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TUTORIAL REVIEW

Two-dimensional difference gel electrophoresis applied for analytical proteomics: fundamentals and applications to the study of plant proteomics

Sandra Cristina Capaldi Arruda,[a] Herbert de Sousa Barbosa,[bc] Ricardo Antunes Azevedo[a] and Marco Aurélio Zezzi Arruda*[bc]

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The present review reports the principles, fundamentals and some applications of two-dimensional difference gel electrophoresis for analytical proteomics based on plant proteome analysis, also emphasizing some advantages of 2-D DIGE over 2-D PAGE techniques. Some fluorescent protein labeling reagents, methods of protein labeling, models of 2-D DIGE experiments, and some limitations of this technique are presented and discussed in terms of 2-D DIGE plant proteomes. Finally, some practical applications of this technique are pointed out, emphasizing its potentialities in plant proteomics.

1. Introduction

The word proteomics was coined in 1996 by Wilkins *et al.*[1] and is defined as the study of the overall protein composition of an organism, cell or organelle. In this context, one of the main targets of studies of proteomics is to establish possible biomarkers in biological systems.[2] In this way, studies of protein changes in biological organisms are frequently carried out through polyacrylamide gels by evaluation of their images, providing relevant information for comparative proteomic studies[3] as well as using appropriate mass spectrometric techniques for evaluating the identity of the studied proteins.[4,5]

[a]*Laboratory of Genetics and Biochemistry of Plants, Department of Genetics, Escola Superior de Agricultura Luiz de Queiroz, ESALQ, USP, 13400-970 Piracicaba, SP, Brazil*
[b]*Spectrometry, Sample Preparation and Mechanization Group-GEPAM, Institute of Chemistry, University of Campinas – UNICAMP, P.O. Box 6154, 13083-970 Campinas, SP, Brazil*
[c]*National Institute of Science and Technology for Bioanalytics, Institute of Chemistry, University of Campinas – UNICAMP, P.O. Box 6154, 13083-970 Campinas, SP, Brazil*

Sandra Cristina Capaldi Arruda

Dr Sandra Arruda received her degree in Forestry from the Univesity of São Paulo (ESALQ/USP) in 1996. In the period between 1993 and 1995 made part of undergraduate reserach at the Botanical Garden (Córdoba, Spain). In 2000 she received the MSc degree in Sciences from ESALQ/USP, and the PhD in Sciences (2004) at Center for Nuclear Energy in Agriculture (CENA/USP). Currently she is developing a Post-Doctoral program in plant proteomics and enzymology at the Laboratory of Genetics and Biochemistry of Plants (ESALQ/USP).

Herbert de Sousa Barbosa

Dr Herbert Barbosa received his degree in Chemistry from Federal University of Piauí (2004), Master in Analytical Chemistry, Federal University of Piauí (2007) and Ph.D. in Analytical Chemistry by the University of Campinas (Unicamp). He has experience in Analytical Chemistry, with emphasis on Bioanalytics, acting on the following topics: electrophoresis gels, mass spectrometry molecular types (MALDI-MS and ESI-MS) and atomic (ICP-MS) and proteomics and metallomics comparatives. Currently developing a Post-Doctoral project at the Spectrometry, Sample Preparation and Mechanization Group – GEPAM in Department of Analytical Chemistry, Institute of Chemistry, UNICAMP-SP.

Within this scenario, analytical proteomics is an area of utmost importance within the analytical context.[6] For comparative proteomic studies, two-dimensional polyacrylamide gel electrophoretic (2-D PAGE) separations are the most used platform, due to their high resolution, allowing either high efficiency protein separation or the identification of potential protein spots with differences in concentration or expression in the gels evaluated.[2,6–8] The earliest applications of 2-D PAGE were attempted *ca.* 30 years ago.[9,10]

However, comparison between different separations on these gels, which is made by analyzing their digitized images, requires constant attention by the analyst, due to the personalized interpretation of the data obtained.[3] Another issue involved in proteomic studies that use comparative 2-D PAGE analysis of gels images lies in the large variation that exists between gels, resulting in poor reproducibility as well as in the difficulty in making accurate measurements of the proteins resolved in the gels.[11] These variations can be attributed to the sample preparation method employed, natural variations when considering biological samples, and variations related to the electrophoretic system used, among others.[3,12,13]

