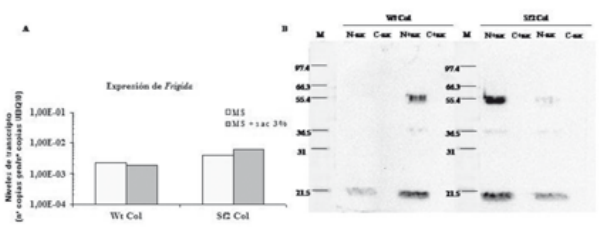

Figura 2. *Estudio de los niveles de expresión de FRIGIDA y detección de la proteína en plántulas de A. thaliana, en respuesta a sacarosa. A) Análisis, por QPCR, de los niveles de transcripto de FRIGIDA en plántulas (Wt col y Sf2 col) crecidas en medio MS y medio MS suplementado con 3% de sacarosa. B) Análisis, mediante Western blot, de la presencia de FRIGIDA en extractos nucleares y citosólicos de plántulas Wt col y Sf2 col crecidas en las mismas condiciones de ensayo. Cabe mencionar que FRIGIDA se detecta exclusivamente en las fracciones nucleares (banda de 57 Kda aprox.) y que en presencia de sacarosa parece aumentar su producción. La banda de 20 Kda corresponde a la Histona H3, utilizada como control de la integridad y pureza de las fracciones.*

representado corresponde a proteínas implicadas en mecanismos de señalización y regulación de la expresión génica, como es el caso de FRIGIDA cuya expresión parece aumentar en presencia de sacarosa. Una situación similar puede comprobarse a nivel del perfil de expresión (Figura 1b). Sin embargo, y en el caso particular de FRIGIDA, no parece existir una relación directa entre gen-proteína ya que, en presencia de sacarosa, una mayor producción de proteína (Figura 2b) no parece reflejarse a nivel del correspondiente transcrito (Figura 2a). Mediante el uso combinado de estas herramientas (Proteómica y Transcriptómica) hemos mostrado que podemos aumentar la comprensión de los mecanismos biológicos, ya que permite la integración y la intersección de la información transcripcional, traduccional y post-traduccional.

Referencias

[1] Hochholdinger F, Sauer M, Dembinsky D, Hoecker N, Muthreich N, Saleem M, Liu Y.Proteomic dissection of plant development. Proteomics 2006; 6:4076-4083.

[2] Chen S y Harmon AC. Advances in plant proteomics. Proteomics 2006; 6:5504-5516.

[3] Casal, JJ. Fankhauser C, Coupland G, Blázquez MA. Signalling for developmental plasticity. Trends in Plant Science 2004; 9 (6):309-314.

A DIGE proteomic analysis of wheat flag leaf treated with TERRA-SORB® foliar, a free amino acid-based biostimulator

María José Martinez-Esteso[1], Mayte Vilella-Antón[1], Susana Sellés-Marchart[2], Anna Botta-Català[3], Rafael Piñol-Dastis[3], Roque Bru-Martinez[1]

[1]Grupo de Proteómica y Genómica Funcional de Plantas. Dpto. Agroquímica y Bioquímica, IMEM Ramon Margalef, Universidad de Alicante; [2]Unidad de Genómica y Proteómica, SSTTI, Universidad de Alicante; [3]Departamento I+D Fisiología Vegetal, BIOIBERICA, S.A.

Abstract

In this work, we have undertaken a proteomic approach using DIGE in order to explore molecular mechanisms potentially involved in the biostimulating effect of Terra-Sorb® foliar on wheat applied at flag leaf stage. Proteins identified up- and down-regulated suggest an improvement of wheat productivity by a combination of an enhanced CO_2 fixation, a more active protein synthesis and a decrease of oxidative stress.

