

# Proteomic capacity of recent fluorescent dyes for protein staining

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## Abstract

Staining of two-dimensional gel constitutes a crucial step in comparative proteome analysis with respect to both the number of proteins analysed, the accuracy of spot quantification and reproducibility. In this work, we compared the efficiency of recent fluorophores to stain *Arabidopsis* total protein extract: Sypro Ruby® (SR), Deep Purple® (DP) and 5-hexadecanoylamino-fluorescein (C16-F). In addition, classical visible dyes, colloidal Coomassie blue (CCB) and silver nitrate (SN), were also included. High quality images were obtained for the three fluorescent dyes, DP giving the cleaner background, whereas spikes were observed with SR and a rough background with C16-F. On the other hand, saturation occurred for abundant spots with SR and DP. For a same protein load the number of detected spots ranged between 250 for CCB and 800 for SR in the sequence  $SR > DP \approx SN > C16-F > CCB$ . These differences were shown to rely mainly on the sensitivity between dyes leading to the detection of additional spots belonging to classes of lower abundance. Analysis of the distribution of variation coefficients for spots from replicates showed differences in the staining reproducibility between dyes that ranged in the order  $SR > C16-F > DP > SN > CCB$ . The implications of these results for the selection of a convenient stain are discussed according to specific objectives as well as practical aspects.

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## 1. Introduction

Protein separation by electrophoresis is largely used in proteomic approaches because of both high resolution, availability of powerful image analysis software for gel comparison and compatibility with subsequent protein characterization by mass spectrometry (MS) (Van Wijk, 2001; Patton, 2002). For these various aspects, the selection of the protein staining procedure is of major importance. Classically, Coomassie blue was the most widely used dye. However, it suffers from a low sensitivity in protein detection, including in the improved colloidal version (Neuhoff et al., 1990). By opposition, the other most classical protein stain, silver nitrate, displays an excellent sensitivity but could interfere with protein analysis by MS (Mortz et al., 2001). In the last years, different fluorescent dyes were introduced and proved to combine high sensitivity and compatibility

with mass spectrometry. These encompass both commercially available stains, such as the series of Sypros, and Ruthenium red-based dyes for which synthesis procedures were published (Berggren et al., 1999, 2000, 2002; Steinberg et al., 2000; Rabilloud et al., 2001). However, their present use remains relatively limited, probably due to their cost and/or technical difficulties. Very recently, alternative molecules were proposed. These include both epicocconone, a natural fungal product (Mackintosh et al., 2003; Bell and Karuso, 2003), that is now commercially available (Deep Purple® (DP), Amersham Biosciences) and fluoresceine derivatives (Kang et al., 2003). However, most available information to date concerns their efficiency to stain standard proteins in SDS-PAGE, with little focus on two-dimensional (2D) gels, and their compatibility with MS. Therefore, little is known concerning the power of these recent dyes when used with complex biological samples resolved in series of 2D gels.

Large-scale proteome comparison from 2D gels is now widely used in plant sciences for various goals, such as, for instance, the characterization of genetic diversity

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(Thiellement et al., 1999; Marquès et al., 2001), the analysis of developmental processes (Santoni et al., 1997; Gallardo et al., 2002) or the study of interactions with both abiotic and biotic environment (Costa et al., 1998; Bestel-Corre et al., 2002). The present work was undertaken in order to help the selection of a protein stain convenient for large-scale comparison of 2D gel electrophoresis patterns by image analysis. For this purpose, we selected a panel of staining procedures reported to be compatible with subsequent analysis by MS. Accordingly, using a same extract of soluble proteins from *Arabidopsis thaliana*, we compared the most classical visible stains, colloidal Coomassie blue (CCB) and silver nitrate (SN), with recent fluorescent dyes, Sypro Ruby® (SR), Deep Purple® and C16-fluoresceine (C16-F).

## 2. Results and discussion

### 2.1. Detection of protein patterns in SDS-PAGE

The sensitivity of a staining procedure is usually assessed by searching for the minimal amount or standard proteins that are detectable in SDS-PAGE. However, depending on the nature of the protein used, uneven

staining intensity is commonly observed for a same protein amount (Berggren et al., 2000; Rabilloud et al., 2001; Patton, 2002). Although sound for quantitative comparison, such approach can be misleading for biological samples that contain complex mixtures of proteins in very different amounts and for which the detection in terms of pattern is of interest. Therefore, in a first step, the sensitivity of the various dyes was compared from the patterns obtained with different quantities of a total extract of *Arabidopsis* proteins. In terms of sensitivity, assuming that the whole protein pattern was detected for 4 µg proteins (Fig. 1), SR appeared to be able to evidence a complex pattern down to ca. 0.5 µg, whereas the two other fluorescent dyes required ca. 1 µg. At the same time, this pattern was detected for ca. 1 µg protein with the SN staining procedure used and ca. 2 µg with CCB. These differences in pattern detection are in quantitative agreement with differences in sensitivity previously reported for the detection limit of purified proteins, as well between SR and SN as between SN and CCB (Berggren et al., 2000).