In this context, a promising alternative to these limitations is two-dimensional difference gel electrophoresis (2-D DIGE), which is detailed in this text. This technique,[14] which is based on fluorescent cyanine dyes, allows comparisons between two exact quantitative proteomic samples, which are resolved on the same gel, minimizing the problems mentioned above.[15] Moreover, there is the advantage of the high sensitivity of these dyes (*ca.* 1 fmol of protein), which enables the detection of low abundance proteins when compared to other dyes used in the detection of protein spots, such as Coomassie Brilliant Blue (CBB) and silver staining.[16] Even though excellent results can be achieved when 2-D DIGE is used, the necessity of protein identification through mass spectrometric techniques is imperative for making sense of these proteomics studies.[17,18]

Thus, by describing the potentialities of 2-D DIGE, using a few studies involving this technique in plant proteomics, this review emphasizes the fundamentals of 2-D DIGE in order to present some of its characteristics to the readers, as well as some applications in plant proteomics, also emphasizing the importance of analytical procedures within the context of proteomics.

2. Fundamentals of the 2-D DIGE technique

In 1997, Ünlu *et al.*[14] first described a modification of two-dimensional (2-D) polyacrylamide gel electrophoresis, showing that in a single gel reproducible differences between two protein samples are detected. The system was accomplished by the fluorescent tagging of two samples with two distinct dyes. These samples were then run on the same 2-D gel, with post-run fluorescence imaging of the gel into the two images. The images were superimposed. The authors introduced the amine reactive dyes in order to insure that the same relative mobility could be achieved for common proteins for both samples, regardless of the dye used. This technique was named difference gel electrophoresis (DIGE) and *Drosophila* embryo extracts were used as samples. The authors indicated that DIGE was reproducible and sensitive at the nanogram level.

The principle involved in the DIGE technique is that different dyes provide different fluorescence wavelengths for detection, allowing two or more differentially labeled samples to be combined before IEF as well as to be resolved using the same 2-D gel. Frequently, three samples are labeled in 2-D DIGE: two of them are experimental samples whereas the third sample is composed of a mixture of equal amounts of all experimental samples (*i.e.*, a pooled internal standard). This creates a standard for each protein during analysis. The protein samples are then visualized using fluorescence imaging, which enables the detection of differences between protein abundances in the samples. Therefore, the image processing software divides corresponding pixels into separate images after background subtraction and normalization. The resulting image ratio enables the visualization of the differences between the two samples.[16] As well as

Ricardo Antunes Azevedo

Dr Ricardo Azevedo receives the degree in Biological Sciences from the Catholic University of Campinas (1983), Master in Plant Genetics from the University of Campinas (1988) and Ph.D. (1992) and post-doctoral (1997) in Plant Biochemistry from the University of Lancaster, England. Since March 2001 he works as Research Fellow at the University of Lancaster, England. He is Professor at the University of São Paulo since 1993, and Full Professor since 2009. The main research lines in Physiological and Biochemical Genetics of Plants with an emphasis in Botany (Plant Physiology) refers to amino acids and nitrogen metabolism, and oxidative stress by heavy metals.

Marco Aurélio Zezzi Arruda

Dr Marco Arruda received his degree in Industrial Chemistry from the Methodist University of Piracicaba (1987), Master in Nuclear Energy in Agriculture by the Center for Nuclear Energy in Agriculture (1990), Ph.D. in Analytical Chemistry from the University of Cordoba (1995) and post-doctoral fellow at the Center for Nuclear Energy in Agriculture by the Center for Nuclear Energy in Agriculture (1995–1996). He is currently Full Professor at the University of Campinas. He has experience in Analytical Chemistry, acting on the following topics: Bioanalytics, sample preparation, mass and atomic spectrometry and mechanization.

reducing the number of gels that need to be run, the differentially labeled samples are subjected to the same handling procedures.[15] Consequently, variation in spot intensities due to gel-specific experimental factors, for example, protein loss during sample insertion into the IPG strip, will be the same for each sample within a single DIGE gel. In this sense, the relative amount of a protein in a gel in one sample compared to another one will be unaffected, raising the confidence to which protein changes can be detected and quantified.[15,19]

This system may analyze up to 0.25 mg of proteins per sample and two samples are compared in each gel at the same time. When detectivity is required, fluorescence scanning may provide better detection than silver staining, making detectional proteins with <2-fold expression differences possible.

