. While transcription isn't strictly necessary for germination, translation is, and blocking translation will prevent germination entirely [26, 27]. The dried, mature seeds lie dormant before they germinate. When seeds are soaked, their metabolism quickly starts up again, leading to significant changes in both biochemistry and physiology. When the metabolism gets back on track, the reserves can be accessed, which means there will be enough energy and substrates for the subsequent seedling growth. Granules of starch are stored in the endosperm of rice, which is structured into semicrystalline arrays. Attacking the surface of the granules should be the initial step in degrading starch. Only alpha-amylase, an enzyme unique to plants, can facilitate this process. To facilitate further breakdown, alpha-amylases in germinating cereal endosperm break the a-1,4 links in the surface-exposed glucose polymers of the starch granules. This process releases soluble glucans. Debranching, which is catalysed by isoamylase, is the second stage. The branching polymers' a-1,6 links are hydrolyzed by this enzyme. Two other paths exist for the continued breakdown of linear and soluble glucans, which are byproducts of the initial two stages of degradation. Two enzymes are involved in this process: glucan phosphorylase catalyses the first, which can release glucose 1-phosphate; and maltose phosphorylase converts maltose to glucose 1-phosphate and glucose. Beta-amylase catalyses the second. The second route in chloroplasts was found to be responsible for the degradation of the linear glucans. Germinating rice seeds contained enzymes from both pathways, suggesting that starch could be digested in two ways throughout the germination process. Other enzymes involved in starch breakdown remained steady throughout this process, with the exception of alpha-amylase, which experiences a substantial rise during germination [28-31]. There must to have been some preprogramming of starch breakdown during seed development and maturation for germination to occur. Cereal seeds primarily generate alpha-amylase in the aleurone layer, which is stimulated by the phytohormone gibberellic acid (GA). Therefore, GA is crucial for starch breakdown. Proteomic investigation of mutants lacking GA in Arabidopsis revealed that GA does not influence the release of stored lipids and proteins, but it does influence the loosening of cell walls. The seed-germination-regulating phytohormones ABA and GA are hostile to one another. While GA encourages germination, ABA helps keep seeds in dormancy. Proteomic research shown that ABA inhibits the germination of rice seeds by reducing the expression of certain GA-responsive proteins [32, 33]. Sulfuric acid also induces alpha-amylase in rice seeds, suggesting that treating the seeds with sulfuric acid can improve their germination rate. The process of starch degradation begins in the endosperm after alpha-amylase has been produced. In grain seeds, proteomic research revealed the presence of both full-length and fragmented alpha-amylase. It is unclear at this time whether either stage serves a useful purpose.

Environmental Stress and Its Impact on Yield Proteomics

Among grains, rice has a vast range of cultivars that can withstand varying degrees of moisture in lowland and mountainous regions. Genetic, morphological, physiological, and environmental factors are among those that impact the quantitative trait of rice yield. Examining the primary components of grain yield is a common method for doing yield trait analysis. The key factors that determine rice yield are the number of panicles per hill, the number of filled spikelets per panicle, and the weight of the grain. While these traits are mostly under hereditary control, how well they function might change depending on factors including development conditions and environmental pressures. Important environmental stresses include low oxygen levels, flooding, salinity, extreme heat or cold, and drought. In the end, the crop yield is reduced since the stresses have a significant impact on the yield components. Many things can change the primary yield components. For example, according to Liu et al. (2005), spikelet fertility and grain weight of rice under drought stress are significantly impacted by the fl ag leaf, peduncle, and anther. Consequently, the regulatory mechanism of environmental influences can be better understood by analysing plant traits associated with seed setting and fertility throughout the reproductive stage. Under drought and salinity, morphological assessments of rice showed a considerable decrease in filled spikelet per panicle and grain weights. During anthesis, the quantity of viable spikelets could be drastically reduced by temperature stressors like heat or cold [34-37]. The rice harvest is sensitive to changes in spikelet fertility and seed setting. Several approaches were used to thoroughly assess the molecular pathways of rice's reaction to stress. It is believed that proteomics may help shed light on how different