In order to get information about the linearity of responses, the intensities of both high- and low-abundance protein bands (bands 1 and 2, Fig. 1) were compared (Fig. 2). An almost linear relationship, with regression coefficients ranging between 0.96 and 0.99,

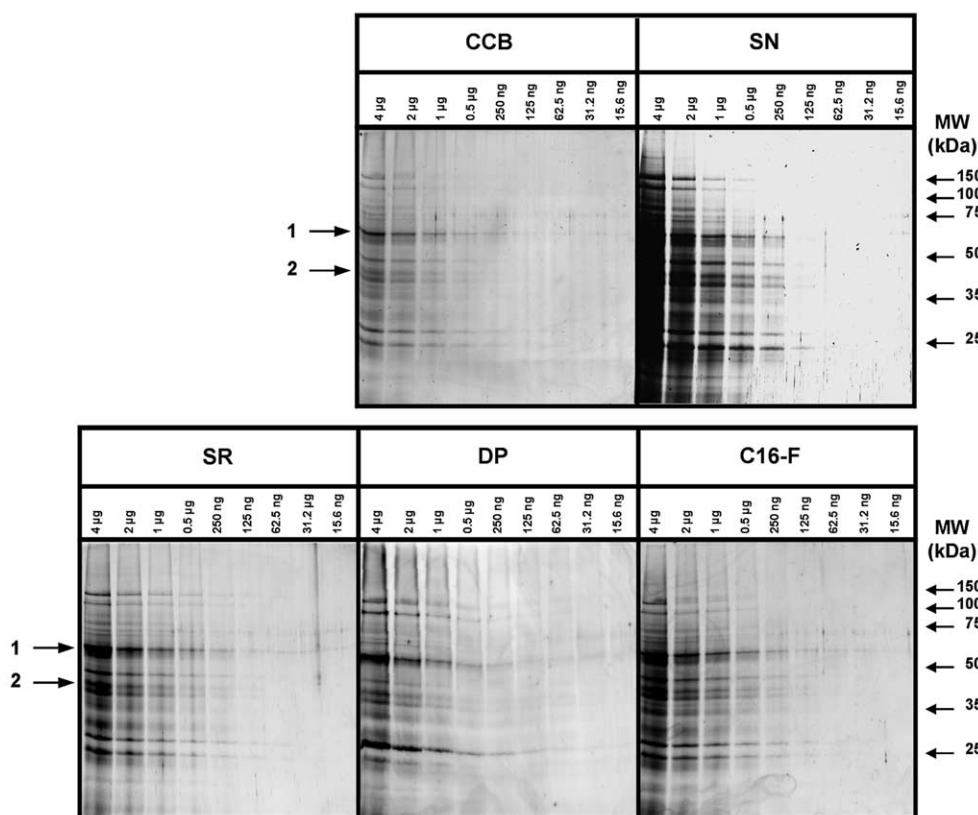


Fig. 1. Comparison of protein patterns by SDS-PAGE. Total protein extracts from *Arabidopsis* cells was submitted to serial dilutions from 15.6 ng to 4 µg. All gels are shown using identical grey scale range. Arrows refer to the bands quantified in Fig. 2.

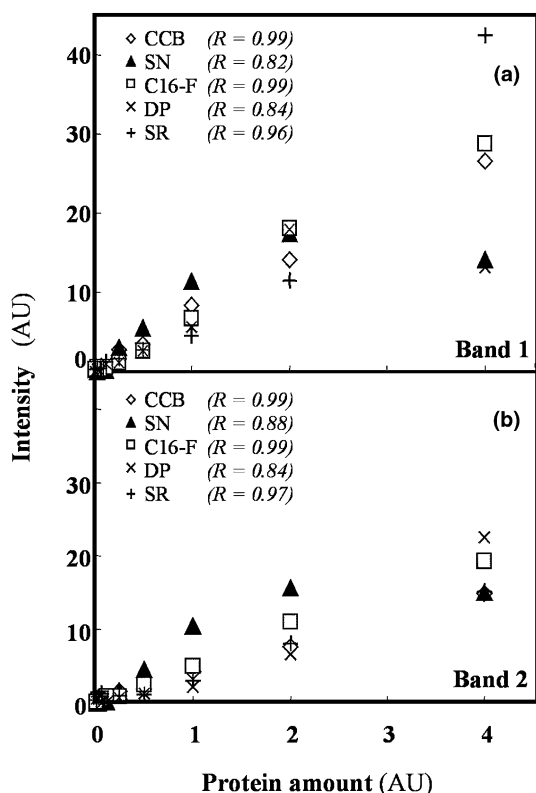


Fig. 2. Relationship between band intensity and protein amount. Intensity of bands “1” (a) and “2” (b) from Fig. 1 was measured according to the total amount of proteins loaded per lane (from 15.6 ng to 4  $\mu$ g). Insets: correlation coefficients between band intensity and protein amount.