Comparing the advantages of DIGE over traditional 2-D PAGE, some interesting characteristics can be pointed out:

- **Multiplexing**: the labeled samples are mixed and then separated on the same 2-D PAGE gel. Thus, for samples on the same DIGE gel, gel-to-gel variation is completely eliminated and the number of gels needed for one experiment can be decreased by 2–3-fold.
- **Gel-to-gel comparison**: as already mentioned, one of the three samples labeled on a DIGE gel can be a mixture of equal amounts of all experimental samples, creating a standard for each protein during analysis. Therefore, comparisons across different samples can be made with a high degree of confidence.
- **User-friendly manipulation**: large format gels are cumbersome to handle. Since proteins in DIGE are pre-labeled, no DIGE gel manipulation after electrophoresis is necessary. Additionally, the scanner used for imaging accepts gel-sandwiches, including the glass plates, with a further reduction of the variation between gels as well as the risk of them being damaged.

2.1 Fluorescent protein labeling reagents

The protein extracts labeled with different fluorescent dyes can then be visualized separately by exciting the different dyes at their specific excitation wavelengths.[16] The most commonly used fluorescent protein labeling reagents for DIGE are synthetic *N*-hydroxysuccinimidyl (NHS) ester derivatives of the cyanine dyes (Cy2, Cy3, Cy5), which will be discussed later. In addition, other fluorescent dyes are the Alexa Fluor dyes (*e.g.*, 488, 546, 594). These dyes are sulfonated compounds and are significantly more fluorescent and photostable than the Cy3 and Cy5 dye groups.[20,21] However, the literature has few reports of these dyes being applied for the DIGE technique, taking into consideration that these dyes are more expensive compared to cyanine dyes. Moreover, the negative charge of these fluorophores may cause non-specific electrostatic interactions with positively charged molecules.[22] Other fluorophores that are also available include the maleimide-conjugated infrared dyes DY-680 and DY-780, where only cysteine-containing proteins are labeled, thus excluding proteins without cysteines. The application of these dyes requires the need for a minimum quantity of 200 μg of protein mass, which can be difficult in samples with low amounts of protein.[20,23] As mentioned earlier for Alexa fluor dyes, the literature has few reports of these dyes being applied with the DIGE technique. A schematic representation of the 2-D DIGE protocol for minimal lysine labeling used as an internal standard is shown in Fig. 1.

Among the dyes cited, the cyanine dyes (Cy2, Cy3, Cy5) are the most widely used.[24–27] These fluorophore dyes are structurally similar and react with primary amine groups of lysine residues, forming amide in a nucleophilic substitution reaction. The dyes are positively charged to offset the charge of lysine, which is lost during the labeling reaction. At this point, the samples should be labeled in denaturing 2-D electrophoresis lysis buffer in the absence of primary amines (*e.g.*, buffers and carrier ampholytes) at pH values between 8.0 and 8.5.[15] The charge and mass matching ensures that all the samples co-migrate to the same point during electrophoresis. It is important to note that, during the labeling reaction, the dye : protein ratio is extremely low, showing that protein molecules are only labeled with a single dye molecule.[28]

After 2D separation, the different protein samples labeled can be visualized separately by exciting the different dyes at their specific excitation wavelengths. Therefore, from the images generated for each dye, the signals from labeled protein spots are determined and the normalized intensities or spot volumes for each spot from different dyes (Cy2, Cy3, Cy5) are compared in order identify differentially expressed protein between the samples.[15,16] This is achieved by using an image containing appropriate laser wavelengths for exciting the different dyes, and filters for collecting the light emitted. Each dye generates digital images of an individual sample.[16]

For quantitative protein analysis, an internal standard is used, which is marked with a dye (generally Cy2) and applied to gel electrophoresis, along with two samples to be analyzed (labeled with Cy3 and Cy5).[28] The internal standard is prepared from

