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UTILIZATION OF PROTEOMICS APPROACH TO UNDERSTAND GENES ASSOCIATED WITH THE OCCURRENCE OF BIOTIC STRESS IN PLANTS

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ABSTRACT

Of the most fundamental fields of modern biology is transcriptomics, with a focal point on the expression pattern of plants under various conditions by assessing ribonucleic acid. So far, this approach has been a game-changer in revealing the gene structure, function, and most importantly, their cellular and biological role. Considering the criticality of pathogens for crop plants, understanding plant defense mechanisms against them is in high demand. This study aimed to review the principles of these approaches and their recent application in the plant. An Important method to address this gap is transcriptomics, which can effectively provide insight into plants against pathogens. This field has covered different aspects of plant biology besides the plant-pathogen relationship. Identifying pathogens in infected plants and the series of reactions they provoke at the gene level is crucial to finding the responsible gene (s). Finding the gene associated with resistance or vulnerability to a specific pathogen paves the way to differentiate the potential genotypes. Thus, the breeding attempts would be more successful. The advancement in biotechnology has revolutionized this field with some of the methods that have been commonly applied in studies on the plant-pathogen relationship, for instance, Northern blotting, microarray, real-time polymerase chain reaction.

Keywords: *plant-pathogen relationship, gene expression, polymerase chain reaction, microarray method, SAGE*

1. INTRODUCTION:

The precise and controlled pattern that causes different genes to be expressed in other cells and at different times is called the gene expression pattern. Different expression of genes in cells causes differences in leaf and root cells in plants or humans, causes differences in liver and muscle cells, and differentiates a healthy cell from a cancer cell. However, the question that arises is how researchers find out genes that are on and when these genes are turned on or off. Gene expression is an active phenomenon, and the same genes may function differently under various conditions. Simply put, it can be said that two organisms have identical genotypes but indicate different phenotypes, which is due to differences in the expression of various genes (Gibson, 2005; Anderson and Kedersha, 2009). Researchers often use laboratory methods such as Northern Blot or serial analysis of gene expression (SAGE) to answer various gene expression questions, especially defense genes in plants under stress. Most of these methods make it possible to identify genes that are turned on and off in the cell; consequently, this information can indicate the conditions that led to these genes expression (Ohtsu *et al.*, 2007; Weber *et al.*, 2008). The main groups of pests and diseases that damage plants include fungi, bacteria, nematodes, viruses, and insects. Plants protect themselves through various tools, including structural barriers that act as physical barriers and produce secondary metabolites with antimicrobial activity (Ambrose and Belanger, 2012). Plants can activate their immune system after understanding the pathogen. This speed of these processes is one of the most critical factors in the success of resistance in plants (Fregene *et al.*, 2004).

Activation of resistance genes leads to subsequent changes at the infection site and systematically throughout the plant, causing physical changes in the cells such as more lignin production and higher plant strength (Venu *et al.*, 2007). The essential part of effective plant defense against pathogens is the rapid induction of plant defense genes. A significant group of defense genes is genes encoding pathogenic proteins (PR). While the function of some PR protein genes is still unclear, some of them have chitinase and

glucanase activity, which are cell wall compounds. Other induced defense genes include diphenhydramines, enzymes involved in the biosynthesis of phytoalexins, plant protection enzymes, and messaging components such as specific transcription factors (Q. Zhu *et al.*, 1994; El Ghaouth *et al.*, 2003; Suryadi *et al.*, 2014). Induction of defense genes in plants responding to pathogens occurs primarily at the transcriptional level. Secondly, the regulation of temporal and spatial expression patterns of defense genes is an essential part of plant defense. Identifying message transmission components for the expression of plant defense genes, including crucial key transcription factors, has raised the hope that these factors are a reliable tool for increasing plant resistance to a wide range of pathogens. The studies have mainly been on the model plant, Arabidopsis, which will eventually lead to the transfer of these results to crops using recombinant DNA methods (Bolwell, 1999; K. B. Singh *et al.*, 2002; De Palma *et al.*, 2019).