was obtained for CCB, SR and C16-F, in agreement with previous observations (Berggren et al., 1999; Kang et al., 2003). A propensity to saturation, more pronounced for the high abundance protein band 1, was observed for SN and to a smaller extent for DP. Taken together, those of these results concerning previously characterized dyes were in agreement with available data for dynamic range (Patton, 2002; Yan et al., 2000).

## 2.2. Staining of 2D gels

In a second step, the 2D patterns were analysed using the same *Arabidopsis* protein extract. Owing to the differences in length and thickness between analytical 1D gels and large 2D gels, a 100  $\mu$ g protein amount was used for loading all the 2D gels. Well-resolved patterns were observed for the five dyes on the pI 4–7 range and 15–150 kDa range (Fig. 3). Direct inspection of gel images showed substantial differences concerning the background. DP and SN gave a very clean background with few precipitates. A clear contrast was obtained with SN and SR but some spikes were observed with SR. In the case of CCB, a non-uniform background was detected between the top and the bottom of the gel. Finally, C16-F gave a very rough background but without consequence on the spot detection by the software.

On the other hand, different relative staining intensities were obtained for the same spots depending on the dye (Fig. 4). Simultaneously, staining saturation was observed for SR, DP and SN. This process was

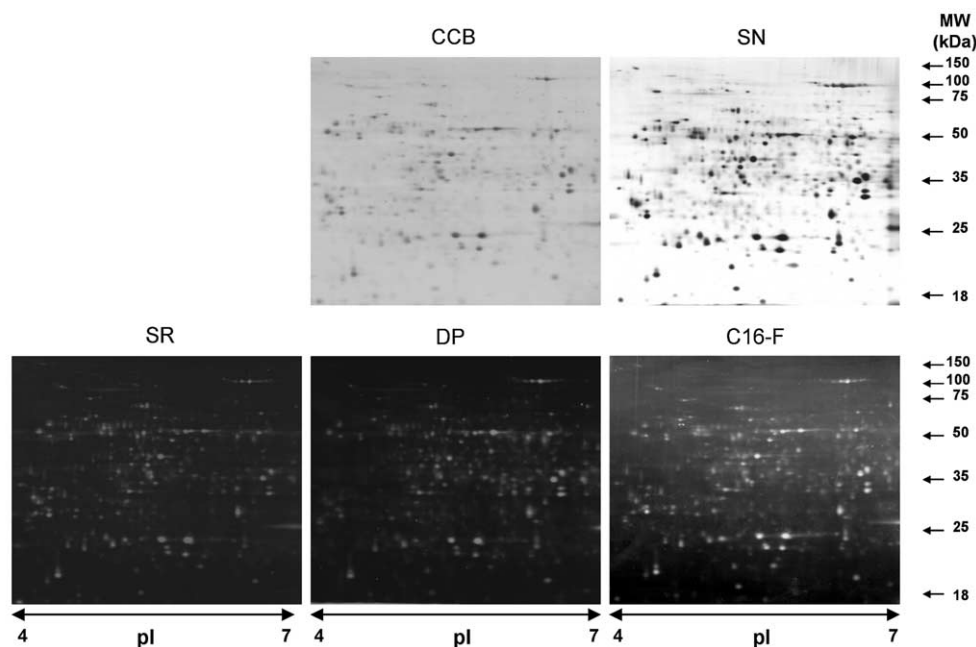


Fig. 3. Comparison of 2D protein patterns. 100  $\mu$ g of total protein extracts from *Arabidopsis* were focused on pI 4–7 range and separated on gels covering the 15–150 kDa range.

