Proteomic analyses of the effect of nitrogen assimilation in wheat cultivars under different fertilization regimes

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Proteomic analyses of the effect of nitrogen assimilation in wheat cultivars under different fertilization regimes

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Abstract. Nitrogen nutrition is one of the major factors that limits growth and production of crop plants. It affects many processes, such as development, architecture, flowering, senescence and photosynthesis. Although the improvement in technologies for protein study and the widening of gene sequences have made poss ble the study of the plant proteomes, only limited information on proteome changes occurring in response to nitrogen amount are available up to now. In this work, two-dimensional gel electrophoresis (2-DE) has been used to investigate the protein changes induced by different nitrogen sources of wheat plants.

Keywords. Nitrogen assimilation – Proteomics – Wheat – Fertilization regimes.

Analyses protéomiques de l'effet de l'assimilation de l'azote chez les cultivars de blé sous différents régimes de fertilisation

Résumé. *La fertilisation azotée est l'un des principaux facteurs qui limitent la croissance et la production des plantes cultivées. Elle intervient dans de nombreux processus tels que le développement, l'architecture, la floraison, la sénescence et la photosynthèse. Bien que l'amélioration des technologies pour l'étude des protéines et l'élargissement des séquences de gènes ait permis d'étudier les protéomes de plantes, peu d'informations sont disponibles à présent sur les modifications du protéome induites par la quantité d'azote apportée. Dans ce travail, l'électrophorèse bidimensionnelle sur gel (2-DE) a été utilisée pour étudier les changements des protéines induits de par différentes sources d'azote chez les plantes de blé.*

Mots-clés. *Assimilation de l'azote – Protéomique – Blé – Régimes de fertilisation.*

I – Introduction

Wheat (*Triticum aestivum* and *T. turgidum*) is one of the three most important cereal crops worldwide. Its cultivation presents a dominant position in the European agriculture due to its adaptability and large consumption in all the Mediterranean countries. A number of projects worldwide have been focusing on understanding the uptake, assimilation and utilization of nitrogen to improve the efficiency of nitrogen recovery in the grain. Whilst the physical processes of nitrogen and sulphur remobilization have been studied in detail, the genetic control of these processes and their contribution to agronomic productivity are less well understood. The peculiarity of the flag leaf allows for an efficient translocation of assimilates until the very late stages of leaf senescence, and the relative contribution of the flag leaves to the final grain nitrogen level is essential.

Although generally low, soil nitrogen availability can fluctuate greatly in both space and time due to factors such as precipitation, temperature, soil type and pH. Therefore, the preferred form in which N is taken up depends on plant adaptation. Nitrate uptake occurs at the root level and two nitrate transport systems have been shown to coexist in plants and to act co-ordinately to take up nitrate from the soil solution and distribute it within the whole plant (Tsay *et al*., 2007).

The analysis of the protein profile of plant tissue is an optimal method for quantifying changes in protein abundance caused by cropping systems. Proteomics is the study of the expression genes that have physiological effects on the plant. By identifying these proteins we can then link the protein back to the gene. In this way, candidate genes for agronomic traits can be identified, leading to the development of functional molecular markers for accelerating and assisting crop breeding practices (Varshney *et al*., 2005).

Transcriptomics has previously been used to directly identify genes involved in N metabolism and storage protein synthesis which are differentially expressed in response to organic and conventional fertilisers (Lu *et al*., 2005).

The main advantage of using a proteomics approach allows the observation of post-transcriptional changes to gene products that would not be identified in the transcriptome, such as protein degradation involved in important plant physiological processes, including N remobilization.

The objectives of the study presented here were to compare the effect of contrasting components of organic and conventional cropping systems on a) agronomic/physiological traits, b) the wheat flag leaf proteome, c) the association between the flag proteome and agronomic/physiological traits. This is a first step towards identifying functional molecular marker for subsequent marker-assisted breeding of wheat.