The pathways involved in the expression of plant defense genes are regulated by several defense messenger molecules, including salicylic acid, ethylene, nitric oxide (NO), jasmonic acid, and reactive oxygen species (ROS) such as hydrogen peroxide. ROS and NO are critical signaling molecules during the hypersensitivity reaction, and their activity together seems to cause local death of plant cells (Ma *et al.*, 2018; De Palma *et al.*, 2019; N. Li *et al.*, 2019). A significant increase in salicylic acid levels at the site of infection and to a lesser extent in other parts of the plant and salicylic acid as a spray on the plant increases the plant resistance to many pathogens. Transgenic plants unable to accumulate salicylic acid due to the lack of a bacterial enzyme that converts salicylic acid to its inactive form cannot develop systemic acquired resistance (SAR) and induction of defense genes (A. Singh *et al.*, 2017; Kamle *et al.*, 2020). Rapid defense genes and their changes are obtained in different stress conditions with the pathogen (Yao *et al.*, 2020). In the early days of the study of gene expression, researchers mostly studied the expression of a single gene or a few of genes over a period of time. With the help of new methods, methods that make it possible to study many genes have recently been used. Fortunately, new techniques have made it possible to study gene expression on a large scale (H. Chen and Vierling, 2000; López-Maury *et al.*, 2008). It is essential to study the expression of plant defense genes during stress concerning pathogens. The results of this research increase information related to the mechanism of tolerance and resistance to pathogens and facilitate the production of tolerant cultivars through biotechnological methods (Nelson *et al.*, 2004; Gutierrez *et al.*, 2008).

Numerous reviews exist on plant response to abiotic and biotic stress. Still, only a few systematic reviews have considered the application of proteomics techniques to unravel the underlying genes involved with these defense responses. Therefore, various popular proteomics methods for analyzing gene expression profiles in plants against pathogens and pests will be discussed in this review focusing on plant defense responses.

2. MATERIALS AND METHODS:

The focal point of this review was proteomics methodologies that had been utilized for elucidating the genes underlying the response mechanisms involved with biotic stress, excluding abiotic stress. The application of mitigating substances in plants against pests and pathogens is not covered in the review. However, the criticality of the topic is appreciate — a period of 1990 to 2020 covered in a systematic literature review. Given the richness of the literature in this area, specificity was taken to be more focused. This review is based on published works that have been conducted on plants, mainly crops or model species.

As it is a critical area of biology, several reviews have been published. However, the level of focus on plant defense systems is quite a different one. Scopus, ScienceDirect, ResearchGate and Google Scholar databases were used. The search was done in the English language. Specific keywords have opted to retrieve many results after identifying proteomics methodology to understand the abiotic stress-associated genes. The chosen keywords were: “abiotic stress”, “plant-pests relationship”, “plant-pathogen relationship”, “genetic mechanism in plants”, “influence of pests and pathogens on plants”, “microarray”, “real-time PCR”, “SAGE method”, “proteomics of plant”, “plant signaling against pathogens”, “inducing plant defense system”, “simultaneous defense systems in plants”, “gene families”, “northern blotting”, “southern blotting”, “northern blotting hybridization”, “rice”, “arabidopsis”, “wheat”, “maize”. The terms mentioned above were searched combinations with AND. The keywords were only searched in English.

Major peer-reviewed published papers were used in this paper; however, technical journals, books, and conference proceedings were

included in a couple of cases. After screening 550 abstracts and titles, the 75 references in this paper were selected. Some of the topics chosen were: (1) Northern blotting variants and their application detecting miRNAs expression (2) Seq—quantitative evaluation of gene expression (3) serial analysis of gene expression (SAGE) technology (4) characterization of bacterial isolates producing in plants (5) plant signaling against pathogens and pests (6) gene-level responses to plant-pathogen relationships and (7) plant defense system induction by pests and pathogens.

3. RESULTS AND DISCUSSION:

3.1. Methods of studying the expression of defense genes in plants

There are different methods for determining the quantity and quality of transcripts (Southern, 2006), divided into four general forms: the Northern blot and Northern ballet Reverse; Microarray; Real-time reverse transcription PCR; and the Serial Gene Expression Analysis Method (SAGE).

3.1.1. Methods based on hybridization of Northern blot and reverse Northern blot

The number of mRNA copies of each gene accurately indicates the expression of that gene. Tracing the expression of a gene ultimately suggests the intensity of transcription and expression of that gene. The Northern blotting method is one of the first methods to show differences in the number of mRNAs produced by each gene. This method was invented by James Alvin and Jog Stark at Stanford University in 1979. It was first used to track specific RNA sequences. The name is derived from Southern blotting (Brown *et al.*, 2004; Josefsen and Nielsen, 2011; Rio, 2015). In this method, mRNA is first extracted from biological samples, and then mRNA is isolated from cellular materials, including DNA, proteins, lipids, and other cellular organs. The different mRNA pieces are separated by gel electrophoresis (a method that separates molecules based on weight and electrical charge) and then transferred to a membrane called blotting (Figure 1).