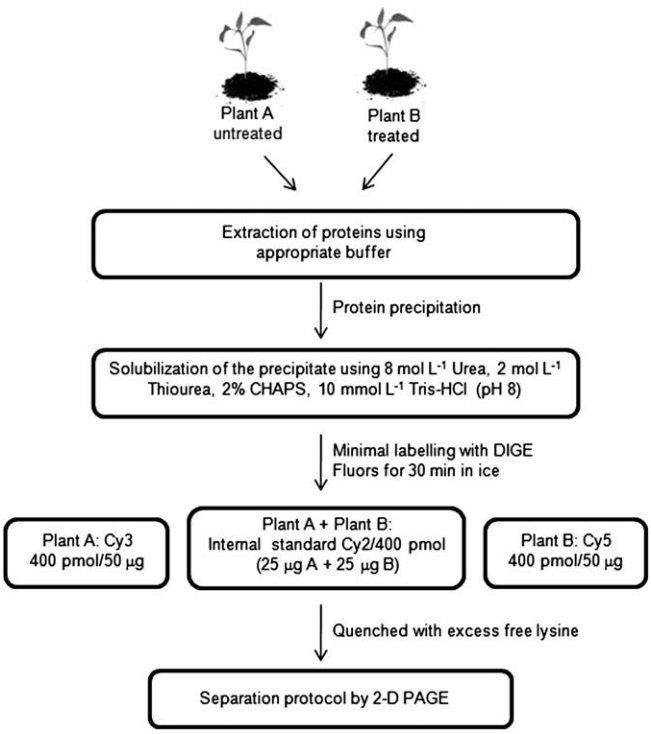

Fig. 1 Schematic representation of the 2-D DIGE protocol for minimal lysine labeling.

a mixture of equal amounts of proteins from two samples and therefore contains all proteins that are present in each sample.[15,28] Thus, each protein has a single signal in the internal standard, which is used for a direct quantitative comparison within each gel and for normalizing the abundance values for each protein when comparing different gels. As a consequence, the abundance of each protein spot in a biological sample can be measured as a ratio (not a volume) to its corresponding spot present in the internal standard. In this way, as each sample spot map is co-detected with a standard spot map, all of the spots are compared in the gel to the same pooled standard. This enables accurate quantification and accurate spot statistics between gels and, most importantly, separation of experimental gel-to-gel variation from biological variation in studying protein spot abundance. Each sample will contain an image with a similar spot pattern, simplifying and improving the confidence of inter-gel spot matching. This is not possible with conventional 2-D PAGE because of the high level of variation associated with all samples running on individual gels.[16,28]

A study of the effect of the internal standard on experimental variation was carried out by Alban *et al.*[28] In this work, the authors show that the coefficient of variation was larger for the non-standardized abundance spot (spot volumes) than the standardized abundance spot (spot volume ratios). In conclusion, the authors say that comparisons of in-gel spot volume ratio measurements from different gels results in less experimental variation than when comparing spot volumes from different gels, showing that the inclusion of the pooled standard in the experimental design does greatly reduce experimental variation.

Kieffer *et al.*[27] showed an example of the use of the internal standard in which 2-D DIGE and mass spectrometry (MALDI-QTOF) were used to investigate the effects of Cd exposure in a hydroponic culture of young poplar leaves, due to the deleterious effects of this metal on the growth and development of plants. The control and Cd-treated samples were labeled with Cy3 and Cy5 dyes, respectively. The internal standard, formed by pooling equal quantities of the two samples, was labeled with Cy2. The results pointed out that 125 spots were identified with an absolute variation of at least 1.5-fold between control and treated samples.

Regarding the process of labeling proteins, two alternative concepts are involved: minimum labeling by attaching the dye to the amino group of lysine residues and saturation labeling of all cysteine residues. The main commercial reagents are produced by GE Healthcare and named CyDye DIGE fluor minimal (Cy2, Cy3 and Cy5) and CyDye DIGE fluor saturation dyes (Cy3 and Cy5 maleimide derivatives). These methods of protein labeling are discussed below.