environmental pressures affect rice development, growth, and yield. In particular, it will be useful to assess the reproductive-stage proteome of rice under environmental stress and to analyse the components of yield. When it comes to environmental challenges like drought, salt, and cold, the fertility of the spikelet is the most vulnerable component of rice output. The yield can be significantly reduced if the number of unfilled spikelets per panicle increases since spikelets are sterile. At the heading stage, a proteome comparison of rice anther from genotypes that were tolerant to drought and those that were sensitive to it revealed that drought stress and re-watering significantly affected two classes of proteins [38, 39], reversible and irreversible. In contrast to drought-tolerant cultivars, drought-sensitive ones have a substantially higher number of irreversible proteins controlled by rewatering. Additionally, the drought-sensitive cultivar was the only one in which cysteine protease, an enzyme involved in the breakdown of the anther wall, was downregulated. The proteomics data showed that in a sensitive cultivar, the anther development was stopped by dryness, which led to poor anther dehiscence and reduced pollen density. Thus, dehydration during the heading stage impacts the rice anthesis process, and rewatering is not enough to completely counteract the negative consequences. During the reproductive stage of rice growth, extremely high temperatures have a deleterious impact. Pollen sterility, caused by low temperatures, is one of the primary causes of reduced yield. Pollen that is unable to develop could be produced if the conveyance of complete sucrose is inhibited by cold.

One of the most important economic properties of rice is its grain weight. A genetic regulatory mechanism regulates the filling of grains in rice. One transcriptomic study found that the panicle of a high-yielding hybrid rice variety expressed more genes involved in carbohydrate biosynthesis and starch metabolism compared to its parental lines. These genes include starch biosynthesis, ADP-glucose pyrophosphorylase, sucrose-P synthase, and invertase. Both the germination process and the nutritional value of seeds are enhanced when storage proteins build up in them [40, 41]. Various globulins, prolamins, and glutelins, among other storage proteins, begin to build up in rice seeds fourteen days following anthesis, according to a proteomic survey. Endosperm cell division in rice seed is reduced by environmental stressors. The pressures also have an adverse effect on photosynthetic efficiency, which changes the source-sink dynamic and remobilization mechanisms. The main reasons for the decrease in grain filling are a lack of assimilates and a decrease in the capacity to accumulate reserves. A proteome analysis of rice plants grown at various stages revealed that the leaves play an important role in nutrient mobilisation to the grains, and that increased photosynthetic activity in the leaves speeds up grain filling. Environmental pressures can cause changes in source and sink capacities, which in turn affect grain filling.

The Use of Proteomics in the Study of Stress Tolerance

Research into stress ultimately aims to reduce yield loss. How much environmental stressors rice can withstand depends on a lot of different things. There are three main categories into which these components fall: the plant's physiological state, the stress condition, and the genetic background. It is well-known that various elements, including fertiliser supply, developmental stage, and others, impact the physiological status of plants. Too much fertiliser used could reduce rice's stress tolerance. Researchers found a correlation between nitrogen supply and spikelet sterility under cold stress. They reasoned that growing rice plants in environments with high nitrogen levels would cause physiological damage to the pollen grains, making them less viable. Understanding the mechanisms of stress tolerance—and, by extension, how to increase rice yields under stressful conditions—requires first identifying the external elements that can impact the amount of stress tolerance. A plant's physiological susceptibility to stress is also affected by its developmental stage. The review by Neilson et al. (2010) compared the stress reactions of plants in their vegetative and reproductive phases. Researchers found that rice and other essential crops were more vulnerable to challenges caused by cold, drought, salinity, heat, and flooding during the early stage of male gametophyte development. Physiological and proteomic investigations further demonstrated that reproductive-stage rice is susceptible to abiotic stress. One common indicator of stress damage to plant growth is infertility of the spikelet, which could be caused by most abiotic stresses. Plant tolerance can be influenced by the severity, length, and kind of stress. Researchers Han et al. (2009) looked at how different high temperature stressors, ranging from 35 to 45 degrees Celsius, affected the proteome of young rice plants. Their findings suggested that proteins involved in different antioxidant pathways and protective machinery could be inducibly upregulated by raising the temperature. Changing the stress duration could also produce similar effects. Protein expression patterns under extended drought stress were different from those exposed to brief stress, according to a proteomic analysis of rice seedlings subjected to varying