To identify copies of mRNA produced by a specific gene, such as under pathogen stress, the samples would be incubated with a small piece called a probe from single-stranded RNA or DNA sequences. These molecules are labeled using radioactive molecules (Brown *et al.*, 2004; Wang and Yang, 2010). The probe or detector is designed based on the mRNA sequence of the target gene and then bound to the desired sequence. After the probe is bonded in the desired sequence, the probe is exposed to X-rays, and the radiation emitted from it causes a stain on the radiological film. The intensity of the signal on the photographic film shows the researchers how much mRNA was present in the prototype, and the intensity of the stain determines this; in this method of a gene that is expressed during stress, It always remains constant and is used as a fixed gene (Seki *et al.*, 2001; Rabbani *et al.*, 2003; Forment *et al.*, 2005). Another method used to study gene expression is reverse northern blotting, where the nucleic acid is permanently stained on a membrane.

In contrast, the stain is a collection of amplified cDNA fragments or the target gene (Figure 1). In this method, after DNA is stained on the membrane, a hybridization or two-vein reaction is performed using cDNA probes made from RNAs extracted from tissues. Therefore, this method is commonly used to study the expression profile of genes and study the expression of many genes in an organism (Fraser *et al.*, 1994; Barrett and Kawasaki, 2003; Brown *et al.*, 2004).

Some advantages of Northern blotting include a standard and reproducible method for studying gene expression; identify mRNA size; ability to study RNA splicing; ability to study the half-life of RNA; preliminary probes can also be used; and membrane can be held after the reaction is completed and reviewed years later (Dallman *et al.*, 1991). On the Other hand, however, the disadvantages of Northern blotting are: it is difficult to identify multiple probes; if the RNAase enzyme sample is slightly damaged, the quality of the answers and the amount expressions have a false negative problem; and the Standard Northern blotting method is less sensitive than other methods such as Real-time PCR (Dallman *et al.*, 1991; Iskandar *et al.*, 2004).

Navabpour *et al.* (2011) studied the response of spinach and rapeseed to induced stress. In this study, three treatments of methyl viologen, silver nitrate, and 3-Amino-1,2,4-triazole with four different concentrations were used along with control and combination treatment (ascorbic acid + stress treatments). TBARM assay was performed to assess the level of cellular oxidation. Sampling was performed at 48, 24, 12, 6, and 72 hours after all treatments. Study of gene expression and Northern blot hybridization 48 h after the treatment took place. The results showed that all experimental treatments affected the

percentage of dead cells, the amount of TBARM, and the expression of genes through a relative increase in the level of reactive oxygen species (ROS). The Rubisco photosynthetic gene (RBCS) decreased activity with increasing concentration of stress treatments. Ascorbic acid pretreatment spray increased the relative expression of this gene by 51%. Although differences in expression patterns were observed for the other genes studied depending on the plant type and experimental treatments, they generally showed a positive response to stress treatments.

With increasing the concentration of treatments, a linear increase in gene expression was observed (Gholamnezhad *et al.*, 2016). In a study conducted by Ray *et al.* (2013) on the expression of genes involved in resistance to *Septoria tritici* blotch (STB) wheat, it was revealed that resistant cultivars express protein isomerase disulfide 3 hours after exposure to the disease while the increase in the expression of the same gene occurred 96 hours after infection in sensitive cultivars (Maskos and Southern, 1992).

3.2. Microarray method

This approach has been one of the first throughput-high tools to study transcripts over the past two decades (Kuhn, 2001). This method, by creating thousands of profiles of genes, simultaneously improves the study of transcript analysis (Gechev *et al.*, 2004; J. Y. Zhu *et al.*, 2011). Microarray technology can be divided into three general parts:

3.2.1 Step I. Chip design and preparation

At this stage, the necessary information must first be collected from the sequence of genes to be examined, and then, a fragment of a unique gene sequence with a length of 25 bp must be made. By placing these probes, about 1,000 spots can be placed on a chip and test the input, although the chips are readily available for the genes of several organisms on the market (Slonim and Yanai, 2009).

3.2.2 Step II: Reaction Preparation

This involves extracting mRNA and building a cDNA and simultaneously marking it with a fluorescent dye, the steps of hybridization, washing and drying the chip, and finally scanning the microbial chip. At this stage, after selecting the cell or tissue to be studied, mRNA is extracted from it, and then cDNA is made from the extracted mRNAs, then two populations of labeled cDNAs are mixed and hybridized with a DNA chip (Figure 2). Hybridization conditions and the regulation of different temperatures are critical (Nguyen *et al.*, 2002).