2.2 Minimal and saturation labeling

The choice of the dye for protein labeling takes into account the mass of protein available for labeling, where the minimal dyes are suitable for detecting differences between protein abundance when 50 μg of protein is used, since the saturation dyes are suitable for detecting protein abundance when only 5 μg of protein is used.[16,29] These two DIGE labeling options, commented upon above, provide rapid methods for preparing differentially labeled samples for comparative proteomics studies based on fluorescence.[30]

The minimal labeling of lysine residues due to the very low ratio between dye and protein, where the only protein molecules visualized on the gel, are those labeled with a single dye molecule. Only 3–5% of the total protein present in the sample is labeled using these dyes, where the minimal dyes add *ca.* 500 Da to the labeled protein. This shows that the increase in protein mass makes a negligible impact on the apparent molecular mass of proteins.[30] It is important to note that because the Cy2, Cy3 and Cy5 dyes are matched in terms of charge and molar mass, the same protein labeled with any of these dyes will migrate to the same position on a 2-D gel.[16] In this sense, this labeling method is applied in most studies reported in the literature.

An experimental procedure for minimal labeling using Cy2, Cy3 and Cy5 dyes uses 400 pmol of each dye to be added to 50 μg of protein of each sample and the pooled standard, this ratio being recommended by the manufacturer.[29] Considering the high cost of these dyes, another option would be the reduction of the amount of dye applied to the sample, increasing the number of samples that can be analyzed. For example, Di Carli *et al.*[26] used this strategy for comparing the leaf proteome of transgenic plants expressing antiviral antibodies, where about 2000 spots were detected on gels. Thus, when a sample has a relatively large amount of proteins, a decrease of the amount of dye applied to the sample is possible, without loss of protein visualization in the gel.

The saturation dyes label all available cysteine groups in each protein, where the reagents for saturation labeling dyes comprise Cy3 and Cy5 maleimide derivatives,[31] which are designed to form a covalent bond with the thiol group of cysteine residues in a protein *via* a thioether linkage.[16] This is based on the principle that, generally, there are fewer cysteines residues *per* protein, so that higher stoichiometric labeling of protein cysteines can be achieved without compromising sample solubility.[15] These dyes are suitable for saturation labeling of all cysteine residues, allowing an improvement in detectivity for protein detection and enabling successful 2-D DIGE analysis of samples with low protein concentrations.[16,30] However, due to the relative low prevalence of cysteine residues in proteins, high amounts of dye are necessary for this labeling strategy.

The literature reports these dyes are being used for labeling samples with low protein contents, such as infected ticks[32] and tissues samples isolated by laser micro-dissection.[33–35] Furthermore, a study to identify cysteine modifications in proteins has also been reported.[36] Curiously, so far there are no studies in the literature reporting the application of these dyes in plant samples, considering that this type of sample has relatively high amounts of proteins.

2.3 Models of 2-D DIGE experiments

The great interest in the field of analytical proteomics has enabled scientists to obtain a large amount of information from different samples. The 2-D DIGE technique allows the identification of protein expression changes in proteomes of biological samples with higher accuracy. The introduction of an internal standard led to an increase in accuracy, and multiplexing of

samples using different fluorescent dyes reduces systematic variation.[14,28,30]

In addition, 2-D DIGE also enables the use of experimental designs that would not be possible using conventional 2-D PAGE to enable an accurate statistical analysis of protein expression, taking into account the biological variations that are expected between experiments. This is possible, because the pooled control sample labeled with Cy2, and run on each gel, acts as an internal standard to ensure that each protein in the samples should appear in the entire quantity of the gels. The Cy3 or Cy5 labeled sample is then compared to the same internal standard and measurements are taken relative to the internal Cy2 standard, reducing gel-to-gel variation and increasing statistical confidence in this type of analysis.[14–16] A proteomic analysis model employing the technique of 2-D DIGE for the detection of differential spots can be seen in Fig. 2.

Ndimba *et al.*[25] applied an experimental design, possible with 2-D DIGE, to investigate the effects of salinity and hyperosmotic stress on *Arabidopsis thaliana* cellular proteins using two treatments: NaCl and sorbitol. Five independent experimental replicates were carried out for each treatment as well as for the control. A total of 2949 spots were detected and matched in all eight analytical proteome gels, where 266 protein spots showed a significant change in abundance. Mass spectrometry analysis identified 75 spots, where these proteins were subdivided into ten functional categories: citrate cycle, glycolysis and carbohydrate metabolism, detoxifying enzymes, proton transporting ATPases, heat-shock proteins, transcription and translation, signal transduction, amino acid and purine biosynthesis, proteolytic enzyme and cytoskeleton-related protein.