durations of drought stress [42-44]. Proteome study of rice exposed to different stresses across different time periods suggests that plant cells may adapt to different harsh environments. Another aspect that can influence how plants react to abiotic stress is the kind of stress itself. There are several other environmental stressors that can inhibit growth, including toxicity from ions and water, extreme heat, and other causes. When trying to understand how severe environmental stresses including drought (Ali and Komatsu, 2006), salt, cold, heat, flooding, and anoxia affect rice throughout its vegetative stage, many proteome studies have been conducted. Despite the classification and description of sensitive proteins to different abiotic stimuli, the reproductive stage stress tolerance mechanism of rice has received less attention. A survey of the literature was used to classify the major physiological and cellular responses of rice to drought, salinity, cold, heat, flooding, or anoxia, and to summarise the key categories of differentially expressed proteins. But when we looked at how cells responded and which proteins were differentially regulated in response to abiotic stressors, we saw that the photosynthetic apparatus was the most commonly affected [45, 46]. Previously, it has been documented that photosynthesis electron transport, photosystems I and II, and RuBisCO activity are all downregulated in response to drought, salt, cold, and heat stress. The decrease in photosynthetic efficiency, modification of the source-sink connection, and remobilization processes could be responsible for the reduction of grain weight and yield under abiotic stress. The grain filling is impacted by the reduction in photosynthetic efficiency, which in turn limits assimilation.

Evaluation of food safety and screening of transgenic plants

The use of proteomics methods to assess food safety and screen transgenic plants is on the rise. The endosperm proteome expressing the hGM-CSF protein was screened using the iTRAQ approach since it is more crucial to adopt a quantitative approach to establish reliable biomarkers and confident screening systems. One great way to test the safety of new foods is with a rice variety that is low in phytic acid, or lpa. If this field continues to advance, it may one day be possible to quickly quantify food allergen proteins. An ideal quantitative approach for this would be one that is quick, easy, and does not require labels. The field of rice proteomics has come a long way in the decade from its inception with a few scattered studies in the year 2000; it has now expanded to influence many areas of rice biology and even plant biology more generally. During normal growth and development and under various biotic and abiotic conditions, proteomes have been generated for the majority of rice tissues, organs, and organelles [47, 48]. Listing the components of these proteomes with relative and absolute quantitative data has been, and will continue to be, the major goal for the last decade. The fields of comparative and functional proteomics are flourishing in tandem with advances in comprehensive proteome analysis. The identification of phosphorylation sites genome-wide and protein secretion into the ECS are two instances of functional proteomics in rice. In a similar vein, the vast resources for comparative proteomics have been generated by bacterial, human, and plant species. Recent developments in rice and plants pave the way for more in-depth studies that use mutant resources and gene editing approaches to answer data-driven biological issues. It is true that low-throughput (i.e. focused proteomics) functional analysis of rice proteins discovered utilising proteomics methods has been carried out to reveal their biology [49, 50]. Applications of proteomics have been investigated for use in screening transgenic plants and determining the safety of rice meals, in addition to basic research. These accomplishments are a result of well-planned tactics and developments in proteomics. Proteomes generated using 1-DE, 2-DE, or shotgun methods (such MudPIT) show that the proteins they identify are highly complimentary, and when used together, they give a virtually full picture of the target proteome or subproteome.