3.2.3 Step III: Data Analysis

Analysis of data is possible with the help of relevant software, hardware, and databases. By measuring the intensity difference between these two colors for each point, the results can be analyzed, and finally, the pattern of gene expression in each cell type can be drawn (Walter *et al.*, 2001). These three parts are entirely interdependent and should be tried to be done correctly because otherwise, the test result will not be good. Due to the variety of models and different types of microchips, the micro-data analysis section provides many algorithms and methods of analysis (Wilhelm and Landry, 2009). Micro-analysis allows biological researchers to perform their experiments in the shortest time and on a large scale. With this vast amount of information on gene expression, the relationship between DNA, RNA, and protein can be understood and compared with other organisms. There are pre-prepared kits for different organisms such as humans, mice, Arabidopsis, and many other organisms. Microbiology is used in various branches of science such as medicine, pharmaceutical, food industries, and agricultural sciences. This method is very useful in medical science in diagnosing cancers as well as microbial and viral pathogens. It also has applications in agriculture to diagnose plant pathogens and track changes in the expression of genes involved in the disease resistance process (K. Singh *et al.*, 1990). In 2018, a study was conducted to identify defense genes in rapeseed using microbial cDNA related to Arabidopsis. In this study, the changes in the frequency of 2000 expression labels or EST of Arabidopsis in rapeseed interactions with necrotrophic fungi *Alternaria brassicicola* were examined.

The results of studies on rapeseed were compared with previous results obtained on Arabidopsis. Search for homology using canola plant expression sequence tags from a database with about 6000 unique clones identified defense genes in canola. Genes identified in connection with rapeseed-pathogen interaction included genes involved in active oxygen metabolism, plant resistance genes, regulatory genes, and genes involved in secondary metabolism (Primrose *et al.*, 2001). Kumar *et al.* (2014) used the microarray method with 1,000 sequences of expression tags,

or ESTs, compared the genes involved in the SAR pathway in Arabidopsis. Besides, by knowing the genomic sequences of this plant, they were able to identify the promoters of genes that were expressed in the SAR reaction. Also, they used microarrays that had 1,000 sequences of expression tags, or ESTs.

Some of the limitations of the microarray method include the inability to detect new transcripts; the low dynamic range for transcript recognition; and problems with reproducibility and comparisons between experiments (Bumgarner, 2013).

3.3. Real-time Reverse transcription PCR (Real-time qPCR)

Real-time qPCR means moment-by-moment observation of a process. Along with the need to accurately quantify gene expression, the many problems in semi-quantitative PCR paved the way for a new arena in PCR. The initial design of Time-Real PCR was first carried out by Higuchi et al. (1993). In this diagnostic system, a fluorescent substance is released during the reaction in proportion to the number of products per cycle. The amount of fluorescent is identified and recorded by an indicator (Hadi et al., 2012). The real-time RT-PCR is a suitable and accurate method for studying gene expression. The basis of this method is based on the quantitative measurement of copies amplified in the exponential stage of PCR reaction by measuring the amount of fluorescence light (Huggett et al., 2005).

This method has undergone many changes since its introduction, so that it is now one of the most accurate and fastest methods. Diagnostics are used in many fields of science. The product of this reaction is marked using labeled materials. The sensitivity of this method is higher than the electrophoresis (Klein, 2002), and the dynamic range of detection is increased. There is no need to quickly and hastily perform the PCR process because, in conventional PCR, samples should be stained with ethidium bromide immediately. This method has a high-resolution power to detect changes less than twice as much, while agarose gel resolution is inferior. This method is used to evaluate the exact amounts of DNA and RNA, while it does not have the difficulties of the conventional method (Dallas et al., 2005).

3.3.1 Real-Time PCR Assay

In general, there are several methods for performing quantitative PCR using the Real-time PCR method as follows:

3.3.1.1 The non-specific form

This method is performed using SYBR green binding agents such as DNA-binding agents (Figure 3). This dye is attached by being placed in a small DNA gap. The advantages of this method include cheap, convenience, and sensitivity. One of its major disadvantages is the connection of green SYBR to two strands, such as dimer primer and other non-specific bands that estimate the concentration higher than the original amount. Therefore the optimization of the reaction conditions should be such that the primer Dimmer and non-specific product should be created to a minimum. A melting curve is used to confirm the results of this experiment (Primrose *et al.*, 2001; Pantchev *et al.*, 2010). One of Time-Real PCR advantages is drawing the melting curve, carried out after the PCR process. It is specific for this molecule and depends on DNA structure and factors such as length and number of nucleotides, probe concentration, ambient salt content, and percentage of GC since SYBR Green can differentiate different products using curve melt. After the PCR is completed, the device can draw a melting diagram of each sample by measuring the fluorescence changes at different temperatures (Capote *et al.*, 2012).

3.3.1.2 The specific form

This model uses the FRET mechanism where the probes are designed so that at the beginning of the probe, there is a fluorescent dye called the reporter, and at the end, there is another fluorescence called quencher. When the reporter and the quencher are at a molecular distance close to each other (when connected to a probe), the light coming into the reporter creates an emission whose wavelength is in the quencher excitation region, and it absorbs this light as radiation. Emits at longer wavelengths that the device cannot measure. After the separation of quencher and reporter, the light emitted by the reporter is not absorbed by the quencher, and in this case, the device measures the emitted light fluorescently. In this method, several different probes can be used (Figure 3), including the TaqMan probe, Beacons probe, Scorpion probe, and hybridization probe. Of the essential Real-time PCR applications are Absolute Quantification and Relative Quantification (Mackay *et al.*, 2002; Löfström *et al.*, 2015; Kralik and Ricchi, 2017; X.