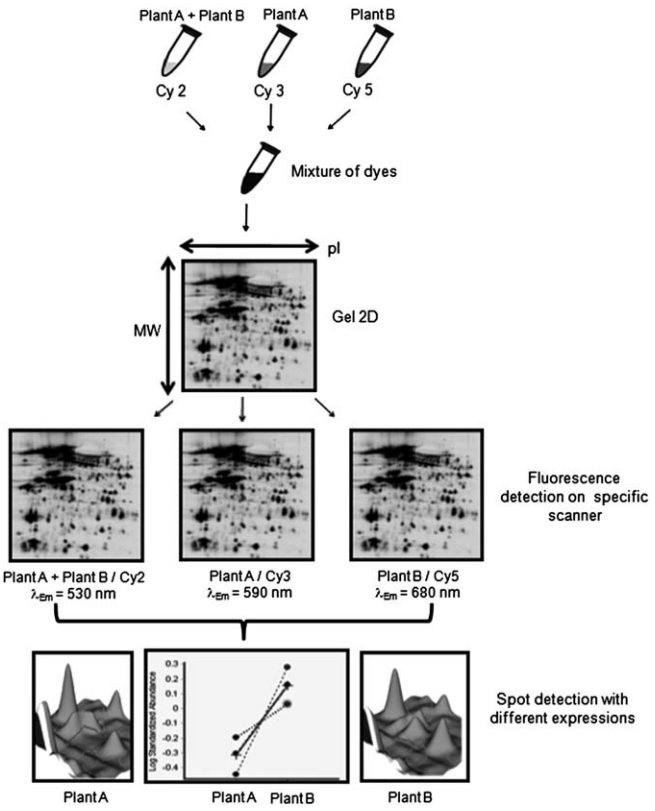

Fig. 2 Proteomic analysis by 2-D DIGE.

Alvarez *et al.*[24] also used the experimental design of the 2-D DIGE technique to investigate the effect of Cd on *Brassica juncea* roots. The 2-D DIGE gels of control and Cd-treated root proteins were prepared in triplicate. In one of the replicates, the dyes were swapped in order to correct possible differences in the fluorescence characteristics of acrylamide at the different wavelengths of excitation of Cy3 and Cy5. The internal standard labeled with Cy2 consisted of a pooled sample comprised of equal amounts of each control and Cd-treated sample used for each replicate. As a result, 69 spots were differentially expressed in response to Cd-treatment, with 43 decreased and 26 increased in abundance, where all proteins were identified by mass spectrometry (nano-LC-ESI-MS/MS).

2.4 Limitations of the 2-D DIGE technique

Some limitations associated with the 2-D PAGE, such as restriction in molar mass separation range (high protein and low molar mass), extremely acidic or basic proteins, hydrophobic proteins and low abundance proteins (proteins characterized by codon bias values <0.1)[37–39] may also be associated with the 2-D DIGE technique, limiting the proteomic analysis of most biological sample types.

The limitations related to the 2-D DIGE technique are mainly associated with the labeling chemistry required for attaching the dye to the proteins. One source of systematic variation using DIGE comes from the different absorptivities of the dyes. Because Cy5 has a higher absorptivity than Cy3, it causes higher volume values than the Cy3 dye under similar illumination settings.[30] Thus, some proteins appear to be preferentially labeled with one dye over the other, despite their similar structures, identical reactive groups and the fact that labeling is carried out under denaturing conditions.[15] A scaling factor and a background offset are used to adjust the dye-specific gain in order to calibrate the spot volumes.[19]

Other factors to be considered are related to high abundance protein spots in a conventional gel system (2-D PAGE), which present a medium or low abundance protein spot in the DIGE system. This is probably due to the low lysine content of some proteins, taking into account that, with minimal labeling, proteins with a high percentage of lysine residues could be more efficiently labeled when compared with proteins containing few lysine residues. In addition, the technique is not applicable to those proteins without lysine (when labeling with the minimal dyes) or cysteine (when labeling with the saturation dyes).[16]