Genetically Modified Feed Safety Evaluation

Food insecurity is becoming more of a problem as the world's population rises and agricultural output is strained. The majority of the world's 870 million malnourished people live in underdeveloped nations in Asia, Africa, and South America. The availability of farmland is decreasing due to environmental deterioration and climate change, which further complicates efforts to meet the rising demand for food. One strategy to lessen human impact on the environment is to increase productivity and enhance food quality through the use of contemporary biotechnology, which includes genetic modification techniques. Because of its high protein content, animal products play an important role in human health and nutrition. Especially in arid regions, it is vital to the rural economy of the majority of emerging nations. Farming practices are putting more strain on land, water, and biodiversity conservation efforts to meet the protein needs of a rapidly expanding global population. Since forage farming uses up around 80% of the

world's arable land and supplies the grazing feed-base for the dairy and red meat sectors, there is an immediate need for solutions to address climate change and the enormous demand for natural resources. A more productive, resource-efficient, and environmentally friendly agricultural sector is possible with the use of molecular breeding technology applied to forage crops. Forage quality limits, pest and disease resistance, nutrient acquisition efficiency, tolerance to abiotic stresses, and targeted growth and development modifications are all areas that breeding programmes aim to address genetically. The classical DNA modification technique of transgenesis allows for the cultivation of crops endowed with specific features conferred by the transgene(s) inserted. In contrast to genetic modification (GM), which results in random gene insertion, genome editing technologies allow for the modification of DNA to create defined multination(s) and/or insert foreign gene(s) at the targeted site(s). Changing an organism's DNA in a way that doesn't happen naturally is the standard definition of genetic modification. Plant varieties engineered by transgenesis are thus considered genetically modified (GM) organisms. Based on the structure and approach for transgenesis, transgenic crops can be further classified into four types [51, 52]. This initial group of transgenics, sometimes known as single trait transgenics, accounts for the vast majority of genetically modified (GM) crops on the market today. The 35s promoter sequence of cauliflower mosaic virus and the nopaline synthase terminator (nos-T) from Agrobacterium are two examples of frequent transgenic components found in these crops. Most second-class transgenics are hybrids of first-generation GM crop varieties; they have stacked changed features. The hybrid cross process has the potential to minimise development costs while increasing the economic values of the GM variety. Genetically modified (GM) crops that are so-called near-intragenics fall into the third category of transgenics; in these cases, the transgene structure is derived from the host with very minor adjustments. The final category is more closely associated with cisgenic or true intragenic technologies; in these cases, the transgene differs from its conventional counterpart solely in terms of the specific order and insertion sites, and it contains no alterations whatsoever; all it contains are products and elements from the host. Genome editing technologies, on the other hand, make use of biological tools like sequence-specific nucleases to create changes in plant genomes that scientists want. These changes can include removing unwanted DNA from the host, introducing transgenes at specific loci, or controlling the expression of synthetic or endogenous genes. Safety concerns for human ingestion have largely been studied since the commercialisation of early-generation transgenic crops was approved [53, 54]. Genetically modified (GM) crops used as animal feed and GM forage are not the main focus of the current regulatory regime. There are a lot of plants and plant parts that aren't eaten by people but are used as feed for cattle. Some animal feedstuffs, like grain, are also eaten by humans. Based on the existing data on the product's exposure to current safety assessment practices, this review article seeks to summarise and examine the components necessary for the safety evaluation of genetically modified crops for use as animal feed.

RISK EVALUATION

The level of scientific proof needed to evaluate the safety of genetically modified crops can change depending on the jurisdiction in question. Nevertheless, environmental studies to permit coexistence frameworks, the creation of tracking and tracing procedures to guarantee legality and traceability, and a thorough molecular characteriszation of the transgene insertion are frequent studies in the safety assessment of GM crops. Toxicological, allergenicity, nutritional, and horizontal transfer studies, among others, have taken a case-by-case approach, taking into account the latest scientific findings and technological developments. Feeding animals genetically modified crops can alleviate worries about their impact on human health while highlighting benefits like nutritional equivalency and feeding value. From a business perspective, there are two distinct types of assessments needed by regulatory bodies: pre- and post-marketing. Molecular characteriszation and the creation of tracking and tracing tools for traceability purposes are examples of the reasonably typical technologies that fall under the former category and are applicable to all genetically modified foods and feed. Environmental, food/feed, toxicological, and allergenicity safety investigations are only a few examples of the case-by-case technologies that are part of pre-marketing concerns. Regulatory oversight concerns post-marketing, including genetically modified (GM) labelling and traceability.