Chen *et al.*, 2020).

3.3.2. Applications of Real-time PCR

By using this method, differentiation of plant defense gene expression patterns under various conditions can be made. Differences between different growth stages stressed plants, or between infected plant samples vs. healthy plants have been studied (Gholamnezhad *et al.*, 2016). in a study using the real-time RT-PCR method based on scorpion probe and specific primers, leaf blight virus disease in grapes or nematode carrying them were detected (Hussain and Singh, 2016). In most studies using real-time PCR, the method is based on two methods, the first based on the SYBR Green fluorescence and the other based on the TaqMan detector. These two methods have helped identify plant viruses in different hosts (Santala and Valkonen, 2018). Owing to the absence of protein in the structure of viroids, one of the plant pathogens, real-time PCR and RT-PCR are considered two very reliable approaches for identifying these pathogens (Oliveira *et al.*, 2011).

Real-time PCR based on the fluorescent substance Green-SYBR was used to identify citrus exocortis viroid as well as citrus viroid IIb (Almeida *et al.*, 2018). Control of plant diseases caused by bacterial agents requires very accurate identification methods; using various real-time PCR has accelerated the specificity and sensitivity of identifying plant bacterial agents (Q. Li *et al.*, 2011).

A specific PCR was developed using Green SYBR dye to detect the bacterium Xanthomonas axonopodis citrus canker in 2014 specifically. Another study by Adhikari *et al.* (2020) on the expression of 14 candidate genes in resistance to wheat leaf blight on susceptible and resistant wheat cultivars by real-time PCR showed that the expression of these 14 genes in resistant and sensitive cultivars at different time points. Four genes of chitinase, phenylalanine ammonia-lyase, 1-PR, and peroxidase were expressed in the first 24 hours after infection. These results in seedlings wheat resistance to *M. graminicola* indicated the response is completed 24 days after infection and continues after that, especially in resistant cultivars. So, analysis of the expression pattern of these genes can be a reliable and rapid way to distinguish resistant and susceptible cultivars (Tomlinson *et al.*, 2010). Bilodeau et al. (2 017) evaluated the effects of three different chemical methods, including Green SYBR, TaqMan, and beacons molecules, using beta-tubulin, ITS, and elicitine gene sequences to identify *Phytophthora ramorum*, the cause of sudden death syndrome in elm trees. This study showed that all three methods could separate the isolate 65 of the pathogen from other pathogen species in all infected samples (Feau *et al.*, 2019).

3.4. Serial analysis of gene expression (SAGE)

This method was first used by Velculescu *et al.* (1995) at Johns Hopkins University in the United States. This method is used to generate gene expression profiles for a particular cell or tissue and to identify specific genes expressed under specific cellular conditions. SAGE is also widely used in the study of microorganisms, cancer, and evolution. SAGE method is based on three essential principles (Marioni *et al.*, 2008).

The first principle of using short oligonucleotide sequences (tags) (Figure 4), is about 11 to 27 bp, derived from a specific part of the cDNA and sufficient to identify an mRNA transcript uniquely. The second principle is the sequential connection of tag sequences, which allows serial analysis of transcripts. Tags about 25 to 51 are connected and placed in a vector structure and then sequenced automatically. As a result, the information will be obtained for more than 31-35 different genes by performing a sequencing reaction. The third principle states that the number of times a particular tag is viewed accurately reflects the level of expression of the associated copies. Instead of studying the complete cDNA, a short sequence of 12 bp is provided, each of which is representing an mRNA in the transcript. The basis of this method is the same 12-bp sequences, despite their small size, are sufficient to identify mRNA-encoding genes.

In the next step, the mRNA is converted to a double-stranded cDNA and then treated with a restriction enzyme with a quadruple recognition site such as AluI to cleave the cDNA at many sites. The end-restriction fragments remain attached to the cellulose granules, and the other pieces can be washed to remove them from the column (Matsumura *et al.*, 1999; Yamamoto *et al.*, 2001; Gowda *et al.*, 2004; Hu and Polyak, 2006). A short linker is then attached to the free end of each cDNA. This linker has a BsmEI enzyme recognition site. This enzyme is a unique restriction enzyme that, in addition to cutting its recognition site, also cuts below its identification site at a distance of 11 to 14 nucleotides. Therefore, it treats BsmEI and separates fragments with an average length of 12 bp from each cDNA end. These pieces are collected and connected head-to-tail in a chain and sequenced,

and separate sequences can be identified in the chain; Because BsmEI sites separate them. At the end of the SAGE reaction, the polymer is made up of sequentially linked nucleotide tags and identifiers by locating the enzyme to which they are cleaved. The resulting sequence analysis by the software leads to a list of gene identities expressed in the cell or tissue under study, the frequency of which will be an estimate of their expression. There is now a wealth of SAGE project information in databases (Noureddine *et al.*, 2005; Anisimov, 2008).