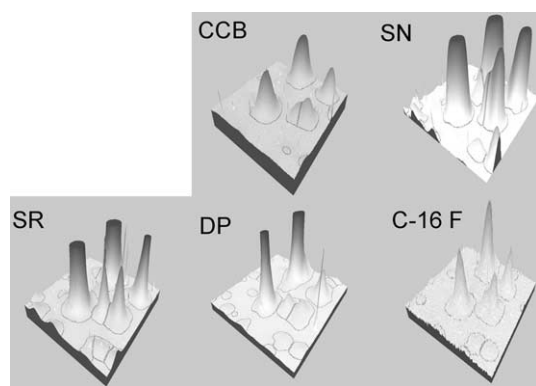


Fig. 4. Local comparison of gel staining. The same area was selected using the three-dimensional viewer of Progenesis® software.

spot-dependent and appeared to be more rapidly observed with DP than SR and SN. Taken together, these qualitative observations indicated the occurrence of quite large differences in dynamic ranges, as already stressed from 1D gel data. For SN, this behaviour is likely to be linked to its poor linearity which is known to result in many saturated and negatively stained spots (Rabilloud, 1992). This is more surprising as well for SR which display an enhanced linearity on 1D gels (Steinberg et al., 2000), although saturation of abundant spots has also been observed (Yan et al., 2000), as for DP which was shown to give linear responses up to four orders of magnitude (Mackintosh et al., 2003). It should

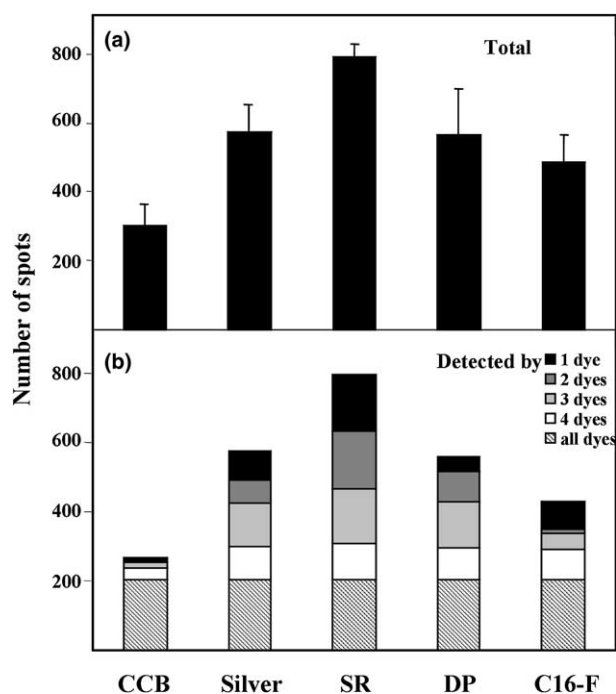


Fig. 5. Typology of spots detected by the various dyes. The number of spots matching between gels stained with the various dyes was calculated from triplicate gels. (b) (hatched area), spots detected by all dyes; (black area), spots detected by only 1 dye; (a) total number of spots stained with the various dyes.

be emphasized that gel imaging constitutes a compromise between the detection of minor spots and the limitation of the number of saturated abundant spots. Present data were recorded using single imaging conditions, with no effort to acquire different images specifically optimized for different subsets of proteins according to their abundance. Therefore, the occurrence of saturated spots should be taken mainly as reflecting the very large natural differences in protein abundance within a total extract. No systematic determination of the number of saturated spots was made here. Nevertheless, on a quantitative point of view, the observations above suggest that, for complex protein extracts, the

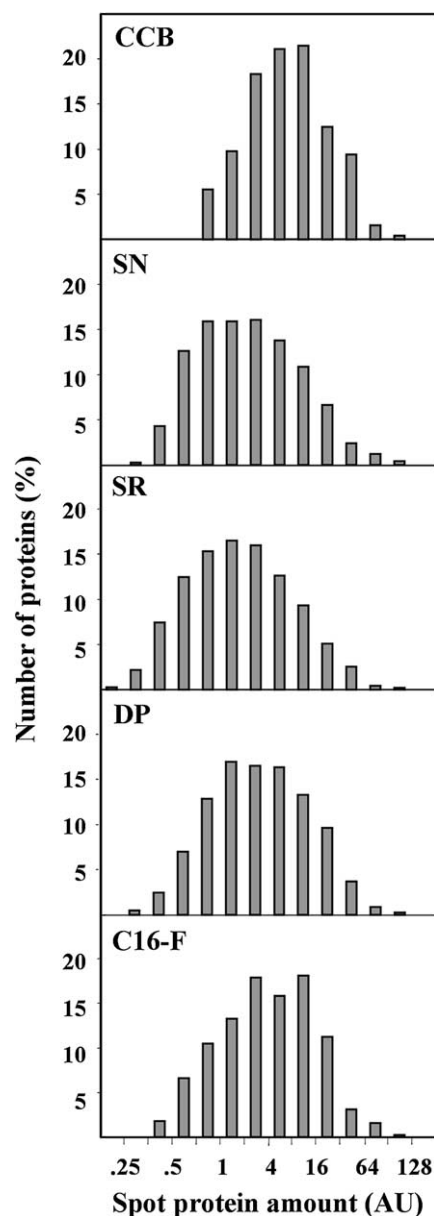


Fig. 6. Distribution of spots in classes of protein amount. Raw data from triplicates of each stain were normalized with respect to the cumulated signal of spots detected by all dyes (hatched area, Fig. 4), taken as an internal standard.

pertinence of data obtained for abundant spots by using SN, SR and DP should be ascertained when aiming to perform comparative analysis.