Analysis at the molecular level

Molecular characteriszation of genetically modified (GM) crops provides a detailed account of the transgene's structure and the trait's stability. It is used as a starting point for developing detection and identification technologies to meet traceability and labelling regulations, and it is also the basis for all safety studies of genetically modified

products before they are commercialised. Genetically modified (GM) feed and food producers are required by law to provide details on the transgene's insertion site, flanking areas, copy number, genomic locus(es) altered, and other relevant information. In order to better characterise risks and hazards, it is preferable to use DNA transformants with a low insertion copy number in the following safety evaluation process. South blot analysis and polymerase chain reaction (PCR) in its many forms, including real-time PCR (qPCR), have become the most popular ways to determine the number of transgenes incorporated. In order to do a Southern blot analysis, a wide variety of restriction enzymes must be carefully considered, screened extensively, and probes must be designed. In certain instances, this process is dependent on previous sequencing knowledge regarding the transgene insertion. Having said that, the method does involve a manual interpretation procedure and is thus rather difficult and time-consuming. The outcome might also be misleading when it comes to the transgene copy number if there have been sequence rearrangements that have changed where the restriction enzyme recognition sites are located in the inserted transgene [55-57]. An easier alternative to Southern blot analysis, a qPCR-based assay can compare to an endogene, which allows for a more precise quantification of the copy number of transgenes.

But sometimes it's hard to identify a single copy reference gene in crop species because of polyploidy, which causes complex structures and genetic redundancy, or because of ancestral whole genome duplications (Ren et al., 2018). For the purpose of determining the GM copy number, droplet digital PCR (ddPCR) has been suggested as an alternative to DNA calibrations and reference gene identification. This approach determines the absolute DNA copy number in a sample. Once low-copy number transformants have been located, the next step is to pinpoint exactly where the transgene(s) are located in the crop genome. In this regard, DNA sequencing methods have been employed; nevertheless, it is possible that the process may also reveal backbone sequences that were not meant to be transferred from the transformation vector to the host genome. Sanger sequencing formed the foundation of the method that was previously employed for this objective. Molecular characteriszation of genetically modified (GM) crops may soon be possible with the use of second-generation sequencing (SGS) technologies, which offer greater sequencing capacity and, maybe, more accurate assembled sequences. In order to make post-transformation screening easier, SGS methods can automate, scale, and speed up the process of selecting potentially useful events based on their molecular profile.

The small read lengths of these technologies (50-400 base pairs) make it impossible to precisely determine the location of the insertion(s) in native DNA; in contrast, transgenic constructions often consist of thousands of base pairs. Alignment and assembly of short sequencing reads is a computational process that is crucial for molecular characteriszation. However, the alignment and assembly procedure might be problematic due to repeated motifs typically found in plant genomes. Read lengths can now reach tens of thousands of bases per read because to the commercialisation of single-molecule sequencing, often known as third-generation sequencing (TGS) technologies. Despite reports of read lengths exceeding 300 kb, the actual limit is imposed by the size of the input DNA fragment. A more accurate GM characteriszation may be possible with an increase in read lengths into the tens of thousands, which allows for longer sequence readings of the flanking regions in the collected fragments and may even solve alignment issues. Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT Oxford Science Park) produce the majority of the TGS platforms used today. For more precise results, PacBio employs a sequencing-by-synthesis approach that involves capturing a single DNA molecule and a circular consensus sequence (CCS). To create a continuous long read, the CCS ligates hairpin adaptors to both ends of target double-stranded DNA, creating a circular DNA template. This template is then sequenced many times. When DNA bases pass through a nanopore, they interrupt the electric current to different levels with distinct nucleotides; this process is known as nanopore sequencing, and it employs nanopores to sequence native single-stranded DNA. Due to its inexpensive setup costs, relative ease of manipulation, and real-time raw data delivery, nanopore sequencing may be preferable to PacBio for molecular characteriszation of GM products. One portable gadget that has been tested and found to be effective in detecting illegal genetically modified (GM) items is the MinION from ONT. Additional testing proved that the MinION could ascertain the complete molecular profile of three transgenic crops (clover, ryegrass, and canola) in just 48 hours. Nevertheless, new regulations are being developed by regulatory agencies to establish pre-market data submission requirements for whole genome sequencing (Health Canada, 2019) and next-generation sequencing (NGS) (UCD Centre for Food Safety et al., 2018). During the risk evaluation of genetically modified (GM) crops, sequencing methods are often used in combination, such as Sanger sequencing with single-gene sequencing (SGS) or single-gene sequencing (SGS) with tandem genomic sequencing (TGS). A few examples of genetically modified fodder crops that