Some of the advantages of the SAGE method are: the SAGE allows extensive analysis of mRNA transcripts without prior knowledge of the organism transcriptome; and the sequence of each tag is sufficient to search for it in the database, and the frequency of each tag directly indicates the frequency of the relevant copy (Anisimov, 2008).

3.4.1 Applications of SAGE in plant studies

Biological and non-biological, the study of toxin metabolism and the analysis of tissue or organ expression profiles. However, these studies mainly have been carried out on plant models such as rice and Arabidopsis. The application of SAGE method in studying other plants is expanding (Westermann *et al.*, 2012). a study using the SuperSAGE method attempted to elucidate the interaction of plant host and pathogen. The gene expression profile in both rice and pathogenic fungi was investigated simultaneously. Genes were identified that increased and decreased in expression in response to the pathogen elicitor. Reports have indicated that a large number of genes whose expression is reduced are related to proteins involved in photosynthesis (Matsumura *et al.*, 2005). This review showed that is an advantageous method to check cell transcriptome is the interaction of host and pathogen, especially in organisms whose genome yet to be known (Venu *et al.*, 2007).

4. CONCLUSIONS:

It is imperative to study the expression of defense genes associated with pathogens. The results of the current research available can enable scholars to elucidate the mechanism of tolerance and resistance to pathogens and facilitate the production of tolerant cultivars through biotechnological methods. Pathogenic proteins are produced by molecules such as ethylene, salicylic acid, and phytoalexins, which activate plant defense responses and increase cell wall strength and increase lignin formation. Together, these responses lead to the development or growth in resistance to pathogenic fungi. Specific transcription factors regulate the expression of these proteins. Transcription factors are essential and critical components in controlling gene expression in all living tissues and cause phenotypic diversity and adaptation of organisms during evolution. Thus, obtaining a reliable understanding from the controlling genes involved in defense response to biotic stress requires robust mythologies in transcriptomics that tools such as northern blot, microarray, real-time qPCR, and SAGE can provide. Fortunately, the advancement and introduction of sequencing techniques have been accelerated during the last decade. Therefore significant progress has been predicted, particularly in developing cultivars capable of resisting deadly pathogens.

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6. OPEN ACCESS:

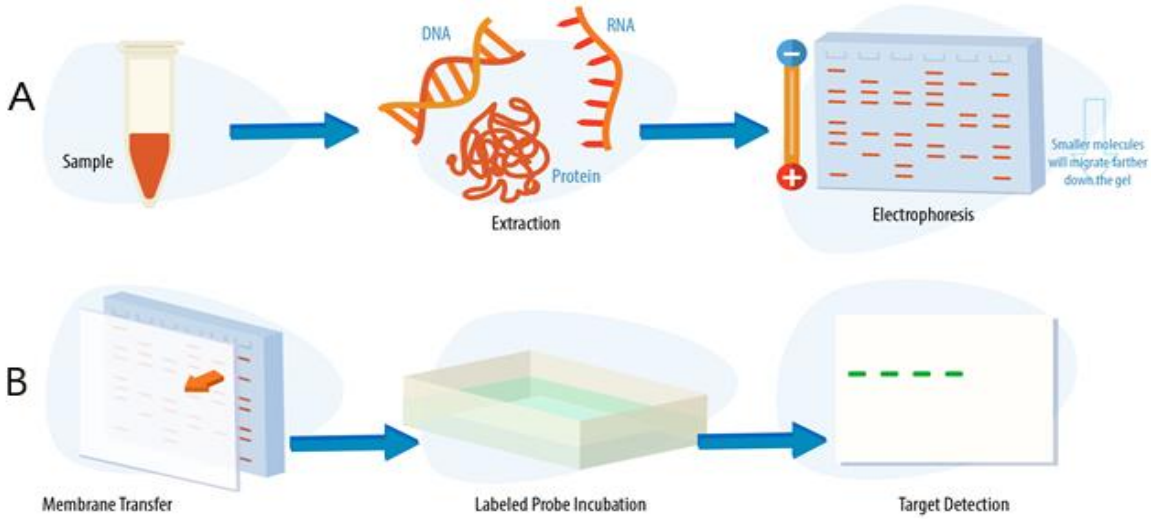

Figure 1. *A schematic of the processes of two types of Northern blot. A) Northern blotting and B) Reverse northern blot. (Retrieved from https://www.labmanager.com/insights/southern-vs-northern-vs-western-blotting-techniques-854).*