### 2.3. Spot detection

Depending on the dye, the number of stained spots spanned between ca. 250 for CCB and ca. 800 for SR (Fig. 5(a)). This result is in quite close agreement with 1D gel data above suggesting that the observation of a full pattern required ca. four times more protein with CCB than with SR. On the other hand, it appeared that the three dyes detecting the highest number of spots corresponded to those showing propensity to saturation. More detailed analysis revealed that most spots from CCB-stained gels matched with spots also detected by the four other dyes (Fig. 5(b)). Simultaneously, each

from these four detected additional spots. Globally, SR appearing to stain nearly all the spots that were visualized by other dyes as well as an extra set of spots not detected by any other one. Therefore, this suggests that the observed differences in 2D pattern result mainly from differences in the sensitivity of the dyes rather than from differential specificity of the interactions between dyes and protein. This view was corroborated by the fact that additionally detected spot covered the whole pI and MW ranges, suggesting that no bias was introduced by physico-chemical properties of proteins. In order to get more insights into this point, the relationship between the number of spots detected and their abundance was investigated. For this purpose, the cumulated signal from the same ca. 200 spots from each gel that were stained by all the five dyes together was used as an internal reference to normalize the data. Following clas-

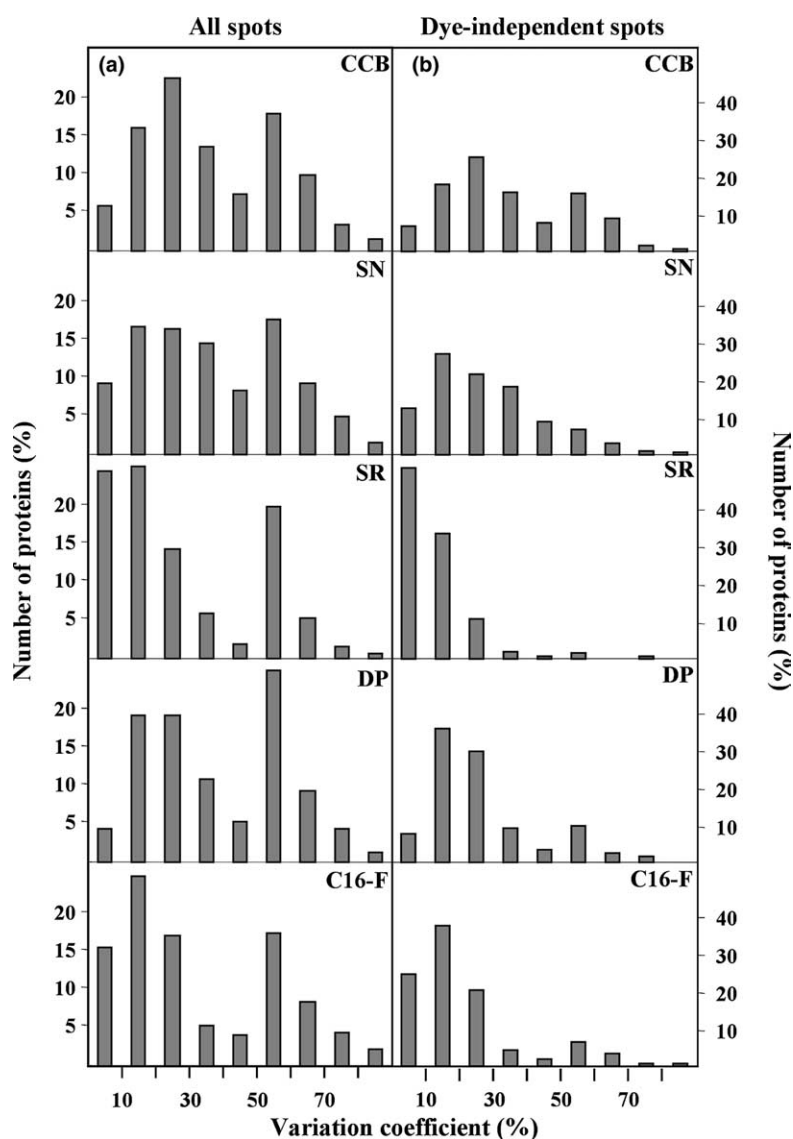


Fig. 7. Distribution of spots in classes of variation coefficient. Data were computed from triplicates: (a) for all detected spots or (b) for spots detected by all dyes (hatched area, Fig. 4).

sification of spots into abundance classes, CCB appeared to detect mainly medium- and high-abundance spots (Fig. 6). C16-F displayed a similar distribution, but covering, in addition, some classes of less abundant spots. Both DP and SR covered the largest range of abundance classes, whilst SN displayed an intermediate behaviour. For these three dyes, however, it should be pointed out that the size of classes grouping high-abundance spots was likely under-estimated due to staining saturation. Nevertheless, the distributions observed support the hypothesis that the differences in terms of number of spots reflect mainly the differences in sensitivity between the dyes. This interpretation is further supported by the observation that the 160 additional spots detected with SR are all distributed in low-abundance classes (data not shown).