II – Material and methods

In the present research, we analysed two durum wheat cultivars (Creso and Dylan) under different fertilization regimes (Table 1) normally used in organic and conventional agriculture. A 2-dimensional electrophoresis gel coupled with mass-spectrometry approach was used, according to Vita *et al*. (2013), on wheat leaf samples and significant differences in expressed proteins were detected. To confirm these data, analyses related to transcript levels on nitrogen transporter genes through qPCR were performed. Primer pairs were designed using conserved sequences in related species (e.g. *Brachypodium distachyon*, *Triticum aestivum*).

Table 1. Nitrogen fertilization (Kg/ha) applied.

N treatment	Pre-sowing(P)	Emergence (E)	Coverage ©
Control	0	0	0
Synthesis	40	40	40
Leather	40	40	40
Protein hydrolysate 1	0	60	60
Protein hydrolysate 2	0	60	60
Rhizovit	0	60	60

III – Results

1. Effect of crop management on protein expression in flag leaves

About 72 2-DE gels (Fig. 1) have been analyzed by using Progenesis Samespot (software version 3.2.3). Six gels were realized for each experimental condition. Bioinformatics analyses revealed that contrasting fertilization regimes resulted in significant differential expression of 30 protein spots of interest, which distinguish cultivars among them and between treatments (some examples in Fig. 2), selected for the protein identification through mass-spectrometry analysis. The selection of spots was made on the basis of fold change (>1,3) and ANOVA values (p value <0,05).

2. Creation of subgroups for analysis

To compare the protein profile of different cultivars and treatments, 4 subgroups of analysis were created.

1. Subgroup Creso-Dylan,
2. Subgroup Creso (6 treatment),
3. Subgroup Dylan (6 treatment),
4. Each treatment (Creso vs. Dylan).

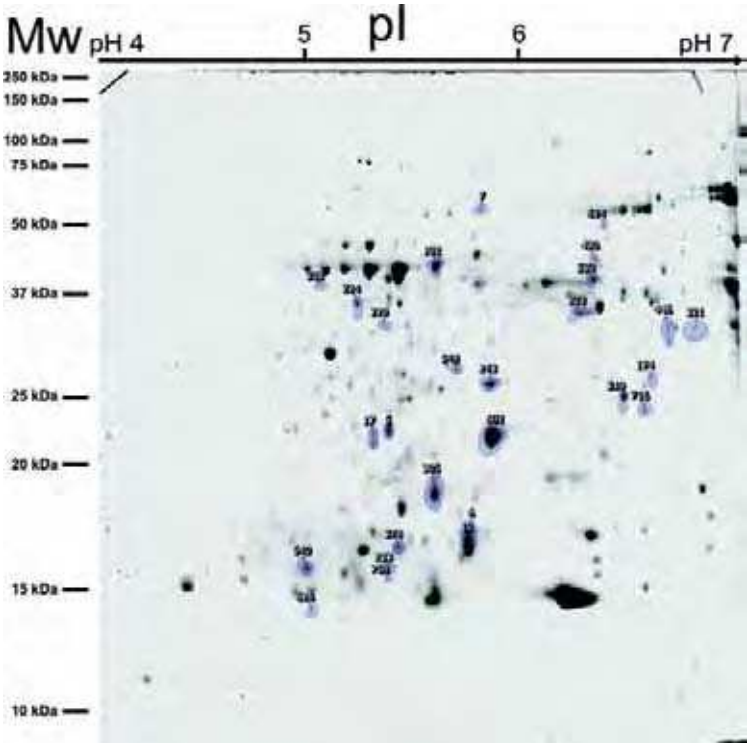

Figure 1. Reference Gel.