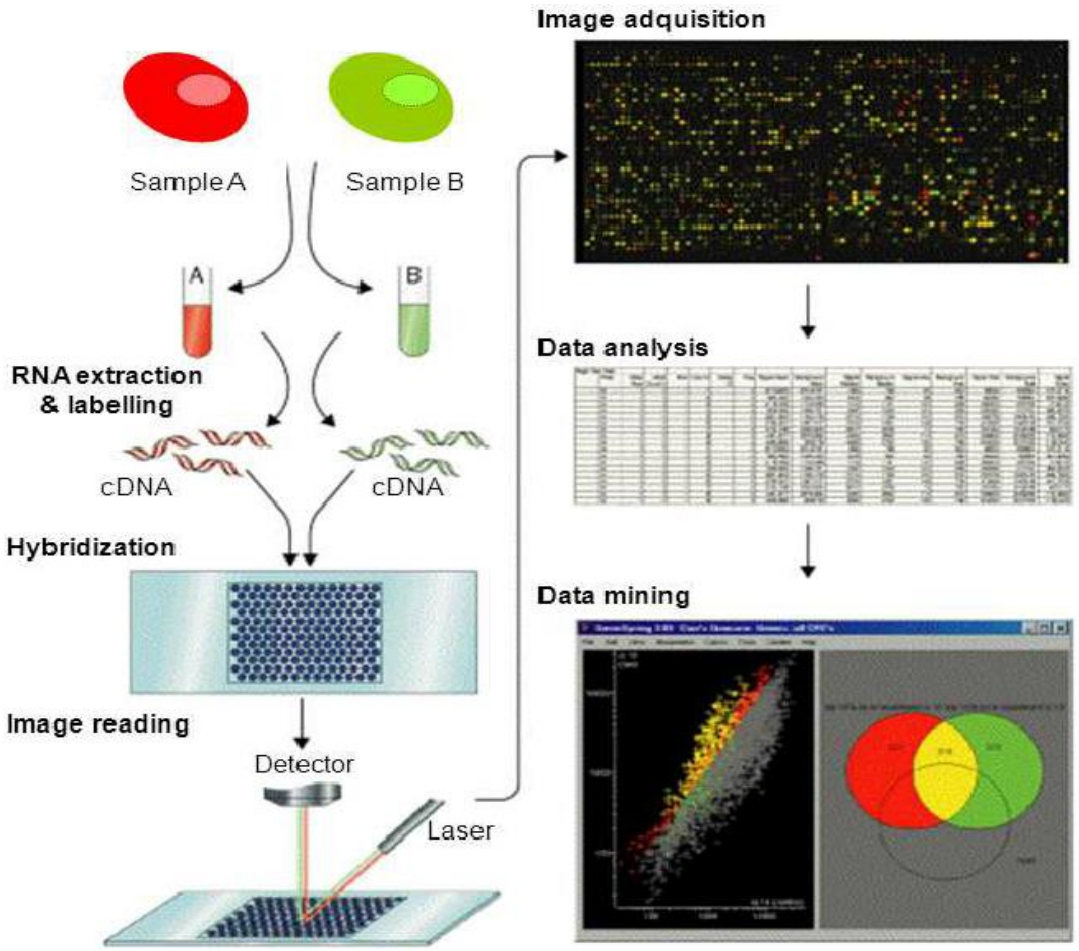

Figure 2. *Schematic displaying the DNA microarray methodology. This method often utilizes for identifying messenger RNAs (mRNA), so-called expression profiling. The approach composed of the fluorescently labelling of RNA while the RNA is transformed into complementary DNA (cDNA) (Lamas et al., 2012).*