have been studied using the same methods as genetically modified food crops are Alfalfa, Switchgrass, and Sorghum [58]. But it's important to think about how introduced features might manifest in plant components that aren't eaten. To avoid insect attack and human exposure to the substance when eating its grains, a GM plant can be engineered to manufacture the Bt toxin exclusively in its leaves. Molecular characteriszation must account for the fact that in such instances, animals' exposure to the GM toxin would be significantly amplified if they consumed those plant parts.

Studies on Toxicology

Toxicological investigations seek to identify active agents or compounds that may cause unintended harm to species that are not specifically targeted, while also characterising the intended alterations. Toxicological evaluations of genetically modified (GM) materials should always be case-specific and take into account the known toxicological characteristics of newly introduced chemicals. Animal studies, taking into account the species of interest and the severity of their effects, are typically the only way to determine the in-vivo toxicity of a chemical. But there are new ways to find toxicants or anti-nutrients in genetically modified (GM) feed by studying the in-planta metabolic pathway. This includes using "-omics" approaches, which could help us understand the pleotropic effects of new plant varieties better. It is also possible to do in vivo investigations on animals, as well as in vitro tests using stomach enzymes, cultured cell lines, and receptor proteins. One nontargeted strategy for finding GM crop AEs is high-throughput "-omics" profiling methods that include metabolomics, transcriptomics, and proteomics. Researchers used omics methods to profile GM glyphosate-tolerant soybean, which differed from the isogenic line in terms of several metabolites; these differences were accounted for by changes in the regulation of the shikimate pathway. When comparing the proteome profiles of conventionally bred and naturally occurring rice varieties with GM stacked rice that carried the herbicide-resistant gene bar and insect-resistance cry, the results were almost identical.

Transgenic plants do not exhibit the same level of transcriptome change as mutagenized plants, according to another study. Fungal or secondary metabolite analysis is a common part of toxicological studies involving genetically modified feed that employ omics approaches. Mycotoxins are one example of an undesirable compound produced by fungus that is associated with crops. Plants in Bt maize hybrids, which are a key ingredient in cattle feed, have lower fumonisin concentrations than isoline. Given that insect damage may assist fungal spore migration and colonisation, it was speculated that the reduced fumonisins in GM maize were a result of the pest reduction in maize. Genetically modified (GM) maize may reduce fumonisin contamination risks but does not increase animal toxicological risk. Similarly, an analysis of endophytic fungus-produced secondary metabolites known as alkaloids in transgenic high-energy perennial ryegrass revealed that the transgenic plants' alkaloids concentration was either the same as or lower than that in the isogenic line. The reduced concentration of alkaloids may be explained, in part, by the fact that transgenic plants grow faster, which dilutes the effect of fungal biomass modulation (Giraldo et al., 2018). There are different types of in vivo toxicology investigations, such as acute (14 days), subacute (28 days), chronic (90 days), or specific (reproductive, mutagenicity, etc.) research. Cry genes and the tnos promoter were found in the intestines of mice in a long-term trial that fed them crushed Bt cotton seeds; they were absent from the brain, blood, liver, kidneys, and stomach. Cattle and hens given Bt maize did not show any harmful effects in experiments that lasted more than 100 days.