#### 2.4. Quantification reproducibility

Beside the sensitivity, another main feature of importance in large-scale proteome analysis is the reproducibility of the staining across gels. Three replicates were compared for each staining procedure, in order to calculate the variation coefficient of the signal recorded for each spot, and to analyse the distribution of these coefficients (Fig. 7(a)). For all the dyes, two populations of spots were observed. The first one was centred around ca. 20% and accounted roughly for the two-thirds of spots. For this main population, quite large differences were noticed depending on the dye. The more reproducible results were observed for SR, with most variation coefficients below 20%. By opposition, for CCB and SN, this population covered a larger range up to 40%. The two other fluorescent dyes displayed an intermediary distribution. On the other hand, the second population showed higher variability with variation coefficients amounting to 50–60% whatever the dye. This minor population included spots covering all the range of molecular weight, and was constituted by different spots according to the dye. It should be noticed that, except for CCB, its size decreased to less than 10% of spots when the distribution was calculated only on those spots that were stained by all the dyes (Fig. 7(b)). Therefore, according to the preceding analysis in terms of abundance of proteins, this population can be hypothesized to correspond to proteins of relatively low abundance and/or to proteins having lower affinity for the dyes. Taken together, none of the dyes appears to lead to weakly dispersed results, when looking at the global pattern. By opposition, when overlooking minor proteins (Fig. 7(b)), lower and very different variability can be expected following the dye used. In terms of proteome comparisons, analysis are often based on an arbitrary selected ratio factor to detect variations in abundance between different samples. The results above indicate that true statistical analysis should be preferred

to the likely poorly informative use of such unique factor.

### 3. Concluding remarks

Several features are of importance to select a 2D gel staining procedure in projects aiming at large-scale quantitative and differential proteome analysis. These include: (i) the sensitivity which determines the number of spots detected for a given protein amount; (ii) the occurrence of saturation in the staining which precludes the production of quantitative data for abundant spots; (iii) the reproducibility of the staining which affects the statistical significance in comparisons. In terms of number of detected spots, with the protocols used here, the tested dyes range in the order  $SR > DP \approx SN > C16-F > CCB$ , and this was shown to be due to the capacity of SR to detect additional spots of low abundance. Unfortunately, the three more sensitive dyes display also a propensity to saturation. In terms of reproducibility, the dyes range in the order  $SR > C16-F > DP > SN > CCB$ . The SR appears therefore particularly suitable to detect reproducibly proteomes covering both minor and abundant proteins. On the other hand, C16-F, which is weakly subject to saturation, could be a good candidate when the detection of very minor spots is not a main goal, whereas DP constitutes a valuable compromise especially in terms of sensitivity.

Beside the basic criteria above, other features of practical importance include the ease of the staining procedure and its cost. On this point of view, the two not saturating dyes (CCB and C16-F) involve very simple manipulations and inexpensive molecules. Therefore, according to the features above, the new dye C16-F appears to constitute a promising alternative when the amount of protein samples is not strongly limiting.

### 4. Experimental

#### 4.1. Plant material

Cell suspensions of *A. thaliana* (ecotype Columbia) were maintained at 23 °C under agitation and 12 h photoperiod. Cells were grown in Gamborg's B5 basal like medium supplemented with 2-naphthaleneacetic acid ( $186 \mu\text{g l}^{-1}$ ) and sucrose ( $18 \text{ g l}^{-1}$ ) (Axelos et al., 1992). They were harvested in exponential phase, seven day after inoculation, and washed thoroughly with MilliQ deionized water before extraction of proteins.

#### 4.2. Chemicals

Urea was from VWR; CHAPS, Triton X100, iodoacetamide, bromophenol blue and silver nitrate were

from Sigma-Aldrich; glycerol, glycine, DTT and Tris were from Euromedex. High quality SDS (USB), IPG strips and Deep Purple<sup>®</sup> were from Amersham Biosciences. Coomassie brilliant blue G-250 and Sypro Ruby<sup>®</sup> were from Biorad; 5-hexadecanoylamino-fluorescein (C16-F) was from Molecular Probes.