Table 1. *Proteins de-regulated in wheat flag leaf treated with Terra-Sorb® Foliar. Normalised spots volumes for CONTROL and TREATED samples.*

Protein Information				Norm. Vol.	
Ref. Spot	Protein	Description	Accession No.	CONTROL	Terra-Sorb
73	EF-Tu Chl-1	translational elongation factor Tu	125540125	1	1,412
10	EF-Tu Chl-2	translational elongation factor Tu	125540125	1	1,371
14	EF-Tu Chl-3	translational elongation factor Tu	125540125	1	1,301
18	RUBISCO LBP-B-1	RuBisCO large subunit-binding protein subunit beta, chloroplast	2493650	1	1,256
122	RUBISCO LBP-B-2	RuBisCO large subunit-binding protein subunit beta, chloroplast	2493650	1	1,231
30	RUBISCO LBP-B-3	RuBisCO large subunit-binding protein subunit beta, chloroplast	2493650	1	1,176
8	RUBISCO LBP-A-1	RuBisCO large subunit-binding protein subunit alpha, chloroplast precursor	134102	1	1,4
12	RUBISCO LBP-A-2	RuBisCO large subunit-binding protein subunit alpha, chloroplast precursor	134102	1	1,328
11	RUBISCO LBP-A-3	RuBisCO large subunit-binding protein subunit alpha, chloroplast precursor	134102	1	1,284
56	RUBISCO-L (fragmento?)-1	RuBisCO large subunit. Ribulose bisphosphate carboxylase large chain, N-terminal domain.	548677	1	1,825
62	RUBISCO-L (fragmento?)-2	RuBisCO large subunit. Ribulose bisphosphate carboxylase large chain, N-terminal domain.	548677	1	1,611
5	RUBISCO A-1	RuBisCO activase A chloroplast precursor	12643756	1	1,43
9	RUBISCO A-2	RuBisCO activase A chloroplast precursor	12643756	1	1,413
89	RUBISCO A-3	RuBisCO activase A chloroplast precursor	12643756	1	1,274
170	RUBISCO A-A (Precursor?)	RuBisCO activase A chloroplast precursor	12643756	1	1,184
40	PRK-1	Phosphoribulokinase, chloroplast precursor	125580	1	1,087
46	PRK-2	Phosphoribulokinase, chloroplast precursor	125580	1	1,037
71	HSP90	Heat schock protein 90kDa	556673	1	1,47
33	EF-G Chl	Elongation factor G, chloroplast precursor (EF-G)	125549171	1	1,113
36	EF-G Chl	Elongation factor G, chloroplast precursor (EF-G)	125549171	1	1,108
32	PGM	phosphoglycerate mutase	32400802	1	-1,264
34	GA3PDH	glyceraldehyde-3-phosphate dehydrogenase	15222111	1	-1,27
29	ATP-CF1-A	ATP synthase CF1 alpha subunit	14017569	1	-1,273
22	pEF-G Chl	putative Elongation factor G, chloroplast precursor (EF-G). Fragmento?	125549171	1	-1,289
131	Cu/Zn SOD	Cu/Zn superoxide dismutase	1568639	1	-1,34
13	Cu/Zn SOD	Cu/Zn superoxide dismutase	1568639	1	-1,476

Communication

Proteomics has been shown as successful approach to analyze the response of plants to external stimulus [1]. We performed a proteomic approach in order to study the effects of Terra-Sorb ® foliar on wheat leaves at the flag leaf stage (39 BBCH). Terra- Sorb ® foliar, a L-α-amino acid-based product from enzymatic hydrolysis with a high ratio of free-to-total amino acids, was applied two and three days following foliar by spraying treatment. Flag leaf samples were taken from independent control (C) and treated plots (T).

A quantitative DIGE approach was used to compare the proteomes of T vs C plants in four biological replicates. After SameSpots v3.0 gel analysis, 37 deregulated spots were selected out of 918 detected, with an ANOVA $p<0.05$. As shown in table 1, 26 protein spots encoded by 12 different genes were successfully identified by nLC-MS/MS in a XCTplus IT mass spectrometer (Agilent) (SpectrumMill Proteomics Workbench as search engine; NCBInr as database).