Research on Allergens

Since both the allergenicity and toxicological studies aim to identify newly expressed chemicals, they can be conducted simultaneously. Toxic effects are predictable and reproducible among individuals since they impact the majority of exposed people with only slight variations in sensitivity, whereas allergenic reactions typically only impact a small percentage of the population and can cause more severe symptoms. Anxieties over genetically modified (GM) food crops have surfaced in the United States on multiple occasions. To improve the nutritional value of soybeans, scientists implanted a 2S albumins gene from Brazil nuts into a specific type of soybean. Transgene products were found to pose dangers to humans, particularly those with Brazil nut allergies, and as a result, research into genetically modified soybeans came to a halt (Moreno and Clemente, 2008; Delaney, 2015). There were also concerns about the Cry9C protein, which is a bacillus-derived insect pest resistance protein with a better heat stability and the potential for a longer digesting time. The US government thus did not authorise the consumption of StarLink maize, an illegal kind of maize that contained the Cry9C transgene. In order to determine the safety of genetically

modified (GM) feed, it may be necessary to conduct an allergenicity research on both humans and animals. Applying the same methodology to an animal allergenicity test as to a human one is possible. But even in humans, there isn't a tried-and-true method for predicting allergic responses to proteins that aren't naturally present. A case-by-case assessment of the sensitising potential of new proteins should be conducted using animal models, according to the European Food Safety Authority (EFSA).

Studies on rats, mice, and pigs were published with the intention of evaluating the risk of allergies in humans. The animals used in these studies were primarily food allergy models. Amino acid sequence homology, in vitro digestibility tests, serum screening, and animal models are the most frequent ways to evaluate allergenicity (Van Haver et al., 2003). One way to find out if a new protein poses a cross-reaction risk is by comparing its amino acid sequence to that of recognised allergens. This is done using bioinformatic approaches. It may be necessary to employ additional approaches such as in vitro digestibility testing, serum screening, and animal models in addition to bioinformatic methods in order to forecast the probability that the novel protein could develop into a de novo allergy. One of the most popular ways to find out how easily a new protein breaks down in the body is to run it through an in vitro pepsin resistance test. Since the immunogenicity of dietary proteins can be affected by gastrointestinal digestion, this assay can be utilised as additional evidence of potential adverse reactions to GM food or feed. These reactions can be IgE or non-IgE related. Another option for evaluating endogenous allergens is to use sera from people who have relevant allergies; this method is called serum screening or immunoassay. While these assays are now the gold standard for determining which proteins are allergenic in vitro, they have limitations when it comes to evaluating the safety of genetically modified feed due to the prevalence of allergic animals. Alternative studies for comparing the endogenous allergenicity of GM plants to non-GM plants can be conducted using additional analytical and molecular profiling methods. There has to be more research on immunological responses, especially allergic reactions, in cattle that are fed GM products. Currently, there is very little available information on this topic. However, unlike with GM food in humans, the benefit of performing allergenicity assessments on GM feed is the ability to conduct direct evaluations in target species.

Vertical Gene Transfer

Human genetic transfer (HGT) is the process of transferring DNA from one species to another, allowing it to be incorporated into a living cell or organism. Concerns about the potential effects on human and animal health as well as environmental factors have prompted investigations into the possibility of transgene migration into other species, particularly microorganisms or naturally occurring populations of taxonomically related species. Since the introduction of recombinant DNA into other creatures might impact the well-being of people, animals, and the environment, this part pertains to studies concerning environmental, feed safety, toxicology, and allergenicity. Although in vitro gastric or intestinal fluid-based digestion systems can be used to evaluate HGT in bacteria, studies evaluating the digestion process of feed-stuff recombinant DNA and proteins have also frequently used cattle species. Take calves as an example. While recombinant DNA has been discovered in their ruminal solid phase and duodenal digesta, it has not been detected in their liquid ruminal and duodenal phases, milk, blood, or faeces. According to their findings, the transgene is broken down quickly during the initial phases of digestion. Researchers were unable to find any signs of recombinant DNA in the milk, blood, muscles, kidneys, liver, or spleen of cows that were given transgenic corn. Additionally, milk from cows fed a diet that included as much as 26% transgenic glyphosate-tolerant soybean did not contain any recombinant DNA. Eggs, liver, kidneys, and muscle did not contain any recombinant DNA in trials on fowl that were given transgenic maize. While some studies have shown a small chance of recombinant DNA insertion into the genomes of animals or humans with digestive organs, the vast majority have found no such danger of horizontal transgene transfer. With an even reduced chance of recombinant DNA integration into germ cells, the likelihood of passing the gene on to subsequent generations should be negligible. Because humans indirectly eat genetically modified (GM) feed items through cattle, concerns about their effects on human health should be considered as well. Based on the studies mentioned earlier, it appears that the indirect dangers to human health from recombinant DNA are minimal due to its quick digesting process. Animal products made from crops that have been genetically modified have also been the subject of extensive safety testing; nevertheless, the vast majority of these studies have failed to detect the presence of recombinant DNA in the tested meat and dairy products. On the other hand, milk tested positive for trace amounts of recombinant DNA in two instances. But the writers saw their