#### 4.3. Preparation of total soluble proteins extracts

Cells were grinded in liquid nitrogen, and the fine powder was mixed with 90% acetone (v/v), 10% TCA (v/v) and 0.07% 2-mercaptoethanol (v/v), and incubated at –20 °C for 30 min. Insoluble material was centrifuged at 42,000g. Pellets were washed three times with pure acetone containing 0.07% 2-mercaptoethanol (v/v), air dried and then solubilized in 9 M urea, 4% CHAPS (w/v), 0.05% Triton X100 (v/v) and 65 mM DTT. Protein amount was estimated according to Bradford (1976). All manipulations were performed at 4 °C.

#### 4.4. Gel electrophoresis

##### 4.4.1. SDS-PAGE

Proteins sample were prepared by mixing 1 volume of sample in solubilization buffer and 1 volume of 2X loading buffer (80 mM TRIS (pH 6.8), 0.7 M 2-mercaptoethanol, 20% (v/v) glycerol, 2% (w/v) SDS, 0.01% (w/v) bromophenol blue). They were then heated 5 min at 95 °C. SDS-PAGE was performed with 11% acrylamide gels (1.5 mm thick) according to Laemmli (1970). A twofold serial dilution of protein was used (from 4 µg to ≈15 ng). Electrophoresis was carried out at 10 mA per gel in the stacking gel, and 20 mA per gel in the running gel.

##### 4.4.2. 2D gel electrophoresis

2-DE was performed using 18 cm linear pH 4–7 IPG strips. Protein samples were supplemented with 0.5% (v/v) IPG buffer 4–7 (Amersham Bioscience) and 0.002% (w/v) bromophenol blue. Strips were hydrated directly with protein solution. Isoelectric focusing was performed using an IPG-Phor device (Amersham Bioscience) until 100,000 kV h<sup>–1</sup>. Before the second dimension, proteins were reduced (50 mM Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 130 mM DTT) and alkylated in the same buffer containing 130 mM iodoacetamide instead DTT for 15 min. Strips were then embedded using 0.6% (w/v) low-melt agarose on the top of a 11% acrylamide gel. SDS-PAGE was carried out overnight, using a DALT system (Amersham Bioscience), at 15 mA per gel and 10 °C.

#### 4.5. Gels staining and imaging

For CCB, gels were stained following the method of Neuhoﬀ et al. (1990) during five day, and digitalized at 300 dpi with a GS 710 densitometer (Biorad).

For SN, gels were stained according to the method developed by Mortz et al. (2001) for subsequent MS analysis. Gels were digitalized at 300 dpi with the GS 710 densitometer.

For SR, gels were stained according to instructions of the manufacturer (<http://www.probes.com/media/pis/mp12000.pdf>). Gels were visualized with a FLA-5000 image analyser (Fuji) using the 473 nm excitation laser and the long pass filter Y510. Image were acquired at a 100 µm resolution with a 700 V voltage applied to the photomultiplier tube.

For DP, gels were stained according to instructions of the supplier (<http://www.amershambiosciences.com/>). Images were acquired with the FLA-5000 analyser using the 532 nm excitation laser and the long pass filter O575 with the same setting as for SR.

For C16-F, gels were stained according to Kang et al. (2003). Image were acquired as for SR.

#### 4.6. Image analysis

Gel images were analysed with the Progenesis<sup>®</sup> Workstation software (Perkin–Elmer). Triplicate gels were grouped and the same area of each gels was selected for spot detection. Excepted background removal, no filtering was used. When spikes were observed, specific attention was paid to ensure that no additional and artefactual spot was introduced. The gels with the highest number of spots were selected as reference gels and a combined warping and matching algorithm was used to create an average gel including spots detected at least two times in the triplicates. Spot volume was expressed as percentage of the total volume of all spots detected on the gels.

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#### References

- Axelos, M., Curie, C., Mazzolini, L., Bardet, C., Lescure, B., 1992. A protocole for transient gene expression in *Arabidopsis thaliana* protoplasts isolated from cell suspension cultures. *Plant Physiol. Biochem.* 30, 123–128.
- Bell, P.J., Karuso, P., 2003. Epicocconone, a novel fluorescent compound from the fungus *epicoccumnigrum*. *J. Am. Chem. Soc.* 125, 9304–9305.
- Berggren, K., Steinberg, T.H., Lauber, W.M., Carroll, J.A., Lopez, M.F., Chernokalskaya, E., Zieske, L., Diwu, Z., Haugland, R.P., Patton, W.F., 1999. A luminescent ruthenium complex for ultra-sensitive detection of proteins immobilized on membrane supports. *Anal. Biochem.* 276, 129–143.