Four spots identified as RUBISCO activase A chloroplast precursor (RBA) were up-regulated by treatment with Terra-Sorb ® foliar. This enzyme produces ATP-dependent conformational changes in RUBISCO, making the inactivated enzyme to re-enter the catalytic cycle. Phosphoribulokinase has been detected with a slight increase in abundance, which is consistent with an enhancement of carbon fixation. In addition, RUBISCO large subunit-binding protein subunits alpha and beta (RUBISCO LBP A and B), that assists RUBISCO folding, have been found up-regulated in treated leaves. These results together suggest that Terra-Sorb ® foliar promotes RUBISCO activity, and thus enhanced carbon fixation.

Three up-regulated spots identified as chloroplast elongation factors Tu (EF-Tu Chl), G (EF-G Chl) and heat shock protein 90 (HSP-90) indicate a potentially enhanced protein synthesis and folding to produce functional proteins upon Terra-Sorb ® foliar treatment. Plants invest an important amount of the photosynthetic energy in biosynthesis of amino acids, thus the application of exogenous amino acids via foliar may give rise to an enhancement of protein synthesis.

Among those down-regulated spots four proteins have been identified: chloroplast ATPase alpha subunit, phosphoglyceromutase (PGM), glyceraldehyde-3-phosphate dehydrogenase (GA3PDH) and Cu/Zn superoxide dismutase (Cu/Zn SOD). There is a slight decrease of the three first proteins consistent with a decrease in consumption of the product of photosynthesis 3-phosphoglycerate for catabolism, thus this product might be accumulated in a reservoir as a sugar. The decrease of a superoxide dismutase specific for scavenging of the superoxide generated by photooxidation of O_2 in PS-I (Cu/Zn SOD) is consistent with a lower oxidative stress state.

According to results, the three major molecular effects found of spraying wheat with Terra-Sorb ® foliar on the flag leaf proteome support the physiological effects described upon treatment of other crops with Terra-Sorb ® foliar foliar such as increase of plant photosynthetic activity and chlorophyll content, promotion of rapid recovery from stress and improvement of nutrient absorption [2].

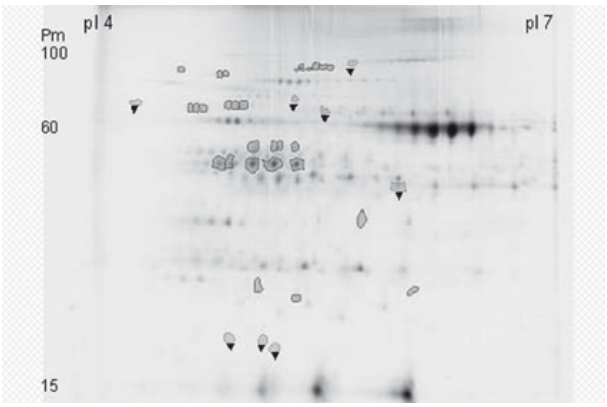

Figure 1. *2D reference gel image showing the location of selected spots down- regulated (upwards arrowhead) and up-regulated.*

Acknowledgements

The technical support and helpful discussions of Ramón Sánchez Pérez and Cándido Marín Garrido are very acknowledged. The DIGE and protein identification analysis were carried out at Servicio de Proteómica de la Universidad de Alicante, a PROTEORED member (www.proteored.org). This work was supported by Bioibérica, S.A.

References

[1] Martínez-Esteso MJ, Sellés-Marchart S, Vera-Urbina JC, Pedreño MA, Bru-Martinez R. J. Proteomics 2009 ;73 (2): 331-341.

[2] Gordon LK, Daniel PK, Thomas LW. Plant Growth Regulation Society of America 2005; 33 (4): 134-141.