existence as feed particle contamination of airborne or faecal debris. So far, the majority of scientific research has failed to identify any substantial dangers associated with eating genetically modified (GM) foods. These results can be applied to other types of crops as well. Although dietary DNA is not completely digested and, in rare instances, minute bits might be discovered in animal organs, proteins generated from recombinant DNA, like any protein, undergo degradation in the gastrointestinal tract.

CONCLUSION

Farmers may tackle climate change, sustainability, and food insecurity by using molecular breeding techniques like genome editing and genetic manipulation in fodder crop species. Although the majority of genetically modified (GM) products and biomass end up in livestock feed, there is a dearth of data about the safety evaluation of GM forage crops designated solely for animal consumption. Taking into account the varied risk profiles of the two types of results, a comparable methodology developed for genetically modified (GM) food might be used to the evaluation of forage crops under the current regulatory review framework. Genomic modification (GM) feed and food can benefit from the same methods employed in environmental safety assessments, genetic engineering (GE), molecular characteriszation, and genetic tracking (HGT). Nevertheless, methodological tweaks may be necessary due to the fact that various sections of the genetically modified plant that animals consume may have varying amounts of the new proteins, hence altering their exposure. No clear guidelines exist for the conduct of nutritional, toxicological, or allergenicity research on genetically modified (GM) feed specifically for animals. The regulatory cost of ensuring the safety of genetically modified (GM) feed can be reduced by the development of more tailored measures that protect the species that will eat the crop. More effective use of resources and elimination of superfluous evaluation call for a rethinking of the risk assessment process for genetically modified (GM) food and feed. Ultimately, we need a better system to evaluate genetically modified (GM) innovative crops so that we can speed up the commercialisation of goods that may improve people's health and the economy. Extensive research in rice proteomics since 2000 has led to the discovery and elucidation of tissue, organ, and organelle proteomes in both typical and pathological environments. Additionally, well-established proteomes have been important in re-annotating the rice genome and uncovering the novel function of previously identified proteins. Recent developments in rice proteomics have established this grain as a foundational crop for the cereal family. The groundbreaking proteomics studies conducted on rice have ensured its status as a crop model system. In order to adapt to a dynamic environment, research into rice proteomics should lead to improvements in crop plants, and vice versa. Overall, environmental pressures will always be a part of growing rice, but we may lessen their negative impacts by learning more about how cells respond to and tolerate stress. This problem may be amenable to proteomics analysis of stress-related cellular responses, which could provide a solution. We still don't know enough about how stresses affect yield and yield-related features, despite the fact that rice has been examined extensively for their effects on important environmental stresses such drought, salt, cold, heat, flooding, and anoxia. In addition, it is currently difficult to understand how rice's molecular systems responded to multiple pressures at once. As a result, it may inspire researchers to work on more stress-tolerant rice types in the hopes of finding answers to these issues. Proteomics has many applications, including the cloning of novel genes by differential analysis and the study of post-translational changes that change a protein's function in the cell by influencing its binding and activity. Expectedly, proteomics will have a significant influence on rice lineage improvement through the usage of fully analysed genes and analyses of embedded protein activities. Integrating proteomics data with other comprehensive analysis data should soon be possible with a high degree of accuracy. With these results, we can better understand the rice proteome and its subcellular compartments in the future, which could lead to the discovery of novel rice crop enhancement targets.

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