- Berggren, K., Chernokalskaya, E., Steinberg, T.H., Kemper, C., Lopez, M.F., Diwu, Z., Haugland, R.P., Patton, W.F., 2000. Background-free, high sensitivity staining of proteins in one- and two-dimensional sodium dodecyl sulfate-polyacrylamide gels using a luminescent ruthenium complex. *Electrophoresis* 12, 2509–2521.
- Berggren, K.N., Schulenberg, B., Lopez, M.F., Steinberg, T.H., Bogdanova, A., Smejkal, G., Wang, A., Patton, W.F., 2002. An improved formulation of SYPRO Ruby protein gel stain: comparison with the original formulation and with a ruthenium II tris (bathophenanthroline disulfonate) formulation. *Proteomics* 2, 486–498.
- Bestel-Corre, G., Dumas-Gaudot, E., Poinot, V., Dieu, M., Dierick, J.F., van Tuinen, D., Remacle, J., Gianinazzi-Pearson, V., Gianinazzi, S., 2002. Proteome analysis and identification of symbiosis-related proteins from *Medicago truncatula* Gaertn by two-dimensional electrophoresis and mass spectrometry. *Electrophoresis* 23, 122–137.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* 72, 248–254.
- Costa, P., Bahrman, N., Frigerio, J.M., Kremer, A., Plomion, C., 1998. Water-deficit-responsive proteins in maritime pine. *Plant Mol. Biol.* 38, 587–596.
- Gallardo, K., Job, C., Groot, S.P., Puype, M., Demol, H., Vandekerckhove, J., Job, D., 2002. Proteomics of *Arabidopsis* seed. *Plant Physiol.* 129, 823–837.
- Kang, C., Kim, H.J., Kang, D., Jung, D.Y., Suh, M., 2003. Highly sensitive and simple fluorescence staining of proteins in sodium dodecyl sulfate-polyacrylamide-based gels by using hydrophobic tail-mediated enhancement of fluorescein luminescence. *Electrophoresis* 24, 3297–3304.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Mackintosh, J.A., Choi, H.Y., Bae, S.H., Veal, D.A., Bell, P.J., Ferrari, B.C., Van Dyk, D.D., Verrills, N.M., Paik, Y.K., Karuso, P., 2003. A fluorescent natural product for ultra sensitive detection of proteins in one-dimensional and two-dimensional gel electrophoresis. *Proteomics* 3, 2273–2288.
- Marquès, K., Sarazin, B., Chané-Favre, L., Zivy, M., Thiellement, H., 2001. Comparative proteomics to establish genetic relationships in the Brassicaceae family. *Proteomics* 1, 1457–1462.
- Mortz, E., Krogh, T.N., Vorum, H., Gorg, A., 2001. Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. *Proteomics* 1, 1359–1363.
- Neuhoff, V., Stamm, R., Pardowitz, I., Arold, N., Ehrhardt, W., Taube, D., 1990. Essential problems in quantification of proteins following colloidal staining with coomassie brilliant blue dyes in polyacrylamide gels, and their solution. *Electrophoresis* 11, 101–117.
- Patton, W.F., 2002. Detection technologies in proteome analysis. *J. Chromatogr. B* 771, 3–31.
- Rabilloud, T., 1992. A comparison between low background silver diammine and silver nitrate protein stains. *Electrophoresis* 13, 429–439.
- Rabilloud, T., Strub, J.M., Luche, S., van Dorselaer, A., Lunardi, J., 2001. A comparison between Sypro Ruby and ruthenium II tris (bathophenanthroline disulfonate) as fluorescent stains for protein detection in gels. *Proteomics* 1, 699–704.
- Santoni, V., Delarue, M., Caboche, M., Bellini, C., 1997. A comparison of two-dimensional electrophoresis data with phenotypical traits in *Arabidopsis* leads to the identification of a mutant (cri1) that accumulates cytokinins. *Planta* 202, 62–69.
- Steinberg, T.H., Chernokalskaya, E., Berggren, K., Lopez, M.F., Diwu, Z., Haugland, R.P., Patton, W.F., 2000. Ultrasensitive fluorescence protein detection in isoelectric focusing gels using a ruthenium metal chelate stain. *Electrophoresis* 21, 486–496.
- Thiellement, H., Bahrman, N., Damerval, C., Plomion, C., Rossignol, M., Santoni, V., de Vienne, D., Zivy, M., 1999. Proteomics for genetic and physiological studies in plants. *Electrophoresis* 20, 2013–2026.
- Van Wijk, K.J., 2001. Challenges and prospects of plant proteomics. *Plant Physiol.* 126, 501–508.
- Yan, J.X., Harry, R.A., Spibey, C., Dunn, M.J., 2000. Postelectrophoretic staining of proteins separated by two-dimensional gel electrophoresis using SYPRO dyes. *Electrophoresis* 21, 3657–3665.