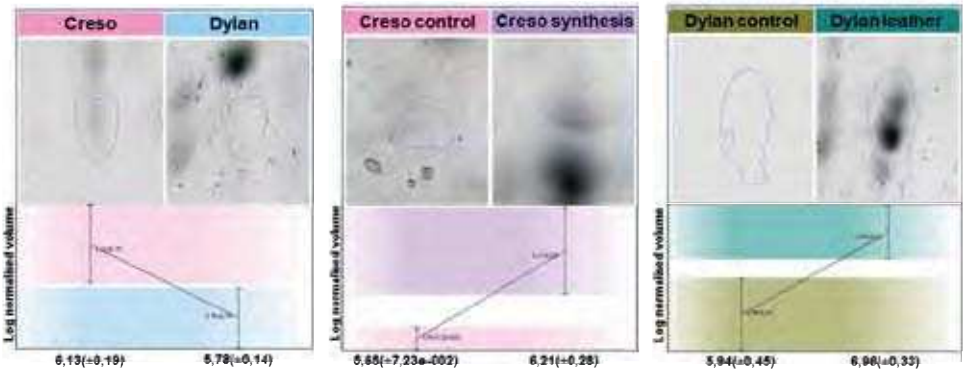

Figure 2. Examples of differential expressed spots: 1. Subgroup Creso – Dylan, 2. Subgroup Creso, 3. Subgroup Dylan.

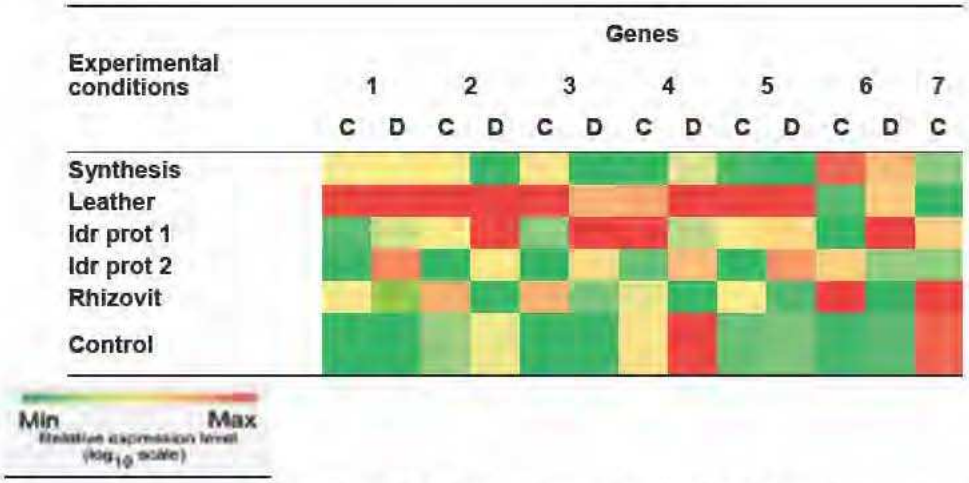

Figure 3. Pathway of nitrogen uptake for primer selection qPCR of the genes related. C=Creso; D=Dylan. 1. Nitrate transporter 2 (NRT 2); 2. Ferredoxin nitrite reductase (E.C. 1.7.7.1); 3. Low affinity nitrate transporter (NRT 1.2); 4. Glutamine synthetase isoform a (GS2a) (E.C. 6.3.1.2); 5. Nitrate transporter 2.6 (NRT 2.6); 6. Asparagine synthetase (AS) (E.C. 6.3.5.4); 7. Aspartate aminotransferase (AspAT) (E.C. 2.6.1.1).

IV – Conclusion and future perspectives

It clearly ensues from RT_PCR analysis, that treatment with leather induces both high and low affinity nitrate transporters and moreover this treatment, in general, induces the entire pathway of nitrogen uptake in both cultivars (Creso and Dylan). *Arabidopsis thaliana* NRT 1.2 (NTL1) represents one of the most highly expressed nitrate genes in shoots (Okamoto *et al.*, 2003).

Otherwise, the use of Rhizovit as nitrogen source seems to be responsible for the high expression levels of asparagine synthetase (AS) and aspartate aminotransferase (AspAT) in Creso cultivar. Rhizovit stimulates plant nitrogen uptake because it contains a microbial activator which stimulates the activity of microorganisms that transform nitrogen into a form easily taken up by plants. Works are in progress to identify those proteins of the nitrogen uptake pathway more influenced by treatments.

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