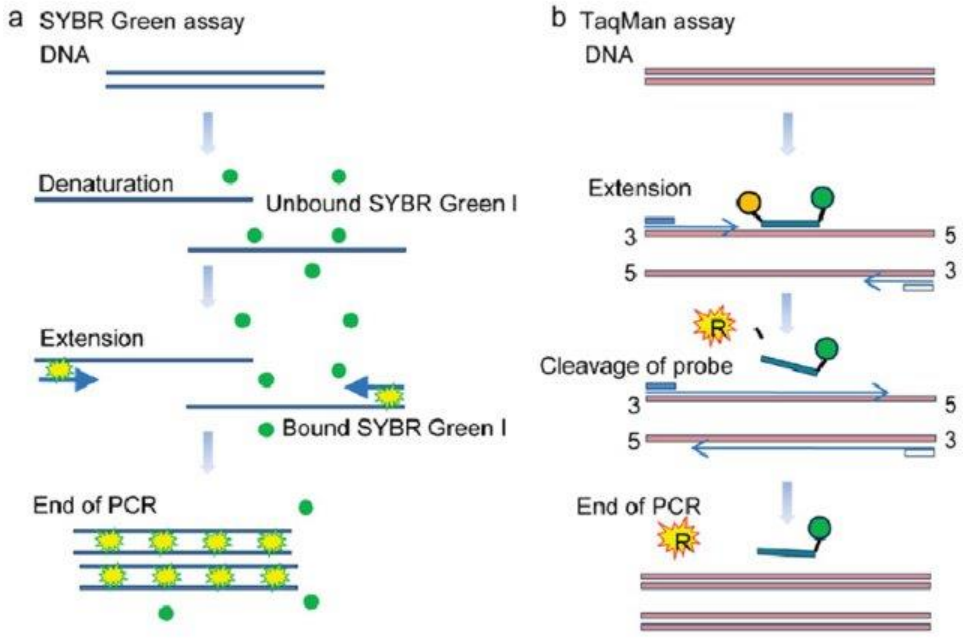

Figure 3. *Real-time polymerase chain reaction (PCR) chemistry: (a) SYBR Green assay, and (b) TagMan (Bae et al., 2013).*

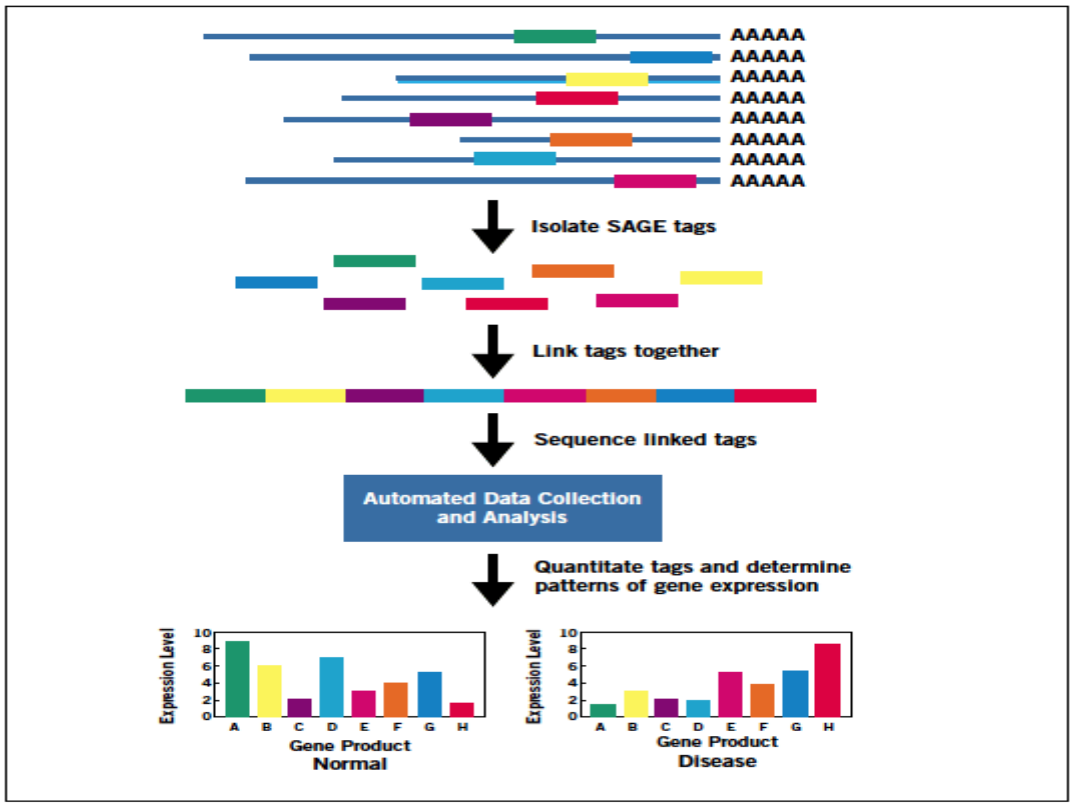

Figure 4. *The SAGE process starts with splicing and transcribing the genes to generate mature mRNA transcripts, then isolate the SAGE tags. The extracted mRNA turns into stable double-stranded–cDNA. Restriction enzymes digest the ds-cDNA to produce 11 linked 'tag' fragments. These tags are then sequenced using long-read Sanger sequencing (Different colors indicate different tags). The transcription of the genes of interest can be reported using their tag frequency and comparing the gene product of the infected sample with the control (Shafee and Lowe, 2017).*