2.5 2-D DIGE technique applications in plant proteomics studies

The development of new techniques has distinct impacts, depending on the research field. The use of proteomic techniques is critical for plant studies and it may help to elucidate several aspects of metabolic regulation of key essential processes. Plants are naturally subjected to stressful conditions[40] and their responses may vary dramatically depending on the type of stress, plant species, tissue/organ, and a series of other factors.[41] Arruda and Azevedo[42] have already commented on the urgent need for a more clear integration on the use of bioanalytical and more sophisticated methods to study plant responses to stress by

metals. The proteomic approach using 2-D DIGE is a major step forward so that new advances in terms of gathering information are achieved.

An increase in 2-D DIGE applications is achieved in the recent literature as a supporting proteomic method in expression profiling. This technique has been widely used to assess proteome changes as well as to find biomarkers in a wide variety of situations, such as cancer and clinical proteomics studies.[43–46] Due to development in plant physiological, proteomic and biochemical areas, as well as in order to contribute to increasing progress obtained in plant proteomics and genomics studies, an overview is provided in this section related to the uses and benefits of 2-D DIGE methodology in plant proteomics.

The majority of the examples follow a similar pattern in terms of 2-D DIGE analysis, in accordance with the manufacturer's indication, and the samples are treated as in the 2-D PAGE technique. In order to make the text more amenable to the readers, the discussion will focus on the objective and on the results obtained, which can include, or not, the use of the 2-D DIGE with other techniques such as: differential isotope tagging strategies coupled to non-gel-based LC-MS, MS-MS analysis, MALDI-TOF, μLC-ESI-IT-MS-MS, ESI-MS-MS analysis, and RNA isolation followed by RT-PCR, among others.

Marouga *et al.*[16] commented about the development of 2-D DIGE and pointed out the advantage of this technique over the traditional 2-D PAGE. According to these authors, the great advantage is related to the ability of 2-D DIGE to deal with complex lysates and to simultaneously visualize large parts of the interested proteome. Because of this, the authors commented that the development and the use of new techniques continue to complement rather than replace 2-D DIGE.

In order to emphasize the use of 2-D DIGE in proteomic studies, particularly for those related to cell organelles, Lilley and Dupree[47] discussed some methods for quantitative proteomics analysis in plant organelle characterization in a review paper. Among all the discussed topics, they concluded that, due to the necessity of knowing the subcellular localization of proteins, 2-D DIGE as well as differential isotope tagging strategies coupled to non-gel-based LC-MS were proven to be useful in this area of research.

For berries, an important culture for the economy of many countries, changes in the metabolome during the maturation process have been well documented, but the biological events which occur at the protein level have yet to be fully investigated. According to Di Carli *et al.*,[48] in order to gain new insights into the post-harvest withering process, they studied the protein expression profiles of grape (Corvina variety) development, focusing on withering. The authors decided, using 2-D DIGE and to avoid artifacts due to preferential labeling and to ensure statistical significance, to apply a random design with a dye-swap approach. A total of 12 gels were run, 11 analytical gels representing 3–4 biological replicates according to the experimental design. One preparative gel was used for transgenic plant and protein interaction studies. According to the authors, the detection of 90 proteins differentially expressed was possible by using the 2-D DIGE during grape ripening/withering. From these 90 proteins, 72 were identified by MS-MS analysis. These results represent an important insight into the withering process in terms of both *Vitis* germplasm characterization and knowledge.

In another example in which the 2-D DIGE technique was successfully employed, Di Carli *et al.*[26] investigated whether the expression in plants of recombinant antibodies directed against viral proteins may influence the host leaf proteome. The authors utilized two transgenic plant models, *Lycopersicon esculentum* cv. MicroTom and *Nicotiana benthamiana*. Results revealed that, among the 2000 spots, about 10 were differentially expressed in both transgenic models. The spots were identified by MALDI-TOF PMF and μLC- ESI-IT-MS-MS procedures. The results presented by the authors suggest that the expression of recombinant antibodies in both evaluated transgenic systems does not significantly alter the leaf proteomic profile expressing antiviral antibodies. As a special aid, the authors emphasize that this work represents the first demonstration, based on proteome analysis, that engineered plants expressing low levels of antibodies can be safely and effectively used to obtain protection against viruses. Moreover, the authors referred to other transgenic systems as a 'new model of study' since they pointed out that 'every new genetically modified plant has to be considered as unique and, therefore, analyzed as a result of an independent DNA integration event'.

Amme *et al.*[49] applied 2-D DIGE in order to evaluate the proteome analysis of cold stress response in *Arabidopsis thaliana*. In this report, protein labelling was performed using the CyDyes DIGE Fluors according to the manufacturer's recommended protocol. The results revealed that many of the proteins analyzed were previously reported in cold-stress response studies, indicating that the employed methodology was valid. As a conclusion, authors emphasized the capacity of proteome approaches to analyze cellular mechanisms at the protein level, which can contribute to understanding the complexity of plant defense responses.

Delaplace *et al.*,[50] working on the post-harvest ageing of potato (*Solanum tuberosum* L.) tubers stored under realistic agronomical conditions (270 days, 4 °C), applied 2-D DIGE for proteomic analyses. The authors observed during ageing that the differentially expressed proteins were involved mainly in starch catabolism, control of protein conformation, protein recycling, and stress response. Moreover, 14 breakdown products of patatin increased during ageing, indicating enhanced patatin proteolysis. They concluded that all the changes observed during ageing appeared to allow the potato tubers to maintain their radical scavenging activity until the end of the storage period, as no increase of oxidative damage was observed. Besides this, the authors commented that the all data are interpreted considering the impact of reactive oxygen species (ROS) on the development and the behavior of other plant systems undergoing ageing or senescence processes. The results showed that using 2-D DIGE, five spots were found to vary in intensity during the post-harvest ripening process and that it was possible to identify two proteins, that are related to Ca^{2+}-calmodulin-dependent kinases. The authors commented that these proteins are good candidates for future studies in post-harvest ripening.

In order to better understand the early development of *Cunninghamia lanceolata* [(Lamb.) Hook] seed embryos from a proteomic point of view, Shi *et al.*[51] carried out a proteomic analysis of *Cunninghamia* seeds in six developmental stages during early embryogenesis. Results revealed 136 spots that differed in kinetics of appearance. A posterior analysis by liquid

chromatography coupled to tandem mass spectrometry and MALDI-TOF mass spectrometry identified proteins represented by 71 of the spots. According to the data, these proteins are involved in programmed cell death and chromatin modification. The authors commented that the proteins may play a central role in determining the number of zygotic embryos generated and controlling embryo patterning and shape remodeling. These results could provide important information for plant cloning procedures and for the establishment of platforms for research into plant development/regulation and *in vitro* transgenic studies as well as to better understand the somatic embryogenesis process.

Two ongoing projects in our laboratories are using 2-D DIGE: one to study plant–pathogen interactions using sugarcane *Leifsonia xyli* subsp. *xyli*, and the second to evaluate possible alterations in the proteome map in transgenic and non-transgenic soybean (*Glycine max* L. Merril) seeds, leaves and roots, and its correlation with oxidative stress. Studies of metal-induced stress signaling in plants can also dramatically benefit from these refined techniques.

As a matter of fact, the results reported in the papers cited in this section are just a few examples that confirm how important the systems biology approach is, an interdisciplinary study field that focuses on complex interactions in biological systems.

3. Conclusions

This review points out the importance of the 2-D DIGE technique in order to contribute to improving analytical proteomics research in plant proteomics. When considering the totality of 2-D DIGE applications in the literature (from Web of Science), only 3.7% of the published manuscripts refer to plant proteomics. This is a curious situation, since different models frequently used in human and clinical studies are taken from ones based on plant studies.

Additionally, it is important to mention that 2-D DIGE studies show the necessity of transdisciplinary work, since not only biochemical, physiological and biological concepts are important, but also those based on analytical ones, again supporting the idea of systems biology. Only with a well optimized method, which involves good sample preparation, well-defined 2-D DIGE parameters, skilled analysts for obtaining good labeling, among others, can results be accurately obtained, thus making sense in terms of proteomic analysis.

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